

## CONCISE REPORT

# Total Lymphoid Irradiation and Cyclophosphamide as Preparation for Bone Marrow Transplantation in Severe Aplastic Anemia

By Norma K. C. Ramsay, Taehwan Kim, Mark E. Nesbit, William Krivit, Peter F. Coccia, Seymour H. Levitt, William G. Woods, and John H. Kersey

**A new combination of total lymphoid irradiation and cyclophosphamide was used prior to bone marrow transplantation in an attempt to achieve decreased rejection rates and graft-versus-host disease. Nine previously transfused patients with severe aplastic anemia received marrow from an HLA-identical, MLC-compatible sibling following this preparative regimen. There were no episodes of graft rejection, and only one patient developed graft-versus-host disease. Of the 9 patients, 7 (78%) are surviving with a median follow-up of 400 days. The excellent results of this pretransplant combination of total lymphoid irradiation and cyclophosphamide warrants application of this regimen to a larger series of patients.**

**B**ONE MARROW transplantation is the therapy of choice for patients with severe aplastic anemia who have matched donors. Major obstacles to allogeneic marrow grafting are graft rejection and graft-versus-host disease. The incidence of graft rejection is reported to be from 25% to 60% in various centers using preconditioning with the most frequently utilized regimen, cyclophosphamide alone.<sup>1-4</sup> Total body irradiation in combination with other agents prior to transplantation is associated with decreased graft rejection, however, significant morbidity is experienced with the use of total body irradiation.<sup>4</sup> Graft-versus-host disease continues to occur in 25%–50% of patients who receive bone marrow transplants for aplastic anemia.<sup>1,2</sup>

Selective irradiation of the lymphoid system has been reported to provide excellent conditioning for bone marrow grafting in mice, rats, and dogs with very low rates of rejection and no graft-versus-host disease.<sup>5-7</sup> Based on these data, we developed a new method of pretransplant immunosuppression using total lymphoid irradiation combined with cyclophosphamide for patients with aplastic anemia. The main objective of this regimen was to reduce the rejection rate seen with cyclophosphamide alone without increasing the morbidity experienced with total body irradiation.

We describe the results of nine patients who received total lymphoid irradiation combined with

cyclophosphamide prior to transplantation for severe aplastic anemia at the University of Minnesota. The results indicate reduced graft rejection and graft-versus-host disease from previously reported experience.

## MATERIALS AND METHODS

Nine consecutive patients with severe aplastic anemia, using the criteria of the International Aplastic Anemia Study Group,<sup>8</sup> were transplanted at the University of Minnesota Hospitals from 1977 to early 1979. The patients ranged in age from 1 to 18 yr, with a median of 11 yr. Seven patients had idiopathic aplastic anemia, one patient had Fanconi anemia, and one patient had posthepatic aplasia. The duration of disease prior to transplantation was 1–8 mo, with a median of 2 mo. All patients had received multiple transfusions from non-family donors prior to transplantation.

The preparative regimen for transplantation included 4 days of cyclophosphamide, 50 mg/kg/day intravenously (days –6, –5, –4, –3), a day of rest (day –2), and total lymphoid irradiation (day –1) followed by marrow infusion (day 0). The radiation field shown in Fig. 1, was essentially the same field that is used for treatment of patients with Hodgkin disease and includes all major lymphoid organs, including the thymus, spleen, and lymph nodes. The dose of radiation was 750 rads administered at 26 rads/min in a single dose through anterior and posterior fields using a 4 MeV linear accelerator.<sup>9</sup>

The marrow donor was a sibling matched at the major histocompatibility complex in all 9 cases. The marrow cell dose was 2.4–5.2 × 10<sup>8</sup> cells/kg with a median dose of 3.9 × 10<sup>8</sup> cells/kg. Following transplantation, all patients were randomized to receive one of two regimens of prophylaxis for graft-versus-host disease. Six patients received methotrexate, 15 mg/sq m intravenously (i.v.) day 1, 10 mg/sq m i.v. days 3, 6, 11, and weekly to 100 days, and 3 patients received identical methotrexate therapy in combination with prednisone 40 mg/sq m orally days 7–21 and Minnesota antithymocyte globulin 15 mg/kg i.v. every other day × 7 doses starting on day 8.

