Long-Term Follow-Up of Allogeneic Marrow Transplants in Patients With Aplastic Anemia Conditioned by Cyclophosphamide Combined With Antithymocyte Globulin

To the Editor:

In 1994 we reported on a novel regimen consisting of cyclophosphamide (CY) combined with antithymocyte globulin (ATG) to condition 39 patients with aplastic anemia for marrow transplants from HLA-identical family members.[1] The regimen was patterned after a successful second transplant regimen[2] that, in turn, was developed on the basis of animal studies showing synergistic immunosuppression when an alkylating agent was alternated with ATG.[3] The regimen's purpose was to reduce the risk of marrow graft rejection in aplastic anemia patients, most of whom had been previously transfused. Two of the 39 patients rejected their grafts, and both were successfully retransplanted. Three patients died. One, who had a 4-year history of aplastic anemia, died on day 5 with massive candidiasis that preceded the transplant. The second, who had a 13-year history of dyskeratosis congenita and a 2-year history of pneumonia, died on day 72 with pneumonia. The third, who had chronic graft-versus-host disease (GVHD), died on day 180 with a mixed *Pseudomonas aeruginosa* and cytomegalovirus pneumonia. Actuarial survival at 3 years was 92%, which compared favorably with the 72% survival rate in 39 historical patients conditioned with CY only who were matched for age and risk factors for rejection of GVHD. The current update with follow-up ranging from 3.2 to 8.2 years among surviving patients shows that the early survival advantage has persisted.

Thirty-nine consecutive patients were entered onto study from July 13, 1988 to April 17, 1993, and the results were analyzed as of January 1997. Patient ages ranged from 2 to 52 years (median, 24.5 years), 87% of the patients had been previously transfused, the median time from diagnosis to transplant was 2.4 months, and 41% of transplanted patients had previous unsuccessful treatment of their aplastic anemia by immunosuppressive therapy. Patients were conditioned for transplant by CY, 50 mg/kg intravenously on each of 4 successive days and, after the first, second, and third dose of CY, patients received ATG at 30 mg/kg intravenously per dose. Thirty-six hours after the last dose of CY, marrow was infused. Immunosuppression for prevention of GVHD consisted of a short course of methotrexate and at least 180 days of cyclosporine, as previously described.[4] For each CY/ATG-treated patient, 1 historical patient receiving CY alone was chosen to be comparable in terms of age, laminar airflow room isolation, postgrafting immunosuppression, transfusion status, and the combination of patient and donor sex and donor parity. A major difference between the two groups of patients was that 62% of the historical patients received buffy coat cell infusions in addition to the marrow in an attempt at reducing the previously observed high graft rejection rate in multiply transfused individuals.[5] Although this approach was successful with regard to rejection, patients receiving buffy coat cell infusions experienced a higher than usual incidence of chronic GVHD.[6]

Graft rejection was seen in 5% of current and 8% of historical patients ($P = .96$), and acute GVHD was seen in 15% of current and 20% of historical patients ($P = .64$). Figure 1 updates the data on chronic GVHD and on survival. The prevalence curves for chronic GVHD describe both the times of onset of this complication and of its disappearance, along with discontinuation of its therapy. 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 ($P = .01$) between the prevalence curves for the two groups of patients, which began diverging at approximately 6 months after transplant. At 4 years after transplantation, 8% of current patients compared with 21% of historical patients still required treatment for chronic GVHD. The Kaplan-Meier estimate of survival at 8 years posttransplant was 92% in current patients compared with 72% among historical patients ($P = .02$).

Since the original report, 16 additional patients have been transplanted with this protocol. None of the 16 rejected the graft. Three patients died. One of them died on day 83 from respiratory syncytial virus pneumonia during a community based epidemic. The second died on day 210 from bilateral idiopathic pneumonia. The third died on day 223 from septicemia secondary to GVHD of gut and liver. Overall survival among the 55 patients was 89% at 8 years.

