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ORIGINAL ARTICLE

Elevations of tumor necrosis factor receptor 1 at day 7 and acute graft-versus-host disease after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning

E Willems[1,2,4], S Humblet-Baron[2,4], O Dengis[1,2], L Seidel[3], Y Beguin[1,2] and F Baron[1,2]

[1]*Division of Hematology, Department of Medicine, CHU of Liége, Liége, Belgium;* [2]*Groupe Interdisciplinaire de Génoprotéomique Appliquée (Giga)-Research, Hematology Unit, University of Liége, Liége, Belgium and* [3]*Department of Statistics, University of Liége, Liége, Belgium*

Acute GVHD has remained a significant cause of nonrelapse mortality after allogeneic hematopoietic cell transplantation (HCT) with nonmyeloablative conditioning. The role of TNF-α in the biology of acute GVHD after nonmyeloablative conditioning has not been studied thus far. Here, we measured TNF receptor 1 (TNFR1) as a surrogate marker for TNF-α in 106 patients before the start of the conditioning regimen (baseline) and 7 days after allogeneic HCT with nonmyeloablative conditioning. The nonmyeloablative regimen consisted of 2 Gy TBI alone ($n = 15$), 2 Gy TBI plus fludarabine 90 mg/m^2 ($n = 73$), or 4 Gy TBI plus fludarabine 90 mg/m^2 ($n = 18$). TNFR1 levels increased significantly from baseline to day 7 after nonmyeloablative HCT ($P < 0.0001$). Patients conditioned with 4 Gy TBI had higher TNFR1 day 7/baseline ratio than those conditioned with 2 Gy TBI (median 1.65 versus 1.25; $P = 0.01$). In a multivariate Cox model, high TNFR1 day7/baseline ratio was associated with grades II–IV (HR = 2.2, $P = 0.01$) and grades III–IV (HR = 2.9, $P = 0.007$) acute GVHD, but had no impact on overall survival ($P = 0.8$). In summary, our data suggest that nonmyeloablative conditioning induces the generation of TNF-α, and that the magnitude of TNF-α generation depends on the conditioning intensity (2 Gy versus 4 Gy TBI). Further, assessment of TNFR1 levels before and on day 7 after nonmyeloablative HCT provided useful information on subsequent risk of experiencing acute GVHD.

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Introduction

Nonmyeloablative allogeneic hematopoietic cell transplantation (HCT) has been increasingly used in patients with hematological malignancies who are too old or too sick to tolerate a myeloablative allogeneic HCT,[1–4] as well as in patients who had failed a high-dose transplant.[5] This approach relies on optimization of pre- and post transplant immunosuppression to prevent host-versus-graft (rejection) and excessive graft-versus-host reactions,[6] and on graft-versus-tumor effects mediated by donor T (and perhaps NK) cells for tumor eradication.[7,8]

Myeloablative conditioning has been shown to contribute to the physiopathology of GVHD, presumably by inducing tissue damage and the release of a 'cytokine storm' that activates donor T cells.[9,10] TNF-α production by activated donor T cells as well as activated macrophages has been associated with acute GVHD both in mice and in humans.[11–13] Further, TNF receptor 1 (TNFR1) has been used as a surrogate marker for TNF-α because its concentration is closely correlated to that of TNF-α,[14,15] and because it has superior stability to TNF-α in long-term storage.[16,17] Choi *et al.* and Kitko *et al.* recently reported that TNFR1 plasma elevation from baseline to day 7 after transplantation with myeloablative conditioning correlated with GVHD severity and overall survival,[16,17] whereas no correlation was observed between GVHD and baseline or day 7 TNFR1 levels.[16,17]

