Published in final edited form as:
Biol Blood Marrow Transplant. 2015 February ; 21(2): 259–265. doi:10.1016/j.bbmt.2014.09.016.

Toll like receptor polymorphisms in allogeneic hematopoietic cell transplantation

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Abstract

To assess the impact of the genetic variation in toll-like receptors (TLR) on outcome after allogeneic myeloablative conditioning hematopoietic cell transplantation (HCT) we have investigated 29 single nucleotide polymorphisms (SNP) across 10 TLRs in 816 patients and donors. Only donor genotype of TLR8 rs3764879, which is located on the X chromosome, was significantly associated with outcome at the Bonferroni corrected level P≤0.001. Male hemizygosity and female homozygosity for the minor allele were significantly associated with disease free survival (DFS) (hazard ratio (HR) 1.47 (95% confidence interval (CI) 1.16–1.85); *P*=0.001). Further analysis stratified by donor sex due to confounding by sex, was suggestive for associations with overall survival (male donor: HR 1.41 (95% CI 1.09–1.83), P=0.010); female

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AUTHOR CONTRIBUTIONS

BK analyzed and interpreted data and drafted and revised the manuscript and. CE genotyped samples, analyzed and interpreted data and revised the manuscript. SS, MH, TW and SJL provided patient and donor material, performed statistical analyses, interpreted data and revised the manuscript. KM planned and designed the study, interpreted data and revised the manuscript.

FINANCIAL DISCLOSURES

The authors report no potential conflicts of interest.

donor: (HR 2.78 (95% CI 1.43–5.41), P=0.003), DFS (male donor: HR 1.45 (95% CI 1.12–1.87), P=0.005; female donor: HR 2.34 (95% CI 1.18–4.65), P=0.015) and treatment related mortality (male donor: HR 1.49 (95% CI 1.09–2.04), P=0.012; female donor: HR 3.12 (95% CI 1.44–6.74), P=0.004).

In conclusion our findings suggest that the minor allele of TLR8 rs3764879 of the donor is associated with outcome after myeloablative conditioned allogeneic HCT.

Keywords

Toll-like receptors; Toll-like receptor 8; Allogeneic hematopoietic cell transplantation; single nucleotide polymorphism

INTRODUCTION

After allogeneic hematopoietic cell transplantation (HCT), both innate and adaptive immune mechanisms are involved in the immune reactions that result in graft versus host disease (GVHD) and the curative graft versus tumor effect (GVT) (1;2). Although the importance of human leukocyte antigen (HLA) matching on outcome after allogeneic HCT is well recognized (3), the significance of genetic variation in immune response genes outside the major histocompatibility complex system has become increasingly evident (4–8).

The toll-like receptors (TLR) are germline encoded pattern recognition receptors which recognize specific microbial pathogen associated molecular patterns (PAMP) and endogenous alarmins (9–12). TLRs are mainly expressed on antigen presenting cells, and play a central role in immune surveillance and in the initiation of the inflammatory response (9;13). Ten different TLR (TLR1–10) have been identified in humans. After allogeneic HCT TLRs may not only be involved in the immune response to infections, but also in acute GVHD, where tissue damage induced by high dose conditioning may lead to a milieu with ample supply of TLR ligands (14;15). Furthermore, TLRs are thought to be involved in GVT, as administration of TLR agonists has been shown to induce antitumor immunity and tumor regression in in-vivo tumor models and clinical trials (16–19).

In the setting of allogeneic HCT the TLR genes have been studied in terms of the impact of their variation on outcome and susceptibility to infection. Although single nucleotide polymorphisms (SNPs) in several TLR genes have been investigated, the most extensively studied are two functional SNPs in *TLR4*, Thr399Ile and Asp299Gly. These SNPs have been associated with acute GVHD, invasive aspergillosis and hemorrhagic cystitis (20–23).

The objective of the current study is to investigate associations between 29 SNPs across 10 TLR genes (Supplemental table S1) and outcome in a cohort of 816 patients and donors undergoing myeloablative conditioning matched unrelated donor allogeneic HCT for advanced hematological malignancies.

