Published in final edited form as:
Biol Blood Marrow Transplant. 2010 December ; 16(12): 1700–1706. doi:10.1016/j.bbmt.2010.06.003.

TNF Polymorphism Affects Transplant Outcome in Patients with MDS but not with CML, Independent of the Presence of HLA-DR15

Laura F. Newell[1,2], **Ted Gooley**[1,3], **John A. Hansen**[1,2], **Derek L. Stirewalt**[1,2], **Effie W. Petersdorf**[1,2], and **H. Joachim Deeg**[1,2]

[1] Clinical Research Division, Fred Hutchinson Cancer Research Center, University of Washington School of Medicine, Seattle, WA

[2] Department of Medicine, University of Washington School of Medicine, Seattle, WA

[3] Department of Biostatistics, University of Washington School of Medicine, Seattle, WA

Abstract

Both presence of HLA-DR15 and tumor necrosis factor-α (TNF) levels have been reported to affect outcome after hematopoietic cell transplantation (HCT). Patients with myelodysplastic syndromes (MDS) show a high prevalence of HLA-DR15 and express high levels of TNF in the bone marrow. The present analysis involving 7,950 patients showed an HLA-DR15 frequency of 31% in patients with MDS, compared to only 23% in patients with chronic myeloid leukemia (CML). HLA-DR15 was more prevalent in Caucasian than in non-Caucasian patients (p=0.01). Numbers of non-Caucasian subgroups were too small for further analysis. Among Caucasian patients with MDS and CML, the presence of HLA-DR15 did not significantly affect the occurrence of graft-versus-host disease, relapse, non-relapse mortality (NRM), or survival. However, there was a significant correlation of DR15 and TNF polymorphisms at position -308 among patients with MDS, and the TNF -308 AG genotype conferred an increased risk of NRM compared to GG (hazard ratio [HR] 1.49, p=.02), even after adjusting for DR15. Conversely, the TNF -863 AA genotype correlated with decreased overall mortality and NRM compared to the CC genotype (HR 0.36, p=.04, and HR 0.13, p=.04, respectively), even after adjusting for DR15. There was no significant association between TNF -308 or -863 polymorphisms and transplant outcomes in CML patients. These results suggest that TNF polymorphisms, rather than DR15 affected transplant outcome in a disease-dependent manner.

Keywords

MDS; TNF polymorphism; HLA-DR15; hematopoietic cell transplantation

INTRODUCTION

Major histocompatibility complex (MHC) class II genes (HLA-DR, DQ and DP in humans) were originally described in the mouse as immune response genes [1] and subsequently, many examples of disease association with class II HLA antigens/alleles in humans were recognized. In the setting of hematopoietic cell transplantation (HCT) the presence of the class II antigen

Corresponding Author: H. Joachim Deeg, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue N, D1-100, jdeeg@fhcrc.org.

HLA-DR2 (subsequently split into DR15 and DR16) was reported to affect transplant outcome, in particular, the occurrence of graft-versus-host disease (GVHD) and survival [2–4]. Centromeric to the HLA gene complex on chromosome 6, and closely linked to HLA-DR, is the gene encoding the proinflammatory cytokine tumor necrosis factor α (TNF), which is also thought to play a role in transplant outcome [5,6]. As we and others had shown, significant upregulation of TNF in the marrow of patients with myelodysplastic syndromes (MDS) and, furthermore, HLA-DR15 (DR2) has been shown to be associated with MDS [7–10]; we were interested in determining whether transplant outcome in patients with MDS was dependent upon the presence of HLA-DR15, TNF genotype or both.

TNF shows single nucleotide polymorphisms (SNPs) at positions -308 and -863. The TNF-308 position consists of a guanine (G) or an adenine nucleotide (A). The TNF-308 GG genotype was shown to result in higher TNF levels than the AA genotype [11]. Kroeger et al noted that the -308 polymorphism altered the affinity of nuclear factor binding and differentially affected transcription of the TNFα gene: The presence of an adenine nucleotide at -308 increased transcription at least two-fold above levels seen with guanine [12]. The TNF-863 cytosin (C) and adenine (A) polymorphism, implicated in several autoimmune disease [13,14], has been speculated to influences TNFα expression through differential binding of NF-kB complexes and through allele-specific chromatin remodeling [15]. The -863 A allele ultimately is associated with increased TNFα production [16].

