Cyclophosphamide Combined With Antithymocyte Globulin in Preparation for Allogeneic Marrow Transplants in Patients With Aplastic Anemia

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Graft rejection has been a problem after marrow grafts for patients with aplastic anemia who were conditioned with cyclophosphamide (CY). Rejection lessened when patients were given the marrow donor's peripheral blood buffy-coat cells in addition to the marrow, but this result was achieved at the price of more chronic graft-versus-host disease (GVHD). Results with second transplants suggested that CY alternating with antithymocyte globulin (ATG) was more immunosuppressive than CY alone. Therefore, the current study explored CY and ATG without buffy-coat cell transfusions in 39 patients with aplastic anemia given marrow transplants from HLA-identical family members (siblings in 38 cases, father in 1 case). We hoped both to minimize the risks of graft rejection and of chronic GVHD and to improve survival. Patients were 2 to 52 years of age (median, 24.5); 87% had received previous transfusions, and 41% had therapy with immunosuppressive agents before transplant. They were administered four daily doses of CY (total, 200 mg/kg) alternating with three doses of ATG (total, 90 mg/kg) followed by an HLA-identical marrow graft. Methotrexate and cyclosporine were administered to prevent GVHD. Two patients rejected their grafts (5%), and both were successfully retransplanted. Acute (grade 2 or 3) GVHD occurred in 15% and chronic GVHD in 34% of patients. The actuarial survival rate at 3 years was 92%, which compares favorably to the 72% survival rate in 39 historical patients who were matched with current patients for age and risk factors for rejection and GVHD. CY/ATG is a well-tolerated and effective conditioning program for marrow grafting in aplastic anemia that, when combined with GVHD prevention by methotrexate/cyclosporine, results in excellent survival. © 1994 by The American Society of Hematology.

The feasibility of treating severe aplastic anemia by marrow transplantation from HLA-identical sibling donors has now been well established, following initial reports in the 1970s. A major problem with transplants has been graft rejection, which was observed in 30% to 60% of patients treated before 1975. Rejection is thought to result from sensitization of patients to minor histocompatibility antigens of their donors through prior blood transfusions or perhaps also pregnancy. Early transplantation before transfusion has reduced the risk of graft rejection to 10%, and nearly 80% of untransfused patients survive. The fact remains that most transplant candidates have had transfusions, thereby incurring the risk of rejection. Different approaches to condition patients for transplantation have been explored in hopes of both reducing the incidence of graft rejection and increasing survival. All conditioning programs include the immunosuppressive agent cyclophosphamide (CY), but other features vary, including the use of total body irradiation, total lymphoid irradiation, and thoracoabdominal irradiation.

At the Fred Hutchinson Cancer Research Center, CY was used alone, and viable donor buffy-coat cells were infused along with the marrow since the donor's peripheral blood is a potential source of additional pluripotent hematopoietic stem cells and/or lymphoid cells capable of overcoming rejection. With many of these approaches, rejection has become less frequent and survival has increased. Most centers now report survival rates of 60% to 70%.

While effective in reducing the incidence of rejection, most conditioning programs have associated risks. Irradiation may cause late cancer and problems with growth, development, and fertility. The addition of buffy-coat cells increases the risk of chronic graft-versus-host disease (GVHD) to greater than 60%. Animal studies have shown synergistic immunosuppressive effects between antithymocyte globulin (ATG) and alkylating agents, including procarbazine and CY, as assessed by the criteria of skin graft prolongation and reduction of the risk of marrow graft rejection. Initial attempts at using an alternating regimen of procarbazine and ATG to prevent graft rejection gave mixed results. Smith et al reported a rejection incidence of 10% and survival rate of 61%, a result that was better than observed in their earlier patients. A randomized study performed at the Fred Hutchinson Cancer Research Center failed to show an advantage of a procarbazine/ATG-containing regimen over CY alone. However, in other studies, we had devised a regimen of CY alternating with ATG to prepare patients for second marrow transplants after rejection of the first transplants. The regimen was well-tolerated, and three quarters of the patients so treated had successful second grafts. This result suggested that the combination is more immunosuppressive than CY alone. Because of its apparent effectiveness, we now have used CY/ATG to condition patients with aplastic anemia for first transplant. In this way, we hoped to minimize the risk of graft rejection and to avoid the necessity for buffy-coat cell transfusions, thereby decreasing the risk of chronic GVHD. In the present study...

