Engraftment of Allogeneic Hematopoietic Progenitor Cells With Purine Analog-Containing Chemotherapy: Harnessing Graft-Versus-Leukemia Without Myeloablative Therapy

By Sergio Giralt, Elihu Estey, Maher Albitar, Koen van Besien, Gabriela Rondón, Paolo Anderlini, Susan O'Brien, Issa Khouri, James Gajewski, Rakesh Mehra, David Claxton, Borje Andersson, Miloslav Beran, Donna Przepiorka, Charles Koller, Steve Kornblau, Martin Körbling, Michael Keating, Hagop Kantarjian, and Richard Champlin

The immune-mediated graft-versus-leukemia effect is important to prevent relapse after allogeneic progenitor cell transplantation. This process requires engraftment of donor immuno-competent cells. The objective of this study was to assess the feasibility of achieving engraftment of allogeneic peripheral blood or bone marrow progenitor cell after purine analog containing nonmyeloablative chemotherapy. Patients with advanced leukemia or myelodysplastic syndromes (MDS) who were not candidates for a conventional myeloablative therapy because of older age or organ dysfunction were eligible. All patients had an HLA-identical or one-antigen–mismatched related donor. Fifteen patients were treated (13 with acute myeloid leukemia and 2 with MDS). The median age was 59 years (range, 27 to 71 years). Twelve patients were either refractory to therapy or beyond first relapse. Eight patients received fludarabine at 30 mg/m^2/d for 4 days with idarubicin at 12 mg/m^2/d for 3 days and ara-c at 2 g/m^2/d for 4 days (n = 7) or melphalan at 140 mg/m^2/d (n = 1). Seven patients received 2-chloro-deoxyadenosine at 12 mg/m^2/d for 5 days and ara-C 1 at g/m^2/d for 5 days. Thirteen patients received allogeneic peripheral blood stem cells and 1 received bone marrow after chemotherapy. Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine and methyl-prednisolone. Treatment was generally well tolerated, with only 1 death from multiorgan failure before receiving stem cells. Thirteen patients achieved a neutrophil count of greater than 0.5 × 10^9/L a median of 10 days postinfusion (range, 8 to 17 days). Ten patients achieved platelet counts of 20 × 10^9/L a median of 13 days after progenitor cell infusion (range, 7 to 78 days). Eight patients achieved complete remissions (bone marrow blasts were <5% with neutrophil recovery and platelet transfusion independence) that lasted a median of 60 days posttransplantation (range, 34 to 170+ days). Acute GVHD grade ≥2 occurred in 3 patients. Chimerism analysis of bone marrow cells in 6 of 8 patients achieving remission showed ≥90% donor cells between 14 and 30 days postinfusion, and 3 of 4 patients remaining in remission between 60 and 90 days continued to have ≥80% donor cells. We conclude that purine analog-containing nonmyeloablative regimens allow engraftment of HLA-compatible hematopoietic progenitor cells. This approach permits us to explore the graft-versus-leukemia effect without the toxicity of myeloablative therapy and warrants further study in patients with leukemia who are ineligible for conventional transplantation with myeloablative regimens either because of age or concurrent medical conditions.

HIGH-DOSE CHEMO-radiotherapy with allogeneic bone marrow transplantation (BMT) has been extensively used to treat patients with hematologic malignancies.[1,2] This procedure has generally been limited to younger patients in good general medical condition due to the increased risk of regimen related toxicities and graft-versus-host disease (GVHD) that occurs with age and poor performance status.[3-8] Improvements in supportive care and GVHD prophylaxis have now enabled many centers to treat older patients, but few consider patients greater than 55 years of age.[4,6] However, acute myeloid leukemia (AML) is most common in older adults. These patients have a poor prognosis and novel therapeutic options need to be explored.

The curative potential of allogeneic BMT is mediated in part by an immune mediated graft-versus-leukemia effect.[9-14] Evidence supporting the graft-versus-leukemia effect includes a lower relapse rate in patients with GVHD and a higher risk of relapse after syngeneic or T-cell–depleted transplants.[9-14] The most striking evidence for this phenomenon is the observation that infusions of donor lymphocytes can reinduce remissions in many patients.[15] Patients with chronic myelogenous leukemia (CML) are most likely to respond, but selected patients with acute leukemia, chronic lymphocytic leukemia (CLL), myeloma, and lymphoma relapsing after allogeneic BMT have also responded.[15-17] This finding suggests an alternative strategy of inducing graft-versus-leukemia after standard-dose nonablative chemotherapy as primary treatment for susceptible malignancies in patients ineligible for high-dose chemotherapy or total body irradiation.

