Dysregulation of MS4A3 and PRDX5 Gene Expression in Multiple Myeloma Patients

Ilknur SUER[1], Aynur ADAY[2], Melda SARIMAN[3], Mesut AYER[4], Ipek Yonal HINDILERDEN[2], Sema Sirma EKMEKCI[5], Neslihan ABACI[5], Sukru PALANDUZ[1], Kıvanc CEFLE[1], Sukru OZTURK[1]

[1] Istanbul University, Istanbul Medical Faculty, Department of Internal Medicine, Division of Medical Genetics
[2] Istanbul University, Istanbul Medical Faculty, Department of Internal Medicine, Division of Hematology,
[3] Istinye University, Molecular Cancer Research Center
[4] University of Health Sciences, Haseki Training and Research Hospital, Hematology Clinic
[5] Istanbul University, Department of Genetics, Aziz Sancar Institute of Experimental Medicine, İstanbul, TURKEY

ABSTRACT

Multiple myeloma (MM) is a disease in which plasma cells increase clonally. We aimed to investigate the comparison of our transcriptome data in MM, MGUS (Monoclonal gammopathy of undetermined significance) and control groups in our research. Analysis of transcriptome data revealed that CD74, FUS, MS4A3, PTPN6, PRDX5 and UNC45B genes were significantly different in the MM group compared to control group. Pathway analyzes of these genes have shown that they are associated with certain pathways such as the cellular response and immunological system. In this study, we aimed to examine the expression levels of these genes among the MM (n= 50), MGUS (n= 15) and control (n= 14) groups using the Quantitative Real-Time Polymerase Chain Reaction. According to the consequence of the study, it was determined that MS4A3 gene expression decreased significantly in the MM patient group compared to MGUS and control group, while PRDX5 gene expression was significantly increased. Also ROC (Receiver Operating Characteristic) analysis showed that MS4A3 gene has a significant diagnostic power between MM and MGUS group (area= 0.727; p= 0.008). Since multiple myeloma is more common in men than in women, it was statistically evaluated whether there is no difference in gene expression between women and men. However, it was determined that there was no statistically significant difference between the groups. As a result, the MS4A3 and PRDX5 genes, which are important in various diseases such as cancer, may shed light on new treatment options for MM disease.

Keywords: Transcriptome, Multiple Myeloma, Gene Expression, MS4A3, PRDX5

INTRODUCTION

Multiple myeloma (MM) is a lymphoproliferative disease characterized by uncontrolled B lymphocyte-derived plasma cells in the bone marrow. It is the most frequent second hematological malignity (10-13%) and it is responsible for 15-20% of all the deaths related to hematological malignancies.[1-3] The malignant accumulation of plasma cells causes damage in the bone marrow, kidney and other organs. The M proteins secreted by the plasma cell clone can be detected in blood and urine.[4]

Multiple myeloma is more common in men than women (3:2) and although it commonly occurs in older ages (60-70 years), it can be seen in patients as young as 40 years old (less than 5%).[5-6] It has been reported that environmental factors, chemical factors, obesity, radiation exposure, viral factors (HIV, etc.) and/or genetic factors may play a role in etiopathogenesis. The main clinical findings are anemia, monoclonal protein in serum and urine, lytic lesions in bones, bone pain, hypercalcemia and renal failure.[7-10]

The pathways involved in the molecular mechanism of cancer and the detection of specific genes in these pathways are of great importance as they contribute to the development of new treatment strategies. Likewise, it may be possible to clarify the relationship between the new candidate genes in the pathogenesis of the disease and increase survival by developing effective target specific strategies for treatment.[11]

In this study, we aimed to compare the expression levels of CD74, FUS, MS4A3, PRDX5, PTPN6, UNC45B genes between whole bone marrow samples of multiple myeloma patients (n= 50), monoclonal gammopathy of undetermined significance (MGUS) samples (n= 15) and control samples (n= 14) (healthy bone marrow transplantation donors).

MATERIALS AND METHODS

Collection of Patient and Control Samples

Newly diagnosed and untreated MM and MGUS samples were enrolled in this study. Bone marrow samples of MM and MGUS were gathered at the time of diagnosis. While selecting MM samples among the cases with a bone marrow plasma cell level of more than 10% to be included in the study, SMM samples were excluded from the study and only MM samples were included. The MM (50 samples), MGUS (15 samples) and control groups (14 bone marrow healthy donors samples) gave written informed consent before the study by the Declaration of Helsinki. The study was approved by Istanbul Medical Faculty Clinical Research Ethics Committee (Date: 23.12.2016, No: 1521) and was carried out in Istanbul University, Medical Genetics Division of Internal Medicine Department.