In the present study, we first analyzed the prevalence of HLA-DR-15 in 7950 patients who underwent hematopoietic cell transplantation for various hematologic diseases, including MDS. Results confirmed a high prevalence in patients with MDS. We then selected the cohort of patients with the lowest prevalence of HLA-DR15, chronic myeloid leukemia (CML), as a comparison group, and used these two cohorts to measure linkage disequilibrium between HLA-DR-15 and the TNF -308 and -863 SNPs in our patient population, and to analyze the effects of the HLA-DR15 alleles and TNF polymorphisms on transplant outcome.

METHODS

Patient, Disease, and Transplant Characteristics

This retrospective analysis involved 7950 patients undergoing first hematopoietic cell transplantation at the Fred Hutchinson Cancer Research Center, for aplastic anemia, acute myeloid and lymphoblastic leukemias, chronic lymphocytic leukemia, CML, Hodgkin disease, multiple myeloma, non-Hodgkin lymphoma, and MDS. Details of the conditioning regimens, GVHD diagnosis, prophylaxis and therapy, infection prophylaxis, supportive measures, and assessment of relapse have been reported elsewhere [17–22]. All patients had provided informed consent for ongoing research studies according to the requirements of the Institutional Review Board of the Fred Hutchinson Cancer Research Center.

HLA-typing

Related donors were HLA-identical siblings or mismatched relatives as determined by family study prior to hematopoietic cell transplantation [23]. Unrelated donors were characterized for HLA-A, B, C, DRB1, DQB1 alleles using sequence-specific oligonucleotide probe hybridization or sequencing methods [23]. All patients were classified as DR15+ if they expressed at least one copy of a DR15 allele.

Genotyping of TNF single-nucleotide polymorphisms (SNP)

Genotyping of the SNPs of TNF-alpha involving positions -308 and -863 was carried out by multiplex polymerase-chain-reaction (PCR) assay, as previously described, and no TNF typing in addition to that performed for a previous study was carried out [24].

Statistical analysis

The chi-square test was used to compare the frequency of DR15 across various diagnoses as well as the association between the presence of DR15 and TNF genotype. Cox regression was used to examine the association between both the presence of DR15 and TNF genotype and the time-to-event outcomes overall mortality, relapse, and non-relapse mortality (NRM). Logistic regression was used to examine the same associations with the outcome of grades II–IV acute GVHD. Regression models were adjusted for patient age, disease severity (high vs. low), and type of donor (HLA identical sibling vs. others). Because of the linkage disequilibrium between DR15 and TNF, both DR15 and each of the TNF **polymorphisms** were included in all regression models in order to evaluate the association of one after adjustment for the others. Two-sided p-values from regression models were estimated from the Wald test; no adjustments were made for multiple comparisons [25].

RESULTS

Frequencies of HLA-DR15 and TNFα polymorphisms depend upon diagnosis and racial origin

A total of 6866 Caucasian and 1084 known non-Caucasian patients undergoing first HCT at our Center had HLA data available that allowed categorization in regards to expressing or not expressing at least one copy of a DR15 allele. The frequency of DR15 among Caucasians was higher than among non-Caucasians (25.8% vs. 22.3%; p=.01). Among 765 Caucasian patients with a diagnosis of MDS, 237 (31.0%), had at least one DR15 allele, compared to 393 of 1644 patients with CML (23.9%). Frequencies for other diagnoses are listed in Table 1. A global test of the equality of the proportion of patients positive for DR15 across all diseases yielded p=0.001, supporting the hypothesis that the prevalence of DR15 differed significantly between diagnoses. Characteristics for patients with MDS or CML are summarized in Table 2.

Among Caucasians, the frequency of the TNF -308 genotypes was different from that among non-Caucasians. Specifically, the GG genotype occurred in 68.5% of Caucasians and 85.9% of non-Caucasians; the AG genotype was present in 29.2% and 13.0% of Caucasians and non-Caucasians, and the AA genotype in 2.6 and 1.1 %, respectively (p<.0001). The differences in frequencies of the TNF -863 genotypes were not as marked, but were still statistically significant (p=.02). Because of these differences, the subsequent analyses were restricted to the Caucasian population. Summarized in Table 3 are the allele and genotype frequencies for the TNF -308 and -863 SNPs among Caucasians, and the correlation with DR15, which was significant for TNF -308 (p=.003). The number of patients in any particular non-Caucasian population was not sufficiently large to carry out separate analyses.

