Lower overall survival in male patients with advanced disease undergoing allogeneic hematopoietic stem cell transplantation is associated with *CYP1B1* Leu432Val polymorphism

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Abstract

Human cytochrome P450 1B1 (CYP1B1) is an extrahepatic key enzyme involved in estrogen metabolism, steroid synthesis, and pro-carcinogen activation. In a single-center retrospective study, 382 patients who underwent allogeneic hematopoietic stem cell transplantation and their donors were genotyped for *CYP1B1* C432G polymorphism by reverse transcription polymerase chain reaction. One hundred and sixty-nine patients (44%) were homozygous wild-type (wt) gene CC, 157 (41%) heterozygous CG and 56 (15%) homozygous gene mutated GG. Of interest, mutated *CYP1B1* was more common in male (62%) than in female patients (48%) *P*=0.006, unlike in donors. Five-year estimate for overall survival (OS) was 58±4% (CC) *versus* 48±3% (CG and GG), *P*=0.048. Surprisingly, this difference was only evident in males (*P*=0.024): OS 58±6% *versus* 42±4%, whereas it was virtually absent in females. Importantly, this difference was only evident in male patients with advanced disease (AD) (n=118, *P*=0.002): OS 44±8% (CC) *versus* 32±6% (CG) *versus* 6±6% (GG), whereas it was virtually absent in male patients with early disease. One-year non-relapse mortality in male patients with AD was 8±4% (CC) *versus* 21±5% (CG) *versus* 50±12% (GG), *P*=0.002. Three-year relapse rate in male patients with AD was 31±7% (wt) *versus* 42±6% (mut), *P*=0.04. Multivariate analysis for OS in male patients with AD revealed *CYP1B1* polymorphism as the only prognostic factor: RR 1.78, *P*=0.001. In conclusion, these results suggest that male patients with AD and mutant *CYP1B1* polymorphism have lower OS after allogeneic hematopoetic stem cell transplantation due to a higher non-relapse mortality and a higher relapse rate.

Introduction

Hematopoietic stem cell transplantation (HSCT) offers the only cure for many hematological neoplasms; however, the mortality rate remains high. Complications after allogeneic HSCT include relapse, graft-*versus*-host disease (GVHD), graft rejection, organ damage, and infection. The outcome of HSCT is influenced both by clinical and genetic factors. Human leukocyte antigen (HLA) compatibility is a well-known limiting factor for the success of allogeneic HSCT.[1] In addition, genes other than those of the HLA system (both minor HLA and non-HLA antigens including KIR), in particular those that are also polymorphic, have been shown as potential factors affecting the success of HSCT.[2] The polymorphic expression of drug-metabolizing enzymes is one of the major factors which causes interindividual variability in drug metabolism, and thereby in pharmacologic and toxicologic responses. Single nucleotide polymorphisms (SNP) within non-HLA genes that are involved with an individual's capability to mount an immune response to infectious pathogens, residual leukemia, alloantigens or genes involved in drug metabolism, have been studied for their association with acute GVHD,[3] host organ function, cancer cells, and immune cells, and thus HSCT outcome.[4] These include chemokines and cytokines,[5]

and other predictive biomarkers like *CYP2C19*,[6] *TLR9, NOD2* and *IL23R*,[7] *TGFB1*,[8] and *NOD2/CARD15*.[9,10]

Human cytochrome P450 1B1 (CYP1B1) is an extrahepatic key enzyme overexpressed in many tumors. It is involved in the production of reactive metabolites, and in the bioactivation of environmental carcinogens.[11-14] CYP1B1 is also involved in sex hormone and estrogen metabolism, and steroid and lipid synthesis. It specifically catalyzes the 4-hydroxylation of 17β-estradiol into 4-hydroxyestradiol, which enables the formation of free radicals that cause DNA damage through redox cycling from compounds such as hydroquinones.[15] The enzyme is primarily found in the endoplasmatic reticulum, and its gene is located on the human chromosome 2p,[16,17] whereas the genes for the HLA system are located on chromosome 6p. The *CYP1B1* gene is transcriptionally activated by polycyclic aromatic hydrocarbons, which act via the dioxin inducible Ah receptor complex.[18]

CYP1B1 is strongly overexpressed in multiple human malignancies[18,19] and has been used as a target for cancer chemotherapy[20,21] and immunotherapy.[22,23] Several anticancer agents interact with CYP1B1,[20,24] and CYP1B1 inhibitors have been investigated for their anticancer effects.[25] CYP1B1 is expressed in human lymphocytes, variable and highly inducible,[26] and also in monocytes and macrophages.[27] It plays a role in hematopoietic stem and progenitor cell (HSPC) expansion.[28] *CYP1B1* is also overexpressed in acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), lymphoma, and myeloma.[29,30] Methylation of *CYP1B1* was associated with worse overall survival (OS) and disease-free survival in young adults with ALL and correlated with decreased *CYP1B1* expression.[31] Several polymorphisms were identified in the *CYP1B1* gene causing interindividual variability[32,33] and being associated with a variety of cancers.[13,34,35] The *CYP1B1* polymorphism C432G = Leu432Val also called rs1056836 causes an amino acid exchange at codon 432, where the C-allele codes for Leu432 (wild-type [wt]), and the G-allele codes for Val432 (variant). Amongst others, this affects the conversion of estradiol to the carcinogenic metabolite 4-hydroxyestradiol.[32,33] Mutant (mut) *CYP1B1* Val432Leu is possibly linked with myeloid leukemia.[36] We hypothesized that altered polymorphic drug metabolism by CYP1B1 in patients who underwent allogeneic HSCT, might influence host organ function, cancer cells and donor immune cells, and thus transplant outcome. The aim of our study was to evaluate whether the Leu432Val (or C432G) polymorphism of CYP1B1 enzyme in patients influences the outcome of allogeneic HSCT, in particular: OS, non-relapse mortality (NRM), relapse of disease, and incidence of GVHD.

