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Cutting Edge Communication

Nonmyeloablative Stem Cell Transplantation with CD8-Depleted or CD34-Selected Peripheral Blood Stem Cells

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ABSTRACT

To decrease the incidence of graft-versus-host disease (GVHD) observed after nonmyeloablative stem cell transplantation (NMSCT), we studied the feasibility of CD8-depleted or CD34-selected NMSCT followed by CD8-depleted preemptive donor lymphocyte infusion (DLI) given in incremental doses on days 40 and 80. Fourteen patients with high-risk malignancies and an HLA-identical sibling (*n* = 8) or alternative donor (*n* = 6) but ineligible for a conventional transplant were included. Nonmyeloablative conditioning regimen consisted in 2 Gy total body irradiation (TBI) alone, 2 Gy TBI and fludarabine (previously untreated patients) or cyclophosphamide and fludarabine (patients who had previously received ≥12 Gy TBI). Patients 1–4 (controls) received unmanipulated peripheral blood stem cells (PBSC) and DLI and patients 5–14 CD8-depleted or CD34-selected PBSC followed by CD8-depleted DLI. Post-transplant immunosuppression was carried out with cyclosporine A (CsA) and mycophenolate mofetil (MMF). Initial engraftment was seen in all patients, but 1 patient (7%) later rejected her graft. The actuarial 180-day incidence of grades II–IV acute GVHD was 75% for patients 1–4 versus 0% for patients 5–14 (*p* = 0.0019). Five of 14 patients were in complete remission (CR) 180 days after the transplant and 6/14 had partial responses. The 1-year survival rate was 69%, and nonrelapse and relapse mortality rates were 16 and 18%, respectively. We conclude that CD8-depleted or CD34-selected NMSCT followed by CD8-depleted DLI is feasible and considerably decreases the incidence of acute GVHD while preserving engraftment and apparently also the graft-versus-leukemia (GVL) effect. Further studies are needed to confirm this encouraging preliminary report.

INTRODUCTION

Allogeneic hematopoietic stem cell (HSC) transplants are classically performed after myeloablative regimens consisting of high-dose chemotherapy with or without total body irradiation (TBI) (1,2). The aims of this conditioning are to eliminate host (1) hematopoiesis to "make space" for donor HSC; (2) immune system to prevent rejection; and (3) cancer or leukemic cells (3). However, it has been recently demonstrated that the graft itself, most likely through subclinical graft-versus-host disease (GVHD) reactions, is capable to create these marrow spaces without chemotherapy or bone marrow irradiation (4). In addition, tumor cells resistant to standard doses of chemotherapy are unlikely to be completely eliminated by tolerable higher-intensity doses of chemo-

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radiotherapy alone (5). Moreover, donor alloreactivity against tumor cells has been recognized as a major factor in the success of HSC transplantation (6–10). This graft-versus-leukemia (GVL) effect may be so potent that leukemia relapses after standard allogeneic transplantation can be effectively [70% long-term complete remission (CR) in chronic myeloid leukemia (CML)] treated with donor lymphocyte infusions (DLI) (10–17). Taken together, these findings suggested that the potential for cure after allogeneic HSC transplantation could be obtained by induction of host-versus-graft tolerance with low-dose highly immunosuppressive regimens, allowing in a second step the implantation of donor T and natural killer (NK) (18,19) cells (DLI) to eradicate host tumor cells (3,5,20–32).

The Seattle team has recently proposed an original approach to nonmyeloablative stem cell transplantation (NMSCT) with a conditioning regimen based on single-dose (2 Gy) TBI followed by post-transplant immunosuppression with cyclosporine A (CsA) and mycophenolate mofetil (MMF) (3,33,34). They observed a powerful GVL effect but also a significant incidence of GVHD (33). Recently, we have reported a prospective study of transplantation of CD34-selected allogeneic peripheral blood stem cells (PBSC) after myeloablative conditioning, followed by pre-emptive CD8-depleted DLI (35). Compared to unmanipulated marrow transplantation, this approach significantly decreased the incidence of acute and extensive chronic GVHD without compromising the GVL effect. Therefore, we undertook to investigate the feasibility of applying such a strategy of CD8-depletion or CD34-selection of the graft followed by pre-emptive CD8-depleted DLI in the context of NMSCT after a conditioning and immunosuppression regimen based on the Seattle approach.

PATIENTS AND METHODS

Patient eligibility

Patients with hematologic malignancies were eligible for this program if they were deemed poor candidates for conventional myeloablative therapy because they were older than 55 years ($n = 4$), had concurrent medical conditions ($n = 0$), had failed a previous autograft ($n = 4$), or for a combination of these factors ($n = 3$). Patients with metastatic renal cell carcinoma refractory to interferon-α were also eligible ($n = 3$). Written informed consent was obtained from patients and donors and our institution's Ethical Committee approved the protocol.

Patients and donors

Fourteen patients with malignancies, 12 males and 2 females, aged 22–65 (median 58) years were included. Their clinical characteristics are summarized in Table 1. The number of previous lines of therapy ranged from 1 to 6. Eight patients had undergone a previous autotransplant with 6/8 failures. Only those with CML or renal cell carcinoma (RCC) did not receive a previous autograft. Eight patients had HLA-identical sibling and 6 alternative donors. Conditioning (Table 2) consisted of 2 Gy of single-dose TBI on day 0 ($n = 6$). For patients not heavily pretreated or unrelated transplants, TBI was combined with 30 mg/m^2 per day fludarabine for 3 days ($n = 5$). The first patient was an exception to this rule because the high rejection rate in CML patients with the original Seattle protocol was not yet published. Finally, 3 patients received a combination of fludarabine and cyclophosphamide 1 g/m^2 per day for 3 days (Fluda-Cy) because they had previously received 12 Gy TBI as conditioning regimen for an autotransplant (Table 1). Post-transplant immunosuppression was carried out orally with CsA (6 mg/kg b.i.d. from day -1 to day 120 or longer in case of alternative donor or chronic GVHD) and MMF (15 mg/kg b.i.d. from day -1 to day 28).

Clinical management

The trigger values for red blood cell (RBC) and platelet transfusion were 8.0 g/dl and 15×10^9/L, respectively. Granulocyte colony-stimulating factor (G-CSF) (5 μg/kg/d) was administered when the granulocyte count was below 1.0×10^9/L. The diagnosis and grading of acute and chronic GVHD was established as previously reported (36,37). Disease evaluation were routinely carried out on days 40, 100, 180, and 365. Polymerase chain reaction (PCR) for cytomegalovirus (CMV) was performed weekly until day 100 and every 2–4 weeks thereafter. Patients with a positive PCR received preemptive ganciclovir for a minimum of 4 weeks and generally up to day 100.

Stem cell mobilization, collection, and selection

Donors received human G-CSF (Neupogen®, kindly provided by Amgen, Brussels, Belgium) at 10 μg/kg from day -5 through day -1 before transplant. Collection of PBSC was carried out on days -1 and 0, using a continuous flow blood cell separator (CS3000+, Baxter-Fenwall Laboratories, Deerfield, IL, or Cobe Spectra, Lakewood, CO). The volume of blood processed was 12–16 liters. The PBSC from the first day of harvest were stored overnight in the patient's own plasma. Patients 1–4 received unmanipulated PBSC. In patients 5–11, PBSC were CD8-depleted and in patients 12–14 PBSC were CD34-selected (Fig. 1). Immediately after the second harvest, PBSC from the first and second harvests were pooled. CD8 depletion as well as CD34$^+$ cell selection were carried out using the Isolex 300i© magnetic cell sep-

