Association of functional polymorphisms of the transforming growth factor B1 gene with survival and graft-*versus*-host disease after unrelated donor hematopoietic stem cell transplantation

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ABSTRACT

Background
Many genetic factors play major roles in the outcome of hematopoietic stem cell transplants from unrelated donors. Transforming growth factor β1 is a member of a highly pleiotrophic family of growth factors involved in the regulation of numerous immunomodulatory processes.

Design and Methods
We investigated the impact of single nucleotide polymorphisms at codons 10 and 25 of *TGFB1*, the gene encoding for transforming growth factor β1, on outcomes in 427 myeloablative-conditioned transplanted patients. In addition, transforming growth factor β1 plasma levels were measured in 263 patients and 327 donors.

Results
Patients homozygous for the single nucleotide polymorphism at codon 10 had increased non-relapse mortality (at 3 years: 46.8% *versus* 29.4%, *P*=0.014) and reduced overall survival (at 5 years 29.3% *versus* 42.2%, *P*=0.013); the differences remained statistically significant in multivariate analysis. Donor genotype alone had no impact, although multiple single nucleotide polymorphisms within the pair were significantly associated with higher non-relapse mortality (at 3 years: 44% *versus* 29%, *P*=0.021) and decreased overall survival (at 5 years: 33.8% *versus* 41.9%, *P*=0.033). In the 10/10 HLA matched transplants (n=280), recipients of non-wild type grafts tended to have a higher incidence of acute graft-*versus*-host disease grades II-IV (*P*=0.052). In multivariate analysis, when analyzed with patients' genotype, the incidences of both overall and grades II-IV acute graft-*versus*-host disease were increased (*P*=0.025 and *P*=0.009, respectively) in non-wild-type pairs.

Conclusions
We conclude that increasing numbers of single nucleotide polymorphisms in codon 10 of *TGFB1* in patients and donors are associated with a worse outcome following hematopoietic stem cell transplantation from unrelated donors.

Key words: stem cell transplantation, TGF-β1, polymorphisms, survival, graft-*versus*-host disease.

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Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) using stem cells from volunteer unrelated donors is a life-saving intervention for patients with hematologic malignancies and a therapeutic approach when an HLA-identical sibling donor is not available.[1] The outcome of recipients of such transplants has improved significantly over the last decade. This is due, in part, to improved understanding of HLA matching and refinement in the resolution of tissue-typing techniques, which has lead to better donor selection,[2] to the increased safety in the procedure resulting from changes in the conditioning regimens[3] and immunosupression, and to better supportive care.

It is well recognized that numerous genetic factors in both the patient and donor play significant roles in the recipient's outcome. Apart from the well-defined HLA class I (HLA-A, -B and -C) and class II (HLA-DR, -DQ and -DP) genes,[4] several non-HLA genes have also been associated with differences in outcome.[5]

Transforming growth factor β1 (TGF-β1) is a member of a highly pleiotrophic family of growth factors that are involved in the regulation of numerous immunomodulatory processes. The main sources of TGF-β1 are T cells and platelets, although it can be secreted by other cell types including epithelial, endothelial and connective tissue cells.[6] The pro-fibrotic properties of TGF-β1 have been well defined.[7] Studies have shown that TGF-β1 can inhibit effector T-cell proliferation and activation and decrease Th1 and Th2 cell differentiation, while promoting differentiation of Th17 cells.[8,9] Moreover, TGF-β1 is essential for the proliferation of induced T regulatory (Treg) cells[10,11] and up-regulation of FOXP3[12] and plays a role in the immunosuppressive function of Treg due to cytokine secretion or membrane bound expression.[9,11,13]

Several functional polymorphisms in *TGFB1*, the gene encoding for TGF-β1, have been identified, such as a single nucleotide polymorphism (SNP) at codon 10 (coding(c).29T>C, protein(p).L10P) and another at codon 25 (c.75G>C, p.A74P) of exon 1. Both polymorphisms are part of the signal sequence peptide[10,14-16] and could, therefore, cause alterations in cytokine secretion. While codon 25 SNP have been consistently associated with decreased plasma levels,[17,18] conflicting data have been published regarding the impact of codon 10 SNP on plasma levels of TGF-β1.[17-22] These polymorphisms have been associated with an increased incidence of breast and colorectal neoplasia[20,23] and worse outcome following solid organ transplantation[24] and sibling HSCT.[5,25,26]

To date there is no published evidence on the possible role of these SNP in unrelated donor HSCT, or on plasma TGF-β1 levels and clinical outcomes following transplantation. Given the pathways in which TGF-β1 is involved, we hypothesized that these polymorphisms may influence the outcome of unrelated donor HSCT by modulating the immune response through changes in the secretory processes of the cytokine.

