HLA-Matched Unrelated Donor Hematopoietic Cell Transplantation after Nonmyeloablative Conditioning for Patients with Chronic Myeloid Leukemia

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ABSTRACT

We evaluated 10/10 HLA antigen–matched unrelated hematopoietic cell transplantation (HCT) after nonmyeloablative conditioning with fludarabine 3×30 mg/m^2 and 2 Gy of total body irradiation as treatment for patients with chronic myeloid leukemia who were ineligible for conventional HCT. Data from 21 consecutive patients in first chronic phase (CP1; n = 12), accelerated phase (AP; n = 5), second CP (CP2; n = 3), and blast crisis (n = 1) were analyzed. Stem cell sources were bone marrow (n = 4) or granulocyte colony-stimulating factor–mobilized peripheral blood mononuclear cells (G-PBMCs; n = 17). The patient who underwent transplantation in blast crisis died on day 21 (too early to be evaluated for engraftment) from progressive disease. Sustained engraftment was achieved in 5 of 12 patients who underwent transplantation in CP1, 4 of 5 patients who underwent transplantation in AP, and 2 of 3 patients who underwent transplantation in CP2, whereas 9 patients rejected their grafts between 28 and 400 days after HCT. Specifically, 1 of 4 marrow recipients and 10 of 17 G-PBMC recipients achieved sustained engraftment. Graft rejections were nonfatal in all cases and were followed by autologous reconstitution with persistence or recurrence of chronic myeloid leukemia. Seven of 11 patients with sustained engraftment—including all 5 patients in CP1, 2 of 4 patients in AP, and neither of the 2 patients in CP2—were alive in complete cytogenetic remissions 118 to 1205 days (median, 867 days) after HCT. Two of the remaining 4 patients died of nonrelapse causes in complete (n = 1) or major (n = 1) cytogenetic remissions, and 2 died of progressive disease. Further efforts are directed at reducing the risk of graft rejection by exclusive use of G-PBMC and increasing the degree of pretransplantation immunosuppression.
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INTRODUCTION

Despite promising results with imatinib mesylate [1], allogeneic hematopoietic cell transplantation (HCT) has remained the only proven curative therapy for chronic myeloid leukemia (CML) [2,3]. Cures have been attrib-

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uted to both the myeloablative conditioning regimens and the graft-versus-tumor effects [4,5] mediated by donor immune cells. Myeloablative conditioning regimens for HCT have evolved remarkably during the last decade. Adjustment of busulfan plasma concentrations on the basis of pharmacokinetic studies has avoided both (1) undertreatment and the risk of relapse and (2) overtreatment and the risk of excessive toxicity [6,7]. With tar-

geted busulfan and cyclophosphamide conditioning, survival rates of 86% at 3 years have been achieved in relatively young (median age, 43 years) and otherwise healthy patients with CML in first chronic phase (CP1) given HLA-matched related HCT [8].

Additionally, considerable experience has been obtained with unrelated HCT. Five-year overall survival has been 74% for CP1 patients younger than 50 years who received HCT within 1 year of diagnosis [9]. However, 2-year survival has been <40% in patients 50 to 55 years of age [9], and transplantations have generally not been performed in patients older than 55 years [10,11]. This is unfortunate, because the median patient age at diagnosis for CML is 67 years.

Graft-versus-tumor effects have been well documented for CML, as evidenced by the ability of donor lymphocyte infusions (DLIs) to induce durable complete remissions in patients who relapse after allogeneic HCT [12,13]. To extend the use of allogeneic HCT to include older patients with CML and those with comorbid conditions, several groups of investigators have introduced reduced-intensity [14-17] or truly nonmyeloablative [18-22] conditioning regimens. Those regimens have fewer toxicities than ablative ones but are sufficiently immunosuppressive for allogeneic engraftment and rely on graft-versus-tumor effects for tumor eradication [23]. On the basis of initial experimental canine studies [24] and subsequent studies in HLA-identical sibling recipients [18], we have developed a nonmyeloablative regimen that uses 2 Gy of total body irradiation (TBI) and fludarabine 3×30 mg/m^2 as conditioning for unrelated HLA-matched HCT [25,26]. Postgrafting immunosuppression with mycophenolate mofetil (MMF) and cyclosporine (CSP) is aimed at both enhancing engraftment and controlling graft-versus-host disease (GVHD). Three phase I/II studies have explored variations in the duration and dosing of postgrafting immunosuppression for unrelated HLA-matched HCT in patients with various hematologic malignancies [25,26]. Here, we retrospectively analyzed outcomes among 21 consecutive patients with CML who were included in those studies.