Performing Pathway Analysis Using Bioinformatics Analysis Results

The previous study supported by Istanbul University Scientific Research Projects Unit and titled "Investigation of gene expressions of myeloma cells in the bone marrow of multiple myeloma patients by transcriptome analysis" contained very rich data (Grant No: 7348).[12] In the present study the transcriptome data obtained from (Grant No: 7348) were analyzed by software such as CLC Genomics Workbench (v6.01), NextGene (v2.3.2). The expression levels of all transcribed genes were determined and RPKM (Reads per kilobase of the exon model per million mapped reads) values were calculated to normalize the expression levels of these genes. 77 genes were determined by filtering RPKM values (It was filtered in 1 RPKM in patient samples and different RPKMs out of 1 value in controls) (Table 1). These 77 genes were analyzed using pathway analysis tools such as Reactome, GeneMania, Panther, Pathway Commons and KEGG. Assessment of all data revealed that the CD74, FUS, MS4A3, PRDX5, PTPN6, UNC45B were found to be expressed at significantly different levels compared to control samples. We aimed to confirm the expression levels of these genes by the qRT-PCR technique (Grant no: TDK-2017-24651).

RNA Isolation and cDNA Synthesis

Total RNA was isolated from MM, MGUS and control group samples using QIAamp RNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The purity and concentration of RNA samples were determined spectrophotometrically using NanoDrop ND-2000c (Thermo Fisher Scientific). 1 μg of total RNA was reverse transcribed with QIAGEN RT2 First Strand Kit (Qiagen) according to the manufacturer's protocol for all samples.

Quantitative Real-Time PCR (qRT-PCR)

qRT-PCR was carried out using SYBR Green Master Mix (Roche Life Science, Mannheim, Germany) in a LightCycler480-II real-time thermal cycler (Roche). Primer sequences used for qRT-PCR are provided in Table 2.[13] Gene expression data were normalized to ß-actin. Each experiment was performed in duplicate. The relative quantification analysis was done by the delta-delta-Ct method.[14]

Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS version

Table 1. Comparison RPKM values of MM patient and control group

Gene name	RPKM value Control Group	RPKM value Patient Group	Gene name	RPKM value Control Group	RPKM value Patient Group	Gene name	RPKM value Control Group	RPKM value Patient Group
UNC45B	252.54	1	ATG16L1	2.66	1	DTWD2	0.43	1
RPS14	110.91	1	PID1	2.64	1	ARCN1	0.43	1
RPL13	66.19	1	REPIN1	2.48	1	NEK6	0.41	1
LCN2	55.4	1	LDHB	2.32	1	C1orf151	0.4	1
RPL28	46.63	1	COMMD9	2.28	1	GYG1	0.36	1
FUS	36.23	1	PSAP	2.23	1	MAP3K3	0.35	1
CITED2	27.29	1	PHKG2	2.03	1	ZNF667	0.34	1
PRDX5	24.58	1	SH2D3C	1.93	1	KIAA1715	0.34	1
RPL32	21.62	1	EDEM2	1.63	1	RGS12	0.31	1
CD74	21.09	1	TMEM41B	1.53	1	DGKG	0.3	1
HNRNPA2B1	20.42	1	NFKBIZ	1.18	1	THUMPD2	0.3	1
PTPN6	18.58	1	ARFGAP3	1.07	1	RIN3	0.22	1
RCC1	17.26	1	C5orf34	0.92	1	GOLGA3	0.22	1
HMGB2	17	1	C14orf43	0.91	1	DIDO1	0.22	1
PKM2	15.74	1	OLA1	0.81	1	TMCC1	0.21	1
MS4A3	15.31	1	IKZF2	0.77	1	NLRP5	0.21	1
NSUN5	10.27	1	PTK2B	0.71	1	SMEK2	0.19	1
GLUL	8.69	1	PIK3R1	0.69	1	GALNT14	0.17	1
ATP2A3	8.36	1	UBE2E3	0.63	1	GIGYF2	0.16	1
HNRNPK	8.3	1	SPAG1	0.62	1	CNOT1	0.12	1
HDGF	7.57	1	MTL5	0.59	1	CLTCL1	0.12	1
ADRA2C	6.66	1	WAC	0.59	1	MAP4K4	0.07	1
HFM1	6.16	1	C13orf18	0.57	1	ARID1B	0.06	1
CLEC11A	5.49	1	BAZ1A	0.53	1	ZFHX3	0.05	1
EIF4G2	4.35	1	PHACTR2	0.53	1	PARK2	0.01	1
HNRNPL	4.09	1	CENPN	0.49	1			