Design and Methods

Patients' characteristics

We genotyped, for the presence of a SNP at codons 10 and 25, a cohort of 427 patient/donor pairs: the patients had undergone HSCT, in a UK transplant center, with stem cells from unrelated donors found through the Anthony Nolan Trust. High resolution HLA typing for six HLA genes (*HLA-A, -B, -C, -DRB1, -DQB1, -DPB1*) was carried out for all transplant pairs. The transplants took place between 1997 and 2006 and the median follow-up time was 4.8 years (range, 0.23-11 years). The characteristics of the patients and donors are shown in Table 1. The diagnoses were chronic myeloid leukemia (n=115; 26.9%), acute lymphoid leukemia (n=125; 29.3%), acute myeloid leukemia (n=123; 28.8%), myelodysplastic syndrome (n=24; 5.6%), lymphoproliferative disorders (n=8; 1.9%) and other (n=32; 7.4%). Two hundred and ten patients (49.2%) were classified as having 'early stage' disease (defined as myelodysplastic syndrome, acute leukemia in first complete remission or chronic myeloid leukemia in chronic phase). All patients received myeloablative conditioning regimens; T-cell depletion was included in 86.4% of conditioning protocols, *in vivo* alemtuzumab (Campath; Schering Health Care Ltd., West Sussex, UK) being the most common method. Post-transplant immunosuppression was used in 90.8% of recipients; 60% of these received cyclosporine A and methotrexate and 28.6% cyclosporine A alone. Two hundred and eighty (65.6%) of the transplants were 'fully' HLA matched (10/10 *HLA-A, -B, -C, -DRB1, -DQB1*), and 17.1% were matched for 12/12 alleles (including *HLA-DPB1*) (Table 1).

Ethical approval

All research samples and data were collected according to institutional review board-approved guidelines and protocols of each participating institution. Written informed consent was obtained from all recipients and donors.

Genotyping

Genomic DNA was extracted from whole blood using the salting out technique.[27] The genotyping was carried out using a polymerase chain reaction (PCR) with sequence-specific primers. We used the primers described by Perrey *et al.*[28] The internal control product for the PCR was human growth hormone, as also described by Perrey *et al.* The reagents came from Bioline Ltd., London, UK. The PCR reaction was carried out in a final volume of 12.5 μL, containing 0.5 μL of genomic DNA (100-200 ng/μL), 0.25 μL of each primer (25 μM), 1.25 μL of NH_4 buffer (10x), 0.35-0.4 μL of $MgCl_2$ (50 μM) depending on the reaction, 0.2 μL of dNTP (12.5 μM each), 0.25 μL of internal control primers, 0.1 μL of DNA polymerase (5 U/μL) and 9.1-9.2 μL of s.H_2O (depending on the reaction). The thermocycler procedure consisted of initial denaturation at 95°C for 2 min, followed by 37 cycles of 95°C for 30 s and different annealing temperatures depending on the reaction (66.5°C for codon 10T and 25G, 63.6°C for codon 10C and 61.1°C for codon 25C) for 40 s, followed by a final extension at 72°C for 30 s and +4°C for 4 min. The products were analyzed by 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining. The target band sizes were 233 bp for codon 25, 241 bp for codon 10, and 429 for the internal control.

DNA sequencing of the polymorphic region of TGFB1 exon 1

In order to confirm the accuracy of the genotyping data from

the sequence-specific primers, we sequenced a part of exon 1 that includes codons 10 and 25 in some of the samples. We used the following primers: sense 5´-GTAAAACGACGGCCAGTTC-CGTGGGATACTGAGACAC and antisense 5´-CAGGAAACA-GCTATGACCCAGTTTCTTCTGCCAGTCA. The sequences were tagged with M13 tails (M13 forward 5´-GTAAAACGACG-GCCAGT and M13 reverse 5´-CAGGAAACAGCTATGAC). All results correlated with the sequence-specific primer findings.