The purine analogs fludarabine and 2-chlorodeoxyadenosine (2-CDA) have been shown to be active against a variety of hematologic malignancies.[18] These compounds are also immunosuppressive, effectively inhibiting the mixed lymphocyte reaction in vitro.[19,20]

We performed a pilot trial to determine whether purine analog-containing nonmyeloablative chemotherapy could be sufficiently immunosuppressive to enable engraftment of allogeneic hematopoietic progenitor cells in patients considered ineligible for myeloablative therapy either because of age or medical condition.

PATIENTS AND METHODS

Eligibility criteria. Patients between 55 and 70 years of age with acute leukemia or myelodysplasia beyond first complete remission

From the Department of Hematology and the Department of Laboratory Medicine, The University of Texas M.D. Anderson Cancer Center, Houston, TX.

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Address reprint requests to Sergio Giralt, MD, The University of Texas, M.D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030.

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were considered eligible for this study. Patients less than 55 years of age who were not eligible for a conventional myeloablative transplant protocol because of a concurrent medical condition were also eligible. Patients required an HLA-identical or one-antigen–mismatched related donor, a serum bilirubin level of less than 3.0 mg/dL, a serum creatinine level of less than 2.0 mg/dL, a cardiac ejection fraction of 40% or greater, and a Zubrod performance status of ≤2. Patients and donors signed written informed consents and the treatment was approved by the institutional review board.

Stem cell collection. Donors received filgrastim at 6 μg/kg subcutaneously twice daily starting 4 to 5 days before the first collection. Leukapheresis were performed daily using conventional techniques for blood progenitor cell collection until greater than 3×10^6 $CD34^+$ cells/kg recipient were collected.[21] Donor cells were cryopreserved using standard techniques. If insufficient number of cells were collected or if peripheral blood stem cell collection was not feasible, donor bone marrow was harvested.

Chemotherapy. Fifteen patients were treated. Seven patients without prior exposure to fludarabine received fludarabine at 30 mg/m^2/d intravenously at the same time over 30 minutes for 4 days with either ara-C at 2 g/m^2 administered intravenously over 4 hours starting 4 hours after the beginning of the fludarabine infusion and idarubicin at 12 mg/m^2 intravenously for 3 days.[22] One patient received the same fludarabine schedule with melphalan at 140 mg/m^2 administered once at the end of chemotherapy. Seven patients with prior fludarabine exposure received ara-C at 1.0 g/m^2/d over 2 hours intravenously for 5 days and 2-CDA at a dose of 12 mg/m^2/d by continuous intravenous infusion for 5 days beginning 4 hours after the first dose of ara-C.[23] Cells were infused 2 days after the last dose of chemotherapy.

Supportive care. All patients were treated as inpatients in private rooms. Patients received antibacterial and antifungal prophylaxis with intravenous vancomycin at 1 g daily or oral penicillin at 250 mg four times daily in addition to norfloxacin at 400 mg orally twice daily and fluconazole at 200 mg/d. Broad spectrum antibiotics were begun for temperature greater than 38.3°C or clinical signs of infection.

Patients received filgrastim at 5 μg/kg/d from the day of the transplantation (day 0) until achievement of an absolute neutrophil count (ANC) of greater than 1.5×10^9/L. Hemoglobin was maintained at a level of ≥8 g/dL and the platelet count was maintained at $\geq 20 \times 10^9$/L with filtered and irradiated blood products.

GVHD prophylaxis consisted of cyclosporine at a dose of 3 mg/kg/d by continuous intravenous infusion changed to oral as soon as tolerated and methylprednisolone at 1.0 mg/kg/d beginning on day +5 tapered over the following 8 weeks. Patients without signs of GVHD on day 50 had their cyclosporine tapered by 10% to 20% weekly. Patients developing GVHD received methylprednisolone at 2 mg/kg/d intravenously and tapered upon response as tolerated.