The biology of graft-versus-host reactions after nonmyeloablative conditioning differs from what occurs after myeloablative conditioning in several aspects.[18] First, nonmyeloablative conditioning often leads to an initial state of mixed donor–host chimerism that might favor graft-versus-host tolerance and thus limit GVHD.[19,20] Second, the intensity of the preparative regimens is by definition greatly reduced in the setting of nonmyeloablative conditioning, potentially reducing or preventing the 'cytokine storm,'[21,22] and in particular the release of large amounts of TNF-α by activated macrophages and donor T cells. In addition, the number of recipient-derived APC might be higher after nonmyeloablative than myeloablative conditioning. As recipient-derived APC are thought to have a major role in the initiation of acute GVHD,[23] their

Correspondence: Dr F Baron, Department of Hematology, University of Liège, CHU Sart-Tilman, 4000 Liége, Belgium.
E-mail: F.Baron@ulg.ac.be
[4]These authors contributed equally to this work.
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enhanced persistence after a nonmyeloablative regimen might favor acute GVHD. These observations prompted us to investigate the role of TNF-α in the physiology of acute GVHD after nonmyeloablative conditioning. Specifically, we analyzed the impact of plasma TNFR1 elevation from baseline to day 7 after HCT on acute GVHD and overall survival in a cohort of 106 patients given PBSC from related or unrelated donors after nonmyeloablative conditioning.

Patients and methods

Patients

Data from 106 patients transplanted from November 1999 to October 2008 at the University of Liége were included in the study. Results were analyzed as of February 2009. Included in the analysis were patients with hematological malignancies who were considered ineligible for conventional allogeneic HCT because of age and/or co-morbidities, or preceding extensive therapies such as a myeloablative autologous or allogeneic HCT, as well as four patients with metastatic renal cell carcinoma. Patient characteristics are summarized in Table 1. Median patient age was 58 (range, 10–72) years. Twenty-seven patients had low-risk disease, 38 standard-risk disease, and 41 high-risk disease as defined by Kahl *et al*.[24] Thirty-three patients received unmanipulated PBSC from HLA-matched-related donors, 31 from 10/10 HLA allele-matched-unrelated donors, and 42 from HLA-mismatched-related or -unrelated donor (1/10 HLA allele mismatch ($n=13$), 2/10 HLA allele mismatches ($n=3$), 1/10 HLA antigen mismatch ($n=16$), 1/10 HLA antigen plus 1/10 HLA allele mismatches ($n=7$), 2/10 HLA antigen mismatches ($n=3$)). Written informed consent was obtained from each patient to undergo nonmyeloablative HCT and to collect, store, and analyze blood samples for research purposes. The Ethics Committee of the University of Liege approved the consent form as well as the current research study protocol.

Treatment and evaluation. The nonmyeloablative conditioning regimens consisted of 2 Gy TBI alone ($n=15$; patients considered to be at low risk of graft rejection because they had previously received chemotherapy and were given PBSC from HLA-identical siblings), 2 Gy TBI with 90 mg/m^2 fludarabine ($n=73$, standard regimen), or 4 Gy TBI with 90 mg/m^2 fludarabine ($n=18$, patients considered to be at high risk of early disease progression and/or graft rejection because of prior failed HCT ($n=9$), advanced B cell malignancy ($n=4$), or myelodysplastic syndrome ($n=5$)). Post-grafting immunosuppression combined mycophenolate mofetil with a calcineurin inhibitor for all patients, as described earlier.[25,26] Specifically, the calcineurin inhibitor was given at full dose starting from day −3, and until day 120 and then tapered off to stop by day 180 in patients given grafts from HLA-identical siblings, or until day 180 and then tapered off to stop by day 365 in patients given grafts from alternative donors. Mycophenolate mofetil was given from day −1 to day 28 in patients given grafts from HLA-identical siblings, and from day −1 to day 42 in those given grafts from alternative donor. The duration of calcineurin inhibitor prophylaxis was extended in patients with GVHD.

