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ORIGINAL ARTICLE

Intensive postgrafting immune suppression combined with nonmyeloablative conditioning for transplantation of HLA-identical hematopoietic cell grafts: results of a pilot study for treatment of primary immunodeficiency disorders

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This study was designed to determine the safety of a nonmyeloablative regimen in patients with primary immunodeficiency disorders (PID) who had infections, organ dysfunction or other risk factors that precluded conventional hematopoietic cell (HC) transplant. Fourteen patients received HLA-matched related ($n=6$) or unrelated ($n=8$) HC grafts from marrow ($n=8$), peripheral blood mononuclear cells ($n=5$) or umbilical cord blood ($n=1$), either without conditioning ($n=1$), or after 200 cGy total body irradiation alone ($n=3$) or with 90 mg/m^2 fludarabine ($n=10$). All patients were given postgrafting immunosuppression with mycophenolate mofetil and cyclosporine. Mixed ($n=5$) or full ($n=8$) donor chimerism was established in 13 patients, and one patient rejected the graft. Eight patients developed acute grade III ($n=1$) and/or extensive chronic GVHD ($n=8$). With a median follow-up of 4.9 (range, 0.7–8.1) years, the 3-year overall survival, event-free survival and transplant-related mortality were 62, 62 and 23%, respectively. Correction of immune dysfunction was documented in 8 of 10 patients with stable donor engraftment. These preliminary results indicated that this approach was associated with stable donor engraftment and a low incidence of early mortality and, thus, can be considered for certain high-risk patients with PID. However, there was a risk of GVHD, which is an undesirable outcome for this group of patients.
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Introduction

Allogeneic hematopoietic cell transplantation (HCT) offers curative therapy for most patients with primary immunodeficiency disorders (PID). Successful HCT requires overcoming the bi-directional immunologic barrier composed of host-versus-graft (HVG) and graft-versus-host reactions. To accomplish this, conventional transplant regimens have employed intensive cytotoxic conditioning phases to eliminate HVG reactions, followed by the hematopoietic cell graft used both to rescue the patient from lethal myeloablation and to correct the immunodeficiency disorder. However, intensive cytotoxic conditioning regimens cause profound pancytopenia and increase the risk for severe organ toxicity. In addition, children treated with myeloablative regimens often suffer late effects such as infertility, hormonal dysfunction, growth failure and secondary malignancies.[1–4] Certain factors, including older age and coexisting chronic infections or organ dysfunction, confer greater risks for toxicity or death following myeloablative conditioning regimens.

In 1997, a novel approach to overcome the bi-directional allogeneic graft barriers was reported in a canine model, which combined a nonmyeloablative conditioning regimen using low-dose total body irradiation (TBI, 200 cGy) with post-grafting immunosuppression consisting of mycophenolate mofetil (MMF) and cyclosporine (CSP).[5] The study results showed that potent immunosuppression given after marrow grafting in a major histocompatibility identical model could facilitate the development of bi-directional immunologic tolerance, prevent graft rejection and control GVHD. Subsequently, this regimen with or without fludarabine (90 mg/m^2) was studied as a means to establish donor engraftment in older human patients and those with comorbidities for treatment of hematologic malignancies. Early (day 100) mortality rates ranged from 4.5 to 11% depending upon donor type and extent of co-morbidities.[6–8]

We hypothesized that a similar approach could be effective for treatment of PID through the establishment of stable mixed or full donor chimerism. Supportive evidence that mixed chimerism could ameliorate certain immune deficiency states has been reported in retrospective

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studies of patients primarily given myeloablative conditioning.[9,10] The primary objectives of the current study were, first, to determine the safety of intensive post-transplant immune suppression after nonmyeloablative conditioning in patients with life-threatening co-morbidities and second, to test whether donor engraftment could be established in a variety of PID. A secondary objective was to determine whether mixed chimerism could improve the immunologic deficiencies in various disorders.

Methods

Patients

Fourteen patients with PID, ranging from 0.5 to 30 years of age, underwent HCT at the Fred Hutchinson Cancer Research Center (FHCRC) between February 1998 and April 2006 (Table 1). Results were analyzed as of 17 January, 2006. Patients were eligible for the study if all of the following criteria were present: (1) a PID treatable with HCT; (2) factor(s) that would increase the risk of early transplant-related mortality (TRM, death before day 100) following conventional HCT; and (3) identification of a related or unrelated donor matched for HLA-A, -B, -C, -DRB1 and -DQB1 alleles. Factors considered to increase the risk of mortality included: (1) infection—defined as fungal or viral infections involving one or more organs present at time of transplant or a life-threatening opportunistic infection diagnosed within 3 months of HCT; (2) organ dysfunction—defined as any of the following: liver dysfunction (transaminase >3 times the upper limit of normal), renal dysfunction (biopsy-proven renal disease or glomerular filtration rate $<50\%$), cardiac dysfunction (cardiac ejection fraction $<40\%$), or pulmonary dysfunction (diffusing capacity of the lung for carbon monoxide of $<60\%$); (3) older age (>5 years)—applicable only in cases for which previous studies have documented age to be associated with higher risk of TRM;[10,11] (4) unrelated donor (URD)—applicable only in disorders for which previous studies have documented disease-free survival of $<50\%$ or if the reported experience has not been sufficient to justify a conventional URD HCT.[10] Excluded from this analysis were patients with severe combined immunodeficiency disorder who received grafts from HLA-matched related donors ($n=1$). Patients and/or their parents signed forms approved by the FHCRC Institutional Review Boards documenting informed consent to participate in the clinical trials.

