Selected Genetic Polymorphisms Associated with Hypoxia and Multidrug Resistance in Monoclonal Gammopathies Patients

Vybrané genetické polymorfizmy asociované s hypoxií a multilékovou rezistencí u pacientů s monoklonálními gamapatiemi

Almasi M.[1], Besse L.[2], Brozova L.[3], Jarkovsky J.[3], Bezdekova R.[1], Pour L.[4], Minarik J.[5], Kessler P.[6], Pavlicek P.[7], Roziakova L.[8], Penka M.[1], Hajek R.[1,9], Vasku A.[10], Sevcikova S.[1,11]

[1] Department of Clinical Hematology, University Hospital Brno, Brno, Czech Republic
[2] Department of Oncology and Hematology, Cantonal Hospital St. Gallen, Switzerland
[3] Institute of Biostatistics and Analyses, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[4] Department of Internal Medicine, Hematology and Oncology, University Hospital Brno and Faculty of Medicine Masaryk University, Brno, Czech Republic
[5] Department of Hematooncology, University Hospital Olomouc and Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic
[6] Department of Hematology and Transfusion Medicine, Hospital Pelhrimov, Pelhřimov, Czech Republic
[7] Department for Internal Medicine and Haematology, 3rd Faculty of Medicine, Charles University in Prague and Faculty Hospital Kralovske Vinohrady, Prague, Czech Republic
[8] Department of Hematology and Transfusion Medicine, University Hospital, School of Medicine, Comenius University Bratislava, Slovak Republic
[9] Department of Hematooncology, University Hospital Ostrava, Ostrava, Czech Republic
[10] Department of Pathological Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[11] Babak Myeloma Group, Department of Pathological Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic

Summary

Background: Adaptive response to hypoxia is regulated by several mechanisms and transcription factors, including hypoxia-inducible factors (HIFs). Activation of *HIF-1α* is associated with increased expression of P-glycoprotein and multidrug resistance in cancer cells. In this retrospective study, we analyzed candidate single-nucleotide polymorphisms (SNPs) in *HIF-1α* and *HIF-1β* associated with risk of monoclonal gammopathy of undetermined significance (MGUS) or multiple myeloma (MM). ***Patients and Methods:*** Genotypes of SNPs associated with hypoxia were determined in an independent cohort of monoclonal gammopathies (MG) (275 MM and 228 MGUS patients) and in 219 cancer-free controls by real time polymerase chain reaction allelic discrimination. ***Results:*** When MM patients were compared to controls, protective role of CG genotype compared to CC in *HIF-1β* (rs2228099) for MM development was observed (OR = 0.65; CI 0.45–0.95; p = 0.026). Even after adjustment for patients' age and body mass index (BMI), there were significantly lower odds (OR = 0.55; p = 0.045) of developing MM patients of CG genotype in comparison to CC genotype. Log-rank test confirmed association of GT haplotype (rs11549467, rs2057482) in *HIF-1α* with better overall survival (median 41.8 months; (CI 35.1–48.5)) for "none GT" and median 93.8 months (CI 31.3–156.4) for "at least one GT" haplotype (p = 0.0500). Further, significant associations between SNPs in *MDR1* and outcome of MM were found in 110 MM patients that underwent bortezomib-based treatment. ***Conclusion:*** Our study showed a genetic predisposition for risk of MG development and/or outcome of MM patients; nevertheless, further studies are needed to confirm our initial analysis.

Key words

multiple myeloma – hypoxia – genotype – polymorphism – qPCR

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doc. RNDr. Sabina Sevcikova, Ph.D.
Babak Myeloma Group
Department of Pathological Physiology
Faculty of Medicine
Masaryk University
Kamenice 5
Brno
Czech Republic
e-mail: sevcik@med.muni.cz

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Souhrn

Východiska: Přirozená reakce organizmu na hypoxii je regulována různými mechanizmy a transkripčními faktory, zahrnujícími hypoxií indukovatelné faktory (HIFs). Aktivace *HIF-1α* je u nádorových buněk spojována se zvýšenou expresí P-glykoproteinu a multilékovou rezistencí. V této retrospektivní analýze jsme sledovali kandidátní jednonukleotidové polymorfizmy (single-nucleotide polymorphisms – SNP) genů *HIF-1α* a *HIF-1β* a jejich spojení s rizikem vzniku onemocnění monoklonální gamapatie nejasného významu (monoclonal gammopathy of undetermined significance – MGUS) nebo mnohočetného myelomu (MM). ***Soubor pacientů a metody:*** Genotypy jednonukleotidových polymorfizmů spojovaných s hypoxií byly určovány pomocí real time polymerázové řetězové reakce alelické diskriminace u nezávislé skupiny pacientů s monoklonální gamapatií (MG) (275 pacientů s MM a 228 s MGUS) a u 219 kontrol bez nádorového onemocnění. ***Výsledky:*** Při porovnání pacientů s MM a kontrol jsme pozorovali příznivější vliv genotypu CG genu *HIF-1β* (rs2228099) oproti genotypu CC (OR 0,65; CI 0,45–0,95; p = 0,026). Obdobně i při zohlednění věku pacientů a jejich indexu tělesné hmotnosti byla signifikantně nižší šance (OR 0,55; p = 0,045) rozvoje onemocnění MM u genotypu CG oproti CC. Log-rank test potvrdil souvislost GT haplotypu (rs11549467, rs2057482) genu *HIF-1α* s lepším celkovým přežitím (medián 41,8 měsíce; (CI 35,1–48,5) u haplotypu „žádné GT" a medián 93,8 měsíce (CI 31,3–156,4) u haplotypu „nejméně jeden GT" (p = 0,0500). Dále byla zjištěna významná souvislost mezi jednonukleotidovými polymorfizmy v genu *MDR1* a léčebným účinkem u 110 pacientů s MM léčených bortezomibem. ***Závěr:*** Naše studie ukázala možnou genetickou predispozici k riziku rozvoje MG a/nebo k léčebné odpovědi pacientů s MM, nicméně je třeba provést další studie k potvrzení naší počáteční analýzy.

Klíčová slova

mnohočetný myelom – hypoxie – genotype – polymorfizmus – qPCR

Introduction

Monoclonal gammopathies (MG) are a group of disorders characterized by proliferation of malignant monoclonal plasma cells (PCs) [1]. Multiple myeloma (MM) is a clonal PCs malignancy that accounts for more than 10% of malignant hematologic disorders [2]. MM is further characterized by presence of clonal PCs in the bone marrow (BM), monoclonal immunoglobulin (mIg, M-protein) in serum and/or urine and presence of other clinical symptoms, such as hypercalcemia (C), renal insufficiency (R), anemia (A) and bone involvement (B), commonly known as CRAB symptoms [3]. While the introduction of novel drugs, such as proteasome inhibitors (PIs) and immunomodulatory drugs (IMiDs), has improved clinical response of MM patients, MM remains a hard-to-treat disease. MM is always preceded by precancerosis monoclonal gammopathy of undetermined significance (MGUS). MGUS is defined by serum M protein level lower than 3 g/dL, less than 10% of clonal PCs in the BM, and absence of end-organ damage [4].

Hypoxia is a common feature in cancer affecting important biological properties of tumor cells. Among others, it activates hypoxia-inducible transcription factors (HIFs) that play an important role in MM biology. HIFs are heterodimers consisting of labile HIF-α subunits (*HIF-1α*, HIF-2α or HIF-3α) and a constitutive *HIF-1β* subunit (also known as aryl hydrocarbon receptor nuclear translocator or ARNT) [5]. In hypoxia, *HIF-1β* binds *HIF-1α*, preventing proteasomal degradation. The complex is transported to the nucleus where it binds hypoxia-responsive elements (HREs). This way, HIFs activate genes associated with angiogenesis, osteoclast recruitment and activation, cell-cycle regulation, moreover, they also cause disease progression into active symptomatic MM [6]. Hypoxia is a critical factor in MM as interactions between MM PCs and the microenvironment play a crucial role in MM progression, dissemination, angiogenesis and drug resistance. Moreover, cancer stem cell (CSC) phenotype is a plastic state induced in cancer cells depending upon microenvironmental signals, such as hypoxia [7]. As *HIF-1α* protein is highly expressed in MM cells and involved in BM angiogenesis [8], it could serve as a potential therapeutic target. In fact, the HIF-1 family is expressed in response to acute hypoxia, while HIF-2 family is expressed in response to chronic lack of oxygen [9]. Both HIFs and vascular endothelial growth factor (VEGF) are up-regulated in MM patients, strongly related to each other and linked to increased angiogenesis [10]. These findings indicate that targeting HIFs and the VEGF/receptor autocrine loop may be important in MM treatment. Furthermore, a number of studies concluded that *HIF-1α* inhibition may be a novel therapeutic strategy for MM treatment [11–15]. Activation of *HIF-1α* is associated with increased expression of P-glycoprotein and multidrug resistance in cancer cells as was described in breast cancer and non-small cell lung carcinoma [16,17]. Indeed, key players in multidrug resistance are P-glycoprotein, the best-characterized drug efflux transporter system encoded by *MDR-1* (also known as *ABCB1*), and multiple drug resistance protein-1 encoded by *MRP-1* (also known as *ABCC1*). In tumor cells, high expression of P-glycoprotein decreases the effect of chemotherapy [18].

