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Low T-cell chimerism is not followed by graft rejection after nonmyeloablative stem cell transplantation (NMSCT) with CD34-selected PBSC

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Summary:

We investigate the feasibility of CD34-selected peripheral blood stem cell (PBSC) transplantation followed by preemptive CD8-depleted donor lymphocyte infusions (DLI) after a minimal conditioning regimen. Six patients with advanced hematological malignancies ineligible for a conventional myeloablative transplant ($n=5$) or metastatic renal cell carcinoma ($n=1$), and with an HLA-identical ($n=4$) or alternative ($n=2$) donor were included. The nonmyeloablative conditioning regimen consisted in 2 Gy TBI alone ($n=4$), 2 Gy TBI and fludarabine (RCC patient, $n=1$) or cyclophosphamide and fludarabine (patient who had previously received 12 Gy TBI, $n=1$). Post transplant immunosuppression was carried out with cyclosporin (CyA) and mycophenolate mofetil (MMF). Initial engraftment was achieved in all patients. One out of six patients (17%) experienced grade $\geqslant 2$ acute GVHD only after abrupt cyclosporin discontinuation and α interferon therapy for life-threatening tumor progression. T-cell chimerism was 23% (19–30) on day 28, 32% (10–35) on day 100, 78% (49–95) on day 180 and 99.5% (99–100) on day 365. Three out of four patients who had measurable disease before the transplant experienced a complete response. We conclude that CD34-selected NMSCT followed by CD8-depleted DLI is feasible and preserves engraftment and apparently also the graft-versus-leukemia (GVL) effect. Further studies are needed to confirm this encouraging preliminary report.
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Keywords: hematopoietic stem cell transplantation; nonmyeloablative; T-cell depletion; GVHD; immunity

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In an attempt to reduce mortality associated with allogeneic myeloablative hematopoietic stem cell transplantation (HSCT) in elderly patients or in patients relapsing after a previous transplant, nonmyeloablative conditioning regimens have been developed with the aim of obtaining donor engraftment and using the graft-versus-leukemia (GVL) effect to eradicate underlying malignancies.[1–3] After extensive preclinical studies,[4–8] the Seattle team developed a nonmyeloablative HSCT approach combining 2 Gy TBI + 90 mg/m^2 fludarabine as the conditioning regimen and postgrafting immunosuppression with cyclosporin (CyA) and mycophenolate mofetil (MMF).[5] This approach was recently shown to be feasible (even in patients who were ineligible for a conventional transplant) with a low transplant-related mortality (TRM) that was most often attributed to graft-versus-host disease (GVHD) and/or infections.[9,10] Thus, reduction in the incidence of GVHD is a major challenge to improve the outcome of NMSCT recipients.

In animal models, two conditions are required to obtain powerful GVL effects without GVHD after allogeneic HSCT. The first condition is the absence of GVH-reactive T cells in the initial donor graft, and the second is to allow sufficient time for the recipient to recover from conditioning-induced inflammation before administering donor lymphocyte infusions (DLI).[11,12] In humans, several reports have demonstrated the feasibility of such an approach in the myeloablative transplant setting.[13] The aim of this pilot study was to examine the feasibility of CD34-selected NMSCT followed by preemptive CD8-depleted DLI.

Study design

Patients

Six consecutive male patients aged 35–65 (median 61) years and ineligible for a myeloablative allogeneic transplant were included. Their clinical characteristics are summarized in Table 1. Written informed consent was obtained from patients and donors and our institution's Ethical Committee approved the protocol.

Conditioning and transplant procedure

Donors (HLA-identical siblings ($n=4$) or one-mismatch related donor ($n=2$)) received human G-CSF at 10 μg/kg from day −5 to day −1 before transplant. Collection of peripheral blood stem cell (PBSC) was carried out on days −1 and 0, using a continuous flow blood cell separator (CS3000+, Baxter-Fenwall Laboratories, Deerfield, IL, USA or Cobe Spectra, Lakewood, CO, USA). Immediately after the second harvest, PBSC from the first and second harvests were pooled and CD34$^+$ cell selection was carried out using the Isolex 300i© magnetic cell separator (Baxter), according to the manufacturers' recommendations as previously reported.[14,15] Nonmyeloablative conditioning regimens consisted of 2 Gy TBI alone ($n=4$), 2 Gy TBI and 30 mg/m^2/day fludarabine for 3 days (RCC patient) or cyclophosphamide (1 g/m^2/day for 3 days) and fludarabine (an NHL patient who had previously received 12 Gy TBI). Post transplant immunosuppression was carried out with CyA (CyA, from day –1 to day 180 or longer in case of alternative donor or chronic GVHD) and mycophenolate mofetil (MMF, 15 mg/kg b.i.d. from day −1 to day 28) as previously described.[14]

