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LETTER TO THE EDITOR

Non-myeloablative transplantation with CD8-depleted or unmanipulated peripheral blood stem cells: a phase II randomized trial

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Allogeneic hematopoietic cell transplantation (HCT) following non-myeloablative conditioning has been an effective treatment for many patients with malignancies. This approach relies on graft-versus-tumor effects for tumor eradication.[1] Following non-myeloablative conditioning, acute graft-versus-host disease (GVHD) has not been associated with significant graft-versus-tumor effects, but has rather been associated with a high risk of non-relapse mortality,[1] indicating that efforts aimed at decreasing the incidence of acute GVHD are needed. On the basis of previous studies suggesting that CD8 depletion of the graft could reduce the incidence of acute GVHD without apparently impairing graft-versus-tumor effects,[2] and that CD8-depleted donor lymphocyte infusion (DLI) induced strong graft-versus-tumor effects with a low incidence of acute GVHD ($<10\%$),[3] we designed a phase II prospective randomized trial evaluating the impact of CD8 depletion of peripheral blood stem cells (PBSCs) on HCT outcomes after non-myeloablative conditioning. The protocol was approved by the local Ethics Committee, and written informed consent was obtained from each patient. The trial started in March 2002, and the study was retrospectively registered in ClinicalTrials.gov (protocol no. NCT00693927) in June 2008. Immune recovery in these patients has been previously reported.[4]

After being stratified for donor type, patients were randomized 1:1 between the 'unmanipulated' ($n=28$) and 'CD8-depleted' ($n=25$) arms. Conditioning regimen consisted of 2 Gy total body irradiation with or without fludarabine ($90\,mg/m^2$). Postgrafting immunosuppression included cyclosporine plus mycophenolate mofetil. Clinical management and chimerism analyses were performed as reported earlier.[4] In total, 23 patients received DLI for disease persistence or progression (1 in the unmanipulated group and 2 in the CD8-depleted group), or poor T-cell chimerism with or without persistent disease (12 in the unmanipulated group and 8 in the CD8-depleted group), including 5 patients in the unmanipulated group and 4 patients in the CD8-depleted group who received DLI preceded by 2 Gy total body irradiation as part of a prospective study evaluating this strategy to prevent graft rejection in patients with low donor T-cell chimerism levels. Results were analyzed as of 29 May 2008. Survival and progression-free survival were estimated using the Kaplan–Meier method. The incidences of acute and chronic GVHD, relapse and non-relapse mortality were calculated using cumulative incidence estimates as reported earlier.[1] Patients who received DLI were censured for acute GVHD at the time of DLI, patients with graft rejection were censured at the time of graft rejection for GVHD analyses. Multivariate logistic regression models were fitted with the SAS LOGISTIC procedure (SAS Institute, Cary, NC, USA) for graft rejection and GVHD. Multivariate Cox models were fitted for analyzing progression-free and overall survivals, non-relapse mortality and relapse risk.

In total, 53 patients transplanted between March 2002 and May 2004 were included (Table 1). Scheduled CD8 depletion was not performed in three patients with number of $CD34^+$ cells collected $<4.0\times10^6$ cells/kg. These patients received unmanipulated grafts, but were nevertheless included in the CD8-depleted group (intent-to-treat analysis). Median follow-up for surviving patients was 56 months. Median donor T-cell chimerism levels in CD8-depleted versus unmanipulated recipients were 46% (range, 0.2–100) versus 71% (range, 15–100) on day 28 ($P=0.035$), and 93% (range, 49–100) versus 84% (range, 50–100) on day 365 ($P=0.9$), respectively. Confirming previous observations,[6] four of seven patients with day 28 T-cell chimerism levels between 5 and 30% versus none of the 38 patients with $>30\%$ day 28 donor T-cell chimerism levels ($P=0.0002$) experienced graft rejection. Eight (four initial and four late) graft rejections were observed in the CD8-depleted group (including one of the three patients with low $CD34^+$ cell count given unmanipulated PBSC) versus none in the unmanipulated group ($P=0.001$). In multivariate analysis, human leukocyte antigen (HLA)-mismatched donor was associated with a trend for higher risk of graft rejection (hazard ratio (HR) 4.1; $P=0.09$), whereas the association between study group and graft rejection was present, although not statistically significant (HR 1.9; $P=0.32$). In agreement with our results, a randomized study including 38 patients given unmanipulated or CD8-depleted marrows from HLA-identical siblings following myeloablative conditioning suggested a higher risk of graft failure (2 of 19 patients versus 0 of 19 patients) following CD8 depletion of the grafts, although the difference was not statistically significant.[2]