Luminex assay

The active form of TGF-β1 was measured by luminex assay (Panomincs, Inc., Fremont, CA, USA). Samples were obtained prior to the transplant. Plasma from patients and donors was extracted from whole blood after centrifugation for 5 min at 1600 rpm. Samples were stored at –70°C. After thawing, the samples were spun at 1300 rpm for 7 min at +4°C; the supernatant was filtered through a filter plate with 0.45 µm pores (Millpore Corporation, MA, USA) at 1400 rpm for 5 min. For the activation process, we added 10 µL of 1N HCl to 40 µL of the processed sample and, after incubation for 10 min at room temperature, we added 8 µL 1.2N OHNa/0.5M Hepes. The luminex assay was run according to the manufacturer's instructions. We ran a standard curve and two blank samples for each batch.

Statistical analysis

Statistical analyses were performed using SPSS version 11.0.4 (SPSS Inc, Chicago, IL, USA), R version 2.6.2 (http://r-project.org) and Prism version 5.0a (GraphPad Software, Inc., CA 92037, USA). In univariate analysis, the overall survival and disease-free survival probabilities were compared using log-rank statistics and calculated using the Kaplan-Meier method. For relapse, non-relapse mortality and chronic graft-*versus*-host disease (GvHD) we used Gray's test and analyzed these outcomes using the cumulative incidence method. The competing event for non-relapse mortality was relapse, that for relapse was death without relapse and that for chronic GvHD was death and/or relapse without chronic GvHD. For the analysis of acute GvHD, engraftment and cause of mortality we used χ^2 statistics. Non-parametric tests were employed to compare mean plasma levels (Kruskal-Wallis test for three-group comparisons and the Mann-Whitney test for two-group comparisons). For multivariate analysis we used the Cox regression model for survival factors and logistic regression for acute GvHD analysis. We included all factors that were significant in univariate analyses with a *P* value of 0.2 or less. For all the analyses the *P* value was two-sided, and differences in outcomes were considered to be statistically significant when the *P* value was less than 0.05, while *P* values of 0.05 to 0.1 were taken to indicated a statistical trend.

Results

The observed frequencies of codon 10 genotypes in the patients were TC 52%, TT 37.2% and CC 10.8%; those in the donors were 49.4%, 38.2% and 12.4%, respectively. For codon 25 genotype, the frequencies in the patients were GG 86.7%, GC 12.6% and CC 0.7% and those in the donors were 84.1%, 15.5% and 0.5% respectively. These frequencies were similar to those previously described.[29]

Survival analysis

The overall survival and non-relapse mortality rates in the entire cohort at 5 years were 40.9% and 41%, respectively. The main causes of death were infection (21.8%), relapse (21.3%) and GvHD (4.9%).

The patients' codon 10 genotype had a significant impact on survival outcomes. Patients homozygous for the SNP at *TGFB1* codon 10 (CC) had a worse overall survival compared to patients with other genotypes (TT or TC) (Figure 1A) (at 2 and 5 years 31.8% and 29.3% *versus*

Table 1. Characteristics of the patients and donors.

	Patients and donors wt* (n=74)		Patients and/or donors SNP° (n=353)		*P*
	N.	%	N.	%	
Age of patients					
Older than 40 years	15	20.3	78	22.1	0.43
40 years or younger	59	79.7	275	77.9	
Age of donors					
Older than 30 years	22	29.7	104	29.5	0.53
30 years or younger	52	70.3	249	70.5	
Sex, male					
Patient	44	59.4	237	67.1	0.12
Donor	53	71.6	263	74.5	0.35
Sex matched					
Male patient/female donor	12	16.2	51	14.4	0.40
Total body irradiation					
Yes	61	95.3	285	91.1	0.32
HLA matching					
HLA 10/10	45	60.8	235	66.5	0.20
HLA 12/12	14	18.9	59	16.7	0.37
HLA-A mismatched	10	13.5	27	7.6	0.08
HLA-B mismatched	5	6.8	26	7.4	0.54
HLA-C mismatched	19	25.7	66	18.7	0.11
HLA-DRB1 mismatched	1	1.4	9	2.5	0.45
HLA-DQB1 mismatched	4	5.4	30	8.0	0.26
Disease					
Chronic myeloid leukemia	20	27.1	95	26.9	0.29
Acute myeloid leukemia	24	32.4	99	28	
Acute lymphoblastic leukemia	16	21.6	109	30.8	
Other	14	18.9	50	14.1	
Stage of disease					
Early	36	48.6	174	49.3	0.40
Late	29	39.2	156	44.2	
Unknown	9	12.2	23	6.5	
Stem cell source					
Bone marrow	51	68.9	269	76.2	0.11
Peripheral blood stem cells	23	31.1	83	23.5	
T-cell depletion					
Yes	57	77.1	312	88.4	0.03
No	14	18.9	38	10.8	
Unknown	3	4	3	0.8	
Immunosuppression					
Cyclosporine A	24	32.4	98	27.8	0.24
Cyclosporine A + methotrexate	46	62.2	210	59.5	
Other	0	–		10	2.8
None	2	2.7		23	6.5
Unknown	2	2.7		12	3.4

