Treatment of Non-Hodgkin's Lymphoma With Marrow Transplantation in Identical Twins

By Frederick R. Appelbaum, Alexander Fefer, Martin A. Cheever, C. Dean Buckner, Philip D. Greenberg, Henry G. Kaplan, Rainer Storb, and E. Donnall Thomas

Eight patients with disseminated non-Hodgkin's lymphoma who failed conventional combination chemotherapy were treated with high-dose chemotherapy, a supralethal dose of total-body irradiation, and a bone marrow transplant from a normal identical twin. Seven patients experienced complete remissions. Four of the seven patients (two with diffuse poorly differentiated lymphocytic lymphoma, one with composite lymphoma, and one with diffuse moderately well differentiated lymphocytic lymphoma) remain in complete unmaintained remission 12–126 mo from transplantation. One patient relapsed after 10 mo but was retreated and is alive in unmaintained complete remission 73 mo from transplantation. One patient died of Pseudomonas pneumonia while in complete remission and one patient relapsed and died of progressive lymphoma. These results demonstrate that intensive chemoradiotherapy and twin marrow transplantation can induce frequent and enduring remissions in patients with disseminated non-Hodgkin's lymphoma who have failed conventional therapy.

Patients with disseminated non-Hodgkin's lymphoma (NHL) who fail conventional combination chemotherapy are rarely cured of their disease. The availability of normal identical twin marrow for transplantation provides the opportunity for aggressive chemoradiotherapy without regard to marrow toxicity. Based on this concept, patients with acute leukemia resistant to chemotherapy have been treated with high-dose chemotherapy, supralethal total-body irradiation, and marrow transplantation from a normal genetically identical twin.1–3 The results demonstrated that such therapy can cure some patients with acute leukemia considered to be in the end-stages of their disease.

To determine if a similar approach would benefit patients with disseminated NHL who had failed conventional combination chemotherapy, eight such patients were treated with high-dose chemoradiotherapy and twin marrow transplantation. This report presents the therapeutic results and toxicities observed in these patients.

MATERIALS AND METHODS

The clinical characteristics of the patients are presented in the case histories and Table 1. Identity between twins was established by physical resemblance, by HLA typing, and mixed leucocyte culture tests, by erythrocyte antigen typing and erythrocyte enzyme electrophoretic determinations, and, when available, by pathologic reports of the placenta.

All risks of the treatment protocol were fully explained to patients, donors, and relatives. Informed consent was obtained using forms approved by the Human Subjects Review Committee of the University of Washington or the Fred Hutchinson Cancer Research Center.

The technique of marrow transplantation has been described previously.4 Preparation for engraftment varied according to the protocol in use at the time of transplantation, but usually included high-dose cyclophosphamide and total-body irradiation from opposing cobalt-60 sources. The complete transplant regimens are included in Table 2.

After marrow transplantation, 4 of the 8 patients received immunotherapy as part of a clinical trial. The treatment consisted of the transfusion of normal twin buffy-coat cells in all 4 cases and injections of killed autologous tumor cells in 2 of the 4 cases by methods previously described.5

During the period of pancytopenia, all patients were managed with conventional hospital reverse isolation, except cases 1 and 2 who were managed in laminar air flow rooms. Platelet transfusions were given to maintain the platelet count above 20,000/cu mm. Blood products from nontwin donors were always irradiated with 1500 rad to avoid a possible graft-versus-host reaction. No antitumor maintenance chemotherapy was administered after transplantation.

CASE REPORTS

Cases 1 and 7 have been included in previous reports.5,6 Their pathologic material has been reexamined and classified according to the system recommended by Rappaport.3

Case 1 (Unique Patient Number [UPN] 50)

In February 1969, a 19-yr-old man developed hip pain due to diffuse poorly differentiated lymphocytic lymphoma. Local radiotherapy, chlorambucil, and prednisone relieved the pain. Hypercalcemia then developed and therapy was switched to methotrexate, vincris-
Table 1. Pretransplant Clinical Characteristics

