

To the editor:

Evidence for increased risk of secondary graft failure after in vivo depletion of suicide gene–modified T lymphocytes transplanted in conjunction with CD34⁺-enriched blood stem cells

Suicide gene transfer into donor T lymphocytes is a promising strategy to avoid severe graft-versus-host disease (GvHD) after allogeneic stem cell transplantation (SCT).^{1,2} We conducted a phase 1/2 study³ combining infusion of CD34-enriched peripheral blood stem cells (PBSCs) with suicide gene–modified (SGM) donor T lymphocytes. Before our study was put on hold (after the leukemia cases in France⁴), 3 patients had been treated (Table 1).

T lymphocytes were transduced⁵ with the retroviral vector Mo3TIN,⁶ selected with G418,² cryopreserved, and safety-tested in a good manufacturing practice (GMP) facility (European Institute for Research and Development of Transplantation Strategies [EUFETS]). CD34 cells were enriched using the Miltenyi CliniMACS (Miltenyi, Bergisch Gladbach, Germany) (Table 1). Following myeloablative conditioning, each patient received more than 4×10^6 CD34⁺/kg and approximately 5×10^6 /kg body weight (BW) SGM donor T lymphocytes. The latter were immediately detectable in peripheral blood (PB) by quantitative polymerase chain reaction⁵ (Table 1; Figure 1). Transplantation was well tolerated without acute toxicity; all patients engrafted quickly (Table 1).

Patient 2, with stable numbers of SGM T cells for more than 3 months, is alive and well more than 2.5 years after transplantation (Table 1).

Patient 1 early on showed a strong increase of SGM cell counts (Figure 1), without GvHD. Thereafter, PB was cleared of SGM T lymphocytes within a few days. This patient received a second dose of SGM T cells (day 65), which vanished within 2 days (Figure 1), strongly indicative of their immune rejection. Indeed, *MLR*⁷ data (not shown) confirmed anti-SGM reactivity of this patient's peripheral blood lymphocytes, possibly related to a herpes simplex virus (HSV) reactivation during transplantation.

Patient 3 showed a similar early sharp rise in SGM T cells (Figure 1) associated with acute skin GvHD grade II. Treatment with ganciclovir led to complete resolution of GvHD and rapid disappearance of SGM donor T cells.

Most important, patients 1 and 3 developed secondary graft failure (Table 1). Late graft failure may occur in more than 10% of T-cell–depleted hematopoietic SCTs,⁸ the amount of CD3⁺ cells in the transplant being most critical. In our study, numbers of “contaminating” T cells in the CD34⁺ grafts (Table 1) were far

Table 1. Patients' characteristics and important clinical data

	Case 1	Case 2	Case 3
Age, y	53	22	58
Sex, patient/donor	Female/female	Male/male	Male/female
Diagnosis	CML	CML	MDS
Conditioning, mg/kg BW			
Busulfan	14	14	12
Cyclophosphamide	120	120	120
VP16	—	—	30
PBSCT type	Matched related donor	Matched related donor	Matched related donor
Mismatches	1: HLA-A	No	No
CD34 ⁺ dose/kg	4.22×10^6	4.6×10^6	4.3×10^6
Contaminating CD3 ⁺ cells	2×10^3 /kg	1×10^4 /kg	3.6×10^4 /kg
SGM T cells, CD3⁺/kg	4.8×10^6	5.46×10^6	4.9×10^6
Second infusion (d)	4.8×10^6 (65)	5.46×10^6 (58)	No*
GvHD prophylaxis	Cyclosporin A	Cyclosporin A	Cyclosporin A
Beginning d	−1	−1	−1
GvHD (overall grade/d)	Yes (I/23)	No	Yes (II/14)
GCV treatment (d)	No	No	Yes (17-26)
Outcome	Resolved spontaneously	NA	Resolution
Engraftment (chimerism)	Yes (full)	Yes (full)	Yes (full)
Neutrophils, d	11	12	13
Platelets, d	10	13	16
SGM T cells in vivo	Early loss (d 23) after 2nd infusion; only present for 2 d	Present for more than 3 mo	Loss after GCV treatment (d 25)
Special remarks	HSV infection during transplantation	Increasing bcr/abl levels at ≈ 1 y	—
Clinical outcome	Secondary graft failure (d 156); 2nd allo-PBSCT; severe complications; patient died	DLI for MRD (1×10^7 kg) at 1 y 3 m, alive/well	Secondary graft failure (d 119); 2nd allo-PBSCT; severe complications; patient died

CML indicates chronic myelogenous leukemia; MDS, myelodysplastic syndrome; PBSCT, peripheral blood stem cell transplantation; GCV, ganciclovir; NA, not applicable; DLI, donor leukocyte infusion; MRD, minimal residual disease.

*The protocol allowed a second infusion of 1 to 5×10^6 /kg SGM donor T cells on day 60 only if no GvHD higher than grade 1 had developed.

