Pneumologia
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The role of biomarkers in the management of non-small cell lung cancer

Rolul biomarkerilor în managementul cancerului pulmonar fără celule mici

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Abstract

Lung cancer is an important public health problem, and worldwide it became the leading cause of cancer-related death in both men and women; the survival rate at 5 years after diagnosis is about 18%. The predominant histological type (85%) is non-small cell lung cancer - adenocarcinoma, squamous cell carcinoma, and large cell carcinoma; due to the lack of symptoms in most cases it is diagnosed late, and the patients cannot benefit of a curative treatment. Lack of a sustained response to standard chemotherapy and poor prognosis of patients in advanced stages of the disease, with metastases, led to the intensive studies of the molecular profile of this cancer type in order to develop innovative targeted therapies with a favorable impact on prognosis. Mutations are somatic alterations of the genome which can occur in cancer cells, in the genes responsible for synthesis of proteins essential for cell growth, differentiation, survival or apoptosis, representing the basis of tumorigenesis process. Identification of these gene mutations (EGFR gene mutations, ALK gene translocations, ROS gene mutation), and also determination of PD-L1 expression rate - the so-called biomarkers - represent the basis of targeted molecular therapy and immunotherapy, with a role in the selection of the patients. In conclusion, the most accurate characterization of the tumor type from a histological and biomarker point of view, together with the judicial use of targeted innovative therapies led to the improvement of the prognosis of patients in advanced/metastatic stage of the disease, especially by increasing the progression-free survival and the overall response rate.

Keywords: ***biomarker, NSCLC carcinoma, targeted molecular therapy***

Rezumat

Cancerul bronhopulmonar reprezintă o importantă problemă de sănătate publică, devenind la nivel global principala cauză de deces prin cancer atât în rândul bărbaților, cât și al femeilor, rata de supraviețuire la 5 ani de la diagnostic fiind de aproximativ 18%. Tipul histologic predominant (85%) este reprezentat de carcinomul pulmonar fără celule mici (non-small cell lung cancer – adenocarcinomul, carcinomul scuamos și carcinomul cu celulă mare), care în majoritatea cazurilor, în lipsa unor simptome, este diagnosticat tardiv, fără ca pacienții să poată beneficia de tratament curativ. Lipsa unui răspuns susținut la chimioterapia standard și prognosticul rezervat al pacienților în stadiul avansat al bolii, cu metastaze, au determinat aprofundarea studiilor profilului molecular al acestui tip de cancer, cu scopul de a dezvolta terapii inovatoare țintite cu impact favorabil asupra prognosticului. Mutațiile sunt alterări somatice ale genomului, care pot să apară în celulele canceroase, la nivelul genelor responsabile de sinteza unor proteine esențiale pentru creșterea, diferențierea, supraviețuirea sau apoptoza celulară, reprezentând baza procesului de tumorogeneză. Identificarea acestor mutații genice (mutațiile genei EGFR, translocația genei ALK, mutația genei ROS), precum și determinarea ratei de expresie a PD-L1, așa-numiții biomarkeri, reprezintă baza terapiei moleculare țintite și a imunoterapiei, având rol în selecția pacienților. În concluzie, caracterizarea cât mai precisă a tipului de tumoră din punct de vedere histologic și al biomarkerilor, alături de utilizarea cu discernământ a terapiilor inovatoare țintite au determinat îmbunătățirea prognosticului pacienților aflați în stadiul avansat/metastatic de boală, în special prin creșterea duratei supraviețuirii în absența progresiei bolii (progression-free survival) și creșterea ratei globale de răspuns.

Cuvinte-cheie: ***biomarker, carcinom NSCLC, terapie moleculară țintită***

Lung cancer is considered the leading cause of cancer-related death worldwide, in both men and women, with a poor five-year survival rate (18%)[1,2], most patients presenting in advanced-stage disease at the diagnosis, with low response at conventional chemotherapeutic agents.

Non-small cell lung cancer (NSCLC) accounts for 85% of primary lung cancer. Despite an improvement in overall survival with platinum-based chemotherapy, the prognosis remains poor for patients with advanced-stage NSCLC, with a median survival between 8 and 12 months.

This fact was an impulse for developing new targeted treatment strategies according to specific tumor's features: histological type (adenocarcinoma versus squamous carcinoma) and molecular profile (oncogenic driver mutation, immune checkpoints), resulting a personalized therapeutic approach with substantially improved outcomes for patients.

The first step in establishing the histopatological type is classical haematoxillin-eosine stain of biopsy specimens.

Immunohistochemistry is used to differentiate primary adenocarcinoma from pulmonary metastases of other type of carcinoma (e.g., prostate, breast, colorectal) and to distinguish from squamous cell carcinoma, large cell carcinoma, malignant mesothelioma and neuroendocrine tumors[1].

The current panel of immunohistochemical markers used to confirm **primary pulmonary adenocarcinoma** are[1]:

- **TTF-1** (thyroid transcription factor) – a nuclear transcription protein expressed in embryonal and mature epithelial cells of lung and thyroid tissue. It is positive in 70-100% of non-mucinous adenocarcinoma subtypes. Usually, pulmonary metastases of adenocarcinoma are negative for TTF-1, except for metastatic thyroid carcinoma, which is also positive for tireoglobuline.
- **Napsine A** – a proteinase found in normal type II pnemocytes and in proxymal and distal renal tubules, which is positive in more than 80% of primary lung adenocarcinoma. It can be used complementarily to TTF-1 determination.
- **CK-7** (cytokeratin 7) positive and CK-20 (cytokeratin 20) negative, in contrast with metastatic colorectal adenocarcinoma, which is CK-7 negative and CK-20 positive.