## RESULTS

Of the nine patients who received the cyclophosphamide–total lymphoid irradiation combination prior to transplantation of matched sibling marrow, 7 (78%) are alive from >200 to >650 days, with a median follow-up of 400 days as of August 1979 (Fig. 2). Two patients died. One patient had *Candida albicans* sepsis

---

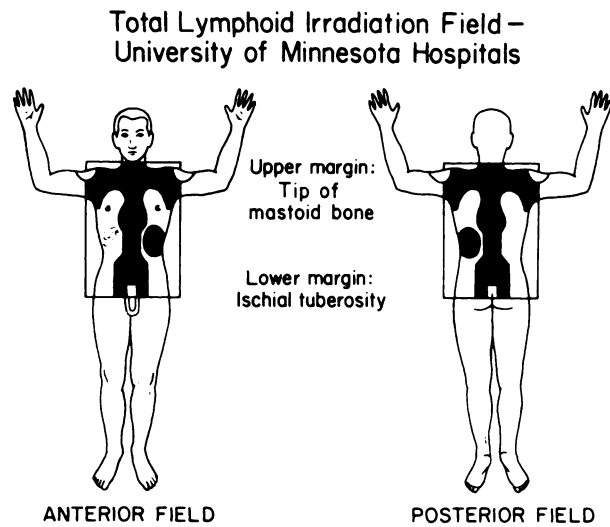
*From the Departments of Pediatrics, Therapeutic Radiology, and Laboratory Medicine and Pathology, University of Minnesota Health Sciences Center, Minneapolis, Minn.*

*Supported in part by USPHS Grants CA 19527, CA 21737, CA 15548, HL 5T32, and HL 07145.*

*Submitted August 24, 1979; accepted October 2, 1979.*

*Address reprint requests to Norma K. C. Ramsay, M.D., Department of Pediatrics, University of Minnesota, Box 366, Mayo Memorial Bldg., Minneapolis, Minn. 55455.*

© 1980 by Grune & Stratton, Inc.  
0006-4971/80/5502-0002\$01.00/0

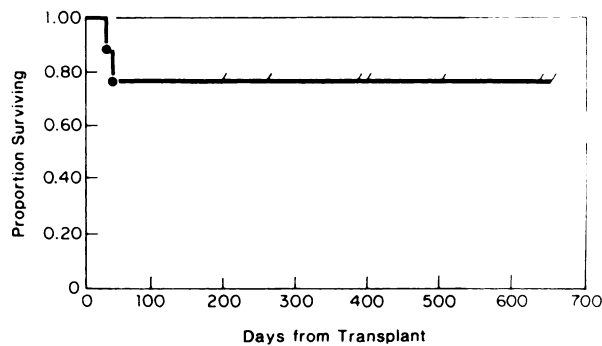


**Fig. 1.** Radiation field of total lymphoid irradiation for pretransplant conditioning for severe aplastic anemia. Black areas are irradiated and include major lymphoid organs, e.g., thymus, spleen, lymph nodes. White areas in the rectangular field are shielded.

prior to transplantation and died 13 days posttransplant from *Candida* infection. The second death occurred at 20 days in the patient with Fanconi anemia who developed sepsis and acute graft-versus-host disease. The surviving patients had prompt engraftment with donor cells, confirmed by utilizing red cell and chromosome markers.

Marrow rejection did not occur in any of the patients. In our previous group of 10 matched patients who received cyclophosphamide alone or cyclophosphamide in combination with other agents, rejection occurred in 6 of 10.<sup>4</sup> With the exception of the one patient with Fanconi anemia, who developed acute graft-versus-host disease, no other patients developed any evidence of acute or chronic graft-versus-host disease.

The morbidity associated with the regimen included stomatitis in one patient, interstitial pneumonia that



**Fig. 2.** Survival curve of 9 patients with severe aplastic anemia who received bone marrow transplants following cyclophosphamide and total lymphoid irradiation.

resolved in one patient, and four episodes of sepsis. The surviving patients have normal hematologic values.

**DISCUSSION**

Bone marrow transplantation is accepted therapy for treatment of patients with severe aplastic anemia with matched donors. Graft rejection, infection, and graft-versus-host disease remain areas of significant morbidity and mortality.<sup>1-4</sup>

Cyclophosphamide, when utilized alone for pretransplant immunosuppression, has been associated with a high bone marrow rejection rate, especially in patients that have received previous transfusions,<sup>10</sup> as in the group reported here, with retransplantation unsuccessful in most cases.<sup>11</sup> The need for further immunosuppression to reduce this rejection rate has led to the addition of agents, such as procarbazine and antithymocyte globulin, without significant improvement.<sup>12</sup> Patients with aplastic anemia and malignancies who receive total body irradiation as part of their pretransplant immunosuppression have an extremely low rejection rate; however, morbidity in this group is significant.<sup>13</sup>

We elected to use total lymphoid irradiation with cyclophosphamide as pretransplant immunosuppression in an attempt to take advantage of the immunosuppressive qualities of irradiation, while sparing organs associated with significant morbidity (lung, oral pharynx, etc.) in patients receiving total body irradiation. Morbidity was minimal with the present regimen. No patient who received matched sibling marrow following total lymphoid irradiation and cyclophosphamide experienced graft rejection. Radiation may, of course, be associated with late effects a number of years posttransplant and long-term follow-up is necessary. We are interested in the possibility that some patients may not require irradiation and a recent report suggests that nontransfused patients may be successfully grafted following conditioning with cyclophosphamide alone.<sup>10</sup>

