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REVIEW



# BIOMARKERS FOR NON-SMALL CELL LUNG CANCER IMMUNOTHERAPY

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## ABSTRACT

The discovery of immune checkpoint inhibition has revolutionized the treatment of many solid malignancies, including non-small cell lung cancer (NSCLC). Immune checkpoint inhibitors (ICI) can restore the antitumor immune response by blocking the inhibition of T-cell activation. Anti-programmed death-ligand 1 (PD-L1) is currently the main biomarker of the effectiveness of anti-PD-1 / PD-L1 blockade in the treatment of NSCLC without driver mutations. High tumor mutational burden suggests an increased neoantigens load and has been associated with the effectiveness of ICI therapy. Microsatellite instability, a biomarker approved for immunotherapy across solid tumors, but it is uncommon in NSCLC. Primary resistance to ICIsis characteristic of NSCLC with driver mutations, acquired is associated with immunoediting resulting in the depletion of potentially immunogenic neoantigens. The review discusses recent advances and future directions for predicting the results of immunotherapy in patients with NSCLC.

**Keywords:**
non-small cell lung cancer, immunotherapy, biomarkers, checkpoint inhibitor, anti-programmed death-ligand 1, tumor mutation burden.

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ОБЗОР

# БИОМАРКЕРЫ ДЛЯ ИММУНОТЕРАПИИ НЕМЕЛКОКЛЕТОЧНОГО РАКА ЛЕГКОГО

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## РЕЗЮМЕ

Открытие ингибирования иммунных контрольных точек произвело революцию в лечении многих солидных злокачественных новообразований, включая немелкоклеточный рак легкого (НМРЛ). Ингибиторы иммунных контрольных точек (ИИКТ) обладают способностью восстанавливать противоопухолевый иммунный ответ, блокируя торможение активации Т-лимфоцитов. Anti-programmed death-ligand 1, трансмембранный белок, лиганд к рецептору PD-1(PD-L1) в настоящее время является основным биомаркером эффективности анти-PD-1/ PD-L1 препаратов лечении НМРЛ без драйверных мутаций. Высокая мутационная нагрузка опухоли, предполагающая повышенную продукцию неоантигенов, также ассоциируется с эффективностью иммунотерапии. Микросателлитная нестабильность – другой биомаркер, одобренный для иммунотерапии при солидных опухолях, – редко наблюдается при НМРЛ. Первичная резистентность к ИИКТ характерна для онкодрайверного НМРЛ, приобретенная связана с иммунноредактированием в результате истощения потенциально иммуногенных неоантигенов. В обзоре обсуждаются последние достижения и будущие направления прогнозирования результатов иммунотерапии у больных НМРЛ.

**Ключевые слова:**
немелкоклеточный рак легкого, иммунотерапия, биомаркеры, ингибиторы иммунных контрольных точек, anti-programmed death-ligand, опухолевая мутационная нагрузка.

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## INTRODUCTION

The discovery of immune checkpoint inhibition has revolutionized the treatment of many solid malignancies, including non-small cell lung cancer (NSCLC). Anti-programmed death-ligand 1, transmembrane protein, ligand to the PD-1 receptor (PD-L1) is currently the main biomarker of the effectiveness of anti-PD-1/ PD-L1 drug blockade in the treatment of NSCLC without driver mutations.

To date, the biomarkers approved by the Food and Drug Administration (FDA) for determining indications for immunotherapy of progressive NSCLC are: the proportion of a tumor proportion score (TPS) expressing PD-L1 on tumor cells and microsatellite instability. Other promising markers studied for immunotherapy are: tumor mutation burden (TMB), tumor-infiltrating lymphocytes (TILs), and the density of CD8+T cells in the tumor microenvironment. The genomic landscape of a tumor affects its immunogenicity and response to immunotherapy. The review discusses the latest achievements and future directions for predicting the results of immunotherapy in NSCLC patients.