*wt: wild-type; SNP: single nucleotide polymorphism; HLA 12/12, matched at 12 out of 12 HLA antigens including DPB1 in a compatible way. Notes: *refers to all wt pairs (both patient and donor). °refers to all pairs with at least one SNP (the patient, the donor, or both).*

48.7% and 42.2%, respectively; log-rank *P*=0.013) and higher non-relapse mortality (Figure 1B) (at 1 and 3 years 46.8% and 46.8% *versus* 26.4% and 29.4%, Gray's test *P*=0.014). In the analysis according to the three possible genotypes, there is a suggestion of dosage effect of the SNP with regards to overall survival (Figure 1C) (the 2- and 5-year overall survival rates for patients with the CC, TC and TT genotypes were 31.8% and 29.3%, 47.2% and 40%, and 50.8% and 45.8%, respectively, log-rank *P*=0.033) and non-relapse mortality (Figure 1D) (the 1- and 3-year non-relapse rates for patients with the CC, TC and TT genotypes were 46.8% and 46.8%, 27.8% and 31.4%, and 24.6% and 26.7%, respectively, Gray's test *P*=0.036). Multivariate analysis for overall survival was performed including the following factors: patients' age, donors' age, patients' cytomegalovirus (CMV) status, donors' CMV status, disease classification, stage of disease, use of post-transplant immunosuppression, HLA incompatibility, T-cell depletion and patients' *TGFB1* codon 10 genotype. As well as the patients' age (older than 40 years) and stage of disease (late stage), we found that patients with the CC genotype had a significant reduction in overall survival [hazard ratio (HR): 1.51; 95% confidence interval (CI), 1.00 to 2.28; *P*=0.048] (Table 2). The analogous multivariate analysis for non-relapse mortality, again including in the analysis patients' and donors' age, patients' CMV status, T-cell depletion, HLA incompatibility and patients' codon 10 genotype, showed that patients with the CC genotype had a significant increase in non-relapse mortality (HR: 1.91; 95% CI, 1.23 to 2.96; *P*=0.004) (Table 3).

We found no statistically significant differences due to the donor codon 10 genotype alone; however, when we analyzed the impact of the total number of SNP present in the patient-donor pair, we found that multiple SNP (in both the patient and the donor) (3-4 SNP *versus* 2 or less) were associated with significantly decreased overall survival (at 2 and 5 years: 33.8% and 33.8% *versus* 48.9% and 41.9%, log-rank *P*=0.033) (Figure 1E) and a higher non-relapse mortality (at 1 and 3 years: 42% and 44% *versus* 26% and 29%, respectively, Gray's test *P*=0.021) (Figure 1F).

Analyzing the different causes of mortality, we found that the presence of the SNP at codon 10 in the patient significantly affected the outcome. Patients homozygous for the SNP had a statistically significantly higher probability of dying because of an infection compared to patients with other genotypes (CC 34.8% *versus* TC/TT 20.3%, *P*=0.024).

