Journal of Research in Applied and Basic Medical Sciences 2023; 9(4): 193-199

Original Article

RABMS Journal of Research in Applied and Basic Medical Sciences

Serum levels of interleukin-10 and its receptor in the multiple myeloma type I patients

Mahdi Rajabzade[1], Mohammad Hassan Khadem Ansari[1]*, Fatemeh Kheradmand[1], Yousef Rasmi[1], Zafar Gholinejad[2*]

[1] *Department of Biochemistry, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran*

[2] *Department of Medical Laboratory Science, Urmia Branch, Islamic Azad University, Urmia, Iran*

***Corresponding authors:** Dr. Mohammad Hassan Khadem Ansari, Zafar Gholinejad, **Address**: Urmia Branch, Islamic Azad University, Urmia, Iran, **Email**: ansari_mh@umsu.ac.ir, zafar.gholinejad777@gmail.com, **Tel**: +989109209986

Abstract

***Background & Aims*:** Multiple myeloma is a malignancy of plasma cells that is associated with high morbidity rate**.** Inflammation is a common feature in malignancy and plays a pivotal role in the cancer pathogenesis. Interleukin-10 is a cytokine with anti-inflammatory properties. The aim of this study was to evaluate the importance of interleukin-10 and its receptor in multiple myeloma in order to find their pathogenic roles and diagnostic value.

***Materials & Methods*:** In this case-control study, 30 multiple myeloma patients and 40 healthy subjects were enrolled. Serum IL-10 and IL-10R1 levels were determined using ELISA method. Data analysis was performed by SPSS software version 23 including descriptive analysis, mean compression and correlation tests.

***Results*:** The mean IL-10 levels were 50.93 ± 8.76 and 52.56 ± 10.47 pg/ml in control and patient groups, respectively. The serum IL-10R levels of multiple myeloma patients were significantly lower than those of the healthy control ($p = 0.041$), where mean serum levels were 2.51 ± 0.86 and 2.1 ± 0.76 pg/ml, respectively. We found a positive correlation between IL-10 and IL-10R levels ($p = 0.042$).

***Conclusion*:** These results demonstrated that IL-10R, but not IL-10, is increased in the patient's serum and they are proposed as a new diagnostic biomarker. In other words, downregulation of the IL-10 receptor and attenuation of IL-10's bio-effect are associated with further inflammation in multiple myeloma type I patients.

***Keywords*:** Biomarker, Interleukin 10, Interleukin 10 Receptor, Multiple Myeloma

Received 12 March 2023; accepted for publication 28 May 2023

Introduction

Multiple myeloma is a malignancy of plasma cells that is characterized by the presence of neoplastic plasma cells in the bone marrow and elevated monoclonal paraprotein levels (1). Multiple myeloma is the second most common hematologic malignancy and is associated with a high morbidity rate due to its end-organ failure (2). The evaluation of serum and urine proteins by capillary, electrophoresis, and bone marrow biopsy analysis, are the main diagnostic tools.

However, the pathogenesis and molecular mechanisms of multiple myeloma are not well understood (3). Inflammation is a common feature in malignancy and plays a pivotal role in the cancer pathogenesis (4). The role of inflammation and pro-inflammatory cytokines in multiple myeloma were reported frequently (5). Bone marrow stromal cells produce pro-inflammatory cytokines by autocrine and paracrine mechanism, which predict the duration of disease-free survival. Furthermore, cytokines have been proposed as biomarkers for the diagnosis of multiple myeloma, patient's prognosis, and tools for immunotherapy (6). The serum levels of TNF-α, IL-6, IL-6R, and IL-1β are increased in multiple myeloma patients (7-11). Interleukin-10 (IL-10) is a cytokine with immunomodulatory and potent anti-inflammatory properties that is produced by activated T cells, macrophages , dendritic cells, and B-lymphocytes (12, 13). This cytokine belongs to class II cytokine family and plays a critical role in blood malignancy and other diseases (14-16). The biological function of IL-10 is mediated by the IL-10 receptor (IL-10R), which has two isoforms, including IL-10R1 and IL-10R2. The IL-10R1 is a cell membrane associated receptor with a transmembrane and extracellular domain that has higher affinity to IL-10 than IL-10R2 (17, 18). These receptors are expressed in several cell lines. Activation of IL-10R activates JAK/STAT signaling pathway as a downstream mediator. The interaction of IL-10 and receptors leads to the overexpression of anti-inflammatory cytokines and induces cell proliferation in CD8+ T and natural killer cells (19).