RPKM= reads per kilobase of the exon model per million mapped reads; MM= Multiple myeloma

Table 2. Sequences of primer pairs used in qRT-PCR study

Primer Name	Primer sequence	Base Reference	
CD74- F	5'-TGTGATGCACCTGCTCCAG-3'	19	*
CD74- R	5'-GGAAGCTCCCCTTCAGTGG-3'	19	*
FUS- F	5'-TTCCCAGCAGAGCAGTCAG-3'	19	*
FUS- R	5'-TGGGGAGTTGACTGAGTTCC-3'	20	*
MS4A3- F	5'-TGTCTACCAGCCCATAGATGG-3'	21	*
MS4A3- R	5'-TGGTATGGGTATTGCAAGGAAC-3'	22	*
PRDX5- F	5'-GTTCAAGGGCAAGAAGGGTG-3'	20	*
PRDX5- R	5'-CCACCTGGACTCCCTTGG-3'	18	*
PTPN6- F	5'-GTCATCGTCATGACCACCC-3'	19	*
PTPN6- R	5'-CAGAGTAGGGCCCATAAGCA-3'	20	*
UNC45B- F	5'-CTCAAGGTGGTGGCAAGG-3'	18	*
UNC45B- R	5'-GATTGGAGACAGCAGCGATC-3'	20	*
ßeta actin- F	5'-GCCTCGCCTTTGCCGATC-3'	18	[13]
ßeta actin-R	5'-CCCACGATGGAGGGGAAG-3'	18	[13]

** It refers to primers designed for this project using various internet programs such as Ensemble, Primer Blast, SNP Check, UCSC in silico PCR, Oligo analyzer (IDT).*

Table 3. Clinico-pathological information of the patients (MM) and features of MGUS and control samples

MM samples	Age	≤ 60	16
		> 60	34
	Gender	Female	18
		Male	32
	Multiple myeloma type	IgA/Kappa Multiple Myeloma	5
		IgA/Lambda Multiple Myeloma	3
		IgG/Kappa Multiple Myeloma	10
		IgG/Lambda Multiple Myeloma	8
		Ig D/Kappa Multiple Myeloma	1
		Ig D/Lambda Multiple Myeloma	1
		Kappa Light Chain Myeloma	5
		Lambda Light Chain Myeloma	3
		NA	14
	Bone marrow plasma cell ratio	10-30%	31
		30-60%	13
		>60%	6
	Durie Salmon's Stage	Stage 1A	17
		Stage 1B	2
		Stage 2A	18
		Stage 2B	10
		Stage 3A	1
		NA	2
	Cytogenetic and molecular characteristics	46,XX	15
		46,XY	22
		46XX/ Hyperdiploidy	1
		46,XY and iFISH-normal	4
		46,XY and iFISH-monosomy 8, del13q14.3	1
		iFISH- del 13q14	1
		t(8;14) (q24;q32), t(4;14) (p16;q32), 46,XX/ Hypodiploidy	1
		Cyclin D1 +	4
		Cyclin D1 -	1
MGUS samples	Age	≤ 60	3
		> 60	12
	Gender	Female	9
		Male	6
	Bone marrow plasma cell ratio	1-4%	9
		5-9%	6
Control samples	Age	≤ 60	12
		> 60	2
	Gender	Female	6
		Male	8

NA= stands for not available

21, SPSS Inc, Chicago, IL, USA). In the statistical analysis, the Kruskal-Wallis test was used to compare gene expression between MM patients, MGUS and control groups. The data were plotted as a box plot and the results of changes were shown as median, lower quartile and upper quartile (Q1 and Q3). To determine groups in which differences in expression were statistically significant, a post hoc Tukey test was performed. The statistical significance was accepted when p values were less than < 0.05. Spearman's rho Correlation Test was used to evaluate whether or not gene expressions are related to each other. ROC (Receiver Operating Characteristic) analysis was performed to determine the discriminating power between the MM-MGUS group, MM-control group and MGUS-control group. SPSS 21 and Graph Pad Prism 5 programs were used in the graphical drawings.

