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ORIGINAL ARTICLE

A NEW CLOCK IS RUNNING FOR MULTIPLE MYELOMA: CIRCADIAN CLOCK PROTEIN-PERIOD 3 (PER-3) POLYMORPHISM

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ABSTRACT

Circadian Clock Protein PERIOD 3 (*PER-3*) is situated on chromosome 1p36.23 and has a polymorphic domain that expresses 4 or 5 copies of the 54-bp tandem repeat sequence. *PER-3* gene polymorphisms play a role in the dysregulation of the immune system. This study intended to investigate the distributions and clinical effectiveness of the *PER-3* gene polymorphism in multiple myeloma (MM) patients. One hundred fifty patients diagnosed between January 2007-2009 and 100 healthy individuals were included in this study. All patients were suitable for autologous stem cell transplantation (ASCT) at first evaluation, and after 4 courses of VCD at least partial remission, ASCT was carried out. Later, LD was used as maintenance. Genotypes of *PER-3* gene of patients and healthy controls were statistically compared before treatment. In addition, these genotypes' effects on overall and progression free survival (OS and PFS) were investigated. Median PFS in the 5R/5R genotype was found to be significantly longer, albeit low, at 86% ($p = 0.046$). In the statistical analysis performed between the 4R/4R genotype and others, the PFS of patients with 4R/4R was found to be significantly shorter at 40.4 months ($p = 0.026$). Patients with the 4R/4R genotype would have a risk of 2.049 times of a shorter PFS ($p=0.009$). With this first study investigating the effect of a circadian gene in MM, the net effect of *PER-3* gene polymorphism on PFS was revealed, and it will be a guide for future studies.

Keywords: Multiple myeloma, epigenetics, circadian clock, PER3, prognosis, survival

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INTRODUCTION

Multiple myeloma (MM) makes up 10% of hematological malignancies and 1% of all cancers. It is mostly seen in men, and the median age is reported to be about 65 years old [1]. MM, which causes excessive production of monoclonal light chain and heavy chain, is a malign disease of plasma cell [2]. Autologous stem cell transplantation (ASCT) after high dose chemotherapy is the favored standard treatment in fit patients diagnosed with MM. The International Staging System (ISS) was made based on levels of serum albumin and beta-2 microglobulin. Yet in the revised ISS, in addition to the ISS, added factors such as serum lactate dehydrogenase (LDH) and deletion of 17p, t (4; 14), t (14; 16) are evaluated by interphase fluorescence in situ hybridization (FISH) [1- 3].

The circadian clock (CC), which is governed via the main center of mammalian physiology in the superior chiasmatic nucleus, plays a role in the arrangement of behavior of biological and physiological, as per the light cycle and dark cycle in the daily period [4, 5]. This center forms a link with a complex neurohumoral network via temperature daily rhythms, photic signals by retina, social stimuli and diet. The circadian rhythm (CR) is regulated by CR pathway genes, the mammalian CC mechanism has interlocking transcription-translation feedback loops, controlled at the molecular level by a set of genes, including *NPAS2, BMAL1, CLOCK*, period genes (*PER-1, -2*, and *-3*), cryptochrome circadian regulator 1 (*CRY-1* and *-2*), *NR1D-1* and *-2*. Previous studies showed that changes of expression in these genes play a role in immune cells and in the functions and expression of cytokines [4, 5]. The physiology of the immune system has a 24-hour CR, and most of these cells express genes of CR. Circadian gene expressions affect many mechanisms including cellular and cytolytic functions and cytokine synthesis. Thus, dysregulation or mutation of PER-3 or other clock genes has been related with some cancers [6].

The CC Protein *PER-3* (*rs57875989*) is situated on chromosome 1p36.23 and has a polymorphic domain that expresses 4 or 5 copies of the 54-bp tandem repeat sequence (variable number tandem repeat, VNTR). This variation, resulting from the deletion or insertion of 18 amino acids, is linked to mood and sleep disturbances and circadian preference in humans [6]. The *PER-3* polymorphism plays an important role in CR disorders, dysregulation of immune system and expression of cytokines [4-8]. It has been suggested that immune dysregulation associated with the *PER-3* polymorphism contributes to chronic inflammatory processes or diseases, and cancer biogenesis [6]. A previous study demonstrated interaction between *PER-3* polymorphism and markers associated with chronic inflammation, including interleukin (IL)-1, -6, interferon-gamma (IFN-Ɣ), and tumor necrosis factor-alpha (TNF-α), and IL-6 expressions. These were higher in patients with 4/5 or 5/5 *PER-3* gene polymorphism compared to patients with the 4/4 polymorphism [6]. It is suggested that the 4/5 or 5/5 *PER-3* genotype is more common in premenopausal breast cancer [9]. Thus, there is increasing interest in studies investigating the relationship between *PER-3* gene polymorphisms and pre-cancerous processes and auto-inflammatory/-immune diseases.

