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Common gene variants within 3′-untranslated regions as modulators of multiple myeloma risk and survival

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Abbreviations: 3′UTR, 3′-untranslated region; ANOVA, analysis of variance; CEU, Caucasian population; CI, confidence intervals; ECACC, European Collection of Cell Cultures; EQTL, expression quantitative trait loci; GERP, Genomic Evolutionary Rate Profiling; GWAS, genome-wide association studies; HWE, Hardy-Weinberg equilibrium; IMMEnSE, International Multiple Myeloma rESEarch; IMWG, International Myeloma Working Group; LD, linkage disequilibrium; MAF, minor allele frequency; MM, multiple myeloma; OR, odds ratios; p3UTR, polymorphic 3′-UTRs; RFP, red fluorescent protein; RFU, relative fluorescence units; SNPs, single nucleotide polymorphisms.

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Abstract

We evaluated the association between germline genetic variants located within the 3′-untranlsated region (polymorphic 3′UTR, ie, p3UTR) of candidate genes involved in multiple myeloma (MM). We performed a case-control study within the International Multiple Myeloma rESEarch (IMMEnSE) consortium, consisting of 3056 MM patients and 1960 controls recruited from eight countries. We selected p3UTR of six genes known to act in different pathways relevant in MM pathogenesis, namely *KRAS* (rs12587 and rs7973623), *VEGFA* (rs10434), *SPP1* (rs1126772), *IRF4* (rs12211228) and *IL10* (rs3024496). We found that *IL10*-rs3024496 was associated with increased risk of developing MM and with a worse overall survival of MM patients. The variant allele was assayed in a vector expressing eGFP chimerized with the *IL10* 3′-UTR and it was found functionally active following transfection in human myeloma cells. In this experiment, the A-allele caused a lower expression of the reporter gene and this was

also in agreement with the in vivo expression of mRNA measured in whole blood as reported in the GTEx portal. Overall, these data are suggestive of an effect of the *IL10*-rs3024496 SNP on the regulation of *IL10* mRNA expression and it could have clinical implications for better characterization of MM patients in terms of prognosis.

KEYWORDS

3′-untranslated region, multiple myeloma, overall survival, risk, single nucleotide polymorphisms, susceptibility

1 | INTRODUCTION

Multiple myeloma (MM) is a plasma cell malignancy originating from the bone marrow. The incidence of this hematological neoplasm is rising worldwide, and despite improvements of therapeutic treatments, it remains incurable. Immunodeficiency and exposure to viruses or chemical agents are considered potential risk factors.[1] Convincing evidences suggest that MM has a solid genetic background, given the increased risk (from 2-fold to 4-fold) in first-degree relatives of MM patients.[2] Genome-wide association studies (GWAS) have allowed a better understanding of the genetic component of MM susceptibility and survival, leading to the identification of several *loci*, some of which are involved in complex biological process such as cell proliferation, cell cycle and DNA repair.[3-9]

The 3′-untranslated region (3′-UTR) of genes plays a crucial role in regulating the diverse fate of mRNAs and therefore in determining the phenotypic diversity.[10] For example, single nucleotide polymorphisms (SNPs) within 3′-UTRs have been shown to affect miRNA-binding sites[11] or stability[12] of mRNAs, thereby modulating the rate of translation. More in general, polymorphic 3′-UTRs (p3UTR) could affect gene expression with a variety of phenotypic effects, including a differential predisposition to cancer or a prolonged survival of cancer patients, as it has been shown for glioma, and for breast, gastric, renal and colorectal carcinoma.[13-15]

In the present study, we extended the investigation to a set of candidate genes (namely *KRAS*, *VEGFA*, *SPP1*, *IRF4*, and *IL10*) involved in pathways relevant for MM pathogenesis, such as apoptosis, B cell differentiation, bone resorption and immunoglobulin production. To this end, we performed a case-control association study within the framework of the International Multiple Myeloma rESEarch (IMMeNSE) consortium, and we found that the p3UTR of *IL10* was associated with MM risk and prognosis. We also performed in vitro assays to better characterize the effects of the p3UTR on *IL10* gene expression.

What's new?

The polymorphic 3′-untranslated region (p3UTR) has been shown to affect gene expression and lead to differential predisposition or prolonged survival in a variety of cancer types. Here, the authors evaluate the association between germline genetic variants within the p3UTR of candidate genes involved in multiple myeloma. The results show that *IL10*-rs3024496 might affect immune homeostasis through the modulation of *IL10* mRNA expression. Overall, the findings suggest that the inclusion of personal genetic background information into the clinical evaluation criteria could help to improve the stratification of patients with multiple myeloma in terms of risk progression and prognosis.