<table>
<thead>
<tr>
<th>Unique Patient Number</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Duration of Disease</th>
<th>Previous Therapy†</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>19</td>
<td>M</td>
<td>DPDLL</td>
<td>14 mo</td>
<td>CHLOR, PRED, Mtx, VCR, Local RT</td>
</tr>
<tr>
<td>846</td>
<td>30</td>
<td>M</td>
<td>CL</td>
<td>6 mo</td>
<td>CY, VCR, PRED, PRO, BLEO, ADR</td>
</tr>
<tr>
<td>1001</td>
<td>21</td>
<td>M</td>
<td>DPDLL</td>
<td>7 mo</td>
<td>CY, VCR, PRED, PRO, DNR, L-ASP</td>
</tr>
<tr>
<td>1143</td>
<td>51</td>
<td>F</td>
<td>DMWDLL</td>
<td>20 mo</td>
<td>CY, VCR, PRED, ADR, BLEO, CHLOR</td>
</tr>
<tr>
<td>380</td>
<td>16</td>
<td>M</td>
<td>DPDLL</td>
<td>14 mo</td>
<td>CY, VCR, PRED, PRO, Local RT</td>
</tr>
<tr>
<td>534</td>
<td>36</td>
<td>M</td>
<td>NPDLL</td>
<td>57 mo</td>
<td>CY, VCR, PRED, VEL, BLEO, ADR, Local RT</td>
</tr>
<tr>
<td>276</td>
<td>10</td>
<td>M</td>
<td>DML</td>
<td>19 mo</td>
<td>HN₂, VCR, PRED, CY, Local RT</td>
</tr>
<tr>
<td>1017</td>
<td>44</td>
<td>M</td>
<td>DPDLL</td>
<td>10 mo</td>
<td>CY, VCR, PRED, ADR, BLEO, VEL, DTIC</td>
</tr>
</tbody>
</table>

*DPDLL denotes diffuse poorly differentiated lymphocytic lymphoma; CL, composite lymphoma; DMWLL, diffuse moderately-well differentiated lymphocytic lymphoma; NPDLL, nodular poorly differentiated lymphocytic lymphoma; and DML, diffuse mixed lymphoma.

†CHLOR signifies chlorambucil; PRED, prednisone; Mtx, methotrexate; VCR, vincristine; PRO, procarbazine; BLEO, bleomycin; ADA, adriamycin; DNA, daunomycin; L-ASP, L-asparaginase; VEL, velban; HN₂, nitrogen mustard; CY, cyclophosphamide; RT, radiation therapy; DTIC, dacarbazine.

tine, and prednisone. He was referred for marrow transplantation in July 1970, with hepatosplenomegaly, osteolytic lesions of the pelvis, femurs, and vertebrae, and pancytopenia due to marrow replacement by tumor. Marrow transplantation was performed on July 24, 1970. Complications of the procedure included Pseudomonas septicemia and severe herpetic stomatitis which resolved, and the patient was discharged from the hospital on the 55th day. Bilateral cataracts were detected in November 1972, and subsequently removed. The patient is active, asymptomatic, and free of disease 126 mo after transplantation.

Case 2 (UPN 846)

A 30-yr-old man developed a parasternal mass in December 1977. Evaluation revealed stage IV composite lymphoma with elements of diffuse histiocytic lymphoma and Hodgkin's disease, mixed cellularity, involving the lymph nodes of the neck and mediastinum and the lung parenchyma. He was treated with cyclophosphamide, vincristine, prednisone, and procarbazine (C-MOPP) for 2 mo, but because of disease progression, therapy was altered to include bleomycin and adriamycin with cyclophosphamide, vincristine, and prednisone (BACOP). Despite 2 mo of therapy with BACOP, adenopathy increased and the patient was referred for marrow transplantation with enlarged lymph nodes in the neck, axillae, celiac axis, and retroperitoneum. On June 17, 1978, the patient underwent marrow transplantation. The patient's only complication of the procedure was mucositis, and he was discharged from the hospital 22 days after the transplant. He remains well in complete remission 32 mo after transplantation.