PATIENTS AND METHODS

The study cohort consisted of 816 donor/recipient pairs with acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML) or myelodysplastic syndrome (MDS) undergoing myeloablative hematopoietic cell transplantation with bone marrow or granulocyte colony stimulating factor (G-CSF) mobilized peripheral blood stem cells (PBSC) from 10/10 allele (HLA-A, B, C, DRB1 and DQB1) matched unrelated donors. Early stage disease was defined as AML and ALL in first complete remission, CML in first chronic phase and MDS with refractory anemia with or without ringed sideroblasts. Intermediate stage disease was defined as AML and ALL in second or subsequent complete remission or in first relapse, CML in accelerated phase or greater than first chronic phase. Advanced stage disease was defined as AML or ALL in second or subsequent relapse or primary induction failure, and CML in blast phase, MDS subtype refractory anemia with excess blasts or in transformation, or MDS not otherwise specified. Transplantation demographics are shown in table 1. The median follow-up was 11.1 (range 0.8–22) years.

Transplantations were facilitated through the National Marrow Donor Program (NMDP) and performed between 1988 and 2004. Data collection and analysis was performed under the auspices of the Center for International Blood and Marrow Transplant Research (CIBMTR). Pre-transplant donor and patient research samples were provided by the NMDP/CIBMTR Research Repository.

Observational studies conducted by the CIBMTR are performed in compliance with the privacy rule (HIPAA) as a Public Health Authority and in compliance with all applicable federal regulations pertaining to the protection of human research participants as determined by continuous review of the Institutional Review Boards (IRB) of the NMDP. A standardized modeling process was used, as previously described (24), to adjust for any bias introduced by the exclusion of non-consenting survivors in the NMDP cohort.

GENOTYPING

SNPs were genotyped using a previously developed in-house assay (25) based on representative SNPs for TLR1-10. SNPs where selected randomly among primarily amino acid changing SNPs, but also potentially regulatory SNPs (e.g. promotor, 3′UTR) and SNPs with previously reported functional effects from the dbSNP database (26) at the time of assay development. Twenty-nine bialleic SNPs observed in persons of European ancestry were included in the analyses (Supplemental table S1). Briefly, allele-specific primers were labelled in an allele-specific primer extension (ASPE) reaction, using polymerase chain reaction-amplified SNP-sites as their target sequences. The labelled ASPE-primers were subsequently hybridized to MicroPlex-xTAG beadsets for detection and counting on the Luminex platform (Luminex Corporation, Austin, TX, USA). All genotypings were carried out randomized and blinded to the technician performing the genotyping.

STATISTICS

Probability of leukaemia-free survival and overall survival were calculated using the Kaplan-Meier estimator. Cumulative incidences were estimated for other endpoints to accommodate competing risks. Comparison of survival curves was done using the log-rank test.

Multivariate analyses were performed using Cox proportional hazards models, which model the hazard functions for overall and leukaemia-free survivals while model the cause-specific hazards for competing risks such as TRM, relapse, aGVHD and cGVHD. All clinical variables were tested for proportional hazards assumptions using time-dependent covariate approach. Factors violating the proportional hazards assumption were adjusted through stratification. Stepwise model building procedures were performed to select the adjusted variables at 0.05 significance level for both entry and retaining in the models. Each SNP was tested for association with the clinical outcomes by forcing it into the model with the selected adjusted variables. The multivariate models are shown in Supplemental Table S2. Only 23 SNPs with minor allele frequencies ≥ 5% were included in the statistical analyses. Based on the Bonferroni criterion (23 SNPs for both patients and donors; 0.05/46) P≤0.001 was used for statistical significance in order to adjust for multiple testing. All the analyses were performed using SAS version 9.3 (The SAS Institute, Cary, NC).

RESULTS

Genotyping

The Hardy-Weinberg equilibrium (HWE) and genotype distributions were analyzed separately for patients and donors (Supplemental Table S1). All SNPs adhered to the HWE expectations at the P=0.001 level.

Six SNPs (rs3923647 in *TLR1*, rs5743704 in *TLR2*, rs5743813 in *TLR6*, rs5743781 in *TLR7* and rs4129008 and rs466657 in *TLR10*) were excluded from further analyses due to minor allele frequencies < 5%. Of the remaining 23 SNPs only *TLR8* rs3764879 donor type showed a significant association between genotype and outcome at the P≤0.001 level in the multivariate models (Table 2) (Supplemental table S3).