MM is a heterogeneous disease not only with respect to tumor PCs and BM microenvironment, but also regarding genetic variability of each individual patient, which can significantly affect a patient's outcome. Several familial studies support the hypothesis of a genetic predisposition for developing MG [19–24]. Evidence for inherited genetic susceptibility is provided by the two-fold increased risk of the disease observed in first-degree relatives of MM patients [25].

Therefore, in our retrospective study, we analyzed genetic variability in genes associated with hypoxia. We analyzed candidate single-nucleotide polymorphisms (SNPs) in *HIF-1α* (rs11 549467 and rs2057482) and *HIF-1β* (rs2228099) associated with risk of MGUS or MM. Consequently, we evaluated assoc-

iation of SNPs in *HIF-1α*, *HIF-1β*, three SNPs and their haplotypes in *MDR1* (rs1045642, rs2032582 and rs1128503) and one in *MRP1* (rs4148356) with outcome of MM patients.

Material and methods

Clinical samples and studied polymorphisms

Altogether, 228 MGUS patients, 275 MM patients and 219 age-matched cancer-free controls were included in the hypoxia study. Two potentially functional polymorphisms in *HIF-1α* (rs11549467, rs2057482) and one in *HIF-1β* (rs22 28099) were genotyped and their association with MGUS and MM risk in a case-control study was assessed. Peripheral blood (PB) samples from all patients were obtained from centers of the Czech Myeloma Group – University Hospital Brno, Faculty Hospital Kralovske Vinohrady Praha, University Hospital Olomouc, University Hospital Ostrava and Hospital Pelhrimov, Czech Republic and from University Hospital Bratislava, Slovak Republic. As a control group, cancer-free cases from the University Hospital Brno were used. Basic characteristics of MGUS and MM patients as well as controls are described in Tab. 1, and clinical characteristics of MGUS and MM patients are summarized in Tab. 2. Further, association of three SNPs and their haplotypes in *MDR1* (rs1045642, rs2032582 and rs1128503) and in *MRP1* (rs4148356) was evaluated with outcome in MM patients. In total, 110 MM patients that underwent bortezomib-based treatment (cyclophosphamide, bortezomib, dexamethasone – CVD regimen) were included in the evaluation of pharmacogenetic association with clinical outcome. Clinical characteristics of MM patients at the start of CVD treatment (n = 110) are shown in Table 3.

All patients were enrolled into the study only after signing the informed consent form approved by the Ethics committees of the hospitals in accordance with the current version of the Helsinki Declaration.

Tab. 1. Basic characteristics of MGUS and MM patients in comparison to controls (n = 722).

Characteristics[1]	Controls (n = 219)	MGUS (n = 228)	MM (n = 275)
sex			
female	101 (46.1%)	103 (45.2%)	119 (43.3%)
male	118 (53.9%)	125 (54.8%)	156 (56.7%)
p[2]	–	0.841	0.527
age			
≤ 55	9 (4.1%)	63 (27.6%)	36 (13.1%)
56–60	20 (9.1%)	41 (18.0%)	47 (17.1%)
61–65	146 (66.7%)	33 (14.5%)	53 (19.3%)
66–70	35 (16.0%)	37 (16.2%)	61 (22.2%)
70	9 (4.1%)	54 (23.7%)	78 (28.4%)
p[2]	–	< 0.001	< 0.001
median (5–95%)	64.0 (57.0–71.0)	63.0 (41.0–81.0)	67.0 (51.0–81.0)
p[2]	–	0.069	< 0.001
BMI (n = 288)			
underweight (≤ 18.5)	0 (0.0%)	–	1 (1.4%)
ideal weight (18.6–25.0)	41 (19.2%)	–	22 (29.7%)
overweigh (25.1–30.0)	105 (49.1%)	–	38 (51.4%)
mild obesity (30.1–35.0)	58 (27.1%)	–	11 (14.9%)
intermediate obesity (35.1–40.0)	7 (3.3%)	–	2 (2.7%)
morbid obesity (> 40)	3 (1.4%)	–	0 (0.0%)
p[2]	–	–	0.064
median (5–95%)	29.0 (22.0–35.0)	–	26.1 (21.0–32.3)
p[2]	–	–	< 0.001

MGUS – monoclonal gammopathy of undetermined significance, MM – multiple myeloma

[1] absolute and relative frequency for categorical variable; median, percentiles (5–95%) for continuous variable

[2] p-values marked with bold are statistically significant; ML Chi square test or Mann-Whitney U test were used for comparison with the control group

SNPs analyses

Genomic DNA (gDNA) was isolated from whole PB using MagNA Pure DNA Isolator (Roche, Switzerland) and its concentration was measured by Nanodrop ND-1000 (Thermo Fisher Scientific, MA, USA). For gene polymorphisms analyses in *HIF-1α* (rs11549467, ID: C_34492744_10; rs2057482, ID: C_8549084_20), *HIF-1β* (rs2228099, ID: C_11846736_20), *MDR1* (rs1045642, ID: C_7586657_20; rs2032582, ID: C_11711720D_40 and C_11711720C_30; rs1128503, ID: C_7586662_10), and *MRP1* (rs4148356, ID: C_25614385_20), quantitative polymerase chain reaction (qPCR) for allelic discrimination was performed. Standard TaqMan genotyping assays were used run on Step-One Real-Time PCR instrument (Applied Biosystems, CA, USA) as previously described [26]. In brief, qPCR was performed in 10 µL

Tab. 2. Clinical characteristics of MGUS and MM patients at diagnosis (n = 722).

Characteristics[1]	MGUS (n = 228)	MM (n = 275)
D–S stage		n = 275
I	–	46 (16.7%)
II	–	48 (17.5%)
III	–	181 (65.8%)
D–S substage		n = 275
A	–	218 (79.3%)
B	–	57 (20.7%)
ISS classification		n = 275
stage 1	–	87 (33.3%)
stage 2	–	89 (34.1%)
stage 3	–	85 (32.6%)
progression of MGUS into	n = 228	
MM	17 (7.5%)	–
other lymphoid malignancy	6 (2.6%)	–
no progression	205 (89.9%)	–
M-protein type	n = 226	n = 275
IgG	159 (70.4%)	165 (60.0%)
IgA	26 (11.5%)	52 (18.9%)
LC only	5 (2.2%)	42 (15.3%)
IgM	29 (12.8%)	4 (1.5%)
other	7 (3.1%)	12 (4.5%)
light chain type	n = 227	n = 272
kappa	129 (56.8%)	158 (58.1%)
lambda	96 (42.3%)	113 (41.5%)
biclonal	2 (0.9%)	1 (0.4%)
immunoparesis	n = 208	n = 197
one Ig lower	30 (15.1%)	65 (63.7%)
both Ig lower	17 (9.0%)	95 (72.0%)
any Ig lower	40 (19.2%)	160 (81.2%)
serum M-protein quantity (g/dL)	0.8 (0.0–2.5) (n = 225)	3.1 (0.0–7.5) (n = 269)
kappa/lambda ratio	1.1 (0.1–17.3) (n = 208)	1.4 (0.0–736.4) (n = 171)
bone marrow infiltration (%)	2.0 (0.0–8.4) (n = 215)	29.6 (11.6–72.0) (n = 218)
normal PC – CD19 (%)	22.7 (1.0–80.0) (n = 170)	0.5 (0.0–53.4) (n = 153)
abnormal PC – CD56 (%)	25.1 (1.5–96.8) (n = 164)	94.8 (0.3–99.7) (n = 152)
hemoglobin level (g/dL)	13.5 (10.4–15.8) (n = 226)	10.7 (7.6–14.3) (n = 270)
thrombocyte count (10E9/l)	232.0 (137.0–374.0) (n = 226)	214.0 (91.0–386.0) (n = 270)
calcium total level (mmol/l)	2.3 (2.1–2.6) (n = 225)	2.4 (2.0–3.3) (n = 269)
albumin level (g/dL)	4.4 (3.5–4.9) (n = 223)	3.8 (2.4–4.8) (n = 268)
creatinine level (umol/l)	80.0 (56.0–189.0) (n = 226)	93.8 (58.0–492.0) (n = 270)
β2 microglobulin (mg/l)	1.9 (1.2–5.9) (n = 219)	3.8 (1.7–21.0) (n = 261)
LDH (ukat/l)	3.4 (2.5–6.7) (n = 220)	3.4 (2.0–7.4) (n = 269)
CRP (mg/l)	2.9 (0.0–27.9) (n = 215)	4.3 (0.3–58.0) (n = 266)

MGUS – monoclonal gammopathy of undetermined significance, MM – multiple myeloma, LDH – lactate dehydrogenase, CRP – C-reactive protein

[1] absolute and relative frequency for categorical variable; median, percentiles (5–95%) for continuous variable

reactions containing 10 ng of gDNA, 0.5 µL of TaqMan Genotyping Assay Mix and 8 µL of PCR master mix (all Applied Biosystems, CA, USA).