DR15 and TNF -308 and -863 polymorphisms in MDS and CML

The associations between SNP genotypes and DR15 are summarized in Table 3. The frequency of DR15 differed across TNF-308 genotypes among patients with MDS (p=.003), and the presence of at least one DR15 allele was suggestively but not statistically significantly different across TNF-863 genotypes (p=.11). Among patients with CML the presence of DR15 showed no statistically significant correlation with the TNF-308 genotype (p=.54), while such an association did exist between DR15 and the TNF-863 genotype (p=.03).

Associations of DR15 positivity and TNF -308 and TNF -863 SNPs with transplant outcome in MDS patients

After adjusting for **TNF -863 and** the presence of DR15, the TNF -308 AG genotype in patients with MDS was associated with an increased risk of NRM relative to the GG genotype; overall mortality was also increased, but not significantly so (Table 4). The AA genotype also tended

to be associated with increased failure rates relative to the GG genotype for each of the above endpoints, but none of the associations was statistically significant (Table 4), as there were only 9 patients with this genotype. There was no statistically significant association between TNF -308 SNPs and relapse (Table 4). Patients with the TNF – 863 AC genotype had similar overall and NRM as those with the CC genotype (Table 4), while the group with the AA genotype had suggestively reduced overall mortality and NRM compared to patients with the CC genotype, although the small number of patients with the AA genotype (n=14) mandates a cautious interpretation of the results. The associations between TNF and overall mortality, NRM, and relapse did not appear to depend on the presence of DR15 (interaction p-values ranging from **.39** to **.95**). Moreover, the magnitudes of the associations between TNF and each of these outcomes after adjusting for the presence of DR15 were virtually the same as the magnitudes of associations between TNF and outcomes without adjusting for the presence of DR15.

There was no statistically significant association between TNF -863 SNPs and acute GVHD (Table 4). The impact of the presence of TNF -308 SNP on grades II–IV acute GVHD was dependent on DR15 status (p=.04, test of interaction). In DR15-negative patients, the TNF -308 AG genotype was associated with an increased probability of acute GVHD compared to patients with the GG genotype (OR 2.**25**; 1.**12**–4.**49**; p=.0**2**), but **the** association of the TNF -308 AG genotype with acute GVHD in DR15-positive patients **was in the opposite direction** (OR 0.**37; 0.12–1.11; p=.08**). Furthermore, there was no statistically significant association between the presence of an HLA-DR15 allele per se and transplant outcomes (overall mortality, relapse, NRM, or grades II–IV acute GVHD, Table 4).

Association of DR15 positivity and TNF -308 and TNF -863 SNPs with outcome in CML patients

Among CML patients, there were no statistically significant associations between either TNF -308 or -863 SNPs and overall mortality, relapse, NRM, or grades II–IV acute GVHD, nor was there a statistically significant association between the presence of a DR15 allele and outcome (Table 5).

HLA-B, TNF polymorphism, and transplant outcome

Given the location of the TNF gene within 200 kb of HLA-B, we also evaluated possible associations between HLA-B and TNF. Several HLA-B antigens showed an association with the TNF SNPs examined, but none of the associations between TNF and transplant outcome was qualitatively changed after adjustments were made for the presence of these HLA-B antigens (data not shown).

DISCUSSION

This analysis of data from patients undergoing HCT for lymphoid or hematologic disorders showed significant disease-dependent differences in the frequency of the presence of HLA-DR15, with 31% of patients with MDS and 23% of patients with CML carrying at least one DR15 allele. A second analysis, restricted to Caucasian patients with MDS or CML for whom molecular information on TNF polymorphism was available, showed a statistically significant correlation of the TNF-308 genotype with DR15 in MDS, but not in CML Patients. The TNF-863 genotype showed a suggestive correlation with DR15 in MDS, and a statistically significant correlation in CML patients. In patients with MDS, the TNF-308 AG genotype was associated with an *increased* risk of NRM relative to the GG genotype, while, conversely, the -863 AA genotype was associated with a **suggestively** *decreased* risk of overall mortality and NRM relative to the CC genotype**, although the number of patients with the TNF -863 AA genotype was small, and these results therefore need to be interpreted with caution.**

Importantly, these associations were seen after adjusting for the presence of DR15 alleles, suggesting that it was not the presence or absence of DR15 that explained the association of the TNF SNPs with transplant outcome. In contrast to the MDS cohort, no statistically significant effects of TNF -308 and -863 SNPs on transplant outcomes were observed among patients with CML. Neither MDS nor CML cohorts showed statistically significant associations between DR15 itself and transplant outcome.