Association between donor TLR8 rs3764879 genotype and outcome

Eight-hundred-two donors were successfully genotyped for rs3764879 which is located on chromosome X. The minor allele frequency was 25% both among male and female donors. As *TLR8* is located on the X chromosome male donors can only be hemizygous for a *TLR8* gene, and therefore only present 2 genotypes, namely presence or absence of the major allele. Of 508 male donors 383 (75%) were hemizygous for the rs3764879 major allele while 125 (25%) were hemizygous for the minor allele. Of 294 female donors 159 (54%), 122 (42%) and 13 (4%) were homozygous for the major allele, heterozygous and homozygous for the minor allele, respectively.

In the multivariate analyses male donor hemizygosity and female donor homozygosity for the rs3764879 minor allele was an independent risk factor significantly associated with DFS

(hazard ratio (HR) 1.47 (95% confidence interval (CI) 1.16–1.85); *P*=0.001), ++++which translated into trends towards lower OS (HR 1.44 (95% CI 1.14–1.82), *P*=0.002) and increased TRM (HR 1.59 (95% CI 1.19–2.12), *P*=0.002) (Supplemental table S3). There were no significant associations between rs3764879 genotype and relapse or GVHD (Supplemental table S3).

Due to the location of *TLR8* on the X chromosome the multivariate analyses of the whole cohort were confounded by the minor allele disparity conferred by gender. When male and female donor rs3764879 genotypes were analyzed separately results were similar to the whole cohort (Table 2). Although not significant at the Bonferroni corrected P≤0.001 level, both male donor hemizygosity and female donor homozygosity for the minor allele were associated with OS (male donor: HR 1.41 (95% CI 1.09–1.83), P=0.010); female donor: (HR 2.78 (95% CI 1.43–5.41), P=0.003), DFS (male donor: HR 1.45 (95% CI 1.12–1.87), P=0.005; female donor: HR 2.34 (95% CI 1.18–4.65), P=0.015) and TRM (male donor: HR 1.49 (95% CI 1.09–2.04), P=0.012; female donor: HR 3.12 (95% CI 1.44–6.74), P=0.004), (Table 2 and Figure 1). Causes of death are shown in detail in table 3. Thirty-four percent of patients transplanted with a male donor hemizygous for the minor allele died of causes unrelated to relapse or GVHD, namely interstitial pneumonia, infection or organ failure, while only 27% of patients transplanted with a donor hemizygous for the major allele died of these causes (p=0.64). In the group of patients transplanted with a female donor homozygous for the minor allele, heterozygous or homozygous for the major allele, 39%, 33% and 28% (p=0.68), respectively, died of interstitial pneumonia, infection or organ failure.

No associations between genotype and relapse or GVHD were observed. The complete multivariate analyses including all covariates are shown in Supplemental table S4). Of note the data from female donors should be interpreted cautiously as the number of female donors homozygous for the minor allele was low (Table 2).

TLR1, 4, 6 and 9 SNPs previously associated with outcome in allogeneic HCT

Heterozygosity for the *TLR4* SNP rs4986790 has previously been associated with hemorrhagic cystitis and invasive aspergillosis (23;27;28). Due to low numbers of homozygous minor allele carriers both in the patient (n=1) and donor (n=3) cohorts these were included in the group of heterozygotes for analysis. Patient minor allele carriage of the TLR4 rs4986790 SNP tended to be associated with increased risk of TRM (HR 1.15 (95% CI 1.12–2.04); P=0.007) translating into lower DFS (HR 1.36 (95% CI 1.05–1.77); P=0.0214) and OS (HR 1.42 (95% CI 1.09–1.86); P=0.010) (Figure 2 and Table 4). There were no associations between the rs4986790 genotype and relapse or GVHD.

For *TLR1* SNP rs5743611 which has previously been associated with invasive aspergillosis, our data were suggestive of an association between patient heterozygosity and grade III–IV acute GVHD was observed (HR 1.55 (95% CI 1.05–2.29); P=0.026), with no impact on survival (Table 4).

In contrast to previous reports no significant associations between the *TLR6* SNP rs5743810 (29) or the *TLR9* SNP rs187084 (30) and outcome were observed (Table 4).

Of note, none of the previously investigated SNPs described in this section were significantly associated with outcome in the current study as the level for statistical significance was set at the conservative Bonferroni corrected P≤0.001.