In this study, we intend to investigate the distributions and clinical efficacy of the *PER-3* gene polymorphism in MM patients.

MATERIAL AND METHODS

One hundred fifty patients (over 18 years old), diagnosed with MM in Hematology Clinic of Gaziantep University between January 2007 - 2009, and 100 healthy persons were included in this study. Demographic characteristics, first-line treatments, clinical scores (Durie-Salmon stages, ISS score, Eastern Cooperative Oncology Group (ECOG) score), laboratory variables, status of survival (overall (OS) and progression-free (PFS)) were recorded.

All patients were suitable for ASCT at first evaluation and following four courses on bortezomib-cyclophosphamide and dexamethasone (VCD) at partial remission (PR), ASCT was carried out. Following 24 months, lenalidomide-dexamethasone (LD) was used as maintenance.

The genotypes of 4R/4R-5R, 5R/5R and alleles of 4R and 5R on *PER-3* gene of patients with ASCT and healthy controls; were statistically compared before treatment. In addition, these genotypes, the effects of on PFS and OS were investigated. Study approval was obtained from Gaziantep University Ethics Committee (07-2007/40).

Isolation Method

DNA isolation via peripheral blood leukocytes was measured by the saline precipitation method [10]. Genotypes of *PER-3* gene polymorphism were studied by polymerase chain reaction- (PCR) and agarose gel electrophoresis method [11].

Statistical Analysis

According to the Kolmogorov-Smirnov test, quantitative variables that fit the normal distribution were shown as mean±standard deviation, but those that did not fit were shown as median (minimum and maximum). Comparisons between qualitative measures expressed as numbers and percentages (%) were made using Chi-square or Fisher's exact tests. Post-hoc analysis was evaluated with Bonferroni correction for intergroup comparisons. Variables associated with different gene variants were determined in the multivariate logistic regression model adjusted for gender and age. Regression results are shown with odds ratio (OR) and 95% confidence interval (CI). Hardy Weinberg Equilibrium (HWE) was performed via the De-Finetti program (online HWE and Association Testing- Institut für Humangenetik, Munich, Germany). All analyses were performed with IBM SPSS version v21.0 (IBM Corp, Armonk, NY, USA), and a p-value less than 0.05 was considered statistically significant.

RESULTS

The median age was 56 years (range: 32-70). The 10-years OS was 79%, while the 10-years PFS was 47% with a median of 52.7 months. The mortality rate was 15.3% (n:23) (Table 1.).

According to *PER-3* gene variants, 4R/4R, 4R/5R and 5R/5R polymorphisms and 4R and 5R alleles did not differ between the MM and control groups (Table 2.).

Table 3 shows the results of evaluating prognostic factors in terms of PFS and OS. In the 10-year survival analysis, the rate was shown as a percentage if it was above 50%, and as a month if it was below 50%. Median PFS in the 5R/5R genotype was found to be significantly longer, albeit low, at 86% ($p = 0.046$). In the statistical analysis performed between the 4R/4R genotype and other genotypes, the PFS of patients with this genotype was found to be significantly shorter with 40.4 months ($p = 0.026$).

Table 4 shows the multivariate analysis. It was observed that patients with the 4R/4R genotype would have a risk of 2.049 times of shorter PFS ($p= 0.009$) (Figure 1.).

DISCUSSION

This study is the first to report on the association between CR and MM. Literature data on CR and hematological malignancies are also limited; there are related studies on solid malignancies.