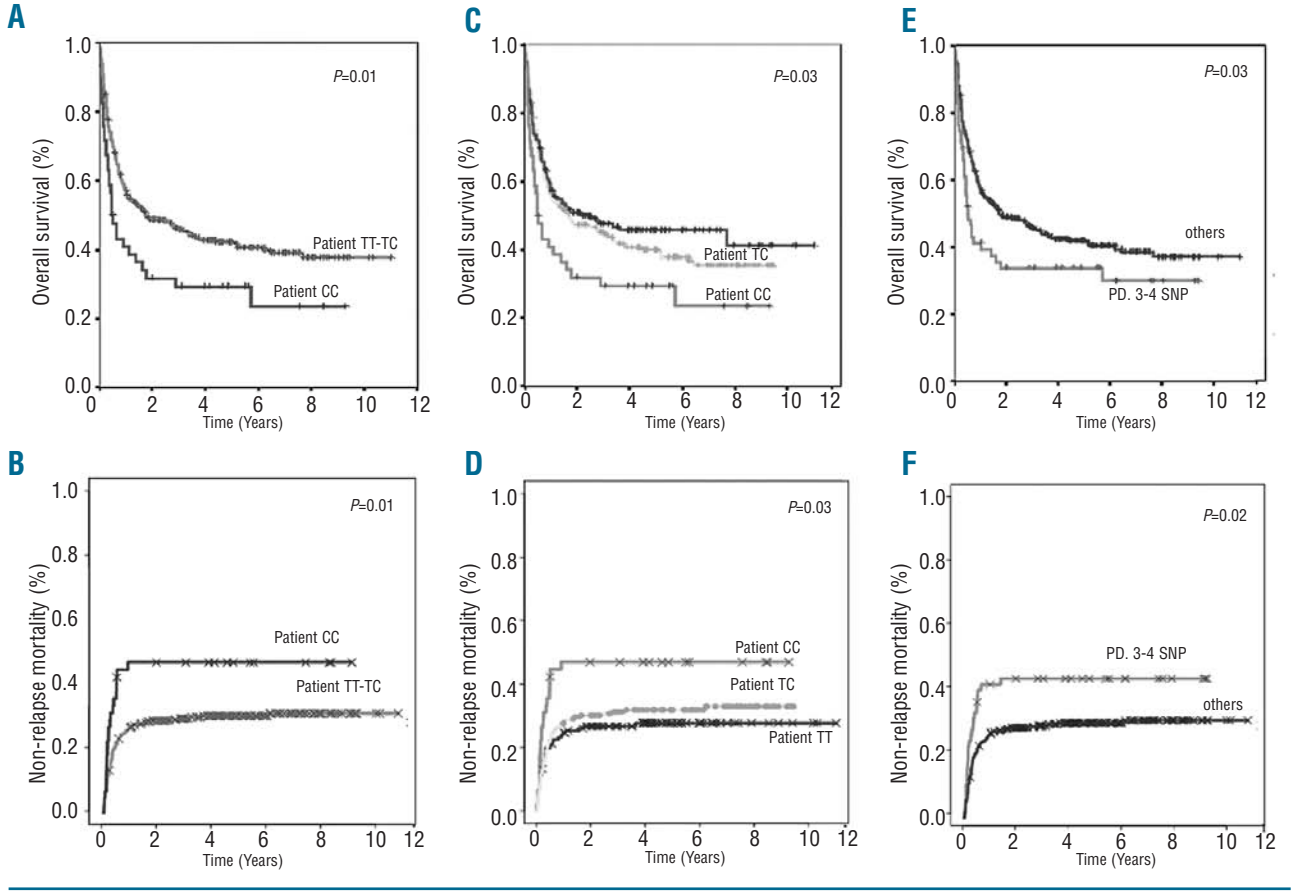

Figure 1. Survival analysis depending on *TGFB1* codon 10 genotype. Compared to patients with TT or TC genotypes, patients homozygous for the SNP at *TGFB1* codon 10 (CC) had worse overall survival (A) and higher non-relapse mortality (B). There is a suggestion of a dosage effect of the SNP with regards overall survival (C) and non-relapse mortality (D). The presence of three or four SNP within the patient-donor pair (PD.3-4 SNP) is associated with a significant reduction in overall survival (E) and increase in non-relapse mortality (F).

Acute graft-versus-host disease

Acute GvHD was graded as absent (grade 0), clinically significant (grades II-IV) and severe (grades III-IV). The overall incidence of acute GvHD was 50.4%, the incidence of acute GvHD grades II-IV was 27.4% and that of grades III-IV was 6.8%.