Acute GVHD of grades II–IV was seen in eight (32%), one (4%) and 0 (0%) in the CD8-depleted group versus in seven (25%), one (4%) and four (14%) in the unmanipulated group, respectively. The 180-day cumulative incidences of grades II–IV and III–IV acute GVHD were 37.6 and 4.0% in the CD8-depleted group versus 43.2% ($P=0.7$) and 18.1% ($P=0.13$) in the unmanipulated group, respectively. The 2-year cumulative incidence of chronic GVHD of National Institutes of Health (NIH) grades II–III was 29% in the CD8-depleted group versus 57% in the unmanipulated group, respectively ($P=0.09$). Possible explanations for the similar incidence of GVHD in the two groups could be the limited number of patients in each arm limiting the power to detect statistically significant differences, or that the number of $CD8^+$ T cells infused does not correlate with occurrence of acute GVHD after non-myeloablative conditioning. In support of the latter hypothesis, two retrospective studies failed to demonstrate an association between the numbers of $CD8^+$ T cells transplanted and acute GVHD incidence.[7,8] However, it should be stressed that none of these studies used CD8 depletion of PBSC, and the median number of $CD8^+$ T cells infused in the current CD8-depleted group was 2.5–5 times lower than the minimal number of transfused $CD8^+$ T cells in these studies.[7,8] In contrast to our results, CD8 depletion of the grafts has been associated with decreased incidence of grade II–IV acute GVHD in a

Table 1 Characteristics of patients

	CD8-depleted[a] *PBSC*	*Unmanipulated PBSC*
Number of patients	25	28
Median age (range) (years)	57 (36–69)	57 (41–65)
Gender (male/female), no. of patients	13/12	19/9
Disease at transplantation; no. of patients		
AML in CR/>CR	0/0	2/1
CML in AP	2	0
CLL in PR/refractory	1/4	0/1
NHL in CR/in PR/>PR	4/2/1	6/1/1
MDS[b] RA/RAEB/RAEB-t	6/1/1	2/3/1
MM in CR/not in CR	0/1	2/5
Myeloproliferative disorder	2	1
Metastatic RCC	0	2
Disease risk[c]: low/standard/high; no. of patients	7/6/12	12/6/10
Donor type; no. of patients		
HLA-identical sibling/child	10/1	12/0
10/10 HLA-allele-matched or 1 HLA-allele-mismatched URD	6	8
2 HLA-allele-mismatched URD	1	0
1 HLA antigen-mismatched URD	5	6
>1 HLA antigen-mismatched donor	2	2
Conditioning regimen; no. of patients		
2 Gy TBI	6	7
2 Gy TBI+fludarabine	19	21
No. of cells transplanted ($\times 10^6$/kg); median (range)		
CD34	3.6 (0.7–12.2)	4.2 (0.8–20.2)
CD3	111 (56–500)	314 (80–631)
CD8	4.6 (0.4–195)	130 (42–272)

Abbreviations: AML, acute myeloid leukemia; AP, accelerated phase; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; CR, complete remission; HCT, hematopoietic cell transplantation; HLA, human leukocyte antigen; MDS, myelodysplastic syndrome; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; PR, partial remission; RA, refractory anemia; RAEB, refractory anemia with blast excess; RAEB-t, refractory anemia with blast excess in transformation; RCC, renal cell carcinoma; TBI, total body irradiation; URD, 10/10 HLA-allele-matched unrelated donor.

[a]CD8 depletion was carried out using the CD8-HDM (high-density microparticles, BB-IDE 6969, generously given by BioTransplant Inc., Charlestown, MA, USA) device, as recommended by the manufacturer.

[b]Status at HCT.

[c]As defined by Kahl *et al.*,[5] low risks include CLL in CR, low-grade NHL, MM in CR, mantle cell lymphoma, myeloproliferative disease, high-grade NHL in CR and acute lymphoblastic leukemia in first CR; standard risks include CLL or MM not in CR, MDS refractory anemia/refractory anemia ringed sideroblasts, AML in CR, and CML in first chronic phase; and high risks include MDS refractory anemia with excess of blasts (RAEB)/RAEB in transformation (RAEB-t), AML after MDS or AML not in CR, high-grade NHL not in CR, Hodgkin lymphoma, MDS after chemotherapy, CML in second CP or accelerated phase/blast crisis, acute lymphoblastic leukemia in second or later CR, chronic myelomonocytic leukemia (CMML), and RCC.

randomized study including 38 patients given marrows from HLA-identical siblings following myeloablative conditioning and cyclosporine alone as GVHD prophylaxis (20 versus 80%, $P<0.01$).[2] Obviously, there were many discrepancies between the two randomized studies, such as the intensity of the conditioning, the stem cell source and the postgrafting immunosuppression, that could all have had an impact on the efficacy of CD8 depletion for preventing acute GVHD.