TABLE 1. PATIENTS AND DONORS

Patients						Donors		
Number	*Age/sex*	*Diagnosis*	*Status at transplant*	*Previous autograft*	*Previous regimens (other than autograft)*	*Relationship*	*Age/sex*	*HLA*
1	58/F	CML	1st CP	No	Hydroxyurea, interferon-α	Sibling	67/M	HLAid
2	64/M	RCC	Metastatic	No	Vinblastine, interferon-α	Sibling	58/F	1 Mismatch
3	65/M	NHL	RR	Yes	Chlor, CVP × 6, CHOP × 7, DHAP, E-Cy	Sibling	58/F	1 Mismatch
4	50/M	NHL	RR	Yes	CVP × 6, CHOP × 6, DHAP × 2, abdominal irradiation	Sibling	42/F	HLAid
5	22/M	HD	2nd CR	Yes	MOPP-ABV × 8, E-Cy × 2, mediastinal irradiation	Sibling	15/F	1 Mismatch
6	58/M	NHL	RR	Yes	ACVBP × 4, VIM-Ara-C, E-Cy, CHOP × 2, abdominal irradiation	Sibling	44/M	HLAid
7	49/M	RCC	Metastatic	No	Vinblastine, interferon-α	Sibling	39/F	HLAid
8	62/M	CML	1st CP	No	Hydroxyurea	Sibling	68/F	HLAid
9	57/F	AML	1st CR	Yes	Dnr + Ara-C × 2, HiDAC	Sibling	68/F	HLAid
10	25/M	ALL	2nd CR	Yes	Dnr + Cy + V, HAM, Mtx + ASP × 2, VAD × 4, Mtz + Ara-C × 2	Unrelated	40/F	HLAid
11	40/M	NHL	RR	Yes	CHOP × 6, E-Cy, PDN-Ri × 4, Mtz + Cy + Vds + PDN, DHAP	Unrelated	48/M	HLAid
12	61/F	CLL	Refractory	No	Chlor + Pdn, CVP × 6, fludarabine × 6	Sibling	61/F	HLAid
13	43/M	NHL	1st CR	Yes	MOPP-ABV × 7, DHAP × 2, E-Cy for HD; ACVBP × 4 for NHL	Sibling	40/M	HLAid
14	64/M	RCC	Metastatic	No	Vinblastine, interferon-α	Child	44/M	1 Mismatch

Chlor, Chlorambucil; CVP, cyclophosphamide, vincristine, prednisone; CHOP, vincristine, adriamycin, cyclophosphamide, prednisone; DHAP, dexamethasone, cytosine arabinoside, cisplatin; E, etoposide; Cy, cyclophosphamide; ACVBP, adriamycin, cyclophosphamide, vincristine, bleomycin, prednisone; VIM, etoposide, ifosfamide, mitoxantrone; MOPP, nitrogen mustard, vincristine, procarbazine, prednisone; ABV, adriamycin, blemomycin, vinblastine; Dnr, daunorubicine; Mtz, mitoxantrone; V, vincristine; HAM, high-dose cytosine arabinoside, mitoxantrone; Mtx, methotrexate; Asp, asparaginase; Ara-C, cytosine arabinoside; HiDAC, high-dose cytosine arabinoside; NHL, non-Hodgkin's lymphoma; HD, Hodgkin's disease; CP, chronic phase; RR, refractory relapse; CR, complete remission; HLAid, HLA-identical; M, male; F, female.

TABLE 2. CONDITIONING REGIMEN AND COMPOSITION OF PBSC GRAFTS

		Cells collected						Cells infused				
Number	*Conditioning regimen*	*$CD34^+$ cells $\times 10^6$/kg*	*$CD3^+$ cells $\times 10^6$/kg*	*$CD4^+$ cells $\times 10^6$/kg*	*$CD8^+$ cells $\times 10^6$/kg*	*$CD56^+$ cells $\times 10^6$/kg*	*Manipulation of PBSC*	*$CD34^+$ cells $\times 10^6$/kg*	*$CD3^+$ cells $\times 10^6$/kg*	*$CD4^+$ cells $\times 10^6$/kg*	*$CD8^+$ cells $\times 10^6$/kg*	*$CD56^+$ cells $\times 10^6$/kg*
1	TBI (2 Gy)	14.85	212	156	79	55	Unmanipulated	14.85	212	156	79	55
2	Fludarabine + TBI (2 Gy)	6.91	382	271	99	61	Unmanipulated	6.91	382	271	99	61
3	Fludarabine + Cyclophosphamide	10.57	323	223	107	140	Unmanipulated	10.57	323	223	107	140
4	TBI (2 Gy)	10.59	318	197	133	44	Unmanipulated	10.59	318	197	133	44
5	TBI (2 Gy)	4.59	270	154	112	30	CD8-depletion	4.54	136	121	2	24
6	TBI (2 Gy)	9.09	206	144	88	50	CD8-depletion	5.65	63	61	4	30
7	Fludarabine + TBI (2 Gy)	5.08	333	256	109	56	CD8-depletion	3.80	206	181	7	52
8	Fludarabine + TBI (2 Gy)	4.47	168	123	62	50	CD8-depletion	2.65	63	55	1	24
9	TBI (2 Gy)	5.09	432	309	106	39	CD8-depletion	4.39	211	185	2	28
10	Fludarabine + TBI (2 Gy)	15.30	753	424	496	225	CD8-depletion	10.43	249	177	10	66
11	Fludarabine + Cyclophosphamide	7.76	227	92	138	94	CD8-depletion	6.21	94	65	22	78
12	TBI (2 Gy)	2.64	374	195	203	87	CD34-selection	1.83	0.08	0.04	0.05	0.05
13	Fludarabine + Cyclophosphamide	8.32	365	216	160	44	CD34-selection	5.71	0.11	0.06	0.06	0.06
14	Fludarabine + TBI (2 Gy)	8.30	340	245	135	54	CD34-selection	7.28	0.08	0.03	0.03	0.07

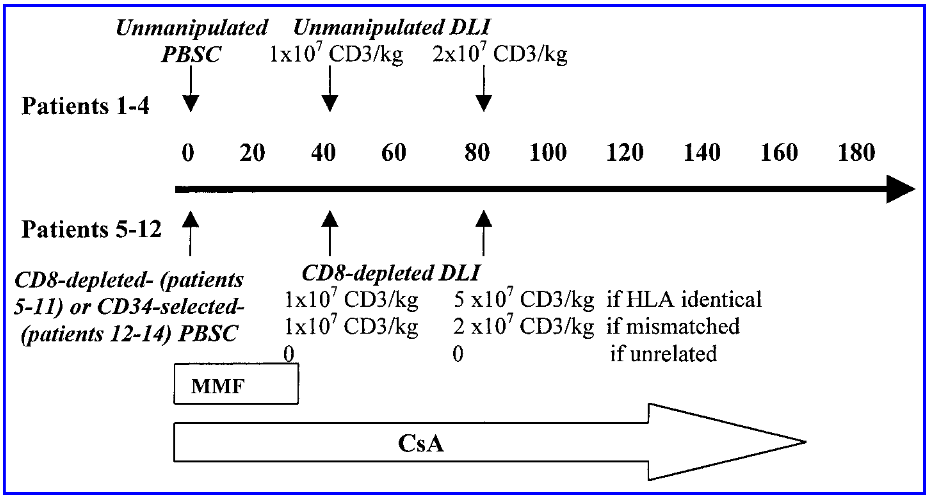


FIG. 1. Schedule of the study. MMF, mycophenolate mofetil.

arator (Nexell International, Wemmel, Belgium), according to the manufacturer's recommendations.

Donor lymphocyte infusions

Around day 40 post-transplantation, donors underwent 12- to 16-liter leukophereses on 2 consecutive days to collect lymphocytes. The collection from the first day of harvest was stored overnight in the patient's own plasma and pooled with the second harvest. Patients 1–4 (unmanipulated PBSC) were assigned to receive unmanipulated DLI (1×10^7 and 2×10^7 CD3$^+$ cells/kg recipient around days 40 and 80, respectively) whereas patients 5–14 (manipulated PBSC) were assigned to receive CD8-depleted DLI (1×10^7 and 5 (2 in mismatched transplants) $\times 10^7$ CD3$^+$ cells/kg recipient around days 40 and 80, respectively) (Fig. 1). CD8-depletion of DLI was carried out with Nexell Isolex 300i© as previously reported. The first DLI was infused fresh whereas the following ones were cryopreserved and thawed. DLI were not to be given in case of an antecedent grade III–IV acute GVHD or active GVHD at time of scheduled infusions nor in recipients of unrelated transplants. Patients with mixed chimerism on day 100 received a third DLI around day 120.

