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Low incidence of acute graft-versus-host disease after non-myeloablative stem cell transplantation with CD8-depleted peripheral blood stem cells: an update

We examined the effect of CD8-depletion of the graft in transplant recipients conditioned with low-dose total body irradiation +/- fludarabine. Ten patients received unmanipulated peripheral blood stem cells (PBSC) (control group) and 16 CD8-depleted PBSC (CD8-depleted group). The 100-day incidence of grade II-IV acute graft-versus-host disease was 70% in the control group versus 0% in the CD8-depleted group (p=0.001).

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We prospectively analyzed the impact of CD8-depletion on the incidence of acute graft-versus-host disease (GVHD) as well as on T-cell chimerism after non-myeloablative stem cell transplantation (NMSCT). Twenty-six patients with hematologic malignancies but ineligible for a conventional myeloablative transplant or patients with metastatic renal cell carcinoma (RCC) were included in this study. 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 consisted in 2 Gy single dose total body irradiation (TBI) on day 0 (N=6). For patients not heavily pre-treated or those with an unrelated donor, TBI was combined with 30 mg/m^2/day fludarabine for 3 days (N=13). Finally, 7 patients received a combination of fludarabine and cyclophosphamide 1 g/m^2/day for 3 days (Fluda-Cy) because they had previously received 12 Gy TBI as the conditioning regimen for an autotransplant (Table 1). Post-transplant immunosuppression was carried out with oral cyclosporine (CsA, 6 mg/kg b.i.d. from day −1 to day 120 or longer in case of an alternative donor or chronic GVHD) and mycophenolate mofetil (MMF, 15 mg/kg b.i.d. from day -1 to day 28). Stem cell mobilization, collection and CD8-depletion using the Baxter Isolex 300i© were performed as previously described.[1,2] Patients 1-10 (unmanipulated PBSC) were assigned to receive unmanipulated donor lymphocyte infusions (DLI) (1×10^7 and 2×10^7 CD3$^+$ cells/kg recipient at about day 40 and day 80, respectively) whereas patients 11-26 (CD8-depleted PBSC) were assigned to receive CD8-depleted DLI (1×10^7 and 5 (2 in mismatched transplants) $\times10^7$ CD3$^+$ cells/kg recipient at about day 40 and day 80, respectively).[2] CD8-depletion of DLI was also carried out with Baxter Isolex 300i© as previously reported.[2] DLI were not to be given in the case of an antecedent grade III-IV acute GVHD or active GVHD at time of the scheduled infusions nor in recipients of unrelated transplants. Patients with mixed chimerism on day 100 received a third DLI at about day 120. Chimerism among total peripheral blood white blood cells (WBC), T cells and myeloid cells as well as in unfractionated marrow was assessed on days 28, 60, 100, 180 and 365 after HSCT using fluorescence in situ hybridization (FISH) to detect X and Y chromosomes for recipients of sex-mismatched transplants and polymerase chain reaction (PCR)-based analysis of polymorphic microsatellite regions for recipients of sex-matched transplants, as previously reported.[2,3] The probabilities of GVHD and graft rejection were studied by life-table analyses and Wilcoxon rank tests were used for comparisons between groups.

The 100-day actuarial incidence of grade I-IV (II-IV) acute

Table 1. Characteristics of the patients.

	Unmanipulated PBSC	*CD8-depleted PBSC*	*p value*
Number of patients	10	16	
Age [Median (range)]	58 (39-64)	51 (22-62)	NS
Sex (male/female)	7/3	14/2	NS
Disease at transplantation			
NHL beyond CR2	2	5	NS
Metastatic RCC	2	3	
Refractory multiple myeloma	1	2	
MDS	2	1	
HD in CR	0	1	
ALL in CR	0	1	
AML in CR	1	1	
Refractory AML	0	1	
CML in CP	1	1	
CML in AP	1	0	
Prior autologous HSCT (yes/no)	4/6	10/6	NS
Nonmyeloablative conditioning regimen			
2 Gy TBI alone	2	4	NS
2 Gy and fludarabine	5	8	
Fludarabine and cyclophosphamide	3	4	
Donor			NS
HLA identical sibling	5	5	
Related with 1 mismatch	2	2	
HLA identical unrelated	3	9	
Stem cell source			
PBSC	9	16	NS
BM	1	0	
Mean (+SD) cells collected ($\times10^6$)/ kg recipient			
CD34	7.7+3.4	7.7+3.6	NS
CD3	305+99	328+170	NS
CD4	189+67	192+106	NS
CD8	122+41	140+110	NS
Mean (+SD) cells grafted ($\times10^6$)/ kg recipient			
CD34	7.7+3.4	5.5+2.2	0.06
CD3	305+99	142+85	0.001
CD4	189+67	111+63	0.009
CD8	122+41	8+9	0.001

NS: not significant.