Donor lymphocyte infusions

Around day 40 post transplantation, donors underwent 12–161 leukaphereses on two consecutive days to collect lymphocytes. CD8-depletion was carried out with Baxter Isolex 300i© as previously reported.[14] Patients received CD8-depleted DLI at doses of 1×10^7 and 5 (2 in mismatched transplants) $\times10^7$ CD3$^+$ cells/kg recipient around days 40 and 80, respectively. We chose that schedule of pre-emptive CD8-depleted DLI because we had previously shown that it was safe after a myeloablative CD34-selected HSCT.[14,16] Patients with mixed chimerism on day 100 or with progressive disease received additional DLI (Table 1). The first DLI was infused fresh while the following ones were cryopreserved and thawed.

Clinical management

The diagnosis and grading of acute and chronic GVHD was established as previously reported.[17,18] Disease evaluation was routinely carried out on days 40, 100, 180 and 365.

Laboratory analyses

Aliquots of the pooled PBSC as well as the CD34-selected fractions were incubated with phycoerythrin (PE)-conjugated anti-CD34, CD3, CD4, CD8 and CD56 monoclonal antibodies for 20 min at 20°C, washed and fixed. A total of 1×10^5 cells/condition was analyzed using an 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).

Chimerism among peripheral blood T cells and myeloid cells as well as in unfractionated marrow was assessed on days 28, 42, 60, 80, 100, 120, 180, 240, 365 and 730 after HCT. For recipients of sex-mismatched transplants ($n=2$), chimerism was assessed by fluorescence *in situ* hybridization (FISH) to detect X and Y chromosomes. For recipients of sex-matched transplants ($n=4$), chimerism was assessed by a PCR assay with multiplex amplification of nine STR-loci and fluorescence detection as described by Thiede *et al*.[19] CD3 (T cells) and CD13/CD33 (myeloid cells) selection was carried out with an FACStar Plus sorter (Becton-Dickinson) or with RosetteSep (StemCell technologies, Vancouver, Canada). Mixed chimerism (MC) was defined as between 5 and 94% donor cells and full chimerism (FC) as $\geqslant$95% donor cells.

Statistical analyses

The probability of GVHD, TRM and survival were studied by life-table analyses. Statistical analyses were carried out with the Graphpad Prism (Graphpad Software, San Diego, CA, USA).

Results

Toxicities and engraftment

None of the six patients developed grade 2 or higher regimen-related toxicities.[20] Donor cells engrafted in all patients. The neutrophil nadir occurred on day 7 and was 1.3 (0.12–1.32) $\times10^9$/l. Three out of six patients received a median of four (0–7) doses of G-CSF for treatment of neutropenia. The median platelet nadir was 67 (10–165) $\times10^9$/l and only 1/6 patients (17%) required one platelet transfusion. Finally, the median Hb nadir was 9.8

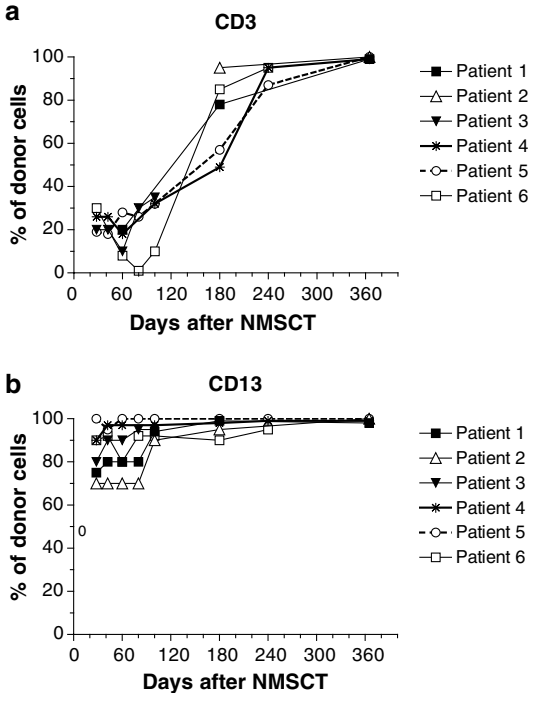

Figure 1 Evolution of T (**a**) and myeloid (**b**) cell chimerism.

(9.2–13.5) g/dl and one patient required RBC transfusion during the first month after HSCT because of angina pectoris.

Chimerism

The evolution of chimerism is shown in Figure 1. Median T cell (CD3+) and myeloid cell (CD13+ or CD33+) chimerisms were 23% (19–30) and 85% (70–100), respectively, on day 28; 32% (10–35) and 94% (90–100), respectively on day 100; 78% (49–95) and 98% (90–100), respectively on day 180 and 99.5% (99–100) and 99.5% (99–100), respectively on day 365. CD3 and CD13 chimerisms 2 years after the transplant were 100 and 100%, respectively, in patient 1 and 100 and 100%, respectively, in patient 2.