Methods

Patients

We included a total of 382 patients (and donors) for the *CYP1B1* gene polymorphism analysis, who were transplanted at the University Hospital of Essen, Department of Bone Marrow Transplantation. Patient characteristics are outlined in Table 1.

Selection of patients was performed purely on the basis that genetic material (peripheral blood) from both the recipient and the donor were available. Genotyping was performed without knowledge of clinical data, or outcome of the patients analyzed. This study was conducted in accordance with the

Table 1. Patient demographics, disease and transplant characteristics.

Characteristics	N=382
Age in years, median (range)	42 (15-67)
Sex, M/F, %	53/47
Diseases, N	
AML	142
ALL	53
CML	91
MDS	35
OMF	14
MM	14
NHL	12
CLL	6
CMML	5
Other	9
Acute leukemias, %	51
Advanced disease, N (%)	
M	118/204 (58)
F	97/178 (54)
Donor HLA-type, %	
Matched	80
Mismatched	20
Stem cell source, %	
PBSC	82
BM	18
Donor (F to M)	
% of all patients	11
% of male patients	21
Conditioning, %	
MAC	95
RIC	5
TBI	74
Chemotherapy only	26
GVHD prophylaxis, %	
CSA-MTX only	64
T-cell depletion	36
Years of HSCT, range (median)	1993-2003 (2002)
Median follow-up in months (last follow-up)	76 (April 2014)

AML: acute myeloid leukemia; ALL: acute lymphocytic leukemia; CML: chronic myeloid leukemia; MDS: mylelodysplastic syndrome; OMF: osteomyelofibrosis; MM: multiple myeloma; NHL: non-Hodgkin lymphoma; CLL: chronic lymphocytic leukemia; CMML: chronic myelomonocytic leukemia; HLA: human leukocyte antigen; M: male; F: female; PBSC: peripheral blood stem cells; BM: bone marrow; MAC: myeloablative conditioning; RIC: reduced-intensity conditioning; TBI: total body irradiation; GVHD: graft-*versus*-host disease; CSA-MTX: cyclosporin and methotrexate; HSCT: hematopoietic stem cell transplantation.

amended Declaration of Helsinki. All aspects of this study were approved by the Institutional Review Board on Medical Ethics at the University Hospital of Essen (BO 06-3057), and all patients and donors gave their written informed consent.

Early disease *versus* advanced disease

Early disease (ED): patients with acute leukemia (AL) in first complete remission (CR), chronic myloid leukemia (CML) in first chronic phase, osteomyelofibrosis, severe aplastic anemia, paroxysmal nocturnal hemoglobinuria, hypereosinophilic syndrome, refractory anemia (RA) and refractory anemia with ring sideroblast (RARS) (both untransformed myelodysplastic syndrome [MDS]).

Advanced disease (AD): patients with AL in partial remission (PR) or second CR, secondary AML, relapsed AL, CML with accelerated phase or blast crisis, refractory anemia with excess blasts (RAEB), chronic myelomonocytic leukemia, transformed MDS, multiple myeloma, non-Hodgkin lymphoma, chronic lymphocytic leukemia stage ≥3.

Conditioning regimen and clinical study endpoints

All protocols and procedures are described in detail in the *Online Supplementary Appendix*.

Isolation of genomic DNA

DNA was prepared from peripheral blood mononuclear cells obtained from the donor and patient before the transplant, using the phenol/chloroform method.[43]

Genotyping for *CYP1B1* codon 432 polymorphism

CYP1B1 Leu432Val polymorphism (rs1056836) was analyzed by a LightCycler (Roche Diagnostics; Mannheim, Germany) protocol using the polymerase chain reaction (PCR) primers and hybridization probes published by Brüning *et al.*[44] The primers for the PCR (and the hybridization probes) were synthesized by MWG Biotech (Ebersberg, Germany). Polymorphisms of *CYP1B1* at codon 432 were determined by use of the hybridization probe format. The following genotypes were determined by reverse transcription (rt)-PCR: CYP1B*1/*1 = CC (wt), *CYP1B*1/*2* = CG, *CYP1B*2/*2* = GG (homozygous mut). PCR and subsequent melting curve analysis were performed using the LightCycler device and software (Roche Applied Science; Mannheim, Germany). Control samples confirmed by sequencing were included in each run.

Statistical analysis

Probabilities of OS were calculated using the Kaplan-Meier method.[45] Differences between time-to-event distribution functions were compared by a log-rank test (SPSS).[46] Cumulative incidence was used to estimate the endpoints of NRM, relapse and GVHD to accommodate for competing risks (R statistic).[47] Relapse and NRM were analyzed as competing risks.[48] Combined data are shown as mean ± standard error. In order to study acute and chronic GVHD, we considered relapse and death to be competing events. Comparisons between categorical covariates were conducted using the χ^2 test where appropriate. *P* values are always two-sided.

A Cox proportional hazards model was used for multivariate regression to evaluate the prognostic significance of different covariates on the endpoints of OS, NRM, and relapse. Covariates analyzed - see the *Online Supplementary Appendix*. All interactions between patient sex, AD and other covariates were tested. Relative risk (RR) estimates and their 95% confidence intervals (95% CI) were derived from Cox regression after adjustment for all other covariates in the model. Statistics Software used are presented in the *Online Supplementary Appendix*.