Statistical analyses

According to the position of SNPs on chromosome, haplotypes were derived as rs11549467, rs2057482 for the *HIF-1α* and in order as rs1045642, rs2032582, rs1128503 for *MDR1*. Haplotypes were inferred in the PHASE software, ver. 2.1 [27,28]. The default setting was left and the "-x15" was added to provide 15 repetitions of algorithm and choice of the best one. To test the Hardy-Weinberg equilibrium and linkage disequilibrium, the package "genetics" in R software was used [29].

Basic characteristics were described by absolute and relative frequencies for categorical variables and median (5–95 percentile) for continuous variable. Comparison between groups was performed using Maximal-Likelihood Chi-square test in case of categorical variables and Mann-Whitney U test in case of continuous variable. Association of marker with status control vs. MGUS or control vs. MM was expressed by odds ratio (OR) with 95% confidence interval (CI) and p-value of Wald's test. The most frequent genotypes/haplotypes for the control group were set as reference. Significant associations were adjusted by age and body mass index (BMI) as next predictors

Tab. 3. Clinical characteristics of MM patients at the beginning of CVD treatment regimen (n = 110).

Characteristics[1]	Total (n = 110)	New diagnosis (n = 47)	Relapse (n = 63)
sex	n = 110	n = 47	n = 63
female	49 (44.5%)	24 (51.1%)	25 (39.7%)
male	61 (55.5%)	23 (48.9%)	38 (60.3%)
age	70.0 (57.0–80.0)	70.0 (60.0–80.0)	70.0 (57.0–79.0)
D–S stage	n = 109	n = 47	n = 62
I	13 (11.9%)	12 (25.5%)	1 (1.6%)
II	16 (14.7%)	8 (17.0%)	8 (12.9%)
III	80 (73.4%)	27 (57.4%)	53 (85.5%)
D–S substage	n = 109	n = 47	n = 62
A	90 (82.6%)	38 (80.9%)	52 (83.9%)
B	19 (17.4%)	9 (19.1%)	10 (16.1%)
ISS classification	n = 102	n = 45	n = 57
stage 1	34 (33.3%)	12 (26.7%)	22 (38.6%)
stage 2	38 (37.3%)	19 (42.2%)	19 (33.3%)
stage 3	30 (29.4%)	14 (31.1%)	16 (28.1%)
M-protein type	n = 109	n = 47	n = 62
IgG	65 (59.6%)	26 (55.3%)	39 (62.9%)
IgA	18 (16.5%)	4 (8.5%)	14 (22.6%)
LC only	18 (16.5%)	11 (23.4%)	7 (11.3%)
IgM	3 (2.8%)	3 (6.4%)	0 (0.0%)
other	5 (4.5%)	3 (6.4)	2 (3.2%)
light chain type	n = 86	n = 46	n = 40
kappa	48 (55.8%)	22 (47.8%)	26 (65.0%)
lambda	37 (43.0%)	23 (50.0%)	14 (35.0%)
biclonal	1 (1.2%)	1 (2.2%)	0 (0.0%)
serum M-protein quantity (g/dL) (n = 108)	2.4 (0.0–7.7)	2.4 (0.0–5.0)	2.5 (0.0–7.8)
Bone marrow infiltration (%) (n = 98)	20.4 (0.8–62.2)	15.2 (2.4–49.6)	28.8 (0.4–65.6)
hemoglobin level (g/dL) (n = 109)	10.8 (7.9–14.3)	10.7 (7.9–14.6)	10.8 (8.2–13.6)
thrombocyte count (10E9/l) (n = 109)	200.0 (75.4–353.0)	223.0 (90.0–388.0)	169.0 (45.6–312.0)
calcium total level (mmol/l) (n = 109)	2.3 (2.0–2.7)	2.3 (2.0–2.7)	2.3 (2.1–2.7)
albumin level (g/dL) (n = 108)	3.8 (2.8–4.7)	3.5 (2.5–4.5)	4.0 (3.1-4.7)
creatinine level (umol/l) (n = 108)	92.0 (57.0–395.0)	89.0 (53.0–366.0)	99.0 (61.0–395.0)
β2 microglobulin (mg/l) (n = 101)	3.7 (1.9–13.7)	3.5 (1.9–13.7)	3.8 (1.9–19.3)
LDH (ukat/l) (n = 107)	3.4 (2.1–6.5)	3.3 (2.5–6.5)	3.4 (2.1–6.4)
CRP (mg/l) (n = 107)	4.9 (1.0–111.5)	4.5 (1.0–111.5)	5.5 (0.0–63.1)

MM – multiple myeloma, CVD – cyclophosphamide, bortezomib, dexamethasone regiment, LDH – lactate dehydrogenase, CRP – C-reactive protein

[1] absolute and relative frequency for categorical variable; median, percentiles (5–95%) for continuous variable

in multiple logistic regression. Association of marker with treatment intervals was evaluated using Cox proportional hazards model. When mentioned, overall survival (OS), time to progression (TTP), progression free survival (PFS) were assessed within 2 years after diagnosis/treatment beginning; events after this period were set as censored. Kaplan-

Tab. 4. Univariate and adjusted (age and BMI) association of rs2228099 (HIF-1β) with MM and control status.

			Univariate		Age and BMI adjusted	
***HIF-1β*[1]**	**controls (n = 219)**	**MM (n = 275)**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**
rs2228099						
CC	87 (39.7%)	136 (49.5%)	reference			
CG	108 (49.3%)	110 (40.0%)	0.65 (0.45–0.95)	**0.026**	0.55 (0.30–0.99)	**0.045**
GG	24 (11.0%)	29 (10.5%)	0.77 (0.42–1.41)	0.403	0.51 (0.19–1.41)	0.194
CC	87 (39.7%)	136 (49.5%)	reference			
CG, GG	132 (60.3%)	139 (50.5%)	0.67 (0.47–0.97)	**0.031**	0.54 (0.31–0.95)	**0.032**

MM – multiple myeloma
[1] described by absolute and relative frequencies
p-values marked in bold are statistically significant

Tab. 5. Association of rs2228099 (HIF-1β) with MM and control status separately for BMI categories (</≥ 25 kg/m²).

	BMI					
	< 25 (n = 51)			**≥ 25 (n = 237)**		
	controls (n = 28)	**MM (n = 23)**	**p[2]**	**controls (n = 186)**	**MM (n = 51)**	**p[2]**
***HIF-1β*[1]**						
rs2228099						
CC	11 (39.3%)	9 (39.1%)	0.117	75 (40.3%)	30 (58.8%)	**0.018**
CG	16 (57.1%)	9 (39.1%)		89 (47.8%)	20 (39.2%)	
GG	1 (3.6%)	5 (21.7%)		22 (11.8%)	1 (2.0%)	
CC	11 (39.3%)	9 (39.1%)	1.000	75 (40.3%)	30 (58.8%)	**0.025**
CG, GG	17 (60.7%)	14 (60.9%)		111 (59.7%)	21 (41.2%)	

MM – multiple myeloma
[1] described by absolute and relative frequencies
[2] p values marked in bold are statistically significant; obtained by ML Chi-square test

-Meier curves were plotted for significant associations. Log-rank test was used to evaluate the statistical significance of difference between the curves. P-values less than 0.05 were considered statistically significant.

Joint prediction of *MDR1* and *HIF-1α* was evaluated using multivariate Cox proportional hazards model with treatment intervals as the dependent and all genetic markers as independent variable. Receiver operating characteristic (ROC) analysis was performed with the binary endpoint from OS, TTP and PFS. The results include sensitivity and specificity values with p-value for the area under the curve (AUC).

Analysis was performed in the SPSS software (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Hardy-Weinberg equilibrium and linkage disequilibrium were tested in R software (http://www.r-project.org/).

Results

SNPs in *HIF-1α* and *HIF-1β*

Genotypes of SNPs associated with hypoxia (*HIFs*) were determined in an independent cohort of 722 samples (219 cancer-free controls, 228 MGUS and 275 MM patients).