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we report the results among the first 39 patients conditioned with CY/ATG. Two patients rejected their graft; the cumulative incidence of chronic GVHD among surviving patients was 34%, and the 3-year actuarial survival was 92%.

**MATERIALS AND METHODS**

From July 13, 1988 to April 17, 1993, 39 consecutive patients were referred to Seattle and entered onto the study. Patient characteristics are listed in Table 1. Marrow donors were genotypically HLA-identical siblings in 38 cases, and a phenotypically HLA-identical father in one case.

Patients were referred after consultation with their physicians and admitted after outpatient and inpatient conferences that fully outlined the advantages and disadvantages of the transplant procedure. Protocols and consent forms were approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center.

Before transplantation, 38 patients received CY, 50 mg/kg, intravenously (IV) on each of 4 successive days.1,2 One patient had Fanconi anemia and was given CY 35 mg/kg per IV on each of 4 days.32 Twelve hours after the first, second, and third dose of CY, patients were also given ATG (Atgam; Upjohn, Kalamazoo, MI) at 30 mg/kg IV per dose infused over a period of 10 to 12 hours.12 Marrow was infused 36 hours after the last dose of CY. The day of marrow infusion was designated day 0.

Postgrafting immunosuppression consisted of methotrexate/cyclosporine.26 Methotrexate was administered at a dose of 15 mg/m² IV on day 1 and 10 mg/m² on days 3, 6, and 11 after transplant. Cyclosporine was begun on the day before transplant and was scheduled to be administered at a dose of 1.5 mg/kg IV every 12 hours until recovery from chemotherapy-induced gastrointestinal toxicity. At that time, cyclosporine was administered orally at 6.25 mg/kg every 12 hours. The full dose of cyclosporine was given until day 50, but it was reduced if renal toxicity developed. The dose was reduced by 50% if the serum creatinine value doubled above baseline values and was temporarily withheld if the creatinine value exceeded 2 mg/dL. After day 50, cyclosporine therapy was decreased by 5% per week and stopped 6 months after transplant.

**Table 1. Patient Data**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Current Patients</th>
<th>Historical Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients studied</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>Transplant year</td>
<td>1988-93</td>
<td>1980-88</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>24.5</td>
<td>25.3</td>
</tr>
<tr>
<td>Range</td>
<td>2-52</td>
<td>2-46</td>
</tr>
<tr>
<td>Sex (F/M) (no. of patients)</td>
<td>18/21</td>
<td>19/20</td>
</tr>
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<td>Possible causes of aplastic anemia (no. of patients)</td>
<td>29</td>
<td>33</td>
</tr>
<tr>
<td>Unknown</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Drug or chemical</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Dyskeratosis congenita</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fanconi anemia</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>No. of untransfused/transfused patients*</td>
<td>5/34</td>
<td>5/34</td>
</tr>
<tr>
<td>Preceding transfusions (U)</td>
<td>Patient positive</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Low†</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Moderate‡</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>High**</td>
<td>38</td>
</tr>
<tr>
<td>Refractory to random donor platelets (no. of patients)</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>History of treatment (no. of patients)§</td>
<td>Range</td>
<td>1.1-9.8</td>
</tr>
<tr>
<td>ATG</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Androgens</td>
<td>10</td>
<td>6</td>
</tr>
</tbody>
</table>

* Two of the five current patients, one of five previous untransfused patients, four of the multiply transfused current patients, and one multiply transfused previous patient had previously been pregnant.
† Exact data were available in 28 current and 29 previous patients; 11 current and 10 previous additional patients were reported to have multiple transfusions.
‡ Exact data were available in 26 current and 32 previous patients; 13 current and seven previous additional patients were reported to have multiple transfusions.
§ Overall, 16 (41%) current and 15 (38%) historical patients had treatment with ATG, cyclosporine, corticosteroids, or vincristine, given either alone or in combination.
¶ Statistically significantly longer in current patients than in historical patients (P = .027 by Wilcoxon rank-sum test).