To the best of our knowledge, there is currently no comprehensive study on the relationship between IL-10 and its receptor with multiple myeloma. The aim of this case-control study was to determine the serum levels of IL-10 and IL-10R in multiple myeloma patients and healthy subjects in order to describe the role of this cytokine and its receptor in the pathogenesis and their predictive value as biomarkers.

Materials & Methods

Sampling:

In this case-control study, inclusion criteria was patients with a confirmed multiple myeloma and 30 patients were enrolled. For the control group, 40 age- and sex-matched subjects were selected. Exclusion criteria were subjects who had inflammatory disorders, infections and immunodeficiency, or who had received NSAIDs or anti-inflammatory drugs. Blood samples were obtained and sera were separated by centrifugation at room temperature and were stored at -70°C refrigerator.

ELISA Assay:

Serum IL-10 and IL-10R1 levels were determined using ELISA kits (bioassay technology laboratory Shanghai). The ELISA assays were performed according to the manufacturers' instructions.

Statistical Analysis:

Data analysis was performed using SPSS software version 23. Descriptive analysis was performed and represented as charts, tables, and figures. Parametric and non-parametric statistical tests were used to compare the mean of variables between the case and control groups. The findings were expressed as mean ± SD, and $p < 0.05$ was considered as statistically significant.

Results

Serum IL-10 Levels:

The data analysis results of IL-10 are shown in Table 1 and the Figure 1A depicted IL-10 levels. The mean IL-10 levels did not show a significant difference between the patients and control groups.

Table 1. Serum IL-10 levels in patients and control groups

IL-10(pg/ml)	Minimum	Maximum	Mean	SD	Range	p value
Control	37.30	86.60	50.93	8.76	49.30	
Multiple myeloma	23.30	71.90	52.56	10.47	48.60	0.481

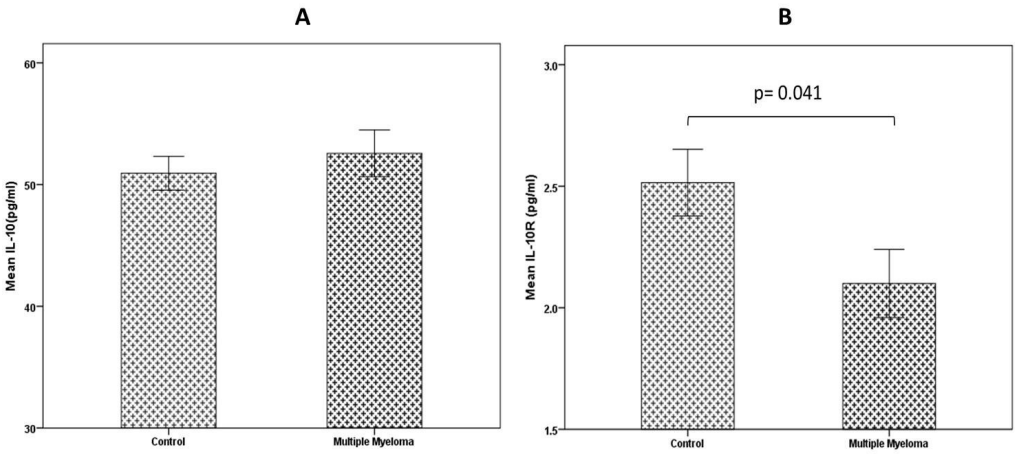

Fig. 1. A: Comparison of serum IL-10 levels in multiple myeloma and normal groups. There was no significant difference, B: Serum IL-10R levels in multiple myeloma was lower than normal groups significantly (p = 0.041).

IL-10R Levels:

The descriptive analysis results of IL-10R are shown in Table 2 and the Figure 1B depicted IL-10R levels. The mean IL-10R levels showed significant difference between the patients and control groups (0.041). The mean IL-10R levels were lower in multiple myeloma patients.