### PD-L1 tumor expression

PD-L1 is a transmembrane protein encoded by the PD-L1 gene found on chromosome 9 in humans. Constitutive expression of low-level PD-L1, characteristic of resting lymphocytes, antigen-presenting cells of other tissues, is necessary for maintaining homeostasis in anti-inflammatory conditions [1]. The inhibitory PD-1 molecule present on B-lymphocytes, activated T-lymphocytes and natural killer cells binds to the ligands PD-L1 (B7-H1 or CD271+) and PD-L2 (B7-DC or CD273+) [2]. The interaction of the PD-1 molecule with the PD-L1 sligand inhibits the proliferation, survival and activity of cytotoxic T-lymphocytes, induces apoptosis of tumor-infiltrating lymphocytes (TILs) and the accumulation of immunosuppressive regulatory T cells (T-reg.) in the tumor microenvironment [3]. With advanced NSCLC, approximately 40 to 58 % of patients have PD-L1-negative tumors, 28 to 31 % have tumors with low (1-49 %) expression of PD-L1, and only 10 to 32 % have tumors with high (50 % or more) expression of PD-L1 [4, 5]. Antibody blockade of immune control points of the PD-1/PD-L1 axis revolutionized the treatment of advanced and metastatic NSCLC, becoming the standard of first-line treatment of patients both in isolation and in combination with chemotherapy [6].

The expression of PD-L1 is determined by the immunohistochemical method. 5 different anti-PD-L1 immunoglobulins of the IgG1 class are used for testing in clinical trials: 22C3, 28-8, SP142, SP263 and 73-10. The percentage of expression is most often measured using the TPS indicator, which is estimated by quantifying viable tumor cells with partial or complete staining of cell membranes [7].

Numerous clinical studies [8] of the use of anti-PD-1 and anti-PD-L1 antibodies have shown the value of studying the expression of PD-L1 as a predictive biomarker. A randomized clinical trial of KEYNOTE-010, which compared the effectiveness of pembrolizumab at two different doses of 2 or 10 mg/kg every 3 weeks with docetaxel chemotherapy in previously treated patients with progressive NSCLC with a TPSPD-L1 index of ≥1 %. The main endpoints of the study were determined by the overall survival (s) and progression – free survival (PFS-progression-free survival). Patients treated with pembrolizumab had a significantly longer median S: 10.4 months. when prescribing pembrolizumab at a dose of 2 mg/kg (HR0, 71, $p$=0.008) and 12.7 months at a dose of 10 mg/kg (HR 0.61 $p$<0.00001) compared with patients receiving only docetaxel-8.5 months. After 1 year, most of the patients receiving pembrolizumab were alive: in the group of pembrolizumab at a dose of 10 mg/kg, OV was 52.3 %, and at a dose of 2 mg/kg – 43.2 %, compared with those receiving docetaxel – 34.6 %. A subgroup analysis revealed that a higher PD-L1 TPS is a predictor of longer survival. The median OV of patients with TPS PD-L1≥50 % was 14.9 months in the group of patients receiving pemrolizumab at a dose of 2 mg/kg versus 8.2 months. In the docetaxel group (HR 0.54; 95 % [CI] 0.38-0.77; $p$=0.0002) and 17.3 months. In the group of patients receiving pemrolizumab at a dose of 10 mg/kg versus 8.2 months in the docetaxel group (HR 0.50; 95 % [CI] 0.36-0.70; $p$<0.0001) [8].

In a study by Reck M, Rodriguez-Abreu D, Robinson AG, et al. PHASE 3 KEYNOTE-024 The efficacy of pembrolizumab immunotherapy compared to standard two – component platinum – containing chemo-

therapy in the first line for EGFR-and ALK-negative advanced NSCLC with PD-L1 TPS expression ≥ 50 % was studied. As a result, the study demonstrated clear advantages in patients receiving immunotherapy in terms of median PFS, S and the frequency of objective responses to treatment. The median response duration in the pembrolizumab group was not reached [5].