Results

CYP1B1 genetic polymorphism in patients and donors

Among recipients (R) 169 (44%) were genotyped as homozygous wt gene CC, 157 (41%) as heterozygous genotype CG, and 56 (15%) had the homozygous gene mutation GG. As shown in Table 2, regarding demographic and transplant characteristics, there was no difference between the three *CYP1B1* C432G polymorphism groups CC, CG and GG in male patients with AD. With exception to age group >50 years, where there appeared to be a higher percentage of wt R SNP. The same holds true when looking at all male patients and the patient cohort overall (*data not shown*).

Interestingly, male R were more likely to have the mut SNP

Table 2. Male patients with advanced disease - demographic and transplant characteristics (N=118).

Characteristics	Genotype			*P*
	CC	CG	GG	
Patients, N (%)	39 (33)	63 (53)	16 (14)	
Age in years, median (range)	47 (19-64)	42 (19-65)	44 (23-63)	NS
>40, N	25	39	12	0.62
>50, N	17	12	4	0.03
Patients, %	52	36	12	
Donor type				
Unrelated, N (%)	18 (26)	40 (57)	12 (17)	0.09
HLA-mismatched, N	10	15	5	0.83
Female, N	10	12	3	0.71
Transplant source, N PBSCT	34	58	14	0.69
Treatment regime, N				
MAC	36	58	15	0.97
TBI consisting	25	45	12	0.65
T-cell depletion	16	22	4	0.52

Male patients with advanced disease characteristics are balanced for the 3 *CYP1B1* allele frequencies (in %) between wild-type (CC genotype), heterozygous mutant (CG genotype), and homozygous mutant (GG genotype). Statistical analysis was performed by χ2 test. Genotype indicates *CYP1B1* C432G polymorphism; NS: not significant; PBSCT: peripheral blood stem cell transplantation; MAC: myeloablative conditioning; TBI: total body irradiation.

compared to female R (62% *vs*. 48%), whereas the wt SNP was more common in females (52% *vs*. 38%), *P*=0.006 (χ^2), see Table 3A. This difference was even more pronounced in AD: the prevalence of mut SNP in male R was 67% *versus* 50% in female R.

Among donors (D) 167 (44%) were genotyped as homozygous wt gene CC, 162 (42%) as heterozygous genotype CG, and 53 (14%) had the homozygous gene mutation GG. Calculated genotype frequencies were comparable to the R group. Within the donor group however, there was no sex difference in distribution between wt and mut SNP, as shown in Table 3B, in contrast to the R group. Our data with donor *CYP1B1* polymorphism or D SNP yielded interesting results. However, D SNP data did not influence the outcome results presented in this paper. Due to clarity and space constraints, they are not included here.

Across all patients with wt R SNP (n=169), 52% of women, and 51% of men had AD (*P*=0.843). Whereas across all patients with mut R SNP (n=213), 56% of women, and 62% of men had AD (*P*=0.351). Of all male patients (n=204), mut R SNP was 56% in ED, and 67% in AD (*P*=0.105). Whereas of all female patients (n=178), mut R SNP was 46% in ED, and 50% in AD (*P*=0.626).

Table 3. *CYP1B1* polymorphism (Leu432Val) of recipients (A) and donors (B), N=382.

A			B		
Recipient	**Genotype wt N=169**	**Genotype mut N=213**	**Donor**	**Genotype wt N=166**	**Genotype mut N=216**
Male, N (%) N=204	77 (38)	127 (62)	Male, N (%) N=237	102 (43)	135 (57)
Female, N (%) N=178	92 (52)	86 (48)	Female, N (%) N=145	64 (44)	81 (56)

Wild-type (wt) single nucleotide polymorhism (SNP) equals CC genotype and mutant (mut) SNP stands for CG and GG genotype. Percentages indicate the prevalence of wt and mut SNP in the respective recipient or donor group: each row equals the sum of 100%. (A) *P*=0.006 (χ^2); (B) *P*=0.833 (χ^2).

Overall survival

Across all patients (n=382) the 5-year estimate for OS was 58±4% for the CC group, 50±4% for the CG, and 46±7% for the GG group (*P*=0.141), whereas it was 58±4% for wt R SNP, and 48±3% for the mut R SNP (CG and GG combined) (*P*=0.048). Multivariate analysis was performed. In all patients the following factors available at transplant influenced the OS: AD (RR 2.70; 95% CI: 1.94-3.77; *P*<0.001), mismatch donor (RR 1.64; 95% CI: 1.19-2.27; *P*=0.003), and age >40 years (RR 1.54; 95% CI: 1.16-2.06; *P*=0.003), and R SNP (RR 1.22; 95% CI: 1.01-1.49; *P*=0.044).

Importantly, this difference was evident especially in male patients (n=204) where the group with *CYP1B1* mutations did significantly worse: 5-year OS was 58±6% *versus* 44±5% *versus* 37±9% (*P*=0.062) (Figure 1A), whereas it was 58±6% for wt R SNP *versus* 42±4% for mut R SNP (*P*=0.024). In sharp contrast, OS of female patients was almost identical between the three groups (5-year OS: 58±5% *vs*. 58±6% *vs*. 58±10%) (Figure 1B). Multivariate analysis was performed. In male patients only the following factors available at transplant influenced OS: AD (RR 2.52; 95% CI: 1.59-4.00; *P*<0.001), R SNP (RR 1.46; 95% CI: 1.12-1.91; *P*=0.005), and age >40 years (RR 1.50; 95% CI: 1.03-2.19; *P*=0.034).