Table 2. Serum IL-10R levels in patients and control groups

IL-10R(pg/ml)	Minimum	Maximum	Mean	SD	Range	p value
Control	1.2	5.6	2.51	0.86	4.40	0.041
Multiple myeloma	1	4.40	2.1	0.76	3.40	

Correlation Analysis:

There was a significant positive correlation between IL-10 and IL-10R in patients, healthy subjects, and in the total population. Figure 2 showed this correlation analysis between IL-10 and IL-10R levels.

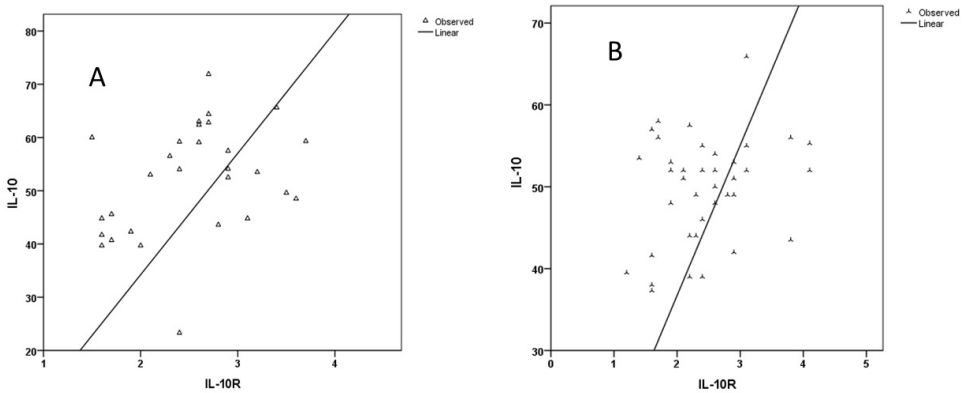

Fig. 2. Correlation between serum IL-10 and IL-10R levels in patients (A) and healthy control (B)

Discussion

About two centuries ago, multiple myeloma was characterized, but the molecular mechanisms of pathogenesis remain unclear (20, 21). Current knowledge supports the pathological role of inflammation in multiple myeloma. Pro-inflammatory cytokines have double-edge effects on multiple myeloma, which increase cancer progression by induction of cell growth and apoptosis inhibition and anticancer effect by CD4+ T cells and natural killer cells (22).

In our study, IL-10 levels showed no significant difference between patients and healthy control. However, a previous study by Pappa et al. showed increased levels of IL-10 in multiple myeloma (23). This inconsistency probably may be due to the patient's disease stage. Wang et al. showed that upregulation of IL-10 is associated with poor prognosis (24). So, in the patient population where IL-10 levels remain unchanged, there may be a good prognosis that may be due to ethnicity and genetic properties (25).

In the multiple myeloma patients, IL-6 induces cell proliferation and cancer progression via IL-6R in bone marrow stromal cells (26).

The promoter sequences regulate the gene expression by modulation of transcription factors interaction. Mazur et al. showed that IL-10 promoter gene polymorphism does not associate with the susceptibility for multiple myeloma, which is in agreement with our finding (27). However, the evaluation of serum IL-10 levels in multiple myeloma patients with poor and good prognoses showed that this cytokine is associated with better survival and outcome (24). In Wang et al.'s study, the mean IL-10 was 201.96 pg/ml, which was four times higher than our report (24). Because race and ethnicity affect the cytokine levels, and studied populations are different, we interpret this difference as racial variation (28).

The biological effects of ligands depend on ligands concentration and receptors levels (29). Therefore, we conclude that the biological effects of IL-10 in multiple myeloma patients are reduced due to the downregulation of receptors (30-32). We observed that IL-10R down-regulated in multiple myeloma patients and probably be a good biomarker for diagnosis. The reductions of biological effects of an anti-inflammatory cytokine lead to inflammation, that would trigger the cancer progression. There are reports showing that IL-2R and IL-6R, the pro-inflammatory cytokines receptors, were unregulated in multiple myeloma (6). These findings are in agreement with our findings because IL-10R is an anti-inflammatory signaling (33). Besides, Kasamatsu et al. found an association between IL-10R gene polymorphism and .multiple myeloma (34). However, another study found no significant changes in IL-10R gene expression (35).