All observed genotype frequencies in controls (n = 219) conformed to the Hardy-Weinberg equilibrium (p = 0.304, 0.080 and 0.284 for rs2228099, rs11549467 and rs2057482, resp.). Linkage disequilibrium analysis between the two SNPs (n = 722; D'=0.970, r^2 = –0.053; p = 0.043 for rs11541467 vs. rs2057482) was confirmed as well. Genotype and haplotype frequencies were compared between MM and MGUS cases and controls. Frequencies of all polymorphisms in the entire cohort (n = 722) are presented in supplementary tables (Supplementary tab. 1).

Evaluation of MM patients vs. controls showed a statistically lower frequency of CG genotype in *HIF-1β* (rs2228099) in MM (40.0% in MM vs. 49.3% in controls;

OR 0.65; 95% CI 0.45–0.95; p = 0.026) in comparison to the CC genotype. Even after adjustment for patients' age and BMI, there were significantly lower odds (OR 0.55; 95% CI 0.30–0.99; p = 0.045) for MM development in patients with CG genotype in comparison to CC genotype. Further, significant results were observed also after recoding into CC vs. CG + GG genotype not adjusted for age and BMI (50.5% in MM vs. 60.3% in controls; OR 0.67; 95% CI 0.47–0.97; p = 0.031) and adjusted for age and BMI (OR 0.54; 95% CI 0.31–0.95; p = 0.032) (Table 4). However, we did not observe any significant associations for SNPs in *HIF-1α*.

Further, significant association of SNP in *HIF-1β* (rs2228099) in MM patients vs. controls for the BMI status ≥ 25 kg/m^2 (p = 0.018 for CC vs. CG vs. GG genotype; p = 0.025 for CC vs. CG + GG genotype) was observed (Tab. 5).

Association between genotypes/haplotypes and risk group of MGUS patients

MGUS patients with available clinical parameters (n = 205) were divided into four groups according to the previously described Mayo Clinic risk stratification model [30] as follows low risk (n = 74), low-intermediate risk (n = 76), high-intermediate risk (n = 46) and high risk (n = 6). Association between genotypes/haplotypes and risk group was evaluated. For SNP genotype rs2057482 (*HIF-1α*) and *HIF-1α* haplotype (rs11549467 and rs2057482), significant association with risk group was observed. When the risk groups were aggregated into "low risk" vs. "none-low risk", higher odds of "none-low risk" category for CT genotype of rs2057482 in *HIF-1α* was observed (OR 2.48; 95% CI 1.15–5.35; p = 0.021) in comparison to CC genotype. Further, comparison of "low risk" vs. "none-low risk" categories of MGUS showed higher odds of "none-low risk" category for "at least one GT" haplotype in comparison to "none GT" (OR 2.62; 95% CI 1.22–5.63; p = 0.014). Next, higher odds of "none-low risk" category for GC/GT haplotype was observed in comparison to GC/GC haplotype (OR 2.40; 95% CI 1.10–5.21; p = 0.027) (Tab. 6). However, there was no association between such

Supplementary tab. 1. Frequencies for the polymorphisms and haplotypes in studied groups (n = 722).

	Total[1] (n = 722)	Controls[1] (n = 219)	MGUS[1] (n = 228)	MM[1] (n = 275)
***HIF-1β* (rs2228099)**				
CC	325 (45.0%)	87 (39.7%)	102 (44.7%)	136 (49.5%)
CG	313 (43.4%)	108 (49.3%)	95 (41.7%)	110 (40.0%)
GG	84 (11.6%)	24 (11.0%)	31 (13.6%)	29 (10.5%)
CC	325 (45.0%)	87 (39.7%)	102 (44.7%)	136 (49.5%)
CG, GG	397 (55.0%)	132 (60.3%)	126 (55.3%)	139 (50.5%)
***HIF-1α* (rs11541467)**				
AA	2 (0.3%)	1 (0.5%)	0 (0.0%)	1 (0.4%)
GA	32 (4.4%)	7 (3.2%)	10 (4.4%)	15 (5.5%)
GG	688 (95.3%)	211 (96.3%)	218 (95.6%)	259 (94.2%)
GG	688 (95.3%)	211 (96.3%)	218 (95.6%)	259 (94.2%)
GA, AA	34 (4.7%)	8 (3.7%)	10 (4.4%)	16 (5.8%)
***HIF-1α* (rs2057482)**				
CC	579 (80.2%)	176 (80.4%)	178 (78.1%)	225 (81.8%)
CT	134 (18.6%)	39 (17.8%)	48 (21.1%)	47 (17.1%)
TT	9 (1.2%)	4 (1.8%)	2 (0.9%)	3 (1.1%)
CC	579 (80.2%)	176 (80.4%)	178 (78.1%)	225 (81.8%)
CT, TT	143 (19.8%)	43 (19.6%)	50 (21.9%)	50 (18.2%)
***HIF-1α haplotype* (rs11549467–rs2057482)**				
none GC	14 (1.9%)	6 (2.7%)	4 (1.8%)	4 (1.5%)
once GC	160 (22.2%)	44 (20.1%)	54 (23.7%)	62 (22.5%)
twice GC	548 (75.9%)	169 (77.2%)	170 (74.6%)	209 (76.0%)
at least one GC	708 (98.1%)	213 (97.3%)	224 (98.2%)	271 (98.5%)
none GT	579 (80.2%)	176 (80.4%)	178 (78.1%)	225 (81.8%)
once GT	134 (18.6%)	39 (17.8%)	48 (21.1%)	47 (17.1%)
twice GT	9 (1.2%)	4 (1.8%)	2 (0.9%)	3 (1.1%)
at least one GT	143 (19.8%)	43 (19.6%)	50 (21.9%)	50 (18.2%)
none AC	688 (95.3%)	211 (96.3%)	218 (95.6%)	259 (94.2%)
once AC	32 (4.4%)	7 (3.2%)	10 (4.4%)	15 (5.5%)
twice AC	2 (0.3%)	1 (0.5%)	0 (0.0%)	1 (0.4%)
at least one AC	34 (4.7%)	8 (3.7%)	10 (4.4%)	16 (5.8%)
GC/GC	548 (75.9%)	169 (77.2%)	170 (74.6%)	209 (76.0%)
GC/GT	131 (18.1%)	38 (17.4%)	46 (20.2%)	47 (17.1%)
GC/AC	29 (4.0%)	6 (2.7%)	8 (3.5%)	15 (5.5%)
other	14 (1.9%)	6 (2.7%)	4 (1.8%)	4 (1.5%)

MGUS – monoclonal gammopathy of undeterminated significance, MM – multiple myeloma

[1] described by absolute and relative frequencies

Tab. 6. Association of the SNPs (*HIF-1α*) and the risk stratification of MGUS patients according to MAYO model (n = 205).

	Risk			
HIF-1α[1]	**low (n = 74)**	**none low (n = 131)**	**OR (95% CI)**	**p**
rs2057482				
CC	64 (86.5%)	93 (71.0%)	reference	
CT	10 (13.5%)	36 (27.5%)	2.48 (1.15–5.35)	**0.021**
TT	0 (0.0%)	2 (1.5%)	–	**0.516**
CC	64 (86.5%)	93 (71.0%)	reference	
CT, TT	10 (13.5%)	38 (29.0%)	2.62 (1.22–5.63)	**0.014**
Haplotype (rs1154967–rs2057482)				
none GT	64 (86.5%)	93 (71.0%)	reference	
at least one GT	10 (13.5%)	38 (29.0%)	2.62 (1.22–5.63)	**0.014**
GC/GC	62 (83.8%)	88 (67.2%)	reference	
GC/GT	10 (13.5%)	34 (26.0%)	2.40 (1.10–5.21)	**0.027**
GC/AC	2 (2.7%)	5 (3.8%)	1.76 (0.33–9.37)	0.507
other	0 (0.0%)	4 (3.1%)	–	0.149

SNPs – single-nucleotide polymorphisms, MGUS – monoclonal gammopathy of undetermined significance
[1] described by absolute and relative frequencies
p-values marked in bold are statistically significant

Tab. 7. Cox regression model for overall survival from diagnosis for MM patients (n = 275).

HIF-1α	**n**	**Number of deaths**	**Hazard ratio (95% CI)**	**p**
rs2057482				
CC	225	117	reference	
CT	47	19	0.64 (0.39–1.04)	0.073
TT	3	1	0.41 (0.06–2.96)	0.379
CC	225	117	reference	
CT, TT	50	20	0.62 (0.39–1.01)	0.052
haplotype (rs1154967 and rs2057482)				
none GT	225	117	reference	
at least one GT	50	20	0.62 (0.39–1.01)	0.052
none GT	225	117	reference	
once GT	47	19	0.64 (0.39–1.04)	0.073
twice GT	3	1	0.41 (0.06–2.96)	0.379
GC/GC	209	109	reference	
GC/GT	47	19	0.63 (0.39–1.02)	0.062
GC/AC	15	7	0.70 (0.33–1.51)	0.368
other	4	2	0.75 (0.18–3.03)	0.682

MM – multiple myeloma

haplotypes and time to progression from MGUS into MM.