Histocompatibility

Methods used for histocompatibility testing have been reported previously.[12,13] DRB1 and DQB1 alleles were determined by hybridization of sequence-specific oligonucleotide probes.[14] Direct automated fluorescent methods were used to identify HLA-A, -B and -C alleles.[15] Related and unrelated donors were matched for alleles at HLA-A, -B, -C, -DRB1 and -DQB1.

Preparative regimen

Post grafting immunosuppression with high-dose CSP and MMF was given after a nonmyeloablative conditioning regimen. Since CSP and MMF could exacerbate infections present pretransplant, the regimen was intensified by stages in patients as follows: patients received no conditioning ($n=1$), 200 cGy TBI (7 cGy/min; day 0, $n=3$) or 200 cGy TBI plus fludarabine (30 mg/m^2/day; days -4 to -2; $n=10$). Patients received unmanipulated bone marrow ($n=8$), granulocyte colony-stimulating factor (G-CSF)—mobilized peripheral blood mononuclear cells (G-PBMC, $n=5$) or umbilical cord blood ($n=1$) collected according to established methods and infused through a central venous catheter on day 0.[16,17] The median total nucleated, CD34, and CD3 cell doses were 6.5 (range, 1.4–30) $\times 10^8$, 12.8 (range, 2.0–25) $\times 10^6$ and 2.2 (range, 0.2–14.2) $\times 10^8$ per kilogram recipient weight, respectively. Patients who achieved $>5\%$ donor CD3-cell chimerism levels were evaluated for disease responses. A second donor cell infusion was planned if the immune deficiency was not corrected following establishment of mixed chimerism (see definition of study end points).

Post-grafting immunosuppression

All patients received CSP and MMF for postgrafting immunosuppression. CSP was initiated on day -3 at doses of 2 mg/kg intravenously every 8 h for children less than 6 years of age or 1.5 mg/kg intravenously every 12 h for older children. The dose of CSP was adjusted to achieve serum trough levels between 400 and 600 ng/ml through day 28 after HCT; after stable therapeutic levels were achieved, the intravenous form was converted into the oral form (Neoral). The duration of CSP prophylaxis after transplant was extended to 180 days with the aims of reducing both the incidence and severity of GVHD.[18] MMF was administered to the first six patients at a dose of 15 mg/kg orally or intravenously every 12 h, commencing 4 h after the marrow infusion and continuing until day $+27$ after HCT. Subsequently, MMF was given every 8 h after pharmacokinetic studies in adult patients established the half-life.[7,19,20] Patients received MMF from day 0 to day 40 followed by a taper of 11% per week if there was no evidence of GVHD. If patients developed GVHD, treatment with CSP and/or MMF was extended.

GVHD grading and treatment

Diagnosis and clinical grading of acute and chronic GVHD were performed according to established criteria[21,22] and treated as described previously.[23,24] Patients were not considered evaluable for acute GVHD if they died before engraftment or for chronic GVHD if they died before day 80.

Prophylaxis against infection

Measures to prevent infection included intravenous immunoglobulin, prophylactic fluconazole, trimethoprim–sulfamethoxazole or other prophylaxis for *Pneumocystis carinii* pneumonia (PCP), acyclovir if indicated for prevention of herpes simplex virus reactivation, and pre-emptive ganciclovir or foscarnet if cytomegalovirus (CMV) antigen was detected.[25,26] Reactivation of CMV and Epstein–Barr virus (EBV) was detected using PCR amplification of nucleic acids from serum samples, as described pre-

Table 1 Characteristics and outcomes of 14 patients who received nonmyeloablative conditioning followed by allogeneic grafts for high-risk PID

