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The scientific monograph presents the theoretical and practical aspects of the development of modern scientific research. General questions of economics and enterprise management, regional economics, marketing, technical sciences, technology of food and light industry, and so on are considered. The publication is intended for scientists, educators, graduate and undergraduate students, as well as a general audience.

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LUNG CANCER BIOMARKERS

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Abstract. According to CLOBOCAN, in 2040 the incidence of cancer will increase by 47% compared to 2020. Lung cancer is the leading cause of death from malignant neoplasms in both men and women. In 2020, 2.2 million new cases of lung cancer and 1.8 million deaths from the disease were registered. It is unlikely that this problem will be solved soon. Therefore, doctors are faced with the question of early diagnosis of malignant neoplasms, determination of prognostic factors and personification of treatment. In Ukraine, about 42% of lung tumors are diagnosed at an advanced stage. Such patients have a poor prognosis and an overall 5-year survival of no more than 5%. This is because non- small cell lung cancer has a low sensitivity to classical cytotoxic therapy. Therefore, it is important to know which part of the pathological process can be targeted so that the patient receives the maximum effect from treatment and the minimum number of side effects. For patients whose cancer can be completely removed surgically, personalization of treatment is also important. Patients usually receive adjuvant chemotherapy after radical surgical treatment for stage IV-IIIA lung cancer. Unfortunately, in some of them in the coming years after surgery there is a recurrence of the disease. Therefore, the study of molecular genetics and other biological markers is important because it allows you to choose the path for targeted, immunotherapy or intensification of classical chemotherapy by increasing the total number of cycles. The review presents the main molecular genetics and immunohistochemical markers of lung cancer, which determine the prognosis and treatment tactics. The expediency of testing tumor tissue for the most common genetic mutations (EGFR,

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B-RAF) and gene rearrangements (ALK, ROS1) is substantiated. The expediency of performing immunohistochemical research in comparison with molecular genetics and its influence on treatment tactics is considered. A comparative analysis of the reliability and informativeness of the results of immunohistochemical studies. Values of immune checkpoint inhibitors on the tumor cell surface (PD-1, PD-L, CTLA-4), intracellular immune checkpoint inhibitors (CISH), protein 53 and proliferation marker Ki-67 for the diagnosis of lung cancer are described. This review considers the choice of optimal treatment option based on data obtained during testing for the most common diagnostic markers of lung cancer.

Introduction

According to CLOBOCAN, incidence of cancer in 2040 will increase by 47% compared to 2020. Malignant neoplasms are one of the reasons that do not increase human life expectancy. At the same time, appearance of malignant tumors is a consequence of increased life duration. It is unlikely that problem of neoplasms will be solved in the nearest future. Therefore, doctors are faced with the problems of early detection of malignant neoplasms, determination of prognostic factors and treatment personification [1, p. 15].

Lung cancer is the leading cause of death from malignant neoplasms in both men and women. Over 2.2 million new cases of lung cancer and 1.8 million deaths from the disease were registered in 2020. Among men population, lung cancer is most diagnosed in 36 countries, although it is the leading cause of death in 93 countries. The highest rates detected in Micronesia / Polynesia, Eastern and Southern Europe, East, and West Asia. This disease is especially common among men in Turkey. Among women, lung cancer is the leading cause of death in 25 countries in North America, Northern and Western Europe, Micronesia / Polynesia, Australia / New Zealand. Hungary has the highest incidence rate of lung cancer in women [1, p. 16].

The incidence rate of lung cancer among men and women can vary significantly. Usually, men get sick 2-3 times often, but in some countries the incidence rate among women is 6-7 times lower. This feature can be observed in North Africa. In North America incidence rates in men and woman are equal. In these countries, lung cancer in men is found only 20% more often than in women [1, p. 16; 2, p. 95].

Appearance of lung cancer can be the resulted by various risk factors, such as harmful working conditions, unhealthy lifestyle, the presence of occupational (eg, asbestosis) or chronic lung disease during life, genetic predisposition. Also influence on development of lung cancer have ethnicity, gender, race, geographical location [3, p. 8; 4, p. 330; 5, p. 570; 6, p. 776; 7, p. 6]. All of factors that were mentioned previously are still very important, but the main cause of lung cancer is smoking. The World Health Organization in its report noted the correlation between the prevalence of smoking among people over 15 and the incidence of lung cancer in low-income countries [8, p. 466; 9, p. 10]. Since 2000, amount of women who smoke has increased significantly. Compared to 1970, morbidity and mortality among this population has doubled. It is interesting, but it was found that level of economic development of the country did not influence on the mortality from lung cancer among men. Conversely, cancer mortality is much higher among women in industrialized countries. In developing countries, lung cancer among women has significantly lower incidence than breast tumors [3, p. 8].