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With eight exceptions, patients were treated in laminar airflow isolation rooms with skin and gut decontamination.

Documentation of hematopoietic engraftment and assessment, grading and treatment of acute and chronic GVHD were performed as described previously.1,2,3,5,6,7 Significant acute GVHD was treated according to protocols that usually included methylprednisolone, 2 mg/kg/d, either IV or orally in divided doses for a period of 7 to 14 days.34 Chronic GVHD was treated with methylprednisolone either alone or combined with cyclosporine.38 Marrow aspirates were usually performed on days 28, 56, 84, and 365 after transplant to assess the quality of engraftment. To determine the origin of the hematopoietic cells, cytogenetic studies and in situ hybridization using probes for repetitive DNA sequences on the X and Y chromosomes were performed on marrow and peripheral blood cells from the 19 patients in whom there was a sex difference with the donor. In additional patients, amplified genomic DNA segments containing a variable number of tandem repeats were tested to identify polymorphisms that distinguished the host and donor. Finally, 17 donor-recipient pairs had a RBC group difference, which served as a marker.

Results of the study were analyzed as of September 1993. In an effort to better understand the posttransplant experience of these patients, we compared current results with those of a group of historical patients. The historical patients were chosen from all previous aplastic anemia patients given HLA-identical marrow grafts in Seattle who received CY in preparation for marrow grafting along with postgrafting immunosuppression by methotrexate and cyclosporine.11 Eleven of the 39 current and 12 of the 39 historical patients received less than 100% of the calculated methotrexate dose, either because of renal or liver dysfunction or mucositis. For each study patient, one historical patient was chosen to be comparable in terms of age and known risk factors for graft rejection and GVHD. Factors included isolation in laminar airflow room,35 transfection status,5,12 and the combination of patient and donor sex and donor parity.13 Three current and three historical patients received prophylactic immunoglobulin infusions, and no patient in either group was given ganciclovir for cytomegalovirus infection prophylaxis. Sixty-two percent of the 39 historical patients received buffy-coat cell transfusions and 38% did not, either because they were untransfused or because they were children with pediatric donors who were not suitable for leukapheresis.13

Tables 1 and 2 list characteristics of the current and historical patients.

Survival, graft rejection, and chronic GVHD were the primary outcomes of interest. We also compared the incidence of acute GVHD in the two groups. Survival curves were plotted using the methods of Kaplan and Meier.29 Comparisons between these curves were based on the log-rank test.39 The probabilities of acute GVHD and of rejection were determined with cumulative incidence estimates.40 For some comparisons, Wilcoxon rank-sum tests were also used.

The probability of chronic GVHD at each time was calculated for patients who did not reject and who had not died of other causes by that time. Differences between the conditional probability curves were tested using the methods described by Pepe and Mori.45 Prevalence curves for chronic GVHD were calculated according to the method described by Pepe et al.45 The prevalence curves take into account not only the time of onset of chronic GVHD, but also the time of its resolution in response to therapy. The median follow-up duration was 2.5 years among current patients and 7.3 years among historical patients. Statistical comparisons between the two groups pertain to the first 3 years after transplant, because many current patients have been monitored for no longer than 3 years.

RESULTS

Table 1 lists the data before and at the time of transplant, and Table 2 lists the results after transplant. Three differences in the current patients compared with the historical controls were a significantly longer time from diagnosis to transplant, the addition of ATG to the conditioning program, and the absence of buffy-coat cell transfusions. ATG toxicity was limited to transient reactions in the form of skin rashes, fevers above 38.5°C, or arthralgias and myalgias in 22 of 39 current patients. No differences in infections after transplant