Case 3 (UPN 1001)

Diffuse poorly differentiated lymphocytic lymphoma, stage IV, was diagnosed by scalene node and bone marrow biopsy in November 1978 in a 21-yr-old man. The patient was begun on combination chemotherapy with C-MOPP. After 4 mo of therapy, he developed a malignant pleural effusion and for the first time was noted to have malignant cells in his peripheral blood, at which time he was referred to the University of Washington. On March 24, 1979, the patient was begun on therapy with daunomycin, L-asparaginase, vincristine, and prednisone, which cleared the malignant cells from the peripheral blood and bone marrow. On May 14, 1979, while in apparent complete remission, the patient underwent marrow transplantation. The patient's major complication of the procedure was fever treated presumptively with antibiotics, and he was discharged from the hospital 38 days after transplantation. He remains well in complete remission 21 mo after transplantation.

Case 4 (UPN 1143)

A 49-yr-old woman presented in June 1978 with pancytopenia, and bone marrow biopsy revealed moderately well differentiated lymphocytic lymphoma. She initially improved with cyclophosph-

Table 2. Treatment and Result

<table>
<thead>
<tr>
<th>Unique Patient Number</th>
<th>Preparation</th>
<th>Radiation†</th>
<th>Buffy Coat</th>
<th>Tumor Cells</th>
<th>Result as of 2/21/81</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>CY</td>
<td>1000</td>
<td>+</td>
<td>+</td>
<td>Alive in CR at 126 mo</td>
</tr>
<tr>
<td>846</td>
<td>BCNU HN₂</td>
<td>200 × 6</td>
<td>-</td>
<td>-</td>
<td>Alive in CR at 32 mo</td>
</tr>
<tr>
<td>1001</td>
<td>CY</td>
<td>200 × 7</td>
<td>-</td>
<td>-</td>
<td>Alive in CR at 21 mo</td>
</tr>
<tr>
<td>1143</td>
<td>CY</td>
<td>1000</td>
<td>-</td>
<td>-</td>
<td>Alive in CR at 12 mo</td>
</tr>
<tr>
<td>380</td>
<td>CY</td>
<td>1000</td>
<td>+</td>
<td>-</td>
<td>Recurrent lymphoma at 10 mo but alive in CR at 73 mo</td>
</tr>
<tr>
<td>534</td>
<td>BCNU CY</td>
<td>1000</td>
<td>+</td>
<td>+</td>
<td>Died or Pseudomonas pneumonia at 6 mo (no evidence of lymphoma)</td>
</tr>
<tr>
<td>276</td>
<td>CY</td>
<td>1000</td>
<td>+</td>
<td>-</td>
<td>Recurrent lymphoma at 7 mo and died at 16 mo</td>
</tr>
<tr>
<td>1017</td>
<td>BCNU CY</td>
<td>200 × 7</td>
<td>-</td>
<td>-</td>
<td>Died of refractory lymphoma in 3 mo</td>
</tr>
</tbody>
</table>

*CY signifies cyclophosphamide, 120 mg/kg; BCNU, carmustine, 360 mg/sq m; HN₂, nitrogen mustard, 1 mg/kg.
†Rad; if fractionated then rad × days.
phamide, vincristine, and prednisone, but after 8 mo her disease progressed. Therapy was changed to adriamycin, bleomycin, and prednisone, but after initial improvement, disease progression was noted by August 1979. Therapy with daily chlorambucil was begun but had no effect, and she was referred for marrow transplantation.

Evaluation at the University of Washington revealed splenomegaly and pancytopenia with a marrow largely replaced by tumor. On February 21, 1980, she underwent marrow transplantation. Complications of the procedure included fever and anogenital herpes simplex, and the patient was discharged 40 days after transplantation in complete remission. The patient continues well without evidence of disease 12 mo after transplantation.

**Case 5 (UPN 380)**

In November 1973, a 15-yr-old man was found to have stage II diffuse, poorly differentiated lymphocytic lymphoma involving the mediastinum. He was treated with 4500-rad mantle irradiation but relapsed in April 1974 with recurrent mediastinal tumor and a malignant pleural effusion. Therapy with C-MOPP was begun and continued until November 1974, when the patient relapsed with bone marrow and central nervous system disease. He was then referred for transplantation, which was performed on January 7, 1975. The patient had no complications of the procedure and was discharged 20 days after transplantation.