Lung cancer belongs to a heterogeneous group of diseases. Histological
variants of tumors in lung cancer have clinical importance. Most often non- small cell lung cancer is diagnosed. Non-small cell lung cancer is divided into 3 subtypes: squamous cell, adenocarcinoma, large cell. During last decades scientists mentioned tendency in changing of proportion between different histological variants in the whole world. Thus, until 90s, squamous cell lung cancer predominated among men, while now the incidence of adenocarcinoma is increasing among both men and women.

Last years, more and more attention is paid to molecular genetic testing of tumor tissues and determining levels of expression of biological markers on the surface of malignant cells. In the eighth edition of the TNM classification, that was published in 2017, these markers were identified as new and promising prognostic factors. In addition, examination of this factors is extremely important for determination of treatment tactics.

In Ukraine, about 42% of lung tumors are diagnosed at the last stage. Such patients have a poor prognosis and overall, 5-year survival is not more than 10-15% [10]. This is because non-small cell lung cancer has a low sensitivity to classical cytotoxic therapy. Therefore, it is important

to know which part of the pathological process can be targeted for patient to receive maximum effect from treatment and minimum number of side effects.

For patients whose tumor can be completely removed surgically, personalization of treatment is also important. Patients usually receive adjuvant chemotherapy after radical surgical treatment for lung cancer IB-IIIA stages. Unfortunately, in some cases after surgery there is a recurrence of the disease. Therefore, the study of molecular genetics and other biological markers is important because it allows to choose the path for targeted, immunotherapy or intensification of classical chemotherapy by increasing the total number of cycles. Below, main molecular, genetic and immunohistochemical markers of lung cancer that influence on the prognosis and treatment options will be described.

1. Markers for targeted therapy

1.1 Epidermal growth factor receptor (EGFR)

Epidermal growth factor receptor is one of the most common mutations in patients with non-small cell lung cancer. At first it was described in 2004. It has a huge impact on the proliferation and survival of malignant cells [11, p. 50213]. EGFR mutation occurs mainly in patients with adenocarcinoma. For people with squamous cell tumors, it is very rare. Due to this, diagnostic study for this group of the patients is not performed [12, p. 669]. EGFR is a member of the tyrosine kinase receptor family. They can be activated by binding to the dimerized ligand and the receptor. This leads to the activation of signaling pathways that stimulate angiogenesis, proliferation, cells migration, inhibit apoptosis and increase the survival of atypical cells.

There are many types of EGFR activating mutations. Deletion of exon 19 and point mutation of L858R in exon 21 are clinically significant. The presence of these mutations is a predictor of a good prognosis in patients after the treatment with targeted therapy. About 80% of patients have satisfactory treatment results after the use of tyrosine kinase inhibitors. The recurrence- free period lasts on average about 13 months [13, p. 288]. Several clinical studies have shown that the overall survival of patients after targeted therapy is significantly higher among patients with a 19-exon deletion. For the L858R mutation, such a pattern was not observed [14, p. 729; 15, p. 38].

The main reason for the recurrence of the disease and the development of resistance to treatment is the appearance of a specific mutation T790M. In 60% of patients, it occurs after 9-13 months of treatment with first- generation tyrosine kinase inhibitors (erlotinib, gefitinib, and afatinib) [16, p. 289; 17, p. 923]. Some patients are insensitive to specific targeted therapy. Resistance may be due to the presence of additional mutations such as KRAS or rearrangements in ALK and ROS1 genes.

Treatment with tyrosine kinase inhibitors has opened completely new horizons for patients with activating EGFR mutations. Back in the middle of the 20th century scientists noted that lung cancer was sensitive to chemotherapy, but the median survival of patients remained low. After the FDA approved the use of first-generation of tyrosine kinase inhibitors (erlotinib, gefitinib, and afatinib), they were recommended as first-line therapy in patients with EGFR-activating mutations [18, p. 73].

The next challenge is treatment of patients who had a recurrence of the disease after treatment with first-line drugs. Due to this, arise need to influence on T790M mutation, which led to resistance to treatment. After a detailed study of the biological features of this mutation, several specific pyrimidines were isolated. There have been many clinical trials investigating the effectiveness of pyrimidines WZ3146, WZ4002 and WZ8040, but none of them have been approved by the FDA for use. Osimertinib is the only drug available for use. It is a representative of third-generation tyrosine kinase inhibitors. Osimertinib is prescribed in case of recurrence of the disease in patients previously taking first-generation tyrosine kinase inhibitors. It should be noted that this drug has low efficacy against wild-type tumors.