Table 1 Patient characteristics

Characteristic	*Value*
Median patient age, years (range)	58 (10–72)
Median donor age, years (range)	41 (18–70)
Female donor to male recipient, # pts (%)	23 (22)
Diagnosis, # pts (%)	
Acute myeloid leukemia	21 (20)
Myelofibrosis	3 (3)
Chronic myeloid leukemia	3 (3)
Chronic lymphocytic leukemia	8 (7)
Myelodysplastic syndrome	15 (14)
Multiple myeloma	25 (24)
Non-Hodgkin lymphoma	23 (22)
Hodgkin disease	4 (4)
Renal cell carcinoma	4 (4)
Disease risk, # pts (%)	
Low	27 (25)
Standard	38 (36)
High	41 (39)
Comorbidity (HCT-CI score)	
0–1	30 (28)
2–3	52 (49)
4–9	24 (23)
Donor, # pts (%)	
Related	36 (34)
HLA identical	33 (31)
1 HLA allele mismatch	1 (1)
1 HLA antigen mismatch	2 (2)
Unrelated	70 (66)
10/10 HLA allele match	31 (29)
1 HLA allele mismatch	12 (11)
>1 HLA allele mismatch	27 (26)
Conditioning regimen, # pts (%)	
2 Gy TBI	15 (14)
2 Gy TBI + fludarabine	73 (69)
4 Gy TBI + fludarabine	18 (17)
Cell dose, median (range) ($\times 10^6$/kg recipient)	
CD34$^+$ cells	4.6 (0.8–20.0)
T cells	334 (80–1215)
Acute GVHD, # pts (%)	
Grade	
0/I	61 (57.5)
II	28 (26.5)
III	8 (7.5)
IV	9 (8.5)
Day of onset of grades II–IV acute GVHD; median (range)	38 (4–341)
3-year overall survival (%)	44

Abbreviation: HCT = hematopoietic cell transplantation.

The diagnosis, clinical grading, and treatment of acute GVHD were performed according to established criteria for nonmyeloablative HCT.[7,18] Specifically, patients with signs/symptoms of acute GVHD without signs of chronic GVHD beyond day 100 were classified as having acute GVHD. Diagnosis and grading of chronic GVHD were performed using the NIH consensus criteria.[27] Treatment was given for grades II–IV acute GVHD and for extensive chronic GVHD, according to established guidelines.[28]

None of the patients received anti-TNF agents. Standard prophylaxis against infections was used.[26] Disease evaluation was routinely carried out on days 40, 100, 180, 365 and then at least yearly thereafter.

Samples and TNFR1 measurement

Blood samples were prospectively collected before the start of the conditioning regimen, then on days 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, and 98 after HCT, and then generally once every 2 weeks up to day 180. The serum component of each blood sample was separated and frozen for later analysis on the day of sample acquisition. TNFR1 serum concentration was retrospectively assessed using a cytokine enzyme-linked immunoabsorbent assay (R&D, Minneapolis, MN, USA) according to the manufacturer's protocol. All samples and standards were run in duplicate.

Chimerism analysis

Chimerism among total peripheral blood T cells was assessed on days 28, 40, 100, 180, and 365 after HCT using fluorescence *in situ* hybridization to detect X and Y chromosomes for recipients of sex-mismatched transplants and PCR-based analysis of polymorphic microsatellite regions for recipients of sex-matched transplants.[25,26,29,30] CD3 (T cells) selection was carried out with RosetteSep (StemCell Technologies, Vancouver, BC, Canada) as described earlier.[26]