Recently, the results of the follow-up study of the KEYNOTE-024 study were published [9]. The median OS in the group of patients receiving pembrolizumab in the first line was 30.0 months versus 14.2 months in the chemotherapy group [9]. The presented results ultimately led to the approval of pembrolizumab monotherapy in patients with metastatic NSCLC without activating mutations with high PD-L1 expression (Table 1).

A phase 3 clinical trial of KEYNOTE-042 led to the approval of pembrolizumab for PD-L1 – positive progressive NSCLC with any level of PD-L1 expression. The KEYNOTE-042 protocol is a randomized open-label international double-blind study of pembrolizumab immunotherapy compared to standard chemotherapy in patients with untreated metastatic PD-L1-positive (TPS≥1 %) NSCLC. Patients who started treatment showed significantly longer GS in the group receiving pembrolizumab compared to first-line chemotherapy in all PD-L1 positive groups: PD-L1 TPS≥50 % – HR 0.69, 95 % [CI] 0.56-0.85, $p$=0.0003; PD-L1 TPS≥20 % – HR 0.77, 95 % [CI] 0.64-0.92, $p$=0.002 and PD-L1 TPS≥1 %-HR 0.81, 95 % [CI] 0.71-0.93, $p$=0.0018 [10]. The median GS was 17.7 months in the pembrolizumab group versus 12.2 months in the chemotherapy group; among patients with PD-L1 TPS≥50 %, the median GS reached 17.7 months versus 16.7 months in the group with PD-L1 TPS≥20 % and against 12.1 months. in the group with PD-L1 TPS ≥1 %, respectively [10].

It was shown that smoking or quit smoking patients with progressive non-squamous NSCLC who received nivolumab had better GS indicators compared to non-smoking patients [12]. Two studies have linked the history of smoking with an increase in TPS PD-L1 [11, 12]. The group of patients with the nicotine addiction gene had a higher level of objective response – 56 % compared to the group of patients without it – 17 % ($p$=0.03) [12]. In addition, the clinical study of KEYNOTE-024 demonstrated an increase in the survival rate when quitting smoking during immunotherapy [5].

In most clinical studies of IT immunotherapy in EGFR – and ALK-negative progressive NSCLC, high levels of PD-L1 expression correlated with better GS, PFS indicators and the frequency of objective responses to treatment compared to first-line chemotherapy [9, 13]. However, for patients with metastatic NSCLC, whose disease progressed on platinum-containing two-component chemotherapy, both nivolumab and atezolizumab are approved in the second line regardless of PD-L1 expression [14-17].

### Microsatellite instability and MMR-deficient malignant tumors

The defective DNA repair process is known to lead to hypermutation genomic status, otherwise called high microsatellite instability (MSI-H). Mismatch repair (MMR) DNA repair proteins are represented by: MutL homolog 1 (MLH1), MutS homolog 2 (MSH2), MutS homolog 6 (MSH6) and PMS1 Homolog 2 (PMS2). Inactivation of any of the genes encoding

**Table 1. Approved biomarkers for IT immunotherapy in NSCLC**

| Biomarkers approved by FDA | Drug | Therapy outcomes | Evidence-based clinical studies |
|---|---|---|---|
| PD-L1 ≥ 50 % | Pemrolizumab in the first line against chemotherapy | The best indicators of GS and PFS in the pemrolizumab group | KEYNOTE-024 [9] |
| PD-L1 ≥ 50 % | Pemrolizumab in the first line | The best indicators of GS and PFS in the pemrolizumab group | KEYNOTE-042 [10] |
| MSI-H | Pemrolizumab for any morphological subtype | The best indicators of GS and PFS | D.T.Le et al, 2015 [22] |

Note: MSI-H – microsatellite instability high.

these proteins occurs in 80 % of cases as a result of somatic mutations, and in 20 % is secondary to germinal mutations, followed by a second inactivating somatic damage in the remaining wild-type allele [18]. MMR-deficient colorectal cancer carries 100 times more somatic mutations than MMR-deficient adenocarcinomas. MMR-deficient cancers and among them NSCLC have pronounced lymphocytic infiltrates that correlate with the immune response [19].