Table 1. Clinical features and treatment regimens of MM patients

		Multiple Myeloma		**Control**	**Control**	**p**
		median	**n[a] (%)**	**median**	**n[b] (%)**	
Age		56 (32-70)		53 (28-68)		0.140*
Gender	Female/Male		77/73 (51.3/48.7)		55/45 (55/45)	0.367[&]
MM Subtypes	κ/λ		83/39 (68/32)			
	G/A		79/18 (65/15)			
	Light chain		25 (20)			
Stage (Durie-Salmon)	II/III		38/84 (25/75)			
	A/B		91/31 (79/21)			
IPI	I		39 (32)			
	II/III		34/50 (28/40)			
ECOG	>1		12 (10)			
Hemoglobin	gr/dL	10.4 (6.2-15)				
Leukocyte	mm^3	7200 (2760-18500)				
Platelet	$10^3/\mu L$	172 (69-406)				
C-reactive protein	mg/dL	8 (2.1-352)				
LDH	IU/L	212 (93-1037)				
B2-microglobulin	mg/L	4.9 (1.5-48)				
Albumin	gr/L	3.5 (1.6-5.1)				
Treatment	VCD, ASCT, LD					
OS (10-years, %)		(79)				
PFS (10-years, %)		(47) – 52.7 months				
Relapse			63 (42)			
Mortality			23 (15.3)			
Follow-up duration, months, (range)		36.1 (4.1-155.2)				

n^a = 150; n^b = 100; *median test, [&]Pearson Chi-Square
**MM: Multiple myeloma, IPI:International Prognostic Index, ECOG:Eastern Cooperative Oncology Group, CRP:C-reactive protein, LDH: Lactate dehydrogenase, OS: Overall survival, PFS: Progression free survival, VCD: Bortezomib-cyclophosphamide and dexamethasone, LD: Lenalidomide- dexamethasone, ASCT: Autologous stem cell transplantation

Table 2. Comparison of frequencies of **PER-3** gene variants between patients with MM and healthy controls

PER3	**Genotype**	**Multiple Myeloma**	**Healthy Controls**	**OR Exp(B)**	**95% CI**	**p**
Genotypes		n=[a] (%)	n=100 (%)			
PER-3	4R/4R	70 (46.7)	42 (42)	0.657*	0.285-1.517*	0.325*
	4R/5R	64 (43.3)	44 (44)	0.745*	0.320-1.734*	0.494*
	5R/5R	16 (10.7)	14 (14)	1.363[&]	0.633-2.935[&]	0.434[&]
Allele						
	4R	204 (67.5)	128 (64)			
	5R	98 (32.5)	72 (36)	1.159[&]	0.796-1.689[&]	0.443[&]

[a]n= 150, *:OR (95%CI) was adjusted by age and sex, [&]Fisher's Exact Test.
PER3: Circadian Clock Protein PERIOD 3

Table 3. Comparison of PFS and OS with prognostic factors of patients with MM

			PFS*	Log Rank p-value	OS*,#	Log Rank p
		n	52.7		77#	
Gender, n	*Female / Male*	73/77	89.3 /39.2	0.080	66# / 69#	0.690
Age, n	*<65 / ≥65*	130/20	54.3 / 40.4	0.230	56# / 54#	0.058
Stage (Durie-Salmon), n	*II/III*	38/84	28.5 / 54.3	0.257	87.1 / 99.1	0.229
	A/B	91/31	40.4 / 65.0	0.277	55# / 51#	0.301
IPI, n	*I*	39	69.3		99.1	
	II	34	40.4		70#	
	III	50	52.7	0.764	88.2	**0.026**
ECOG, n	*≤ 1 / >1*	109/12	47.1 / 28.5	0.959	53# / 31.4	0.301
LDH (IU/L), n	*<480 / ≥480*	116/7	52.7 / 17	0.086	53# / 17	**0.001**
CRP (mg/L), n	*<5 / ≥ 5*	51/71	54.3 / 52.7	0.686	85# /99.1	0.057
PER3	4R/4R	70	40.4		74#	
	4R/5R	64	69.3		57#	
	5R/5R	16	86#	**0.046**	100#	0.349
PER-3	4R/4R	70	40.4		63#	
	4R/5R -5R/5R	80	89.6	**0.028**	99.1	0.738

*: median months, #:10-yrs %
MM: Multiple myeloma, IPI:International Prognostic Index, ECOG:Eastern Cooperative Oncology Group, CRP:C-reactive protein, LDH: Lactate dehydrogenase, OS: Overall survival, PFS: Progression free survival, PER3: Circadian Clock Protein PERIOD 3

Table 4. Multivariate analysis of 150 MM patients (Cox proportional hazard model backward)

	PFS			
		Exp (B)	**%95 CI**	**p**
PER-3	4R/4R	0.488	0.284-0.837	**0.009**
LDH	≥480	0.441	0.170-1.141	0.091
Gender	Female/Male	0.604	0.357-1.022	0.060