The low incidence of graft-versus-host disease in our matched patients is encouraging and is lower than that reported in patients prepared for transplantation using cyclophosphamide alone or with other agents.<sup>1,2,4</sup> Slavin et al., have reported that total lymphoid irradiation when given in fractionated doses results in a low incidence of graft-versus-host disease in mice that receive allogeneic bone marrow.<sup>7</sup> The mechanism responsible for the low incidence of graft-versus-host disease in the animal experiments is in part due to active suppression of graft-versus-host reactive cells. Studies are presently underway to determine whether a suppressive mechanism is responsible for the low

incidence of graft-versus-host disease in our patient population.

In conclusion, this new pretransplant immunosuppressive regimen has resulted in a low incidence of graft rejection and graft-versus-host disease with minimal morbidity in a high-risk group of patients. The present survival is 78% for patients who received marrow from matched sibling donors and warrants

application to a larger group of patients with aplastic anemia.

#### ACKNOWLEDGMENT

The authors want to acknowledge the excellent nursing care provided by Sharon Roell, R.N., Jeanette Mefford, R.N., and the transplant nurses, and the secretarial assistance of Maureen Zielinski.

#### REFERENCES

1. Storb R, Thomas ED, Weiden PL, Buckner CD, Clift RA, Fefer A, Fernando LP, Giblett ER, Goodell BW, Johnson FL, Lerner KG, Neiman PE, Sanders JE: Aplastic anemia treated by allogeneic bone marrow transplantation: A report on 49 new cases from Seattle. *Blood* 48:817, 1976
2. U.C.L.A. Bone Marrow Transplant Team: Bone marrow transplantation in severe aplastic anemia. *Lancet* 2:921, 1976
3. Gluckman E, Devergie A, Marty M, Bussel A, Rottembourg J, Dausset J, Bernard J: Allogeneic bone marrow transplantation in aplastic anemia: Report of 25 cases. *Transplant Proc* 10:141, 1978
4. Kersey JH, Kim T, Levitt SH, Krivit W, Nesbit M, Coccia P, Warkentin P, O'Leary M, Ramsay NKC: Combined immunosuppression using cyclophosphamide plus total lymphoid irradiation in preparation for allogeneic marrow transplantation in humans, in Thierfelder S (ed): *Immunobiology of Bone Marrow Transplantation*. New York, Springer-Verlag, 1979
5. Slavin S, Strober S, Fuks Z, Kaplan HS: Induction of specific tissue transplantation tolerance using fractionated total lymphoid irradiation in adult mice: Long-term survival of allogeneic bone marrow and skin grafts. *J Exp Med* 146:34, 1977
6. Slavin S, Reitz B, Bieber CP, Kaplan HS, Strober S: Transplantation tolerance in adult rats using total lymphoid irradiation: Permanent survival of skin, heart and marrow allografts. *J Exp Med* 147:700, 1978
7. Slavin S, Fuks Z, Kaplan HS, Strober S: Transplantation of allogeneic bone marrow without graft-versus-host disease using total lymphoid irradiation. *J Exp Med* 147:963, 1978
8. Camitta BM, Thomas ED, Nathan DG, Santos G, Gordon-Smith EC, Gale RP, Rapoport JM, Storb R: Severe aplastic anemia: A prospective study of the effect of early marrow transplantation on acute mortality. *Blood* 48:63, 1976
9. Kim TH, Kersey J, Khan FM, Sewchand W, Ramsay N, Krivit W, Coccia P, Levitt SH: Single dose total lymphoid irradiation combined with cyclophosphamide as immunosuppression for human marrow transplantation in aplastic anemia. *Int J Radiat Oncol Biol Phys* 5:993, 1979
10. Storb R, Thomas ED, Buckner CD, Clift RA, Deeg J, Fefer A, Goodell BU, Sale GE, Sanders J, Singer J, Stewart P, Weiden PL: Marrow transplantation in 30 "untransfused" patients with severe aplastic anemia. *Ann Intern Med* (in press)
11. Wright SE, Thomas ED, Buckner CD, Clift RA, Fefer A, Neiman PE, Storb R: Experience with second marrow transplants. *Exp Hematol* 4:221, 1976
12. Weiden PL, Storb R, Thomas ED, Graham TC, Lerner KG, Buckner CD, Fefer A, Neiman PE, Clift RA: Preceding transfusions and marrow graft rejection in dogs and man. *Transplant Proc* 8:551, 1976
13. Gale RP for U.C.L.A. Bone Marrow Transplant Team: The role of cyclophosphamide and total-body irradiation in marrow transplantation for severe aplastic anemia, in Baum S, Ledney G (eds): *Experimental Hematology Today 1978*. New York, Springer-Verlag, 1978, pp 157-162