Laboratory analyses

Aliquots of the pooled PBSC as well as the CD8-depleted or CD34-selected fractions were incubated with phycoerythrin (PE)-conjugated anti-CD34, CD3, CD4, CD8, and CD56 monoclonal antibodies (HPCA2; Becton-Dickinson, Palo-Alto, CA) for 20 min at 20°C, washed, and fixed. A total of 1×10^5 cells/condition was analyzed using a FACS-scan analyzer (Becton-Dickinson). The percentage of CD34$^+$ cells was defined with dot plot analysis using the whole nucleated cell population. The percentage of positive cells in the isotype control was subtracted from the CD34$^+$ percentage to give the final percentage of CD34$^+$ cells. Data acquisition was performed with the Cellquest software (Becton-Dickinson). Donor lymphocytes (before and after CD8 depletion) are well as patient peripheral white blood cells (on days 28, 42, 60, 80, 100, 120, 180, and 365) were similarly examined using double labeling with fluorescein isothiocyanate (FITC)- and PE-conjugated antibodies after treatment with a lysing solution.

Chimerism among peripheral blood white blood cells (WBC), T cells, and myeloid cells as well as in unfractionated marrow was assessed at days 28, 42, 60, 80, 100, 120, 180, and 365 after HCT using fluorescence in situ hybridization (FISH) to detect X and Y chromosomes for recipients of sex-mismatched transplants and PCR-based analysis of polymorphic minisatellite or microsatellite regions for recipients of sex-matched transplants (38). CD3 (T cells) and CD13 (myeloid cells) selection was carried out with a FACStar Plus sorter (Becton-Dickinson). Mixed chimerism (MC) was defined as between 1% and 94% donor cells and full chimerism (FC) as ≥95% donor cells.

Statistical analyses

The probability of GVHD, transplant-related mortality (TRM), and survival were studied by life-table analyses, and Wilcoxon rank tests were used for comparisons between groups. Statistical analyses were carried out with Graphpad Prism (Graphpad Software, San Diego, CA).

RESULTS

Collection of PBSC, CD8 depletion, and CD34 selection

A median of 8.0 (2.6–15.3) $\times 10^6$ CD34$^+$ cells/kg, 328 (168–753) $\times 10^6$ CD3$^+$ cells/kg, 207 (92–424) $\times$

10^6 $CD4^+$ cells/kg, and 108 (62–496) $\times$ 10^6 $CD8^+$ cells/kg were collected (Table 2). After CD8 depletion, a median of 4.54 (2.65–10.43) $\times$ 10^6 $CD34^+$ cells/kg, 136 (63–249) $\times$ 10^6 $CD3^+$ cells/kg, 121 (55–185) $\times$ 10^6 $CD4^+$ cells/kg, and 4 (1–22) $\times$ 10^6 $CD8^+$ cells/kg were infused (Table 2). The procedure reduced the number of $CD3^+$, $CD4^+$, and $CD8^+$ cells infused by 0.38, 0.22, and 1.7 log, respectively. After CD34 selection, a median of 5.71 (1.83–7.28) $\times$ 10^6 $CD34^+$ cells/kg, 0.08 (0.08–0.11) $\times$ 10^6 $CD3^+$ cells/kg, 0.04 (0.03–0.06) $\times$ 10^6 $CD4^+$ cells/kg, and 0.05 (0.03–0.06) $\times$ 10^6 $CD8^+$ cells/kg were infused (Table 2). The procedure reduced the number of $CD3^+$, $CD4^+$, and $CD8^+$ cells infused by 3.6, 3.7, and 3.6 log, respectively.

Toxicities and engraftment

None of the 14 patients developed grade 2 or higher regimen-related toxicities (39). Initial engraftment was observed in 100% of the patients, and nonfatal graft rejection occurred only in 1 CML patient (patient #1, whose conditioning did not include fludarabine). The actuarial 180-day incidence of graft rejection was 9%. The neutrophil nadir occurred on day 7 and was 0.72 (0.09 to 2.33) $\times$ 10^9/L (Fig. 2A). Ten out of 14 patients (75%) received a median of 4 (0–8) doses of G-CSF for treatment of neutropenia (Fig. 2C). The median platelet nadir was 88 (8–191) $\times$ 10^9/L (Fig. 2B), and only 3/14 patients (21%) required 1 single (n = 2) or 6 (n = 1) platelet transfusions (Fig. 2C). Finally, the median hemoglobin (Hb) nadir was 10.2 (7.3–11.8) g/dL and 4 patients (29%) required RBC transfusions (1, 2, 3, and 9 units, respectively) during the first month after HSCT (Fig. 2C).

Infections, CMV reactivation, and hospitalization

Six patients (that received only 2 Gy TBI as conditioning regimen) were eligible for outpatient transplantation. Four of the 6 patients did not require hospitalization within 100 days of HSCT. The other patients were hospitalized before transplant for administration of fludarabine with (n = 3) or without (n = 5) cyclophosphamide. They were discharged on day 1 (range 0–9) and 6 of them were rehospitalized within 100 days of HSCT for a median duration of 7 (range 0–35) days.

Forty-three percent of the patients experienced bacterial infections within 100 days of transplant that were successfully managed by antibiotics except in patient 10 who died of pseudomonas septic shock and lung aspergillosis on day 51. In addition, 1 patient died of (presumably viral) encephalitis on day 186. PCR CMV reactivation was detected in 8 of 11 (73%) CMV-seropositive patients and was successfully managed by preemptive ganciclovir in all of them (Table 3).

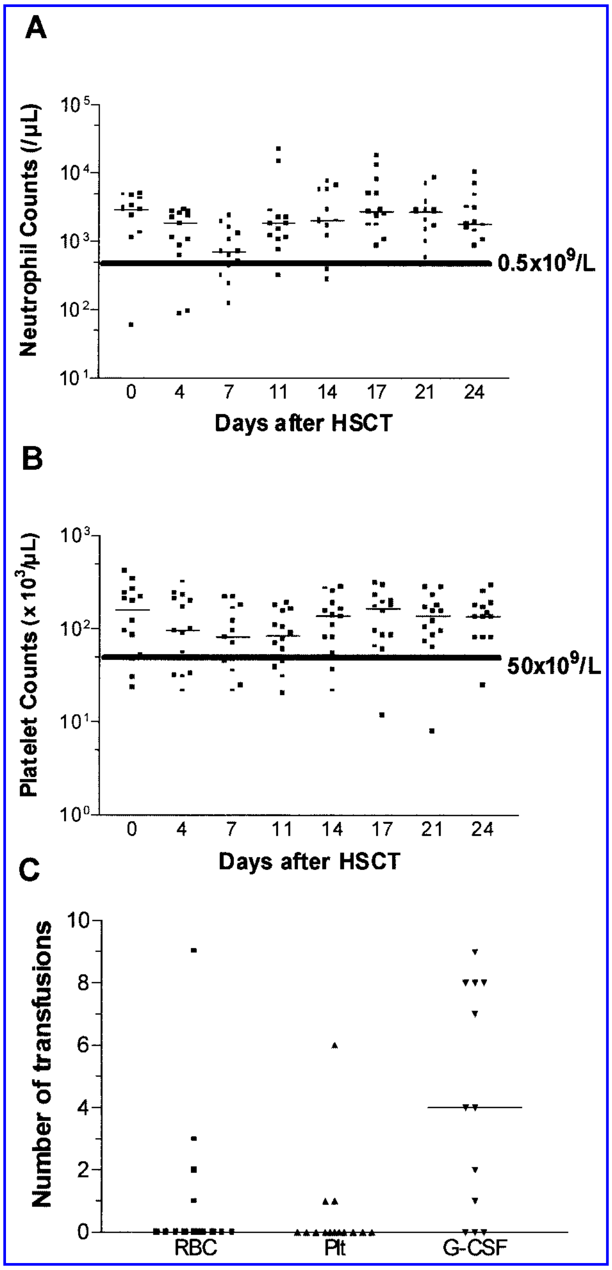


FIG. 2. Evolution of neutrophil (**A**) and platelet (**B**) counts in the immediate post-transplant period. (**C**) Number of RBC and platelet (Plt) transfusions and of G-CSF doses administered per patient.