MM: Multiple myeloma, PER3: Circadian Clock Protein PERIOD 3, LDH: Lactate dehydrogenase

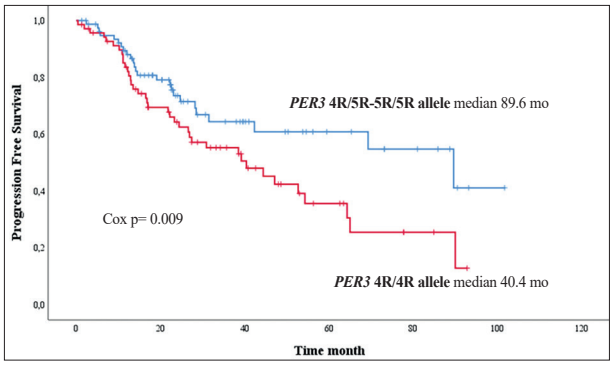

Figure 1. Progression free survival curves: 4R/4R and 4R/5R-5R/5R

In a current study from 2021 by Dagmura et al. [12], CR and pancreatic cancer (PC) were studied. The frequency of the 4R/3R, 3R/3R genotypes, and 3R allele of *PER-2* VNTR in patients with PC were higher than in the healthy controls ($p < 0.001$, for all). *PER-2* VNTR 4/5 genotype was found to be related to perineural invasion ($p = 0.040$). The genotype and allelic distributions of the *PER-3* VNTR variant were similar between the groups ($p > 0.05$). In this study, a significant relationship between *PER-2* and perineural invasion, which can be a marker of poor prognosis, was demonstrated.

Geng et al. [13] examined the association of *PER-3* gene variants (*rs1012477*, 4/5-repeat) with solid malignancies. In the meta-analysis, data were collected from a total of 8 separate studies, and no relationship was found between *PER-3* gene variants and cancer susceptibility. The articles covered in the study were related to prostate, breast cancer, glioma and colorectal cancer (CRC). In another study from 2013 [14], the relationship between CRC and CR was investigated, and the polymorphism of 311T > C on *CLOCK-1* gene did not affect the outcome of patients with CRC, yet it increased the risk of developing CRC. The evaluation of 4/5 repeat allele polymorphism in *PER-3* was similar in terms of allele and genotype distributions between the control group and the CRC group. A previous study showed that the 5- repeat *PER-3* VNTR sequence is related with increase of colorectal adenoma development [15]. Although there was no difference in

cancer susceptibility in our study, the fact that the 4R/4R genotype was associated with shorter PFS is an important point for treatment responses.

Clinical data on hematological malignancies and *PER-3* polymorphisms is limited. In a study on diffuse large B-cell lymphoma patients from 2015 [16], *PER-3 rs10462020* variant was significantly different in OS between groups of mutated and non-mutated genotypes ($p = 0.047$). In another study in the same year [17], 9 different CR gene expressions were examined in cases of newly diagnosed acute myeloid (AML) and lymphoblastic leukemia (ALL). In AML patients, the expressions of *PER-1, -2, CRY-1, -2*, *timeless*, muscle and brain aryl hydrocarbon receptor nuclear translocator (*ARNT*) -like 1 (*BMAL-1*) were down-regulated, while *CK1ε* was up-regulated. In patients with ALL, the expressions of *PER-3* and *CRY-1* were down-regulated, while *CK1ε* and *timeless* were up-regulated. Improvement in *PER-3* expression was detected in attained remission patients with AML and ALL, while this was not detected in relapsed patients after treatment. The CC genes were changed in acute leukemia patients, and *PER-3* up-regulation is related with an improved clinical outcome.

The relationship of CR genes with sleep disturbance has been extensively studied. Guess et al. [6] found that the 4/4 genotype showed more physical fatigue and sleep disturbances. Sleep disturbance and increased inflammation or inflammatory markers have been the subject of many studies [18–20]. In these studies there is a positive correlation between sleep disturbance and increased vascular endothelial growth factor (VEGF) concentration. VEGF contributes to the pathogenesis of MM. It plays a role in myeloma development, bone marrow microenvironment interaction, disease progression and drug resistance. VEGF and VEGF receptor expression is increased in myeloma bone marrow [21]. Anti-VEGF treatment strategies are also discussed in the treatment of myeloma. In addition, proteosome inhibitors, immunomodulatory drugs (IMIDs) and bisphosphonates, which are the cornerstones of MM treatment, have also been shown to reduce VEGF levels [21]. In our study, the 4R/4R genotype, which is significantly associated with sleep disorder and physical fatigue, high VEGF level is thought to be related. Therefore, a significant short PFS constitutes the most important result of the study.