DISCUSSION

The current study is the largest investigation of associations between TLR SNPs and outcome after allogeneic HCT at present. Our cohort of 816 patient and donor pairs was genotyped for 29 SNPs across the 10 known human TLR genes. To achieve sufficient statistical power SNPs with minor allele frequencies <5% were excluded, leaving 23 SNPs to be tested in the multivariate models for clinical outcome variables. Although conservative, the Bonferroni method was used to adjust for multiple comparisons to lower the risk of false positive associations. As *TLR8* is located on the X chromosome and males only carry one of these they can only present phenotypes corresponding to either major or minor allele homozygosity, while heterozygosity also exists in the female population. Hemi- or homozygosity for the rs3764879 minor allele in male and female donors was significantly associated with lower DFS with trends towards lower OS and TRM. No association with GVHD or relapse was observed. Although the rs3764879 genotype is inherently confounded by gender no interactions between gender and genotype were observed. When male and female donors were analyzed separately, patterns similar to that seen in the whole cohort were observed, but levels of significance were lower in the separate male and female donor subsets, in line with the reduced power of the stratified analyses. Especially in the female subset, data should be interpreted with caution due to low numbers of minor allele homozygous donors. However the data do suggest a clinical impact of to the absence of phenotypical expression of the rs3764879 major allele.

Althougn non significant, we observed increased frequencies of death due interstitial pneumonia, infection or organ failure in patients transplanted with minor allele hemi-or homozygous donors, which is iin agreement

No associations were observed between rs3764879 and relapse or GVHD, indicating that the poorer outcome was conferred by other causes. Although not significant, we did observe higher frequencies of death related to interstitial pneumonia, infection or organ failure. The finding is in line with *TLR8* being part of the anti-viral immune response where it recognizes non-self nucleic acids and subsequently stimulates the release of pro-inflammatory cytokines (31). The rs3764879 SNP is located in the promoter region of *TLR8* and the minor allele has been associated with lower cytokine production (32). *TLR8* is expressed mainly by haematopoietically derived cells (monocytes and myeloid derived dendritic cells) (33;34) and in keeping with this, the significant associations between outcomes and genotypes were not found for recipient genotypes of the TLR8 SNP.

rs3764879 is in complete linkage disequilibrium with the codon 1 TLR8 SNP rs3764880 SNP, which introduces a frame-shift mutation leading to a truncated final protein (35;36). Only few studies have adressed the association between *TLR8* genotype and disease, and in agreement with our data they have shown associations between the minor allele of the promoter SNP (rs3764879) or the codon 1 SNP (rs3764880) and progression of HIV

infection, susceptibility to mycobacterium turberculosis, hepatitis C infection and asthma (36–40).

Several polymorphisms across most TLRs have been studied in a variety of disease settings (41). The most extensively investigated are the functional non-synonymous TLR4 SNPs D299G (rs4986790) and T399I (rs4986791), which are in linkage disequilibrium (21). TLR4 recognizes microbial cell wall components from gram negative bacteria and fungal ligands such as candida mannan, glucuronoxylomannan and Aspergillus fumigatus antigens (9;42;43). In the setting of allogeneic HCT both D299G and T399I have been associated with invasive aspergillosis. Koldenhoff et al. observed that patient or donor carriage of the minor allele of either SNP was associated with invasive aspergillosis (27), while Bochud et al. in a large discovery/validation cohort observed a significant association between donor carriage of the minor allele and invasive aspergillosis and TRM (21). In contrast Kesh et al. did not observe an association between TLR4 D299G and T399I genotype and invasive aspergillosis. The same study also included SNPs in TLR1 and TLR6 which heterodimerize with TLR2 to mediate responses to lipopeptides from several pathogens. Presence of either the minor allele of TLR1(rs5743611) or TLR1 (rs4833095) and TLR6 (rs5743810) in the recipient were associated with increased risk of invasive aspergillosis (29).

Presence of the minor allele of TLR4 D299G in patient and donor has also been associated with hemorrhagic cystitis in a pediatric cohort transplanted after myeloablative conditioning (23).

Elmaagcli et al. investigated 2 SNPs in TLR9, which is located intracellularly and senses single stranded DNA from microbial pathogens containing CpG motifs, in a cohort of AML patients treated with high dose allogeneic HCT (30). They observed that patient homozygosity for rs187084 conferred a lower 5 year probability of relapse and increased OS.

