Influence of genetic polymorphisms in *CYP3A4*, *CYP3A5*, *GSTP1*, *GSTM1*, *GSTT1* and *MDR1* genes on survival and therapy-related toxicity in multiple myeloma

We investigated the role of single nucleotide polymorphisms in genes encoding for drug-metabolizing enzymes in 209 newly diagnosed multiple myeloma patients included in a clinical trial comparing single with double intensive therapy. We observed no significant association between polymorphisms in *CYP3A4, CYP3A5, MDR1, GSTM1* and *GSTT1* and outcome either after treatment with induction chemotherapy or after high-dose therapy.

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Currently the standard therapy for patients ≤65 years of age with multiple myeloma (MM) consists of high-dose melphalan supported with autologous peripheral blood stem cell (PBSC) transplantation.[1,2] Even with high-dose treatment, patients cannot be cured and the majority of them will experience treatment failure and relapse. Cancer chemotherapy is associated with significant inter-subject variations in response and toxicity, as was observed in the HOVON-24 trial.[3] Variations may occur due to genetic alterations, such as single nucleotide polymorphisms (SNP), affecting drug-metabolizing enzymes and thus resulting in altered pharmacokinetics of therapeutic agents, and are likely to influence the response to certain chemotherapeutic agents.[4] The development of prognostic factors that take such genetic variations into account could aid in defining a more individualized prognosis and help to identify patients who are at risk of treatment failure.

We analyzed the influence on outcome (partial and complete remissions, progression-free, event-free and overall survival and toxicity) of several polymorphisms in genes involved in the metabolism of chemotherapeutic agents in newly diagnosed patients with MM, who were included in a prospective randomized clinical trial of single versus double intensive treatment (HOVON-24). We examined SNP in phase I metabolism and phase 2 metabolism catalyzed by cytochrome P450 enzymes and gluthatione-S-transferases, respectively. Both groups of enzymes are involved in the metabolism of many chemotherapeutic agents, of which the alkalylating agents, vinca-alkaloids and antibiotic antitumor agents are of our main interest since they are part of most therapeutic regimens in multiple myeloma.

For phase I metabolism, the *CYP3A4* polymorphism (290A→G) and *CYP3A5* (6986A→G) polymorphisms were analyzed together because of the close linkage of *CYP3A4* AA (*1B/*1B) and *CYP3A5* GG (*3/*3), using a restriction fragment length polymorphism (RFLP)-polymerase chain reaction (PCR).

With respect to phase II metabolism, *GSTT1* and *GSTM1* polymorphisms were analyzed simultaneously using a multiplex PCR, whereas the *GSTP1* polymorphism was determined using RFLP-PCR. This method was also used to determine the *MDR1* (*ABCB1*) gene polymorphism. The effect of the SNP in *CYP3A4*1B* is controversial and the polymorphism in the *MDR1* gene is associated with altered protein expression. The SNP in the other described genes result in a less active or absent enzyme.

The patients' characteristics have been described previously.[3] The *CYP3A4* genotype was assessed in 197 patients. A *CYP3A5* genotype was successfully assigned to 205 patients. No significant differences were observed in responses on protocol (partial and complete remissions) after VAD and high dose therapy between patients carrying the wild-type allele or SNP (Table 1). In univariate analysis event-free and overall survival were significantly better in patients with the *CYP3A5* mutant, however, in multivariate analysis including all clinical variables this effect was lost (Figure 1). There was no evidence of differences in hematologic or non-hematologic toxicity between patients with all the analyzed genotypes (*data not shown*).

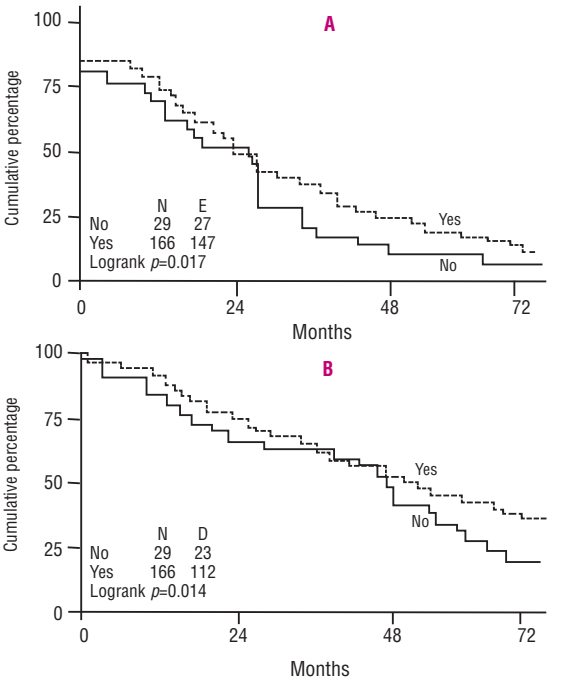

Figure 1. Kaplan-Meier plots of EFS and OS for the *CYP3A4* (290A→G) and *CYP3A* (6986A→G) polymorphisms. The solid line represents patients with the combined genotype *CYP3A4* AA (*1B/*1B) and *CYP3A5* GG (*3/*3) and the dotted line represents patients without this genotype combination. A. Event-free survival. B. Overall survival.