PATIENTS AND METHODS

Patients

Data from 21 consecutive patients with CML in CP1 (n = 12), CP2 after blast crisis (BC) (n = 3), accelerated phase (AP; n = 5), or BC2 (n = 1) and who underwent transplantation between January 2000 and March 2004 under Fred Hutchinson Cancer Research Center (FHCRC) multi-institutional protocols 1463 (n = 14), 1641 (n = 5), and 1668 (n = 2) were analyzed as of June 31, 2004 (Table 1). The median patient age was 54 years (range, 33-66 years). The median time from diagnosis to HCT was 26 months (range, 5-121 months). Two patients had experienced treatment fail-

ure with myeloablative allogeneic HCT as the result of either disease progression or graft rejection. Six patients had experienced treatment failure with imatinib therapy because of intolerance (n = 3) or lack of cytogenetic responses to the drug given at 800 mg/d (n = 3). Four patients (all included in protocol 1463) received marrow and 17 received granulocyte colony-stimulating factor–mobilized peripheral blood mononuclear cells (G-PBMCs) as stem cell sources. Two patients each had single HLA class I allele mismatches with their donors, whereas the remaining 19 patients were matched for 10/10 HLA antigens/alleles. Participating institutions included the FHCRC, the University of Washington Medical Center, the Veterans Affairs Medical Center (all in Seattle, WA; n = 12), Stanford University (Palo Alto, CA; n = 4), the University of Colorado (Denver, CO; n = 2), the University of Utah (Salt Lake City, UT; n = 2), and Baylor University (Dallas, TX; n = 1). Patients were considered ineligible for conventional allogeneic HCT because of age (older than 50 years; n = 17), medical comorbidities (n = 2), or failed preceding myeloablative allogeneic HCT (n = 2). The study protocols were approved by the institutional review boards at FHCRC and each of the collaborating sites. All patients signed consent forms approved by the local institutional review boards.

Conditioning Regimen, Postgrafting Immunosuppression, and Follow-up

Patients were conditioned with fludarabine 30 mg/m^2/d on days -4, -3, and -2 and with 2 Gy of TBI on the day of HCT [26]. Postgrafting immunosuppression included MMF and CSP in all 3 protocols. Protocol 1463 administered MMF 15 mg/kg orally twice a day from the evening of day 0 until day +40, with taper through day +96. CSP was administered at 6.25 mg/kg orally twice a day, from day -3 to day +100, with taper through day 180 [26]. Protocol 1641 included MMF 15 mg/kg orally thrice a day from the evening of day 0 until day +40, with taper through day +96, and CSP was given identically to protocol 1463. In protocol 1668, MMF was given at 15 mg/kg orally thrice a day from the evening of day 0 until day +30 and at 15 mg/kg orally twice a day from day +31 until day 150, with a subsequent taper through day 180, whereas CSP was given at 6.25 mg/kg orally twice a day from day -3 to day +80.

Diagnosis, clinical grading, and treatment of GVHD were performed as previously reported [26,27]. Standard prophylaxis against *Pneumocystis carinii*, fungal infections, toxoplasmosis, and cytomegalovirus infections was used [26].

Pathology, cytogenetics, and reverse transcriptase–polymerase chain reaction (RT-PCR) monitoring were performed on days 28, 56, 84, and 180 and yearly thereafter. In patients who underwent transplantation in Seattle, quantitative RT-PCR was performed by