Study endpoints. The primary objectives of this study were to evaluate the engraftment potential and antileukemic effects of allogeneic hematopoietic progenitor cells in patients with acute leukemia or myelodysplastic syndromes (MDS) receiving intensive, but nonmyeloablative purine analog-containing chemotherapy. Chimerism and evidence of minimal residual disease were determined by conventional cytogenetics and restriction fragment length polymorphisms (RFLPs) using published techniques.[24,25] Additional endpoints included toxicity as defined by the criteria of Bearman et al[26] and hematologic recovery defined as the first of 3 consecutive days with an ANC greater than 0.5×10^9/L. Acute and chronic GVHD were scored according to standard criteria.[27,28] Complete remission was defined as less than 5% blasts in bone marrow with normal maturation, an ANC greater than 1.5×10^9/L, and platelet transfusion independence. Survival was calculated as of August 8, 1996 from the day of transplantation according to the methods of Kaplan and Meier.[29]

RESULTS

Patient and disease characteristics. From July 1995 to July 1996, 15 patients with acute leukemia or MDS were treated. Patient and disease characteristics are summarized in Table 1. In brief, the median age was 59 years (range, 27 to 71 years). Thirteen patients had AML (3 preceded by a prior hematologic disorder), 1 patient had chronic myelomonocytic leukemia (CMML), and 1 patient had refractory anemia with excess blasts in transformation (RAEB-T). The median time to transplantation from diagnosis was 391 days (range, 50 to 1,315 days). Nine patients were refractory to salvage chemotherapy, 3 patients were in untreated relapse (first, second, and third relapse), 1 patient was in a third remission, 1 patient had an untreated secondary AML 4 years after an autologous BMT, and the patient with CMML had received hydroxyurea chemotherapy. The median number of prior therapies was 2 (range, 0 to 4).

The median donor age was 57 years (range, 25 to 68 years). All donors were either fully HLA-compatible siblings (n = 13) or one-antigen–mismatched siblings (n = 2). Six donors were women and 9 were men; 6 donor-recipient pairs were sex-mismatched. Donors received a median of 5 days of treatment with filgrastim. Eight underwent one apheresis procedure and 5 required two procedures. The median $CD34^+$ cell yield expressed as number of $CD34^+$ cells per liter of blood processed during the first collection was 21.3×10^6 (range, 6.2 to 45.5×10^6). The median number of $CD34^+$ cells infused was 4.5×10^6/kg (range, 1.7 to 9.9×10^6/kg).

Toxicity. The chemotherapy was well tolerated in 13 of the 15 patients. One patient (unique patient no. [UPN] 1100), who had secondary AML and progressive pneumonia, died from multiorgan failure after the second dose of fludarabine, idarubicin, and ara-C. She developed progressive respiratory failure with renal and hepatic deterioration. Further chemotherapy was discontinued, and she died 48 hours later without receiving her donor cells. The only other instance of grade 3 toxicity was a patient with extensive anthracycline exposure and an ejection fraction of 54% before therapy (UPN 6108) who developed congestive heart failure that responded to diuretics and inotropic support.

Engraftment. Thirteen patients achieved an ANC of greater than 0.5×10^9/L a median of 10 days posttransplantation (range, 8 to 17 days). One patient never cleared his peripheral blood blasts and failed to recover normal hematopoiesis. Ten patients achieved a platelet count of 20.0×10^9/L or greater without transfusions a median of 13 days postinfusion (range, 7 to 78 days).

GVHD. Acute GVHD occurred in 3 patients, 1 limited to skin alone (grade 1) and the other 2 involving skin and gut (grade 2). GVHD responded to steroid therapy alone in 2 patients and required antithymocyte globulin in another. Chronic GVHD has not occurred, but only 5 patients are evaluable for this complication.

Response and chimerism. Thirteen of 15 patients cleared their peripheral blood blasts. Eight patients achieved remis-

Table 1. Patient and Disease Characteristics

UPN	Diagnosis/Stage at Transplantation	Age/Sex	Time to Transplantation (d)	Regimen	$CD34^+$ Dose (10^6/kg)	No. of Prior Therapies
5175	AML/Ref	61/F	791	Flag/Ida	4.4	2
5213	AML/Ref	61/M	123	Flag/Ida	4.9	1
5264	AML/Rel#1	57/F	439	2CDA/AraC	4.5	1
6044	CMML Chronic	57/M	318	Flag/Ida	4.4	1
6055	MDS/Rel#3	60/F	1315	2CDA/AraC	1.7	3
5105	AML/Ref	35/M	391	Flu/Mel	4.0	4
1100	Secondary AML Untreated	52/F	50	Flag/Ida	NA	0
6151	AML/Ref	65/M	502	Flag/Ida	3.2	3
6108	AML/Ref	64/F	252	2CDA/AraC	4.5	2
6127	AML/Ref	71/M	297	Flag/Ida	5.2	3
6115	AML/Ref	60/F	319	2CDA/AraC	9.9	2
6117	AML/Ref	51/F	624	2CDA/AraC	5.2	3
6201	AML/CR#3	59/F	916	2CDA/AraC	6.2	3
6176	AML/Ref	59/M	317	2CDA/AraC	3.6	3
6123	AML/Ref	27/F	538	Flag/Ida	4.7	2