Interestingly, this difference in survival rate was observed primarily in male patients with AD (n=118), where the group with *CYP1B1* mutations did significantly worse: their 5-year OS was 44±8% *versus* 32±6% *versus* 6±6% (*P*=0.002) (Figure 2A). In contrast, regarding male patients with ED, OS was almost identical between the three groups (5-year OS: 74±7% *vs*. 69±8% *vs*. 69±12%) (Figure 2B). Multivariate analysis was performed (Table 4). In male patients with AD, only the following factor available at transplant influenced OS: R SNP with RR 1.78; 95% CI: 1.25-2.52; *P*=0.001.

Non-relapse mortality

One-year estimate for NRM in male patients with AD was 8±4% for wt/wt *versus* 21±5% for mut/wt *versus* 50±12% for mut/mut *CYP1B1* R SNP (*P*=0.002) (Figure 3A). In contrast, there was no difference in the outcome concerning male patients with ED (NRM 13% *vs*. 13% *vs*. 19%) (Figure 3B). Multivariate analysis was performed (Table 4). In male patients with AD, only the following factor influenced NRM: *CYP1B1* R SNP (RR 1.98; 95% CI: 1.21-3.25; *P*=0.007). Causes of death (n=15) in the GG group (n=16) of male patients with AD were: relapse n=6, sepsis n=4, invasive fungal infection n=3, and veno-occlusive disease n=2 patients. When looking at the R SNP group GG in male patients with AD, there was no pattern with regards to clinical features (see Table 2), other outcomes or causes of death compared to group CG and group CC. Relapse and infections were a common cause of death.

Occurrence of relapse

When looking at relapse, *CYP1B1* genetic polymorphism in patients (R SNP) at first did not seem to make a difference in either sex. However, in male patients with AD (n=118), mut R SNP was associated with more relapses at 3 years than wt R SNP: 31±7% *versus* 43±6% *versus* 38±12% (*P*=0.08) (Figure 4A). When combining wt/mut and mut/mut the numbers were 31±7% *versus* 42±6% (*P*=0.04). In contrast, male patients with ED did not show this phenomenon (relapse 24% *vs*. 16% *vs*. 19%) (Figure 4B). This was confirmed by multivariate analysis in male patients with AD (Table 4) where R SNP was the strongest predictor for relapse (RR 1.92; 95% CI: 1.21-3.07; *P*=0.006), with mut R SNP performing worse, and graft type (RR 0.38;