Table 1. *Patients and Results of HCT*

Patient No.	Disease Stage	Time between Diagnosis and HCT (mo)	Patient Age (y)/Sex	Comorbidities at HCT	Stem Cell Source	CD34 Cell Doses (×10⁶/kg)	CD3 Cell Doses (×10⁸/kg)	% Day 28 Donor T-Cell Chimerism	Rejection (Day)	Acute GVHD (Grade)	Extensive Chronic GVHD	Survival, days (Disease Status)
1	BC2	52	33/M	Prior HCT with URD; VOD; CRF	Marrow	1.88	0.29	ND	NE	0	NE	21 (PD)*
2	CP2	65	61/M		G-PBMC	3.28	3.62	70	No	III	Yes	233 (PD)*
3	CP2	121	63/F		G-PBMC	4.13	2.04	62	No	III	No	164 (MCR)*
4	CP2	88	60/F		G-PBMC	6.28	1.18	15	Yes (42)	II	No	540 (PD)*
5	AP	5	36/F	Two prior HCT with MRD	Marrow	2.76	0.46	ND	No	II	Yes	241 (PD)*
6	AP	54	66/M		G-PBMC	5.5	2.2	45	No	I	Yes	267 (CCR)†
7	AP	92	63/F		G-PBMC	5.5	2.1	90	No	0	Yes	478 (CCR)*
8	AP	29	58/M		G-PBMC	5.28	1.8	99	No	II	No	115 (CCR)†
9	AP	12	53/F		G-PBMC	3.5	3.69	10	Yes (266)	0	No	271 (PD)*
10	CP1	13	52/F		G-PBMC	7.26	1.7	41	No	II	Yes	1205 (CCR)†
11	CP1	9	54/F		G-PBMC	4.03	3.27	80	No	II	Yes	1126 (CCR)†
12	CP1	9	52/M		G-PBMC	1.18	2.13	66	No	II	Yes	1123 (CCR)†
13	CP1	11	61/M		G-PBMC	2.9	2.27	90	No	II	Yes	867 (CCR)†‡
14	CP1	24	54/M		G-PBMC	9.58	2.84	28	No§	III	NE	118 (CCR)†
15	CP1	26	54/M		Marrow	2.37	0.26	7	Yes (43)	0	No	1188 (CML)†
16	CP1	—	54/F		G-PBMC	5.51	ND	50	Yes (79)	0	No	960 (CML)†
17	CP1	11	38/M	Obesity, AVM	G-PBMC	6.44	1.61	40	Yes (400)	II	Yes	946 (CML)†
18	CP1	24	58/M		G-PBMC	2.29	ND	0	Yes (28)	0	No	806 (PD)*
19	CP1	29	53/M		Marrow	1.3	ND	0	Yes (29)	0	No	698 (CML)†
20	CP1	26	51/M		G-PBMC	3.3	0.9	10	Yes (54)	0	No	734 (CML)†
21	CP1	36	40/F	Obesity	G-PBMC	10.7	5.39	19.5	Yes (211)	II	No	356 (CML)†

BC2 indicates second blast crisis; CP2, second chronic phase; AP, accelerated phase; CP1, first chronic phase; M, male; F, female; HCT, hematopoietic cell transplantation; URD, unrelated donor; VOD, veno-occlusive disease of the liver; CRF, chronic renal failure; MRD, HLA-matched related donor; AVM, arteriovenous malformation; G-PBMC, granulocyte colony-stimulating factor–mobilized peripheral blood mononuclear cells; ND, not done; NE, not evaluable; PD, progressive disease; CCR, complete cytogenetic response; MCR, major cytogenetic response; CML, chronic myeloid leukemia.

*Dead.

†Alive.

‡Had PD after HCT but achieved CCR after 1 course of farnesyl transferase inhibitor and extensive chronic GVHD.

§The patient had 28% donor T-cell chimerism on day 28; this predicted a high risk of subsequent graft rejection. He received pentostatin (4 mg/m²) on day 43 followed by donor lymphocyte infusion 2 days later. This resulted in a significant increase in donor chimerism among all subpopulations, and the patient is currently surviving with sustained graft >118 days after HCT.

using 5′ exonuclease–based fluorogenic real-time RT-PCR to detect *bcr/abl* and β_2-microglobulin messenger RNA. Assays were performed in single tubes in an ABI PRISM 7700 sequence detector (PE Applied Biosystems, Foster City, CA), as previously described [28].

Persistent, progressive, and relapsed CML in the absence of severe manifestations of acute and chronic GVHD was treated by rapid discontinuation of systemic immunosuppression to initiate graft-versus-tumor effects. Percentages of donor-host chimerism levels in marrow, T cells, and granulocytes were determined by using fluorescent in situ hybridization for X- and Y-chromosomes if patients and donors were sex mismatched or by PCR-based amplification of variable number tandem repeat sequences unique to donors and hosts if patients and donors were sex matched [26,29].

Statistical Methods

Survival was estimated by the Kaplan-Meier method. Progression was defined as increases in pretransplantation numbers of metaphases with the Philadelphia chromosome by >25% after discontinuation of immunosuppression, the development of new clonal cytogenetic abnormalities, or evidence of transformation to AP or BC. Complete cytogenetic remission was defined as the disappearance of Philadelphia chromosome and any other clonal abnormalities, and major cytogenetic response was defined as a decrease of Philadelphia chromosome–positive marrow metaphases to ≤25%. Cumulative incidence estimates were calculated for GVHD and nonrelapse mortality. Deaths were treated as competing events in analyses of GVHD. All *P* values were derived from likelihood ratio statistics and were 2 sided.

