УДК 616.24-006.6:616.329-006.61
https://doi.org/10.20538/1682-0363-2022-3-96-104

sPD-1/sPD-L1 proteins in non-small cell lung cancer and esophageal squamous cell carcinoma

Stilidi I.S.[1], Kovaleva O.V.[1], Gratchev A.N.[1], Tchevkina E.M.[1], Podlesnaya P.A.[1], Tsarapaev P.V.[1], Suleymanov E.A.[2], Kushlinskii N.E.[1]

[1] N.N. Blokhin National Medical Research Center of Oncology
24, Kashirskoe Highway, Moscow, 115478, Russian Federation

[2] People's Friendship University of Russia (RUDN University)
21/3, Miklukho-Maklaya Str., Moscow, 117198, Russian Federation

ABSTRACT

Background. Implementation of immunotherapy in clinical oncological practice has significantly improved the results of cancer treatment. It resulted in the need for seeking new markers to assess the effectiveness of therapy and the disease prognosis.

Aim. To analyze the content of soluble forms of PD-1 and PD-L1 immune checkpoint proteins in the blood serum of patients with non-small cell lung cancer and esophageal squamous cell carcinoma and their association with clinical and morphological characteristics of the disease and the disease prognosis.

Materials and methods. The study included tumor samples obtained from 43 patients with non-small cell lung cancer and 21 patients with esophageal squamous cell carcinoma. The concentration of sPD-L1 and sPD-1 in the blood serum was determined using enzyme-linked immunosorbent assay (ELISA). The Mann – Whitney test was used to determine statistically significant differences in independent groups. A correlation analysis was performed using the Spearman's rank correlation coefficient. Overall survival was analyzed by constructing survival curves using the Kaplan – Meier method and a Cox proportional hazards model. The differences were considered statistically significant at $p < 0.05$.

Results. The study showed that sPD-1 and sPD-L1 were found in the blood serum of both cancer patients and healthy donors, and their concentrations did not differ significantly. It was shown that the high concentration of sPD-L1 in the blood serum of patients with non-small cell lung cancer was significantly associated with the late stage of the disease and was an independent unfavorable prognostic factor. It should be noted that for patients with esophageal cancer, an unfavorable prognostic marker was the high concentration of the soluble form of PD-1 protein, and not PD-L1 ligand, as in case of lung cancer.

Conclusion. The content of sPD-1 and sPD-L1 in the blood serum can have different prognostic significance for various types of cancer, and further studies are required to confirm their clinical usability.

Keywords: sPD-1, sPD-L1, immunotherapy, non-small cell lung cancer, esophageal cancer, prognosis

Conflict of interest. The authors declare the absence of obvious or potential conflict of interest related to the publication of this article.

Source of financing. The study was supported by the RFBR grant (project No. 20-015-004790).

Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at N.N. Blokhin National Medical Research Center of Oncology.

✉ *Kovaleva Olga V.,* ovkovleva@gmail.com

For citation: Stilidi I.S., Kovaleva O.V., Gratchev A.N., Tchevkina E.M., Podlesnaya P.A., Tsarapaev P.V., Suleymanov E.A., Kushlinskii N.E. sPD-1/sPD-L1 proteins in non-small cell lung cancer and esophageal squamous cell carcinoma. *Bulletin of Siberian Medicine.* 2022;21(3):96–104. https://doi.org/10.20538/1682-0363-2022-3-96-104.

Белки sPD-1/sPD-L1 при немелкоклеточном раке легкого и плоскоклеточном раке пищевода

Стилиди И.С.[1], Ковалева О.В.[1], Грачев А.Н.[1], Чевкина Е.М.[1], Подлесная П.А.[1], Царапаев П.В.[1], Сулейманов Э.А.[2], Кушлинский Н.Е.[1]

[1] Национальный медицинский исследовательский центр (НМИЦ) онкологии им. Н.Н. Блохина Россия, 115478, г. Москва, Каширское шоссе, 24

[2] Российский университет дружбы народов (РУДН) Россия, 117198, г. Москва, ул. Миклухо-Маклая, 21/3

РЕЗЮМЕ

Введение. Активное внедрение иммунотерапии в клиническую онкологическую практику позволило значительно улучшить результаты лекарственного лечения опухолей. Это привело к необходимости поиска новых маркеров, с помощью которых можно оценить эффективность проводимой терапии и прогноз заболевания.