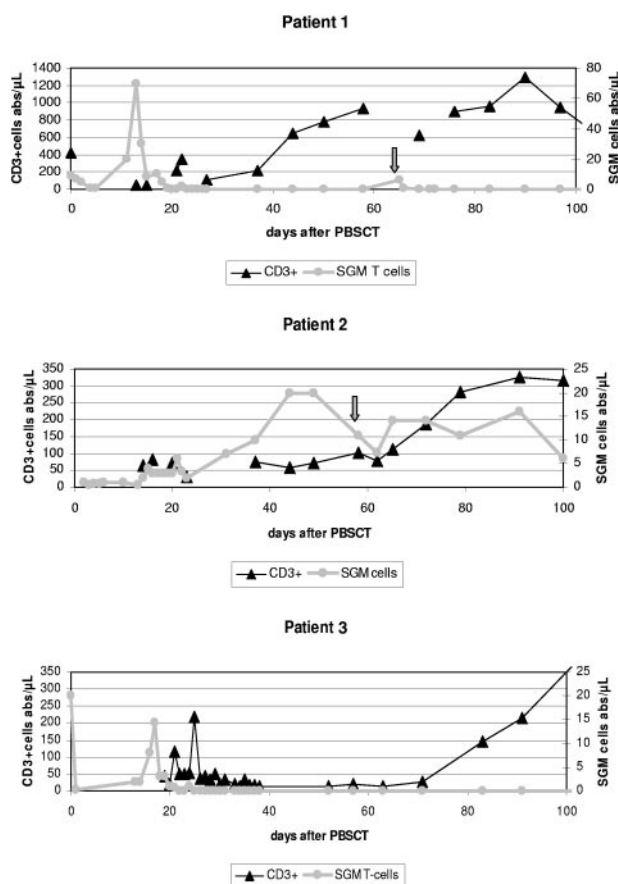


Figure 1. Absolute CD3⁺ T-cell counts (including CD3⁺CD56⁺) and numbers of suicide gene-modified (SGM) donor T lymphocytes during the first 100 days after PBSCT in all 3 patients treated according to our protocol. Patient 2 showed stable numbers of SGM cells for about 3 months accompanied by increasing absolute CD3⁺ counts and full donor chimerism. In contrast, in both patients 1 and 3 early in vivo depletion of SGM donor T lymphocytes was observed, mediated by ganciclovir applied to treat an acute GvHD grade II (patient 3) or most probably as the result of an anti-HSV-thymidine kinase (tk) immune reaction (patient 1). Both patients appeared to have higher absolute CD3⁺ counts on day 100 compared with patient 2, but developed mixed chimerism (not shown) and eventually rejected their grafts at days 156 and 119. Arrows indicate a second donor SGM T lymphocyte infusion in patient 1 (day 65) and patient 2 (day 58). Note that different Y-axes should be applied to CD3⁺ and SGM cells.

below the suggested threshold of $2 \times 10^5/\text{kg}$,⁸ but 5×10^6 SGM T cells/kg was added. Obviously, complete loss of these SGM T lymphocytes about 3 weeks after transplantation in both patients (Figure 1) led to a situation comparable to full T-cell depletion. This, probably in concert with other factors (HLA mismatch +

busulphan conditioning for CML, patient 1; slightly reduced busulphan dose, patient 3⁸), may have facilitated autologous T-cell recovery, as indicated by T-cell chimerism data from patient 3 (not shown).

In conclusion, we confirmed earlier reports^{1,2} that the use of SGM T lymphocytes may allow control of acute GvHD. At the same time, we made the troubling observation that early total in vivo depletion of SGM donor T cells may be associated with an increased risk of transplant rejection. This suggests that minimum numbers of donor CD3⁺ cells are required after transplantation, not only to facilitate engraftment⁸ but also to prevent late rejection. To preclude the rejection risk associated with HSV-tk-mediated depletion of donor T cells, add-back of limited numbers of nonmodified T lymphocytes (eg, 2×10^5 CD3⁺/kg) to the CD34-enriched PBSCs⁸ may be a valuable approach.

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One of the authors (K.K.) is employed by a company (EUFETS) whose (potential) products may be related to the present work.

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To the editor:

Identification of immunodominant alloreactive T-cell epitopes on the Jk^a red blood cell protein inducing either Th1 or Th2 cytokine expression

Although antibodies implicated in red blood cell (RBC) alloimmunization have been studied for many years, little is known about helper T-cell responses that drive their production.¹⁻³ The aim of this work was to determine T-cell antigenic determinants involved in T-cell responses against RBC antigens, and the subsequent patterns of stimulatory cytokine production. The Kidd blood group antigen, Jk^a, was selected as a model. T

lymphocytes from 11 donors whose anti-Jk^a alloimmunization was the result of previous pregnancies were stimulated during 6 hours by 4 overlapping peptides mimicking the Jk^a sequence 266 to 293 (Jk^a1, Jk^a2, Jk^a3, Jk^a4). The real-time reverse-transcriptase-polymerase chain reaction was chosen to quantify T helper 1 (Th1)- and Th2-type cytokines (respectively, interleukin-2 [IL-2] and IL-4). The results showed a clear Th1/Th2 dichotomy in