2 | MATERIALS AND METHODS

2.1 | Study population

The study population consisted of 3056 MM and 1960 controls and patients recruited from eight countries in the context of the International Multiple Myeloma rESEarch (IMMEnSE) consortium (Table 1).[16] Patients were defined by a confirmed diagnosis of MM, according to International Myeloma Working Group (IMWG) criteria. Controls were recruited in the same geographical area of the patients, among blood donors and hospitalized volunteers with diagnoses excluding cancer. For every subject enrolled in the study, information on sex and age at recruitment/diagnosis was collected. For patients, also clinical and pathological characteristics, including disease stage (Durie-Salmon and/or International Staging System), and the type of first-time therapy were retrospectively collected from medical.

2.2 | SNP selection criteria

SNPs were selected through a gene candidate approach in order to pick the ones involved in the most important biological function that are deregulated in MM: apoptosis, B cell differentiation, bone resorption and immunoglobulin production.[1]

In order to identify those genes, the workflow of our study started with the candidate gene selection and was performed using three data mining search tools: Coremine (https://www.coremine.com/), SNPs3d (http://www.snps3d.org/) and Gene Prospector.[17] Each tool adopts a different algorithm to mine information from databases and literature repositories and to connect gene or protein names to keywords of interest. We then intersected the results

TABLE 1 Characteristics of IMMEnSE cases and controls

	Cases	Controls
Geographic origin		
Italy	298	228
Poland	1259	350
Spain	283	324
France	502	176
Portugal	152	195
Hungary	155	101
Denmark	299	489
Israel	108	97
Total	3056	1960
Median age (25%-75% percentiles)	62 (55-68)	52 (41-64)
Sex		
Males	51.8%	52.0%
Females	48.2%	48.0%
Disease stage		
Durie-Salmon[a]		
1	200	—
2	360	—
3	906	—
Total	1466	—
Disease stage ISS[a]		
1	378	—
2	383	—
3	425	—
Total	1186	—
First-line therapy[b]		
New	716	—
Old	747	—
Total	1463	—
Median overall survival (25%-75% percentiles)	39 (20.5-69.47)	

[a]The sum does not add up to the total of subjects due to missing data.
[b]New therapies are those based on proteasome inhibitors and/or immunomodulating drugs; old therapies are all others.

obtained from the three tools and assembled a list of 52 candidate genes. Next, we selected SNPs within the 3′-UTR of the candidate genes with minor allele frequency (MAF) higher than 5% in the Caucasian (CEU) population of the 1000 Genomes project. We further selected the most conserved SNPs, using three tools: jSNPSelector (http://jsnpselector.sourceforge.net, a tool developed at the Department of Biology, University of Pisa, based on the alignment of 46 placental mammal genomes in the Ensembl database), GERP (Genomic Evolutionary Rate Profiling, http://mendel.stanford.edu/SidowLab/downloads/gerp/index.html) and SiPhy-omega (https://www.broadinstitute.org/mammals-models/29-mammals-project-supplementary-info). We chose for genotyping the top six SNPs located in five different genes, selecting only the SNPs resulting conserved in all methods (*KRAS*-rs12587, *VEGFA*-rs10434, *SPP1*-rs1126772, *KRAS*-rs11047885, *IL10*-rs3024496 and *IRF4*-rs12211228), as reported in Supplementary Table 1.

2.3 | SNP genotyping and quality control

Genomic DNA was extracted from peripheral blood of MM patients and controls using the QIAampR 96 DNA QIAcubeR HT Kit. Genotyping was performed using the TaqMan (Applied Biosystems) technique in 384-well format plates, with 10 ng of DNA from each subject. The order of DNAs of patients and controls was randomized on plates in order to ensure that an equal number was analyzed simultaneously. Eight percent of the samples were duplicated for quality control purposes.

2.4 | In silico analysis

For *rs3024496*, we performed bioinformatics analysis in order to gain insight on its functional role. Specifically, we used LDlink (https://ldlink.nci.nih.gov/) to identify SNPs in LD with *rs3024496* and RegulomeDB (http://regulome.stanford.edu) to identify the regulatory potential of the resulting SNPs. Finally, we used GTEx portal (https://gtexportal.org/) in order to identify potential associations between the SNPs and expression levels of nearby genes (eQTL) (Supplementary Table 2).