Цель исследования – анализ содержания растворимых форм белков контрольных точек иммунитета sPD-1 и PD-L1 в сыворотке крови больных немелкоклеточным раком легкого и плоскоклеточным раком пищевода, а также их ассоциации с клинико-морфологическими характеристиками заболевания и прогнозом.

Материалы и методы. В исследование включены образцы опухолей от 43 пациентов с немелкоклеточным раком легкого и 21 пациента с плоскоклеточным раком пищевода. Концентрацию sPD-L1 и sPD-1 определяли в сыворотке крови с помощью иммуноферментного анализа. Для определения статистически значимых различий в независимых группах использовали критерий Манна – Уитни. Корреляционный анализ проводили с помощью определения коэффициента ранговой корреляции Спирмена. Анализ общей выживаемости – путем построения кривых дожития по методу Каплана – Мейера и с использованием модели пропорциональных рисков Кокса. Статистически достоверными считались различия при $p < 0,05$.

Результаты. Показано, что sPD-1и sPD-L1 обнаруживаются в сыворотке крови как у пациентов с онкологическими заболеваниями, так и здоровых доноров, и их концентрации значимо не отличаются. Показано, что высокая концентрация sPD-L1 в сыворотке крови больных немелкоклеточным раком легкого значимо ассоциирована с поздней стадией заболевания и является независимым неблагоприятным прогностическим фактором. Необходимо отметить, что для пациентов с раком пищевода неблагоприятным прогностическим маркером является высокое содержание растворимой формы рецептора PD-1, а не его лиганда PD-L1, как для рака легкого.

Выводы. Содержание в сыворотке крови sPD-1и sPD-L1 может иметь различное прогностическое значение для злокачественных опухолей различных нозологий, и необходимость его анализа для клинического применения требует дальнейшего изучения.

Ключевые слова: sPD-1, sPD-L1, иммунотерапия, немелкоклеточный рак легкого, рак пищевода, прогноз

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Работа выполнена при финансовой поддержке гранта РФФИ (проект № 20-015-004790).

Соответствие принципам этики. Все участники исследования подписали добровольное информированное согласие. Исследование одобрено локальным этическим комитетом (НМИЦ) онкологии им. Н.Н. Блохина.

Для цитирования: Стилиди И.С., Ковалева О.В., Грачев А.Н., Чевкина Е.М., Подлесная П.А., Царапаев П.В., Сулейманов Э.А., Кушлинский Н.Е. Белки sPD-1/sPD-L1 при немелкоклеточном раке легкого и плоскоклеточном раке пищевода. *Бюллетень сибирской медицины.* 2022;21(3):96–104. https://doi.org/10.20538/1682-0363-2022-3-96-104.

INTRODUCTION

Currently, immunohistochemistry is considered to be a gold standard in molecular diagnosis, which is used as the main method for making decisions on immunotherapy. However, available methods for predicting a patient's response to therapy or detection of residual disease using molecular biology research are insufficient. At the same time, the field of application of immunotherapy is constantly expanding, and there is an urgent need for new diagnostic markers or methods of their use that will allow to improve the results of cancer treatment.

According to their functional characteristics, immune checkpoints, which are the targets of immunotherapy, can be divided into two groups: molecules activating the immune response and those inhibiting it. The most well-described checkpoint molecules are PD-1 / PD-L1 and CTLA-4 that belong to the second group. The PD-1 / PD-L1 interaction promotes tumor escape from immune surveillance by suppressing T cell activity. High tissue expression of these proteins may be associated with a poor prognosis for various types of tumors.

In recent years, a large number of new receptors and their ligands involved in immune regulation have been identified and described. In addition to immune checkpoints on the cell surface, soluble forms of these proteins have been identified in the bloodstream. The presence of these proteins in biological fluids is due to proteolysis, alternative splicing, and their presence on the surface of exosomes [1–3]. It has been shown that as a result of proteolysis or alternative splicing, soluble forms of PD-1 and PD-L1 (sPD-1 and sPD-L1, respectively) can be generated [4]. sPD-L1 and sPD-1 can be detected and quantified in the bloodstream of patients with various solid tumors [5–7], which opens prospects for the development of methods for minimally invasive diagnosis and therapy monitoring. However, the role of sPD-L1 and sPD-1 in the pathogenesis of malignant tumors has not been clearly defined. In this regard, the aim of this study was to analyze the content of sPD-1 and sPD-L1 in the blood serum of patients with non-small cell lung cancer (NSCLC) and esophageal squamous cell carcinoma (ESCC) and their association with clinical and morphological characteristics of the disease and the disease prognosis.