Association of SNP in *HIF-1α* and *HIF-1β* with progression and survival

Additionally, for MM patients, association between SNPs in *HIF-1α* and *HIF-1β* and OS or length of the treatment response were evaluated. No significant association between studied SNPs and length of treatment response was observed; however, rs2057482 SNP in *HIF-1α* and *HIF-1α* haplotype (rs11549467 and rs2057482) were associated with better OS specifically in rs2057482 CT genotype (p = 0.073); in rs11549467 and rs2057482 combination – "at least one GT haplotype" (p = 0.052), "once GT haplotype" (p = 0.073), GC/GT haplotype (p = 0.062) (Tab. 7). Log-rank test confirmed association of GT haplotype (rs11549467 and rs2057482) with better OS with median of 41.8 months (95% CI 35.1–48.5) for "none GT" and median of 93.8 months (95% CI 31.3–156.4) for "at least one GT haplotype" (p = 0.050) (Fig. 1).

Predictive value of polymorphisms associated with hypoxia and multidrug resistance in CVD treated MM patients

For this part of study, 110 MM patients treated with CVD regimen were included; all samples were collected prior to CVD treatment (Tab. 3). Association of three SNPs and their haplotypes in *MDR1* (rs1045642, rs2032582 and rs11203), one in *MRP1* (rs4148356), two in *HIF-1α* (rs11549467, rs2057482) and one in *HIF-1β* (rs2228099) with MM patient's outcome was evaluated.

Survival cut-off points were established based on time-dependent ROC analysis (data not shown), which showed suitable AUC for 2-year time period for outcome evaluation; the 2-year treatment intervals (OS, TTP, and PFS) were evaluated from the beginning of CVD treatment. Further, linkage disequilibrium analysis was performed also between three *MDR1* SNPs (n = 110; D' = 0.829, r^2 = 0.663; p < 0.001 for rs1045642 and rs2032582; D'=0.741, r^2 = –0.561; p < 0.001 for rs1045642 and

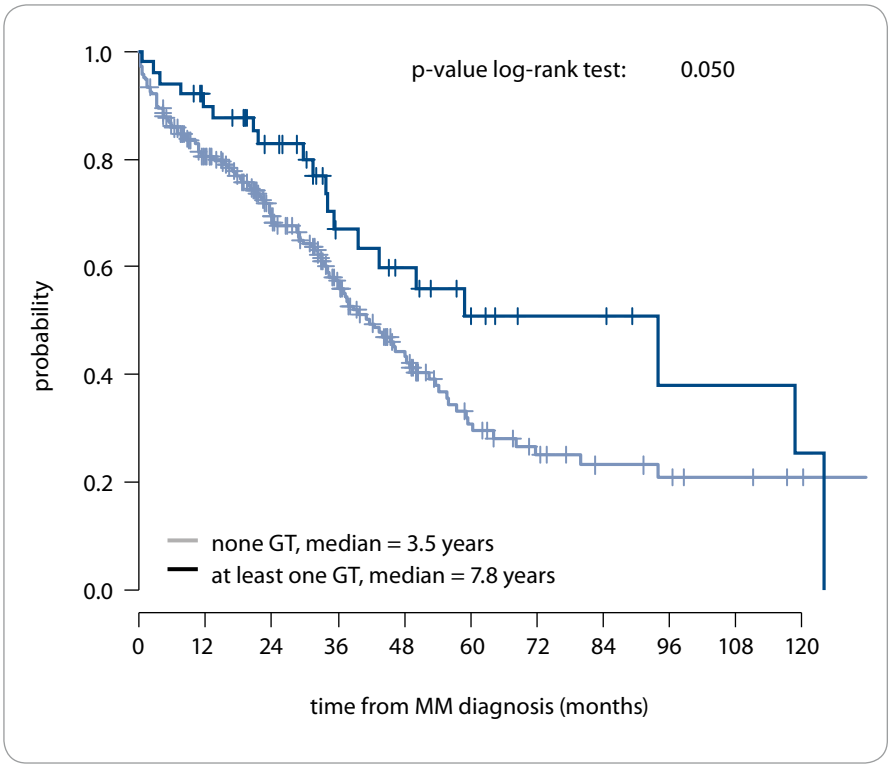

Fig. 1. Overall survival for GT haplotype rs11549467 and rs2057482 (*HIF-1α*) – "none GT" vs. "at least one GT" (n = 275) (10-year interval visualized).
MM – multiple myeloma

Tab. 8. Cox regression model for 2-year OS, TTP and PFS (n = 110).

MDR1	Total (n = 110)		New diagnosis (n = 47)		Relapse (n = 63)	
haplotypes[1]	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
TGT/CGT vs. other						
OS	2.60 (1.22–5.53)	**0.013**	3.56 (1.38–9.18)	**0.009**	1.32 (0.32–5.56)	0.702
TTP	2.05 (0.93–4.49)	0.074	1.28 (0.38–4.31)	0.689	3.62 (1.26–10.41)	**0.017**
PFS	2.45 (1.26–4.76)	**0.008**	1.97 (0.81–4.79)	0.134	3.22 (1.13–9.19)	**0.029**
at least one CWC vs. none CWC[2]						
OS	0.48 (0.27–0.85)	**0.011**	0.42 (0.18–0.97)	**0.042**	0.60 (0.26–1.39)	0.230
TTP	0.48 (0.29–0.79)	**0.004**	0.59 (0.27–1.31)	0.197	0.34 (0.17–0.67)	**0.002**
PFS	0.48 (0.30–0.76)	**0.002**	0.56(0.29–1.11)	0.096	0.38 (0.19–0.74)	**0.005**
at least one TGT vs. none TGT						
OS	1.64 (0.91–2.95)	0.098	1.83 (0.74–4.50)	0.188	1.52 (0.70–3.30)	0.291
TTP	2.07 (1.25–3.44)	**0.005**	3.27 (1.31–8.19)	**0.011**	1.50 (0.81–2.76)	0.194
PFS	1.74 (1.11–2.72)	**0.016**	2.35 (1.14–4.83)	**0.020**	1.35 (0.76–2.38)	0.305

OS – overall survival, TTP – time to progression, PFS – progression free survival
[1] for rs1045642, rs2032582 and rs1128503
[2] allele W summarises allele A or T
p-values marked in bold are statistically significant