Immunohistochemical examination is the most common method of laboratory diagnostic of lung cancer. The main disadvantages of this method are differences in results obtained in different laboratories and the peculiarities of the interpretation of results. It is important to highlight that about 70% of tumors have high levels of expression of EGFR protein. The intensity of expression can depend on the way how tumor tissue was prepared, the method of obtaining antigen or the type of antibody clone. For example, when histological specimens were made from formalin-fixed tumor blocks, the higher level of expression was found in samples fixed with alcohol or CytoLyt solution (Hologic). If slides were air-dried, staining was much less pronounced [19, p. 103].

Specific antibody clones are used for effective diagnostic. The results of such investigation will differ from other immunohistochemical techniques, including total EGFR and phosphorylated EGFR. Specific antibody clones recognize the three-dimensional structure of a protein through a specific mutation. This mutation can turn into another combination of amino acids. It is characterized by the inability to bind to wild-type EGFR protein. Various antibodies have been developed to diagnose the L858R point mutation in exon 21 and the 15 bp deletion in exon 19. For EGFR L858R-mutant antibodies, the sensitivity and efficiency were 95% and 100%, respectively. The mutant-specific EGFR antibody of exon 19 was less effective in detecting exon 19 deletions. Only 15 bp deletions were successfully diagnosed.

According to the CAP / IASLC / AMP Guidelines for Laboratory

Diagnostic of Lung Cancer, antibodies specific for the EGFR mutation may be used only after careful testing for valid molecular analysis under limited material conditions [20, p. 332]. Nowadays in the updated guidelines in 2017, the widespread usage of immunohistochemistry for the selection of patients for therapy with tyrosine kinase inhibitors is not recommended [20, p. 335].

Several studies have shown no correlation between the presence of EGFR mutations and the expression level of wild (total) EGFR. Therefore, it is not advisable to prescribe targeted therapy with tyrosine kinase inhibitors only based on immunohistochemical investigation [21, p. 928]. The value of this method was evaluated during the implementation of the human monoclonal antibody necitumumab for the treatment of patients with squamous cell lung cancer. For the use of this drug in combination with chemotherapy, the expression level of wild-type EGFR protein is very important [22, p. 770]. In the United States, the FDA has approved the use of necitumumab in combination with cisplatin and gemcitabine, regardless EGFR expression status. Necitumumab has also been approved by the European Medicines Agency. This drug is allowed for treatment of patients with last stages squamous cell non-small cell lung cancer, which according to immunohistochemistry expresses wild-type EGFR protein.

Determination of phosphorylated EGFR is useless due to sample
instability. This method is not suitable for determination of treatment
tactics [23, p. 65].

1.2 Anaplastic lymphoma kinase (ALK)

Anaplastic lymphoma kinase (ALK) is one of the members of the receptor tyrosine kinase of the insulin receptor superfamily. The ALK gene is located on the short arm of the second chromosome. For the first time the fact of rearrangement of the ALK gene was detected in anaplastic large cell lymphoma. This rearrangement encodes a chimeric protein that has constitutive kinase activity. This factor stimulates the cell to grow and divide. In addition, approximately 4-7% of patients with non-small cell lung cancer may have an EML4-ALK fusion. This type of tumor is mainly found in young people who have never smoked with a special histological variant of the tumor – squamous cell carcinoma. There may be several options for rearranging EML4-ALK. Fusion of ALK with members of the kinesin family 5B (KIF5B), the fused TRK gene (TFG), the kine light chain 1 (KLC1), and protein 1 (HIP1) has occasionally been reported, leading to malignant cell transformations [24, p. 182]. In very rare cases, there is a combination of mutations in EGFR, KRAS and molecular changes in ALK. The presence of ALK fusion allowed to identify a separate group of patients who in 57-74% cases respond to ALK inhibitors. Crisotinib is a classic representative of this group of drugs. Individuals receiving crizotinib have a significantly higher median survival than patients receiving classical chemotherapy [25, p. 2254]. Nowadays, testing for ALK rearrangement in patients with advanced pulmonary adenocarcinoma is recommended in the NCCN and ASCO clinical guidelines. Despite advances in treatment, over time, in some patients develop resistance to crizotinib. Resistance developing due to the appearance of secondary mutations in the kinase domain of EML4-ALK, such as L1196M, C1156Y and F1174L [26]. In 2017, the FDA approved alectinib as a second-generation ALK inhibitor,
which is considered 2 times more effective than crizotinib.