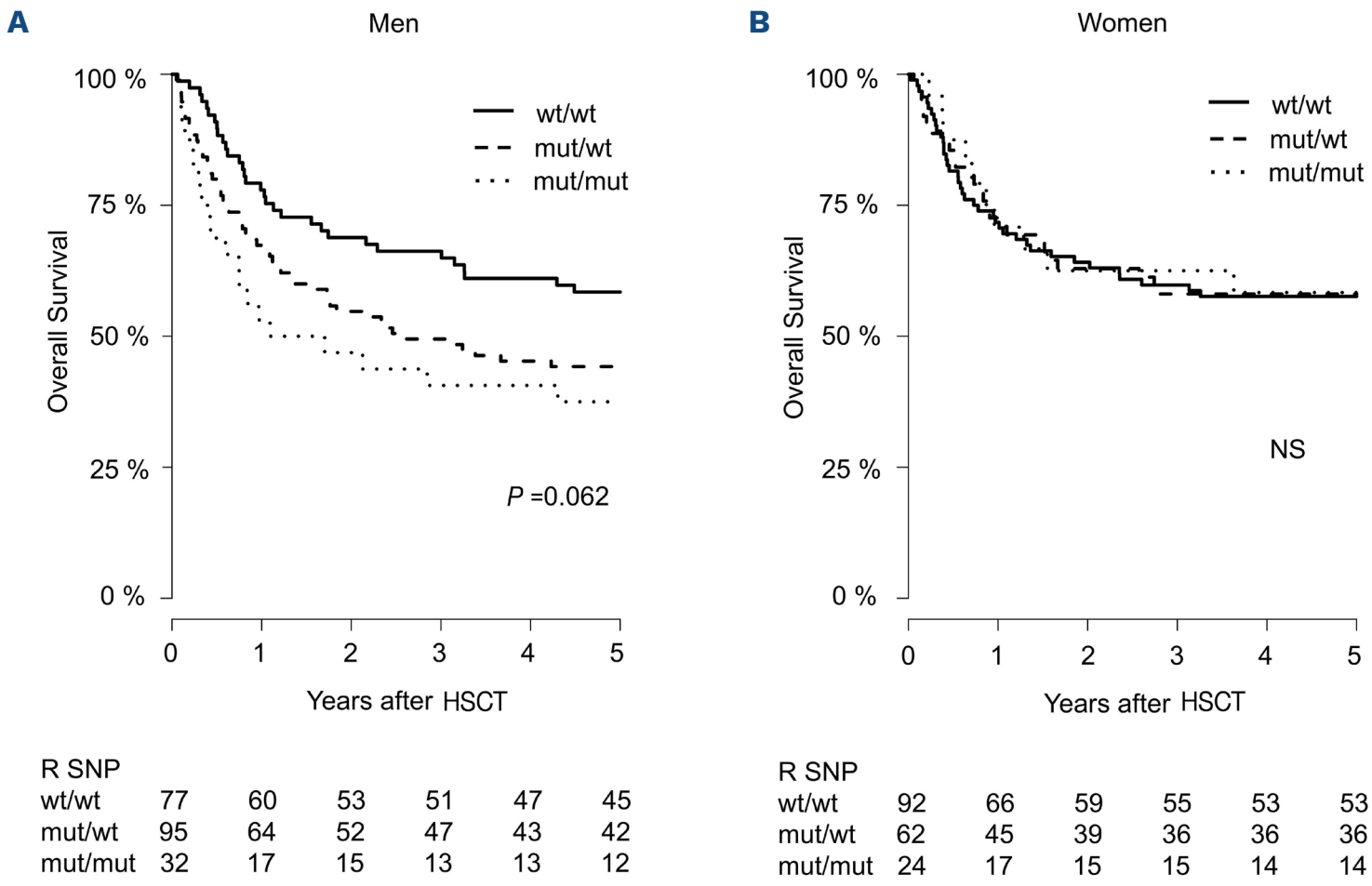

Figure 1. Overall survival estimate by *CYP1B1* polymorphism. (A) Male patients (N=204): patients with mutant (mut) recipient single nucleotide polymorphism (R SNP) (wt/mut and mut/mut) had lower overall survival (OS) than patients with wild-type (wt) R SNP (wt/wt). (B) Female patients (N=178) displaying no difference (not significant [NS]). R SNP here *CYP1B1* C432G polymorphism CC, CG and GG. HSCT: hematopoietic stem cell transplantation.

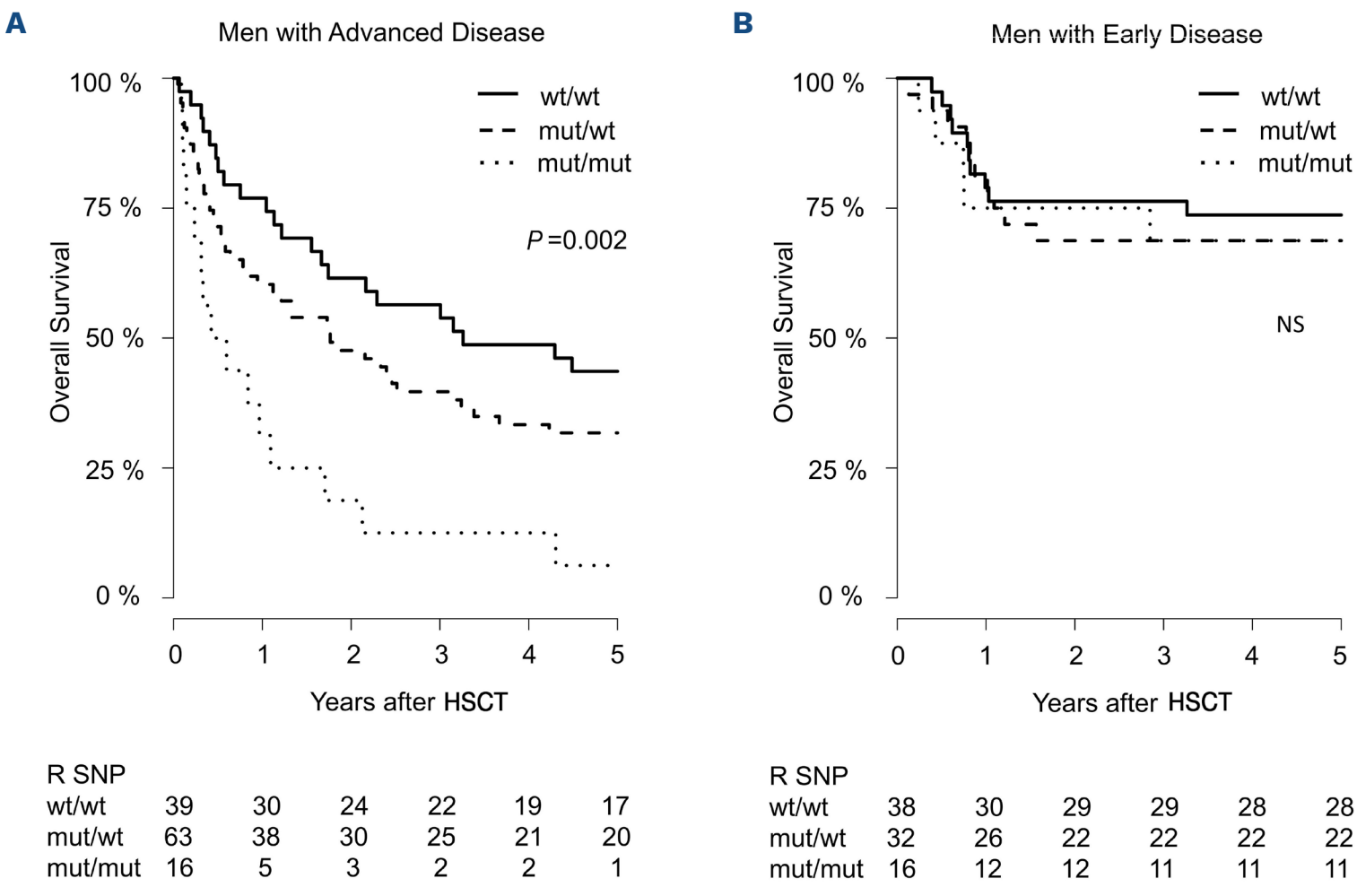

Figure 2. Overall survival estimate in male patients by *CYP1B1* polymorphism. (A) Male patients with advanced disease (N=118): patients with mutant (mut) recipient single nucleotide polymorphism (R SNP) had lower overall survival (OS) than patients with wild-type (wt) R SNP. (B) Male patients with early disease (N=86) displaying no difference (not significant [NS]). HSCT: hematopoietic stem cell transplantation.