Patient	*Diagnosis*		*Age (year)/ gender*	*Pre-transplant risk factors*	*Cond. Reg*	*Donor/cell source*	*Cell dose/(kg)*			*Second HCT/DLI/ stem cell boost*	*Acute GVHD (grade)*	*Chronic GVHD*	*F/U (years)/COD*
	Clinical	*Molecular*					*TNC ($\times 10^8$)*	*CD34 ($\times 10^6$)*	*CD3 ($\times 10^8$)*				
1	SCID	IL2RG; 374del17_373ins18; Frameshift	0.9/M	CMV, FTT, persistent rotavirus	TBI	URD/BM	30	14.0	0.7		II	+	>7.5
2	SCID	IL2RG; 866G>A; R285Q	0.5/M	Pneumocystis carinii pneumonia	TBI	URD/BM	15.4	19.5	NA		II	–	>2.9
3	SCID	RAG2; 461C>T; A154V	0.5/F	Disseminated CMV disease, influenza A	Flu/TBI	URD/PBMC	8.3	9.8	2.2	+	II	–	†0.3 refractory disseminated CMV disease
4	CVID	N/A	30.1/M	Disseminated MAC, severe FTT (40 kg), age, multiple tx	None	MRD/BM	4.3	NA	NA		II	–	>8.1
5	TCSD	Defective transmembrane Ca^{2+} influx into T-cells.[53] Molecular defect unknown	8.1/F	Pulmonary CMV, EBV-LPD, multiple tx intermittent paralysis	TBI	MRD/BM	1.8	6.0	0.2	+	—	–	†1.1, RRT 2nd HCT
6	TCSD	CD3 signaling defect. Molecular defect unknown	1.9/M	Pneumocystis carinii pneumonia, chronic diarrhea	Flu/TBI	MRD/BM	5.0	12.8	0.6		II	–	>5.5
7	TCD	CD3 signaling defect, radiation sensitivity. No antibody response Molecular defect unknown	0.7/M	Pneumocystis carinii pneumonia	Flu/TBI	URD/BM	3.9	2.2	NA		II	+	†1.1, cardiac arrest
8	WAS	WAS; 416T>C; F128L	1.0/M	Pulmonary CMV and para influenza, multiple platelet tx, FTT	Flu/TBI	URD/PBMC	1.4	19.9	14.2	+	II	+	>4.9
9	WAS	WAS; 811+1G>A; splicing	12.8/M	Renal failure, age	Flu/TBI	URD/PBMC	16.0	25.0	5.1		—	+	†1.0, B-cell non-EBV lymphoma
10	XHIM	CD40L; 629G>T; R203I	1.1/M	*Pneumocystis carinii* pneumonia	Flu/TBI	MRD/BM	5.3	10.9	0.5		II	+	>6.2
11	XHIM	CD40L; 412ins5_417ins4; frameshift	3.4/M	URD	Flu/TBI	URD/PBMC	23.9	14.9	8.8		II	+	>4.1
12	IPEX	FOXP3; 1271G>A; C424Y	2.3/M	Membranous GN, IDDM, enteropathy	Flu/TBI	MRD/CB	7.6	2.0	NA	+	—	–	>4.0
13	CGD	Abnormal oxidative burst	25.6/F	Pulmonary aspergillosis/ candidemia	Flu/TBI	URD/PBMC	18.7	16.5	3.4		II	+	†2.7, pneumonia
14	CHH	RMRP gene mutation; 4C>T, 211C>G	2.0/F	EBV-LPD, pulmonary atypical mycobacterial infection	Flu/TBI	MRD/BM	4.89	11.20	NA		III	+	>0.7

Abbreviations: > alive; † dead; CB = cord blood; CD40L = CD40 ligand; CGD = chronic granulomatous disease; CHH = cartilage hair hypoplasia; COD = cause of death; Cond. Reg = conditioning regimen; CVID = common variable immunodeficiency; DLI = donor lymphocyte infusion; EBV–LPD = EBV–lymphoproliferative disease; F = female; Flu = fludarabine; FOXP3 = forkhead box P3; FTT = failure to thrive; GN = glomerulonephropathy; IDDM = insulin dependent diabetes mellitus; IL2RG = interleukin-2 receptor γ chain; IPEX = immune dysregulation-polyendocrinopathy-enteropathy-X-linked; M = male; MAC = *mycobacterium avium* complex; MRD = matched related donor; RRT = regimen related toxicity; TCD/TCSD = T-cell signaling defect; TNC = total nucleated cell count; tx = transfusions; URD = unrelated donor; WAS = Wiskott–Aldrich syndrome; XHIM = X-linked hyper-IgM syndrome.

viously.[27,28] Patients with chronic GVHD were given prophylaxis for PCP and *pneumococcal* infections through the duration of immunosuppressive therapy.