In conclusion, the CY/ATG regimen, when combined with postgrafting immunosuppression by methotrexate/cyclosporine, was well tolerated and was accompanied by low incidences of graft rejection and acute and chronic GVHD. The survival rate was 92%, a result that was significantly better than the 72% survival of a cohort of historical patients who were conditioned with CY alone and, in many cases, received viable donor buffy coat cell transfusions in addition to the marrow graft to reduce the risk of graft rejection. With a follow-up of up to 8.2 years (median, 5.2 years), no unusual long-

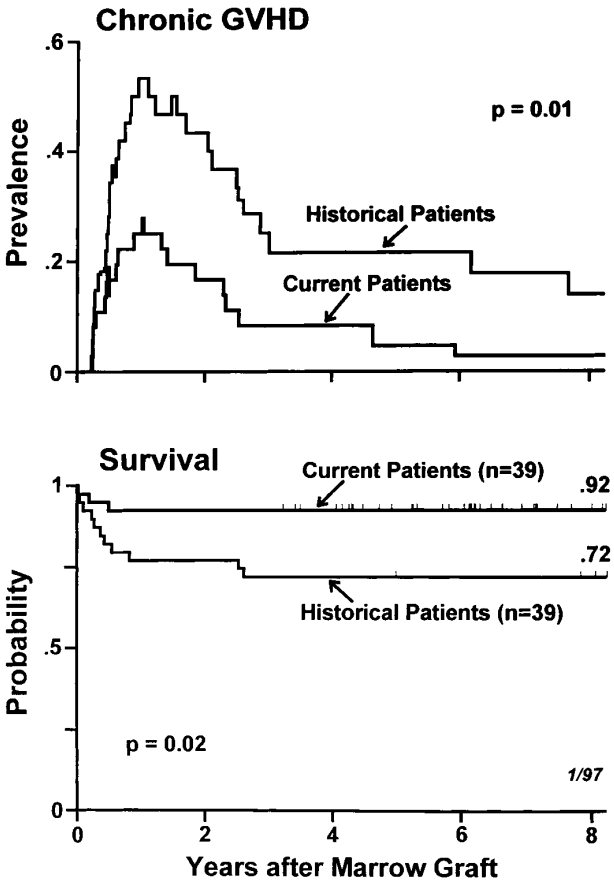

Fig 1. Prevalences of chronic GVHD (top) and Kaplan-Meier estimates of survival (bottom) in 78 patients with aplastic anemia receiving HLA-identical marrow transplants and GVHD prophylaxis with methotrexate/cyclosporine, 39 of whom were conditioned with CY/ATG (current patients) and 39 received CY alone (historical patients). Tick marks indicate surviving patients.

term sequelae from the CY/ATG regimen have been seen among surviving patients.

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REFERENCES

1. Storb R, Etzioni R, Anasetti C, Appelbaum FR, Buckner CD, Bensinger W, Bryant E, Clift R, Deeg HJ, Doney K, Flowers M, Hansen J, Martin P, Pepe M, Sale G, Sanders J, Singer J, Sullivan KM, Thomas ED, Witherspoon RP: Cyclophosphamide combined with antithymocyte globulin in preparation for allogeneic marrow transplants in patients with aplastic anemia. Blood 84:941, 1994

2. Storb R, Weiden PL, Sullivan KM, Appelbaum FR, Beatty P, Buckner CD, Clift RA, Doney KC, Hansen J, Martin PJ, Sanders JE, Stewart P, Witherspoon RP, Thomas ED: Second marrow transplants in patients with aplastic anemia rejecting the first graft: Use of a conditioning regimen including cyclophosphamide and antithymocyte globulin. Blood 70:116, 1987

3. Storb R, Floersheim GL, Weiden PL, Graham TC, Kolb H-J, Lerner KG, Schroeder M-L, Thomas ED: Effect of prior blood transfusions on marrow grafts: Abrogation of sensitization by procarbazine and antithymocyte serum. J Immunol 112:1508, 1974

4. Storb R, Deeg HJ, Farewell V, Doney K, Appelbaum F, Beatty P, Bensinger W, Buckner CD, Clift R, Hansen J, Hill R, Longton G, Lum L, Martin P, McGuffin R, Sanders J, Singer J, Stewart P, Sullivan K, Witherspoon R, Thomas ED: Marrow transplantation for severe aplastic anemia: Methotrexate alone compared with a combination of methotrexate and cyclosporine for prevention of acute graft-versus-host disease. Blood 68:119, 1986