FISH (in situ), immunohistochemistry, polymerase chain reaction, and next-generation sequencing techniques are used to establish the presence of ALK fusion. The US National Cancer Network (NCCN, version 3, 2019) recommends determining the status of ALK by FISH and immunohistochemistry using specific antibodies (clones 5A4 and D5F3). These antibodies have high sensitivity, specificity and in 83-100% of cases confirm the presence of ALK rearrangement in patients with non-small cell lung cancer. According to the NCCN recommendations,

immunohistochemical studies using D5F3 antibodies do not require confirmation by FISH. Prejudice against using of immunohistochemistry was because FISH has been the main method of diagnosing ALK rearrangement for a long time. The method showed almost 100% sensitivity and specificity, but in case of anaplastic large cell lymphoma sensitivity was about 70%. When new clones of antibodies 5A4 (Novocastra) and D5F3 (Ventana) were investigated, diagnostics has reached a new level. However, some large studies have shown that immunohistochemical (IHC) analysis is not always 100% specific and, in some cases, can give faulty results. Despite the high efficiency of both methods, not all scientists agree with the statement about the equivalence of FISH and immunohistochemistry. In some cases, they should be complementary and should not be used alone [27, p. 151].

In conclusion, immunohistochemical method can be used as a screening method for examination of patients with further verification by FISH if it needed.

1.3 Proto-oncogenic tyrosine protein kinase (ROS1)

ROS protooncogene 1, a tyrosine kinase receptor (ROS1) is located on the long arm of chromosome 6 at position 22. This protein plays an important role in embryogenesis, affecting the differentiation of epithelial cells. ROS1 rearrangements were first detected in glioblastoma, gastric and ovarian cancer. Only in 2007 it was described in patients with lung cancer. Among patients with non-small cell lung cancer this mutation occured in 1-2% of cases. A typical patient is a young woman who has lung adenocarcinoma and has never smoked in her life or smoked for a short period of time [28, p. 1124]. Patients with detected ROS1 rearrangement do not have associated mutations, such as EGFR, KRAS, ALK.

Clinical studies have shown that first-generation of tyrosine kinase inhibitors, such as crizotinib, ceritinib, and entrectinib, significantly improve the survival of patients who have not previously received specific drug therapy. Representatives of the next generations of drugs such as lorlatinib, repotrectinib and taletrectinib can penetrate to the brain, so they are more effective in patients with metastases to the central nervous system. They are usually used in the case of acquired resistance after targeted therapy with first-generation drugs. Response to specific treatment is observed in 80%

of patients. ROS1 testing is essential to identify patients who may benefit from crizotinib treatment. The ESMO recommends testing all patients with lung adenocarcinoma and negative EGFR, ALK, and KRAS status for ROS1 rearrangement [29, p. 212].

FISH, RT-PCR, immunohistochemistry, and next-generation sequencing are used in laboratory diagnostics to detect ROS1 rearrangement [30, p. 226]. The only method to confirm the presence of ROS1 in a patient is FISH. This expensive and time-consuming method has been approved by the FDA. In real life, ROS1 rearrangements are rare, so immunohistochemistry may be used to screen patients who are candidates for targeted therapy. Today clones of specific antibodies (eg, D4D6) have been developed and have high a specificity (about 97%) and a sensitivity of almost 100% compared with the FISH method [31, p. 150].

1.4 B-RAF protooncogene, serine / threonine kinase (BRAF)

The B-RAF protooncogene, a serine / threonine kinase oncogene (BRAF), is located on the long arm of chromosome 7 at position 34. It can activate the RAS / RAF / MEK / ERK signaling pathway encoding serine threonine kinase. The processes of cell proliferation, growth and survival occur due to phosphorylation of IEC during activation by oncogenic mutations in BRAF. This type of mutation occurs in patients with colorectal cancer, papillary cancer of the thyroid gland, ovaries. But it is most often diagnosed with malignant melanoma – in about 50% of patients.

In patients with non-small cell lung cancer, BRAF mutation is rare (only 1-3% of patients) [32, p. 161]. Same with ROS1 rearrangement, there are no combinations of BRAF with other driver mutations, such as ALK, KRAS, or EGFR. In 90% of patients with melanoma, there is a variant of the BRAF mutation called V600E (replacement of glutamine by valine in codon 600). Among patients with lung adenocarcinoma, only 50% have this type of mutation. In other cases, mutations G469A (~ 35%) and D594G (~ 10%) may be detected. A portrait of typical patient is a current or former smoker, that is not typical for patients with EGFR gene mutations or ALK rearrangements [33, p. 258].