Abbreviations: Ref, refractory; Rel, relapse; CR, complete remission; Flag/Ida, fludarabine, ara-C, idarubicin, and filgrastim.

sion criteria, with 6 having ≥90% donor cells at that time (between days 14 and 30). One patient achieved remission criteria without evidence of donor cell engraftment by cytogenetics or RFLP, and 1 patient could not be assessed. Five patients relapsed between 43 and 127 days posttransplantation (median, 65 days). Responses and chimerism for each patient are shown in Tables 2 and 3.

Survival. Six patients remain alive between 34 and 175 days posttransplantation (median, 100 days). Two patients remain in remission 34+ and 170+ days posttransplantation; the other 4 have active disease. None of the relapsing patients has responded to cyclosporine withdrawal or filgrastim therapy as treatment of relapse.[30] The median survival was 78 days (range, 0 to 175+ days). The causes of death were

Table 2. Treatment Results and Outcome

UPN	Grade 3-4 Toxicity	Day ANC 0.5×10^9/L	Day Platelet 20.0×10^9/L	GVHD	Outcome and Survival
5175	None	11	12	Skin 2 Gut 2	Achieved CR. Relapsed day 110. Died from progressive disease on day 181.
5213	None	NR	NR	None	Never cleared peripheral blood blasts. Died from progressive disease day 16.
5264	None	8	12	Skin 2	Achieved CR. Relapsed day 81. Died on day 242 in CR from adenovirus infection during a second transplant.
6044	None	17	78	None	Achieved CR, relapsed day 127. Alive day 175, received donor lymphocytes.
6055	None	10	18	None	Achieved CR, alive in continued CR day 170.
5105	None	12	23	None	Never cleared BM blasts, died with disease day 95.
1100	Renal, Hepatic, Pulmonary	NR	NR	NA	Died from multiorgan failure before receiving donor cells.
6151	None	14	NR	None	Never cleared BM blasts. Alive day 83 undergoing salvage therapy.
6108	Cardiac	9		None	Never cleared BM blasts. Alive day 121 with disease.
6127	None	12	NR	None	Never achieved CR, died from disease day 78.
6115	None	12	15	None	Achieved CR, 0% donor cells. Relapsed and died from disease and CMV on day 62.
6117	None	9	7	None	Never cleared BM blasts. Died day 60 from infection during a second transplant.
6201	None	10	10	None	Achieved CR, alive in CR day 34.
6176	None	10	12	Skin 1 Gut 3	Achieved CR, relapsed day 54, alive with disease day 58.
6123	None	10	17	None	Achieved CR, died from progressive aspergillosis day 58.

Abbreviations: CR, complete remission; NR, never reached; BM, bone marrow; CMV, cytomegalovirus.

Table 3. Chimerism Analysis After Stem Cell Transplantation

UPN	% BM Blast Pre-Rx	% BM Blast D14-30	% Donor D14-30	% BM Blasts D60-D90	% Donor D60-D90
5175	15	3.5	95*	5	85*
5213	77				
5264	50	1.8	95*	1	95*
6044	3.8	0	ND	0.7	30*
6055	2.5	1	100*	0.6	100*
5105	23	25	30†		
1100	55				
6151	61	50	0*		
6108	23	12	0†		
6127	76	3.7	95*		
6115	10	0.5	0†	16	0†
6117	27	28	0†		
6201	1	1.4	95†		
6176	29	1	90*		
6123	23	0	95*		

Abbreviations: BM, bone marrow; D, posttransplantation day; ND, not determined.

* Percentage based on results by RFLP.

† Percentage based on results by conventional cytogenetics.

leukemia (n = 5), infection after subsequent therapy (n = 2), multiorgan failure (n = 1), and aspergillosis (n = 1). The actuarial 100 day survival was 66% ± 19% for patients achieving a complete remission and 21% ± 17% for patients not achieving this response (P = .16 log rank).