Regarding the association of *TGFB1* codon 10 genotype and acute GvHD, non-wild-type patient-donor pairs (*i.e.* the presence of a SNP in the patient and/or the donor) had significantly more acute GvHD II-IV than wild-type pairs (31% *versus* 19%, respectively; *P*=0.031). HLA mismatches were also associated with a significant increase in GvHD (*data not shown*). In order to exclude the confounding effect of an HLA mismatch, we analyzed only the 10/10 HLA matched transplants (n=280). In this analysis we found that the non-wild-type donors had a trend to an increased frequency of acute GvHD II-IV (29.9% *versus* 20%, *P*=0.052). As in the survival analysis, there appears to be an additive effect between the patients' and donors' genotype. The non-wild-type pairs had significantly higher incidences of overall acute GvHD (51% *versus* 32%, *P*=0.018) and acute GvHD grades II-IV (30% *versus* 2.5%, *P*<0.001), as well as a trend to a higher incidence of severe acute GvHD (grades III-IV) (6.7% *versus* 0%, *P*=0.077), compared to wild-type pairs (Table 3). In the multivariate analysis, the non-wild-type pairs had a significant increase in overall acute GvHD (HR 2.1; 95% CI 1.06-4.4; *P*=0.025) (adjusted for patients' age, source of transplant and DPB1 incompatibility) and acute GvHD grades II-IV (HR 15.1; 95% CI 1.96-117.4; *P*=0.009) (Table 4).

Codon 10 genotype did not have a significant effect on relapse, disease-free survival, chronic GvHD or engraftment. Codon 25 genotype was not found to have a significant effect on any transplant outcome.

Impact of codon 10 polymorphism on plasma TGF-β1 levels

Given the previously described association of codon 10 with the secretory process of TGF-β1, we analyzed the possible association of the presence of the SNP and plasma levels of this cytokine. We analyzed plasma TGF-β1 levels in 263 patients prior to transplantation. In contrast with previous data, we found no statistically significant differences in plasma levels depending on codon 10 genotype (median levels: CC 697.5, TC 730.0 and TT 537.1, *P*=0.24) (Figure 2). We also measured plasma levels in 327 healthy donors, again finding no statistically significant differences (median levels: CC 737.1, TC 674.1 and TT 765.3, *P*=0.50) (*data not shown*). We then analyzed the possible impact of patient-donor pair plasma levels and clinical outcomes. For a total of 234 pairs analyzed, we found no significant effect of plasma levels of TGF-β1 on any clinical outcome.

Discussion

A better understanding of the factors that are important in the biology of HSCT will help us to improve patients' post-transplant outcome and give them a longer and better quality of life. We have shown in a large number of patient-unrelated donor pairs that increased numbers of

Table 2. Multivariate analysis for overall survival in the whole cohort.

	P value	Hazard Ratio	95% CI for Exp (B) Lower	Upper
Patient 10 CC	0.048	1.51	1.00	2.28
Patient older than 40 years	0.003	1.64	1.18	2.28
Donor older than 30 years	0.044	1.38	1.01	1.88
Patient CMV status	0.291	1.18	0.86	1.60
Donor CMV status	0.647	1.08	0.77	1.50
Disease				
Other disease	0.897			
Chronic myeloid leukemia	0.282	0.65	0.30	1.41
Acute myeloid leukemia	0.510	0.77	0.36	1.65
Myelodysplastic syndrome	0.445	0.70	0.28	1.73
Acute lymphoblastic leukemia	0.470	0.75	0.35	1.62
Lymphoproliferative disorders	0.793	0.85	0.27	2.67
Post-transplant immunosuppression				
Cyclosporine + methotrexate	0.402			
Cyclosporine	0.468	1.12	0.82	1.52
Other	0.230	0.49	0.15	1.56
None	0.330	1.29	0.76	2.18
HLA mismatched	0.334	1.15	0.86	1.53
T-cell depletion	0.256	0.78	0.51	1.19
Late stage of disease	0.013	1.45	1.08	1.95

Table 3. Multivariate analysis for non-relapse mortality in the whole cohort.

	P value	Hazard ratio	95% CI for Exp (B) Lower	Upper
Patient 10 CC	0.004	1.91	1.23	2.96
Patient older than 40 years	0.003	1.73	1.19	2.49
Donor older than 30 years	0.057	1.45	0.98	2.13
Patient CMV status	0.284	1.20	0.85	1.71
T-cell depletion	0.344	1.33	0.73	2.41
HLA mismatched	0.377	1.16	0.82	1.63

Patient 10 CC, patient homozygous for the SNP at TGFB1 *codon 10.*

Table 4. Incidence of acute GvHD depending on the *TGFB1* codon 10 genotype of the patients and donors.