This study had also some important limitations. OS may not have differed significantly due to the narrowing of the patient population when the genotype was divided into gene variant subgroups. In addition, not evaluating synchronous cytokines is another important limitation. There were also deficiencies in patient data in terms of comorbidity or genetic mutation subtypes, and it was therefore not possible to analyze them together.

In conclusion, this study contributes to the literature in terms of MM and *PER-3* gene polymorphism. Although no significant results were obtained in terms of MM pathogenesis, it was revealed that the 4R/4R genotype had a statistically significant short PFS. It is thought that this information will shed light on new studies in terms of the therapeutic effect and pathogenesis in MM.

ABBREVIATIONS

MM: Multiple myeloma
ASCT: Autologous stem cell transplantation
ISS: International Staging System
R-ISS: The revised ISS
LDH: Lactate dehydrogenase
FISH: Fluorescent in situ hybridization
SCN: Superior chiasmatic nucleus
PER: Circadian Clock Protein PERIOD 3
CC: Circadian Clock
CRY: Cryptochrome circadian regulator 1
CR: Circadian rhythm
VNTR: Variable number tandem repeat
TNF-α: Tumor necrosis factor-alpha
IFN-Ɣ: Interferon-gamma
IL-1: Interleukin-1
IL-6: Interleukin-6
CRP: C-reactive protein
OS: Overall survival
PFS: Progression free survival
VCD: Bortezomib-cyclophosphamide and dexamethasone
PR: Partial remission
LD: Lenalidomide-dexamethasone
PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism
OR: Odds ratio
CI: Confidence interval
HWE: Hardy Weinberg Equilibrium
PC: pancreatic cancer
CRC: Colorectal cancer
AML: Acute myeloid leukemia
ALL: Acute lymphoblastic leukemia
BMAL1: Brain and muscle aryl hydrocarbon receptor nuclear translocator (ARNT) - like 1
TIM: Timeless
VEGF: vascular endothelial growth factor
IMID: Immunomodulatory drugs

DECLARATIONS

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No funding has been received.

Conflict of Interest
None to declare.

Availability of Data and Materials
The authors declare that data supporting the findings of this study are available within the referenced articles.

Authors' Contributions
All authors contributed to the editing of the manuscript. IS wrote the manuscript and made tables.

Ethics Approval and Consent to Participate
Ethical committee approval was received (Faculty of Medicine, Gaziantep University, approval year and number: 2018/78) and the patients and control subjects gave informed consent before the beginning of the study. The experimental procedures were based on the Declaration of Helsinki and relevant institutional regulations.

Patient Consent for Publication
Informed consent obtained as well as written forms from all of our patients to publish.

