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ОЦЕНКА ПРЯМЫХ ВЗАИМОДЕЙСТВИЙ ОПУХОЛЕВЫХ КЛЕТОК, МИЕЛОИДНЫХ СУПРЕССОРНЫХ КЛЕТОК И PD-1- И TIM-3-ЭКСПРЕССИРУЮЩИХ Т-КЛЕТОК У БОЛЬНЫХ МНОЖЕСТВЕННОЙ МИЕЛОМОЙ

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Резюме. Уход атипичных плазматических клеток (ПК) из-под иммунного надзора при множественной миеломе (ММ) опосредован разнообразными механизмами, среди которых существенные роли играют индукция Т-клеточного истощения и экспансия миелоидных супрессорных клеток (МСК). При этом в настоящее время все еще нет данных о возможном влиянии МСК на индукцию Т-клеточного истощения. Целью настоящей работы была оценка возможной взаимосвязи относительного содержания атипичных ПК, МСК и фенотипически истощенных PD-1$^+$ и TIM-3$^+$Т-клеток в костном мозге (КМ) и периферической крови (ПК) больных ММ при различных стадиях заболевания. Образцы ПК (n = 88) и КМ (n = 56) были получены у больных ММ (впервые выявленные (n = 6), пациенты в ремиссии (n = 71) и с прогрессирующим течением (n = 11)). Методом проточной цитометрии была проведена оценка относительного содержания Т-клеток, экспрессирующих ингибиторные рецепторы PD-1 и TIM-3, полиморфноядерных МСК (ПМЯ-МСК, Lin$^-$CD14$^-$HLA-DR$^-$CD33$^+$CD66b$^+$), моноцитарных МСК (М-МСК, CD14$^+$HLA-DR$^{low/-}$), ранних МСК (Р-МСК, Lin$^-$HLA-DR$^-$CD33$^+$CD66b$^-$) и атипичных ПК (CD45^{dim}CD38$^+$CD138$^+$CD56$^+$CD19$^-$CD117$^+$CD27$^-$CD81$^-$, в КМ). Циркулирующие и выделенные из КМ отдельные субпопуляции PD-1$^+$/TIM-3$^+$Т-лимфоцитов, Р-МСК КМ, а также атипичные ПК и уровни бета-2-микроглобулина сыворотки последовательно увеличивались в группах больных на различных стадиях ММ, от впервые выявленных до пациентов в ремиссии и с прогрессирующим течением. Несмотря на параллельное увеличение изучаемых показателей, не было выявлено каких-либо ассоциаций между маркерами опухолевого роста (атипичные ПК КМ, концентрация бета-2-микроглобулина сыворотки) и исследуемыми популяциями клеток. В образцах КМ больных с ремиссией ПМЯ-МСК обратно коррелировали с относительным

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содержанием CD4+Т-лимофцитов, CD4+PD-1+ и CD8+TIM-3+ субпопуляциями; также обнаружены позитивные корреляции между резидентными P-МСК и CD4+PD-1+TIM-3+ клетками и между циркулирующими М-МСК и CD8+PD-1+ и (в виде тенденции) CD8+TIM-3+Т-клетками. Не удалось обнаружить каких-либо взаимосвязей между исследуемыми популяциями клеток в образцах КМ и ПК больных с впервые выявленной ММ и у пациентов с прогрессирующим течением. Возможное взаимное влияние атипичных ПК, МСК и PD-1+/TIM-3+Т-лимфоцитов, по всей видимости, не линейно, в особенности в условиях экстенсивного роста опухоли на момент постановки диагноза и прогрессии ММ. Обнаруженные обратные корреляции между относительным содержанием ПМЯ-МСК и субпопуляциями Т-клеток могли быть ассоциированы с супрессорными эффектами МСК как на преимущественно активированные PD-1+ клетки, так и на истощенные TIM-3+ субпопуляции. Прямые корреляции между резидентными P-МСК и CD4+PD-1+TIM-3+ Т-клетками и между циркулирующими М-МСК и PD-1+ и TIM-3+ CD8+Т-клетками могли подтверждать способность МСК индуцировать Т-клеточное истощение.