Donor lymphocyte infusions and chimerism

Four patients (patients 2 and 3, who had active GVHD at that time, as well as patients 10 and 11, who underwent unrelated transplants) did not received preemptive DLI (Table 3). On the other hand, 3 patients received additional DLI for poor chimerism and/or persistence of the underlying disease. DLI were well tolerated and no patient developed significant acute GVHD or marrow aplasia after DLI.

TABLE 3. CLINICAL DATA

	DLI			GVHD			CMV			Follow-up	
				Acute GVHD	Chronic GVHD						
	DLI#1 (d40)	DLI#2 (d80)	DLI#3 (d120)	Grade	Grade (day)	Treatment	Status Don/Rec	Reactivation (PCR+)	Other complications	Disease status	Survival status (day)
1	10	10	—	0	None	—	+/−	Yes (d60)	No	Blast crisis	Death (204)
2	0[a]	0[a]	—	2	Extensive (210)	CsA, steroids	+/+	Yes (d39)	Diabetes, infections	PR>75%	Alive (463)
3	0[a]	0[a]	—	2	Limited (155)	FK506	−/+	Yes (d36)	Encephalitis	CR	Death (186)
4	10	20	—	2	Extensive (158)	CsA, steroid, ECP	−/+	Yes (d74)	Diabetes, infections	CR	Alive (428)
5	10[c]	20[c]	—	0	Limited (180)	Steroids	−/−	No	No	CR	Alive (387)
6	10[c]	50[c]	—	0	Extensive (236)	CsA, steroids	−/+	Yes (d25)	No	CR	Alive (338)
7	Death	Death	—	0	None	—	−/+	No	No	Progression	Death (22)
8	10[c]	50[c]	130[(c)]	0	None	—	+/−	Yes (d52)	No	Maj cytog resp	Alive (280)
9	10[c]	50[c]	—	0	Extensive (219)	—	+/+	No	No	CR	Alive (259)
10	—[b]	—[b]	—	0	None	—	+/+	Yes (d25)	Septic shock, aspergil.	CR	Death (51)
11	—[b]	—[b]	—	1	Extensive (180)	CsA, steroids	−/−	No	Bronchitis	CR	Alive (197)
12	10[c]	50[c]	20	1	None	—	+/−	No	No	PR>25%	Alive (252)
13	10[c]	50[c]	120[(c)]	0	None	—	−/−	No	Endocarditis	CR	Alive (246)
14	10[c]	20[c]	—	0	None	—	−/+	Yes (d26)	No	Too early	Alive (80)

[a]Active GVHD at that time
[b]Unrelated transplant
[c]CD8-depleted
ECP, extracorporeal photopheresis; CR, complete response; PR, partial response; Maj cytog resp, major cytogenetic response; aspergil., aspergillosis; Don, donor; Rec, recipient.

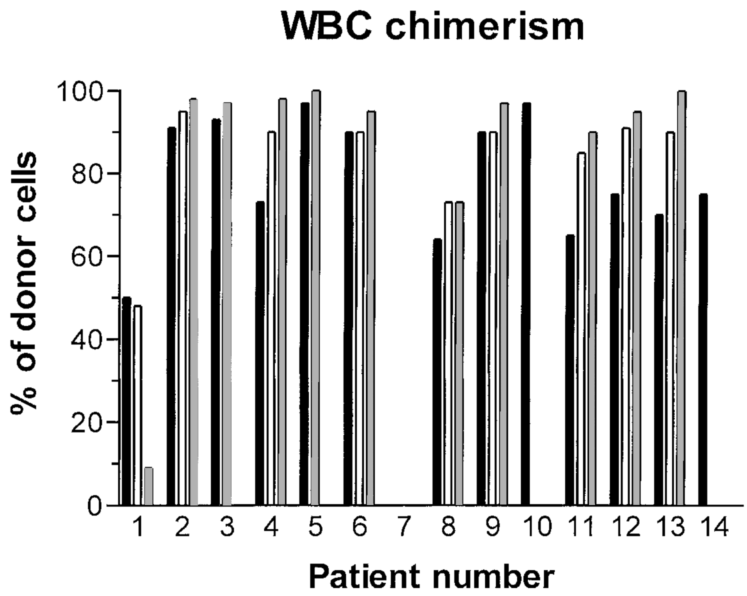
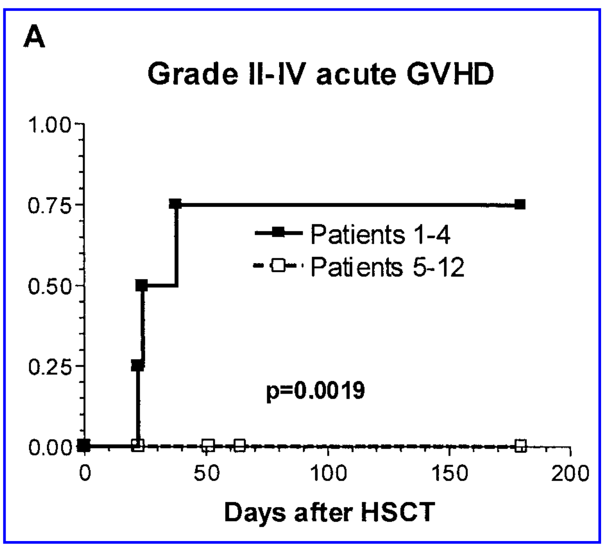


FIG. 3. Evolution of WBC chimerism on days 28 (black bars), 100 (white bars), and 180 (gray bars) after transplantation.

The evolution of chimerism is shown in Fig. 3. Total white blood cell, $CD3^+$ cell, $CD13^+$ cell, and bone marrow (BM) chimerisms were 83% (50–97), 90% (30–98), 95% (50–98), and 90% (50–97), respectively, on day 28; 90% (48–100), 94% (37–100), 95% (71–100), and 90% (7–99), respectively on day 100; and 95% (9–100), 78% (18–98), 98% (3–100), and 97% (60–100), respectively, on day 180. The lowest figures were obtained in patient 1, who rejected her graft after day 100. Two of 3 patients who were full donor chimera (FC) before DLI remained FC after DLI (the third died before DLI). In addition, CD8-depleted DLI converted MC to FC in 5/9 evaluable patients. Thus, FC was achieved in 9/12 evaluable patients. For CD8-depleted or CD34-selected transplant recipients, WBC and BM chimerisms were 83% (64–97) and 89% (70–94), respectively, on day 28; 90% (73–100) and 90% (74–90), respectively, on day 100; and 95% (73–96) and 95% (60–100), respectively, on day 180.

Acute and chronic GVHD

The actuarial 180-day incidence of grade II–IV acute GVHD was 23% (Fig. 4). Three out of 4 patients who received unmanipulated PBSC and DLI (patients 1–4) versus 0/10 patients who received CD8-depleted or CD34-selected PBSC and CD8-depleted DLI (patients



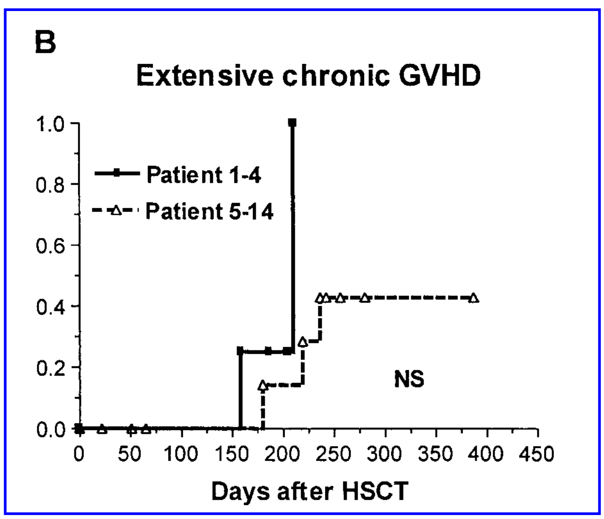


FIG. 4. (**A**) Actuarial 180-day incidence of grades II–IV acute GVHD. (**B**) Actuarial 1-year probability of extensive chronic GVHD.