Flow cytometric analysis, cell sorting and chimerism analyses

Procedures for immunophenotype analyses and sorting of cell subsets by flow cytometry have been described previously.[29,30] Donor chimerism levels were assessed in sorted CD33, CD3, CD4, CD8, CD19 and CD56 cell subsets by FISH to detect X and Y chromosomes for recipients of sex-mismatched grafts, or by PCR-based analyses of polymorphic microsatellite regions for recipients of sex-matched grafts, using methods described previously.[31–34]

Disease response

Recovery of T- and B-cell numbers was evaluated by flow cytometry using specific monoclonal antibodies to CD3, CD4, CD8 and CD19. Lymphocyte function was assessed by measuring lymphocyte proliferation to mitogen (PHA), anti-CD3 antibody and antigens (tetanus toxoid and candida). Proliferation was measured by [H^3]thymidine incorporation according to standard methods.[35] *In vivo* antibody responses were evaluated using immunization with the T cell-dependent neoantigen, bacteriophage ΦX174, as described previously.[36,37] Surface staining and flow cytometry for functional CD40 ligand expression were performed on stimulated lymphocytes as described previously.[38] Neutrophil oxidative burst was determined by flow cytometry using dihydrorhodamine fluorescence as described.[39]

Definition of study end points

The primary end points of the study were safety, TRM and donor engraftment. TRM was defined as death at any time from causes other than the underlying disease. Early TRM was defined as death before day 100. Death due to infection was scored as TRM, including patients who had pre-existing infections. Event-free survival was defined as time to death or rejection, whichever came first. Adverse events were graded by the common toxicity criteria version 3. *Donor engraftment* was defined as achievement of at least 5% peripheral blood CD3 cells of donor origin. *Mixed chimerism* was defined as 5–94% CD3 cells of donor origin, and *full chimerism* was defined as $\geqslant$95% CD3 cells of donor origin. *Graft failure* was defined by failure to achieve at least 5% CD3 cells of donor origin and absolute neutrophil counts $>500/\mu l$ for 3 consecutive days and/or subsequent loss of donor engraftment ($<5\%$ donor CD3).

Statistical methods

TRM and incidence of GVHD were calculated using a cumulative incidence estimate, treating rejection and malignancy as competing risk events for TRM and second transplant, death, DLI and malignancy as competing risk events for GVHD.[40] Survival and event-free survival curves were calculated using the Kaplan and Meier method.[41] Time to death (for overall survival) or rejection (for event-free survival) was censored at the last contact date for each patient. Plots of individual patients' hematologic parameter trajectories included median curves smoothed with cubic spline functions.

Results

Hematologic recovery

For those patients who received conditioning ($n=13$), absolute neutrophil counts (ANC) declined to a median value of 221 (range, 24–930) cells/μl within the first 30 days, with the nadir occurring at a median of 12 (range, 0–30) days after HCT (Figure 1a). The median durations of ANC <500 cells/μl and <1000 cells/μl were 15 (range, 5–23) and 20 (range, 5–35) days, respectively, after HCT. Four patients received G-CSF for treatment of neutropenia (ANC <500 cells/μl). The median duration of platelet counts $<20\,000\,\mu l$ was 12 (range, 6–16) days (Figure 1b). The median number of platelet and packed red blood cell transfusions were 0 (range 0–64) and 4 (range 1–93), respectively.

Donor chimerism

The percent of donor cells among CD33, CD3, CD4, CD8, CD19 and CD56 subsets are provided at days 28, 90, 365 and at last follow-up (Table 2). Full donor CD3-cell chimerism was documented at 7.3, 2.9 and 0.2 years after HCT in the three patients with SCID. Donor CD19 cell chimerism was established in one of two X-SCID patients with sufficient follow-up (patients no. 1 and no. 2), and in a

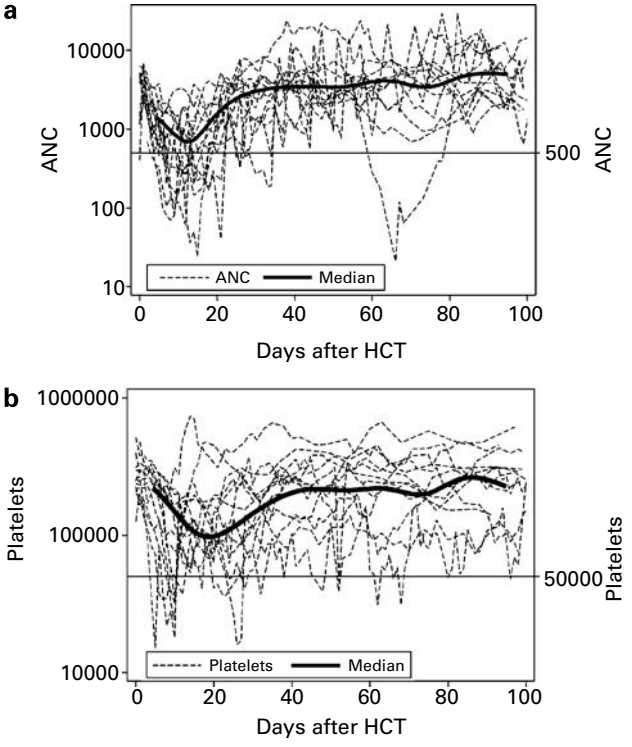

Figure 1 Absolute neutrophil counts (**a**) and platelet counts (**b**) within the first 100 days after HCT in 14 patients with primary immunodeficiency disorders. Individual blood counts (dotted line) and median value (solid line) are shown according to day following HCT.