2.5 | Gene reporter expression assay

The human myeloma U266B1 (RRID: CVCL_0566) cell line was obtained from the European Collection of Cell Cultures (ECACC). The U266B1 cell line has been authenticated using STR profiling within the last 3 years. The cells were grown in RPMI-1640 medium supplemented with 15% heat-inactivated FBS, 100 U/mL penicillin and 100 U/mL streptomycin (all from Euroclone S.p.A.). All cells were kept at 37°C in a constant humidified 5% CO_2 atmosphere. All experiments were performed with mycoplasma-free cells.

For the functional study, we employed the IL10 3′-UTR_GFP vector (Applied Biological Materials Inc.) harboring the common allele of rs3024496 within the 3′-UTR of the human *IL10* gene downstream of a green fluorescent protein (GFP) coding sequence. Site-directed mutagenesis was carried out on the 3′-UTR-IL10_GFP in order to obtain a vector carrying the rare variant of the rs3024496 A>G (from now on referred to as GFP_IL10_A and GFP_IL10_G, respectively). The mutagenesis was performed using QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent Technologies), following the manufacturer's instructions.

To evaluate the functional role of the selected SNP, 3×10^6 cells were electroporated with 7 μg of a control vector expressing a red fluorescent protein (RFP) (HR410PA-1; SBI) and with 14 μg of either

the GFP_IL10_A or GFP_IL10_G. The electroporation was performed using Neon Transfection System (Invitrogen in Life Technologies) following these specific electroporation parameters: voltage, 1450 V; pulse width, 10 ms; pulse number, 3. Fluorescence intensity was measured at single-cell level 48 hours after the electroporation assay, using BD FACS Jazz System (BD Biosciences). All experiments were replicated independently three times.

2.6 | Statistical analysis

Departure from Hardy-Weinberg equilibrium (HWE) was tested among IMMEnSE controls in each population. Analysis of association between SNPs and MM risk was performed with unconditional logistic regression, adjusting for a set of covariates including age (at diagnosis for MM cases, at recruitment for controls), sex and country of origin. The association between SNPs and MM risk was calculated by estimating odds ratios (OR) and their 95% confidence intervals (CI). For all the SNPs, we performed a statistical analysis using the allelic and codominant models, setting the more common allele as reference and a $P < .0042$, calculated with the formula 0.05/12 (6 SNPs × 2 models), as the Bonferroni-corrected threshold for statistical significance.

Survival analysis was performed with Cox regression, calculating hazard ratios (HR) and 95% CI using overall survival (OS) as end point. OS was defined as the time interval between MM diagnosis and death or the last date of follow-up. This analysis was adjusted by age, sex, country of origin, disease stage and type of first-line therapy.

For the in vitro experimental validation, the statistical analysis was limited to the cells that were efficiently transfected with both

TABLE 2 Associations between selected SNPs and MM risk in the IMMEnSE consortium[a]

Gene	SNP	Genotype	Cases	Controls	OR (95% CI)	*P* value	*P* trend
IL10	rs3024496	G	2541	1727	1 (–)	—	**.008**
		A	3357	2029	1.05 (0.96-1.15)	.298	
		G/G	551	424	1 (–)	—	
		G/A	1439	879	**1.20 (1.01–1.44)**	**.042**	
		A/A	959	575	**1.24 (1.02-1.50)**	**.028**	
SPP1	rs1126772	A	4607	2248	1 (–)	—	.227
		G	1267	628	1.01 (0.90-1.13)	.873	
		A/A	1810	877	1 (–)	—	
		A/G	987	494	1.08 (0.92-1.26)	.333	
		G/G	140	67	1.19 (0.84-1.68)	.322	
IRF4	rs12211228	G	5208	2467	1 (–)	—	.518
		C	816	435	**0.87 (0.76-0.99)**	**.036**	
		G/G	2257	1051	1 (–)	—	
		G/C	694	365	0.87 (0.74-1.03)	.118	
		C/C	61	35	0.72 (0.44-1.17)	.187	
VEGFA	rs10434	G	3368	2109	1 (–)	—	.777
		A	2628	1727	0.99 (0.91-1.09)	.906	
		G/G	961	575	1 (–)	—	
		G/A	1446	959	1.00 (0.86-1.16)	.979	
		A/A	591	384	1.03 (0.85-1.24)	.788	
KRAS	rs11047885	A	4661	2321	1 (–)	—	.315
		C	1189	549	1.05 (0.93-1.18)	.44	
		A/A	1859	941	1 (–)	—	
		A/C	943	439	1.06 (0.90-1.25)	.464	
		C/C	123	55	1.01 (0.69-1.50)	.938	
KRAS	rs12587	G	3186	1571	1 (–)	—	.637
		T	2578	1271	0.98 (0.89-1.07)	.623	
		G/G	921	432	1 (–)	—	
		G/T	1344	707	0.91 (0.77-1.08)	.271	
		T/T	617	282	1.04 (0.85-1.28)	.694	