MSI-H (microsatellite instability high) tumors or tumors with high microsatellite instability show increased regulation of control points in the tumor microenvironment, including PD1, PD-L1, LAG3 (lymphocyte activation gene 3) and IDO (indolamine 2,3-dioxygenase). These control points that suppress the activity of CD8+cytotoxic T-lymphocytes that infiltrate the tumor microenvironment are also found in MMR-deficient malignant neoplasms [20]. In a phase 2 clinical study, the results of therapy of patients with MMR-deficient and MMR-surplus solid tumors, including NSCLC, treated with pembrolizumab were compared. WES-whole-exome sequencing revealed approximately 1,782 somatic mutations per tumor in patients with MMR-deficient cancer and an average of 73 mutations per tumor in patients with MMR-surplus cancer ($p$=0.007). The observed objective response rate was 39.6 % in a cohort of 149 patients with 15 different solid tumors, including NSCLC, of which 7 % had a complete response. Four out of 10 patients with MMR-deficient colorectal cancer responded to imunotherapy with pembrolizumab (Table. 1) [21].

Based on the study under discussion, pembrolizumab is approved by the FDA for the treatment of adults and children with unresectable or metastatic, MSI-H – positive or MMR-deficient solid tumors that do not have alternative treatment options after progression [19].

### Mutational load of the tumor

The mutational burden of a tumor (TNB) is a set of somatic non-synonymous mutations: insertions, deletions and substitutions of protein-coding bases in the coding region of the tumor genome. The increased mutational load is the result of exposure to smoking, radiation, ultraviolet rays and other environmental and nutritional factors that lead to inflammation. It is suggested that high TMB enhances immunogenicity by increasing the number of neoantigens expressed by cancer cells, which are recognized by T-lymphocytes as foreign, causing a stronger immune response in the presence of IT (Table. 2) [22, 23].

TMB is measured by various methods, including full-exome sequencing (WES-whole-exome sequencing) and targeted next-generation sequencing (NGS) panels. The use of WES for the determination of TMV in NSCLC patients revealed an association between a higher load of somatic non-synonymous mutations and the clinical efficacy of pembrolizumab in 2 different groups of patients [24]. In the group with high TMB, consisting of 16 patients with a predominant clinical response duration of more than 6 months, the average number of non-synonymous mutations was 302 versus 148 for the group with a short response ($p$=0.02). In patients with a high load of tumors with non-synonymous mutations, an increase in the level of objective response was observed to 63 % versus its complete absence ($p$=0.03) and survival rates to progression with a median of 14.5 versus 3.7 months (HR 0.19, 95 % CI 0.05-0.70; $p$=0.01) [24]. An inde-

**Table 2. Potential biomarkers for IT immunotherapy in NSCLC**

| Studied biomarkers | Medication | Therapy outcomes | Evidence-based clinical studies |
|---|---|---|---|
| TMB high | Nivolumab, Ipilimumab | ORR, PFS indicators improvement | CheckMate-227 [25] CheckMate-026 [23] |
| STK11/LKB1 Mutation | Anti-PD-1 or anti-PD-L1 antibodies or a combination of anti-PD-L1 with anti-CTLA-4 antibodies | Shorter PFS | SU2C and CheckMate-057 [28] |
| HLA class I allele C03:04 | IT | Shorter PFS | M.V.Negrao et al, 2019 [32] |
| Acquired loss of beta-2-microglobulin | Combination of anti-PD-L1 with anti-CTLA-4 antibodies | IT resistance | S.Gettinger et al, 2017 [31] |

pendent set of 18 NSCLC samples from patients receiving pembrolizumab formed a validation group. The average load of non-synonymous mutations was 244 in the tumors of patients with a long-term clinical response, compared with 125 in tumors without one ($p$=0.04). Significantly longer PFS was observed in patients with a non-synonymous mutational load above 200: their median PFS was not reached compared to 3.4 months in the group with low TMV (HR 0.15, 95 % CI0, 04-0.59; $p$=0.006) [24].