RESULTS

Peripheral Blood Cell Changes, Donor Engraftment, and Graft Rejection

Patients experienced declines in both neutrophil and platelet counts after conditioning. Neutrophil and platelet nadirs were 0.1×10^9/L (range, 0.0-0.9×10^9/L) and 43×10^9/L (range, 6-373×10^9/L), respectively. Median numbers of red blood cell and platelet transfusions were 8 (range, 0-32) and 0 (range, 0-10), respectively. Median durations of neutropenia (defined as absolute neutrophil count $<0.5 \times 10^9$/L) and thrombocytopenia (defined as platelet count $<20 \times 10^9$/L) were 7 days (range, 0-20 days) and 0 days (range, 0-17 days), respectively. One patient died on day 21 (too early to be evaluated for engraftment) from BC (Table 1). Initial donor engraftment (defined as ≥5% donor T-cell chimerism) was observed in 18 (90%) of the 20 remaining patients and was sustained in 1 of 4 marrow and 10 of 17 G-PBMC recipients, whereas 9 patients did not engraft or rejected initial grafts 28 to 400 days after HCT. All graft rejections were nonfatal and were followed by autologous hematopoietic reconstitution and persistence or recurrence of CML.

Day 28 T-Cell Chimerism Levels ≤40% Predicted Subsequent Graft Rejection

Day 28 T-cell chimerism was assessed in 19 patients (Table 1). Eight of 9 patients with donor T-cell chimerism levels ≤40% on day 28, compared with 1 of 10 with levels >40%, subsequently rejected their grafts (*P* = .001). Patient 14, who had a day 28 donor T-cell chimerism level of 28%, received pentostatin (4 mg/m²) on day 43, followed by DLI on day 45, in a successful attempt to prevent graft rejection. Donor chimerism levels among T cells, granulocytes, and marrow mononuclear cells increased after pentostatin administration and DLI (Figure 1A), and the patient experienced sustained engraftment.

Outcomes in Patients with Graft Failure or Graft Rejection (n = 9)

Among the 9 patients with graft rejection, 4 in CP1 achieved unsustained complete cytogenetic remissions 56 to 107 days after HCT, whereas 3 in CP1, 1 in AP, and 1 in CP2 had persistence of CML. The 4 patients with cytogenetic remissions eventually experienced disease progression that began in 1 case 198 days before graft rejection was diagnosed and that began in the other 3 cases 112, 113, and 230 days after graft rejection. At the time of the analyses, 3 patients had died of progressive disease, and 6 were alive with CML.

Outcomes in Patients with Sustained Engraftment (n = 11)