[a]All analyses are adjusted for age, sex and country of origin. Results showing $P < .05$ are in bold.

control and IL10_GFP vectors. The relative fluorescent units detected from the red and green channels were used to calculate the ratio GFP/RFP. A *Z*-score statistic was performed to account for the inter-experimental variability. Analysis of variance (ANOVA) was carried out to assess the difference between the two constructs.

3 | RESULTS

Genotype concordance rate was greater than 98%. Samples with call rate lower than 75% were discarded (N = 396). After exclusions, all SNPs had a call rate over 91%, which was uniform between cases and controls.

No deviations from HWE among the controls were observed, with the exception of rs12211228 (*IRF4*), rs11047885 (*KRAS*), rs1126772 (*SPP1*) and rs12587 (*KRAS*) SNPs in the Danish sample set. Therefore, these four SNPs were analyzed excluding samples from Denmark.

Among the SNPs investigated, only *IL10*-rs3024496 SNP showed an association with MM risk at nominal level of $P < .05$ (Table 2). Carriers of the A-allele showed an increased risk (OR = 1.20, 95% CI = 1.01-1.44 for heterozygotes) as compared to the homozygotes for the G-allele. The trend test showed a statistical *P* value close to the Bonferroni-corrected threshold of significance ($P = .008$). The same allele was also associated with a shorter OS at the nominal level of 0.05, with an HR of 1.44 (95% CI = 1.05-1.96, $P = .022$) for the heterozygotes and of 1.42 (95% CI = 1.02-1.99, $P = .04$) for the homozygotes. None of the other SNPs showed noteworthy results, with the exception of *IRF4*-rs12211228, which showed a weak association with MM risk in the allelic model ($P = .036$, Table 2).

Furthermore, the in vitro assay showed that the p3UTR carrying the A-allele of rs3024496 significantly decreased the expression of the GFP reporter gene. In particular, U266B1 MM cells transfected with the GFP_IL10_A vector showed a 6% reduction in relative fluorescence units (RFU), compared with cells transfected with the GFP_IL10_G vector ($P = .019$; Figure 1A). In addition, the *IL10*-rs3024496-A allele was also associated with reduced expression of *IL10* in GTEx (Figure 1B).

4 | DISCUSSION AND CONCLUSION

Our previous data reported significant associations between p3UTRs within *DDR1*, *TCF19* and *POU5F1* genes, and increased risk of developing MM,[18] highlighting the importance of association studies in discovering novel variants associated with MM pathogenesis. Herein, we expanded the investigation by evaluating the association between p3UTRs of candidate genes, namely *KRAS* (rs12587 and rs7973623), *VEGFA* (rs10434), *SPP1* (rs1126772), *IRF4* (rs12211228) and *IL10* (rs3024496) with MM risk and prognosis. The SNPs within MM candidate genes were selected for their highest conservation scores among phylogenetically distant organisms, suggesting a functional role to be likely. Among the investigated p3UTRs, we found a positive signal within *IL10*, a gene coding a cytokine produced primarily by monocytes and to a lesser extent by lymphocytes, with pleiotropic effects on immunoregulation and inflammation. Specifically, we found that AA genotype of *IL10*-rs3024496 was associated with increased risk of developing MM, and also that the A allele in *IL10*-rs3024496 was associated with a worse OS of MM patients. It is interesting to note that *IL10*-rs3024496 and *IL10*-rs2222202 (an SNP in strong LD) adult cancer types, including colorectal[19] and prostate cancers.[20]

Results from the in vitro assay were in agreement with a hypothetical functional role for rs3024496, showing that the A-allele