Subsequently, as part of the CheckMate-026 study, TMV was calculated using tumor WES and compared with blood DNA in 312 patients. The patients were divided into trigroups in accordance with the values of TMV. TMV from 0 to less than 100 mutations was considered a low load, from 100 to 242 mutations were considered an average load, and from 243 or more mutations were considered a high load. Patients with high TMV treated with nivolumab had higher objective response rates – 47 % vs. 28 % and longer PFS with a median of 9.7 months vs. 5.8 months. compared with patients who received chemotherapy [23].

CheckMate-227-an open phase 3 clinical trial compared the results of immunotherapy with nivolumab, nivolumab in combination with ipilimumab – an CTLA-4-anti–cytotoxic Tlymphocyte–associated protein (4anti-CTLA-4 antibody) and nivolumab in combination with platinum-containing two-component chemotherapy in patients with stage IV NSCLC. TMV was calculated using the NGS target panel after applying various filters and, as a result, was divided into a calculated area (0.8 Mb) to calculate the number of mutations per megabase. Among the patients selected for TMV, a predetermined TMV reference point of 10 mutations per mega base was selected for a preliminary study of the effectiveness of combined immunotherapy with nivolumab and ipilimumab in comparison with chemotherapy in the group. Patients who received combined immunotherapy with anti-PD-1 and anti-CTLA-4 antibodies had a higher level of objective response – 45.3 % versus 26.9 % in those who were treated with chemotherapy. The median PFS was 7.2 months. when using nivolumab and ipilimumab against 5.5 months. with chemotherapy (HR-0.58; 97.5 % [CI] 0.41-0.81; $p$<0.001), a1-year PFS: 42.6 % vs. 13.2 %, respectively [25].

It was found that TMV calculated using MSK-IMPACT (Memorial Sloan Kettering-Integrated Mutation Profilingof Actionable Cancer Targets), a clinical diagnostic platform for molecular oncology of solid tumors based on NGS, predicts survival after immunotherapy for several types of cancer. The study also included 350 NSCLC patients who received IT therapy. The reference point determined by the 30 % normalized MSK-IMPACT mutation load for NSCLC was 10.8 mutations per megabase. The patients of this group had the best OV (HR 0.75; $p$<0.032) [26].

Thus, TMV is a new biomarker that, as shown in several clinical studies, predicts the response to IT immunotherapy. A higher TMV of the tumor, apparently, increases the probability that immunogenic neoantigens cause a pronounced antitumor response. Currently, it is necessary to harmonize the methods of measuring TMV for the use of a biomarker in clinical practice in order to optimally select patients who will benefit from the appointment of immunotherapy.

### Tumor infiltrating lymphocytes

The infiltration of the tumor by lymphocytes correlates with the best survival of patients who underwent surgical treatment. In the Lung Adjuvant Cisplatin Evaluation Biomarker (LACE) Bio study, which included patients with localized NSCLC, the degree of tumor infiltration by lymphocytes was studied based on the criterion-intensive or non-intensive, with the primary endpoint chosen by the S, and the secondary endpoint-relapse-free survival. Intensive lymphocytic infiltration was defined as more than 50 % of lymphocytes in the volume of tumor tissue compared to epithelial tumor cells. The study established the lymphocytic infiltration of the tumor as an independent prognostic factor predicting a longer life of patients whose removed tumors had intense lymphocytic infiltration [27].

The above-mentioned effectiveness of IT in MMR-deficient and MSI-H tumors is not least associated with their high lymphocytic infiltration. However, it is important not only the total number of lymphocytes, but also their subpopulation composition and functional activity. Thus, CD8+ infiltration by lymphocytes is associated with a positive effect of immuno-

therapy, and the accumulation of Tregs cells in the tumor is an unfavorable predictor sign. As shown in experimental models, their removal with CD25 antibodies before the use of anti-PD-1 antibodies leads to an increased antitumor response and, in the future, may be one of the therapeutic strategies [28].