None of the previously published associations between TLR genotypes and allogeneic HCT were replicated at the Bonferroni adjusted significance level of $P \leq 0.001$. However in the current study, patient carriage of the TLR4 SNP rs4986790 minor allele was associated with TRM and OS at the $P<0.05$ level, lending support to the probable importance of TLR4 genotype on infection related morbidity and mortality. In contrast to previous reports no association between D299G and acute GVHD were observed (20;28), and no relevant significant associations between previously studied SNPs in TLR1, 6 and 9 were observed (29;30).

The inconsistent results often observed across genetic association studies are due to several factors that make direct interstudy comparisons difficult. The generally small cohorts in transplant studies increase the risk of type I errors as the effect of single genetic variants usually is modest. Furthermore heterogeneity between patient populations, with differences in treatment regimens, diagnoses, racial admixture, other yet unknown risk factors and the possibility of the selected polymorphisms being in linkage disequilibrium with unknown functional polymorphisms all contribute to an unclear picture.

In conclusion the present study is currently the largest and most comprehensive investigation of associations between TLR genotype and outcome after allogeneic HCT. Although none of the previously published associations between TLR SNPs and outcome were validated at the Bonferroni corrected significance levels, similar trends were observed. However a novel association between a *TLR8* promoter polymorphism and survival was observed, and evidence supporting the importance of *TLR4* D299G was presented. To confirm the significance of these findings further experimental and clinical studies are needed to explain their molecular background and assess their impact on outcome in a prospective manner, respectively.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The CIBMTR is supported by Public Health Service Grant/Cooperative Agreement U24-CA076518 from the National Cancer Institute (NCI), the National Heart, Lung and Blood Institute (NHLBI) and the National Institute of Allergy and Infectious Diseases (NIAID); a Grant/Cooperative Agreement 5U10HL069294 from NHLBI and NCI; a contract HHSH250201200016C with Health Resources and Services Administration (HRSA/DHHS); two Grants N00014-13-1-0039 and N00014-14-1-0028 from the Office of Naval Research; and grants from Actinium Pharmaceuticals; Allos Therapeutics, Inc.; Amgen, Inc.; Anonymous donation to the Medical College of Wisconsin; Ariad; Be the Match Foundation; Blue Cross and Blue Shield Association; Celgene Corporation; Chimerix, Inc.; Fred Hutchinson Cancer Research Center; Fresenius-Biotech North America, Inc.; Gamida Cell Teva Joint Venture Ltd.; Genentech, Inc.; Gentium SpA; Genzyme Corporation; GlaxoSmithKline; Health Research, Inc. Roswell Park Cancer Institute; HistoGenetics, Inc.; Incyte Corporation; Jeff Gordon Children's Foundation; Kiadis Pharma; The Leukemia & Lymphoma Society; Medac GmbH; The Medical College of Wisconsin; Merck & Co, Inc.; Millennium: The Takeda Oncology Co.; Milliman USA, Inc.; Miltenyi Biotec, Inc.; National Marrow Donor Program; Onyx Pharmaceuticals; Optum Healthcare Solutions, Inc.; Osiris Therapeutics, Inc.; Otsuka America Pharmaceutical, Inc.; Perkin Elmer, Inc.; Remedy Informatics; Sanofi US; Seattle Genetics; Sigma-Tau Pharmaceuticals; Soligenix, Inc.; St. Baldrick's Foundation; StemCyte, A Global Cord Blood Therapeutics Co.; Stemsoft Software, Inc.; Swedish Orphan Biovitrum; Tarix Pharmaceuticals; TerumoBCT; Teva Neuroscience, Inc.; THERAKOS, Inc.; University of Minnesota; University of Utah; and Wellpoint, Inc. The views expressed in this article do not reflect the official policy or position of the National Institute of Health, the Department of the Navy, the Department of Defense, Health Resources and Services Administration (HRSA) or any other agency of the U.S. Government.

The study was also supported by grants from the Danish Biotechnology Program, the Danish Child Cancer Foundation and the Dagmar Marshall Foundation.