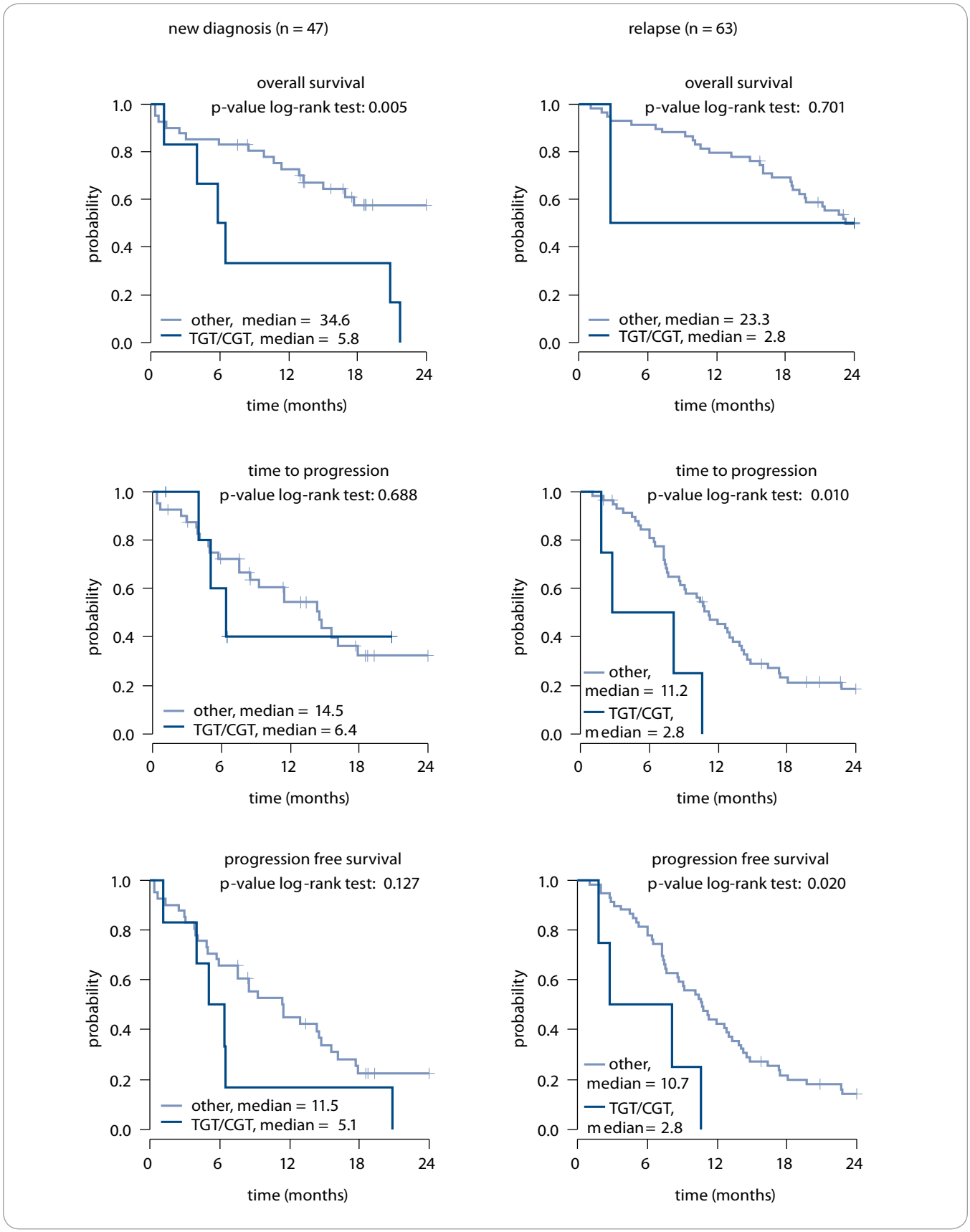

Fig. 2a. Kaplan-Meier curves for the TGT/CGT haplotype rs1045642, rs2032582 and rs1128503 (*MDR1*) which reached significance in Cox regression model.

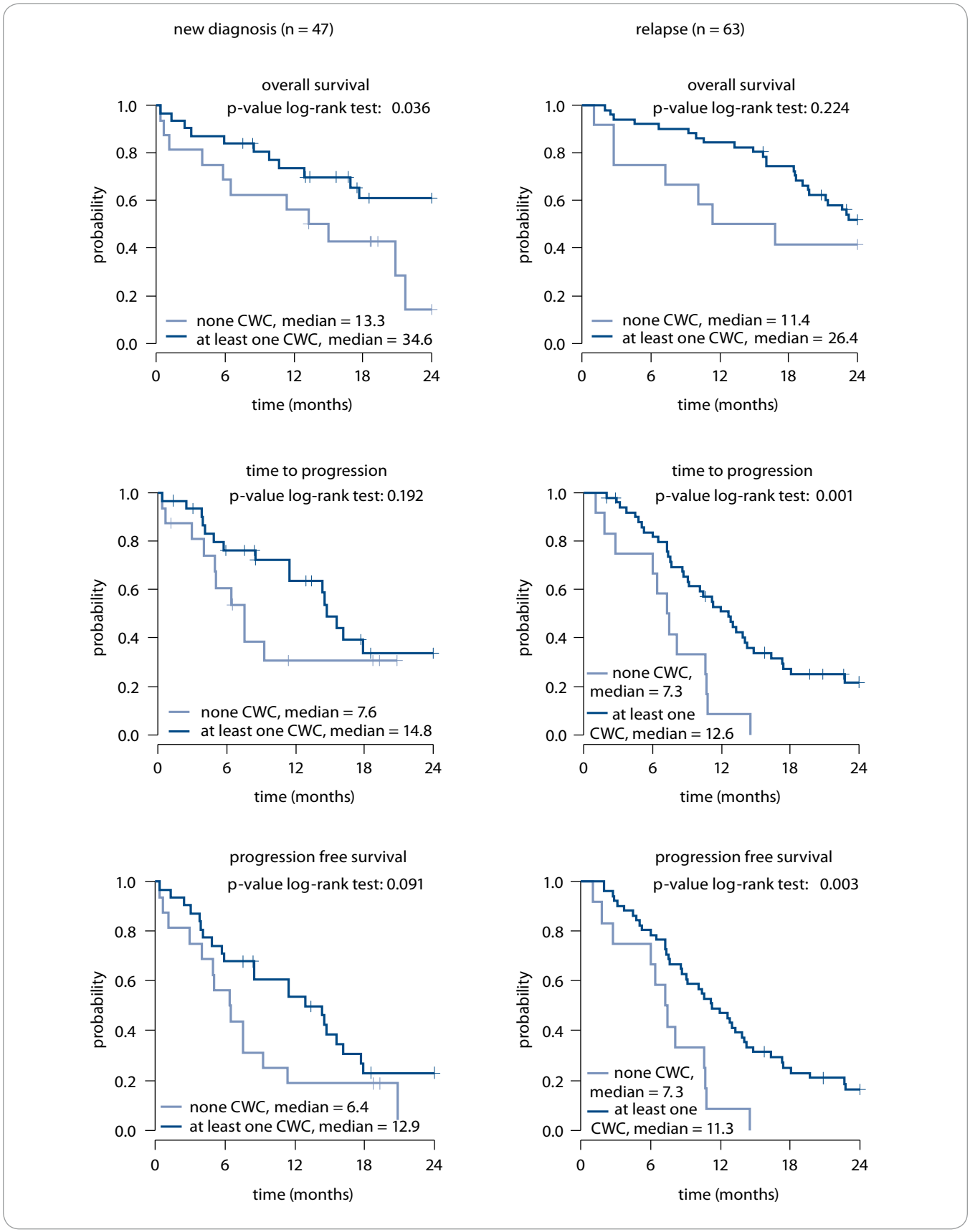

Fig. 2b. Kaplan-Meier curves for the at least one CWC haplotype rs1045642, rs2032582 and rs1128503 (*MDR1*) which reached significance in Cox regression model.

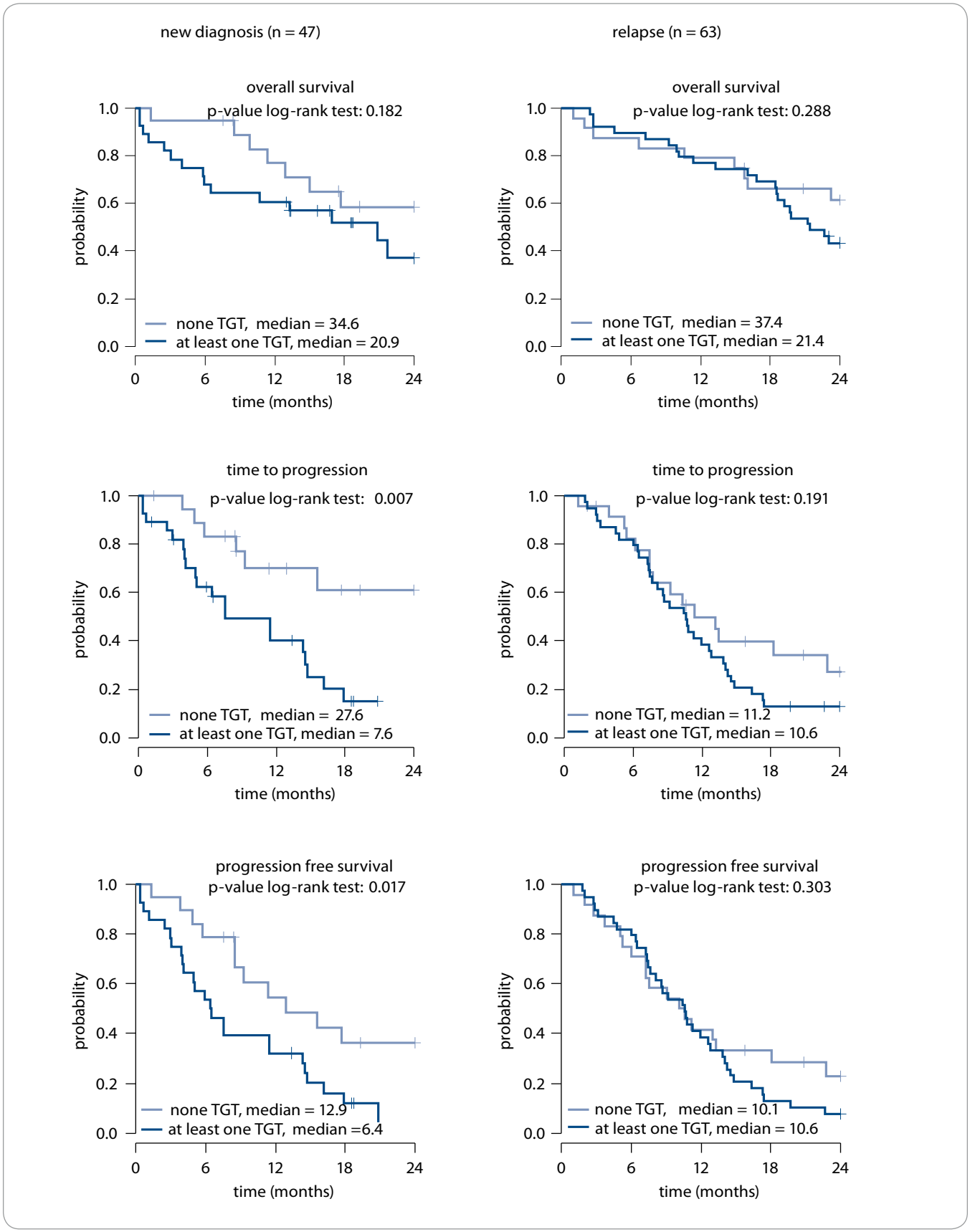

Fig. 2c. Kaplan-Meier curves for the at least one TGT haplotype rs1045642, rs2032582 and rs1128503 (*MDR1*) which reached significance in Cox regression model.

rs1128503; D' = 0.918, r^2 = –0.870; p<0.001 for rs2032582 and rs1128503). Frequencies for significant haplotypes of *MDR1* for patients treated with CVD treatment regimen (n = 110) are presented in Supplementary tab. 2. Since *MDR1* locus (rs2032582) has three alleles, the A and T allele were combined as W, so that this locus could be treated as two alleles.