The 100-day probabilities of septicemia (except those caused by coagulase-negative staphylococcus), cytomegalovirus infection and fungal infection were 22, 54 and 4%, respectively in the unmanipulated group, versus 26% ($P=0.8$), 59% ($P=1.0$) and 5% ($P=0.9$), respectively, in the CD8-depleted group.

The 5-year cumulative incidence of relapse/progression was 46% in the CD8-depleted group versus 47% in the unmanipulated group ($P=0.7$). In multivariate analysis, higher disease risk (see Table 1) was associated with a higher risk of relapse (HR 2.2; $P=0.004$). Here, 100-day and 5-year non-relapse mortalities were 12 and 32% in the CD8-depleted group versus 4 and 26% in the unmanipulated group ($P=0.8$), respectively. In multivariate analysis, patients given HLA-mismatched grafts had higher risk of non-relapse mortality than those given grafts from HLA-identical siblings ($P=0.028$) or from 10/10 HLA-allele-matched unrelated donors ($P=0.083$). Five-year progression-free and overall survival were 23 and 45% in the CD8-depleted group versus 28% ($P=0.5$) and 48% ($P=0.85$; Figure 1a) in the unmanipulated group, respectively. In multivariate analysis, patients given HLA-mismatched grafts had a worse survival than those given grafts from HLA-identical sibling (HR 2.6; $P=0.041$) or from 10/10 HLA-allele-matched unrelated donors (HR 1.8; $P=0.26$; Figure 1b).

In summary, CD8 depletion of PBSC failed to reduce the incidence of acute GVHD but increased the risk of graft rejection after non-myeloablative conditioning. Furthermore, HLA-mismatched PBSC recipients had higher risk of graft rejection, higher risk of non-relapse mortality and shorter survival than those given PBSC from HLA-identical siblings.

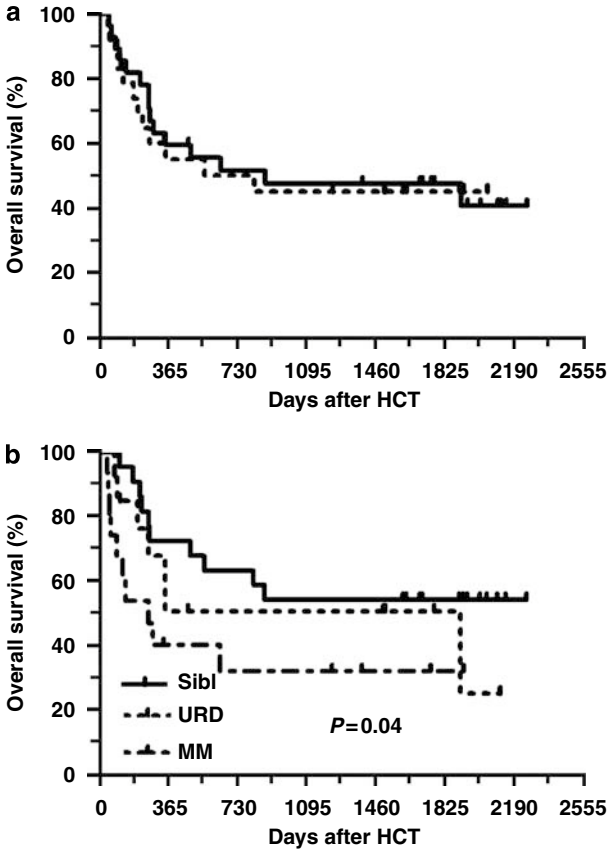

Figure 1 (**a**) Overall survival in CD8-depleted (broken lines) and unmanipulated (solid lines) peripheral blood stem cell (PBSC) recipients. (**b**) Overall survival according to donor type (Sibl, HLA-identical sibling; URD, 10/10 HLA-allele-matched unrelated donor; MM, HLA-mismatched donor).

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