	Acute GvHD(%) any grade	*P*	Acute GvHD II-IV (%)	*P*	Acute GvHD III-IV (%)	*P*
Full cohort						
p WT	50.7	0.18	28.2	0.38	9.8	0.10
p TC-CC	55.8		30		5.8	
d WT	51.3	0.22	28.3	0.39	7.2	0.57
d TC-CC	55.7		30		7.3	
pd WT	46.3	0.10	19.4	0.031	8.9	0.35
Others	55.5		31.4		6.9	
10/10 cohort						
p WT	45.4	0.25	22.7	0.21	6.8	0.38
p TC-CC	50.5		28.1		5.1	
d WT	43	0.10	20	0.052	3	0.17
d TC-CC	52		29.9		7	
pd WT	32	0.018	2.5	<.001	0	0.077
Others	51		30		6.7	

p: patient; WT: wild-type; d: donor; pd: patient-donor.

SNP in codon 10 of *TGFB1* in patients and donors are associated with a worse outcome following transplantation.

TGF-β1 is secreted as a pre-pro-peptide. The mature form is cleaved from the COOH terminal in the trans Golgi by furin type enzymes.[14,30,31] A homodimer of the pro-peptide, known as latency-associated protein (LAP) is non-covalently bound to a homodimer of the mature peptide and is only released before the cytokine makes contact with its receptor.[10,30] The pre-region, located in the N terminus of the peptide, is known as "signal sequence" peptide, and is cleaved during the secretion process. This peptide contains the core, a sequence normally made of 8-15 hydrophobic amino acids,[16,32] which is proposed to participate in the transmembrane orientation of the endoplasmic reticulum membrane. Codon 10 is located in the center of the core, and a change between Leucine (highly hydrophobic) to Proline (an indifferent or less hydrophobic amino acid) may change the tertiary structure of the peptide and, therefore, may alter the process of secretion.

Conflicting data have been published regarding the impact of the codon 10 SNP on plasma levels of TGF-β1, and there is no evidence associating this polymorphism with the membrane expression of the cytokine in T cells, which has been described as a possible way of mediating Treg immunomodulation.[13,33]

We demonstrated that the presence of the SNP in codon 10 in the patient as well as in the patient-donor pair has a deleterious effect on overall survival, due to an increase in non-relapse mortality. Analyzing the different causes of death, we found that patients homozygous for the SNP had a significantly higher probability of death due to infection, probably due to a modification in the immunosuppressive environment secondary to the presence of the SNP. Likewise, although not statistically significant, the probability of death due to hepatic veno-occlusive disease/thrombotic thrombocytopenic purpura was also increased, coinciding with previous findings of an association between veno-occlusive disease and TGF-β1 secretion.[34]

In agreement with a study by Baron *et al.*, who demonstrated that the donor gene-expression profile, in particular the TGF-β1 pathway, has a dominant influence on the development of GvHD,[35] we also found that the presence of the SNP at codon 10 in donors, as well as in patient-donor pairs, was associated with a higher incidence of acute GvHD and its clinically significant forms. This agrees with observations by Hattori *et al.*,[25] who found that the wild-type donor genotype (TT) was protective for acute GvHD in an HLA-identical sibling donor pediatric cohort.

In contrast with previous results,[17-21,36] we did not find a significant difference in plasma levels of TGF-β1 depending on codon 10 genotype in either patients or donors. This lack of effect may be due to the fact that the subjects did not have a homogenous state of immune activation (donors were in a resting state and patients were under different conditions depending on the stage of disease, or recent chemotherapy). Similarly, the plasma levels were not related to clinical outcomes, probably because of the heterogeneous conditions already described. In addition, the polymorphisms may influence the autocrine-paracrine secretion of the cytokine and effects may, therefore, not

Table 5. Multivariate analysis of acute GvHD grades II-IV in the fully HLA-matched (10/10) cohort.

	P value	Odds Ratio	95% CI Lower	Upper
Patient younger than 40 years	0.176	0.54	0.22	1.31
Male recipient	0.261	0.66	0.33	1.35
Donor CMV seronegative	0.173	0.56	0.24	1.28
Disease classification				
Other diseases	(ref)	1	–	–
Chronic myeloid leukemia	0.915	0.90	0.14	5.74
Acute myeloid leukemia	0.456	0.48	0.07	3.25
Myelodysplastic syndrome	0.803	0.76	0.09	6.34
Acute lymphoid leukemia	0.894	1.13	0.17	7.39
Lymphoproliferative disease	0.265	4.88	0.3	79.1
Late stage	0.656	0.85	0.42	1.7
Post-transplant immunosupression				
Cyclosporine A + methotrexate	(ref)	1	–	–
Cyclosporine A	0.423	0.73	0.34	1.57
Other	0.428	2.75	0.22	33.8
None	0.767	0.8	0.18	3.43
DPB1 incompatibility	0.086	1.97	0.9	4.3
T-cell depletion	0.824	0.89	0.33	2.37
Non-WT PD	0.009	15.1	1.96	117.4