5. Storb R, Doney KC, Thomas ED, Appelbaum F, Buckner CD, Clift RA, Deeg HJ, Goodell BW, Hackman R, Hansen JA, Sanders J, Sullivan K, Weiden PL, Witherspoon RP: Marrow transplantation with or without donor buffy coat cells for 65 transfused aplastic anemia patients. Blood 59:236, 1982

6. Storb R, Prentice RL, Sullivan KM, Shulman HM, Deeg HJ, Doney KC, Buckner CD, Clift RA, Witherspoon RP, Appelbaum FR, Sanders JE, Stewart PS, Thomas ED: Predictive factors in chronic graft-versus-host disease in patients with aplastic anemia treated by marrow transplantation from HLA-identical siblings. Ann Intern Med 98:461, 1983

Improved Reconstitution of CD4 T Cells and B Cells But Worsened Reconstitution of Serum IgG Levels After Allogeneic Transplantation of Blood Stem Cells Instead of Marrow

To the Editor:

In a recent issue of *Blood*, Ottinger et al[1] described that the numbers of circulating CD4 T cells and B cells are higher after peripheral blood stem cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). We wish to present data that support the findings of Ottinger et al and to add new findings on B-cell immunity, including the surprising finding of decreased serum IgG levels after PBSCT compared with BMT.

Blood from 57 HLA-identical sibling PBSC (n = 26) or BM (n = 31) transplant recipients was studied at approximately 2 months after grafting. All patients had a hematologic malignancy and were conditioned with high-dose chemotherapy and/or radiation (usually cyclophosphamide [120 mg/kg] with busulfan [16 mg/kg] or fractionated total body radiation [13.2 Gy]). PBSCs were obtained from donors after mobilization with filgrastim (15 to 30 μg/kg/d subcutaneously for 4 days). The PBSC and BM grafts were not cryopreserved or depleted of any subset of nucleated cells. Graft-versus-host disease prophylaxis usually consisted of methotrexate and cyclosporine.[2] All patients underwent a thorough work-up to determine disease status on approximately day 80. Relapse was diagnosed in 7 of the 57 patients; these patients were excluded from analysis. One other patient was excluded because only 25% of his day 80 marrow nucleated cells were of donor origin by variable nucleotide tandem repeats (VNTR).[3] The remaining 49 patients (20 PBSC and 29 BM recipients) had greater than 90% marrow or blood nucleated cells of donor origin by VNTR or Y-chromosome in situ hybridization.[4] Demographic and clinical characteristics of the 49 patients analyzed are given in Table 1.

Because at least some lymphocytes after grafting are derived from the mature lymphocytes transferred with the graft,[5] we also quantified lymphocyte subpopulations in 18 PBSC and 15 BM grafts. For each lymphocyte subset the ratio of median number of cells in a PBSC graft to median number of cells in a BM graft was calculated to show how much more cells of each subset are typically transferred with a PBSC graft compared with a BM graft.

The enumeration of lymphocyte subsets in blood was performed by three-color flow cytometry as described.[6,7] Lymphocyte subsets in the grafts were detected analogically, except for initial (forward $\times$ side scatter) gating on all nucleated cells rather than mononuclear cells; the absolute count of each lymphocyte subset was calculated as the total nucleated cell count multiplied by the proportion of these cells. Serum IgG levels were determined by standard nephelometry.

T cells. As displayed in Table 2, PBSC recipients received greater than 11 times higher numbers of naive and memory CD4 T cells and greater than 7 times higher numbers of CD8 T cells than BM recipients. At 2 months after grafting, circulating naive CD4 T cells were approximately 6 times higher, whereas memory CD4 T cells were only slightly (1.6 times) higher after PBSCT than after BMT, and CD8 T cells were similar in both patient groups. The discrepancy in the PBSC/BM ratio of memory CD4 T cells and CD8 T cells in the grafts versus in the blood after transplantation remains to be explained. It could be due to increased propensity of PBSC graft-derived memory CD4 T cells and CD8 T cells to die,[8] due to