Sustained engraftment was demonstrated in 5 of 12 patients with CP1, 4 of 5 patients with AP, and 2 of 3 patients with CP2. Grade II and III acute GVHD occurred in 6 (55%) and 3 (27%) patients with sustained engraftment, respectively; no grade IV acute GVHD was observed. Extensive chronic GVHD was seen in 8 patients.
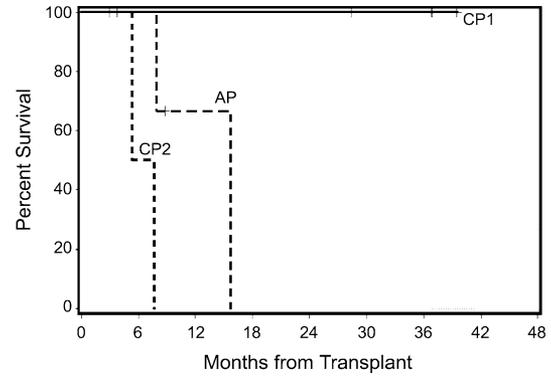
Seven of 11 patients with sustained engraftment, including 4 of 5 in CP1, 3 of 4 in AP, and none of 2 in CP2, achieved sustained complete cytogenetic remissions, whereas 1 patient who underwent transplantation in CP2 achieved a major cytogenetic remission (Table 1). The fifth patient with CP1 developed BC 189 days after HCT but then achieved a complete cytogenetic remission and became BCR/ABL negative by quantitative RT-PCR after a short course of therapy with a farnesyl transferase inhibitor (3 days of lonafarib [SCH66336, Sarasar] given at 200 mg twice daily) and experienced development of chronic exten-
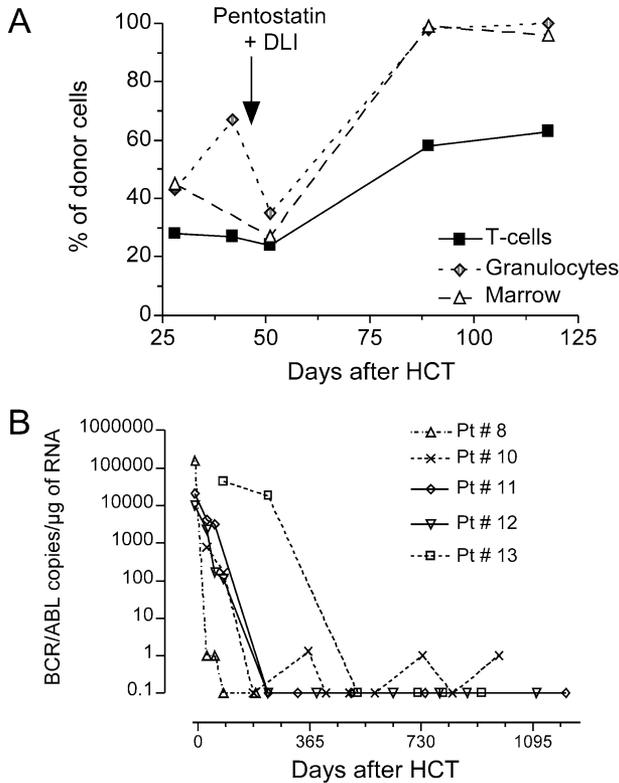
Figure 1. A, Peripheral blood donor T cell, granulocyte, and marrow mononuclear cell chimerism levels in patient 14, with CML in CP1. The patient had 28% donor T-cell chimerism on day 28, and this predicted a high risk of subsequent graft rejection. He received pentostatin (4 mg/m²) on day 43, followed by donor lymphocyte infusion 2 days later. This resulted in a significant increase in donor chimerism among all subpopulations, and the patient is currently surviving with a sustained graft >118 days after HCT. B, Evolution of BCR/ABL messenger RNA in 4 patients with CML in CP1 and 1 patient with CML in AP who had sustained engraftment. Molecular remissions were achieved 84 to 524 days (median, 230 days) after HCT. Values of 0.1 indicate negative quantitative RT-PCR.

sive GVHD. One patient with CP2 and 1 with AP died of progressive disease, and 1 patient each in CP2 and AP died of nonrelapse causes; 1 of the 2 had a major cytogenetic response, and the other was in complete cytogenetic remission. Figure 1B shows the evolution of BCR/ABL messenger RNA testing in 5 patients with sustained engraftment who underwent transplantation for CML in CP1 (n = 4) or AP (n = 1). All 5 patients achieved molecular negativity 84 to 524 days (median, 230 days) after HCT.

The 100-day and 1-year nonrelapse mortalities for patients with sustained engraftment were 0% and 11%, respectively. The 1-year probabilities of overall survival were 100%, 67%, and 0% for patients with sustained engraftment who underwent transplantation in CP1, AP, and CP2, respectively (Figure 2). Figure 3 shows overall and progression-free survivals in all patients (rejectors and nonrejectors) included in this study.


Figure 2. Overall survival in patients with CML in CP1, AP, and CP2 who had sustained engraftment.

DISCUSSION

The patients with CML reported here took part in 3 phase II protocols that explored engraftment, toxicities, and efficacy of a nonmyeloablative conditioning regimen for unrelated HCT in patients with various hematologic malignancies. Although the conditioning regimen of fludarabine and 2 Gy of TBI remained the same, the protocols differed slightly from each other with regard to stem cell sources (marrow versus G-PBMC) and the duration and dosing of postgrafting MMF and CSP.

A main finding among patients with CML has been failure of sustained engraftment, which was observed in
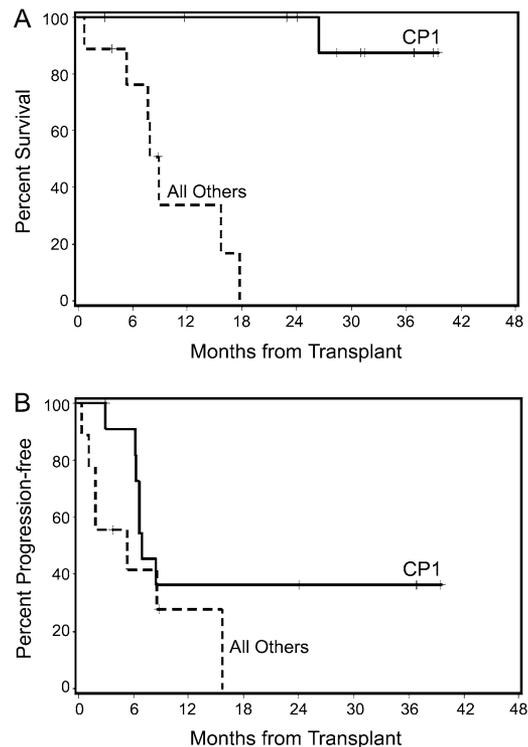
Figure 3. Overall survival (A) and progression-free survival (B) in all patients (n = 21).