5–14) experienced grade ≥ II acute GVHD (Table 3). The actuarial 180-day incidence of grade II–IV acute GVHD was 75% for patients 1–4 versus 0% for patients 5–12 (p = 0.0019) (Fig. 4). The actuarial 1-year probabilities of chronic GVHD and extensive chronic GVHD were 68 and 53%, respectively (Fig. 4). For patients 5–14, the figures were 57% and 43%, respectively. For patients 1–4, the figures were 100% and 100%, respectively.

Immune recovery

$CD4^+$ T cells remained greater than 200/μl in 7/13 evaluable patients (Fig. 5). The $CD8^+$ cell recovery was fast and 9/11 evaluable patients had more than 200 $CD8^+$ T cells/μl on day 40. Patients conditioned with Fluda-Cy had slower $CD4^+$ and $CD8^+$ T cell recovery compared to other patients (Fig. 5A,B). On day 40, the median number of $CD4^+$ T cells/μl was 94 (51–127) for Fluda-Cy recipients versus 315 (156–570) for other patients (p = 0.0160). For $CD8^+$ T cells, the figures were 104 (76–283) and 369 (161–676), respectively (p = 0.07). On the other hand, PBSC manipulation apparently also impacted on immune recovery. Patients 1–4 (unmanipulated PBSC) had a higher $CD8^+$ cell count than patients 5–14 (Fig. 5C,D). Finally, mean $CD4^+$ and $CD8^+$ cell counts remained low despite DLI for at least 180 days after CD34-selected transplants (Fig. 5C,D).

Disease responses

Five patients were grafted for refractory or poor prognosis non-Hodgkin's lymphoma (NHL (see Fig. 6)). Four of them were in CR 6 months after the transplant. Patient 11 who developed NHL after an autologous HSCT for refractory Hodgkin's disease was in CR before and remained in CR >6 months after the transplant. One pa-

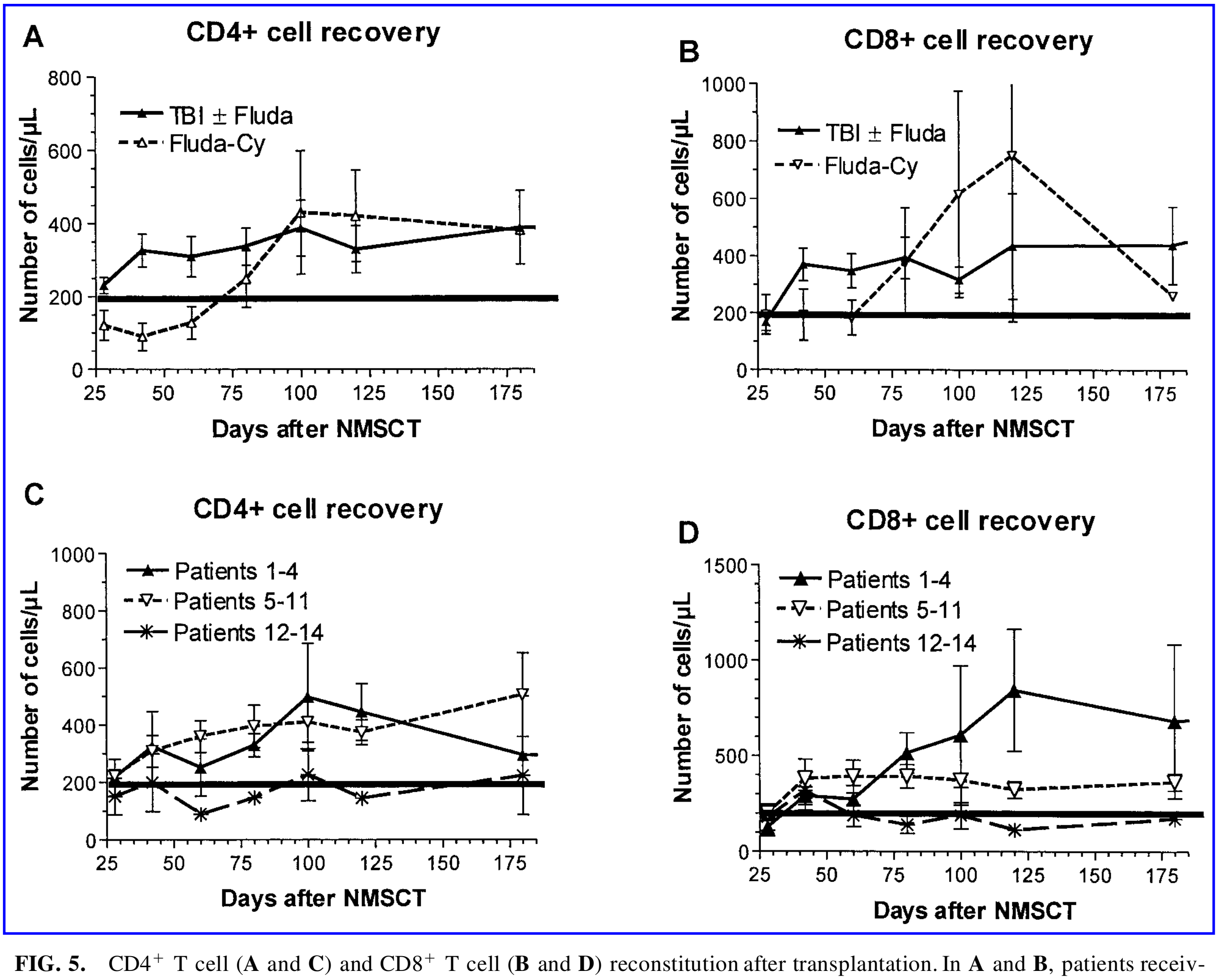


FIG. 5. $CD4^+$ T cell (**A** and **C**) and $CD8^+$ T cell (**B** and **D**) reconstitution after transplantation. In **A** and **B**, patients receiving Fluda-Cy conditioning (broken line) and other patients (continuous line) are shown separately. In **C** and **D**, patients receiving unmanipulated PBSC (patients 1–4; continuous line and closed triangles), CD8-depleted PBSC (patients 5–11; broken line and open triangles), or CD34-selected PBSC (patients 12–14; broken line and stars) are shown separately. The horizontal line indicates the lower limit of normal values.

tient with refractory chronic lymphocytic leukemia (CLL) showed a partial response. The two CML patients achieved hematologic remission and major cytogenetic responses (11 and 14% Ph^+ BM cells on FISH analysis). Unfortunately, the first patient rejected her graft while developing blast crisis on day 141. The second one is currently treated with DLI after cyclosporine discontinuation. Three patients were treated for RCC. The first one (patient 2) achieved partial response on day 180 that further improved on day 365. The second one (patient 7 who had a 15-cm lung metastasis) died of disease progression on day 24. The third one is not yet evaluated for response. The 2 patients with acute leukemia in CR remained in CR 51+ and 180+ days after the transplant. Finally, a patient with Hodgkin's disease (HD) in CR after autologous HSCT and mediastinal radiotherapy remains in CR more than 1 year after the NMSCT. The 1-year probability of relapse mortality was 18%.