Statistical analyses

TNFR1 levels before and on day 7 after HCT were compared with the Wilcoxon-matched pair test. Their association was analyzed with the Spearman test. TNFR1 levels in patients with or without acute GVHD were compared with the Mann–Whitney test. Potential pre-transplant factors affecting donor T-cell chimerism levels (TNFR1 day7/baseline ratio, number of T cells transplanted, number of $CD34^+$ cells transplanted, fludarabine use or not, 2 or 4 Gy TBI, HLA mismatching or not, donor type, patient age) and TNFR1 day7/baseline ratio (number of T cells transplanted, number of $CD34^+$ cells transplanted, fludarabine use or not, 2 or 4 Gy TBI, HLA mismatching or not, donor type, patient age, disease risk) were determined using multivariate linear regression models. Survival was estimated using the Kaplan–Meier method. Comparison between survival curves was carried out with the log rank test. Multivariate Cox models were performed for acute GVHD, progression/relapse, and overall survival (HR and 95% confidence intervals). For acute GVHD, factors introduced in the models included TNFR1 day7/baseline ratio, 2 or 4 Gy TBI, HLA mismatching or not, donor type, donor or recipient CMV seropositivity, patient age, and female donor to male recipient or other gender combination. For relapse/progression and overall survival, factors introduced in the models included TNFR1 day7/baseline ratio, HLA mismatching or not, donor type, donor or recipient CMV seropositivity, patient age, disease risk, comorbidity at HCT, and female donor to male recipient versus other gender combinations. Statistical analyses were done with Graphpad Prism (Graphpad Software, San Diego, CA, USA) or with SAS version 9.1 (SAS Institute, Cary, NC, USA).

Results

Factors affecting TNFR1 day 7/baseline ratio

There was a close correlation between baseline (pre-conditioning) and day 7 TNFR1 levels (Spearman $R = 0.7$, $P < 0.0001$). However, TNFR1 levels increased significantly from baseline (2753 ± 1886 pg/ml) to day 7 ($3781 \pm 2{,}803$ pg/ml) after nonmyeloablative HCT ($P < 0.0001$). Median TNFR1 day 7/baseline ratio was 1.3 (range, 0.8–3.9), with four patients having a TNFR1 day 7/baseline ratio $\geqslant 2.5$. In univariate analysis, patients conditioned with 4 Gy TBI had higher TNFR1 day 7/baseline ratios than those conditioned with 2 Gy TBI (1.65 versus 1.25; $P = 0.01$) (Figure 1). In a multivariate model, the association between TBI dose and TNFR1 day 7/baseline ratios (modeled as a linear continuous variable) remained statistically significant ($P = 0.017$), whereas number of T cells ($P = 0.7$) or $CD34^+$ cells ($P = 0.7$) transplanted, disease risk ($P = 0.4$), donor type ($P = 0.5$), HLA matching ($P = 0.5$), fludarabine use ($P = 0.7$), and patient age ($P = 0.2$) had no statistically significant impact on TNFR1 day 7/baseline ratios.

Factors affecting day 28T-cell chimerism levels

In multivariate analysis, patients given 4 Gy TBI had higher day 28 T-cell chimerism levels than those given 2 Gy TBI ($P = 0.02$), whereas those given grafts from HLA-identical siblings had lower T-cell chimerism than those given grafts from HLA-matched-unrelated ($P = 0.01$) or HLA-mismatched donors ($P = 0.01$). In contrast, there were no associations between day 28 donor T-cell chimerism levels and TNFR1 day 7/baseline ratio (modeled as a linear continuous variable, $P = 0.8$), number of transplanted T cells ($P = 0.6$), number of transplanted $CD34^+$ cells ($P = 0.4$), patient age ($P = 0.1$), and fludarabine use or not ($P = 0.7$).

Day 28 donor T-cell chimerism levels were higher in patients with grades II–IV acute GVHD, than in patients without GVHD (86 ± 15 versus 74 ± 26, $P = 0.04$). After

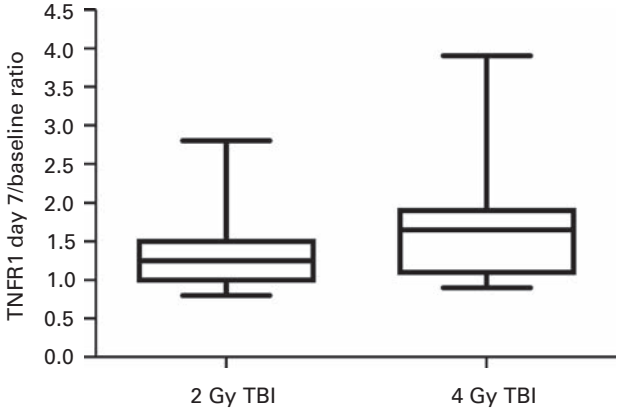

Figure 1 TNFR1 day 7/baseline ratio in patients conditioned with 2 or 4 Gy TBI ($P = 0.01$).