9 of 20 evaluable cases. This observation contrasted with observations in CML patients given related grafts after conditioning with 2 Gy of TBI and fludarabine, all 10 of whom achieved sustained engraftment [30], and also with findings in 64 other patients given unrelated grafts for acute myeloid leukemia, 61 of whom (95%) achieved sustained engraftment [31]. In each case, graft failure was followed by autologous marrow recovery with a return to the status quo. This is different from conventional HCT, for which graft failures have been seen in 5% to 15% of CML patients, and subsequent marrow aplasia was the rule [9,10]. A high rate of graft rejection among CML patients receiving grafts from unrelated donors after reduced-intensity conditioning has also been reported by other investigators. A graft failure rate of 44% (3 of 8 patients) was described after a reduced-intensity conditioning regimen that combined fludarabine, busulfan, and antithymocyte globulin [32], and, recently, Hallemeier [33] observed graft failure in 5 of 22 evaluable patients given unrelated G-PBMC as treatment of CML after conditioning with 5.5 Gy of TBI and cyclophosphamide. A previous analysis of data from the first 89 patients with various hematologic malignancies given unrelated grafts after conditioning with fludarabine and 2 Gy of TBI showed that marrow grafts were associated with a higher incidence of graft failure than G-PBMC grafts [26]. Recent analyses of data in 130 consecutive patients given unrelated grafts after nonmyeloablative conditioning showed that a higher number of grafted $CD8^+$ ($P = .005$) and $CD34^+$ ($P = .01$) cells resulted in a lower probability of graft rejection [34]. Two of the 4 current marrow recipients experienced graft failure, 1 of the 4 died too early for evaluation, and the only patient with engraftment had experienced treatment failure with 2 previous high-dose conventional allogeneic HCTs. Nevertheless, 7 of the 17 G-PBMC recipients also rejected their grafts. Increasing the dosing of postgrafting MMF from 15 mg/kg every 12 hours to 15 mg/kg every 8 hours reduced the rejection rate in G-PBMC recipients from 5 in 10 to 2 in 7, but not convincingly so. Clearly, more effective pretransplantation immunosuppression will be required to ensure uniform engraftment. A dose relationship with respect to TBI and allogeneic marrow engraftment has been demonstrated [35,36] in a preclinical canine model. This led us to conduct a dose-escalation study to determine the minimal dose of TBI required to promote engraftment in ≥90% of CML patients given grafts from unrelated donors.

Eight of 9 current patients with day 28 donor T-cell chimerism levels ≤40% rejected their grafts, thus confirming previous observations in patients with other hematologic malignancies [37]. These findings led to the design of a protocol aimed at preventing graft rejection through administration of pentostatin (to reduce host-versus-graft reactions) followed by DLI, as was used in 1 of the current patients, who had a day 28 donor T-cell chimerism level of 28%. Although the strategy was apparently successful in this patient, more experience will be needed to confirm its efficacy [38].

Sustained complete cytogenetic remissions have been achieved in all but 1 of the patients with CML in CP1 or AP who had stable engraftment. The relatively long time periods required for remissions to be complete were consistent with the concept that remissions were due to graft-versus-tumor effects. Nonrelapse mortality in patients with sustained engraftment was 0% at 100 days and was 11% at 1 year after HCT. This was encouraging given the age of the patients, the median time interval (>2 years; range, 0.5-10 years) between diagnosis and HCT, and the advanced disease stage in 9 of the patients. The 1-year probabilities of overall survival in patients with CP1, AP, and CP2 who had sustained engraftment were 100%, 66%, and 0%, respectively. These observations attested to the relative safety of the current regimen and suggested that HCT should be considered early in the disease course, preferentially when patients were still in CP1. Ideal candidates for this approach would be patients who either are intolerant of imatinib mesylate or do not achieve 3-log BCR/ABL reductions after 1 year of treatment with the drug [1].

In summary, unrelated donor HCT after nonmyeloablative conditioning with fludarabine and low-dose TBI was feasible with relatively low nonrelapse mortality. Sustained complete cytogenetic remissions were achieved in most patients who had stable engraftment, but graft rejection occurred in 45% of patients. Further efforts are directed at reducing the risk of rejection by exclusive use of G-PBMC and by increasing the degree of pretransplantation immunosuppression.