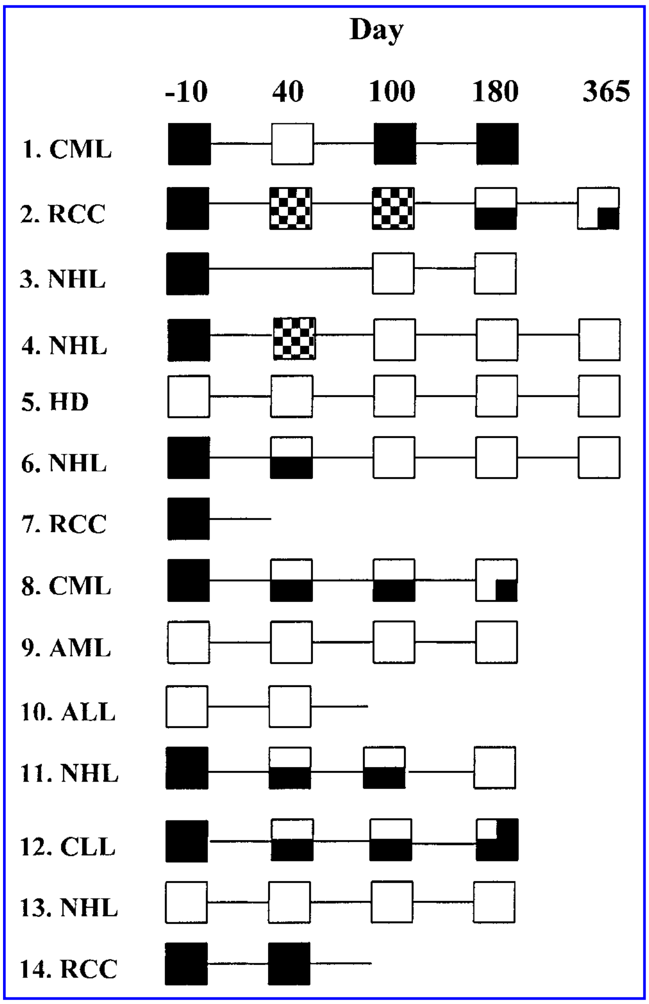


FIG. 6. Evolution of disease response after transplantation. (■) Progression; (▩) stabilization; (◩) partial response ≥ 25%; (⬓) partial response ≥ 50%; (◪) partial response ≥ 75%; (□) complete remission.

Transplant-related mortality and survival

After a median follow-up of 230 (44-561) days, 10 of 14 patients (71%) were alive. The actuarial 1-year probability of survival was 69% (Fig. 7A). Two patients died of progressive disease, 1 of septic shock, and 1 of encephalitis. The actuarial 180-day and 1-year probabilities of transplant-related mortality (TRM) were 8 and 16%, respectively (Fig. 7B).

DISCUSSION

NMSCT is based on a two-step approach: first, induction of mixed chimerism and then, in a second step, eradication of malignant cells by the GVL effect (5,9). The Seattle group has developed a very-low-intensity conditioning regimen with only 2 Gy TBI and post-grafting immunosuppression with CsA and MMF (33,34). This approach allowed the transplant procedure to be performed in an outpatient setting in about 50% of the patients and clearly induced a strong GVL effect with a low TRM (34). However, a high rate of graft rejection was observed in patients who had not previously received intensive chemotherapy.

In our study, initial engraftment was seen in all patients. Chimerism analysis on day 42 in the first patient included [a CML patient in first chronic phase (CP)] evidenced poor chimerism (30% T cell and 50% myeloid as well as BM chimerism). She subsequently rejected her graft around day 100. This observation, as well as the report of similar cases by the Seattle team led us to add fludarabine in the conditioning regimen of patients not heavily pretreated, such as CML and RCC patients. This modified conditioning permitted to achieve durable engraftment in all other patients. To avoid radiation-induced organ damage, patients who had previously received ≥12 Gy TBI had a nonmyeloablative conditioning regimen consisting of cyclophosphamide 3 g/m^2 and fludarabine 90 mg/m^2. This later conditioning regimen also allowed us to conduct the immediate post-transplant course in an outpatient setting, although the degree of myelosuppression was higher. CD8-depletion or CD34-selection of the graft was not associated with initial graft failure or graft rejection, even in mismatched or unrelated transplant recipients. In such patients, initial as well as long-term chimerism was at least as good as in patients receiving unmanipulated PBSC. Although it was thought that a large dose of donor T lymphocytes (and particularly $CD8^+$ lymphocytes) (40) was required to implant an allogeneic graft in the NMSCT setting (4,41), this study demonstrated for the first time that the absence of CD8 lymphocytes, or even of any lymphocytes, in the graft does not impair initial engraftment and sustained chimerism after a very mild nonmyeloablativeregimen and post-transplant CsA and MMF.

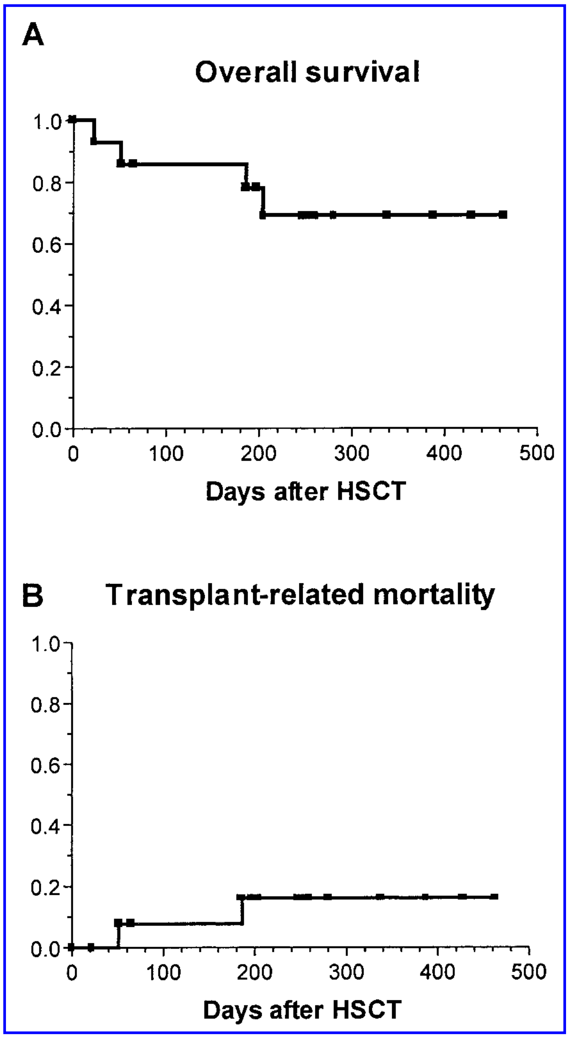


FIG. 7. (**A**) Probability of survival. (**B**) Probability of transplant-related mortality.

Acute and chronic GVHD remained a substantial limitation of the Seattle approach: the incidence of grade II, III, and IV acute GVHD were 33%, 13%, and 4%, respectively (33), and the incidence of chronic GVHD was 74% (34). In animal models, it is now well demonstrated that donor lymphocytes given several weeks after the transplant in mixed chimera induce significantly less GVHD than a similar dose of donor T cells given together with the graft (41,42), without reducing their antitumor efficacy (21,41). More recently, we have reported that after a myeloablative conditioning regimen, the transplantation of CD34-selected allogeneic PBSC followed by pre-emptive CD8-depleted DLI significantly decreased the incidence of acute and severe chronic GVHD compared to transplantation of unmanipulated bone marrow (35). Therefore, we evaluated the feasibility of transplanting T cell-depleted PBSC (by CD8-depletion or by CD34-selection) followed by preemptive CD8-depleted DLI after a mild nonmyeloablative regimen.

Three of the 4 patients (including 2 mismatched transplants) who received unmanipulated PBSC and DLI developed grades II–IV acute GVHD and chronic GVHD. On the other hand, none of the 10 patients who received CD8-depleted or CD34-selected PBSC and pre-emptive CD8-depleted DLI experienced grades II–IV acute GVHD, although this group also included 2 mismatched and 2 unrelated transplants. We chose to give preemptive DLI under cyclosporine prophylaxis because two previous reports have shown that this strategy maintains the GVL effect (35,43). Moreover, in a preliminary report of the French transplant group, the duration of cyclosporine prophylaxis after NMSCT was strongly associated with increased overall survival (44). Unfortunately, despite the short follow-up of our patients, the incidence of extensive chronic GVHD in CD8-depleted transplants was still significant (43%), although lower than that reported by the Seattle team and that observed in our patients receiving unmanipulated PBSC and DLI (in 3 patients).

The small number of patients included as well as their heterogeneity does not allow an accurate estimate of the GVL effect. However, responses were observed in both the unmanipulated and CD8-depleted or CD34-selected groups, suggesting that these latter approaches did not impair the GVL effect, at least when preemptive CD8-depleted DLI are systematically given after the PBSC transplant.