excluding data from patients who had acute GVHD before day 28 after HCT, there was a suggestion that increasing day 28 donor T-cell chimerism levels were associated with a higher risk of subsequent grades II–IV acute GVHD: 8% of patients with day 28 donor T-cell chimerism levels $\leqslant 50$, versus 38% of those with levels $>50\%$ developed grades II–IV acute GVHD ($P=0.053$).

Association between TNFR1 day 7/baseline ratio and acute GVHD

Acute GVHD of grades II, III, and IV was observed in 28, 8, and 9 patients, respectively. Median time for diagnosis of grades II–IV acute GVHD was 38 (range, 4–341) days, with only one patient experiencing acute GVHD before day 7. Eight of 18 patients (44%) given 4 Gy TBI in their conditioning regimen experienced grades II–IV acute GVHD, whereas 37 of 88 patients (42%) conditioned with 2 Gy TBI did so. Factors associated with acute GVHD in univariate and multivariate analyses are listed in Tables 2 and 3. In a multivariate Cox model, high TNFR1 day 7/baseline ratio (modeled as a continuous linear variable) was the only factor statistically significantly associated with a higher risk of grades II–IV (HR 2.2, $P=0.01$) (Figure 2a) and grades III–IV (HR 2.9, $P=0.007$) acute GVHD (Tables 2 and 3). There was also a trend for an association between a high incidence of grades II–IV acute GVHD and HLA disparity between donor and recipient, older patient age, and female donor to male recipient (Tables 2 and 3), although these factors did not reach statistical significance perhaps because of the relatively small number of patients analyzed. Interestingly, day 7 TNFR1 levels were not statistically significantly associated with grades II–IV acute GVHD ($P=0.07$), suggesting that TNFR1 day 7/baseline ratio predicts better for acute GVHD than TNFR1 day 7 alone (as also observed by other groups of investigators[16,17]).

Impact of acute GVHD occurrence on TNFR1 levels

As shown in Figure 2a, compared to those without grades II–IV acute GVHD, patients developing grades II–IV acute GVHD had higher relative TNFR1 levels on day 7 (NS) and on day 35 ($P=0.04$), but not on day 63 after HCT. To further analyze the role of TNF in acute GVHD after nonmyeloablative conditioning, we compared TNFR1 levels at onset of acute GVHD (median, day 38) in patients with grades II–IV acute GVHD, versus TNFR1 levels around day 35 after HCT in patients who never experienced grades II–IV acute GVHD. Patients developing grades II–IV acute GVHD had higher absolute (5957 ± 3498 pg/ml versus 4555 ± 3733 pg/ml, $P=0.0146$), and relative (defined as TNFR1 at the time of GVHD (or day 35) divided by baseline TNFR1 level; 2.5 ± 1.5 versus 1.8 ± 1.0, $P=0.0089$) TNFR1 levels at onset of GVHD than those without acute GVHD around day 35 after HCT (Figure 2b).

Association between TNFR1 day 7/baseline ratio and relapse/progression

One- and 3-year incidences of relapse/progression were 40 and 47%, respectively. In multivariate analysis, high disease risk was associated with a high risk of relapse/progression (HR 2.1, $P=0.001$), whereas TNFR1 day 7/baseline ratio (modeled as a continuous linear variable) was not (HR 0.6, $P=0.2$) (Table 4).

Association between TNFR1 day 7/baseline ratio and overall survival

One- and 3-year overall survival rates were 65 and 45%, respectively. In multivariate analysis, unrelated donor ($P=0.01$), high disease risk ($P=0.05$), and higher patient age ($P=0.03$) were each associated with a higher risk of mortality, whereas TNFR1 day 7/baseline ratio (modeled as a continuous linear variable) was not ($P=0.8$) (Table 4). Specifically, 3-year survival was 46% in patients with TNFR1 day 7/baseline ratio $\leqslant 1.3$, versus 44% in those with TNFR1 day 7/baseline ratio >1.3 (Figure 3).