DISCUSSION

Despite modern combination chemotherapy, most patients with acute leukemia relapse within the first 2 years of achieving complete remission. Durable responses to salvage therapy are rare, with 24% of patients expiring before achieving a response and approximately 33% achieving second remissions; the median survival is 18 weeks. The prognosis with conventional chemotherapy for second or third relapse is worse, with a median survival of 7 weeks and a complete remission rate of less than 10%.[31]

Myeloablative chemoradiotherapy with allogeneic progenitor cells can result in long-term disease-free survival in selected patients with advanced acute leukemia. This therapy is associated with substantial toxicity and the treatment-related mortality rate approaches 40%. This limits the use of transplantation therapies to younger patients with a relatively good performance status.[1,2]

The antileukemic effect seen with donor lymphocyte infusions in patients relapsing after allogeneic transplantation suggests that if engraftment of donor hematopoietic cells can be achieved after standard-dose chemotherapy, it may be possible to exploit the graft-versus-leukemia effect without the potential morbidity associated with myeloablative therapy.[15,16]

The purine analogs fludarabine and 2-CDA produce lymphocytopenia and substantial immunosuppression and could therefore enhance engraftment of allogeneic hematopoietic progenitors.[18-20] Fludarabine and 2-CDA combinations have been extensively studied in combination with ara-C as salvage therapy for patients with acute leukemia.[22,23] The addition of allogeneic hematopoietic progenitor cells could potentially enhance hematopoietic recovery as well as provide a graft-versus-leukemia effect. The presence of stable chimerism would allow subsequent donor lymphocyte infusions in patients with minimal residual disease or a high likelihood of relapse.

This study examined the feasibility of this approach in poor prognosis patients with recurrent or resistent AML or MDS who were not eligible for myeloablative therapy because of advanced age or concomitant medical illness. Thirteen patients recovered granulocytes between 8 and 17 days postinfusion (15 to 24 days after the beginning of chemotherapy), with 6 patients in remission with predominantly donor cells. Moreover, 3 patients in remission 60 to 90 days postinfusion had ≥80% donor cells as assessed by cytogenetics or RFLP. We did not perform studies concerning lineage-specific engraftments and the extent of lymphoid engraftment is unknown. Longer follow-up and specific assays for chimerism in each lineage are required for evaluation of the extent and durability of engraftment. In this study, the durability of the graft was difficult to assess in our patients due to the fact that many of them had recurrent leukemia within a short period of time. However, the observation that 3 of 4 patients in remission had predominantly donor cells between 2 and 3 months posttransplantation without other signs of graft failure suggests the possibility of durable engraftment in some patients.

We were unable to demonstrate the feasibility or efficacy of donor lymphocyte infusions for treating relapse in this setting because many patients relapsed quickly after transplantation with rapidly progressive disease. However, this study suggests that a strategy of purine analog-containing nonmyeloablative chemotherapy followed by infusion of donor lymphocytes for patients with evidence of minimal residual disease or with a high risk of relapse could be feasible in patients with less advanced hematologic malignancies. Such a strategy may be particularly useful for patients with CML, multiple myeloma, or acute leukemia in first remission who have an HLA-compatible donor but are deemed ineligible for a conventional myeloablative preparative regimen due to their age or medical condition.

Many other potential applications exist for a nonmyeloablative regimen that can consistently achieve engraftment of allogeneic hematopoietic progenitor cells. These include treatment of nonmalignant hematologic disorders or of immune or metabolic diseases or induction of tolerance for solid organ transplantation. The results of this study suggest that purine analog-containing nonmyeloablative regimens should be explored in these settings.

In summary, this study shows that purine analog-containing nonmyeloablative chemotherapy followed by allogeneic hematopoietic progenitor cells is feasible in elderly or debilitated patients with advanced leukemia. This therapy can result in engraftment of donor cells with rapid hematologic recovery and little regimen-related toxicity. Further studies with a larger number of patients and longer follow-up will be necessary to determine if this approach will be associated with an acceptable risk of acute and chronic GVHD and if further infusions of donor lymphocytes for

treatment of minimal residual disease or as relapse prevention will improve leukemia-free survival in these patients or in patients with less advanced disease. Further exploration of this strategy as alternative therapy for patients with hematologic malignancies who have an HLA-compatible donor but are ineligible for a conventional myeloablative therapy is warranted.

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