**STK11/LKB1 gene**

The CTK11/LKB1(Serine/threonine-proteinkinase/liverkinaseB1) gene is a tumor suppressor gene inactivated in approximately one-third of KRAS-mutant NSCLC, playing a key role in the primary resistance of NSCLC to IT. The STK11/LKB1 gene encodes serine-threonine kinase, which, when inactivated by mutational or non-mutational mechanisms, affects the immune microenvironment of the tumor, leading to a decrease in the number of tumor-infiltrating cytotoxic CD8+ T lymphocytes both in human tumors and in genetically engineered mouse models [29]. Increased accumulation of T-cell depletion markers and increased production of interleukin-6 by tumor cells, leading to the recruitment of myeloid cells and neutrophils with suppressive properties against T-lymphocytes, was shown in mice with the knockout CTK11/LKB1 gene. Neutrophil depletion and neutralization of interleukin-6 in mouse models with loss of STK11/LKB1 enhanced the function and increased the number of T-lymphocytes in the tumor [30]. STK11/LKB1 is an ascending activator of the AMPK (adenosinemonophosphate-activated proteinkinase) signaling pathway, which, being inactive, cannot block the mTOR (mammalian target of rapamycin) signaling pathway or induce mitochondrial autophagy. The activated mTOR signaling pathway ultimately leads to the growth of tumor cells. Repeated induction of LKB1 restored the level of PD-L1 expression on the surface of tumor cells, stimulating the chemotaxis of T-lymphocytes [31].

Retrospective clinical studies of SU2C and CheckMate-057 of two different groups of patients showed that the alteration of the STK11/LKB1 gene in lung adenocarcinomas makes them less sensitive to IT immunotherapy with a significant decrease in the level of objective response, PFS ($p$<0.001) and OV ($p$=0.0015) compared with lung adenocarcinomas with REDSTK11/LKB1 mutations of the wild type [29].

**HLA class I**

HLA (human leukocyte antigen) of class I plays an important role in the antitumor immune response, and a wider range of molecules is believed to lead to an increase in the chances of presentation of an immunogenic antigen and the probability of a response to IT. The main histocompatibility complex I (MHC-I) in humans is represented by the classical molecules HLA-A, HLA-B and HLA-C. A decrease in the expression of beta-2-microglobulin, which is a component of MHC-I, is described as a mechanism of acquired resistance to IT immunotherapy [32]. However, a recent study conducted in MD Anderson Cancer Center, which compared 3 different groups of patients with progressive NSCLC who received anti-PD-1/PD-L1 immunotherapy, did not reveal differences in results depending on HLA status. The study evaluated the expression of PD-L1, TMB, HLA genotype, mutational status and the presence of STK11 mutations; all these biomarkers were correlated with the results of treatment. After HLA typing, 2 groups were identified: HLA-heterozygous, with heterozygosity of patients in all classes of HLA, and homozygous, with homozygosity of at least 1 locus of the HLAI class. HLA-A and HLA-B were grouped into supertypes. There was no statistically significant difference in PFS between heterozygous and homozygous HLA patients [33].

In recent years, the role of non-classical molecules of the main histocompatibility complex has been considered, among which HLA-G attracts special attention. It is expressed on a number of cells, including tumor cells, and exhibits an immunosuppressive effect by interacting with inhibitory ILT2 and ILT4 receptors expressed on many cells of the immune system (NK, T, B, DC). These receptors bind to HLA-G 3-4 times more strongly than with classical MHCI, which indicates the leading role of this interaction in regulating the activity of T cells and antigen-presenting cells. In addition, ILT2 and ILT4 receptors compete with CD8+ lymphocytes for binding to MHCI, which results in inhibition of their cytotoxicity [34]. Such features of the tumor microenvironment can affect the IT effect.