Discussion

After myeloablative conditioning, several observations have suggested that high-dose chemo-radiotherapy induced TNF-α production/secretion by macrophages in response to TLR ligands and by activated T cells.[9,10,31] Although other cytokines have also been involved in the physiopathology of acute GVHD, the role of TNF-α as a critical mediator of acute GVHD has been well established in murine models.[11,32] Further, persistent elevation of TNF-α levels has been observed before and at onset of acute GVHD in human HCT recipients.[12] The biology of graft-versus-host reactions after nonmyeloablative conditioning

Table 2 Factors predicting acute GVHD in univariate analysis

Factor	*Grades II–IV acute GVHD*		*Grades III–IV acute GVHD*	
	Hazard ratio (95% CI)	P-*value*	*Hazard ratio (95% CI)*	P-*value*
TNFR1 day 7/baseline ratio[a]	**2.1 (1.2–3.8)**	**0.01**	**3.7 (1.8–7.4)**	**0.0002**
Dose of total body irradiation (4 Gy versus 2 Gy)	1.1 (0.7–1.6)	0.7	1.6 (0.9–2.7)	0.08
HLA-identical alternative versus HLA-identical sibling donor	1.6 (0.6–3.9)	0.3	1.5 (0.3–7.2)	0.6
HLA mismatched versus HLA-identical sibling donor	1.2 (0.6–2.4)	0.5	1.6 (0.6–4.6)	0.3
Donor or recipient CMV seropositivity	0.7 (0.4–1.4)	0.3	0.4 (0.2–1.1)	0.07
Patient age[a]	1.02 (0.99–1.05)	0.2	1.03 (0.97–1.08)	0.3
Female donor to male recipient versus other gender combinations	1.4 (0.7–2.7)	0.3	0.4 (0.1–1.8)	0.3

Abbreviation: TNFR1 = TNF receptor 1.
Bold values indicate *P*-value <0.05.
[a]Modeled as a linear continuous variable.

Table 3 Factors predicting acute GVHD in a multivariate Cox model

Factor	*Grades II–IV acute GVHD*		*Grades III–IV acute GVHD*	
	Hazard ratio (95% CI)	P-*value*	*Hazard ratio (95% CI)*	P-*value*
TNFR1 day 7/baseline ratio[a]	**2.2 (1.2–4.2)**	**0.01**	**2.9 (1.3–6.2)**	**0.007**
Dose of total body irradiation (4 Gy versus 2 Gy)	1.0 (0.6–1.5)	0.9	1.3 (0.7–2.3)	0.4
HLA-identical alternative versus HLA-identical sibling donor	1.7 (0.7–4.4)	0.3	1.4 (0.3–7.2)	0.7
HLA mismatched versus HLA-identical sibling donor	1.4 (0.7–2.7)	0.4	1.7 (0.6–5.0)	0.3
Donor or recipient CMV seropositivity	0.7 (0.4–1.4)	0.4	0.4 (0.1–1.1)	0.07
Patient age[a]	1.02 (0.99–1.05)	0.3	1.01 (0.96–1.07)	0.6
Female donor to male recipient versus other gender combinations	2.0 (1.0–4.2)	0.066	0.8 (0.2–3.9)	0.8

Abbreviation: TNFR1 = TNF receptor 1.
Bold values indicate *P*-value <0.05.
[a]Modeled as a linear continuous variable.