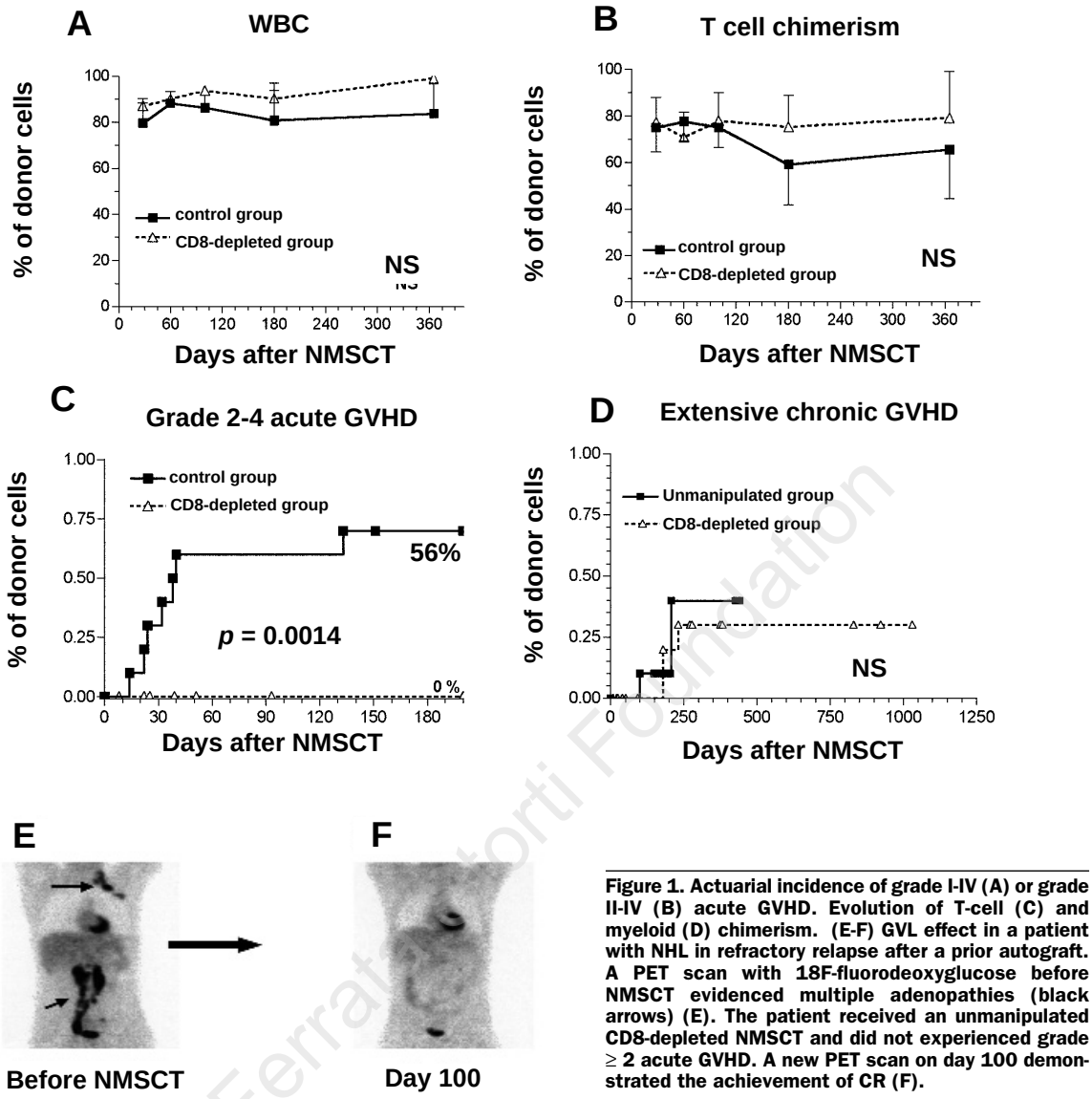

Figure 1. Actuarial incidence of grade I-IV (A) or grade II-IV (B) acute GVHD. Evolution of T-cell (C) and myeloid (D) chimerism. (E-F) GVL effect in a patient with NHL in refractory relapse after a prior autograft. A PET scan with 18F-fluorodeoxyglucose before NMSCT evidenced multiple adenopathies (black arrows) (E). The patient received an unmanipulated CD8-depleted NMSCT and did not experienced grade ≥ 2 acute GVHD. A new PET scan on day 100 demonstrated the achievement of CR (F).

GVHD was 70 (70) % in the control group versus 26 (0) % in the CD8-depleted group, p=0.014 (0.001) (Figure 1A-B). The incidence of extensive chronic GVHD was 40% in the control group versus 30% in the CD8-depleted group (p=NS).