GVHD

Only one out of six patients (17%) experienced grade $\geqslant 2$ acute GVHD. This patient had been transplanted for

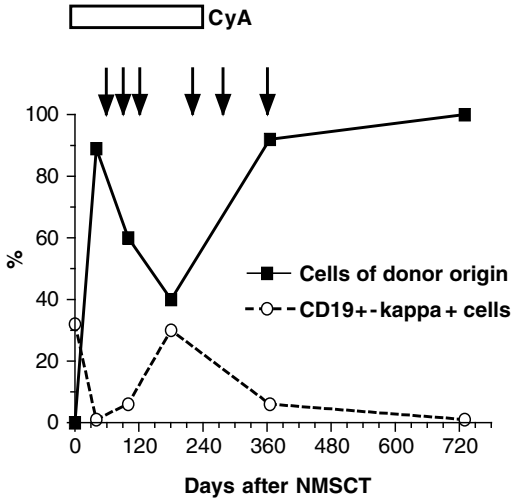

Figure 2 Disease evolution in patient 1. Leukemic cells (broken line) disappeared progressively after DLI (black arrows), as donor cells increased in the bone marrow.

Table 1 Patients, donors and clinical evolution

	Patient 1	*Patient 2*	*Patient 3*	*Patient 4*	*Patient 5*	*Patient 6*
Patient						
Age/sex	65/M	43/M	65/M	65/M	35/M	58/M
Underlying disease	CLL	NHL	RCC	MM	HD	NHL
Status at transplantation	RD	CR[a]	RD	Relapse after a previous auto-HSCT	CR after a previous auto-HSCT[b]	Relapse after a previous auto-HSCT
Number of previous regimens	3	4[a]	1	3	4	3
Donor						
Age/sex	61/F	40/M	44/M	58/F	32/M	24/M
Relationship	Sibling	Sibling	Child	Sibling	Sibling	Child
HLA compatibility	HLAid	HLAid	1 MM	HLAid	HLAid	1 MM
Graft						
CD34+/kg infused ($\times 10^6$)	1.83	5.71	7.28	2.19	8.79	5.36
CD3+/kg infused ($\times 10^6$)	0.08	0.11	0.08	0.05	0.04	0.05
DLI : CD3+/kg infused ($\times 10^6$)						
DLI 1 (day 40)	10	10	10	10	10	10
DLI 2 (day 80)	50	50	20	50	50	20
DLI 3 [dose (day)]	50 (120)	—	—	50 (230)	50 (230)	25 (110)
DLI 4 [dose (day)]	20[c] (220)	—	—	—	—	—
DLI 5 [dose (day)]	50[c] (280)	—	—	—	—	—
DLI 6 [dose (day)]	50[c] (360)	—	—	—	—	—
Graft-versus-host disease						
Acute GVHD (grade)	1	0	3[d]	0	0	0
Chronic GVHD	Limited	Extensive	N/A	No	Limited	Extensive
Disease evolution						
Best response achieved	CR	CR	PD	CR	CR	CR
Current disease status	CR	CR	Death	CR	CR	CR
Survival						
Survival status (day)	Alive (844+)	Alive (838+)	Death (119)	Alive (378+)	Alive (376+)	Alive (336+)
Cause of death	—	—	Progression	—	—	—

[a]Also previous auto-HSCT for HD.
[b]CR1 was achieved only after radiotherapy and CR2 only after auto-transplantation.
[c]Unmanipulated.
[d]After abrupt CyA discontinuation and α-Interferon therapy for life-threatening tumor progression.
RD = refractory disease; CR = complete remission; CLL = chronic lymphoid leukemia; NHL = non-Hodgkin's lymphoma; RCC = renal cell carcinoma, MM = multiple myeloma, HD = Hodgkin's disease, N/A = not applicable.

metastatic RCC and developed grade 3 acute GVHD after abrupt CyA discontinuation and α-interferon therapy for life-threatening tumor progression. Four patients developed limited ($n=2$) or extensive ($n=2$) chronic GVHD.

Response

Three out of four patients who had measurable disease before the transplant achieved CR (Figure 2 and Table 1). The third one, transplanted for refractory metastatic RCC, died of progressive disease 120 days after the transplant (Table 1). The two patients transplanted for poor-risk lymphoma in CR remained in CR (Table 1).