MATERIALS AND METHODS

The study included 43 patients with NSCLC and 21 patients with ESCC, as well as 9 healthy donors who were examined and treated at the N.N.Blokhin National Medical Research Center of Oncology. All procedures involving patients and healthy donors, performed during the study, comply with the ethical principles of the local Ethics Committee and the 1964 Declaration of Helsinki and its subsequent amendments or comparable ethical standards. An informed consent was obtained from each individual included in the study. The clinical diagnosis in all patients was confirmed by the data of the morphological study of the tumor according to the WHO Classification of Tumors of the Digestive System (2019) and Tumors of the Lung (2021). The description of the studied samples is presented in Tables 1 and 2.

Table 1

Clinical and morphological characteristics of NSCLC patients

Parameter	Number of cases (%)
Age	
≤61	22 (51%)
>61	21 (49%)
Gender	
Male	37 (86%)
Female	6 (14%)
Histology	
Adenocarcinoma	18 (42%)
Squamous cell lung cancer	25 (58%)
Stage	
I–II	25 (58%)
III–IV	18 (42%)
Localization	
Central	26 (60%)
Peripheral	17 (40%)
Tumor size (T)	
T1–T2	28 (65%)
T3–T4	15 (35%)
Nodal status (N)	
N0	17 (40%)
N+	26 (60%)
Grade (G)	
G1–G2	27 (63%)
G3	16 (37%)

Table 2

Clinical and morphological characteristics of ESCC patients

Parameter	Number of cases (%)
Age	
≤62	11 (52%)
>62	10 (48%)
Gender	
Male	18 (86%)
Female	3 (14%)
Stage	
I–II	9 (43%)
III–IV	12 (57%)
Tumor size (T)	
T1–T2	6 (29%)
T3–T4	15 (71%)
Nodal status (N)	
N0	10 (48%)
N+	11 (52%)
Grade (G)	
G1–G2	15 (24%)
G3	6 (76%)

The levels of sPD-L1 and sPD-1 were determined in the blood serum obtained according to the standard method before the initiation of specific treatment using PD-L1 Human ELISA and PD-1 Human ELISA kits (Affimetrix, eBioscience, USA) in accordance with the manufacturer's instructions. The measurements were carried out on the automated ELISA analyzer BEP 2000 Advance (Siemens Healthcare Diagnostics, Germany). The concentrations of the markers were expressed in picograms (pg) per 1 ml of the blood serum.

The obtained data were processed using the GraphPad Prizm 9.0 software. To compare the variables and analyze their relationships, we used the nonparametric Mann – Whitney test and the Spearman's rank correlation coefficient. To analyze the overall survival, patients were divided into 2 comparison groups depending on the median content of sPD-1 and sPD-L1 in the blood serum. Overall survival was analyzed by constructing survival curves using the Kaplan – Meier method. Statistical significance of differences was compared using the log rank test. To assess the potential impact of various risk factors on survival, a multivariate analysis was additionally performed using a nonparametric Cox proportional hazards model. The differences and correlations were considered statistically significant at $p < 0.05$.

RESULTS

This study is devoted to the analysis of the content of soluble forms of sPD-1 and sPD-L1 checkpoint proteins in the blood serum of patients with NSCLC and ESCC and their association with the clinical and morphological characteristics of patients and the prognostic value. At the first stage of the study, the diagnostic potential of the studied proteins was assessed. The median concentrations of sPD-1 and sPD-L1 in the blood serum of healthy donors were 30.9 (28.2–42.8) pg / ml and 0.59 (0.40–1.5) pg / ml, respectively, in the group of patients with NSCLC – 34.3 (27.4–45.4) pg / ml and 0.95 (0.33–2.08) pg / ml, respectively, and in the group of patients with ESCC – 30.9 (26.0–53.9) and 0.956 (0–2.45) pg / ml, respectively. The statistical analysis showed that the content of soluble forms of sPD-1 and sPD-L1 did not differ significantly between healthy donors and patients with cancer, therefore, it was concluded that these proteins cannot be used as diagnostic markers. Further, the association between the levels of sPD-1 and sPD-L1 in the blood serum of patients with NSCLC and ESCC and the clinical and morphological characteristics of the diseases was analyzed. The results are presented in Tables 3 and 4.