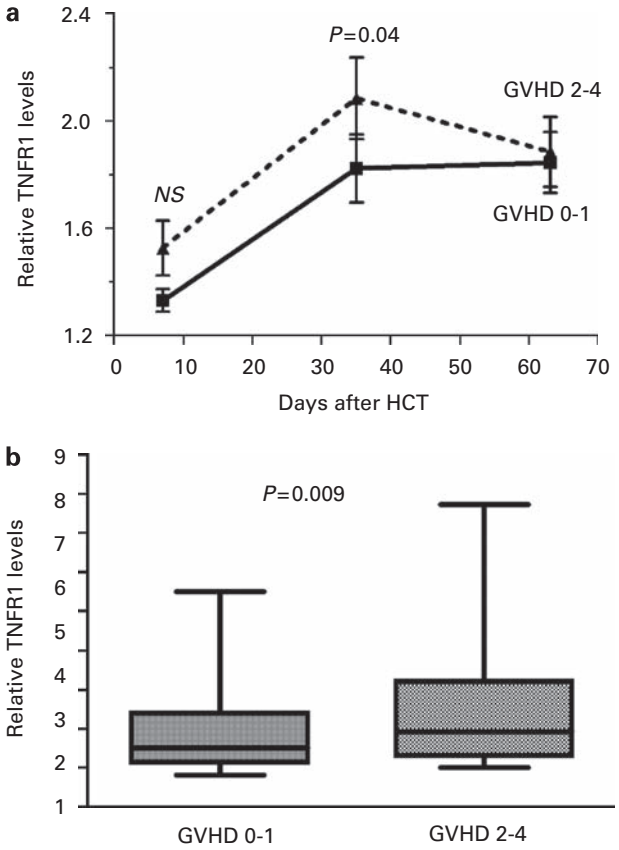

Figure 2 (**a**) Evolution of relative (defined as TNFR1 levels at the observed day divided by baseline TNFR1 levels) TNFR1 levels on days 7, 35, and 63 after HCT in patients with or without grades II–IV acute GVHD. (**b**) TNFR1 levels (mean ± standard deviation) on the day of onset (median 38 days after HCT) of GVHD in patients with grades II–IV acute GVHD, or around day 35 after HCT in patients without GVHD ($P=0.01$).

differs from what occurs after myeloablative conditioning in several aspects.[18] In particular, the intensity of the preparative regimens and transplant-related toxicities are by definition greatly reduced in the setting of nonmyeloablative conditioning.[33] This prompted us to investigate the role of TNF-α in the biology of acute GVHD after nonmyeloablative conditioning. We used TNFR1 as a surrogate marker for TNF-α, given that it is more stable in long-term storage and that previous work had shown that TNFR1 was elevated in patients with GVHD, and correlated with TNF-α levels.[16,17] Several observations were made.

First, TNFR1 levels were significantly higher on day 7 after nonmyeloablative HCT than before transplantation. However, median TNFR1 day 7/baseline ratio seemed to be lower after nonmyeloablative conditioning than has been observed by other groups of investigators in patients given allogeneic grafts after myeloablative conditioning.[16] Indeed, only 4 of 106 patients given nonmyeloablative conditioning in this study had a TNFR1 day 7/baseline ratio $\geqslant 2.5$, whereas 25% of patients given myeloablative conditioning had such a ratio in the Choi study.[16] The impact of conditioning intensity on TNFR1 increases after HCT was also directly shown in this study, as patients given 4 Gy TBI had higher TNFR1 day 7/baseline ratio than those given 2 Gy TBI ($P=0.01$). Nevertheless, our data also suggest that donor/recipient alloreactivity might also influence TNFR1 levels after nonmyeloablative conditioning as TNFR1 levels continue to increase from day 7 to day 35 after HCT (Figure 2b).

The most important observation of our study was the association between high TNFR1 day 7/baseline ratio and the probability of developing grades II–IV and grades III–IV acute GVHD. This is in agreement with previous papers analyzing data from patients given myeloablative conditioning.[16,17] We could not use the TNFR1 ratio $\geqslant 2.5$ as a cutpoint for predicting patients at risk for acute GVHD (as done by other groups of investigators[16,17]) because only four patients in our study had such a ratio. However, interestingly, these four patients all experienced grades II–IV acute GVHD. If confirmed in further prospective studies, the current observation could lead to the development of protocols aimed at preventing severe acute GVHD in patients with high TNFR1 increment on day 7 after nonmyeloablative HCT by maximizing post-grafting immunosuppression or administrating anti-TNF agents.