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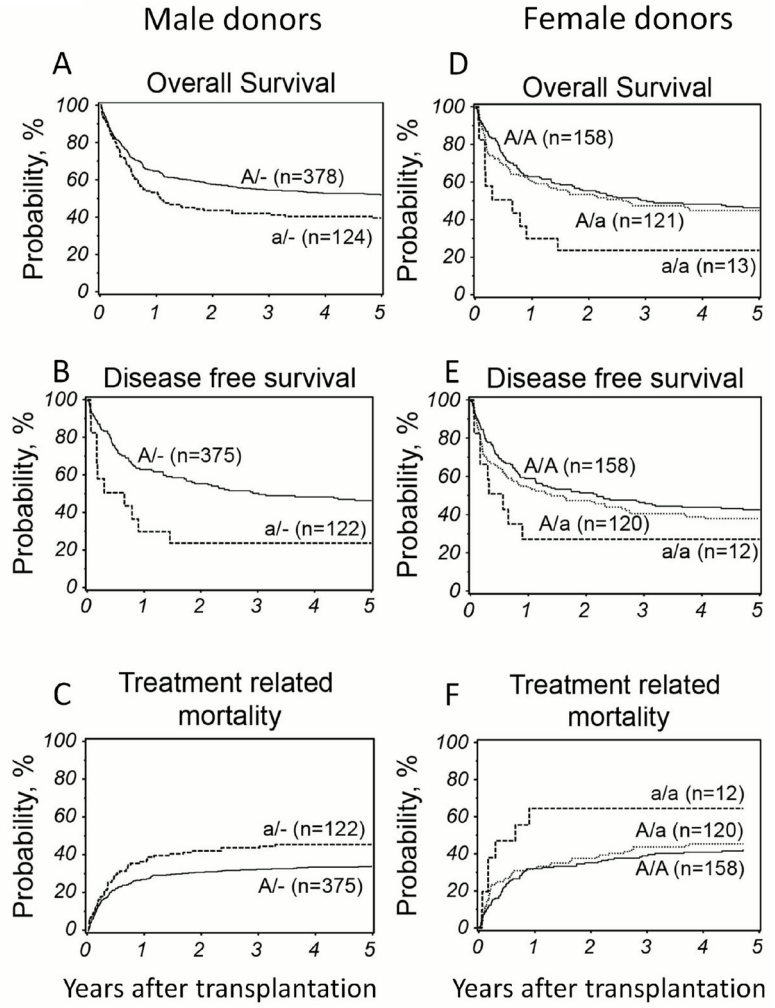

Figure 1.

Adjusted probability of overall survival, disease free survival and treatment related mortality according donor *TLR8* rs3764879 genotype analyzed separately for male (panel A, B and C) and female donors (panel D, E and F). A/−, Major allele hemizygous (solid line); a/−, minor allele hemizygous (dashed line); A/A, major allele homozygous (solid line); A/a, heterozygous (dotted line); a/a, minor allele homozygous (dashed line).

Table 1

Transplantation demographics

Variable	
Number of patients, no.	816
Number of centers, no.	89
Male patient gender	470 (58)
Patient age, median (range), years	37 (<1–65)
0–9 years	61 (7)
10–19 years	75 (9)
20–29 years	130 (16)
30–39 years	206 (25)
40–49 years	236 (29)
≥50 years	108 (13)
Donor age, median (range), years	36 (18–59)
Sex of donor/patient	
Female/male	144 (18)
Other combinations	672 (82)
Karnofsky prior to transplant ≥ 90 (only evaluable for 789 patients)	627 (79)
CMV serostatus of donor/patient	
Negative/negative	328 (40)
Other combinations	461 (56)
unknown	27 (3)
Donor previous pregnancies (female only, n=346)	
0	118 (34)
1	50 (14)
≥2	116 (34)
unknown	62 (18)
Disease at transplant	
Acute myeloid leukemia	126 (15)
Acute lymphoblastic leukemia	138 (17)
Chronic myeloid leukemia	390 (48)
Myelodysplastic syndrome	162 (20)
Disease stage at transplant	
Early	651 (80)
Intermediary	72 (9)
Advanced	85 (10)
Other	8 (1)
Graft Type	
Bone marrow	711 (87)
Peripheral blood stem cells	105 (13)
Graft-versus-host disease prophylaxis	
Tacrolimus ± other	193 (23)

Variable	
Cyclosporine + methotrexate	576 (71)
Other combinations	47 (6)
Use of anti-thymocyte globulin	75 (9)
Transplantation year	
1988–1995	202 (25)
1995–1999	324 (40)
2000–2008	290 (36)