Ключевые слова: миелоидные супрессорные клетки, PD-1, TIM-3, Т-лимфоциты, опухолевые плазматические клетки, множественная миелома

ATTEMPT TO ASSESS DIRECT INTERACTIONS BETWEEN TUMOR BURDEN, MYELOID-DERIVED SUPPRESSOR CELLS AND PD-1- AND TIM-3-EXPRESSING T CELLS IN MULTIPLE MYELOMA PATIENTS

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Abstract. The avoidance of immune surveillance by malignant plasma cells (PCs) in multiple myeloma (MM) is mediated by different mechanisms, among which an induction of T cell exhaustion and expansion of myeloid-derived suppressor cells (MDSCs) appear to play substantial roles, but it is still a lack of data on possible MDSC-mediated induction of T cell exhaustion. The aim of the present work was to evaluate possible relationship between frequencies of MM PCs, MDSCs and phenotypically exhausted PD-1+ and TIM-3+ T cells in bone marrow (BM) samples and peripheral blood (PB) of MM patients at various disease stages. Peripheral blood (n = 88) and BM samples (n = 56) were obtained from MM patients (newly diagnosed (n = 6), patients in remission (n = 71) and with progressive disease (n = 11)). Frequencies of T cells expressing checkpoint receptors PD-1 and TIM-3, polymorphonuclear MDSCs (PMN-MDSCs, Lin−CD14−HLA-DR−CD33+CD15+/CD66b+), monocyte MDSCs (M-MDSCs, CD14+HLA-DR$^{low/-}$), early MDSCs (E-MDSCs, Lin−HLA-DR−CD33+CD15−/CD66b−), and MM PCs (CD45^{dim}CD38+CD138+CD56+CD19−CD117+CD27−CD81−) were assessed with flow cytometry. Circulating and BM-resident PD-1+/TIM-3+T cell subsets, BM E-MDSCs, as soon as MM PCs and serum beta2-microglobulin (B2-M) levels were gradually increased in patients at different stages. Despite that, there were no associations between the markers of tumor load and the studied cell subsets. In patients in remission, BM PMN-MDSCs negatively correlated with CD4+T cells, CD4+PD-1+ and CD8+TIM-3+T cell subsets; there were positive correlations between BM E-MDSCs and CD4+PD-1+TIM-3+ cells and PB M-MDSCs and CD8+PD-1+ and (as a trend) CD8+TIM-3+T cells. We found no associations for the samples of patients at diagnosis and with progression. We can conclude that a possible mutual influence of malignant PCs, MDSCs and PD-1+/TIM-3+T cells is nonlinear, especially during a manifest tumor growth at diagnosis and progression. The detected negative correlations between resident PMN-MDSCs andT cell subsets might be associated with MDSC suppressive function, affecting both predominantly activated PD-1+ cells and exhausted TIM-3+ subsets. The positive correlations between BM E-MDSCs and CD4+PD-1+TIM-3+ cell subset and circulating M-MDSCs and PD-1+ and TIM-3+ CD8+T cells might confirm an ability of MDSCs to induce T cell exhaustion.

Keywords: myeloid-derived suppressor cells, PD-1, TIM-3, T cells, tumor plasma cells, multiple myeloma

Introduction

Multiple myeloma (MM) is an incurable hematological malignancy characterized by the uncontrolled proliferation of a B cell precursor clone that differentiates to plasma cells in the bone marrow (BM). Multiple myeloma pathogenesis involves complex interactions between tumor cells, bone marrow microenvironment, and immune cells with pro- or anti-tumor properties. The avoidance of immune surveillance by malignant B cells in MM is mediated by a variety of mechanisms, among which an induction of T cell exhaustion (TCE) and expansion of myeloid-derived suppressor cells (MDSCs) seem to play crucial roles [10, 14]. T cell exhaustion is characterized by a pronounced decrease in T cell functions and phenotypically recognized by an up-regulation of inhibitory checkpoint receptors (ICRs) PD-1, TIM-3 etc [14]. An increment in T cells expressing ICRs was previously described for MM [1, 2]. Myeloid-derived suppressor cells, which origin and functions are clear from the name, are traditionally divided into three subsets: polymorphonuclear MDSCs (PMN-MDSCs, $Lin^-CD14^-HLA-DR^-CD33^+CD15^+/CD66b^+$), monocyte MDSCs (M-MDSCs, $CD14^+HLA-DR^{low/-}$) and early MDSCs (E-MDSCs, $Lin^-HLA-DR^-CD33^+CD15^-/CD66b^-$) [6].