In November 1975, the patient developed a neck mass and retroperitoneal adenopathy and biopsy revealed an undifferentiated lymphocytic lymphoma. He was treated for 4 mo with cyclophosphamide, adriamycin, vincristine, and prednisone (CHOP) followed by 1 yr of therapy with chlorambucil. He achieved a complete remission and remains in unmaintained complete remission 73 mo after transplantation.

**Case 6 (UPN 534)**

In January 1971, a 31-yr-old man was found to have stage III nodular poorly differentiated lymphocytic lymphoma and was treated with 4000-rad total nodal irradiation. Two years later he relapsed and was treated with cyclophosphamide, vincristine, and prednisone. After failure with that regimen, combinations of velban and bleomycin, and then adriamycin, vincristine, and prednisone were tried without benefit. At the time of referral for transplantation, he had generalized adenopathy and a marrow largely replaced by lymphoma. Transplantation was performed on October 8, 1975. Complications of the procedure included fever, oral mucositis, and cystitis which resolved, and he was discharged 28 days after transplantation in complete remission. Six months later he presented with fever, cough, and dyspnea. A chest x-ray revealed diffuse infiltrates and *Pseudomonas* was recovered from cultures of sputum and blood. Despite broad-spectrum antibiotics and respiratory support, the patient died 188 days after transplantation. At autopsy, no residual lymphoma was found.

**Case 7 (UPN 276)**

An 8-yr-old boy was found to have diffuse mixed lymphoma in 1971 and was treated with mantle irradiation and one course of nitrogen mustard, vincristine, and prednisone. He relapsed in February 1972, and treatment with cyclophosphamide, vincristine, and prednisone was begun. The patient relapsed with lymphadenopathy and circulating lymphoma cells, and he was referred for marrow transplantation which was performed on February 6, 1973. The patient had no complications from the procedure and was discharged 15 days after transplantation. Seven months after transplantation he relapsed with disease in his testicle and central nervous system. Further therapy was unsuccessful in eradicating the disease and the patient died 488 days after transplantation.

**Case 8 (UPN 1017)**

Stage IV diffuse poorly differentiated lymphocytic lymphoma was diagnosed in a 44-yr-old man in September 1978, for which he received 8 mo of chemotherapy with cyclophosphamide, vincristine, and prednisone. After a partial response, the disease progressed and he was subsequently treated with adriamycin, bleomycin, velban, and DTIC without response. He was then referred for marrow transplantation, which was performed on June 28, 1979. Despite this therapy, the patient never achieved a complete response and died of progressive lymphoma 3 mo after transplantation.

**RESULTS**

Table 2 summarizes the results in the 8 patients. Complete remissions were obtained in 7 of 8 patients, with 4 patients still in complete remission after 12–126 mo without further therapy. One patient died of *Pseudomonas* pneumonia while in complete remission, and two patients who achieved complete remission developed recurrent lymphoma. One of these patients went on to die of his disease, but the other was treated with combination chemotherapy and is alive 73 mo after transplantation in unmaintained complete remission. Although the recurrent lymphoma in this last case was histologically more undifferentiated than the original disease, it is not known whether this represented a new tumor or recurrence of the same tumor.

The acute toxicities of the therapy were relatively mild. High-dose cyclophosphamide resulted in the expected nausea and vomiting and two patients developed transient cystitis. Total-body irradiation caused variable degrees of nausea, vomiting, dry mouth, and diffuse skin erythema, which usually resolved in 2–4 wk.

The period of pancytopenia after transplantation was relatively short, with recovery of granulocytes to above 500/cu mm in 10–28 days (median 12) after transplantation. Platelet transfusions were no longer necessary by day 9–45 (median 16) after transplantation. During the period of myelosuppression, 6 of 8 patients became febrile, and in one patient, bacteremia due to *Pseudomonas aeruginosa* was documented. Localized herpes simplex infections were observed in 2 patients. The median duration of hospitalization was 26 days (range 15–55).

Long-term complications have included the development of cataracts in two patients. Aspermia and hypothyroidism were demonstrated in one case each, but not all patients have been screened for these complications.