The *GSTP1* genotype could be determined in 204 patients. No significant differences in toxicity were observed during treatment. No significant associations were found with outcome after VAD and high-dose treatment (Table 1). Genotyping the *MDR1* C3435T polymorphism was successful in 202 patients. There were no significant associations between the genotypes and toxicity, response after VAD or high-dose therapy, or Kaplan-Meier survival estimates. Similarly no associations were found for the *GSTT1/ GSTM1* polymorphisms (Table 1).

We examined the impact of genetic polymorphisms in drug-metabolizing enzymes on survival and toxicity in MM patients. No association between any of the polymorphisms and outcome was observed. An analysis performed in myeloma patients treated in the MRC VII trial, showed an association between the *GSTP1* Val/Val genotype and an improved outcome in patients receiving conventional treatment.[5] An explanation for this could be that high-dose therapy overcomes the effects of polymorphisms. However, in the present study no significant influence of any SNP on response could be demonstrated, whether after induction treatment with VAD or after

Table 1. Genetic polymorphisms in CYP3A4, CYP3A5, GSTP1, GSTM1, GSTT1 and MDR1 genes

		PR on protocol			CR on protocol		
Genotype	*No (%).*	*No. (%)*	*OR (95% CI)*	*p*	*No. (%)*	*OR (95% CI)*	*p*
All patients	209	172 (82)			52 (25)		
GSTP1, n=204							
AA	75 (37)	68 (91)	1	0.0321	28 (28)	1	0.20
AG	98 (48)	76 (78)	0.36 (0.14-0.88)		26 (27)	0.93 (0.47-1.82)	
GG	31 (15)	23 (74)	0.30 (0.10-0.91)		4 (13)	0.38 (0.12-1.22)	
MDR1, n=202							
CC	21 (10)	18 (86)	1	0.17	5 (24)	1	0.96
CT	92 (46)	70 (76)	0.53 (0.14-1.97)		21 (23)	0.95 (0.31-2.89)	
TT	89 (44)	77 (87)	1.07 (0.27-4.19)		22 (25)	1.05 (0.35-3.20)	
CYP3A4 AA & *CYP3A5* GG, n=195							
No	29 (15)	23 (79)	1	0.68	5 (17)	1	0.33
Yes	166 (85)	137 (83)	1.23 (0.46-3.30)		42 (25)	1.63 (0.58-4.53)	
GSTT1, n=204							
Absent	56 (27)	42 (75)	1	0.10	13 (23)	1	0.79
Present	148 (73)	126 (85)	1.91 (0.90-4.06)		37 (25)	1.10 (0.53-2.27)	
GSTM1, n=205							
Absent	103 (50)	82 (80)	1	0.38	24 (23)	1	0.72
Present	102 (50)	86 (84)	1.38 (0.67-2.82)		26 (25)	1.13 (0.60-2.13)	

OR indicates odds ratio; and CI, confidence interval. p values are for the comparison of the PR and CR rates between the different subgroups, unadjusted for Salmon & Durie stage and β2-microglobulin. The analyses with adjustment for these two variables yielded similar results, and are therefore not shown. Correction for multiple testing was applied, which means that only p values ≤0.01 were considered statistically significant.

high-dose therapy.

An important issue is the inter-study differences in the effects of polymorphisms. Since gene polymorphisms exert a small effect, most studies are underpowered and false-positive and false-negative results may occur. This was demonstrated by performing a meta-analysis of multiple studies concerning the effect of polymorphisms.[6] Furthermore, differences in methodology, such as study design, study population, sample size, use of adjustment for multiple testing or differences in chemotherapy regimens used can explain inter-study differences.

Meta-analyses and more extensive analyses in large numbers of patients in controlled clinical trials on the effects of functional polymorphisms in drug-metabolizing enzymes may be required to elucidate the inter-individual heterogeneity of drug response. Research should be further focused on other biological functions of metabolizing enzymes and the influence of polymorphisms on these functions.

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