Table 2 Percent donor chimerism levels of sorted peripheral blood cell subsets at days +28, +90, +365, and last follow-up for 14 patients with PID

Patient no.	*Diagnosis*	*Day*	*% CD33*	*% CD3*	*% CD4*	*% CD8*	*% CD19*	*% CD56*
1	SCID	28	95	100	ND	ND	ND	ND
		90	60	95	ND	ND	50	ND
		365	50	50	50	95	20	30
		2366	1	96	97	96	5	77
2	SCID	28	35	100	ND	ND	ND	ND
		90	60	99	99	99	80	99
		365	46	97	92	95	QNS	77
		682	100	100	100	100	100	100
3	SCID	28	57	98	100	99	ND	QNS
		56[a]	1	99	97	88	QNS	24
4	CVID	28	35	10	35	ND	ND	ND
		90	95	35	35	35	95	ND
		365	100	50	50	50	100	ND
		1194	100	50	50	50	QNS	ND
5	TCSD	28	ND	ND	ND	ND	ND	ND
		90	55	5	ND	ND	ND	ND
		250[a]	5	5	ND	ND	ND	ND
6	TCSD	28	96	4	8	10	ND	ND
		90	68	24	35	28	ND	ND
		365	48	72	80	81	51	71
		1837	25	96	96	97	89	80
7	TCD	28	82	71	83	37	ND	ND
		90	77	89	95	83	ND	ND
		370	11	94	100	75	NE	79
8	WAS	28	100	85	89	96	ND	97
		90	10	95	79	98	QNS	75
		365	0	96	95	99	ND	38
		493[a]	1	95	ND	ND	ND	ND
9	WAS	28	88	94	ND	ND	ND	ND
		90	90	98	94	97	95	97
		258	100	100	100	100	ND	100
10	XHIM	28	24	8	ND	ND	ND	ND
		90	31	4	4	8	10	ND
		365	6	9	10	11	4	ND
		2083	11	12	11	13	9	33
11	XHIM	28	91	60	56	76	63	75
		90	100	60	57	67	66	82
		365	95	79	71	79	52	87
		1114	100	89	89	96	96	99
12	IPEX	28	0	NE	1	1	QNS	1
		191[a]	63	NE	5	9	28	14
13	CGD	28	95	84	80	86	QNS	82
		90	95	78	ND	ND	ND	ND
		355	100	100	100	100	QNS	100
14	CHH	28	99	99	ND	ND	ND	ND
		90	100	100	100	100	ND	100
		253	100	100	100	100	99	100

Abbreviations: DLI = donor lymphocyte infusion; HCT = hematopoietic cell transplantation; ND = not done; NE = not evaluable; QNS = quantity not sufficiency.
[a]Percent donor chimerism before stem cell boost, DLI or second HCT.

RAG2 SCID patient after a planned stem cell boost. Among the 11 patients with PID other than SCID, full donor and mixed donor CD3 chimerism was established in five and five patients, respectively. One patient (no. 5) rejected the graft, and had uneventful recovery of autologous hematopoiesis.

Transplant-related toxicity and mortality

Early TRM (<day 100) was 0% and the 3-year TRM was 23%. One patient died before day 180 of disseminated CMV disease. This patient had ganciclovir-resistant CMV pneumonitis and viremia, with 240 000 copies of CMV by PCR, at time of HCT. Three patients had grade IV non-hematologic organ toxicities before day 100, which included hyperbilirubinemia (not caused by sinusoidal obstructive syndrome), hypotension, hypoxia, hyponatremia/adrenal insufficiency and renal failure. There were no grade V toxicities. Bacterial infections were diagnosed in eight patients, and none of the patients developed a fungal infection. Among the nine patients at risk for CMV reactivation, two reactivated and were treated with pre-emptive ganciclovir until day 100.

GVHD

Of the 14 patients, 10 developed acute grade II GVHD (6/8 recipients of marrow and 4/5 recipients of G-PBMC), and 1 developed acute grade III GVHD (Table 1). No patient developed acute grade IV GVHD. The cumulative incidences of acute GVHD grade II and III were 71 and 7%, respectively. The median onset of grade II–III GVHD was 18 (range, 7–71) days after HCT. Extensive chronic GVHD developed in eight patients (2/6 recipients of related grafts and 6/8 recipients of unrelated grafts; 4/5 recipients of G-PBMC grafts and 4/8 recipients of marrow grafts). The 1-year cumulative incidence of extensive chronic GVHD was 47%. Complications from chronic GVHD were responsible for the death of one (7%) patient. At time of last follow-up, nine patients were alive, and all had a Lansky/Karnofsky performance score of 100. Of the nine patients, six do not require treatment for GVHD, while three patients continue to receive immunosuppression at 0.7, 6.2 and 7.5 years after HCT.