**DISCUSSION**

It is now well established that intensive chemoradiotherapy followed by twin marrow transplantation can cure some patients with refractory acute leukemia. The results reported here demonstrate that some patients with refractory non-Hodgkin’s lymphoma...
may also be cured by this technique. Four of 8 patients are alive in continuous complete unmaintained remission, and the duration of complete remission in 2 of the 4 is sufficiently long that cure is extremely likely. The transplantation procedure was well tolerated in that all patients were successfully engrafted and all survived the first 100 days after grafting.

The pretransplant regimens used in this study varied depending on the protocol in use in Seattle at various times for allogeneic transplantation. Several patients received BCNU in addition to the standard transplant regimen in an attempt to decrease the relapse rate after transplantation. Fractionated total-body irradiation was used for some cases with the hope that fractionation might allow DNA repair of normal tissue, thus reducing toxicity and permitting the use of a higher total dose of irradiation with potentially increased lethality for malignant cells. Some patients received potential immunotherapy in the form of killed autologous tumor cells and/or donor buffy-coat cells. The results presented do not permit conclusions regarding the contribution of any given component of the treatment regimens or the sensitivity of individual histologic categories of NHL to the transplant procedure.

Patients with disseminated NHL who fail conventional combination chemotherapy often respond to second line agents, but few of these patients achieve prolonged complete remissions or are cured. For example, Weick et al. reported that only 3 of 54 patients with NHL resistant to conventional chemotherapy achieved a complete remission with a combination of vincristine, BCNU, adriamycin, and prednisone. Skarin et al. treated 20 patients using high-dose methotrexate with folinic acid and achieved a complete response in 4, but these responses were generally of short duration (median 4.5 mo). Similar results have been reported by others. By comparison, the results reported here are sufficiently encouraging to suggest that marrow transplantation should be used for any patient with an identical twin and disseminated NHL who has failed first-line chemotherapy.

The implications of this study are not limited to patients with identical twins. Marrow transplantation following aggressive chemoradiotherapy should be applicable to adult patients with disseminated NHL and HLA-matched nontwin sibling donors. Preliminary results suggest that allogeneic transplantation may cure occasional children with either Burkitt's lymphoma or T-cell lymphoma. Allogeneic transplantation entails increased risks resulting chiefly from graft-versus-host disease and its complications. However, a recent analysis of a large number of patients with acute leukemia in relapse treated with nontwin marrow transplants revealed a reduced rate of relapse after transplantation in patients with graft-versus-host disease, implying that one target of the graft-versus-host disease is the host leukemic cell. Thus, although increased toxicity would be expected, one might presume an even greater antitumor effect after allogeneic marrow transplantation for NHL than was observed after twin transplantation. We have recently reported that more than 50% of patients with acute nonlymphocytic leukemia transplanted from an HLA-matched nontwin donor during first remission remain disease free more than 2 yr from transplantation. Thus, by performing transplantation early in the disease course, when the body burden of malignant cells would be expected to be minimal and the patients in good clinical condition, the chance of success of allogeneic transplantation should be improved.

It is now established that human bone marrow can be cryopreserved and reinfused, allowing for successful autologous marrow transplantation. Thus, the approach reported here should also be applicable to patients without twin or allogeneic donors. Autologous marrow transplantation has been used in the treatment of patients with Burkitt's lymphoma resistant to conventional chemotherapy. By treating such patients with total-body irradiation and/or very high-dose combination chemotherapy, some could be cured of their disease. Currently, autologous transplantation is limited to those patients whose marrow can be stored while it is free of tumor. The application of methods to "purge" the marrow of tumor cells employing tumor-specific antibodies and/or in vitro treatment of the marrow with chemotherapeutic agents should further extend the utility of autologous marrow transplantation.

Future studies of twin marrow transplantation for NHL should provide information about the relative effectiveness of this approach for the different histologic subtypes of this disease and should allow for the development of more effective chemoradiotherapy regimens. This information, in turn, may have more widespread applicability to the treatment of NHL with allogeneic or autologous marrow grafting.

REFERENCES
MARROW TRANSPLANTS FOR LYMPHOMA


Treatment of non-Hodgkin's lymphoma with marrow transplantation in identical twins

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