Table 3

Association between serum sPD-L1 and sPD-1 levels and clinical and morphological characteristics of NSCLC patients

Parameter	sPD-1, pg / ml			sPD-L1, pg / ml		
	Me	(Q_1–Q_3)	*p*	*Me*	(Q_1–Q_3)	*p*
Age						
≤61	33.81	(27.22–51.69)	0.833	0.965	(0.401–1.835)	0.824
>61	34.60	(27.27–38.63)		1.090	(0.211–2.758)	
Gender						
Male	34.19	(27.17–45.57)	0.458	0.965	(0.464–2.143)	0.738
Female	35.25	(31.85–54.65)		0.776	(0.062–4.252)	
Histology						
Adenocarcinoma	34.47	(27.05–47.58)	0.765	0.903	(0.464–2.481)	0.513
Squamous cell lung cancer	34.19	(27.88–42.22)		0.965	(0.106–2.020)	
Stage						
I–II	33.44	(27.71–41.97)	0.966	0.715	(0.000–1.649)	0.037*
III–IV	34.98	(26.56–47.18)		1.463	(0.652–3.067)	

Table 3 (continued)

Parameter	sPD-1, pg / ml			sPD-L1, pg / ml		
	Me	(Q_1–Q_3)	*p*	*Me*	(Q_1–Q_3)	*p*
Localization						
Central	34.26	27.31–47.18	0.820	0.965	0.306–2.019	0.698
Peripheral	34.60	27.37–41.97		1.090	0.274–2.143	
Tumor size (T)						
T1–T2	33.88	(27.66–38.16)	0.701	0.965	(0.083–1.927)	0.372
T3–T4	35.63	(23.92–51.95)		1.214	(0.464–2.944)	
Nodal status (N)						
N0	35.56	(30.74–56.66)	0.153	0.715	(0.042–1.835)	0.419
N+	30.57	(26.31–40.41)		1.028	(0.464–2.298)	
Grade (G)						
G1–G2	34.33	(27.65–51.60)	0.394	1.214	(0.211–2.575)	0.513
G3	32.96	(24.66–36.84)		0.840	(0.464–1.494)	

The analysis showed that the increased concentration of sPD-L1 in the blood serum of patients with NSCLC was significantly associated with the late stage of the disease. No associations with other tumor characteristics were found for NSCLC, although it should be noted that there was a trend toward a decrease in the sPD-1 concentration in the blood serum of patients in the presence of regional metastasis.

For ESCC, it was found that in the group of patients with T1–T2 tumors, the concentration of sPD-L1 was significantly higher than in the group of patients with T3–T4 tumors (Table 4).

Table 4

Association between serum concentrations of sPD-L1 and sPD-1 and clinical and morphological characteristics of ESCC patients

Parameter	sPD-1, pg / ml			sPD-L1, pg / ml		
	Me	(Q_1–Q_3)	*p*	*Me*	(Q_1–Q_3)	*p*
Age						
≤62	30.98	(29.62–37.15)	0.545	0.965	(0.000–2.821)	0.685
>62	29.08	(24.27–59.24)		0.840	(0.000–2.174)	
Gender						
Male	30.98	(25.29–51.32)	0.406	0.778	(0.000–2.451)	0.836
Female	33.57	(30.23–72.88)		1.090	(0.000–2.944)	
Stage						
I–II	32.28	(29.92–53.90)	0.496	1.463	(0.295–2.698)	0.230
III–IV	29.52	(24.92–63.95)		0.274	(0.000–2.111)	
Tumor size (T)						
T1–T2	53.90	(33.04–78.70)	0.010*	1.772	(0.818–2.852)	0.202
T3–T4	29.62	(24.73–32,28)		0.464	(0.000–2.451)	
Nodal status (N)						
N0	32.92	(29.23–59.24)	0.306	1.277	(0.443–2.574)	0.194
N+	30.23	(24.73–37.15)		0.083	(0.000–2.451)	
Grade (G)						
G1–G2	30.98	(25.56–36.25)	0.436	1.028	(0.000–2.729)	0.473
G3	48.75	(26.09–80.21)		0.464	(0.000–1.772)	

PROGNOSTIC VALUE OF SPD-L1 / SPD-1 IN PATIENTS WITH NSCLC AND ESCC

Depending on the concentration of soluble forms of the studied proteins, for the analysis of survival rates, the patients were divided into 2 groups: with high and low levels of sPD-1 / sPD-L1 relative to the median. Patient survival graphs are shown in the Figure.