In MM, an increase in $CD14^+HLA-DR^{-/low}$ and $CD11b^+CD14^-CD33^+$ MDSC subsets and the absence of quantitative changes compared with healthy individuals have been described [5, 8]. The suppression of the immune response [6, 8], enhancement of angiogenesis [15], support for the survival of tumor cells [8, 11] are currently identified for a tumor-promoting activity of MDSCs. Simultaneously, it is still a lack of data concerning possible MDSC-mediated induction of TCE. The aim of the present work was to evaluate possible relationship between frequencies of MM plasma cells (MMPCs), MDSCs and $PD-1^+$ and $TIM-3^+$T cells in BM specimens and peripheral blood (PB) of MM patients during the induction therapy courses.

Materials and methods

Eighty-eight MM patients who had been treated at the Department of Hematology of Research Institute of Fundamental and Clinical Immunology (Novosibirsk, Russia) were enrolled in the study. All patients gave informed consent in accordance with the Declaration of Helsinki of 1975; the local ethics committee approved the study protocol. The patients were staged according to the Durie-Salmon system (1975). Responses were defined according to the International Myeloma Working Group criteria. Baseline characteristics of patients are described in Table 1.

Peripheral blood (n = 88) and BM (n = 54) samples were obtained during routine diagnostic procedures.

TABLE 1. BASELINE CHARACTERISTICS OF PATIENTS

Characteristic	Value
Age at analysis, years; median (min-max)	52 (35-68)
Sex, female/male	47/41
Types **IgG** **IgA** **Light chain** **Unknown**	 53 18 8 9
Durie-Salmon stage **II** **III**	 25 63
Disease status at analysis: **– newly diagnosed** **– complete remission, partial response** **– progressive disease**	 6 71 11
Chemotherapy regimens before analysis **1** **2** **≥ 3**	 14 45 23
Time from the date of diagnosis to analysis, months; Me ($Q_{0.25}$-$Q_{0.75}$)	8.5 (6.1-12.7)

Mononuclear cells (MNCs) were isolated by density gradient centrifugation and stained with the following mouse anti-human monoclonal antibodies according to the manufacturer`s recommendations: CD3 (FITC, PerCP), CD4 (FITC, PerCP), CD8 (FITC, PE-Cy 7), PD-1 (PE, APC) and TIM-3 (PerCP-Cy 5.5, BV421) − for T cell assessment; Lin (FITC), CD33 (PerCP-Cy 5.5), HLA-DR (FITC, PerCP), CD66b (APC), CD14 (FITC) − for MDSC assessment. Before and after incubation with monoclonal antibodies, samples were washed with PBS 1,500 rpm for 7 min. As controls were used unstained live cells, "fluorescence-minus-one", BD CompBeads Anti-mouse Ig, /Negative Control Compensation Particles Set (BD Biosciences). The evaluated T cell and MDSC subsets were: $CD4^+PD-1^+$, $CD4^+TIM-3^+$, $CD4^+PD-1^+TIM-3^+$, $CD8^+PD-1^+$, $CD8^+TIM-3^+$, $CD8^+PD-1^+TIM-3^+$ and $Lin^-HLA-DR^-CD33^+CD66b^-$ (E-MDSCs), $Lin^-HLA-DR^-CD33^+CD66b^+$ (PMN-MDSCs), $CD14^+HLA-DR^{low/-}$ (M-MDSCs), respectively.

Samples were analyzed on FACSCalibur and FACSCanto II flow cytometers (BD Biosciences) using CellQuest Pro and FACSDiva software, respectively. T cell and MDSC subsets were presented as the percentage of lymphocytes and MNCs, respectively. To assess MMPCs, fresh BM samples were stained with the following mouse anti-human monoclonal antibodies: CD38 (FITC or APC), CD56 (PE), CD27 (PerCP-Cy5.5), CD138 (PerCP-Cy5.5 or APC), CD117 (Pe-Cy7), CD81 (APC-H7), CD19 (V450), CD45 (V450), cytIgLambda (FITC), cytIgKappa (PE-Cy7). For intracellular staining, samples were incubated with BD FACS Permeabilizing Solution 2 (BD Biosciences). Samples were analyzed on FACSCanto II flow cytometer using FACSDiva software. Multiple myeloma plasma cells were indicated as $CD45^{dim}CD38^+CD138^+CD56^+CD19^-CD117^+CD27^-CD81^-$ and were presented as the percentage of all nucleated BM cells.