As shown in Figure 1C-D, CD13 and CD3 chimerism evolution was similar in the two groups, demonstrating that CD8-depletion of PBSC did not impair donor chimerism. Two patients with chronic myeloid leukemia and one with myelodysplastic syndrome rejected their graft. The actuarial 180 (365)-day probability of graft rejection was 10 (10) % in the control group versus 8 (21) % in the CD8-depleted group (p=NS).

The aim of this study was to analyze the effect of CD8-depletion on acute GVHD and on T-cell chimerism and not to assess the graft-versus-leukemia effect. However, 3/7 evaluable recipients in the CD8-depleted group with active disease before NMSCT were in complete remission and 3 others in partial remission on day 100 after the transplant, demonstrating that CD8-depletion of PBSC did not abrogate the graft-versus-leukemia effect. GVHD is the first cause of non-relapse death after NMSCT.[4-7] The incidence of GVHD in our control group was similar to that reported by others,[4,5,8] but it was significantly much higher than in our CD8-depleted group, demonstrating that CD8-depletion was highly efficient in preventing acute GVHD after NMSCT. However, the incidence of extensive chronic GVHD was not statistically different in the two groups, perhaps because of the higher incidence of alternative donors in the CD8-depleted group than in the control group.

CD8-depletion of the graft in the myeloablative bone marrow transplantation setting has been associated with a higher incidence of graft rejection.[9] However, our results indicate that CD8-depletion did not decrease T cell chimerism after NMSCT. This may be due to the use of PBSC instead of bone marrow as the stem cell source. We conclude that NMSCT with CD8-depleted PBSC is feasible with a very low incidence of acute GVHD. Prospective, randomized studies are needed to confirm this important observation.

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Response to mycophenolate mofetil therapy for refractory chronic graft-versus-host disease

Preliminary results of our study suggest that mycophenolate mofetil (MMF) might be a useful treatment in patients who have failed or could not tolerate conventional immunosuppressive therapy for chronic graft-versus-host disease (GVHD). In addition, the favorable toxicity profile and steroid-sparing effect of MMF may be particularly attractive features of this medication.

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Studies in animal models and preliminary clinical trials indicate that mycophenolate mofetil (MMF) might be an effective agent for the treatment of graft-versus-host disease (GVHD).[1-5] We analyzed 21 adult patients given a hematopoietic stem cell transplantation (HSCT) who received MMF for treatment of chronic GVHD.

Peripheral blood stem cell grafts (n=14) or marrow grafts (n=7) were infused from HLA-matched sibling (n=18) or unrelated (n=3) donors. Seventeen patients with hematologic malignancies received conventional myeloablation prior to their transplant, 4 patients with hematologic malignancies (n=3) or solid tumor (n=1) were transplanted after a nonmyeloablative preparative regimen. GVHD prophylaxis consisted of cyclosporine (CSA) and a short course of methotrexate (MTX) in 17 cases. Four patients received CSA and MMF as part of the non-myeloablative protocol; CSA was tapered off by day 100 if no GVHD developed, whereas MMF was discontinued on day +30. Extensive chronic GVHD developed in 15 patients, while limited chronic GVHD occurred in 6 (Table 1). Multiple organ involvement (> 2 organs) was observed in 67% (14 of 21) of the patients.

Eighteen of the 21 patients were evaluable for response to MMF treatment. In 12 cases, MMF was introduced because of ineffectiveness of previous immunosuppressive therapies (i.e. progression or no improvement of chronic GVHD after 6 weeks of previous treatment). Three patients were enrolled in the study because of dependence on steroids (i.e. the need for > 20 mg/day methylprednisolone for more than 6 weeks) and resistant disease; in 3 cases MMF was introduced because of intolerance to previous treatments.

MMF was administered at the dose of 1 g bid. in 16 patients; two patients received MMF at the dose of 500 mg bid. because of low body weight (< 50 Kg) and co-existing nausea and vomiting. Patients were treated with MMF in addition to CSA/tacrolimus and steroids (n=11), CSA alone (n=5) or tacrolimus alone (n=2).

The median time from onset of chronic GVHD to the initiation of MMF treatment ranged between 0 and 2503 days (median 235 days). The duration of therapy has ranged from 51 days to 23 months (median 184 days).

Five complete clinical responses (i.e. complete resolution of all GVHD manifestations) and 8 partial clinical responses (i.e. greater than 50% response in organ involvement, but less than a complete resoponse) were observed, for a clinical response rate of 72% (13 of 18). Nine of 13 evaluable patients with extensive chronic GVHD and 4 of 5 evaluable patients with limited chronic GVHD had a clinical response. The distribution of responses according to organ involved by chronic GVHD is shown in Table 2. Three patients had stable disease and were, therefore, counted as non-responders. Two patients experienced progression of disease while receiving MMF.

Steroid dose was decreased by ≥ 50% in 9 of 11 (82%) evaluable patients, and in 6 cases steroid therapy could be discontinued. The estimated probability of stopping all immunosuppression at 2 years after starting the study was 24% (95% CI, 2%-85%). The most common side effects of MMF