The results of the study showed that the increased concentration of sPD-L1 in NSCLC was significantly associated with a poor disease prognosis. For sPD-L1 in ESCC, a similar pattern was observed, but the data did not reach statistical significance. The concentration of sPD-1 in the blood serum is not a prognostic marker in patients with both NSCLC and ESCC. However, it should be noted that

in patients with a high content of sPD-1 in the blood serum, a trend toward an unfavorable prognosis was noted.

Next, a univariate and multivariate statistical analysis of the prognostic value of the studied markers was carried out. The results are presented in Table 5.

Table 5

Statistical analysis of the prognostic value of sPD-L1 / sPD-1 in patients with NSCLC and ESCC

Parameter	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*	HR	95% CI	*p*
sPD-1 NSCLC (high / low)	0.885	(0.285–2.749)	0.831	1.020	(0.985–1.053)	0.253
sPD-L1 NSCLC (high / low)	3.937	(1.257–12.34)	0.026*	1.214	(0.983–1.464)	0.046*
sPD-1 ESCC (high / low)	2.199	(0.731–6.613)	0.139	1.045	(1.014–1.082)	0.006*
sPD-L1 ESCC (high / low)	1.998	(0.647–6.164)	0.199	1.269	(1.002–1.650)	0.051

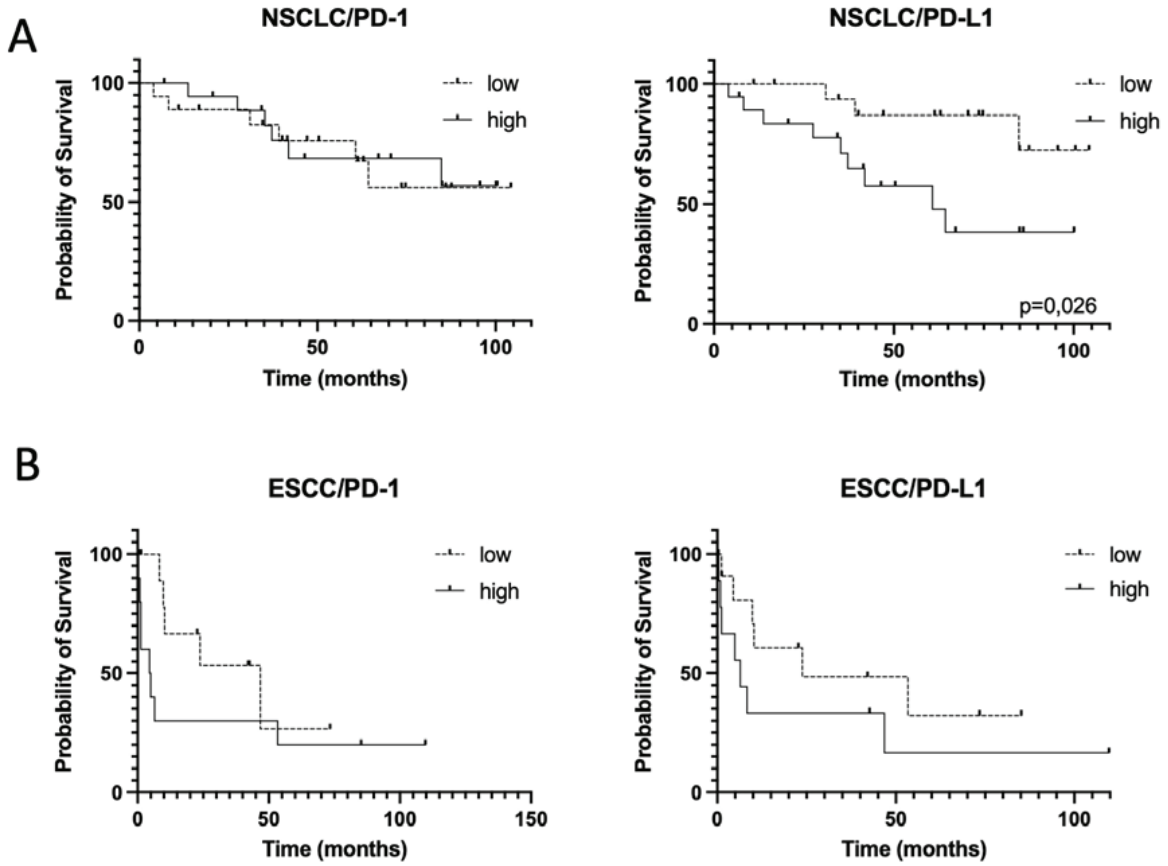

Figure. Analysis of the overall survival in NSCLC and ESCC patients depending on serum concentrations of sPD-L1 and sPD-1

Cox's regression analysis showed that high levels of sPD-1 in ESCC and high levels of sPD-L1 in NSCLC were independent prognostic factors associated with reduced survival. Thus, a high content of the soluble form of the sPD-L1 ligand in the blood serum of patients with NSCLC is an unfavorable prognostic factor, while for patients with ESCC, a high content of the soluble form of the sPD-1 receptor, rather than its ligand, is an unfavorable prognostic marker.