Data presented as no. (%) unless otherwise specified

Table 2

Association between *TLR8* rs3764879 donor genotype and outcome

	Male only				Female only			
Variable	**Genotype**	**n**	**HR (95% CI)**	**p**	**Genotype**	**n**	**HR (95% CI)**	**p**
OS	A	378	1.00		AA	158	1.00	
	a	124	1.41 (1.09–1.83)	0.01	Aa	121	1.13 (0.83–1.53)	0.455
					aa	13	2.78 (1.43–5.41)	0.003
DFS	A	375	1.00		AA	158	1.00	
	a	122	1.45 (1.12–1.87)	0.005	Aa	120	1.23 (0.91–1.68)	0.188
					aa	12	2.34 (1.18–4.65)	0.015
Relapse	A	375	1.00		AA	158	1.000	
	a	122	1.35 (0.86–2.12)	0.193	Aa	120	1.03 (0.55–1.91)	0.935
					aa	12	0.87 (0.20–3.83)	0.849
TRM	A	145	1.00		AA	158	1.00	
	a	58	1.49 (1.09–2.04)	0.012	Aa	120	1.21 (0.86–1.71)	0.280
					aa	12	3.12 (1.44–6.74)	0.004
aGVHD								
Grade II–IV	A	374	1.00		AA	158	1.00	
	a	123	0.89 (0.67–1.19)	0.427	Aa	118	1.16 (0.83–1.61)	0.379
					aa	13	1.27 (0.61–2.65)	0.529
Grade III–IV	A	367	1.00		AA	148	1.00	
	a	121	0.81 (0.51–1.27)	0.357	Aa	114	1.76 (1.04–2.98)	0.036
					aa	13	3.96 (1.57–9.96)	0.004
cGVHD	A	371	1.00		AA	156	1.00	
	a	123	1.05 (0.79–1.41)	0.718	Aa	120	0.99 (0.72–1.36)	0.965
					aa	13	1.22 (0.43–3.50)	0.709

OS, overall survival; DFS, disease free survival; TRM, treatment related mortality; aGVHD, acute graft versus host disease; cGVHD, chronic graft versus host disease; A, Major allele hemizygous; a, minor allele hemizygous; AA, major allele homozygous; Aa, heterozygous; aa, minor allele homozygous; HR, hazard ratio; CI, confidence interval; NA, not available.

Table 3

Causes of death according to *TLR8* rs3764879 donor genotype

	Male donors			Female donors		
Cause of death	**Genotype**	**N (%)**	**p**	**Genotype**	**N (%)**	**p**
Primary disease	A/-	40 (10)	0.76	A/A	17 (11)	0.37
	a/-	17 (14)		A/a	8 (7)	
				a/a	1 (8)	
New malignancy	A/-	5 (1)	0.53	A/A	1 (1)	0.92
	a/-	1 (1)		A/a	1 (1)	
				a/a	0 (0)	
Graft versus host disease	A/-	29 (8)	0.91	A/A	17 (11)	0.72
	a/-	11 (9)		A/a	10 (8)	
				a/a	2 (15)	
Interstitial pneumonia	A/-	21 (6)	0.41	A/A	16 (16)	0.74
	a/-	11 (9)		A/a	16 (13)	
				a/a	2 (15)	
Infection	A/-	45 (12)	0.77	A/A	20 (13)	0.44
	a/-	19 (15)		A/a	10 (8)	
				a/a	2 (15)	
Organ failure	A/-	37 (10)	0.70	A/A	9 (6)	0.19
	a/-	13 (10)		A/a	14 (12)	
				a/a	1 (8)	
Other	A/-	29 (8)	0.50	A/A	16 (10)	0.58
	a/-	9 (7)		A/a	16 (13)	
				a/a	3 (23)	

N, number of deaths; percentage, percent of all deaths; A, Major allele hemizygous; a, minor allele hemizygous; AA, major allele homozygous; Aa, heterozygous; aa, minor allele homozygous.

Table 4

Association between *TLR1, 4, 6* and *9* genotype and outcome after allogeneic HCT