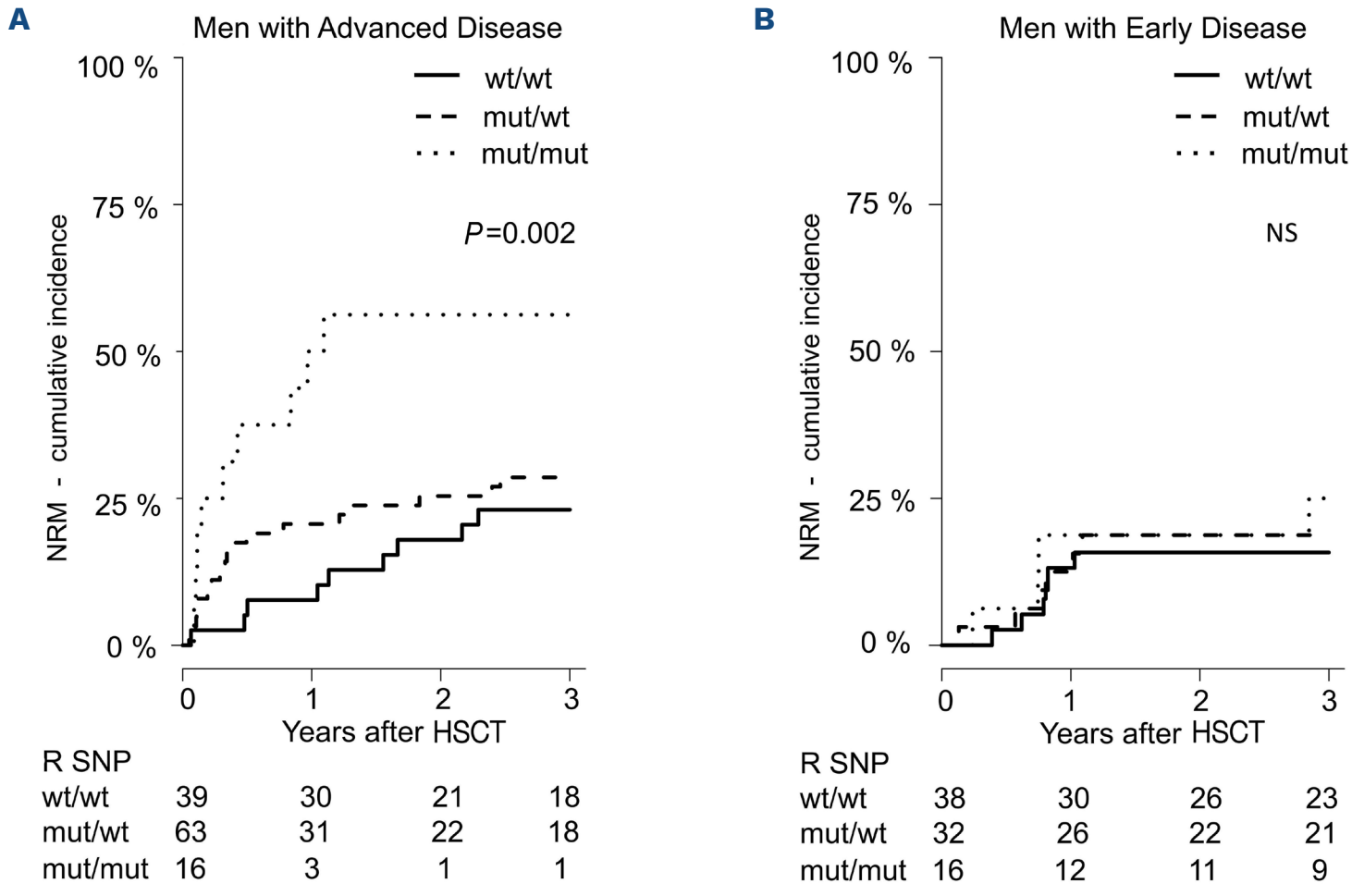

Figure 3. Non-relapse mortality estimate in male patients by *CYP1B1* polymorphism. (A) Male patients with advanced disease (N=118): male patients with mutant (mut) recipient single nucleotide polymorphism (R SNP) had a higher non-relapse mortality (NRM) than those with wild-type (wt) R SNP. (B) Male patients with early disease (N=86) displaying no difference (not significant [NS]). HSCT: hematopoietic stem cell transplantation.