At the final stage of the study, a correlation analysis of the content of sPD-1 / sPD-L1 in the blood serum of patients with NSCLC and ESCC was carried out. The analysis did not reveal a correlation between the content of the studied proteins in ESCC ($r = 0.119$; $p = 0.609$), however, in NSCLC, the content of sPD-1 significantly correlated with the content of sPD-L1 $r = 0.331$; $p = 0.03$). It may indicate that the mechanisms of interaction of these proteins may be tissue independent.

DISCUSSION

Despite the rapid development of immunotherapy in the treatment of cancer, the frequency of objective responses to drugs of this class for patients with ESCC and NSCLC is insufficient. In this regard, the identification and validation of new biomarkers of the effectiveness of immunotherapy drugs is currently extremely relevant. The results of modern studies demonstrate that the assessment of PD-L1 expression is a key factor for evaluating the effectiveness of treatment with immune checkpoint inhibitors in patients with malignant tumors of various localizations. However, the analysis of tissue expression of PD-L1 is not unified and differs depending on the drug used. Thus, there is an evaluation scale that takes into account only tumor cells, while another evaluation method takes into account the expression of PD-L1 on the surface of both tumor and immune cells of the stroma. In addition, currently, when methods for minimally invasive diagnosis and neoadjuvant chemotherapy are being developed, it is of great importance to have a possibility to evaluate the effectiveness of prescribed drugs and the response to ongoing treatment using soluble blood markers [8].

Currently, anti-PD-1/PD-L1 immunotherapy is the preferred second-line, and in some cases, first-line therapy for NSCLC [9]. From the literature data, it is known that sPD-L1 in the blood can be considered as a marker of the effectiveness of treating patients with NSCLC with immune checkpoint inhibitors. It was shown that a high level of sPD-L1 two months after treatment with nivolumab was associated with a poor response to ongoing therapy. It should be noted that, according to the results of this study, the concentration of sPD-L1 did not correlate with the level of tissue expression of this protein [10]. The PD-1 / PD-L1 checkpoint inhibitors nivolumab and pembrolizumab are both FDA and EMA approved for the treatment of esophageal tumors, but the use and efficacy of these drugs, similar to NSCLC, depend on PD-L1 expression in the tumor. There is emerging evidence that sPD-L1 levels also have a predictive potential for evaluating the effectiveness of anti-PD-1/PD-L1 monotherapy in cases of ESCC [8], namely, a higher plasma concentration of sPD-L1 before treatment is a predictor of increased effectiveness of this type of therapy.

The present study analyzed the concentrations of soluble sPD-1 and sPD-L1 in the blood serum of patients with NSCLC and ESCC. The obtained results showed that an increased level of sPD-L1 in the blood serum of patients with NSCLC was associated with a later stage of the disease and a poor disease prognosis. The results obtained are consistent with the literature data [11–13]. Moreover, D. Jovanovic et al. demonstrated that a higher level of sPD-L1 in the blood is typical of patients with NSCLC, compared with lung tumors of other types [13]. It should be noted that the study of the sPD-L1 content in the blood of patients with lung cancer is relevant not only in the context of immunotherapy, but also in the study of the effectiveness of tyrosine kinase inhibitors [14]. In our study, the level of sPD-1 in NSCLC was not prognostically significant, although literature data show that an increase in the concentration of this protein is associated with a rise in overall and disease-free survival rates in patients with advanced NSCLC with *EGFR* mutations treated with erlotinib [15].

The analysis of the association of sPD-1 level with clinical and morphological characteristics of esophageal cancer showed that a higher concentration of this protein is characteristic of T1–T2 tumors. At the same time, it should be noted that our study showed for the first time that sPD-1 is an independent statistically significant marker of a poor ESCC prognosis. High serum levels of PD-L1 in patients with esophageal tumors were also associated with a poor prognosis, but the data did not reach statistical significance. This is consistent with a number of published studies [16, 17]. The obtained results together with the published data indicate that additional studies with larger cohorts are needed making it possible to use sPD-1 and sPD-L1 in clinical practice.