It is proved that individuals with BRAF V600E mutations are very sensitive to therapies with targeted drugs such as BRAF and MEK inhibitors. The median survival of patients who received only platinum-based

cytotoxic therapy remains low and the prognosis is much poorer. The FDA has approved BRAF inhibitors such as vemurafenib and dabrafenib. The kinase V600E is the most sensitive to them. The gold standard of treatment is a combination of BRAF and MEK inhibitors, which have a potentiating effect on each other. The efficacy of the combination of dabrafenib with trametinib has been confirmed in clinical trials [34, p. 360].

2. Markers for immunotherapy

2.1 Cell surface checkpoint inhibitors:

2.1.1 Programmed death-ligand 1 (PD-L) and programmed cell death protein 1 (PD-1)

PD-1 is a membrane protein of the immunoglobulin family that plays a role in the cell differentiation of immune cells. PD-1 is important in the negative regulation of the immune system by preventing the activation of T lymphocytes, which reduces autoimmunity and increases auto tolerance. The inhibitory effect of PD-1 is due to the dual mechanism of stimulation of apoptosis (programmed cell death) of antigen-specific T-lymphocytes in lymph nodes, while apoptosis of regulatory (restrictive) T-lymphocytes, in contrast, is reduced. This protein has two ligands: PD-L1 and PD-L2, which belong to the B7 family of proteins. In response to lipopolysaccharide and granulocyte-macrophage colony-stimulating factor (GM-CSF), the expression of PD-L1 ligand on macrophages and dendritic cells increases. T- and B-lymphocytes express this protein in response to activation of the T-cell and B-cell receptor. PD-L2 is mostly expressed on APCs (a protein encoded by the gene of the same name located in humans on the short arm of chromosome 1), while PD-L1 can be on T lymphocytes, epithelial and endothelial cells. The homology of these ligands is 37%. PD-L1 plays a role in the late phase of the immune response, in inflammatory processes in the tissue regulates the function of T cells and prevents the development of autoimmune processes [35, p. 80]. In tumor tissue, PD-L1 regulates TILs (tumor-infiltrating lymphocytes), which in turn are leukocytes that emerge from the blood and migrate into the tumor. These include T- and B-lymphocytes, which belong to the larger category of “tumor-infiltrating immune cells”, as well as natural killers, macrophages, neutrophilic granulocytes, dendritic cells, eosinophils, basophils, etc. in various proportions. Their number depends on the type and stage of the tumor.

The presence of PD-L1 receptors is often associated with an unfavorable prognosis [36, p. 513].

Therefore, it is important to take an individual approach to each case of lung cancer. Treatment options and testing for biomarkers are constantly changing, but a team of scientists has developed an algorithm that can be used to select the first line of therapy, maintenance therapy, second / third line of therapy depending on the level of PD-L1 expression in tumor tissue and the presence of EGFR, ALK and ROS1 mutations.

PD-L1 expression – evaluation of the proportion of tumor cells TPS (tumor proportion score) stained for all tumor cells in the study section. Stained healthy and necrotic cells are not counted during the evaluation. Depending on the level of PD-L1, tumor expression is divided into three groups: PD-L1 <1% TPS; PD-L1 1–49% TPS; PD-L1> 50% TPS.

For patients with PD-L1 receptor expression greater than 50% TPS, pembrolizumab is recommended as first-line therapy, whereas 1-49% is recommended only as second-line therapy [37, p. 2522]. This applies to both adenocarcinoma and squamous cell carcinoma. Pembrolizumab is a humanized antibody used in immunotherapy. The mechanism of action is to block PD-L1 ligands on T cells, resulting in the activation of tumor- specific cytotoxic T cells and their destruction of cancer cells. The Food and Drug Administration (FDA) first approved it for the treatment of metastatic melanoma, and in 2017 for the treatment of metastatic non-small cell lung cancer. The efficacy and safety of pembrolizumab as a first-line treatment were evaluated in the KEYNOTE-001 study. Recurrence-free and overall patient survival were 6.2 and 22.1 months, respectively. High levels of PD-L1 expression have been associated with prolonged survival. Thus, in patients who participated in a clinical trial and had PD-L1 expression of 50% or more, the relapse-free period was about 12.5 months. On the contrary, at an expression level of 1–49%, this time interval was only 4.2 months [38]. A phase III study for the treatment of metastatic non-small cell lung cancer KEYNOTE-024 showed for the first time the advantage of anti- PD-L1 therapy over standard platinum-based chemotherapy [39, p. 1828]. It is generally believed that for patients with non-small cell lung cancer who do not have EGFR, ALK and ROS1 mutations and have received first or second line chemotherapy, determination of PD-L1 expression is required only with pembrolizumab.