Using multivariate Cox regression model with 2-year TTP as dependent variable and all SNPs in *HIF-1β*, *HIF-1α* (haplotype), *MRP1*, *MDR1* (haplotype) as predictors, only haplotypes of *MDR1* reached p-value lower than 0.1 (specifically "at least one CWC" with p-value 0.022 and "at least one TGT" with p-value 0.068). In case of OS and PFS, p-values lower than 0.1 were not observed.

In the univariate analysis, significant association with 2-year OS, TTP and PFS was observed for these haplotypes of three SNPs in *MDR1* (rs1045642, rs2032582 and rs1128503) – TGT/CGT vs. other, "at least one CWC" vs. "none CWC" and "at least one TGT" vs. "none TGT". All of the analyses were performed together and separately for the categories of newly diagnosed MM (n = 47) and relapsed MM patients (n = 63) and all of the significant associations are depicted in Tab. 8. Kaplan-Meier curves for the haplotypes of three SNPs in *MDR1* that reached significance in Cox regression model are shown in Fig. 2a, b, c.

In newly diagnosed MM patients, there was a significantly worse 2-year OS for TGT/CGT (median 5.8 months; 95% CI 2.8–8.9) compared to other haplotypes (median 34.6 months; 95% CI 11.7–57.5; HR 3.56; 95% CI 1.38–9.18; p = 0.009) (Fig. 2a). Further, in relapsed MM patients with haplotype TGT/CGT, significantly worse 2-year TTP was achieved (median 2.8 months; 95% CI 0.0–8.9) in comparison to other haplotypes (11.2 months; 95% CI = 8.6–13.7; HR 3.62; 95% CI 1.26–10.41; p = 0.017). Similarly, for this haplotype, shorter PFS was observed (median 2.8 months; 95% CI 0.0–8.9) in comparison with other haplotypes (median 10.7 months; 95% CI 8.5–12.9; HR 3.22; 95% CI 1.13–9.19; p = 0.029).

Supplementary tab. 2. Frequencies for the polymorphisms and haplotypes in MM patients with CVD treatment regimen (n = 110).

	Total[1] (n = 110)	New diagnosis[1] (n = 47)	Relapse[1] (n = 63)
***HIF-1β* (rs2228099)**			
CC	46 (41.8%)	19 (40.4%)	27 (42.9%)
CG	53 (48.2%)	21 (44.7%)	32 (50.8%)
GG	11 (10.0%)	7 (14.9%)	4 (6.3%)
***HIF-1α* (rs11549467)**			
GG	104 (94.5%)	44 (93.6%)	60 (95.2%)
GA	5 (4.5%)	2 (4.3%)	3 (4.8%)
AA	1 (0.9%)	1 (2.1%)	0 (0.0%)
***HIF-1α* (rs2057482)**			
CC	88 (80.0%)	38 (80.9%)	50 (79.4%)
CT	19 (17.3%)	8 (17.0%)	11 (17.5%)
TT	3 (2.7%)	1 (2.1%)	2 (3.2%)
***HIF-1α* haplotype (rs1154967-rs2057482)**			
GC/GC	82 (74.5%)	35 (74.5%)	47 (74.6%)
GC/GT	19 (17.3%)	8 (17.0%)	11 (17.5%)
at least one GC	106 (96.4%)	45 (95.7%)	61 (96.8%)
at least one GT	22 (20.0%)	9 (19.1%)	13 (20.6%)
***MRP1* (rs4148356)**			
GG	104 (94.5%)	44 (93.6%)	60 (95.2%)
AG	6 (5.5%)	3 (6.4%)	3 (4.8%)
***MDR1* (rs1045642)**			
CC	39 (35.5%)	16 (34.0%)	23 (36.5%)
CT	59 (53.6%)	25 (53.2%)	34 (54.0%)
TT	12 (10.9%)	6 (12.8%)	6 (9.5%)
***MDR1* (rs2032582)**			
WW	23 (20.9%)	10 (21.3%)	13 (20.6%)
GW	67 (60.9%)	26 (55.3%)	41 (65.1%)
GG	20 (18.2%)	11 (23.4%)	9 (14.3%)
***MDR1* (rs1128503)**			
TT	24 (21.8%)	15 (31.9%)	9 (14.3%)
CT	65 (59.1%)	22 (46.8%)	43 (68.3%)
CC	21 (19.1%)	10 (21.3%)	11 (17.5%)
***MDR1* haplotype (rs1045642-rs2032582-rs11285031)**			
TGT/CWC2	43 (39.1%)	15 (31.9%)	28 (44.4%)
CWC/CWC2	17 (15.5%)	7 (14.9%)	10 (15.9%)
CGT/CWC2	14 (12.7%)	5 (10.6%)	9 (14.3%)
TGT/CGT	10 (9.1%)	6 (12.8%)	4 (6.3%)
at least one CWC2	82 (74.5%)	31 (66.0%)	51 (81.0%)
at least one TGT	67 (60.9%)	28 (59.6%)	39 (61.9%)
at least one CGT	28 (25.5%)	14 (29.8%)	14 (22.2%)

MM – multiple myeloma, CVD – cyclophosphamide, bortezomib, dexamethasone regiment
[1] described by absolute and relative frequencies, [2] allele W summarises allele A or T

Tab. 9. TGT haplotype (*MDR1*) in association with 2-year binary endpoints 1. death, 2. progression or death related to diagnosis, 3. progression or death (not only MM).

At least one TGT vs.	Death[1]	Progression or death related to diagnosis[1]	Progression or death[1]
none TGT haplotype[3]			
total (n = 110)			
AUC (95% CI)	0.59 (0.48–0.69)	0.67 (0.56–0.79)	0.69 (0.56–0.82)
p2	0.121	**0.003**	**0.008**
specificity % (n)	47.4% (27/57)	62.9% (22/35)	70.0% (14/20)
sensitivity % (n)	69.8% (37/53)	72.0% (54/75)	67.8% (61/90)
new diagnosis (n = 47)			
AUC (95% CI)	0.58 (0.42–0.75)	0.69 (0.54–0.85)	0.71 (0.53–0.89)
p[2]	0.343	**0.023**	**0.036**
Specificity % (N)	48.0% (12/25)	61.9% (13/21)	72.7% (8/11)
Sensitivity % (N)	68.2% (15/22)	76.9% (20/26)	69.4% (25/36)
relapse (n = 63)			
AUC (95% CI)	0.59 (0.45–0.73)	0.67 (0.50–0.83)	0.67 (0.47–0.86)
p2	0.224	0.056	0.112
specificity % (n)	46.9% (15/32)	64.3% (9/14)	66.7% (6/9)
sensitivity % (n)	71.0% (22/31)	69.4% (34/49)	66.7% (36/54)

MM – multiple myeloma
[1] events evaluated during first two years from the beginning of the treatment
[2] p-value of AUC (area under the curve), p values marked in bold are statistically significant
[3] for rs1045642, rs2032582 and rs1128503

Using 2-year time-dependent ROC analysis, the "at least one TGT" haplotype within newly diagnosed MM reached significance in TTP and PFS (data shown in Tab. 9).