CONCLUSION

The results of the study indicate that currently there is no unequivocal opinion regarding the clinical and prognostic value of both tissue expression of the key immune checkpoints PD-1 / PD-L1 and their soluble forms. It is also worth noting that most studies have revealed the absence of a correlation between the tissue expression of these proteins and their presence in a soluble form in biological fluids, which certainly indicates the need for further study of their interaction. With further accumulation of data, their spread will decrease, which will ultimately lead to the possibility of their application in clinical practice.

REFERENCES

1. Orme J.J., Enninga E.A.L., Lucien-Matteoni F., Dale H., Burgstaler E., Harrington S.M. et al. Therapeutic plasma exchange clears circulating soluble PD-L1 and PD-L1-positive extracellular vesicles. *J. Immunother Cancer*. 2020;8(2):e001113. DOI: 10.1136/jitc-2020-001113.

2. Chen Y., Wang Q., Shi B., Xu P., Hu Z., Bai L. et al. Development of a sandwich ELISA for evaluating soluble PD-L1 (CD274) in human sera of different ages as well as supernatants of PD-L1+ cell lines. *Cytokine*. 2011;56(2):23–28. DOI: 10.1016/j.cyto.2011.06.004.
3. Daassi D., Mahoney K.M., Freeman G.J. The importance of exosomal PDL1 in tumour immune evasion. *Nat. Rev. Immunol*. 2020;20(4):209–215. DOI: 10.1038/s41577-019-0264-y.
4. Zhou J., Mahoney K.M., Giobbie-Hurder A., Zhao F., Lee S., Liao X. et al. Soluble PD-L1 as a Biomarker in Malignant Melanoma Treated with Checkpoint Blockade. *Cancer Immunol. Res*. 2017;5(6):480–492. DOI: 10.1158/2326-6066.CIR-16-0329.
5. Hofman P., Heeke S., Alix-Panabieres C., Pantel K. Liquid biopsy in the era of immuno-oncology: is it ready for prime-time use for cancer patients? *Ann.Oncol.* 2019;30(9):1448–1459. DOI: 10.1093/annonc/mdz196.
6. Kushlinskii N.E., Gershtein E.S., Goryatcheva I.O. et al. Soluble forms of the immune checkpoint receptor PD-1 and its ligand PD-L1 in blood serum of patients with renal cell carcinoma: clinical and pathologic correlations. *Cancer Urology*. 2019;15(1):15–22 (in Russ.). DOI: 10.17650/1726-9776-2019-15-1-15-22.
7. Kovaleva O.V., Rashidova M.A., Gratchev A.N., Maslennikov V.V., Boulitcheva I.V., Gershtein E.S. et al. Immunosuppression factors PD-1, PD-L1, and IDO1 and colorectal cancer. Dokl Biochem Biophys. 2021;497(1):160–164 (in Russ.). DOI: 10.31857/S2686738921020153.
8. Ji S., Chen H., Yang K., Zhang G., Mao B., Hu Y. et al. Peripheral cytokine levels as predictive biomarkers of benefit from immune checkpoint inhibitors in cancer therapy. *Biomed. Pharmacother.* 2020;129:110457. DOI: 10.1016/j.biopha.2020.110457.
9. Assi H.I., Kamphorst A.O., Moukalled N.M., Ramalingam S.S. Immune checkpoint inhibitors in advanced non-small cell lung cancer. *Cancer*. 2018;124(2):248–261. DOI: 10.1002/cncr.31105
10. Costantini A., Julie C., Dumenil C., Helias-Rodzewicz Z., Tisserand J., Dumoulin J. et al. Predictive role of plasmatic biomarkers in advanced non-small cell lung cancer treated by nivolumab. *Oncoimmunology*. 2018;7(8):e1452581. DOI: 10.1080/2162402X.2018.1452581.
11. Okuma Y., Hosomi Y., Nakahara Y., Watanabe K., Sagawa Y., Homma S. High plasma levels of soluble programmed cell death ligand 1 are prognostic for reduced survival in advanced lung cancer. *Lung Cancer*. 2017;104:1–6. DOI: 10.1016/j.lungcan.2016.11.023.
12. Castello A., Rossi S., Toschi L., Mansi L., Lopci E. Soluble PD-L1 in NSCLC Patients Treated with Checkpoint Inhibitors and Its Correlation with Metabolic Parameters. *Cancers (Basel)*. 2020;12(6):1373. DOI: 10.3390/cancers12061373
13. Jovanovic D., Roksandic-Milenkovic M., Kotur-Stevuljevic J., Ceriman V., Vukanic I., Samardzic N. et al. Soluble sPD-L1 and serum amyloid A1 as potential biomarkers for lung cancer. *J. Med. Biochem*. 2019;38(3):332–341. DOI: 10.2478/jomb-2018-0036.
14. Jia Y., Li X., Zhao C., Ren S., Su C., Gao G. et al. Soluble PD-L1 as a predictor of the response to EGFR-TKIs in non-small cell lung cancer patients with EGFR mutations. *Front. Oncol*. 2020;10:1455. DOI: 10.3389/fonc.2020.01455.
15. Sorensen S.F., Demuth C., Weber B., Sorensen B.S., Meldgaard P. Increase in soluble PD-1 is associated with prolonged survival in patients with advanced EGFR-mutated non-small cell lung cancer treated with erlotinib. *Lung Cancer*. 2016;100:77–84. DOI: 10.1016/j.lungcan.2016.08.001.
16. Shiraishi T., Toyozumi T., Sakata H., Murakami K., Kano M., Matsumoto Y. et al. Soluble PD-L1 concentration is proportional to the expression of PD-L1 in tissue and is associated with a poor prognosis in esophageal squamous cell carcinoma. *Oncology*. 2022;100(1):39–47. DOI: 10.1159/000518740.
17. Fu R., Jing C.Q., Li X.R., Tan Z.F., Li H.J. Prognostic significance of serum PD-L1 level in patients with locally advanced or metastatic esophageal squamous cell carcinoma treated with combination cytotoxic chemotherapy. *Cancer Manag Res*. 2021;13:4935–4946. DOI: 10.2147/CMAR.S312690.