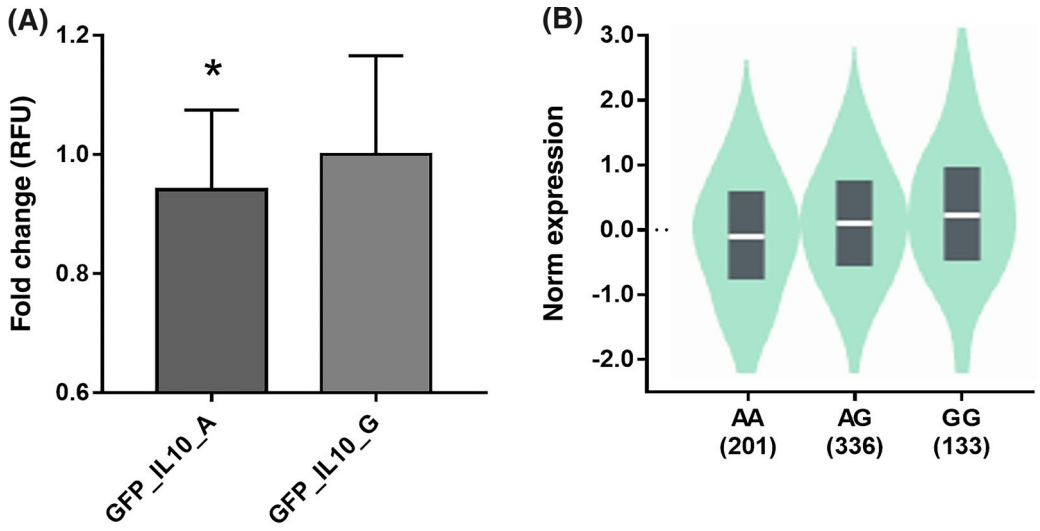

FIGURE 1 IL10-rs3024496 polymorphism affects IL-10 expression levels. A, Bar chart showing the fold change between the average relative fluorescent units (RFU) of cells transfected with the GFP_IL10_A or GFP_IL10_G. The asterisk indicates a statistically significant difference (*P* value = .019; fold change = 0.94). B, Violin plot showing the effect of the rs3024496 variant on the mRNA levels of IL10 in the whole blood of 670 healthy subjects according to GTEx Portal (*P* value = .0017; normalized effect size = 0.12) [Color figure can be viewed at wileyonlinelibrary.com]

significantly decreased the expression of the reporter gene of about 6%. These results paralleled the in vivo data from GTEx reporting a similar association between *IL10* mRNA levels in whole blood and the polymorphism rs3024496, strengthening our observation.

IL-10 has been identified as an anti-inflammatory cytokine. In macrophages, it inhibits the expression of inflammatory cytokines (such as INF-γ, IL-2, IL-3 and TNF-α) and deactivates their functions.[21] It has been shown that IL-10 is an important regulator of the activity of the pro-inflammatory cytokines IL-1β and IL-6 that are aberrantly expressed by MM cells.[22] It was also shown in vivo that IL-1β and IL-6 sustain MM cells survival and play a role in the progression toward MM.[23] Indeed, the targeting of IL-1/IL-6 axis by the use of receptor antagonists in combination with dexamethasone ameliorated the disease progression of MM patients in a phase II clinical trial.[24]

In summary, our results suggest that *IL10*-rs3024496 might affect immune homeostasis through the modulation of *IL10* mRNA expression. Decreased level of IL-10 could be associated with an overproduction of pro-inflammatory cytokines, providing myeloma cells the ideal microenvironment to survive longer and proliferate. Overall, these findings also suggest that the inclusion of the personal genetic background of patients could help to improve the stratification in terms of risk progression and prognosis.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICAL STATEMENT

The IMMEnSE study protocol was approved by the Ethics Committee of the Medical Faculty of the University of Heidelberg (reference number: S-004/2020). Following the guidelines of the Declaration of Helsinki, written informed consent was obtained from each participant.