Another approach (than CD8-depletion) to separate the GVL effect from GVHD would be to infuse specific cytotoxic T cells (CTL) instead of DLI. Donor-derived CTL have been successfully used for the treatment of CMV infections (45) or for prevention or treatment of Epstein-Barr virus (EBV)-associated lymphoma after allogeneic HSCT (46). Remarkably, no significant toxicity nor GVHD were observed with this early post-transplant cell immunotherapy. Recently, the Leiden group reported the achievement of CR by treatment with leukemia-reactive CTL in a patient with accelerated phase CML refractory to standard DLI (47,48). The infusion of donor-derived CTL against specific antigens such as minor histocompatibility antigen (mHA) preferentially expressed in the hematopoietic system (49,50), tumor-specific antigens (51), or antigens overexpressed in tumor cells (52,53) after CD8-depleted or CD34-selected PBSC may permit the GVL effect to be increased greatly while minimizing the risk of GVHD.

In conclusion, our study showed that CD8-depleted and CD34-selected PBSC can engraft after a light conditioning regimen consisting of 2 Gy TBI (and fludarabine for previously untreated patients). The preliminary results suggest that CD8 depletion or CD34 selection of the graft followed by CD8-depleted DLI strongly decrease the incidence of acute GVHD while apparently preserving the GVL effect. Further studies are needed to confirm this preliminary report, to assess the impact of our strategy on the incidence and severity of chronic GVHD, and to investigate the infusion of specific CTL instead of CD8-depleted DLI.

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REFERENCES

1. Storb R. (1995). Bone marrow transplantation. Transplant Proc 27:2649–2652.
2. Buckner CD, RB Epstein, RH Rudolph, RA Clift, R Storb and ED Thomas. (2001). Allogeneic marrow engraftment following whole body irradiation in a patient with leukemia. J Hematother Stem Cell Res 10:201–208.
3. McSweeney PA and R Storb. (1999). Mixed chimerism: preclinical studies and clinical applications. Biol Blood Marrow Transplant 5:192–203.
4. Storb R, C Yu, T Barnett, JL Wagner, HJ Deeg, RA Nash, HP Kiem, PA McSweeney, K Seidel, G Georges and JM Zaucha. (1999). Stable mixed hematopoietic chimerism in dog leukocyte antigen-identical littermate dogs given lymph node irradiation before and pharmacologic immunosuppression after marrow transplantation. Blood 94:1131–1136.
5. Slavin S, A Nagler, E Naparstek, Y Kapelushnik, M Aker, G Cividalli, G Varadi, M Kirschbaum, A Ackerstein, S Samuel, A Amar, C Brautbar, O Ben-Tal, A Eldor and R Or. (1998). Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. Blood 91:756–763.
6. Horowitz MM, RP Gale, PM Sondel, JM Goldman, J Kersey, HJ Kolb, AA Rimm, O Ringden, C Rozman and B Speck. (1990). Graft-versus-leukemia reactions after bone marrow transplantation. Blood 75:555–562.
7. Drobyski WR. (2000). Evolving strategies to address adverse transplant outcomes associated with T cell depletion. J Hematother Stem Cell Res 9:327–337.
8. Barrett J and R Childs. (2000). The benefits of an alloresponse: graft-versus-tumor. J Hematother Stem Cell Res 9:347–354.
9. Morecki S and S Slavin. (2000). Toward amplification of a graft-versus-leukemia effect while minimizing graft-versus-host disease. J Hematother Stem Cell Res 9:355–366.
10. Baron F and Y Beguin. (2000). Adoptive immunotherapy with donor lymphocyte infusions after allogeneic HPC transplantation. Transfusion 40:468–476.
11. Slavin S, E Naparstek, A Nagler, A Ackerstein, J Kapelushnik and R Or. (1995). Allogeneic cell therapy for relapsed leukemia after bone marrow transplantation with donor peripheral blood lymphocytes. Exp Hematol 23:1553–1562.
12. Porter DL. (2001). The graft-versus-tumor potential of allogeneic cell therapy: an update on donor leukocyte infusions and nonmyeloablative allogeneic stem cell transplantation. J Hematother Stem Cell Res 10:465–480.
13. Baron F, P Frère, G Fillet and Y Beguin. (2001). Treatment of leukaemia relapse after allogeneic hematopoietic stem cell transplantation by donor lymphocyte infusion and STI-571. Haematologica 86:993–994.
14. Mattei D, G Saglio, E Gottardi, A Gallamini, N Mordini, and A Bacigalupo. (2001). Persisting molecular remission ten years after donor lymphocyte infusion for hematologic relapse in chronic myeloid leukemia. Haematologica 86:545–546.
15. Kolb HJ, A Schattenberg, JM Goldman, B Hertenstein, N Jacobsen, W Arcese, P Ljungman, A Ferrant, L Verdonck and D Niederwieser. (1995). Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. European Group for Blood and Marrow Transplantation Working Party Chronic Leukemia. Blood 86: 2041–2050.
16. Collins RHJ, O Shpilberg, WR Drobyski, DL Porter, S Giralt, R Champlin, SA Goodman, SN Wolff, W Hu, C Verfaillie, A List, Dalton, N Ognoskie, A Chetrit, JH Antin and J Nemunaitis. (1997). Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. J Clin Oncol 15:433–444.
17. Alyea EP. (2000). Adoptive immunotherapy: insights from donor lymphocyte infusions. Transfusion 40:393–395.
18. Klingemann HG. (2001). Cellular therapy of cancer with natural killer cells: will it ever work? J Hematother Stem Cell Res 10:23–26.
19. Chiorean EG and JS Miller. (2001). The biology of natural killer cells and implications for therapy of human disease. J Hematother Stem Cell Res 10:451–463.
20. Baron F and Y Beguin. (2002). Nonmyeloablative allogeneic hematopoietic stem cell transplantation. J Hematother Stem Cell Res 11:243–263.
21. Spitzer TR, S McAfee, R Sackstein, C Colby, HC Toh, P Multani, SL Saidman, D Weymouth, F Preffer, C Poliquin, A Foley, B Cox, DM Andrews, DH Sachs and M Sykes. (2000). Intentional induction of mixed chimerism and achievement of antitumor responses after nonmyeloablative conditioning therapy and HLA-matched donor bone marrow transplantation for refractory hematologic malignancies. Biol Blood Marrow Transplant 6:309–320.
22. Carella AM, R Champlin, S Slavin, PA McSweeney and R Storb. (2000). Mini-allografts: ongoing trials in humans. Bone Marrow Transplant 25:345–350.
23. Nagler A, S Slavin, G Varadi, E Naparstek, S Samuel and R Or. (2000). Allogeneic peripheral blood stem cell transplantation using a fludarabine-based low intensity conditioning regimen for malignant lymphoma. Bone Marrow Transplant 25:1021–1028.