### Table 1. Marrow Transplantation

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Current Patients</th>
<th>Historical Patients</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients studied</td>
<td>39</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Follow-up of surviving patients (d)</td>
<td>Median</td>
<td>894</td>
<td>2,660</td>
</tr>
<tr>
<td>Range</td>
<td>304-1,847</td>
<td>1,312-4,031</td>
<td></td>
</tr>
<tr>
<td>Probability of marrow graft rejection</td>
<td>5.1%</td>
<td>7.7%</td>
<td>.961</td>
</tr>
<tr>
<td>Acute GVHD (no. of patients)</td>
<td>Grade 0</td>
<td>31 (79%)</td>
<td>28 (72%)</td>
</tr>
<tr>
<td>Grade 1</td>
<td>2 (5%)</td>
<td>3 (8%)</td>
<td>.641</td>
</tr>
<tr>
<td>Grade 2</td>
<td>3 (8%)</td>
<td>4 (10%)</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>3 (8%)</td>
<td>4 (10%)</td>
<td></td>
</tr>
<tr>
<td>Grade 4</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Probability of chronic GVHD at 3 years§</td>
<td>34%</td>
<td>6%</td>
<td>.058</td>
</tr>
<tr>
<td>3-year survival (Kaplan-Meier)</td>
<td>92%</td>
<td>72%</td>
<td>.043</td>
</tr>
<tr>
<td>Causes of death (no. of patients)</td>
<td>Early infection</td>
<td></td>
<td></td>
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<tr>
<td>Infection (≤21 d)</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Infection 21-100 days</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Interstitial pneumonia</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Chronic GVHD and infection</td>
<td>1#</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Graft rejection and infection</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Cardiopulmonary arrest</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Hospital stay (d)</td>
<td>Median</td>
<td>35</td>
<td>36</td>
</tr>
<tr>
<td>Range</td>
<td>24-105</td>
<td>9-94</td>
<td></td>
</tr>
</tbody>
</table>

* Log-rank test.
† Comparison of cumulative incidence curves.
‡ Acute GVHD grade 2 to 3 v 0 to 1.
§ Among patients who did not reject their grafts and who did not die from other causes.
∥ Comparison of conditional probability curves.
¶ One cytomegalovirus pneumonia, one idiopathic pneumonia, two mixed bacterial/fungal pneumonias.
# One mixed Pseudomonas aeruginosa/cytomegalovirus pneumonia.
** Wilcoxon rank-sum test.

The probability of chronic GVHD at each time was calculated for patients who did not reject and who had not died of other causes by that time. Differences between the conditional probability curves were tested using the methods described by Pepe and Mori.45 Prevalence curves for chronic GVHD were calculated according to the method described by Pepe et al.45 The prevalence curves take into account not only the time of onset of chronic GVHD, but also the time of its resolution in response to therapy. The median follow-up duration was 2.5 years among current patients and 7.3 years among historical patients. Statistical comparisons between the two groups pertain to the first 3 years after transplant, because many current patients have been monitored for no longer than 3 years.

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between current and historical patients were noted. The median hospital stay during the first 100 days after transplant was virtually identical among the two patient groups.

**Engraftment and graft rejection.** All 38 current and 37 historical patients who survived beyond the first 2 weeks showed evidence of hematopoietic engraftment as determined by rising peripheral blood counts, marrow cellularity, and blood genetic marker studies.

Two current patients rejected the marrow grafts, one during the first 2 months and one after 6 months. Both had successful retransplants from the same donors and are alive 1.5 and 2.5 years after the initial transplant.

Three historical patients rejected the graft. Two of these had successful second transplants after CY/ATG and one died of infection. The cumulative incidences of rejection for the two patient groups are 5.1% and 7.7%, respectively (P = .38) (Fig 1A).

**Acute GVHD.** Fifteen percent of the current patients developed grade 2 or 3 acute GVHD, compared with 20% of the historical patients, a difference that is not statistically significant (P = .64) (Fig 1B). No patient in either treatment group experienced grade 4 acute GVHD.