Thus, these results are in contrast to some previous reports, which did show correlations of DR15 and transplant outcome in patients with various diagnoses [2–4]. For example, Stern et al. [4] observed a significantly higher 5-year survival rate in DR15-positive than in DR15-negative patients (76% vs 55%, p=.04), and noted a lower 5-year probability of disease-related mortality in DR15-positive patients (5% vs 24%, p=.02). Similar data were presented by Battiwalla et al. [2] who showed a significantly lower incidence of acute GVHD in DR15-positive (23%) than **in** DR15-negative patients (42%; p=.041). However, only 20 patients with MDS were included in that analysis, and patients with serologically determined DR2 (who had not been allele typed) were included in the DR15-negative group. In the study by Davidson et al. [3], DR15-positive adult patients showed improved day 100 survival (p=.03) and overall survival (p=.0143); if patients developed acute GVHD, survival was superior in those who were DR15-positive (p=.02). However, an analysis of results in 88 patients transplanted from HLA-identical unrelated donors, showed inferior survival with DR15 positivity (p=.02), and if acute GVHD developed, DR15-negativity was associated with improved survival (p=.009).

In the present study, no correlation was found between presence or absence of HLA-DR15 and transplant outcome, including GVHD, relapse, NRM, or survival. While it is conceivable that differences of results between reports were related to different composition of the patient cohorts studied, the present analysis found no correlation of DR15 with outcome in patients with either MDS or CML, transplanted from either related or unrelated donors. Similarly, there was no association of specific HLA-B alleles and transplant outcome. In fact, the present data suggest that correlations were with TNF polymorphisms, in particular with the TNF -308G allele. Of course, while 91% of patients with MDS and 85% of patients with CML had donors who were matched for HLA, including HLA-DR, TNF polymorphism data were obtained only in patients and might have differed from those in the donors, certainly in the unrelated donor setting.

Lin et al. analyzed data from 570 patients transplanted at our Center and their HLA-identical sibling donors for an association between GVHD and cytokine SNPs [24]. They analyzed seven different SNPs in five cytokine genes including TNF-308, and found no **statistically** significant association between acute GVHD and the TNF -308 genotype. However, that study included patients with various diagnoses, and in view of the differences between different diagnostic groups observed in the present analysis, those data cannot be globally extrapolated. Middleton et al. [26] determined TNF and Interleukin-10 polymorphic allele frequencies in 80 patients with ALL or CML. They then correlated polymorphism results with incidence and severity of GVHD in 49 patients who subsequently underwent HCT from HLA-identical sibling donors; they found no significant association of outcome with the TNF -308 polymorphism, although they did observe an association of GVHD with a microsatellite near the TNF locus. A significant association between the *donor* TNF -308 SNP (which was not determined in the present study) and severity of acute GVHD (p=.04) was described by Takahashi et al. in patients transplanted primarily from HLA-identical related donors [27]. While patient TNF -308 polymorphism (pre-transplant) was not associated with acute GVHD, there was a significant association with polymorphism in post-engraftment samples (p=.04), suggesting a relationship to donor cell polymorphism. In yet another analysis of results in 77 patients transplanted from HLA-identical sibling donors, Bertinetto et al. observed significant associations between grades II–IV acute GVHD and the TNF -308 or -863 polymorphisms [28]. Finally, Shaw et al.

had shown in patients with CML, acute leukemias or other malignant diseases transplanted from related or unrelated donors that neutrophil engraftment was delayed if either patient (p=0.03) or donor (p=0.02) possessed the TNF -308 AG genotype [29]. No such correlation was found in the present study (not shown).

While both the TNF -308 and -863 SNPs are thought to be associated with increased levels of TNFα production, the present analysis showed quite different effects on post transplant outcomes among MDS patients. The -308 AG genotype conferred increased risk of NRM **relative to the GG genotype**, whereas patients with the -863 AA genotype had **suggestively** decreased risk**s** of overall mortality and NRM relative to the CC genotype. Of course, our analysis, much like other reports, did not include the study of TNFα protein levels, either in blood or marrow [30], which would be desirable to more firmly establish a role of the gene product in transplant outcome.