		Patient *TLR1* (rs5743611)			Patient *TLR4* (rs4986790)		
Variable	**Genotype**	**n**	**HR (95% CI)**	**p**	**n**	**HR (95% CI)**	**p**
OS	AA	666	1.000		696	1.000	
	Aa	114	1.017 (0.779–1.328)	0.902	96	1.424 (1.089–1.863)	0.010
	aa	12	1.432 (0.729–2.813	0.297	0	NA	NA
DFS	AA	662	1.000		689	1.000	
	Aa	112	0.988 (0.758–1.286)	0.927	96	1.363 (1.047–1.774)	0.0214
	aa	11	1.195 (0.585–2.443)	0.625	0	NA	NA
Relapse	AA	662	1.000		689	1.000	
	Aa	112	0.945 (0.584–1.530)	0.819	96	1.034 (0.592–1.805)	0.907
	aa	11	2.054 (0.740–5.704)	0.167	0	NA	NA
TRM	AA	662	1.000		689	1.000	
	Aa	112	0.950 (0.692–1.304)	0.751	96	1.515 (1.123.2.042)	0.007
	aa	11	0.860 (0.316–2.335)	0.767	0	NA	NA
aGVHD							
Grade II–IV	AA	659	1.000		689	1.000	
	Aa	114	1.231 (0.947–0.294)	0.121	96	1.120 (0.839–1.495)	0.442
	aa	12	0.714 (0.294–1.735)	0.457	0	NA	NA
Grade III–IV	AA	638	1.000		668	1.000	
	Aa	112	1.553 (1.054–2.290)	0.026	94	1.071 (0.686–1.672)	0.763
	aa	12	0.267 (0.037–1.935)	0.191	0	NA	NA
cGVHD	AA	650	1.000		682	1.000	
	Aa	112	1.064 (0.807–1.403)	0.660	92	0.789 (0.561–1.109)	0.172
	aa	12	1.026 (0.451–2.336)	0.951	0	NA	NA

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Table 5

	Donor *TLR4* (rs4986790)				Patient *TLR6* (rs5743810)			Patient *TLR9* (rs187084)		
Variable	**n**	**HR (95% CI)**	**p**		**n**	**HR (95% CI)**	**p**	**n**	**HR (95% CI)**	**p**
OS	703	1.000			247	1.000		278	1.000	
	102	1.204 (0.928–1.562)	0.163		409	1.073 (0.871–1.321)	0.510	386	0.877 (0.716–1.073)	0.202
		0	NA	NA	136	1.065 (0.810–1.401	0.652	128	0.920 (0.700–1.208)	0.549
DFS	697	1.000			245	1.000		274	1.000	
	101	1.205 (0.935–1.554)	0.150		404	1.039 (0.846–1.275)	0.716	385	0.865 (0.709–1.056)	0.153
		0	NA	NA	136	1.052 (0.804–1.376)	0.711	126	0.927 (0.709–1.211)	0.580
Relapse	697	1.000			245	1.000		274	1.000	
	101	0.734 (0.428–1.260)	0.262		404	0.705 (0.484–1.028)	0.069	385	0.761 (0.531–1.093)	0.139
		0	NA	NA	136	0.978 (0.614–1.557)	0.925	126	0.622 (0.360–1.073)	0.088
TRM	697	1.000			245	1.000		274	1.000	
	101	1.388 (1.040–1.854)	0.026		404	1.188 (0.929–1.518)	0.169	385	0.961 (0.758–1.220)	0.746
		0	NA	NA	136	1.032 (0.744–1.431)	0.851	126	1.061 (0.779–1.444)	0.709
aGVHD	698	1.000			244	1.000		275	1.000	
Grade II–IV	99	1.018 (0.764–1.358)	0.901		407	0.988 (0.797–1.224)	0.912	283	0.982 (0.792–1.217)	0.865
		0	NA	NA	134	0.812(0.603–1.092)	0.168	127	(0.889–1.555)	0.258
	676	1.000			239	1.000		270	1.000	
Grade III–IV	98	1.286 (0.849–1.946)	0.235		395	0.862 (0.620–1.198)		369	0.809 (0.579–1.128)	0.212
		0	NA	NA	128	0.809 (0.512–1.278)		123	1.071 (0.712–1.613)	0.741
	687	1.000			244	1.000		271	1.000	
cGVHD	99	1.089 (0.806–1.470)	0.580		395	0.973 (0.785–1.207)	0.805	378	1.128 (0.909–1.400)	0.275
		0	NA	NA	135	0.791 (0.586–1.067)	0.125	125	1.193 (0.898–1.585)	0.223

OS, overall survival; DFS, disease free survival; TRM, treatment related mortality; aGVHD, acute graft versus host disease; cGVHD, chronic graft versus host disease; AA, major allele homozygous; Aa, heterozygous; aa, minor allele homozygous; HR, hazard ratio; CI, confidence interval; Rs number, reference single nucleotide polymorphism number; NA, not available.