Second HCT or donor lymphocyte infusion

Three patients met criteria for a second donor cell infusion. Patient no. 3 established full donor CD3 cells, but lost the granulocyte graft, and was given stored peripheral blood stem cells from the original donor following no conditioning, which successfully established full donor CD33 chimerism. Patient no. 8 also established full donor CD3 chimerism, associated with resolution of disseminated infections, but thrombocytopenia persisted. A second marrow graft from the original donor was given after conditioning with fludarabine (total dose 120 mg/m^2) and oral busulfan (BU, total dose 16 mg/kg), which resulted in full chimerism of the CD33 compartment. He survives >4.9 years with 100% donor chimerism of CD3 and CD33 cells and a normal platelet count. Patient no. 12 had persistent low level donor CD4 and CD8 chimerism, which did not improve substantially after DLI was given 5 months after HCT. This patient subsequently received a second transplant following a reduced intensity regimen.[42] One patient (no. 5) was given a second transplant for treatment of graft rejection, using the same donor after a myelo-

ablative regimen (oral BU (total dose 16 mg/kg), cyclophosphamide (CY, total dose 120 mg/kg) and anti-thymocyte globulin (ATG, total dose 90 mg/kg)) which resulted in full donor chimerism, however, the patient died from toxicity related in part to her pretransplant pneumonitis.

Disease responses

Ten of the 14 patients were evaluated for disease response. The four patients who received a stem cell boost, second HCT or DLI (nos. 3, 5, 8, and 12) were not included in this evaluation. Correction of or improvement in the PID was documented in 8 of the 10 patients (Table 3). The two patients with X-SCID (nos. 1, and 2), had significant improvement in T-cell numbers and function (Table 3). Antibody response and antibody class switching normalized in patient no. 2 who had full donor chimerism of B cells. Patient no. 1 has not yet been immunized with bacteriophage ΦX174 to assess B-cell immunocompetence.

Eight patients with PID other than SCID were evaluated clinically and with disease-specific assays to determine disease response. Lymphocyte numbers and function were found to be normal or near normal in the patient with CVID (no. 4) and in 1 of the 2 patients (no. 6 and no. 7) transplanted for T-cell defects, as shown in Table 3. The patient with common variable immunodeficiency (no. 4) was immunized with bacteriophage ΦX174 3 years after HCT and demonstrated a normal primary antibody, however, the patient did not follow-up and the full immunization series was not completed. Clinically at the time of HCT he had persistent fevers, severe failure to thrive weighing 40 kg and suffered from disseminated mycobacterium avium complex (MAC), which resolved following HCT and currently he remains well 8.1 years after HCT. Patient no. 6 also developed a near-normal primary and secondary antibody response to immunization with bacteriophage ΦX174, compared to the pretransplant evaluation (data not shown). Clinically, he remains without significant infectious complications 5.5 years after HCT. Patient no. 7 was evaluated at 1 year after HCT while being treated with immune suppressants for GVHD, which may have contributed to the delay in immune reconstitution. Both patients with Wiskott-Aldrich Syndrome (no. 8 and no. 9) developed normal T cell function after HCT. Table 3 excludes the data for patient no. 8, since the last evaluation was performed after the second graft given to correct the platelet defect. The platelet count in patient no. 9 normalized after HCT, which correlated with full donor chimerism of CD33 cells. Among the two patients with X-linked hyper IgM syndrome (CD40 ligand deficiency (CD40LD)), one (no. 11) was found to have a marked increase in functional CD40 ligand expression on activated T cells (data not shown). Immunization with bacteriophage ΦX174 showed near-normal primary and secondary antibody responses and improved IgM to IgG class switching (data not shown). This patient has remained free of infections >4 years after HCT. The other patient (no. 10) has not been evaluated for immune reconstitution; however he has been treated intermittently for CMV reactivation, suggesting poor T-cell immunity. His donor was subsequently found to be a carrier of the CD40 ligand mutation, which in addition to the low-level chimerism likely explains the persistent T-cell defect. The patient with chronic granulomatous disease (CGD) showed normal neutrophil oxidative burst (patient stimulation index (SI) = 128.8, control SI = 152.7) following HCT compared to before HCT (patient SI 1.2, control SI 152.9) (data not shown). The patient with cartilage hair hypoplasia (no. 14) developed a marked improvement in T-cell function following HCT. (Table 3) Clinically this patient, who was severely neutropenic and dependent on G-CSF before HCT, remains healthy with a normal white blood count 9 months after HCT.