**Chronic GVHD.** Eleven current patients and 19 historical patients developed chronic GVHD. Five of the current patients are currently receiving therapy for chronic GVHD, although therapy is being tapered in two of these after having achieved a complete response. Five historical patients continue to receive therapy for chronic GVHD. The conditional probability of chronic GVHD was 34% in current versus 61% in historical patients, a difference that is strongly suggestive (P = .08; Fig 1C). The conditional probability of chronic GVHD in historical patients who received additional buffy-coat cell infusions was 68% (P = .06, compared with current patients). The prevalence curves describe both the time of onset of chronic GVHD and the time of its disappearance, along with discontinuation of its therapy (Fig 1D). Chronic GVHD was not only less frequent, but also appeared to be more responsive to therapy among current compared with historical patients. This explains the statistically significant difference (P = .043) between the prevalence curves for the two groups of patients, which began diverging at approximately 200 days after transplant.

**Survival.** The Kaplan-Meier estimate of survival was 92% in current patients compared with 72% among historical patients (Fig 2; P = .043).

**Causes of death.** Three of the current patients died. One, who had a 4-year history of aplastic anemia, died on day 5 with massive candidiasis, which preceded the transplant. The second patient had a 13-year history of dyskeratosis congenita and had a pneumonia of unknown etiology 2 years before transplant. At admission, he showed diffuse interstitial thickening of both lungs. He died on day 72 of fungal pneumonia. The third patient, who had both acute GVHD (grade 3) and progressive onset chronic GVHD, died on day 180 with a mixed *Pseudomonas aeruginosa* and cytomegalovirus pneumonia. By comparison, 11 historical patients died. One died on day 3 with cardiopulmonary arrest, one on day 12 with fungal brain lesions and hemorrhage, one on day 36 with idiopathic interstitial pneumonia, three with acute GVHD complicated by interstitial pneumonias on days 84, 99, and 134, respectively, one from infection on day 155 after graft rejection and unsuccessful second transplant, and four from infections complicating chronic GVHD on days 199, 300, 920, and 950, respectively.

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**Fig 1.** Cumulative incidence of (A) graft rejection and (B) developing acute GVHD in patients with aplastic anemia given HLA-identical marrow transplants and GVHD prophylaxis with methotrexate/cyclosporine. (C) Conditional probability and (D) prevalence of chronic GVHD are shown. Data are for 39 current patients conditioned with CY/ATG compared with 39 historical patients given CY alone. Sixty-two percent of the historical patients were given additional buffy-coat cell infusions. The conditional probability calculation describes the proportion of patients who have developed chronic GVHD among those who have not rejected their graft and who have not died from causes other than chronic GVHD.
Marrow grafts for aplastic anemia

Fig 2. Survival of patients with aplastic anemia given HLA-identical marrow transplant and postgrafting immunosuppression with methotrexate/cyclosporine. Shown are data in 39 current patients conditioned with CY/ATG compared with 39 historical patients conditioned with CY alone. Sixty-two percent of the historical patients were given additional donor buffy-coat cell infusions. Tick marks indicate surviving patients. Data were analyzed as of September 15, 1993.

DISCUSSION

Given the nature of the underlying disease, patients with aplastic anemia do not require marrow ablative therapy before allogeneic marrow transplantation. The sole function of the conditioning program is to provide the immunosuppression that enables the marrow to engraft. Cyclophosphamide is well suited for this purpose since it is a strong immunosuppressant and it lacks the adverse long-term side effects of radiation-containing regimens. The early experience using cyclophosphamide showed graft rejection to be a major problem with an incidence of 30% to 60%. Survival rates ranged from 30% to 45%. Animal studies and retrospective analyses of clinical data identified transfusion-induced sensitization to minor histocompatibility antigens expressed on donor marrow cells as the major cause of graft rejection, although, in a minority of patients, an underlying immune etiology of aplastic anemia may cause the transplant to fail. The obvious solution to the problem of rejection is to avoid transfusions whenever possible. After conditioning by CY, 90% of HLA-identical marrow grafts have been successful in untransfused patients, and close to 80% of these patients remain alive with normal hematopoietic function. Ten percent of grafts failed, either due to rejection or perhaps due to mechanisms related to the underlying disease.