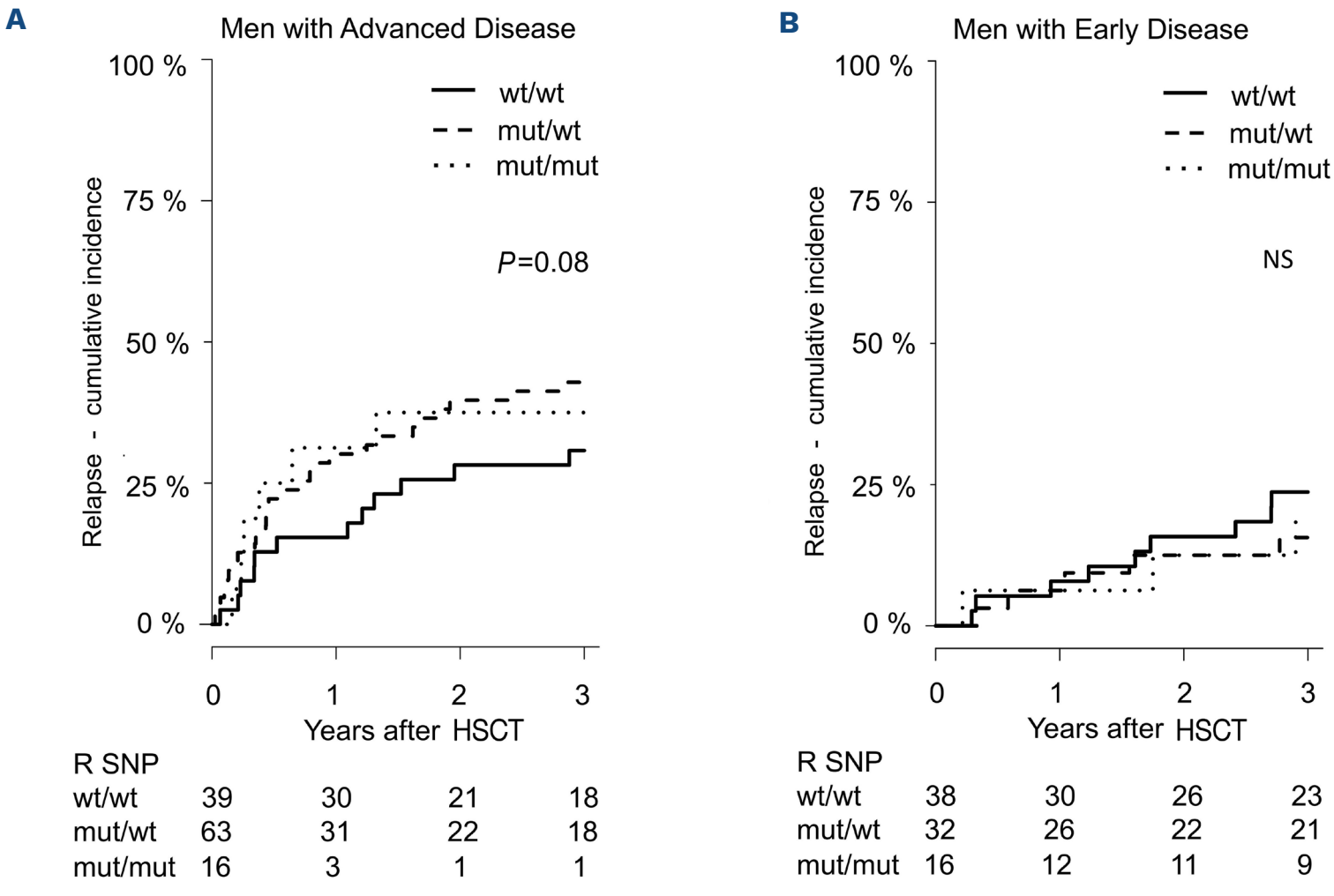

Figure 4. Relapse estimate in male patients by *CYP1B1* polymorphism. (A) Male patients with advanced disease (N=118). Male patients with mutant (mut) recipient single nucleotide polymorphism (R SNP) had a higher relapse rate than those with wild-type (wt) R SNP. (B) Male patients with early disease (N=86) displaying no difference (not significant [NS]). HSCT: hematopoietic stem cell transplantation.

95% CI: 0.16-0.89; *P*=0.026) with PBSC being associated with a lower relapse rate.

Acute and chronic graft-*versus*-host disease

In male patients with AD (n=118) the estimated incidence of acute GVHD, grade 3-4 on day 30 post HSCT was 3±3% *versus* 19±6% *versus* 40±14% for R SNP (*P*=0.177). Whereas for male patients with early disease it was 12% *versus* 13% *versus* 7% (*P*=0.633). Although displaying a trend, the numbers were relatively small and not significant. R SNP was also not significant in multivariate analysis for severe acute GVHD.

The incidence of chronic GVHD (all stages) was similar in the three R subgroups (R SNP), with 64% in CC, 60% in CG, and 44% in GG (χ^2, P=0.367) in male patients with AD. R SNP was also not significant in multivariate analysis for all stages of chronic GVHD.

CYP1B1 is not involved in metabolism of immunosuppressants like cyclosporine, and there was no difference observed between the three subgroups of R SNP when comparing cyclosporine or cortisone doses upon discharge.

For the role of sex mismatch on outcome please see the *Online Supplementary Appendix*.

Engraftment, kidney and liver toxicity

For 198 of 382 patients (52%) additional data was available. This was an unselected group. Male patients with AD, 57 of 198 (29%) displayed no significant difference in engraftment of neutrophils or thrombocyytes between wt and mut R SNP. We also evaluated the maximum creatinine and bilirubin values after transplant. While there was a tendency towards higher mean (not median) creatinine and bilirubin values in patients with mut R SNP, this difference was small and by no means significant.

Discussion

In this retrospective single-center study we evaluated the influence of *CYP1B1* polymorphism (Leu432Val) on the clinical outcome after allogeneic HSCT in adult patients. We found that mut SNP is associated with lower OS in male patients with AD due to increased non-relapse mortality, and a higher relapse rate after HSCT. This outcome difference in male patients is not explained by other prognostic factors available at time of transplant. In contrast, this association has not been seen in male patients with ED or in female patients. Of note, the above has been found in a primarily myeloablative setting.