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REFERENCES

1. Rajkumar SV. Multiple myeloma: 2020 update on diagnosis, risk-stratification and management [published correction appears in Am J Hematol. 2020 Nov; 95(11):1444]. *Am J Hematol*. 2020;95(5):548-567. doi:10.1002/ajh.25791
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin*. 2020; 70(1): 7-30. doi:10.3322/caac.21590
3. Kyle RA, Therneau TM, Rajkumar SV, Larson DR, Plevak MF, Melton LJ. 3rd. Incidence of multiple myeloma in Olmsted County, Minnesota: Trend over 6 decades. *Cancer*. 2004; 101(11) :2667-2674. doi:10.1002/cncr.20652
4. Scheiermann C, Gibbs J, Ince L, Loudon A. Clocking in to immunity. *Nat Rev Immunol*. 2018; 18(7): 423-437. doi:10.1038/s41577-018-0008-4
5. Labrecque N, Cermakian N. Circadian Clocks in the Immune System. *J Biol Rhythms*. 2015; 30(4): 277-290. doi:10.1177/0748730415577723
6. Guess J, Burch JB, Ogoussan K, Armstead CA, Zhang H, Wagner S et al. Circadian disruption, Per3, and human cytokine secretion. *Integr Cancer Ther*. 2009; 8(4): 329-336. doi:10.1177/1534735409352029
7. Ebisawa T, Uchiyama M, Kajimura N, Mishima K, Kamei Y, Katoh M et al. Association of structural polymorphisms in the human period3 gene with delayed sleep phase syndrome. *EMBO Rep*. 2001; 2(4): 342-346. doi:10.1093/embo-reports/kve070
8. Helvaci N, Oguz SH, Kabacam S, Karabulut E, Akbiyik F, Alikasifoglu M et al. Clock gene *PERIOD3* polymorphism is associated with susceptibility to Graves' disease but not to Hashimoto's thyroiditis. *Chronobiol Int*. 2019; 36(10): 1343-1350. doi:10.1080/07420528.2019.1642909
9. Zhu Y, Brown HN, Zhang Y, Stevens RG, Zheng T. Period3 structural variation: a circadian biomarker associated with breast cancer in young women. *Cancer Epidemiol Biomarkers Prev*. 2005; 14(1): 268-270.
10. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*. 1988; 16(3): 1215. doi:10.1093/nar/16.3.1215
11. Nursal A, Cetinay Aydin P, Uysal M, Pehlivan M, Oyacı Y, Pehlivan S. PER3 VNTR variant and susceptibility to smoking status/substance use disorder in a Turkish population. Archives of Clinical Psychiatry (São Paulo). 47. 10.1590/0101-60830000000235.
12. Dagmura H, Yiğit S, Nursal AF, Duman E, Gumusay O. Possible Association of *PER2/PER3* Variable Number Tandem Repeat Polymorphism Variants with Susceptibility and Clinical Characteristics in Pancreatic Cancer. *Genet Test Mol Biomarkers*. 2021; 25(2): 124-130. doi:10.1089/gtmb.2020.0179
13. Geng P, Ou J, Li J, Wang N, Xie G, Sa R et al. Genetic association between PER3 genetic polymorphisms and cancer susceptibility: a meta-analysis. *Medicine (Baltimore)*. 2015; 94(13): e568. doi:10.1097/MD.0000000000000568
14. Karantanos T, Theodoropoulos G, Gazouli M, Vaiopoulou A, Karantanou C, Stravopodis DJ et al. Association of the clock genes polymorphisms with colorectal cancer susceptibility. J Surg Oncol. 2013 Dec; 108(8): 563-7. doi: 10.1002/jso.23434.
15. Alexander M, Burch JB, Steck SE, Chen CF, Hurley TG, Cavicchia P et al. Case-control study of the PERI-

OD3 clock gene length polymorphism and colorectal adenoma formation. *Oncol Rep*. 2015; 33(2): 935-941. doi:10.3892/or.2014.3667

16. Gutiérrez-Monreal MA, Villela L, Baltazar S, Perfecto-Avalos Y, Cardineau GA, Scott SP. A PER3 polymorphism is associated with better overall survival in diffuse large B-cell lymphoma in Mexican population. *Cancer Biomark*. 2015; 15(5): 699-705. doi:10.3233/CBM-150511
17. Yang MY, Lin PM, Hsiao HH, Hsu JF, Lin HY, Hsu CM et al. Up-regulation of PER3 Expression Is Correlated with Better Clinical Outcome in Acute Leukemia. *Anticancer Res*. 2015; 35(12): 6615-6622.
18. Motivala SJ, Levin MJ, Oxman MN, Irwin MR. Impairments in health functioning and sleep quality in older adults with a history of depression. *J Am Geriatr Soc*. 2006; 54(8): 1184-1191. doi:10.1111/j.1532-5415.2006.00819.x
19. Vgontzas AN, Chrousos GP. Sleep, the hypothalamic-pituitary-adrenal axis, and cytokines: multiple interactions and disturbances in sleep disorders. *Endocrinol Metab Clin North Am*. 2002; 31(1): 15-36. doi:10.1016/s0889-8529(01)00005-6
20. Vgontzas AN, Zoumakis M, Bixler EO, Lin HM, Prolo P, Vela-Bueno A et al. Impaired nighttime sleep in healthy old versus young adults is associated with elevated plasma interleukin-6 and cortisol levels: physiologic and therapeutic implications. *J Clin Endocrinol Metab*. 2003; 88(5): 2087-2095. doi:10.1210/jc.2002-021176
21. Ria R, Melaccio A, Racanelli V, Vacca A. Anti-VEGF Drugs in the Treatment of Multiple Myeloma Patients. *J Clin Med*. 2020; 9(6): 1765. Published 2020 Jun 6. doi:10.3390/jcm9061765