As far as a potential impact of HLA-DR15 on treatment outcome is concerned, several hypotheses have been proposed. It has been suggested that the molecule preferentially presents autoantigens on hematopoietic precursor cells, thereby evoking an immune reaction of T cells [4,7]. It has also been suggested that DR15 is associated with an immune profile which increases the responsiveness to immunosuppressive therapy [2]. In view of the present results, which suggest that transplant outcome was affected primarily by TNF polymorphism (in linkage disequilibrium with HLA-DR alleles) rather than by DR alleles themselves, it might be more productive to focus future studies on cytokine profiles [24,26,27].

In summary, the present data show significant differences in the frequency of HLA-DR15 in patients with different hematologic diagnoses, who were referred for hematopoietic cell transplantation. The data in Caucasian patients showed an association of DR15 with TNF -308 and -863 polymorphisms and an effect of these polymorphisms on transplant outcome among patients with MDS, but not in a large comparison group of patients with CML. The data suggest that previously reported correlations of transplant outcome with DR15 may in fact be correlations with TNF polymorphisms. As ongoing clinical studies are testing the effect of TNF blockade on transplant outcome, it might be of interest to determine whether therapeutic results correlate with TNF genotype.

Acknowledgments

Supported in part by NIH grants HL036444, CA18029, AI33484 and CA15704.

We thank Bonnie Larson and Helen Crawford for help with manuscript preparations, and Gary Schoch and Anajane Smith for the update of HLA typing information and clinical outcome data.

References

1. Klein J, Gutknecht J, Fischer N. The major histocompatibility complex and human evolution (Review). Trends Genet 1990;6:7–11. [PubMed: 2183415]
2. Battiwalla M, Hahn T, Radovic M, et al. Human leukocyte antigen (HLA) DR15 is associated with reduced incidence of acute GVHD in HLA-matched allogeneic transplantation but does not impact chronic GVHD incidence. Blood 2006;107:1970–1973. [PubMed: 16282347]
3. Davidson JA, Tate DG, Poulton KV, et al. HLA-DR15, reduced relapse rate and improved survival after HLA identical sibling hemopoietic stem cell transplantation. Biol Blood Marrow Transplant 2007;13:493–494. [PubMed: 17382256]
4. Stern M, Passweg J, Tiercy JM, et al. Human leukocyte antigen DR15 is associated with reduced relapse rate and improved survival after human leukocyte antigen-identical sibling hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2006;12:1169–1175. [PubMed: 17085310]