For a variety of reasons, few patients come to transplantation before transfusions have been administered. For example, only five (13%) of the current patients were untransfused and, of these, two had previous pregnancies, another potential cause of sensitization to minor histocompatibility antigens. Given the difficulty in avoiding transfusions before transplant, three distinctly different approaches have been taken to reduce the risk of graft rejection.

The first approach involved an intensification of the CY conditioning program by adding either total body irradiation or limited-field irradiation that included most lymph node-bearing areas. The use of total body irradiation has been shown to reduce rejection, although at the expense of a dramatically increased mortality rate from GVHD and interstitial pneumonia. As a result, survival with total body irradiation-based regimens was poor. Limited-field irradiation regimens have improved survival rates compared with the early CY experience. The most recently published worldwide results with these alternate transplant regimens for aplastic anemia are shown in Table 3. Surveys by the European and International Bone Marrow Transplant Registries published between 1988 and 1992 reported survival rates of 62% to 63%, in both adult and pediatric patients. Despite the unequivocal success with the irradiation-based regimens, concern has been raised by a recent publication by Socie et al, which drew attention to a high incidence of secondary malignancies associated with these regimens. The estimated 22% incidence of cancer at 8 years in patients treated with the limited-field irradiation regimens far exceeds the 1.4% incidence at 10 years in patients treated with CY alone. Moreover, radiation-based regimens may interfere with fertility, growth, and development. Such interference is minimal in CY-treated patients.

The second approach is based on clinical observations that patients administered large numbers of marrow cells experience significantly less rejection than those receiving low numbers of marrow cells. This observation, together with the knowledge that peripheral blood contains circulating hematopoietic stem cells and also lymphocytes shown to enhance engraftment in experimental animals and in man, formed the rationale for infusing peripheral blood buffy-coat cells from the marrow donor after the transplant. This approach lessened the risk of rejection and increased survival to approximately 70%. While the incidence of acute GVHD remained unchanged, buffy-coat cell recipients experienced significantly more chronic GVHD than patients not given these cells.

The third approach is based on animal studies and involves depleting blood mononuclear cells from transfusions, a technique that is now widely used among blood centers. Mononuclear cells, in particular dendritic cells, may cause sensitization to minor histocompatibility antigens, thereby setting the stage for marrow graft rejection. Animal studies suggest that graft rejection cannot be completely eliminated by buffy-coat cell removal. Even with the best separation techniques, sufficient numbers of mononuclear cells remain in the transfusion products for some sensitization to occur. We have recently demonstrated that sensitization to minor histocompatibility antigens can almost completely be avoided by cesium irradiation of blood products. Whether this experimental finding can be extrapolated to man remains to be determined.

Because of the significant increase in chronic GVHD seen with the added viable buffy-coat cell infusions, we explored an alternative approach to increase immunosuppression without the use of irradiation. The success of CY and ATG in preparing patients with rejection for second transplants prompted us to use the same combination for first transplants. We reasoned that buffy-coat cell infusions could be deleted this way without increasing the risk of rejection. In turn, deletion of buffy-coat cells might reduce the risk of chronic GVHD, thereby improving survival.
Table 3. Worldwide Results of HLA-identical Marrow Grafts for Aplastic Anemia in the 1980s and 1990s