Serum beta2-microglobulin (B2-M) was assessed using latex particle-enhanced turbidimetry kit (Dako Beta-2-Microglobulin PET Kit) on the IMMAGE 800 Immunochemistry System (Beckman Coulter, USA). Statistical analysis was performed using Statistica 6 (StatSoft) package. Data in the text were presented as median and interquartile ranges. The Mann–Whitney U test (two-sided) was used to calculate differences between groups of patients. Spearman's rank correlation was used to evaluate associations for

TABLE 2. COUNTS OF BONE MARROW PD-1⁺ AND TIM-3⁺ T CELL SUBSETS AND MYELOID-DERIVED SUPPRESSOR CELLS IN MULTIPLE MYELOMA PATIENTS, Me ($Q_{0.25}$-$Q_{0.75}$)

Cell subset	Newly diagnosed (n = 6)	Remission (n = 41)	Progressive disease (n = 7)
CD4⁺, %	14.4 (11.3-27.5)	24.3 (17.5-33.4)	19.4 (16.0-24.1)
CD4⁺PD-1⁺, %	2.4 (2.1-3.1)	6.2 (3.7-8.7)*	6.1 (2.0-12.7)*
CD4⁺TIM-3⁺, %	1.1 (0.8-1.7)	2.1 (1.3-3.4)	3.0 (1.3-5.1)*
CD4⁺PD-1⁺TIM-3⁺, %	0.2 (0.1-0.3)	0.7 (0.4-1.2)*	1.2 (0.6-1.4)*
CD8⁺, %	33.9 (21.6-45.6)	18.0 (12.7-25.5)*	15.6 (13.3-29.0)*
CD8⁺PD-1⁺, %	4.3 (2.9-10.9)	5.1 (3.4-7.2)	6.4 (4.6-11.4)
CD8⁺TIM-3⁺, %	2.0 (1.4-2.7)	3.7 (2.6-4.9)*	6.5 (4.7-7.2)* #
CD8⁺PD-1⁺TIM-3⁺, %	0.2 (0.1-0.7)	0.5 (0.4-0.8)	1.5 (1.0-3.4)* #
E-MDSCs, %	0.6 (0.4-0.7)	1.0 (0.6-1.5)*	1.0 (0.8-1.1)*
PMN-MDSCs, %	3.3 (1.5-6.0)	2.2 (0.7-4.0)	1.5 (0.8-2.3)
M-MDSCs, %	1.6 (1.1-3.9)	1.9 (1.2-2.9)	0.3 (0.2-0.4)* #

Note. Relative counts of T cell subsets and MDSCs are presented as the percentages of lymphocytes and mononuclear cells, respectively. p values are assessed with Mann–Whitney U test. *, $p_U < 0.05$ between newly diagnosed patients and treated patients (in remission or with progression). #, $p_U < 0.05$ between patients in remission and patients with progressive disease. E-, M-, PMN-MDSCs indicate early, monocyte, polymorphonuclear myeloid-derived suppressor cells, respectively.

TABLE 3. COUNTS OF CIRCULATING PD-1⁺ AND TIM-3⁺T CELL SUBSETS AND MYELOID-DERIVED SUPPRESSOR CELLS IN MULTIPLE MYELOMA PATIENTS, Me (Q$_{0.25}$-Q$_{0.75}$)