The opposite association with survival was observed for CWC haplotype, as the "at least one CWC" compared to "none CWC" haplotype showed significantly better 2-year OS for newly diagnosed MM patients (median 34.6 months; 95% CI 18.0–51.1) vs. 13.3 months; 95% CI 6.7–19.8; HR 0.42; 95% CI 0.18–0.97; p = 0.042). "At least one CWC" compared to "none CWC" also showed significantly better 2-year TTP (median 12.6 months; 95% CI 10.1–15.1; vs. 7.3 months; 95% CI 5.6–9.0; HR 0.34; 95% CI 0.17–0.67; p = 0.002) and 2-year PFS (median 11.3 months; 95% CI 8.6–13.9 vs. 7.3 months; 95% CI 5.6–9.0; HR 0.38; 95% CI 0.19–0.74; p = 0.005) in relapsed MM patients. (Figure 2b). For newly diagnosed MM patients, "at least one TGT" compared with "none TGT" haplotype was associated with significantly worse TPP (median 7.6 months; 95% CI 0.2–15.0 vs. 27.6 months; 95% CI 15.6–NA; HR 3.27; 95% CI 1.31–8.19; p = 0.011) and PFS (median 6.4 months; 95% CI 3.8–9.1 vs. 12.9 months 95% CI 4.7–21.2; HR 2.35; 95% CI 1.14–4.83; p = 0.020) (Figure 2c).

Further, the differences in clinical parameters between the cohorts of patients with different haplotypes in a group of patients treated with CVD regimen were evaluated. The "at least one CWC" haplotype compared to "none CWC" haplotype in relapsed MM is associated with lower level of calcium (median 2.3 mmol/L; 95% CI 2.1–2.7 vs. 2.5 mmol/L 95% CI 2.2–3.6; p = 0.045). Then, "at least one TGT" haplotype compared to "none TGT" haplotype is associated with worse D–S stadium in newly diagnosed MM (p = 0.015) and also "at least one TGT" haplotype compared to "none TGT" haplotype is associated with higher levels of creatinine (median 113.0 umol/L; 95% CI 64.0–447.0 vs. median 85.0; 95% CI 60.0–170.0; p = 0.020), B2M (median 4.4 mg/L; 95% CI 1.9–19.7 vs. median 2.9; 95% CI 1.9–7.5] p = 0.013) and cytological percentage of BM infiltration by pathological PCs (median 29.6; 95% CI 0.4–72.8 vs. median 20.2; 95% CI 0.0–58.4; p = 0.071) in relapsed MM.

Discussion

Observations from genome-wide association studies in MM [31], Hodgkin's lymphoma [32] and chronic lymphocytic leukemia [33] suggested that genetically determined dysregulation of protooncogenes (such as MYC) may be a common mechanism underlying predisposition to hematological malignancies of the B-cell lineage. Soon after, it was elucidated that not only loci in protooncogenes, but also in other genes or parts of chromosome, such as 3p22.1 (rs1052501 in ULK4), 7p15.3 (rs4487645) and 2p23.3 (rs6746082), are associated with risk of MM, although the last one did not reach genome-wide significance [31]. The association between 3p22.1 (rs1052501) and MM was further validated and its association with MGUS was found as well [34]. Other observations led to identification of MM risk loci at 3q26.2(rs10936599),6p21.33(rs2285803, PSORS1C2), 17p11.2 (rs4273077, TNFRSF13B) and 22q13.1 (rs877529, CBX7) and provided further evidence for genetic susceptibility to MM [35].

So far, many studies assessed association between polymorphisms in genes associated with hypoxia and cancer risk. Therefore, in this study,

we focused on genetic variations in such genes and their effects on the risk of development of monoclonal gammopathy. The most commonly investigated polymorphism associated with hypoxia is C1772T (rs11549465), followed by G1790A (rs11549467), C111A and c*191T > C (rs2057482) in *HIF-1α* [36]. C1772T (rs11549465) and G1790A (rs11549467) polymorphisms were previously associated with susceptibility to develop pancreatic cancer [37,38]. G1790A (rs11549467) polymorphism alone is associated with an increase in tumor-produced *HIF-1α* and in cancer progression [38]. Further, for C1772T (rs11549465) polymorphism, T allele was significantly associated with increased cancer risk. For G1790A (rs11549467), genotype results showed that it is significantly associated with cancers [39]. Unfortunately, in our study, we did not observe any association between these polymorphisms in HIF-1a and MM development. However, for c*191T > C (rs2057482) genotype in *HIF-1α* and *HIF-1α* haplotype (rs11549467, rs2057482), we observed a significant association with higher MGUS risk group, as defined by the MAYO model. Although a previous large meta-analysis showed significant association between *HIF-1α* C1772T (rs11549465) and G1790A (rs11549467), but not c*191T > C (rs2057482) polymorphism with increased risk of cancer [36], it can be still hypothesized that c*191T > C (rs2057482) in *HIF-1α* is associated with progression of MG, as it had been observed to contribute to risk of cervical cancer in a Chinese population [40]. Furthermore, c*191T > C (rs2057482) polymorphism is located in 3'UTR region of *HIF-1α*, which contains regulatory regions that post-transcriptionally influence gene expression, such as binding sites for regulatory proteins and microRNA [41]. Thus, we hypothesized that not only sequence changes, but also impaired regulation of *HIF-1α* expression is involved in susceptibility to MGUS transformation.

Moreover, our results suggest that genotype variation of rs2228099 polymorphism in *HIF-1β* is associated with increased risk of MM. Our data support the hypothesis of protective effect of the G allele in this SNP in MM development. Further, this polymorphism is significantly associated with MM development in a group of obese MM patients. Indeed, the number of cancer cases caused by obesity is estimated to be 20% with increased risk of malignancies influenced by diet, weight change and body fat distribution together with physical activity [42]. Insulin-like growth factor I (IGF-I) produced in adipose tissue induces synthesis of HIF-1a [43], which together with VEGF leads to neovascularization and metastases, as was shown in a colon cancer model [44]. Interestingly, body build or nutritional status may be also involved in the development of MM by various mechanisms that still need to be elucidated [45–54].

In the second part of our study, we focused on polymorphisms in *MRP1* (R723Q, rs4148356), a gene for multiple drug resistance protein, and *MDR1* (3435C > T, rs1045642; 2677G > W, rs2032582 and 1236C > T, rs1128503) encoding P-glycoprotein. There was no significant association between SNP in *MRP1* and patients outcome, which is in discordance with other observations that showed significant association with SNP in *MRP1* (rs4148356) and time to event in 279 patients with relapsed and/or refractory disease treated with bortezomib or bortezomib with pegylated liposomal doxorubicin. In this study, patients with GG genotype had better TTP (median 330 vs. 129 days; p = 0.0008), PFS (median 338 vs. 129 days; p = 0.0006) and OS (p = 0.0045) [55]. Nevertheless, we observed a significant association of patients outcome with *MDR1* haplotypes – TGT/CGT vs. "other", "at least one CWC" vs. "none CWC", "at least one TGT" vs. "none TGT" evaluated separately in the category of newly diagnosed MM (n = 47) and relapsed MM (n = 63). The "at least one CWC" compared to "none CWC" haplotype also showed significantly better 2-year TTP and PFS and is associated with lower level of calcium in relapsed MM patients. For newly diagnosed MM patients, "at least one TGT" compared with "none TGT" haplotype was associated with significantly worse 2-year TPP and PFS and D–S stadium. Moreover, "at least one TGT" haplotype compared to "none TGT" haplotype is associated with higher level of creatinine, B2 microglobulin and cytological percentage of BM infiltration by pathological PCs in relapsed MM patients, but was not associated with significantly worse 2-year patients outcome.

In a previous study of 115 MM patients treated with DAV (dexamethasone, doxorubicin (adryamicin) and vincristine) regimen followed by autologous transplantation, patients with CT and TT genotypes in *MDR1* (rs1045642) achieved longer OS in comparison with those with CC genotype (T allele carriers would show longer OS) [56]. Subsequently, the study of the most frequent haplo/diplotypes in *MDR1* (rs1045642, rs2032582) in 110 MM patients treated with DAV regimen, followed by autologous stem cell transplantation, showed that survival probability was lower for GC/GC patients (55%) than for GC/TT and TT/TT carriers [57]. In contrast to these studies, Jamroziak et al. found comparable allele and genotype frequencies among 111 MM patients and 96 controls [58]. Moreover, patients and control groups did not differ regarding *MDR1* haplotype distribution (rs1045642, rs2032582 and rs1128503), and their results do not support major influence of *MDR1* variants on the risk of MM in Caucasians. Thus, functional importance of SNPs in *MDR1* is controversial as the association between a polymorphism (rs1045642) in *MDR1* and effect of treatment showed contradictory results so far [56–61].

Taken together, we aimed to evaluate 3 SNPs associated with hypoxia in *HIF-1α* (rs11549467, rs2057482) and *HIF-1β* genes (rs2228099) and 4 SNPs associated with multidrug resistance in *MDR1* gene (rs1045642, rs2032582 and rs1128503) and *MRP1* gene (rs4148356) in MGUS and MM patients. Although our results show promising associations, further studies are needed to confirm the usefulness of these polymorphisms in risk prediction of MG and/or outcomes of MM patients that underwent bortezomib-based treatment.

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