As the second / third line of therapy in the presence of PD-L1 expression of 50% and more TPS it is advisable to use standard chemotherapy and ramucirumab. Ramucirumab is a human monoclonal antibody (IgG1) against the vascular endothelial growth factor 2 receptor (VEGFR2), a transmembrane type II tyrosine kinase receptor expressed on endothelial cells. By interacting with VEGFR2, the drug inhibits the binding of its ligands (VEGF-A, VEGF-C and VEGF-D), thereby preventing phosphorylation of the VEGF-stimulated receptor and proliferation and reduces the permeability and migration of human endothelial cells induced by the ligand [40, p. 3872].

For the treatment of patients with PD-L1 receptor expression levels of 1–49% TPS, standard chemotherapy approved for various histological variants of tumors is used as the first line of therapy. In addition, bevacizumab is recommended for adenocarcinoma. This drug is not a representative of immunotherapeutic agents, but a genetically engineered recombinant humanized monoclonal antibody to vascular endothelial growth factor VEGF-A. The mechanism of action of the drug is binding to the factor of vascular endothelial growth, which leads to blocking the binding to its receptor. The consequence of this is the inhibition of vascularization of areas with high growth rates. Because such properties are characteristic of malignant tumors, bevacizumab primarily inhibits tumor vascularization, growth, and metastasis [41, p. 86]. Bevacizumab can be used as a maintenance treatment if the effect is achieved.

Pembrolizumab, nivolumab or atezolizumab are recommended for
second / third line therapy in patients with PD-L1 expression levels of 1–49% TPS. The mechanism of action of nivolumab is like pembrolizumab and is a human immunoglobulin G4 anti-PD-1 monoclonal antibody. Lung cancer tumor cells can produce PD-L1 receptors that protect them from T lymphocytes. Nivolumab blocks the binding of PD-L1 to PD-1, leaving T lymphocytes active. In a phase III study in patients with metastatic non- small cell lung cancer who received docetaxel or nivolumab monotherapy, nivolumab was shown to have significantly better results in terms of one- year survival. It has been proven that with increasing PD-L1 expression, the recurrence-free period is prolonged [42, p. 2199].

In a phase III study in patients with metastatic, pre-treated non-small
cell lung cancer randomized to two groups, overall patient survival was

significantly higher with atezolizumab (13.8 months vs. 9.6 months). The FDA then approved atezolizumab as a second-line treatment for metastatic non-small cell lung cancer [43, p. 261].

Standard chemotherapy (for adenocarcinoma in combination with bevacizumab) is recommended for the treatment of patients with PD-L1 expression levels less than 1% TPS as the first line of therapy, and nivolumab or atezolizumab for the second / third [35, p. 65].

Another representative of PD-L1 receptor blockers is the drug durvalumab. In a phase II study in patients with metastatic non-small cell lung cancer who had already received at least two lines of systemic chemotherapy, it was shown that increasing PD-L1 expression improves one-year survival: 34.5% (with an expression level less than 25%), 47.7% (at an expression level of more than 25%), 50.8% (at an expression level of more than 90%) [44, p. 1012].

Clinical studies have been performed on the efficacy and safety of avelumab. Among patients receiving avelumab as first-line therapy, overall response and control rates were 18.7% and 64%, respectively. Unfortunately, avelumab has not been shown to be effective and has not been approved for the treatment of patients with lung cancer. However, in 2018, the FDA approved the use of the drug cemiplimab, which is also a representative of PD-L1 receptor blockers [45, p. 598].

2.1.2 Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)

CTLA-4 is a protein encoded by a gene located on the short arm of chromosome 2. Cytotoxic T-lymphocyte-associated protein 4, also known as CD152 (differentiation cluster 152), is a protein receptor that functions as an immune checkpoint and reduces the immune response. CTLA-4 inhibits T cell activation by competing with CD-28. CD-28 receptors are located on the surface of antigen-presenting cells. CTLA-4 prevents the binding of CD-28 to B7-1 (CD-80) and B7-2 (CD-86) (14 from the CTLA-4 article).

Monoclonal antibodies that inhibit CTLA-4 block the binding of CTLA-4 to its ligands (CD80 / CD86). This improves the antitumor immune response by activating specific T lymphocytes [46].