Here, we report a positive correlation between IL-10 and IL-10R. Urbańska et al. showed that there is a strong correlation between IL-10 and IL-6 receptor (36). We have no insight into the inducing effects of IL-10 on its receptors and other cytokines levels and their receptors regulation, and need more studies (36). This study is the first report that measured the IL-10R levels in multiple myeloma patients type I. Shekarriz et al found a significant increase in the IL-10 of multiple myeloma patients where it was correlated with disease stage and markers of disease activity (37).

At the molecular level, toll-like receptors are the main upstream receptors which induce the IL-10 production in myeloma and other cell lines (38, 39). Because toll-like receptors are active in multiple myeloma, absence of changes in the IL-10 levels in the type I may be due to the stage of the disease or suppression of genes by other mechanisms such as microRNAs (40). Several studies showed that microRNAs have obvious effects on both the expression of IL-10 and multiple myeloma progression (41).

In the downstream of IL-10 receptors, STAT3 transcription factor is located, which expresses the anti-inflammatory gene (such as Bcl-xl). On the other hand, STAT3 is considered as a target for the treatment of multiple myeloma (42). Therefore, the manipulation and regulation of IL-10 and its receptors could be

considered as targets for the treatment of the disease and need more scrutiny.

Acknowledgments

We gratefully acknowledge the cooperation of all those who played a role in this research.

Conflict of interest

No conflict of interest declaration between the authors.

Funding/support

The Urmia Branch of the Islamic Azad University provided financial support for this study.

References

1. Hideshima T, Mitsiades C, Tonon G, Richardson PG, Anderson KC. Understanding multiple myeloma pathogenesis in the bone marrow to identify new therapeutic targets. Nat Rev Cancer 2007;7(8):585. https://doi.org/10.1038/nrc2189
2. Kazandjian D. Multiple myeloma epidemiology and survival: A unique malignancy. Semin Oncol 2016;43(6):676-81. https://doi.org/10.1053/j.seminoncol.2016.11.004
3. Harris NS, Winter WE. Multiple myeloma and related serum protein disorders: an electrophoretic guide: Demos Medical Publishing; 2012.
4. Schottelius AJ, Dinter H. Cytokines, NF-κB, microenvironment, intestinal inflammation and cancer. The Link Between Inflammation and Cancer: Springer; 2006. p. 67-87. https://doi.org/10.1007/0-387-26283-0_3
5. Mantovani A, Garlanda C. Inflammation and multiple myeloma: the Toll connection. Nature Publishing Group; 2006. https://doi.org/10.1038/sj.leu.2404229
6. Lauta VM. A review of the cytokine network in multiple myeloma: diagnostic, prognostic, and therapeutic implications. Cancer 2003;97(10):2440-52. https://doi.org/10.1002/cncr.11072
7. Hideshima T, Chauhan D, Schlossman R, Richardson P, Anderson KC. The role of tumor necrosis factor alpha in the pathophysiology of human multiple myeloma: therapeutic applications. Oncogene 2001;20(33):4519-27. https://doi.org/10.1038/sj.onc.1204623
8. Jourdan M, Tarte K, Legouffe E, Brochier J, Rossi JF, Klein B. Tumor necrosis factor is a survival and proliferation factor for human myeloma cells. Eur. Cytokine Netw 1999;10(1):65-70.
9. Alexandrakis M, Passam F, Ganotakis E, Sfiridaki K, Xilouri I, Perisinakis K, et al. The clinical and prognostic significance of erythrocyte sedimentation rate (ESR), serum interleukin-6 (IL-6) and acute phase protein levels in multiple myeloma. Clin Lab Haematol 2003;25(1):41-6. https://doi.org/10.1046/j.1365-2257.2003.00492.x
10. Kyrtsonis MC, Dedoussis G, Zervas C, Perifanis V, Baxevanis C, Stamatelou M, et al. Soluble interleukin-6 receptor (sIL-6R), a new prognostic factor in multiple myeloma. Br J Haematol 1996;93(2):398-400. https://doi.org/10.1046/j.1365-2141.1996.4721018.x
11. Lust JA, Donovan KA. The role of interleukin-1 beta in the pathogenesis of multiple myeloma. Hematol Oncol Clin North Am 1999;13(6):1117-25. https://doi.org/10.1016/S0889-8588(05)70115-5
12. Kühn R, Löhler J, Rennick D, Rajewsky K, Müller W. Interleukin-10-deficient mice develop chronic enterocolitis. Cell 1993;75(2):263-74. https://doi.org/10.1016/0092-8674(93)80068-P
13. Ouyang W, Rutz S, Crellin NK, Valdez PA, Hymowitz SG. Regulation and functions of the IL-10 family of cytokines in inflammation and disease. Annu Rev Immunol 2011;29:71-109. https://doi.org/10.1146/annurev-immunol-031210-101312
14. Gazzinelli RT, Wysocka M, Hieny S, Scharton-Kersten T, Cheever A, Kühn R, et al. In the absence of endogenous IL-10, mice acutely infected with Toxoplasma gondii succumb to a lethal immune response dependent on CD4+ T cells and accompanied by overproduction of IL-12, IFN-gamma and TNF-alpha. J Immun 1996;157(2):798-805. https://doi.org/10.4049/jimmunol.157.2.798
15. Sellon RK, Tonkonogy S, Schultz M, Dieleman LA, Grenther W, Balish E, et al. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient

mice. Infect Immun 1998;66(11):5224-31. https://doi.org/10.1128/IAI.66.11.5224-5231.1998

16. Gupta M, Han JJ, Stenson M, Maurer M, Wellik L, Hu G, et al. Elevated serum IL-10 levels in diffuse large B-cell lymphoma: a mechanism of aberrant Janus kinase 2 activation. Blood 2012:blood-2011-10-388538. https://doi.org/10.1182/blood-2011-10-388538
17. Liu Y, Wei S, Ho A, de Waal Malefyt R, Moore KW. Expression cloning and characterization of a human IL-10 receptor. J Immun 1994;152(4):1821-9. https://doi.org/10.4049/jimmunol.152.4.1821
18. Walter MR. The molecular basis of IL-10 function: from receptor structure to the onset of signaling. Interleukin-10 in Health and Disease: Springer; 2014. p. 191-212. https://doi.org/10.1007/978-3-662-43492-5_9
19. Boulland M-L, Meignin V, Leroy-Viard K, Copie-Bergman C, Brière J, Touitou R, et al. Human interleukin-10 expression in T/natural killer-cell lymphomas: association with anaplastic large cell lymphomas and nasal natural killer-cell lymphomas. Am J Clin Pathol 1998;153(4):1229-37. https://doi.org/10.1016/S0002-9440(10)65667-2
20. Kyle RA, Steensma DP. History of multiple myeloma. Multiple Myeloma 2011:3-23. https://doi.org/10.1007/978-3-540-85772-3_1
21. Khadem-Ansari M-H, Faridvand Y, Oskuyi AE. Lipid profile assessment in stage I multiple myeloma. Clin Comm Oncol 2014;1(1):3. https://doi.org/10.4103/2393-8633.132176
22. 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. https://doi.org/10.1155/2017/1852517
23. Pappa C, Miyakis S, Tsirakis G, Sfiridaki A, Alegakis A, Kafousi M, et al. Serum levels of interleukin-15 and interleukin-10 and their correlation with proliferating cell nuclear antigen in multiple myeloma. Cytokine 2007;37(2):171-5. https://doi.org/10.1016/j.cyto.2007.02.022
24. Wang H, Wang L, Chi PD, Wang WD, Chen XQ, Geng QR, et al. High level of interleukin-10 in serum predicts poor prognosis in multiple myeloma. Br J Cancer 2016;114(4):463-8. https://doi.org/10.1038/bjc.2016.11
25. Stowe RP, Peek MK, Cutchin MP, Goodwin JS. Plasma cytokine levels in a population-based study: relation to age and ethnicity. J Gerontol A Biol Sci Med Sci 2010;65(4):429-33. https://doi.org/10.1093/gerona/glp198
26. Hussein MA. Nontraditional cytotoxic therapies for relapsed/refractory multiple myeloma. Oncologist 2002;7(Supplement 1):20-9. https://doi.org/10.1634/theoncologist.7-suppl_1-20
27. Mazur G, Bogunia-Kubik K, Wrobel T, Karabon L, Polak M, Kuliczkowski K, et al. IL-6 and IL-10 promoter gene polymorphisms do not associate with the susceptibility for multiple myeloma. Immunol Lett 2005;96(2):241-6. https://doi.org/10.1016/j.imlet.2004.08.015
28. Brody GH, Yu T, Miller GE, Chen E. Discrimination, racial identity, and cytokine levels among African-American adolescents. J Adolesc Health 2015;56(5):496-501. https://doi.org/10.1016/j.jadohealth.2015.01.017
29. Attie AD, Raines RT. Analysis of Receptor-Ligand Interactions. J Chem Educ 1995;72(2):119-24. https://doi.org/10.1021/ed072p119
30. Zheng MM, Zhang Z, Bemis K, Belch AR, Pilarski LM, Shively JE, et al. The systemic cytokine environment is permanently altered in multiple myeloma. PLoS One 2013;8(3):e58504. https://doi.org/10.1371/journal.pone.0058504
31. Klein B, Bataille R. Cytokine network in human multiple myeloma. Hematol Oncol Clin North Am 1992a;6(2):273-84. https://doi.org/10.1016/S0889-8588(18)30344-7
32. Lauta VM. A review of the cytokine network in multiple myeloma: diagnostic, prognostic, and therapeutic implications. Cancer 2003;97(10):2440-52. https://doi.org/10.1002/cncr.11072
33. Walter MR. The molecular basis of IL-10 function: from receptor structure to the onset of signaling. Curr Top Microbiol 2014;380:191-212. https://doi.org/10.1007/978-3-662-43492-5_9
34. Kasamatsu T, Saitoh T, Ino R, Gotoh N, Mitsui T, Shimizu H, et al. Polymorphism of IL-10 receptor beta