Table 4. Median. lower (Q1) and upper (Q3) quartile of gene expression data

		MM Group	MGUS Group	Control Group	p value
CD74	Median	8.76	11.78	9.36	p= 0.212
	Min (Q1)	1.00	4.31	2.16	
	Max (Q3)	33.13	242.50	240.50	
		MM Group	**MGUS Group**	**Control Group**	**p value**
FUS	Median	8.61	10.53	8.93	p= 0.521
	Min (Q1)	1.89	5.45	1.03	
	Max (Q3)	30.59	91.34	27.10	
		MM Group	**MGUS Group**	**Control Group**	**p value**
MS4A3	Median	18.44	35.91	35.33	p= 0.014
	Min (Q1)	1.00	3.30	5.68	
	Max (Q3)	151.30	121.30	431.10	
		MM Group	**MGUS Group**	**Control Group**	**p value**
PRDX5	Median	3.25	1.77	1.97	p= 0.004
	Min (Q1)	1.02	1.03	1.18	
	Max (Q3)	14.45	22.76	6.64	
		MM Group	**MGUS Group**	**Control Group**	**p value**
PTPN6	Median	2.89	2.75	2.54	p= 0.932
	Min (Q1)	1.03	1.20	1.49	
	Max (Q3)	10.06	16.51	22.47	
		MM Group	**MGUS Group**	**Control Group**	**p value**
UNC45B	Median	10.71	17.00	16.10	p= 0.724
	Min (Q1)	1.00	2.77	1.82	
	Max (Q3)	79.62	430.50	464.10	

RESULTS

Fifty (18 female, 32 male) newly diagnosed and untreated multiple myeloma (MM) patients (mean age 64.16 ± 1.55 years) were prospectively enrolled in this study. The MGUS group consisted of 15 samples (9 female, 6 male) (mean age 65.66 ± 3,75). The control group consisted of 14 samples (6 female, 8 male) (mean age 50 ± 4.78 years). The main features of the MM, MGUS and control groups are summarized in Table 3. Shapiro-Wilk normality test was used to determine whether the variables examined had a normal distribution. In the absence of normal distribution, a non-parametric alternative Kruskal-Wallis Test was applied instead of the ANOVA Test. The results of changes in CD74, FUS, MS4A3, PRDX5, PTPN6 and UNC45B levels in analyzed groups are shown as median, minimum and maximum value Table 4. The relative expression levels of the CD74, FUS, MS4A3, PRDX5, PTPN6 and UNC45B genes and the expression levels of the β-actin gene (as the reference control) were determined.

The ΔΔCt value was calculated and normalized by using the Ct (cycle threshold) data for relative quantification. As a result, the expression of MS4A3 and PRDX5 genes were significantly different in the MM group than in the MGUS group and control group (respectively, p= 0.014 and p= 0.004) (respectively, 1.9 fold decrease and 1.6 fold increase). However, no statistically significant differences were found in CD74, FUS, PTPN6 and UNC45B gene expressions between the multiple myeloma group, MGUS group and control group (respectively, p= 0.212; p= 0.521; p= 0.932; p= 0.724) (Figure 1). Since multiple myeloma is more common in men than in women, it was statistically evaluated whether there is no difference in gene expression between women and men. However, it was determined that there was no statistically significant difference between the groups. Also we compared MM cases with bone marrow plasma cells (BMPCs) ≤ 30% and > 30% for MS4A3 and PRDX5 gene expression levels. Among BMPCs ≤ 30% MM, BMPCs > 30% MM, MGUS and con-

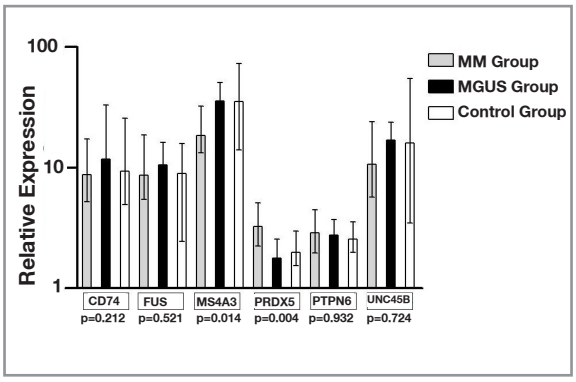


Figure 1. Comparison of median values of CD74, FUS, MS4A3, PRDX5, PTPN6, UNC45B genes expressions in MM, MGUS and control groups