In addition, studies on the effectiveness of CTLA-4 in combination with PD-1 / PD-L1 inhibitors are being actively conducted. The combination of PD-1 and PD-L1 inhibitors with CTLA-4 inhibitors can significantly

improve the treatment outcomes of patients with lung cancer by affecting different immune systems. Thus, it is possible to overcome the resistance that arises during monotherapy of PD-1 and PD-L1 with inhibitors. The combination of ipilimumab and nivolumab showed good results in the clinical study CheckMate 227. According to the study design, the use of ipilimumab and nivolumab as first-line therapy in patients with a positive PD-L1 result was proposed. In contrast, the FDA did not approve the combination of tremelimumab and durvalumab [47, p. 668]. They did not show an advantage over platinum-based chemotherapy. PD-L1 expression is now thought to be a much more important prognostic factor than tumor mutation burden. The stratification factor in the selection of the cohort of patients for combination therapy is the level of expression of PD-L1 receptors. There is some positive news for people with negative PD-L1 status. The CheckMate 227 study showed an increase in the overall survival of this category of patients [48, p. 9500]. Randomized clinical trials are needed for more accurate data. To talk about success in the treatment of patients with lung cancer, it is necessary to consider the effects of side effects, which occur much more often after combination therapy with CTLA-4 and PD-1 / PD-L1 inhibitors.

Therefore, screening of patients with non-small cell lung cancer for
PD-L1 receptors is an important condition for personalizing the treatment of patients. This will improve the quality of diagnosis and treatment of these patients, improve their overall and relapse-free survival.

2.2 Intracellular inhibitors of immune checkpoints and CISH

Currently, the attention of scientists is focused on studying the mechanisms of strengthening immunotherapy. One such method is the blockade of internal immune checkpoints. Some internal regulators include molecules with ubiquitin ligase activity, including CISH. CISH is a new class of internal immunological checkpoints for T cells that can radically enhance adaptive cancer immunotherapy. This protein contains SH2, induced by cytokines. This is another molecule with ubiquitin ligase activity that is induced by T cell receptor binding (TCR). The researchers found that deletion of CISH in effector T cells significantly improves TCR signaling. This process is accompanied by the release of cytokines, negatively affects the survival of atypical cells for their proliferation. This increases the

expression of PD-1 on T cells. In combination with PD-1 antibody blockade, this mechanism results in sustained tumor regression and improved overall survival. Studies have been conducted only on animals, but this area is considered very promising to study [49, p. 2250].

3. Markers for gene therapy

3.1 Protein 53 (p53)

Protein 53 in humans is encoded by the TP53 gene. It is located on the short arm of chromosome 17. Protein p53 acts as a regulator of the cell cycle. Its role in oncogenesis is the suppression of the process of tumor development and preservation of genome stability. This transcription factor prevents cancer [50, p. 61].

The first studies conducted by scientists, on the contrary, showed the oncogenic role of this protein. Adenoviruses and SV40 viruses have been shown to produce specific proteins that bind to it and lead to its accumulation. Later in the laboratories of Oren, Jenkins, Weineberg, Rotter it was found that at high levels of expression of protein 53 is the activation of oncogenesis. On the surface of cancer cells, the concentration of p53 was high, in contrast to normal cells.

The issue was finally resolved after sequencing of the clones used. The researchers compared their nucleotide sequences with the wild-type gene sequence. It became clear that approximately 50% of atypical cells have a mutated variant of the TP53 gene. As a result, mutations in other genes accumulate faster and the tumor becomes more malignant. If the p53 protein functions normally, then in the case of DNA damage, cell division stops, and apoptosis begins. In the case of a mutation in the TP53 gene, the cell acquires the ability to avoid replicative aging and becomes insensitive to chemotherapy and radiation therapy [51, p. 4286].

In patients with Lee-Fraumeni syndrome, cancer occurs during the first 20 years of life. The reason is the presence of one mutant copy of TP53 in the genotype, which excludes a normal copy of the p53 gene [52]. During a person's lifetime, mutations in the TP53 gene can cause tobacco smoke. Polycyclic arenes can cause the replacement of thymine residue by guanine residue. This may be one of the causes of lung cancer in smokers.

Therefore, in general, p53 is a tumor suppressor. But its main function is lost due to various mutations and deletions of genes. This was proved by

the example of colorectal cancer and cancer of other localizations. Given the important role of protein 53 in the development of cancer, scientists have focused their efforts on drug development. The preconditions for the development of gene therapy in this direction have appeared. In 1996, the company Introgen Therapeutics first tried to introduce this method of therapy. He failed. In 2004, gene therapy was first approved for use in oncology for the treatment of neck and head cancer. An adenoviral vector was used for this purpose [53, p. 3].