Authors contribution

Stilidi I.S. – clinical work with patients, editing of the article. Kovaleva O.V. – statistical processing of the data, drafting of the article. Gratchev A.N. – literature review, drafting of the article. Tchevkina E.M. – literature review, drafting of the article. Podlesnaya P.A. – acquisition of the experimental data. Tsarapaev P.V. – acquisition of the experimental data. Suleymanov E.A. – editing of the article. Kushlinskii N.E. – design of the study, coordination of work, final editing and approval of the manuscript for publication.

Authors information

Stilidi Ivan S. – Dr. Sci. (Med.), Professor, Academician of the RAS, Head of the N.N. Blokhin National Medical Research Center of Oncology, Moscow, https://orcid.org/0000-0002-0493-1166

Kovaleva Olga V. – Cand. Sci. (Biology), Senior Researcher, Laboratory for regulation of cellular and viral oncogenes, N.N. Blokhin National Medical Research Center of Oncology, Moscow, ovkovleva@gmail.com, https://orcid.org/0000-0001-6132-9924

Gratchev Alexei N. – Dr. Sci. (Biology), Head of the Laboratory for tumor stromal cell biology, N.N. Blokhin National Medical Research Center of Oncology, Moscow, Alexei.gratchev@gmail.com, https://orcid.org/0000-0003-2137-1866

Tchevkina Elena M. – Dr. Sci. (Biology), Head of the Laboratory for regulation of cellular and viral oncogenes, N.N. Blokhin National Medical Research Center of Oncology, Moscow, tchevkina@mail.ru, https://orcid.org/0000-0001-8837-7969

Podlesnaya Polina A. – Union Researcher, Laboratory for tumor stromal cell biology, N.N. Blokhin National Medical Research Center of Oncology, Moscow, polina.pod@yandex.ru, https://orcid.org/0000-0003-2312-5546

Tsarapaev Pavel V. – Research Laboratory Assistant, Laboratory for Clinical Biochemistry, N.N. Blokhin National Medical Research Center of Oncology, Moscow, pcarapaev96@gmail.com, https://orcid.org/0000-0002-1182-1010

Suleymanov Elkhan A. – Professor, Department of Urology and Operative Nephrology with a Course in Oncourology, RUDN University, Moscow.

Kushlinskii Nikolai E. – Dr. Sci. (Med.), Professor, Academician of the RAS, Head of the Laboratory for Clinical Biochemistry, N.N. Blokhin National Medical Research Center of Oncology, Moscow, biochimia@yandex.ru, https://orcid.org/0000-0002-3898-4127

(✉) **Kovaleva Olga V.,** ovkovleva@gmail.com

Received 07.03.2022;
approved after peer review 06.05.2022;
accepted 25.05.2022