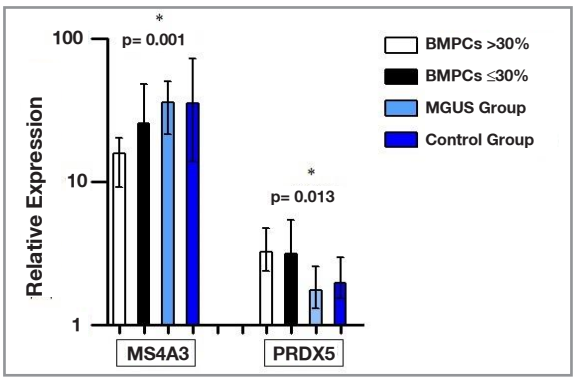


Figure 2. Comparison of median values of MS4A3 and PRDX5 gene expressions between BMPCs ≤30% MM, BMPCs >30% MM, MGUS and control groups

trol groups, the expression changes were found to be statistically significant (p= 0.0015 and p= 0.01 respectively) (Figure 2).

Spearman's correlation coefficients were calculated and the correlation coefficient between MS4A3 and PRDX5 genes was found to be 'r= 0.655' in the MM patient samples (p= 0.000). There was a moderate monotonic correlation between these two genes. There was no significant correlation between these genes for the MGUS samples (r=-0.121; p= 0.666) and control samples (r= 0.255; p= 0.379).

For the MS4A3 and PRDX5 genes which were found to be significantly (p< 0.05) different between the MM, MGUS and control samples in terms of expression, ROC analysis was performed to determine the discriminating power of the test for multiple myeloma. According to the findings obtained by the ROC analysis, MS4A3 gene has a significant diagnostic power between MM and MGUS group (area= 0.727; p= 0.008). Also PRDX5 gene doesn't have a diagnostic power between the MM group-control group (area= 0.280; p= 0.012) and MM group-MGUS group (area= 0.279; p= 0.010).

DISCUSSION

Multiple myeloma (MM) is a clonal B-cell malignancy in the group of plasma cell dyscrasias. The main features of the disease include the accumulation of malignant plasma cells in the bone marrow, excess monoclonal paraprotein in serum/urine, hypercalcemia, renal impairment, lytic bone lesions and anemia.[15]

In cancer therapy, it is not enough to target only cancerous cells but the targeting of micro-environmental changes is also required. Multiple myeloma is one of the diseases that require the consideration of the effects of the bone marrow microenvironment in addition to plasma cells. Therefore, recent research has focused on candidate genes that can be a target for the treatment of multiple myeloma.[16]

In this study, the expression levels of CD74, FUS, MS4A3, PRDX5, PTPN6 and UNC45B genes were compared between multiple myeloma group, MGUS group and control group in whole bone marrow aspirate material. As a result, no statistically significant difference was found in the CD74, FUS, PTPN6 and UNC45B genes between the multiple myeloma group, MGUS group and the control group. However, the MS4A3 and PRDX5 genes were significantly lower and higher expression levels in the MM group than the MGUS group and control group (respectively p= 0.014 and 1.9 fold decrease; p= 0.004 and 1.6 fold increase).

The results of the transcriptome study showed that the expression of the PRDX5 gene in the MM group was lower compared to the control group, whereas the data obtained from the qRT-PCR result was increased in the MM group compared to the MGUS group and the control group. The results of the transcriptome study showed that the expression of the MS4A3 gene in the MM group was lower

compared to the control group and the data obtained from the qRT-PCR result validated this data. In the transcriptome study, bone marrow plasma cells were sorted by flow cytometry and because of the small sample size, the study was carried out by pooling.[12] However, in this study, the plasma cells could not be sorted by flow cytometry and for this reason, the total RNA material obtained from whole bone marrow aspirate was used for confirmation. Although according to the transcriptome data PRDX5 gene was found to be expressed at a lower level in MM group than in the control group, this may be another reason why the expression of the PRDX5 genes is higher in patients in qRT-PCR study.