Another option for gene therapy in patients with malignancies is the use of adenovirus, which is defective in the E1B 55 kDa gene. The virus can bind to p53 and inactivate it. Adenovirus cannot replicate in healthy cells that have functional p53. However, it can affect cancer cells. This method of gene therapy has been approved in China [54, p. 751].

A promising area of treatment is the use of low molecular weight compounds that can restore the activity of p53 and restore its wild-type properties. PRIMA1 is the first representative of this type of compound. Another group of drugs can increase the activity of p53 by disrupting the interaction between MDM2 and p53. This variant can be used only in patients with non-mutated variant p53. Hoffmann-La Roche has developed a special group of substances called Nutlin. These substances bind to the p53-binding pocket of MDM2 and block the interaction of both proteins. During an experiment on animals, scientists were able to achieve a positive response to treatment in animals with malignant neoplasms. Randomized clinical trials are required before these compounds can be used to treat patients with solid tumors [54, p. 754].

4. Marker of proliferation Ki-67

Ki-67 protein is encoded by the gene of the same name. In humans, it is located on the short arm of chromosome 10. By its structure it belongs to the phosphoproteins. Localized in chromosomes and nucleus. The proliferation marker Ki-67 is involved in cell division, alternative slicing, and acetylation. It was first described in 1983. The Ki-67 protein was originally identified as a prototype of the Ki-67 monoclonal antibody. It was obtained by immunizing mice with the nuclei of the Hodgkin's lymphoma cell line L428. The proliferation marker got its name from the number of the original clone in a 96-well plate and from the city of origin (Kiel, Germany) [55, p. 17].

The proliferative activity of malignant cells is directly proportional to the degree of their histological and biological malignancy. Accordingly, the study of Ki-67 expression is widely used in routine diagnosis of cancer. This test allows you to identify atypical cells in the G1-, S-, G2- and M-phases. Ki-67 nuclear antigen is not detected only in the G0 period. Increased expression of Ki-67 begins in the middle or late stage of G1. Then in stages S and G2 its level continues to increase until it reaches a peak in stage M [56, p. 150].

Proliferative activity of cells is the basis of the mechanism of malignant transformation. The size of the primary tumor, the probability of metastasis, the response to drug and radiation therapy depends on the rate of cell division. The rate of cell division is one of the most important biological characteristics of tumors. That is why the proliferative index to some extent determines the treatment tactics for patients with cancer.

Some studies have shown that high Ki-67 expression promotes infiltrative growth and aggressive spread of squamous cell carcinoma of the lungs, larynx, and cervix. In patients with gastric and breast cancer, it is associated with lymph node metastasis. In cases of bladder and prostate cancer, patients have much worse survival [56, p. 150].

Scientists have studied the differences in survival between subtypes of lung adenocarcinoma. After using one-way analysis, a relationship was found between tumor size and Ki-67 expression level. In a multivariate analysis, this proliferation marker was recognized as an independent prognostic factor for pulmonary adenocarcinoma [57, p. 73; 58, p. 2922].

Potentially, Ki-67 may be an indicator of the short-term survival of patients with lung cancer. This marker of proliferation indicates a poor prognosis and the likelihood of rapid disease progression [57, p. 71]. More observations are needed to confirm its prognostic and clinical-pathological role.

Conclusions

Molecular genetic testing of tumor tissue for the diagnosis of mutations (EGFR, B-RAF) and gene rearrangement (ALK, ROS1) is very important for the determination of treatment tactics in patients with non-small cell lung cancer. Immunohistochemistry is a cheaper and more affordable method of diagnosis used in routine practice. Certain antibody clones have high sensitivity and specificity and are not inferior to molecular genetic

research. Unfortunately, immunohistochemistry is not always sufficient to prescribe targeted therapy. In general, this research method is suitable as a screening followed using more accurate diagnostic techniques. The use of immunohistochemistry to determine the expression of PD-L receptors, p53 and Ki-67 is beyond doubt. With the advent of immune checkpoint inhibitors, lung cancer treatment has reached a new level. The use of immunotherapy as the first or second / third line of therapy depends on the level of expression of PD-L receptors. The study of protein expression 53 is important not only in terms of prognosis. Gene therapy drugs are already used in the world, which affect the TP53 gene and make it act as a suppressor in the process of oncogenesis. Therefore, this area is promising in terms of lung cancer treatment. Ki67 should be used as a marker to predict the course of the disease. Its prognostic role has already been proven in several studies.

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