Another important observation in this study was the elevated TNFR1 levels in patients at onset of grades II–IV acute GVHD compared with patients without acute GVHD. This is in full agreement with a prior paper assessing TNFR1 levels in patients with acute GVHD after myeloablative conditioning.[13]

The TNFR1 day 7/baseline ratio had no impact on overall survival in this paper. This is in contrast to what has been observed in patients given myeloablative condition-

Table 4 Factors predicting mortality in a multivariate Cox model

Factor	*Relapse/progression*		*Mortality*	
	Hazard ratio (95% CI)	*P-value*	*Hazard ratio (95% CI)*	*P-value*
TNFR1 day 7/baseline ratio[a]	0.6 (0.3–1.3)	0.2	1.1 (0.6–1.8)	0.8
HLA-identical nonfamilial versus HLA-identical sibling donor	0.7 (0.2–2.1)	0.5	**2.9 (1.3–6.6)**	**0.01**
HLA mismatched versus HLA-identical sibling donor	0.7 (0.3–1.5)	0.3	1.4 (0.7–2.8)	0.4
Disease risk	**2.1 (1.3–3.3)**	**0.001**	**1.5 (1.0–2.2)**	**0.049**
Donor or recipient CMV seropositivity	2.5 (0.9–6.4)	0.07	1.5 (0.7–3.2)	0.35
Comorbidity (HCT-CI score[a])	1.0 (0.8–1.2)	0.8	1.1 (0.9–1.3)	0.3
Patient age[a]	1.02 (0.99–1.05)	0.2	**1.04 (1.00–1.08)**	**0.028**
Female donor to male recipient versus other gender combinations	0.5 (0.2–1.3)	0.16	1.3 (0.6–2.6)	0.5

Abbreviations: HCT = hematopoietic cell transplantation; TNFR1 = TNF receptor 1.
Bold values indicate *P*-value <0.05.
[a]Modeled as a linear continuous variable.

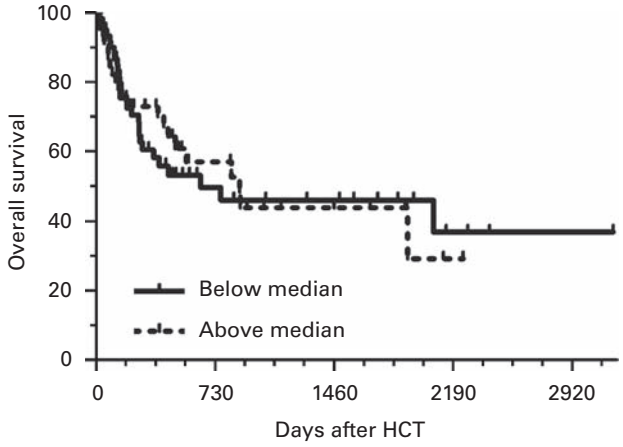

Figure 3 Overall survival in patients with a TNFR1 day 7/baseline ratio below or above 1.3 (median) ($P = 0.88$).

ing.[16,17] This could be due to the fact that only 6 of our 106 patients died from acute GVHD. Interestingly, we did not observe a significant association between TNFR1 day 7/baseline ratio and the risk of relapse/progression. This is in agreement with previous observations showing that acute GVHD is not associated with graft-versus-tumor effects after nonmyeloablative conditioning.[7,34]

In summary, our data suggest that nonmyeloablative conditioning induces the generation of TNF-α, and that the magnitude of TNF-α generation depends on the conditioning intensity (2 Gy versus 4 Gy TBI). Further, assessment of TNFR1 levels before and on day 7 after nonmyeloablative HCT provides useful information on subsequent risk of experiencing acute GVHD.

Conflict of interest

The authors declare no conflict of interest.

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