REFERENCES

1. Hughes TP, Kaeda J, Branford S, et al. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. *N Engl J Med.* 2003;349:1423-1432.

2. Bhatia R, Holtz M, Niu N, et al. Persistence of malignant hematopoietic progenitors in chronic myelogenous leukemia patients in complete cytogenetic remission following imatinib mesylate treatment. *Blood.* 2003;101:4701-4707.

3. Goldman JM. Chronic myeloid leukemia—still a few questions [review]. *Exp Hematol.* 2004;32:2-10.

4. Weiden PL, Flournoy N, Thomas ED, et al. Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. *N Engl J Med.* 1979;300:1068-1073.

5. Truitt RL. The Mortimer M. Bortin Lecture: to destroy by the reaction of immunity—the search for separation of graft-versus-leukemia and graft-versus-host. *Biol Blood Marrow Transplant.* 2004;10:505-523.

6. Slattery JT, Clift RA, Buckner CD, et al. Marrow transplantation for chronic myeloid leukemia: the influence of plasma busulfan levels on the outcome of transplantation. *Blood.* 1997; 89:3055-3060.

7. Andersson BS, Kashyap A, Couriel D, et al. Intravenous busulfan in pretransplant chemotherapy: bioavailability and patient benefit. *Biol Blood Marrow Transplant.* 2003;9:722-724.

8. Radich JP, Gooley T, Bensinger W, et al. HLA-matched related hematopoietic cell transplantation for chronic-phase CML using a targeted busulfan and cyclophosphamide preparative regimen. *Blood.* 2003;102:31-35.

9. Hansen JA, Gooley TA, Martin PJ, et al. Bone marrow transplants from unrelated donors for patients with chronic myeloid leukemia. *N Engl J Med.* 1998;338:962-968.

10. McGlave PB, Shu XO, Wen W, et al. Unrelated donor marrow transplantation for chronic myelogenous leukemia: 9 years' experience of the National Marrow Donor Program. *Blood.* 2000;95:2219-2225.

11. Weisdorf DJ, Anasetti C, Antin JH, et al. Allogeneic bone marrow transplantation for chronic myelogenous leukemia: comparative analysis of unrelated versus matched sibling donor transplantation. *Blood.* 2002;99:1971-1977.

12. Porter DL, Collins RH Jr, Shpilberg O, et al. Long-term follow-up of patients who achieved complete remission after donor leukocyte infusions. *Biol Blood Marrow Transplant.* 1999; 5:253-261.

13. Kolb HJ, Schmidt C, Barrett AJ, Schendel DJ. Graft-versus-leukemia reactions in allogeneic chimeras. *Blood.* 2004;103:767-776.

14. Wong R, Giralt SA, Martin T, et al. Reduced-intensity conditioning for unrelated donor hematopoietic stem cell transplantation as treatment for myeloid malignancies in patients older than 55 years. *Blood.* 2003;102:3052-3059.

15. Khoury H, Adkins D, Brown R, et al. Low incidence of transplantation-related acute complications in patients with chronic myeloid leukemia undergoing allogeneic stem cell transplantation with a low-dose (550 cGy) total body irradiation conditioning regimen. *Biol Blood Marrow Transplant.* 2001;7:352-358.

16. Chakraverty R, Peggs K, Chopra R, et al. Limiting transplantation-related mortality following unrelated donor stem cell transplantation by using a nonmyeloablative conditioning regimen. *Blood.* 2002;99:1071-1078.

17. Or R, Shapira MY, Resnick I, et al. Nonmyeloablative allogeneic stem cell transplantation for the treatment of chronic myeloid leukemia in first chronic phase [comment in Blood 2003;101:5084; author reply 5084-5085]. *Blood.* 2003;101: 441-445.

18. McSweeney PA, Niederwieser D, Shizuru JA, et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. *Blood.* 2001;97:3390-3400.

19. Sloand E, Childs RW, Solomon S, Greene A, Young NS, Barrett AJ. The graft-versus-leukemia effect of nonmyeloablative stem cell allografts may not be sufficient to cure chronic myelogenous leukemia. *Bone Marrow Transplant.* 2003;32:897-901.

20. Lange T, Deininger M, Brand R, et al. *BCR-ABL* transcripts are early predictors for hematological relapse in chronic myeloid leukemia after hematopoietic cell transplantation with reduced intensity conditioning. *Leukemia.* 2004;18:1468-1475.