Non-WT PD, the presence of a SNP at TGFB1 *codon 10 in the patient and/or the donor.*

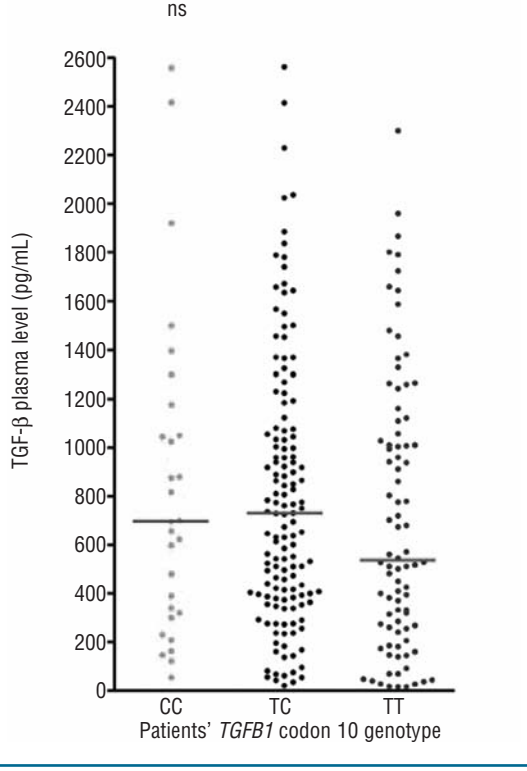

Figure 2. TGF-β1 plasma levels measured using the luminex technique, in 263 patients according to codon 10 genotype. There were no significant differences between the groups. The black lines represent the median.

be seen in plasma. We are currently studying TGF-β1 membrane expression and soluble cytokine in stimulated cells, in relation to *TGFB1* codon 10 genotype. In addition, we are analyzing alterations in T-cell phenotype (especially Treg).

TGF-β1 is critical for Th17 cell differentiation.[9] Th17 cells have been demonstrated to be essential in the immune response against extracellular bacteria and fungi.[37] An alteration in the secretory process of TGF-β1 may, therefore, impair Th17-mediated immune responses to infections with the potential consequences of increased non-relapse mortality and decreased overall survival. Moreover, it has also been demonstrated that TGF-β1 is essential for the generation of induced Treg (a subset known as Th3)[10,11] as well as for their function.[11,13] Therefore, following the cytokine storm observed with myeloablative conditioning for transplants, the presence of the SNP may modify the immunosuppressive capacity of induced Treg, with a subsequent increase in the incidence of acute GvHD.

In conclusion, we have shown in a large number of patient-donor pairs, that increased numbers of SNP in codon 10 of *TGFB1* in patients and unrelated donors is associated with worse outcomes following myeloablative conditioned, unrelated donor HSCT. While an exact functional mechanism remains unclear, our data highlight the importance of pursuing functional analyses of TGF-β1 in this setting. We recognize that our study has limitations, particularly its retrospective nature and the heterogeneity of the patients studied. Furthermore, data on the effect of graft characteristics, such as CD34 and CD3 cell number, are lacking and this could influence the results. Our findings do, therefore, need to be confirmed in prospective studies. The clinical importance of our observations lies in the potential to identify patients at increased risk of developing acute GvHD and non-relapse mortality. If our data our confirmed, information on *TGFB1* genotype could be included in transplant algorithms, to guide alterations in immunosuppressive regimens and/or donor selection.

Authorship and Disclosures

MB, GK, SM, AM and BS designed the study; MB, NM and LC performed the PCR analyses and MB performed the luminex analysis; MB and BS collected the data; MB, HMT and BS analyzed and interpreted the data; MB, NM, SM, AM and BS wrote the manuscript. All the authors contributed to the revision of the article and approved its final version. Each of the authors met the International Committee of Medical Editors (ICMJE) criteria for authorship. The authors report no conflicts of interest.

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