**Predictive multi-ohmic model**

Given the complexity of the interaction between the immune system and the tumor, it is likely that one biomarker will not be enough to determine the

treatment tactics, so it may be necessary to use a combination of biomarkers. It was found that a trivariant multiomic model consisting of TMV, estimatedCD8+T-cell abundance (eCD8T) and fraction ofhigh PD-1 messenger RNA (fPD1) improves the ability to predict the response to IT immunotherapy in various types of malignant tumors [35]. TMB and the level of objective response to anti-PD-1/PD-L1 therapy have a high and statistically significant correlation, eCD8T is also characterized by a strong positive correlation with the level of objective response. Most types of malignant tumors with a higher objective response rate than predicted by the TMB regression model have higher levels of ECD8T and vice versa. The integration of TMB and ECD8T models significantly improved the prediction of the response, demonstrating a significantly improved likelihood function compared to one-dimensional models ($p$<0.001) [35]. The addition of FPD1 to the two-dimensional TMB – eCD8T model showed that the resulting three-dimensional regression model has significantly better prediction accuracy ($p$<0.02). Subtypes of malignant tumors with a higher level of responses than predicted by the bivariant prognostic model have higher levels of fPD1, and those with low levels of response are characterized by lower levels of fPD1 [35].

### NSCLC Mutation Status

Tumors of patients with driver NSCLC give different responses to IT immunotherapy. For example, it is known that tumors carrying the EGFR gene mutation are characterized by feedback with PD-L1 expression, low TMV, absence of T-lymphocytic infiltration and a reduced ratio of PD-L1+ / CD8+tumor-infiltrating lymphocytes ($p$=0.034) [36]. In a promising phase 2 study of the effectiveness of pembrolizumab in patients with EGFR-mutant NSCLC, objective responses to IT therapy with activating EGFR mutations were not observed [37].

An international retrospective study of IMMUNOTARGET examined data from 551 patients with driver mutations, including KRAS, EGFR, ALK, ROS1, BRAF, RET, MET amplification or MET mutation in the 14exone and activating mutationher2. Anti–PD-1 antibodies were received by 94 % of patients and anti-PD-L1 antibodies by 6 %. Only 5 % of patients received IT in the first line of therapy and 40 % in the second line; the rest received immunotherapy as the third line and subsequent lines. The percentage expression of PD-L1 in driver mutations is as follows: HER2-0, EGFR – 3.5 %, ALK – 7.5 %, KRAS – 12.5 %, RET – 26 %, MET – 30 %, BRAF – 50 % and ROS1-90 %. The overall objective response, depending on the driver alteration, was: KRAS – 26 %, BRAF – 24 %, ROS1 – 17 %, MET – 16 %, EGFR – 12 %, HER2 – 7 %, RET – 6 % and ALK – 0 %. For patients with KRAS-mutant NSCLC, no difference in PFS between the subtypes of the KRAS mutation was established. However, PD-L1 positivity was statistically significantly correlated with a longer median PFS: 7.2 months versus 3.9 months ($p$=0.01). Patients with BRAF mutant and HER2-mutant NSCLC smokers had a longer PFS compared to those who had never smoked: 4.1 months versus 1.9 months ($p$=0.03) and 3.4 months versus 2.0 months ($p$=0.04), respectively. PD-L1-positive driver NSCLC with fusion and rearrangements: ALK, ROS1 and RET did not give any response to IT immunotherapy, amedian PFS in never-smokers was equal to 2.6 months. it turned out to be slightly longer compared to smokers-1.8 months ($p$=0.03) [38].

## CONCLUSION

Currently, the considered biomarkers are being studied to determine the relationship of immunotherapy with long-term results. Thus, high PD-L1 expression, high TMB and intensive infiltration of the tumor by CD8+T-lymphocytes are associated with the clinical effectiveness of blocking immune control points. The expression of PD-L1, in turn, correlates with the severity of infiltration by T-lymphocytes and S. The study of the composition of 3 biomarkers suggests a high potential of the multicomic model for predicting the long-term results of treatment of patients receiving immunotherapy. Soluble PD-L1 and TMB in the blood are tested as biomarkers for the selection of candidates who are indicated for immunotherapy. It is even more necessary to identify biomarkers of acquired NSCLC resistance to IT blockade in order to identify patients who need treatment correction to achieve the best results.

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