5. Holler E, Kolb HJ, Möller A, et al. Increased serum levels of tumor necrosis factor a precede major complications of bone marrow transplantation. Blood 1990;75:1011– 1016. [PubMed: 2405918]
6. Wolff D, Roessler V, Steiner B, et al. Treatment of steroid-resistant acute graft-versus-host disease with daclizumab and etanercept. Bone Marrow Transplant 2005;35:1003–1010. [PubMed: 15806135]
7. Sauntharajah Y, Nakamura R, Nam JM, et al. HLA-DR15 (DR2) is overrepresented in myelodysplastic syndrome and aplastic anemia and predicts a response to immunosuppression in myelodysplastic syndrome. Blood 2002;100:1570–1574. [PubMed: 12176872]
8. Sugimori C, Yamazaki H, Feng X, et al. Roles of DRB1 *1501 and DRB1 *1502 in the pathogenesis of aplastic anemia. Exp Hematol 2007;35:13–20. [PubMed: 17198869]
9. Xiao M, Qiong L, Yan Z, et al. Experimental and clinical characteristics in myelodysplastic syndrome patients with or without HLA-DR I 5 allele. Hematol Oncol :9999. prepublished online 10 July 2009. 10.1002/hon.907
10. Maciejewski JP, Follmann D, Nakamura R, et al. Increased frequency of HLA-DR2 in patients with paroxysmal nocturnal hemoglobinuria and the PNH/aplastic anemia syndrome. Blood 2001;98:3513–3519. [PubMed: 11739151]
11. Bouma G, Crusius JB, Oudkerk PM, et al. Secretion of tumour necrosis factor alpha and lymphotoxin alpha in relation to polymorphisms in the TNF genes and HLA-DR alleles: relevance for inflammatory bowel disease. Scand J Immunol 1996;43:456–463. [PubMed: 8668926]
12. Kroeger KM, Carville KS, Abraham LJ. The -308 tumor necrosis factor-alpha promoter polymorphism effects transcription. Mol Immunol 1997;34:391–399. [PubMed: 9293772]
13. Sharma S, Ghosh B, Sharma SK. Association of TNF polymorphisms with sarcoidosis, its prognosis and tumour necrosis factor (TNF)-alpha levels in Asian Indians (Review). Clin Exp Immunol 2008;151:251–259. [PubMed: 18062795]
14. Hirankarn N, Avihingsanon Y, Wongpiyabovorn J. Genetic susceptibility to SLE is associated with TNF-alpha gene polymorphism -863, but not -308 and -238, in Thai population. International Journal of Immunogenetics 2007;34:425–430. [PubMed: 18001298]
15. Skoog T, Hamsten A, Eriksson P. Allele-specific chromatin remodeling of the tumor necrosis factor-alpha promoter. Biochemical and Biophysical Research Communications 2006;351:777–783. [PubMed: 17084384]
16. Udalova IA, Richardson A, Denys A, et al. Functional consequences of a polymorphism affecting NF-kappaB p50-p50 binding to the TNF promoter region. Mol Cell Biol 2000;20:9113–9119. [PubMed: 11094063]
17. Deeg HJ, Storer B, Slattery JT, et al. Conditioning with targeted busulfan and cyclophosphamide for hemopoietic stem cell transplantation from related and unrelated donors in patients with myelodysplastic syndrome. Blood 2002;100:1201–1207. [PubMed: 12149198]
18. Radich JP, Gooley T, Bensinger W, et al. HLA-matched related hematopoietic cell transplantation for chronic-phase CML using a targeted busulfan and cyclophosphamide preparative regimen. Blood 2003;102:31–35. [PubMed: 12595317]
19. Anderson JE, Appelbaum FR, Schoch G, et al. Relapse after allogeneic bone marrow transplantation for refractory anemia is increased by shielding lungs and liver during total body irradiation. Biol Blood Marrow Transplant 2001;7:163–170. [PubMed: 11302550]
20. Flowers, MED.; Vogelsang, GB. Clinical manifestations and natural history. In: Vogelsang, GB.; Pavletic, SZ., editors. Chronic Graft Versus Host Disease: Interdisciplinary Management. New York, NY: Cambridge University Press; 2009. p. 56-69.
21. Mielcarek M, Martin PJ, Leisenring W, et al. Graft-versus-host disease after nonmyeloablative versus conventional hematopoietic stem cell transplantation. Blood 2003;102:756–762. [PubMed: 12663454]
22. Mielcarek M, Storer BE, Flowers MED, Storb R, Sandmaier BM, Martin PJ. Outcomes among patients with recurrent high-risk hematologic malignancies after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant 2007;13:1160–1168. [PubMed: 17889352]
23. Petersdorf EW, Gooley TA, Anasetti C, et al. Optimizing outcome after unrelated marrow transplantation by comprehensive matching of HLA class I and II alleles in the donor and recipient. Blood 1998;92:3515–3520. [PubMed: 9808542]

24. Lin M-T, Storer B, Martin PJ, et al. Relation of an interleukin-10 promoter polymorphism to graft-versus-host disease and survival after hematopoietic-cell transplantation. N Engl J Med 2003;349:2201–2210. [PubMed: 14657427]
25. Fisher, LD.; van Belle, G. Biostatistics: A methodology for the health sciences. New York: John Wiley and Sons; 1993.
26. Middleton PG, Taylor PRA, Jackson G, Proctor SJ, Dickinson AM. Cytokine gene polymorphisms associating with severe acute graft-versus-host disease in HLA-identical sibling transplants. Blood 1998;92:3943–3948. [PubMed: 9808588]
27. Takahashi H, Furukawa T, Hashimoto S, et al. Contribution of TNF-alpha and IL-10 gene polymorphisms to graft-versus-host disease following allo-hematopoietic stem cell transplantation. Bone Marrow Transplant 2000;26:1317–1323. [PubMed: 11223972]
28. Bertinetto FE, Dall'Omo AM, Mazzola GA, et al. Role of non-HLA genetic polymorphisms in graft-versus-host disease after haematopoietic stem cell transplantation. International Journal of Immunogenetics 2006;33:375–384. [PubMed: 16984283]
29. Shaw BE, Maldonado H, Madrigal JA, et al. Polymorphisms in the TNFA gene promoter region show evidence of strong linkage disequilibrium with HLA and are associated with delayed neutrophil engraftment in unrelated donor hematopoietic stem cell transplantation. Tissue Antigens 2004;63:401–411. [PubMed: 15104672]
30. Gersuk GM, Beckham C, Loken MR, et al. A role for tumor necrosis factor-a, Fas and Fas-Ligand in marrow failure associated with myelodysplastic syndrome. Br J Haematol 1998;103:176–188. [PubMed: 9792306]
31. Bornhauser M, Storer B, Slattery JT, et al. Conditioning with fludarabine and targeted busulfan for transplantation of allogeneic hematopoietic stem cells. Blood 2003;102:820–826. [PubMed: 12676781]
32. Socié G, Clift RA, Blaise D, et al. Busulfan plus cyclophosphamide compared with total-body irradiation plus cyclophosphamide before marrow transplantation for myeloid leukemia: long-term follow-up of the 4 randomized studies. Blood 2001;98:3569–3574. [PubMed: 11739158]
33. Slattery JT, Clift RA, Buckner CD, et al. Marrow transplantation for chronic myeloid leukemia: the influence of plasma busulfan levels on the outcome of transplantation. Blood 1997;89:3055– 3060. [PubMed: 9108427]
34. Deeg HJ, Storer BE, Boeckh M, et al. Reduced incidence of acute and chronic graft-versus-host disease with the addition of thymoglobulin to a targeted busulfan/cyclophosphamide regimen. Biol Blood Marrow Transplant 2006;12:573–584. [PubMed: 16635793]
35. Sandmaier, BM.; Storb, R. Nonmyeloablative therapy and hematopoietic cell transplantation for hematologic disorders. In: Blume, KG.; Forman, SJ.; Appelbaum, FR., editors. Thomas' Hematopoietic Cell Transplantation. Oxford, UK: Blackwell Publishing Ltd; 2004. p. 1164-1176.