affects the prognosis of multiple myeloma patients treated with thalidomide and/or bortezomib. Hematol Oncol 2017;35(4):711-8. https://doi.org/10.1002/hon.2322

35. Otsuki T, Yamada O, Yata K, Sakaguchi H, Kurebayashi J, Yawata Y, et al. Expression and production of interleukin 10 in human myeloma cell lines. Br J Haematol 2000;111(3):835-42. https://doi.org/10.1111/j.1365-2141.2000.02413.x
36. Urbanska-Rys H, Wiersbowska A, Stepien H, Robak T. Relationship between circulating interleukin-10 (IL-10) with interleukin-6 (IL-6) type cytokines (IL-6, interleukin-11 (IL-11), oncostatin M (OSM)) and soluble interleukin-6 (IL-6) receptor (sIL-6R) in patients with multiple myeloma. Eur Cytokine Netw 2000;11(3):443-51.
37. Shekarriz R, Janbabaei G, Kenari SA. Prognostic value of IL-10 and its relationship with disease stage in Iranian patients with multiple myeloma. Asian Pac J Cancer Prev 2018;19(1):27.
38. Dillon S, Agrawal A, Van Dyke T, Landreth G, McCauley L, Koh A, et al. A Toll-like receptor 2 ligand stimulates Th2 responses in vivo, via induction of extracellular signal-regulated kinase mitogen-activated protein kinase and c-Fos in dendritic cells. J Immunol 2004;172(8):4733-43. https://doi.org/10.4049/jimmunol.172.8.4733
39. Geijtenbeek TB, Van Vliet SJ, Koppel EA, Sanchez-Hernandez M, Vandenbroucke-Grauls CM, Appelmelk B, et al. Mycobacteria target DC-SIGN to suppress dendritic cell function. J Experim Med 2003;197(1):7-17. https://doi.org/10.1084/jem.20021229
40. Quinn SR, O'Neill LA. The role of microRNAs in the control and mechanism of action of IL-10. Curr Top Microbiol Immunol 2014;380:145-55. https://doi.org/10.1007/978-3-662-43492-5_7
41. Handa H, Murakami Y, Ishihara R, Kimura-Masuda K, Masuda Y. The Role and Function of microRNA in the Pathogenesis of Multiple Myeloma. Cancers 2019;11(11):1738. https://doi.org/10.3390/cancers11111738
42. Chong PSY, Chng WJ, de Mel S. STAT3: A Promising Therapeutic Target in Multiple Myeloma. Cancers 2019;11(5). https://doi.org/10.3390/cancers11050731