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REFERENCES

1. Kumar SK, Rajkumar V, Kyle RA, et al. Multiple myeloma. *Nat Rev Dis Primer*. 2017;3:17046.
2. Vachon CM, Kyle RA, Therneau TM, et al. Increased risk of monoclonal gammopathy in first-degree relatives of patients with multiple myeloma or monoclonal gammopathy of undetermined significance. *Blood*. 2009;114:785-790.
3. Johnson DC, Weinhold N, Mitchell JS, et al. Genome-wide association study identifies variation at 6q25.1 associated with survival in multiple myeloma. *Nat Commun*. 2016;7:10290.
4. Ziv E, Dean E, Hu D, et al. Genome-wide association study identifies variants at 16p13 associated with survival in multiple myeloma patients. *Nat Commun*. 2015;6:7539.
5. Pertesi M, Went M, Hansson M, Hemminki K, Houlston RS, Nilsson B. Genetic predisposition for multiple myeloma. *Leukemia*. 2020;34:697-708.
6. Macauda A, Castelli E, Buda G, et al. Inherited variation in the xenobiotic transporter pathway and survival of multiple myeloma patients. *Br J Haematol*. 2018;183:375-384.
7. Campa D, Martino A, Sainz J, et al. Comprehensive investigation of genetic variation in the 8q24 region and multiple myeloma risk in the IMMEnSE consortium. *Br J Haematol*. 2012;157:331-338.
8. Martino A, Campa D, Buda G, et al. Polymorphisms in xenobiotic transporters ABCB1, ABCG2, ABCC2, ABCC1, ABCC3 and multiple myeloma risk: a case-control study in the context of the International Multiple Myeloma rESEarch (IMMEnSE) consortium. *Leukemia*. 2012;26:1419-1422.
9. Ríos R, Lupiañez CB, Campa D, et al. Type 2 diabetes-related variants influence the risk of developing multiple myeloma: results from the IMMEnSE consortium. *Endocr Relat Cancer*. 2015;22:545-559.
10. Mayr C. Regulation by 3′-untranslated regions. *Annu Rev Genet*. 2017;51:171-194.
11. Xu G-P, Chen W-X, Xie W-Y, Wu L-F. The association between IGF1 gene 3'-UTR polymorphisms and cancer risk: a meta-analysis. *Medicine (Baltimore)*. 2018;97:e13829.
12. Akdeli N, Riemann K, Westphal J, Hess J, Siffert W, Bachmann HS. A 3′UTR polymorphism modulates mRNA stability of the oncogene and drug target polo-like kinase 1. *Mol Cancer*. 2014;13:87.
13. Huang Z, Lu Z, Tian J, Wang G, Gao Z. Effect of a functional polymorphism in the pre-miR-146a gene on the risk and prognosis of renal cell carcinoma. *Mol Med Rep*. 2015;12:6997-7004.
14. Zhou L, Dong S, Deng Y, et al. GOLGA7 rs11337, a polymorphism at the MicroRNA binding site, is associated with glioma prognosis. *Mol Ther Nucleic Acids*. 2019;18:56-65.
15. Wang W, Yuan P, Yu D, et al. A single-nucleotide polymorphism in the 3′-UTR region of the adipocyte fatty acid binding protein 4 gene is associated with prognosis of triple-negative breast cancer. *Oncotarget*. 2016;7:18984-18998.
16. Martino A, Sainz J, Buda G, et al. Genetics and molecular epidemiology of multiple myeloma: the rationale for the IMMEnSE consortium (review). *Int J Oncol*. 2012;40:625-638.
17. Yu W, Wulf A, Liu T, Khoury MJ, Gwinn M. Gene prospector: an evidence gateway for evaluating potential susceptibility genes and interacting risk factors for human diseases. *BMC Bioinformat*. 2008;9:528.
18. Macauda A, Calvetti D, Maccari G, et al. Identification of miRSNPs associated with the risk of multiple myeloma. *Int J Cancer*. 2017;140:526-534.
19. Tsilidis KK, Helzlsouer KJ, Smith MW, et al. Association of common polymorphisms in IL10, and in other genes related to inflammatory response and obesity with colorectal cancer. *Cancer Causes Control*. 2009;20:1739-1751.
20. Wang M-H, Helzlsouer KJ, Smith MW, et al. Association of IL10 and other immune response- and obesity-related genes with prostate cancer in CLUE II. *Prostate*. 2009;69:874-885.

21. Mannino MH, Zhu Z, Xiao H, Bai Q, Wakefield MR, Fang Y. The paradoxical role of IL-10 in immunity and cancer. *Cancer Lett*. 2015;367:103-107.
22. Xiong Y, Donovan KA, Kline MP, et al. Identification of two groups of smoldering multiple myeloma patients who are either high or low producers of interleukin-1. *J Interferon Cytokine Res*. 2006;26:83-95.
23. Musolino C, Allegra A, Innao V, Allegra AG, Pioggia G, Gangemi S. Inflammatory and anti-inflammatory equilibrium, proliferative and antiproliferative balance: the role of cytokines in multiple myeloma. *Mediators Inflamm*. 2017;2017:1852517.
24. Lust JA, Lacy MQ, Zeldenrust SR, et al. Reduction in C-reactive protein indicates successful targeting of the IL-1/IL-6 axis resulting in improved survival in early stage multiple myeloma. *Am J Hematol*. 2016;91:571-574.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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