In our study, the expression of PRDX5 (which has been reported to have a role in the protection of cancer cells against apoptosis) was also found to be increased in the MM group compared to the MGUS group and control group. The findings of our study are the first study for the use of total RNA obtained from whole bone marrow aspirate in multiple myeloma. PRDX5 is a member of the peroxiredoxin family belonging to a group of antioxidant enzymes which are involved in important cellular processes such as apoptosis, cell differentiation and proliferation. This gene encodes an antioxidant enzymatic product that plays a role in oxidizing or detoxification of oxidants such as hydrogen peroxide, nitric acid, hydroxyl, peroxyl, hydroperoxyl, etc.[17] PRDX5 is known to protect the cell against both intracellular and extracellular stress and is involved in immune regulation by eliminating hydrogen peroxide.[18] Aberrant expression of the PRDX5 gene, which is expressed in all mammalian tissues, has been associated with various disorders.[19] In the literature, when the multiple myeloma cell line IM9 cells were exposed to the Epigallocatechin-3-gallate (EGCG) antioxidant, the ROS production (which causes oxidative stress in the cells) was found to be increased. In this way, it had been aimed to increase apoptosis. In that study, it was found that the expression level of PRDX5 decreased in the cells while the EGCG dose was increased. Accordingly, it had been suggested that the anticancer effects of EGCG significantly target PRDX5.[20] It is important to perform functional studies about PRDX5 in multiple myeloma since increased PRDX5 in patients with multiple myeloma may be associated with decreased apoptosis. We think that it is important to elucidate the role of the PRDX5 gene in multiple myelomas, such as other studies in cancer pathogenesis.[21,22]

In our study, the expression of MS4A3 was found to be decreased in the MM group compared to the MGUS group and control group. MS4A proteins are reported to play a role in processes such as cellular growth, survival, differentiation, and the formation of various neoplasms.[23] MS4A3 gene, a member of the MS4A family, has been reported to affect the cell cycle by contributing to signal transduction.[23,24] It has been reported that the expression of the MS4A3 gene, which is expressed in hematopoietic cells and tissues, increases in the hematopoietic stem cell differentiation process.[25,26] In the normal cell proliferation process, MS4A3 (HTm4) regulates the cell cycle to remain in the G0 / G1 phase, preventing excessive phosphorylation of cyclin-dependent kinase 2 (CDK2). It directly modulates the phosphorylation level of CDK2 by binding directly to the cyclin-dependent kinase inhibitor (as CDKN3/KAP). Cyclins and cyclin-related kinases are known to cause cancer through uncontrolled cell growth.[27] It has been reported that targeting various immunotherapeutically activated tumor-associated macrophages with MS4A4A antibody therapy in diseases such as multiple myeloma and mantle cell lymphoma maybe has potential importance.[28] As a result of the gene expression profiling study performed on the U937T human myeloid cell line, decreased expression of the MS4A3 gene has been found. The MS4A3 gene is targeted directly by an oncogene (EVI1 gene). The EVI1 gene has increased expression in hematological malignancies and solid tumors despitenMS4A3 has downregulated expression. This has been reported to play a role in EVI1-mediated aggressive tumor progression.[29] Decreased expression of MS4A3 may be important for multiple myeloma, as it is a gene that has been reported to control the activity of cyclin-dependent kinases and is targeted by the EVI1 oncogene.

The data obtained from next-generation techniques such as whole-exome sequencing and RNA transcriptome studies have the potential to pioneer new strategic developments in treatment and this

can lead to better clinical management of the disease.[30] Cancer-specific markers are informative in the clinical setting at various stages, including the diagnosis of the disease and treatment follow-up. The identification of these markers is also very important for the development of new therapeutic approaches.[31]

CONCLUSION

In conclusion, considering the functions of MS4A3 and PRDX5 genes and their importance in cancer, we suggest that these genes may shed light on new treatment options for multiple myeloma.

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Correspondence:
Dr. Sukru OZTURK
Istanbul Universitesi, Istanbul Tip Fakultesi
Ic Hastaliklari Anabilim Dali
Capa / Fatih
ISTANBUL / TURKEY

Tel: (+90-545) 432 97 58
(+9-0212) 414 20 00/ 33512
e-mail: sozturk@istanbul.edu.tr

ORCIDs:

Ilknur Suer	0000-0003-1954-4190
Aynur Aday	0000-0001-8072-0646
Melda Sariman	0000-0003-0898-529X
Mesut Ayer	0000-0003-1977-0104
Ipek Yonal Hindilerden	0000-0003-1353-2367
Sema Sirma Ekmekci	0000-0002-1201-7542
Neslihan Abaci	0000-0002-9962-4010
Sukru Palanduz	0000-0002-9435-009X
Kivanc Cefle	0000-0002-9420-4543
Sukru Ozturk	0000-0002-8809-7462