Table 1

Incidence of DRB15** allele by disease category in Caucasian patients.

Disease	Number of Patiens with ≥ 1 copy of a DRB15** Allele/Total Number	Percent DRB15 positive
Aplastic Anemia	84/325	25.8
ALL	237/993	23.9
AML	481/1907	25.2
CLL	28/97	28.9
CML	**393/1644**	**23.9**
Hodgkin disease	57/162	35.2
Multiple myeloma	98/343	28.6
Non-Hodgkin's lymphoma	158/630	25.1
MDS	**237/765**	**31.0**

ALL=acute lymphoblastic leukemia; AML=acute myeloid leukemia; CLL=chronic lymphocytic leukemia;

CML=chronic myeloid leukemia; MDS=myelodysplastic syndrome.

Table 2

Patient, Disease, and Transplant Characteristics of Caucasian Patients with MDS or CML

	MDS	CML
Number of patients	**765**	**1644**
Age (years) median (range)	48.3 (0.9 – 78.9)	37.1 (0.7 – 69.7)
Patient-donor gender (No. of pts. [%]):		
Female-Female	150 (20)	307 (19)
Female-Male	168 (22)	343 (21)
Male-Female	186 (24)	401 (24)
Male-Male	253 (33)	588 (36)
Unknown	8 (1)	5 (<1)
Conditioning Regimen (No. of pts [%])[1]:		
High intensity	706 (92)	1626 (99)
Reduced-intensity	59 (8)	18 (1)
Donor (No. of pts. [%]):		
HLA-identical sibling	346 (45)	731 (44)
HLA-matched unrelated donor	349 (46)	676 (41)
Non-sibling relative or mismatched sibling	62 (8)	235 (14)
Autologous*	8 (1)	2 (<1)
Stem Cell Source (No. of pts. [%]):		
Bone marrow	403 (53)	1522 (93)
Peripheral blood	356 (47)	117 (7)
Bone marrow+peripheral blood	4 (<1)	2 (<1)
Cord blood	2 (<1)	3 (<1)
Years transplanted (No. of pts. [%]):		
1969–1985	26 (3)	228 (14)
1986–1990	75 (10)	391 (24)
1991–1995	162 (21)	490 (30)
1996–2000	173 (23)	393 (24)
2001–2005	224 (29)	113 (7)
2006-present	105 (14)	29 (2)

[1] High intensity: CY/TBI; tBU/CY; FLU/BU [31–34];

Reduced intensity: FLU/TBI [35]

* Not further considered in the outcome analysis

Table 3

Allele and genotype frequencies of TNF -308 and -863 SNPs and association between HLA-DR15 and TNF SNPs in Caucasian patients with MDS or CML.