Interestingly, male patients were more likely to have the mut SNP of *CYP1B1* compared to female patients (62% *vs*. 48%). However, in contrast to the R group (which had hematological cancer - therefore, had also been exposed to anticancer drugs and radiation), there was no sex difference in distribution between wt and mut SNP within the donor group. Calculated Leu432Val (or C432G) genotype frequencies of *CYP1B1* did not differ much from that reported earlier for Caucasians: CC 37%, CG 46% and GG 17%.[44,50]

It could therefore be postulated that men with mut SNP have a higher risk of hematological cancer, or are more likely to relapse post induction and consolidation therapy (and thus become transplant candidates more often) than men with wt SNP. This conclusion is corroborated by Tang *et al*.[51] who found that among Caucasians 34% of men with prostate cancer were homozygous for the Val432 polymorphism (or GG in other words), while only 12% of matched control subjects had this genotype. Also by Fritsche *et al*.[50] who reported that genotypes heterozygous and homozygous for the Val432 polymorphism (i.e., CG and GG or mut SNP) were

Table 4. Results of multivariate analysis for main outcomes after hematopoietic stem cell transplantation - male patients with advanced disease only (N=118).

Characteristics	Overall survival		Relapse		NRM	
	RR	*P*	RR	*P*	RR	*P*
Age >40 years	1.57	0.057	1.88*	0.051	1.86	0.073
Donor sex	1.00	0.999	0.90	0.790	1.18	0.693
Relation	1.07	0.803	0.95	0.881	1.11	0.799
Mismatch	1.27	0.353	0.60	0.189	1.64	0.149
Graft	0.66	0.263	0.38	0.026	1.21	0.762
RICMAC	1.15	0.755	1.50	0.489	1.44	0.571
TBI	0.86	0.561	1.00	0.000	0.71	0.340
TC-depletion	1.27	0.332	1.32	0.410	0.88	0.728
R SNP	1.78	0.001	1.93	0.006	1.98	0.007

Cox regression was performed using R. Shown are clinically relevant parameters available at transplant entering the multivariate analysis: see statistics. *Age >50 years was chosen for relapse since it had a better correlation than age >40 years. RR: relative risk; NRM: non relapse mortality; relation: sibling *versus* unrelated; mismatch: HLA-constellation between patient and donor (identical *vs*. mismatch); graft: peripheral blood stem cells *versus* bone marrow; RICMAC: reduced intensity *versus* myeloablative conditioning; TBI: total body irradiation; TC-depletion: *in vivo* T-cell depletion; R SNP: recipient single nucleotide polymorphism (SNP) - here *CYP1B1* C432G polymorphism CC, CG and GG.

more frequent in patients with colorectal cancer compared to controls.

Of interest, even though wt SNP was more common in female patients, it does not appear to be associated with a better outcome. Whereas mut SNP appears to have a clear negative effect in men with AD. Female patients with mut SNP seem to have a better outcome than their male counterparts. It could be speculated that this is due to the presence of estrogens. Sissung *et al.*[52] made a similar observation in male patients with prostate cancer: patients with *CYP1B1*3* polymorphic variant (L432V) GG had a decreased OS after docetaxel-based therapies compared with individuals carrying at least one copy of the *CYP1B1*1 (*allele CC or CG).

NRM was higher in male patients with mut SNP compared to male patients with wt SNP in the AD setting, whereas this difference did not show at all in female patients. An explanation for the NRM increase in male patients with mut SNP could be a higher rate of organ toxicity or more infections due to chemotherapy, radiotherapy or immunotherapy.

Relapse was higher in male patients with AD for mut SNP compared to wt SNP. This could be due to a poor response of cancer cells with mut SNP to chemotherapy, radiotherapy, and immunotherapy, thus leading to a higher relapse rate in patients with AD.

Of note, most patients analyzed received myeloablative transplants in the years 1993-2006, median 2002. The field of transplantation has changed over the years due to advances in donor selection and supportive care, i.e., infection disease and GVHD control, resulting in improved survival.

The sex differences observed in this study comparing wt and mut SNP in recipients were striking. So far, sex differences in outcome after allogeneic HSCT have not been reported with the exception of increased rates of chronic GVHD for female donors and male recipients.[53] T cells of female donors specific for minor histocompatibility antigens encoded by genes on chromosome Y may contribute to GVHD, graft rejection, and graft-*versus*-leukemia effects in hematological malignancies.[54]

In conclusion, our retrospective single-center cohort study provides strong evidence that male patients with mut genetic polymorphism of *CYP1B1* have a lower OS in AD after HSCT, and that this is due to a higher NRM and relapse rate. Genotyping for *CYP1B1* C432G polymorphism might therefore help identify patients with higher risk for allogeneic HSCT in the future, and may perhaps also explain some sex differences in outcome. Validation of the role of *CYP1B1* C432G polymorphism in a prosppective study or confirmation in an independent cohort are warranted before any clinical conclusions are drawn. We also suggest further *in vitro* and *in vivo* studies on hematological cancers and lymphocytes regarding the role of *CYP1B1* and its Leu432Val polymorphism.

Disclosures

No conflicts of interest to disclose.

Contributions

NS was responsible for the analysis of data, interpretation of results, and literature research. He performed the statistical analyses, created tables and figures, and wrote the manuscript. MK was responsible for the design of the study and conduct of the research. He obtained funds, designed the laboratory protocol, supervised experiments, and extracted and collected both clinical and lab data. He also revised and edited the manuscript. Both authors approved the final version of the manuscript.

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Data-sharing statement

The data supporting the findings of this study are available upon request from the corresponding author.

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