24. Nagler A, M Aker, R Or, E Naparstek, G Varadi, C Brautbar and S Slavin. (2001). Low-intensity conditioning is sufficient to ensure engraftment in matched unrelated bone marrow transplantation. Exp Hematol 29:362–370.
25. Giralt S, PF Thall, I Khouri, X Wang, I Braunschweig, C Ippoliti, DF Claxton, M Donato, J Bruton, A Cohen, M Davis, B Andersson, P Anderlini, J Gajewski, S Kornblau, M Andreef, D Przepiorka, NT Ueno, J Molldrem and R Champlin. (2001). Melphalan and purine analog-containing preparative regimens: reduced-intensity conditioning for patients with hematologic malignancies undergoing allogeneic progenitor cell transplantation. Blood 97:631–637.
26. Mohty M, C Faucher, N Vey, AM Stoppa, F Viret, I Chabbert, C Chabannon, R Bouabdallah, P Ladaique, L Collet, C Zandotti, D Maraninchi and D Blaise. (2000). High rate of secondary viral and bacterial infections in patients undergoing allogeneic bone marrow mini-transplantation. Bone Marrow Transplant 26:251–255.
27. Kottaridis PD, DW Milligan, R Chopra, R Chakraverty, S Chakrabarti, S Robinson, K Peggs, DC Linch and S Mackinnon. (2000). In vivo CAMPATH-1H prevents graft-versus-host disease following nonmyeloablative stem cell transplantation. Blood 96:2419–2425.
28. Bornhausser M, C Thiede, U Platzbecker, J Freiberg-Richter, A Helwig, R Plettig, C Röllig and G Ehninger. (2000). Dose-reduced conditioning for allogeneic blood stem cell transplantation: durable engraftment without antithymocyte globulin. Bone Marrow Transplant 26:119–125.
29. Barrett AJ and R Childs. (2000). Non-myeloablative stem cell transplants. Br J Haematol 111:6–17.
30. Lau FY, R Wong, CH Chui and G Cheng. (2001). Successful engraftment in two adult patients with severe aplastic anemia using nonmyeloablative conditioning followed by unrelated hla-mismatched cord blood transplantation. J Hematother Stem Cell Res 10:309–311.
31. Childs R, E Clave, N Contentin, D Jayasekara, N Hensel, S Leitman, EJ Read, C Carter, E Bahceci, NS Young and AJ Barrett. (1999). Engraftment kinetics after nonmyeloablative allogeneic peripheral blood stem cell transplantation: full donor T-cell chimerism precedes alloimmune response. Blood 94:3234–3241.
32. Carella AM, S Giralt and S Slavin. (2000). Low intensity regimens with allogeneic hematopoietic stem cell transplantation as treatment of hematologic neoplasia. Haematologica 85:304–313.
33. Storb R. (2001). Nonmyeloablative preparative regimens: how relevant for acute myelogenous leukemia? Leukemia 15:662–663.
34. McSweeney PA, D Niederwieser, J Shizuru, BM Sandmaier, A Molina, DG Maloney, TR Chauncey, T Gooley, U Hegenbart, RA Nash, J Radich, JL Wagner, S Minor, FR Appelbaum, WI Bensinger, E Bryan, ED Flowers, G Georges, FC Grumet, HP Kiem, B Torok-Storb, C Yu, KG Blume and R Storb. (2001). Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. Blood 97:3390–3400.
35. Baron F, J Siquet, E Baudoux, N Schaaf-Lafontaine, JP Hermanne, G Fillet and Y Beguin. (2002). Pre-emptive immunotherapy with CD8-depleted donor lymphocytes after CD34-selected allogeneic peripheral blood stem cell transplantation. Haematologica 87:78–88.
36. Przepiorka D, D Weisdorf, P Martin, HG Klingemann, P Beatty, J Hows and ED Thomas. (1995). 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant 15:825–828.
37. Margolis J and G Vogelsang. (2000). Chronic graft-versus-host disease. J Hematother Stem Cell Ress 9:339–346.
38. Thiede C, M Bornhausser, U Oelschlägel, C Brendel, R Leo, H Daxberger, B Mohr, M Florek, F Kroschinsky, G Geissler, R Naumann, M Ritter, G Prange-Krex, T Lion, A Neubauer and G Ehninger. (2001). Sequential monitoring of chimerism and detection of minimal residual disease after allogeneic bone marrow transplantation (BSCT) using multiplex PCR amplification of short tandem repeat markers. Leukemia 15:293–302.
39. Bearman SI, FR Appelbaum, CD Buckner, FB Petersen, LD Fisher, RA Clift and ED Thomas. (1988). Regimen-related toxicity in patients undergoing bone marrow transplantation. J Clin Oncol 6:1562–1568.
40. Martin P, S Rowley, C Anasetti, TR Chauncey, T Gooley, E Petersdorf, JO Burik, M Flowers, R Storb, FR Appelbaum, and J Hansen. (1999). A phase I-II clinical trial to evaluate removal of CD4 cells and partial depletion of CD8 cells from donor marrow for HLA-mismatched unrelated recipients. Blood 94:2192–2199.
41. Pelot MR, DA Pearson, K Swenson, G Zhao, J Sachs, YG Yang and M Sykes. (1999). Lymphohematopoietic graft-versus-host reactions can be induced without graft-versus-host disease in murine mixed chimeras established with a cyclophosphamide-based non-myeloablative conditioning regimen. Biol Blood Marrow Transplant 5:133–143.
42. Kolb HJ, W Gunther, M Schumm, E Holler, W Wilmanns and S Thierfelder. (1997). Adoptive immunotherapy in canine chimeras. Transplantation 63:430–436.
43. Barrett AJ, D Mavroudis, J Tisdale and E Read. (1998). T cell-depleted bone marrow transplantation and delayed T cell add-back to control acute GVHD and conserve a graft-versus-leukemia effect. Bone Marrow Transplant 21: 543–551.
44. Michallet M, K Bilger, F Garban, M Attal, A Huyn, D Blaise, N Milpied, P Moreau, P Bordigoni, M Kuentz, A Sadoun, JY Cahan , G Socié, X Thomas, P Arnaud, N Raus, V Lhéritier, A Pigneux and JM Boiron. (2001). Allogeneic hematopoietic stem cell transplantation after nonmyeloablative preparative regimens: impact of pretransplantation and posttransplantation factors on outcome. J Clin Oncol 19:3340–3349.
45. Walter EA, PD Greenberg, MJ Gilbert, RJ Finch, KS Watanabe, Thomas, ED and SR Riddell. (1995). Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. N Engl J Med 333:1038–1044.
46. Rooney CM, CA Smith, CY Ng, SK Loftin, JW Sixbey, Y Gan, DK Srivastava, LC Bowman, RA Krance, MK Brenner and HE Heslop. (1998). Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus-induced lymphoma in allogeneic transplant recipients 92:1549–1555.

47. Falkenburg JH, AR Wafelman, P Joosten, WM Smith, CAM van Bergen, R Bongaerts, E Lurvink, M van der Hoorn, P Kluck, JE Landegent, C Kluin-Nelemans, WE Fibbe and R Willemze. (1999). Complete remission of accelerated phase chronic myeloid leukemia by treatment with leukemia reactive cytotoxic T lymphocytes. Blood 94:1201–1208.
48. Marijt WA and JH Falkenburg. (2001). Specific T cell therapy in leukemia. J Hematother Stem Cell Res 10:493–500.
49. Warren E, P Greenberg and SR Riddell. (1998). Cytotoxic T-lymphocyte-defined human minor histocompatibility antigens with a restricted tissue distribution. Blood 91:2197–2207.
50. Mutis T, R Verdijk, E Schrama, B Esendam, A Brand and E Goulmy. (1999). Feasibility of immunotherapy of relapsed leukemia with ex vivo generated cytotoxic T lymphocytes specific for hematopoietic system-restricted minor histocompatibility antigens. Blood 93:2336–2341.
51. Osman Y, M Takahashi, Z Zheng, T Koike, K Toba, A Liu, T Furukawa, S Aoki and Y Aizawa. (1999). Generation of bcr-abl specific cytotoxic T-lymphocytes by using dendritic cells pulsed with bcr-abl (b3a2) peptide: its applicability for donor leukocyte transfusions in marrow grafted CML patients. Leukemia 13:166–174.
52. Molldrem J, PP Lee, C Wang, K Felio, H Kantarjian, RE Champlin and MM Davis. (2000). Evidence that specific T lymphocytes may participate in the elimination of chronic myelogenous leukemia. Nature Med 6:1018–1023.
53. Gao L, I Bellantuono, A Elsasser, SB Marley, MY Gordon, JM Goldman and HJ Stauss. (2000). Selective elimination of leukemic CD34(+) progenitor cells by cytotoxic T lymphocytes specific for WT1. Blood 95:2198–2203.

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2. Fr??d??ric Baron, Nicole Schaaf-Lafontaine, St??phanie Humblet-Baron, Nathalie Meuris, Emilie Castermans, Etienne Baudoux, Pascale Fr??re, Vincent Bours, Georges Fillet, Yves Beguin. 2004. T-cell reconstitution after unmanipulated, CD8-depleted or CD34-selected nonmyeloablative peripheral blood stem-cell transplantation. *Transplantation* **76**:12, 1705-1713. [CrossRef]
3. F. Baron , Y. Beguin . 2002. Nonmyeloablative Allogeneic Hematopoietic Stem Cell Transplantation. *Journal of Hematotherapy & Stem Cell Research* **11**:2, 243-263. [Abstract] [PDF] [PDF Plus]