Cell subset	Newly diagnosed (n = 6)	Remission (n = 71)	Progressive disease (n = 11)
CD4⁺, %	33.4 (20.0-51.7)	47.8 (37.0-59.7)	41.0 (32.7-58.6)
CD4⁺PD-1⁺, %	4.6 (3.7-9.9)	5.1 (3.3-7.7)	7.0 (4.4-13.2)
CD4⁺TIM-3⁺, %	1.0 (0.5-1.3)	1.3 (0.8-3.1)	2.5 (2.2-5.2)* #
CD4⁺PD-1⁺TIM-3⁺, %	0.1 (0.1-0.3)	0.2 (0.1-0.4)	0.9 (0.4-1.1)* #
CD8⁺, %	26.1 (16.5-34.8)	22.9 (12.4-33.7)	33.7 (14.9-58.3)
CD8⁺PD-1⁺, %	2.8 (1.5-4.4)	2.7 (1.4-4.1)	5.4 (1.6-6.4)
CD8⁺TIM-3⁺, %	4.4 (3.6-6.3)	3.2 (1.8-5.5)	5.8 (2.3-9.6)
CD8⁺PD-1⁺TIM-3⁺, %	0.2 (0.2-0.4)	0.2 (0.1-0.3)	0.8 (0.4-1.4)* #
E-MDSCs, %	0.5 (0.3-0.5)	0.8 (0.5-1.2)	0.6 (0.5-0.8)
PMN-MDSCs, %	0.05 (0.02-0.06)	0.02 (0.01-0.07)	0.05 (0.02-0.6)
M-MDSCs, %	3.9 (2.2-5.1)	2.4 (1.2-5.7)	2.8 (1.7-3.9)

Note. As for Table 2.

continuous variables. p values presented were two-sided. p < 0.05 was considered statistically significant.

Results and discussion

We first comparatively assessed MMPCs in patients with different stages of the disease. Frequencies of multiple myeloma plasma cells in BM samples of patients in remission were significantly lower compared to both newly diagnosed patients and the individuals with progressive disease: 0.12% (0.027-0.875%) *vs* 21.0% (6.8-38.0%); p$_U$ = 0.00010, and 4.2% (1.4-20.6%); p$_U$ = 0.0021, respectively.

The percentages of BM CD4⁺PD-1⁺, CD4⁺PD-1⁺TIM-3⁺ and CD8⁺TIM-3⁺T cell subsets, as well as E-MDSCs were significantly higher in both patients in remission and with progressive disease compared with newly diagnosed MM (Table 2). Besides, CD4⁺TIM-3⁺, CD8⁺TIM-3⁺ and CD8⁺PD-1⁺TIM-3⁺ were increased in BM samples of MM patients with progression compared with individuals in remission and/or newly diagnosed patients (Table 2). On the contrary, BM M-MDSCs were significantly lower in patients with progression (Table 2).

Multiple myeloma PCs and the studied cell subsets were evaluated in same BM samples, and we tried to assess possible direct associations between the tumor burden and frequencies of dysfunctional T cells and MDSCs in MM microenvironment. There were no correlations between malignant PCs and these cell compartments in the each group of patients (data not shown).Then we evaluated associations between BM MDSCs and PD-1⁺/TIM-3⁺T cells. In specimens of patients in remission, PMN-MDSC frequencies negatively correlated with CD4⁺T cells (R$_S$ = -0.39, p = 0.030; n = 31), CD4⁺PD-1⁺ (R$_S$ = -0.56, p = 0.00097; n = 31) and CD8⁺TIM-3⁺T cells (R$_S$ = -0.59, P = 0.00044; n = 31), while E-MDSCs positively correlated with CD4⁺PD-1⁺TIM-3⁺ cell subset (R$_S$ = 0.39, p = 0.040; n = 31). We failed to find such associations for the samples of patients at diagnosis and with progression.

Serum B2-M is used as a surrogate marker of tumor burden. Bone marrow MMPC frequencies correlated with serum B2-M levels: R$_S$ = 0.55, p = 0.000014; n = 56. Serum B2-M levels of patients in remission were significantly lower compared to both newly diagnosed patients and the individuals with progressive disease: 2.4 mg/L (1.97-2.99 mg/L; n = 55) *vs* 5.19 mg/L (2.82-15.8 mg/L; n = 6); p$_U$ = 0.013, and 6.35 mg/L (3.37-7.75 mg/L; n = 11); p$_U$ = 0.000029, respectively. The only association we found was a negative correlation between B2-M and circulating CD4⁺T cells (R$_S$ = -0.31, p = 0.039; n = 45) in patients with remission.