21. Feinstein LC, Sandmaier BM, Maloney DG, et al. Allografting after nonmyeloablative conditioning as a treatment after a failed conventional hematopoietic cell transplant. *Biol Blood Marrow Transplant.* 2003;9:266-272.

22. Uzunel M, Mattsson J, Brune M, Johansson JE, Aschan J, Ringden O. Kinetics of minimal residual disease and chimerism in patients with chronic myeloid leukemia after nonmyeloablative conditioning and allogeneic stem cell transplantation. *Blood.* 2003;101:469-472.

23. Baron F, Maris MB, Sandmaier BM, et al. Graft-versus-tumor effects after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. *J Clin Oncol.* In press.

24. McSweeney PA, Storb R. Mixed chimerism: preclinical studies and clinical applications [review]. *Biol Blood Marrow Transplant.* 1999;5:192-203.

25. Niederwieser D, Maris M, Shizuru JA, et al. Low-dose total body irradiation (TBI) and fludarabine followed by hematopoietic cell transplantation (HCT) from HLA-matched or mismatched unrelated donors and postgrafting immunosuppression with cyclosporine and mycophenolate mofetil (MMF) can induce durable complete chimerism and sustained remissions in patients with hematological diseases. *Blood.* 2003;101:1620-1629.

26. Maris MB, Niederwieser D, Sandmaier BM, et al. HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies. *Blood.* 2003;102:2021-2030.

27. Sullivan KM. Graft-vs.-host disease. In: Blume KG, Forman SJ, Appelbaum FR, eds. *Thomas' Hematopoietic Cell Transplantation.* Oxford, UK: Blackwell Publishing Ltd; 2004, 635-664.

28. Radich JP, Gooley T, Bryant E, et al. The significance of *bcr-abl* molecular detection in chronic myeloid leukemia patients "late," 18 months or more after transplantation. *Blood.* 2001;98:1701-1707.

29. Baron F, Little MT, Storb R. Kinetics of engraftment following allogeneic hematopoietic cell transplantation with reduced-intensity or nonmyeloablative conditioning. *Blood Rev.* In press.

30. Kerbauy FR, Lange T, Gooley T, et al. Non-myeloablative allografting from HLA-identical sibling donors for treatment of CML [abstract 2316]. *Blood.* 2004;104(pt 1):637a.

31. Niederwieser DW, Hegenbart U, Sandmaier BM, et al. Treatment for acute myelogenous leukemia by low dose irradiation based conditioning and hematopoietic cell transplantation from related and unrelated donors [abstract 3074]. *Blood.* 2004;104(pt 1): 840a.

32. Bornhauser M, Thiede C, Platzbecker U, et al. Dose-reduced conditioning and allogeneic hematopoietic stem cell transplantation from unrelated donors in 42 patients. *Clin Cancer Res.* 2001;7:2254-2262.

33. Hallemeier C. Re: High rate of graft failure in 25 patients with chronic myelogenous leukemia conditioned with a reduced-intensity regimen of 550 cGy total body irradiation and cyclophosphamide for unrelated donor transplantation [letter to the editor]. *Biol Blood Marrow Transplant.* 2004;10:726-727.

34. Baron F, Maris M, Storer B, et al. Higher doses of transplanted CD34+, CD3+ and CD8+ cells are associated with better donor T-cell chimerism and less graft rejection, but not with GVHD after nonmyeloablative conditioning for unrelated hematopoietic cell transplantation [abstract 2753]. *Blood.* 2004; 104(pt 1):735a.

35. Yu C, Storb R, Mathey B, et al. DLA-identical bone marrow grafts after low-dose total body irradiation: effects of high-dose corticosteroids and cyclosporine on engraftment. *Blood.* 1995; 86:4376-4381.

36. Storb R, Yu C, Wagner JL, et al. Stable mixed hematopoietic chimerism in DLA-identical littermate dogs given sublethal total body irradiation before and pharmacological immunosuppression after marrow transplantation. *Blood.* 1997;89:3048-3054.

37. Baron F, Baker JE, Storb R, et al. Kinetics of engraftment in patients with hematological malignancies given allogeneic hematopoietic cell transplantation after nonmyeloablative conditioning. *Blood.* 2004;104:2254-2262.

38. Sandmaier BM, Maloney DG, Maris MB, et al. Conversion of low donor chimerism following nonmyeloablative conditioning for hematopoietic cell transplantation (HCT) using pentostatin and donor lymphocyte infusion (DLI) [abstract]. *Blood.* 2004;104:57a.