	MDS	CML
TNF -308 alleles*		
A	120/375 (32%)	181/593 (30.5%)
G	366/375 (97.6%)	577/593 (97.3%)
Genotypes		
AA	9/375 (2.4%)	16/593 (2.7%)
AG	111/375 (29.6%)	165/593 (27.8%)
GG	255/375 (68%)	412/593 (69.5%)
TNF -863 alleles*		
A	115/372 (30.7%)	165/593 (27.8%)
C	358/372 (95.5%)	582/593 (98.1%)
Genotypes		
AA	14/372 (3.8%)	11/593 (1.9%)
AC	101/372 (27.2%)	154/593 (26.0%)
CC	257/372 (69.1%)	428/593 (72.2%)

Proportion of DR15-positive patients

	MDS	CML	Chi-square p-value, MDS, CML
TNF -308			
AA	0/9	2/16 (12.5%)	p=.003, p=.54
AG	26/111 (23.4%)	37/165 (22.4%)	
GG	97/255 (38.0%)	99/412 (24.0%)	
TNF -863			
AA	5/14 (35.7%)	0/11	p=.11, p=.03
AC	25/101 (24.8%)	28/154 (18.2%)	
CC	93/257 (36.2%)	110/428 (25.7%)	

CML=chronic myeloid leukemia; MDS=myelodysplastic syndrome; TNF=tumor necrosis factor α; SNP = single nucleotide polymorphism.

*heterozygotes contribute to both allele categories

Table 4

Association of DR15 and TNF -308 and TNF -863 SNPs with transplant outcome among Caucasian patients with MDS.

Determinant	Overall Mortality	Relapse	NRM	Acute GVHD (II–IV)
TNF -308				
GG	1	1	1	1
AG	1.20 (0.90–1.62, p=.21)	0.76 (0.43–1.35, p=.35)	1.41 (1.01–1.98, p=.05)	1.46 (0.82–2.59, p=.21)
AA	1.57 (0.72–3.42, p=.26)	1.99 (0.59–6.70, p=.27)	1.21 (0.44–3.37, p=.71)	0.69 (0.15–3.14, p=.47)
TNF -863				
CC	1	1	1	1
AC	1.19 (0.88–1.61, p=.26)	1.74 (1.05–2.87, p=.03)	1.12 (0.78–1.60, p=.55)	0.79 (0.45–1.37, p=.97)
AA	0.39 (0.14–1.06, p=.06)	1.24 (0.44–3.52, p=.69)	0.15 (0.02–1.05, p=.06)	0.64 (0.19–2.09, p=.58)
DR15				
absent	1	1	1	1
present	1.03 (0.77–1.38, p=.84)	0.89 (0.53–1.49, p=.66)	1.00 (0.71–1.42, p=.99)	1.29 (0.76–2.21, p=.35)

Values listed are hazard (for overall mortality, relapse, NRM) or odds ratios (for GVHD) (95% CI, p-value).

CI=confidence interval; GVHD=graft-versus-host disease; NRM=non-relapse mortality; TNF=tumor necrosis factor α.

Table 5

Lack of association of DR15 and TNF -308 and TNF -863 SNPs with outcome among Caucasian patients with CML.

Determinant	Overall Mortality	Relapse	NRM	Acute GVHD (II–IV)
TNF -308				
GG	1	1	1	1
AG	0.97 (0.74–1.28, p=.85)	1.31 (0.89–1.91, p=.17)	0.99 (0.71–1.36, p=.93)	0.82 (0.51–1.33, p=.43)
AA	0.85 (0.40–1.83, p=.68)	0.85 (0.27–2.74, p=.79)	0.82 (0.33–2.01, p=.82)	0.86 (0.24–3.05, p=.81)
TNF -863				
CC	1	1	1	1
AC	1.06 (0.81–1.40, p=.67)	1.32 (0.90–1.94, p=.16)	0.97 (0.70–1.36, p=.87)	0.86 (0.53–1.41, p=.56)
AA	1.14 (0.53–2.45, p=.74)	0.89 (0.21–3.66, p=.87)	1.06 (0.43–2.65, p=.89)	0.52 (0.12–2.32, p=.39)
DR15				
absent	1	1	1	1
present	0.92 (0.68–1.23, p=.55)	0.98 (0.65–1.48, p=.91)	0.92 (0.65–1.31, p=.65)	1.14 (0.68–1.89, p=.63)

Values listed are hazard (for overall mortality, relapse, NRM) or odds ratios (for GVHD) (95% CI, p-value).

CI=confidence interval; GVHD=graft-versus-host disease; NRM=non-relapse mortality; TNF=tumor necrosis factor α.