The percentages of circulating CD4+PD-1+, CD4+PD-1+TIM-3+ and CD8+PD-1+TIM-3+T cell subsets were significantly higher in patients with progressive disease compared with both the newly diagnosed MM patients and the individuals in remission (Table 3). Contrary to the BM samples, there were no differences in PB MDSC subsets depending on the stage of the disease (Table 3).

Further we assessed associations between circulating MDSCs and PD-1+/TIM-3+T cells. In PB samples of patients in remission, only CD14+HLA-DR$^{low/-}$ M-MDSCs negatively correlated with CD4+T cells (R_S = -0.34, p = 0.0064; n = 60), and positively correlated with both CD8+PD-1+ (R_S = 0.28, p = 0.029; n = 60) and − as a trend − CD8+TIM-3+T cells (R_S = 0.22, p = 0.098; n = 60). As in the case of BM samples, we found no associations between circulating MDSCs and PD-1+/TIM-3+T cells of patients at diagnosis and with progression.

Conclusion

The present work was devoted to an attempt to find possible direct associations between tumor load, MDSCs and PD-1- and TIM-3-expressing T cells in multiple myeloma patients, as these cell compartments appear to play substantial roles in MM escape from immune surveillance and its clinical progression.

The described gradual increase in circulating and BM-resident PD-1+/TIM-3+T cells and E-MDSCs of patients at different stages are in agreement with the previously published data [2, 3, 7, 13]. The only unexpected finding was the decrease in CD14+HLA-DR$^{low/-}$ M-MDSCs in BM samples of patients with progression. Presumably, M-MDSC decrease was only relative, due to increase in other MNC compartments, or might be caused by intensive treatment or tumor growth itself, i.e., due to unfavorable metabolic changes.

Despite the above-described parallel increment in BM MMPCs, PD-1+/TIM-3+T cells and MDSC subsets at MM diagnosis, treatment and progression, we and others [12] found no correlations between tumor load and the studied cell subsets, although it had been obtained from the same samples. Apparently, a possible mutual influence of these cell compartments is nonlinear, especially during a manifest tumor growth at diagnosis and progression.

The detected negative correlations between resident BM PMN-MDSCs and CD4+T cells, CD4+PD-1+ and CD8+TIM-3+T cell subsets might be associated with MDSC suppressive function, affecting both predominantly activated PD-1+ cells and exhausted TIM-3+ subsets [2]. The positive correlations between both BM E-MDSCs and CD4+PD-1+TIM-3+ cell subset and circulating M-MDSCs and CD8+PD-1+ and CD8+TIM-3+T cells might confirm a poorly described ability of MDSCs to induce TCE. Previously the increase in MDSCs and PD-1+T cells was evaluated for high-risk chronic myeloid leukemia [4]. Additionally, the increment in MDSC counts is associated with the resistance to anti-PD-1 therapy for solid tumors [9]. A role of MDSC populations in the development of TCE in MM requires further investigations.

References

1. Atanackovic D., Luetkens T., Radhakrishnan S., Kroger N. Coinhibitory molecule PD-1 as a therapeutic target in the microenvironment of multiple myeloma. *Curr. Cancer Drug Targets, 2017, Vol. 17, no. 9, pp. 839-845.*

2. Batorov E.V., Aristova T.A., Sergeevicheva V.V., Sizikova S.A., Ushakova G.Y., Pronkina N.V., Shishkova I.V., Shevela E.Y., Ostanin A.A., Chernykh E.R. Quantitative and functional characteristics of circulating and bone marrow PD-1- and TIM-3-positive T cells in treated multiple myeloma patients. *Sci. Rep., 2020, Vol. 10, 20846.* doi: 10.1038/s41598-020-77941-y.

3. Chang Y., Jiang Y., Chen Y., Xing X., Zhou Y., Sang T., Li J., Zhao A., Zhang J., Zhao J., Liu Y., Zheng C. Bone marrow PD-1 positive T cells reflect tumor mass and prognosis in multiple myeloma. *Int. J. Clin. Exp. Pathol., 2018, Vol. 11, no. 1, pp. 304-313.*

4. Christiansson L., Söderlund S., Svensson E., Mustjoki S., Bengtsson M., Simonsson B., Olsson-Strömberg U., Loskog A.S.I. Increased level of myeloid-derived suppressor cells, programmed death receptor ligand 1/programmed death receptor 1, and soluble CD25 in Sokal high risk chronic myeloid leukemia. *PLoS One, 2013, Vol. 8, e55818.* doi: 10.1371/journal.pone.0055818.