Overall and event-free survival

With a median follow-up of 4.9 (range, 0.7–8.1) years, the 3-year overall and event-free survivals were both 62% (Figure 2). Causes of death included infections with or without GVHD ($n = 2$), infectious complications following chemotherapy for primary B-cell non-EBV lymphoma

Table 3 Absolute lymphocyte subset counts and mitogen responses in six patients given nonmyeloablative HCT for correction of PID. Comparison of values before transplant and at last follow-up

Patient no.	*Absolute lymphocyte subset counts*										*Mitogen responses*				
	CD3		*CD4*		*CD8*		*CD19*		*CD56*		*PHA*			*Anti-CD3*	
	Pre	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Dose μg/ml*	*Pre Δ CPM*	*Post Δ CPM*	*Pre Δ CPM*	*Post Δ CPM*
1	19	971	9	527	9	410	1871	164	19	ND	10	0	144 005	ND	121 324
2	146	1389	29	893	119	456	38	516	2	40	10	0	259 904	ND	ND
4	395	1562	188	428	211	1135	4	130	0	74	10	59 850	38 835	1192	18 981
											5	8249	31 972	—	—
											2.5	0	35 590	—	—
6	4225	2086	2158	1145	1699	738	276	305	46	102	10	113 549	103 883	611	12 783
7	1371	315	400	247	955	68	15	5	62	146	10	119 452	10 105	14 377	35 307
14[a]	176	423	144	143	16	286	229	ND	101	90	10	3008	103 744	3689	59 023

Abbreviations: CPM = counts per minute; HCT = hematopoietic cell transplantation; PID = primary immunodeficiency disorders.
[a]Lymphocyte subsets of Patient no. 14's were evaluated around day 80 after HCT, whereas the lymphocyte function studies were evaluated around 8 months after HCT.

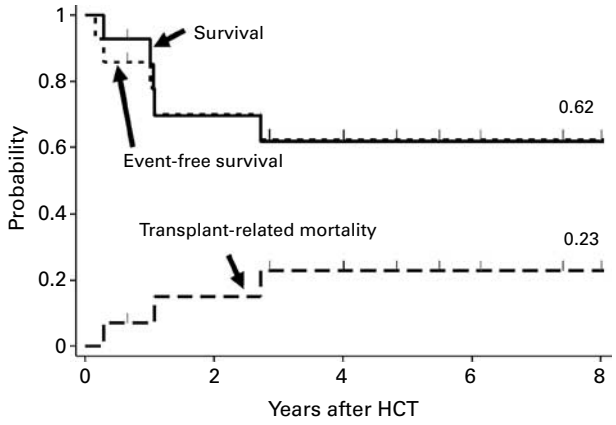

Figure 2 Overall survival, event-free survival and TRM in 14 patients with primary immunodeficiency disorders.

($n=1$), cardiac arrest of unknown etiology ($n=1$) and transplant-related toxicities following second myeloablative HCT ($n=1$) (Table 1).

Discussion

While HCT is effective in the treatment of a variety of PID, it is reasonably common for these patients to have infections or organ dysfunction as a consequence of their disease, which confer higher risk for mortality after HCT. Filipovich *et al*. showed that patients could be defined as 'high-risk' if they developed life-threatening co-morbidities within 3 months of unrelated donor HCT, with an overall survival of 20% compared to 80% survival after HCT for the healthy, average risk patients.[11,43] Our own unpublished data shows 50% mortality by day +100 for high-risk patients with PID, compared to 11% for those without risk factors. Older age has been associated with higher TRM rates following HCT for PID, which may be a consequence of delaying HCT until medically necessary. The risk of mortality also has been correlated with the subtype of PID, for example CGD, CD40LD and TCD consistently have lower survival rates compared to patients given HCT for treatment of other diagnoses.[10,44] Finally, several studies have reported rates of TRM exceeding 30% following unrelated grafts.[10,45] Accordingly, for the subset of high-risk PID patients, the development of a less toxic HCT regimen would provide considerable benefit and perhaps encourage HCT at an earlier age. This study found that the combination of MMF and CSP used as intensive immune suppression following a nonmyeloablative conditioning regimen reduced the incidence of early mortality for patients who would be considered a high risk for TRM, even those with life-threatening infections at the time of HCT.

The aim of this pilot study was to determine the safety of intensive post-grafting immunosuppression in high- risk patients; consequently, the study is limited with respect to analysis for efficacy. Nonetheless, disease responses were observed in patients with partial as well as full donor chimerism. There are several points regarding the efficacy of establishing donor chimerism and disease responses worth considering. First, the level of donor engraftment needed for disease response is disease-specific. Immunodeficiency disorders, in general, are predicted to be correctable with lower level donor chimerism based upon observations of female carriers of X-linked immunodeficiencies with random X-chromosome inactivation who are clinically normal. Moreover, disease responses have been reported in patients with mixed chimerism following conventional HCT.[10] Accordingly, in this study improvement of the immune deficit was observed in most of the patients with mixed chimerism. However, certain PID may require higher level donor chimerism for correction, illustrated by patient 10, who did not have clinical improvement in immune function despite stable chimerism of 10–15% (although the donor cells were from a female carrier, which may in part have contributed to the persistent immune deficiency). Second, disease response will depend upon engraftment of the appropriate cell lineages. This is best illustrated in the patients with Wiskott–Aldrich syndrome, in which donor origin of lymphoid lineage cells corrected the immune defect, whereas thrombocytopenia was corrected only if myeloid lineage cells were of donor origin. Finally, establishment of donor T-cell chimerism may be a bridge to a second transplant. Patients with life-threatening infections could safely be given HCT using this nonmyeloablative regimen to establish donor T-cell chimerism, which established tolerance and enabled recovery from infection. Subsequently, a second infusion of cells could be given with less risk to convert to full chimerism.