Discussion

Two previous preliminary reports have studied the feasibility of NMSCT with CD34-selected PBSC after a nonmyeloablative conditioning regimen combining 2 Gy TBI and fludarabine[21,22] (Table 2). Four out of five patients reported by Kreiter *et al* rejected their transplant 37–210 days after NMSCT. Major differences with our protocol include: (1) no pre-emptive DLI before day 100; and (2) discontinuation of CyA and MMF between days 15 and 50 in patients without GVHD. DLI given early (around day 40) after a T-cell depleted NMSCT have been previously reported to convert mixed into complete donor chimerism.[23,24] Thus, we hypothesized that CD8-depleted pre-emptive DLI given in our patients on days 40 and 80 after the transplant were critical to avoid transplant rejection. Similarly, it has been shown that extending the duration of CyA from 35 to 100 days after the transplant favorably influenced stable donor engraftment in dogs conditioned with 100 cGy TBI and post grafting immunosuppression combining CyA and MMF. Thus, extending the duration of CyA administration in our patients possibly also had a favorable impact.

Gorner *et al* [22] reported the evolution of six patients transplanted with CD34-selected PBSC after a conditioning regimen combining 2 Gy TBI and fludarabine even in previously heavily treated patients. Post grafting immunosuppression was carried out with CyA (extended until day 100 after the transplant) and MMF (Table 2). The authors reported the achievement of stable mixed chimerism in 5/6

Table 2 Previous studies of CD34-selected NMSCT using the Seattle's conditioning regimen

	Kreiter et al[21]	*Gorner et al*[22]	*Baron et al*
Diagnosis			
Multiple myeloma	3	5	1
Lymphoma	0	0	3
Chronic lymphocytic leukemia	1	0	1
Acute myeloid leukemia	0	1	0
Renal cell carcinoma	0	0	1
Chediak–Higashi syndrome	1	0	0
Previous regimens			
Standard-dose chemotherapy: yes/no	4/1	6/0	5/1
Autologous transplantation: yes/no	3/2	5/1	4/2
Conditioning regimen			
2 Gy TBI alone	4	0	3
2 Gy TBI + fludarabine	1	6	2
Cyclophosphamide + fludarabine	0	0	1
Immunosuppression			
CyA + MMF: yes/no	5/0	6/0	6/0
Day of CyA discontinuation, median (in patients without GVHD)	50	100	195 (per protocol)
Donor type			
Matched related	3	2	4
Mismatched related	1	2	2
Matched unrelated	1	2	0
Cells given on day 0 ($\times 10^6$/Kg)			
CD34+	4.6 (3–8)	8.3 (5.5–11.1)	5.5 (1.8–8.8)
CD3+	2 (0.1–2)	0.1 (0.1–0.3)	0.06 (0.04–0.1)
Donor lymphocyte infusion			
Yes/No	2/3	3/3	6/0
Median day of first DLI	115	80	40
CD3+ cells given in first DLI ($\times 10^6$/kg), median	0.1	1	10[a]
Graft rejection: yes (days)/no	4 (37, 125, 150, 210)/1	1 (100)/5	0/6
Acute GVHD $\geqslant$2: yes/no	1/4	1/5	1/5

[a]CD8-depleted.

patients treated. Unfortunately, the follow-up of their patients was quite short and the authors did not report the evolution of T cell chimerism. Our study shows that full donor chimerism is achievable after conditioning with 2 Gy TBI only.

We elected to deplete pre-emptive DLI of CD8 + cells because previous studies have shown that CD8-depletion of DLI decreased the risk of GVHD without impairing the GVL effect nor the ability of DLI to convert mixed chimerism into full donor chimerism.[25–27]

Owing to the low number of patients included in this pilot study, no conclusion can be drawn on the incidence of GVHD or occurrence of the GVL effect. However, the incidence of acute GVHD was low in our study compared to that previously reported in unmanipulated NMSCT recipients.[9,28] Moreover, a GVL effect was observed in three out of four patients who had measurable disease before the transplant.

The evolution of T-cell chimerism in our patients is quite interesting. In unmanipulated NMSCT recipients, low T-cell chimerism on day 28 after the transplant is strongly correlated with graft rejection.[9,29] However, despite of low T-cell chimerism on day 28 (median 23%) in our patients, we did not observe any graft rejection. As mentioned above, we believe that pre-emptive CD8-depleted DLI as well as extension of CyA administration in our patients were critical to avoid transplant rejection. However, it should be noted that no patient in our study was at high risk of transplant rejection (such as CML or MDS patients) and, at our center, we limit this transplantation approach to patients at low/intermediate risk of transplant rejection.

Childs *et al* [30] have previously reported that GVL effects occurred mostly after achievement of full donor chimerism. As the time to achieve full donor T-cell chimerism seems to be markedly delayed in our patients, we believe that this NMSCT approach must be restricted to patients at high risk of GVHD and with a slowly progressing disease.

In conclusion, CD34-selected NMSCT followed by CD8-depleted DLI is feasible, preserves engraftment and apparently also the GVL effect. Further studies are needed to confirm this encouraging preliminary report.

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