5. de Veirman K., van Valckenborgh E., Lahmar Q., Geeraerts X., de Bruyne E., Menu E., van Riet I., Vanderkerken K., van Ginderachter J.A. Myeloid-derived suppressor cells as therapeutic target in hematological malignancies. *Front. Oncol., 2014, Vol. 4, 349.* doi: 10.3389/fonc.2014.00349.

6. Gabrilovich D.I. Myeloid-derived suppressor cells. *Cancer Immunol. Res., 2017, Vol. 5, no. 1, pp. 3-8.*

7. Görgün G., Samur M.K., Cowens K.B., Paula S., Bianchi G., Anderson J.E., White R.E., Singh A., Ohguchi H., Suzuki R., Kikuchi S., Harada T., Hideshima T., Tai Y.-T., Laubach J.P., Raje N., Magrangeas F., Minvielle S., Avet-Loiseau H., Munshi N.C., Dorfman D.M., Richardson P.G., Anderson K.C. Lenalidomide enhances immune checkpoint blockade-induced immune response in multiple myeloma. *Clin. Cancer Res., 2015, Vol. 21, no. 20, pp. 4607-4618.*

8.   Haile L.A., Greten T.F., Korangy F. Immune suppression: the hallmark of myeloid derived suppressor cells. *Immunol. Invest., 2012, Vol. 41, no. 6-7, pp. 581-594.*

9.   Hou A., Hou K., Huang Q., Lei Y., Chen W. Targeting myeloid-derived suppressor cell, a promising strategy to overcome resistance to immune checkpoint inhibitors. *Front. Immunol., 2020, Vol. 11, 783.* doi: 10.3389/fimmu.2020.00783.

10.   Malek E., de Lima M., Letterio J.J., Kim B.G., Finke J.H., Driscoll J.J., Giralt S.A. Myeloid-derived suppressor cells: The green light for myeloma immune escape. *Blood Rev., 2016, Vol. 30, no. 5, pp 341-348.*

11.   Marini O., Spina C., Mimiola E., Cassaro A., Malerba G., Todeschini G., Perbellini O., Scupoli M., Carli G., Facchinelli D., Cassatella M., Scapini P., Tecchio C. Identification of granulocytic myeloid-derived suppressor cells (G-MDSCs) in the peripheral blood of Hodgkin and non-Hodgkin lymphoma patients. *Oncotarget, 2016, Vol. 7, no. 19, pp. 27676-27688.*

12.   Sponaas A.-M., Yang R., Rustad E.H., Standal T., Thoresen A.S., Dao Vo C., Waage A., Slørdahl T.S., Børset M., Sundan A. PD1 is expressed on exhausted T cells as well as virus specific memory CD8^{+} T cells in the bone marrow of myeloma patients. *Oncotarget, 2018, Vol 9, no. 62, pp 32024-32035.*

13.   Tan J., Chen S., Huang J., Chen Y., Yang L., Wang C., Zhong J., Lu Y., Wang L., Zhu K., Li Y. Increased exhausted CD8^{+} T cells with programmed death-1, T-cell immunoglobulin and mucin-domain-containing-3 phenotype in patients with multiple myeloma. *Asia. Pac. J. Clin. Oncol., 2018, Vol. 14, no. 5, pp. e266-e274.*

14.   Wherry E., Kurachi M. Molecular and cellular insights into T cell exhaustion. *Nat. Rev. Immunol., 2015, Vol. 15, pp. 486-499.*

15.   Yazdani Y., Mohammadnia-Afrouzi M., Yousefi M., Anvari E., Ghalamfarsa G., Hasannia H., Sadreddini S., Jadidi-Niaragh F. Myeloid-derived suppressor cells in B cell malignancies. *Tumour Biol., 2015, Vol. 36, no. 10, pp. 7339-7353.*

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