It may be argued that study of this nonmyeloablative regimen in a diverse group of patients will make it difficult to come to conclusions regarding its usefulness. However, these disorders are rare and no single center could accrue enough patients for analysis of efficacy in a single disorder. Moreover, there have been several other reports of reduced toxicity regimens that have been studied in small numbers of patients with PID. Amrolia and colleagues studied fludarabine (150 mg/m^2), melphalan (140 mg/m^2) and anti-lymphocyte globulin (12.5 mg/kg) as conditioning for marrow grafts in eight patients with various PID.[46] Other than myelosuppression and hepatotoxicity, no life-threatening toxicities were observed. Seven of the eight patients were reported to survive 8–17 months after HCT, with high-level donor chimerism in six and mixed donor-host chimerism in two patients. Horwitz and colleagues[47] were able to study 10 patients with CGD who were conditioned with CY (120 mg/kg), fludarabine (125 mg/m^2) and ATG (160 mg/kg), followed by transplant of HLA-matched CD34+-selected PBMC. Delayed DLI was given to increase the level of donor chimerism. Donor chimerism was established in 9 of 10 patients, and GHVD developed in three patients after DLI. Seven patients were reported to have survived from 16 to 26 months, two patients died of transplant-related complications and one patient who rejected the graft died after a second HCT. More recently, Rao *et al*.[48] reported the outcomes of 33 patients with PID (SCID ($n=6$) and non-SCID ($n=27$)) who received unmodified unrelated donor marrow grafts following reduced intensity conditioning consisting of fludarabine (150 mg/m^2), melphalan (140 mg/m^2) and Campath-1 H

(0.2 mg/kg, days −8 to −4, $n=14$) or ATG (2.5 mg/kg, day −2 to +2, $n=19$). All patients had primary engraftment, and at time of last follow-up (>1 year), 55% of the patients had 100% donor chimerism. The incidence of acute GVHD (>grade II) was 9% and 1-year survival was 94%. While these reduced-intensity regimens show promising results, the high degree of immune suppression may not be desirable for those patients with life-threatening infections. As our study demonstrated, a minimal toxicity conditioning regimen combined with intensive post-grafting immune suppression facilitated donor engraftment with low risk of mortality from opportunistic infections.

As described previously in the setting of minimal conditioning, alloreactivity of the donor graft plays an important role in establishing donor hematopoiesis.[5–8] Not unexpectedly we observed GVHD, which in one case contributed to TRM. We believe that, unlike patients with hematologic malignancies, who benefit from the graft-vs-leukemia effect of donor cells, there is no clinical benefit from GVHD in patients with PID. In retrospect, several factors contributed to the high incidence of GVHD in this study. First, we found that the grafts contained large doses of both CD34 and CD3. Kahl *et al*.[49] recently published a study in 81 patients with severe aplastic anemia and found higher numbers of nucleated marrow cell doses (>2.4–3.2×10^8 cells/kg) were associated with an increased risk of developing extensive chronic GVHD (hazard ratio 3.8). Second, higher rates of chronic GVHD were seen among recipients of G-PBMC grafts compared to marrow. This finding is consistent with studies comparing G-PBMC to marrow among recipients of both related and unrelated donor grafts.[50,51] We initiated the study with a preference for G-PBMC as the stem cell source because canine studies and clinical trials in adult patients with malignancies showed a higher risk of graft rejection following marrow grafts.[7,8] However, in this study we could not appreciate a difference in graft rejection according to stem cell source, although this could be explained by the relatively high cell dose of the marrow products. Finally, unrelated donor grafts, which were given to over half of the patients in this study, have been associated with higher incidences of acute grade II–IV and chronic GVHD, compared to related donor grafts in patients with hematologic malignancies conditioned with this nontoxic-conditioning regimen.[52] It should be noted, however, that the quality of life scores were high in our patients, despite the presence of chronic GVHD in many.

This study translated treatment with a minimal toxicity nonmyeloablative HCT, first developed in the dog model and then studied extensively in adult patients with malignant disorders, to high-risk PID with the aim of reducing early TRM. As a first step toward improving survival of high-risk patients, we showed that early TRM could be reduced and immune function could be improved. Current studies are aimed at reducing the incidence of chronic GVHD, the most important obstacle to successful application of this regimen. Nonetheless, our study adds to the emerging field of reduced intensity or nonmyeloablative HCT for treatment of PID, which indicate that the toxic effects of conventional regimens can be reduced without compromising donor engraftment. A minimal-toxicity HCT is a viable treatment approach and may provide the only acceptable transplant option for high-risk patients with life threatening co-morbidities.

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