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Year of Report</th>
<th>Year of Transplant</th>
<th>No. of Patients</th>
<th>Age in Years (median)</th>
<th>Conditioning Regimen</th>
<th>GVHD Prevention</th>
<th>Rejection (%)</th>
<th>GVHD (%)</th>
<th>Surviving Patients (%)</th>
<th>Follow-Up (median)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBMTG—Bacigalupo et al[24]</td>
<td>1988</td>
<td>1981-88</td>
<td>218</td>
<td>1-50</td>
<td>CY ± TLI, TAI, or TBI</td>
<td>MTX or CSP</td>
<td>—</td>
<td>—</td>
<td>69</td>
<td>&lt;1-6 yr</td>
</tr>
<tr>
<td>Hows et al[22]</td>
<td>1989</td>
<td>1979-85</td>
<td>49</td>
<td>3-47 (22)</td>
<td>CY</td>
<td>CSP</td>
<td>17</td>
<td>50</td>
<td>37</td>
<td>69</td>
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<tr>
<td>Champlin et al[25]</td>
<td>1990</td>
<td>1984-88</td>
<td>29</td>
<td>0.7-41 (19)</td>
<td>CY + 300 cGy TLI</td>
<td>MTX/CSP</td>
<td>23</td>
<td>22</td>
<td>—</td>
<td>78</td>
</tr>
<tr>
<td>EBMTG—Locasciulli et al[26]</td>
<td>1990</td>
<td>1970-88</td>
<td>171</td>
<td>1-15</td>
<td>CY ± TLI, TAI, or TBI</td>
<td>MTX or CSP</td>
<td>—</td>
<td>—</td>
<td>63</td>
<td>1 mo-15 yr (4.5 yr)</td>
</tr>
<tr>
<td>IBMTR—Gluckman et al[18]</td>
<td>1992</td>
<td>1980-87</td>
<td>595</td>
<td>1-40</td>
<td>CY ± TLI, TAI, or TBI</td>
<td>MTX, CSP, or MTX/CSP</td>
<td>10</td>
<td>40</td>
<td>45</td>
<td>&gt;2-7 yr</td>
</tr>
</tbody>
</table>

Abbreviations: CY, cyclophosphamide; TBI, total body irradiation; PAPAPA, alternating days of procarbazine and antithymocyte globulin; TLI, total lymphoid irradiation; TAI, thoracoabdominal irradiation; ATG, antithymocyte globulin; MTX, methotrexate; CSP, cyclosporine; Pred, prednisone; EBMT, European Bone Marrow Transplant Group; IBMTR, International Bone Marrow Transplant Registry.
MARROW GRAFTS FOR APLASTIC ANEMIA

that was slightly lower than seen among the historical patients, 62% of whom had been given buffy-coat cell infusions. The patients who rejected were treated successfully with subsequent marrow grafts. While the graft rejection rate has remained low, chronic GVHD decreased from 61% in historical patients to 34% in current patients, and the response to treatment for chronic GVHD appeared to be improved. Only one current patient died from complications associated with chronic GVHD, compared with four deaths with chronic GVHD among historical patients. It is not known whether subtle changes in supportive care may also have contributed to the improvement in the survival rate from 72% to 92%. Because of the historically low incidence of fatal cytomegalovirus pneumonias in CY-conditioned marrow graft recipients, current patients did not receive cytomegalovirus prophylaxis (or treatment) with ganciclovir.

Forty-one percent of current and 38% of historical patients were first given prolonged immunosuppressive therapy before marrow transplantation was considered. The elapsed time from diagnosis to transplant was significantly longer in current compared with historical patients. Fungal infections acquired before the transplant caused the deaths of two current patients and of one historical patient. These deaths might have been avoided by proceeding to transplant as soon as the diagnosis of aplastic anemia was made and an HLA-identical donor identified. The current finding of 92% survival with transplantation emphasizes that immunosuppressive treatments with corticosteroids, ATG, or cyclosporine before transplant are no longer justifiable in patients 55 years of age or younger who have suitable marrow donors. Also, treatment of aplastic anemia with hematopoietic growth factors, unsuccessfully tried in five of the current patients, has been of only limited benefit.55-60 Prolonged delay in referral time from diagnosis to transplant was significantly longer in current patients and of one historical patient. These deaths might have been avoided by proceeding to transplant

by marrow grafting. The incidence of acute GVHD in both current and historical patients was low, on the order of 15% to 20%, owing to postgrafting immunosuppression with methotrexate and cyclosporine. More time is needed to determine whether the current conditioning regimen carries the same low risk of long-term sequelae as previously described for CY alone.

NOTE ADDED IN PROOF

By mid-May 1994, 45 patients have been transplanted with the CY/ATG regimen, and 42 of the patients are alive with functioning grafts between 2.5 months and 5.9 years (median 3 years) after transplant. One patient died of respiratory syncytial virus pneumonia.

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Cyclophosphamide combined with antithymocyte globulin in preparation for allogeneic marrow transplants in patients with aplastic anemia

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