The most important immunohistochemical markers for **squamous cell carcinoma** are p40 and p63 (antibodies of protein P63, a transcription factor expressed in squamous cell carcinoma), both of them being highly specific for squamous cells of bronchial mucosa[3,4].

For **neuroendocrine tumors** there are used: CD56 (neural cell adesion molecule), with high senstivity but less specificity, and the result has to be interpreted in the morphological context, chromogranin A and synaptohysin. The proliferation activity of neuroendocrine pulmonary tumors is assessed by Ki67 antigen imunostaining, which may discriminate between low- and intermediate-grade tumors (typical and atypical carcinoids) and high-grade malignancy tumors (LCNEC, SCLC)[1,3,5].

The immunomarkers relatively sensitive and specific for **malignat mesothelioma** are: calretinin, WT-1, D2-40 (podoplanin antibody), HMBE-1 and cytokeratin 5/6 (negative in adenocarcinoma)[6,7,8].

Immunohistochemistry should be judiciously used in order to preserve tissue for molecular testing, especially in patients with small biopsy samples and advanced disease[1].

The discovery of oncogenic driver mutations and their role in predicting response to targeted therapies which improve outcomes in patients has changed the approach of diagnosis and treatment in lung cancer.

The current recommendations for the treatment of NSCLC are based on the analysis of tumor molecular profile and the detection of targetable oncogenic driver mutations that lead to uncontrolled cell growth and proliferation responsible for tumorigenesis.

The oncogenic driver mutations with targeted therapy commonly observed in NSCLC, especially adenocarcinoma histology, are represened by: **epidermal growth factor receptor (EGFR) gene mutations, anaplastic lymphoma kinase (ALK) gene rearrangement and ROS1 rearrangement**. KRAS mutation is considered a prognostic biomarker associated with short survival with no targeted therapy.

The activating EGFR gene mutations were discovered over a decade ago (2004), with a higher prevalence in adenocarcinoma subtype, women, never-smokers and East Asian patients (50-60% of NSCLC versus 10-20% of NSCLC in Europeans and North Americans)[9,10].

The EGFR gene (located on the short arm of chromosome 7) is responsible for the synthesis of a type 1 transmembrane growth factor receptor (EGFR/erb-b2 receptor tyrosine kinase 1; ERBB1), having tyrosine kinase activity which belongs to the HER/ErbB family tyrosine kinases[11,12].

EGFR is activated after binding of specific ligands to its extracellular domain. It forms dimers with itself and other members of the ErbB family, causing conformational shifts responsible for tyrosine autophosphorylation and kinase activation, leading to the stimulation of intracellular signaling pathways, such as the RAS/RAF/MEK/MAPK pathway, PI3K/PTEN/AKT pathway and STAT pathway[13].

In non-small cell lung cancer, genetic mutations of EGFR (usually within exons 18 to 21) induce constitutive activation of the tyrosine kinase (in the absence of ligand stimulation) and overstimulate the intracellular signaling pathways, leading to increased cell proliferation, angiogenesis, metastasis and decreased apoptosis[14].

The most common EGFR sensitizing mutations are: the **exon 19 deletion** (elimination of 4 amino acids: LREA, downstream of the lysine residue at position 745) and single-point **substitution** (of leucine to arginine: L858R) **in exon 21**[15].

These two mutations are considered to be the "classic mutation", accounting together for almost 85-90% of all EGFR mutations[15].

Generally, the mutations of exons 18, 19 and 21 are associated with sensitivity to EGFR tyrosine kinase inhibitor (TKI) therapy, even though in patients harbouring mutations of exon 18, the response to TKIs is less prolonged compared to those having more common mutations.

The mutation in exon 20 (exon 20 insertions) is an exception, being typically resistant to EGFR TKIs[14].

There are currently four EGFR TKIs (gefitinib, erlotinib, afatinib and osimertinib) available for the treatment of NSCLC harbouring sensitizing mutations.

- **Gefitinib** and **erlotinib** are known as first-generation TKIs, which reversibly bind to the tyrosine kinase domain of the EGFR, competing with ATP and blocking the activation of downstream pathways. In patients with advanced NSCLC harbouring sensitizing EGFR, the use of gefitinib or erlotinib as first-line treatment led to a significantly better response compared to standard chemotherapy containing platinum compounds: a median PFS (progression-free survival) of 10-14 months versus 5-6 months, an OS (overall survival) of 20 to 30 months versus 8-12 months and response rates of 56% to 74% versus 20-35%[16,17,18]. Erlotinib was approved in 2004 by FDA for the treatment of locally advanced or meta-

static NSCLC after progression on at least one chemotherapy regimen[1]. The current guidelines recommend **erlotinib and gefitinib as first-line therapy in patients with metastatic non-squamous NSCLC with active sensitizing EGFR mutations** regardless of their performance status (PS)[1,19].

- Afatinib, a second-generation EGFR TKI, which irreversibly inhibits the entire ErbB/HER family of receptors, including EGFR and HER2, has also been approved for the first-line treatment of metastatic non-squamous NSCLC with common activating EGFR mutations. It has similar efficacy as erlotinib and gefitinib, but the general impression is that it is slightly less safe (regarding serious side-effects of the treatment) than erlotinib or gefitinib[1].

It is well known that the majority of patients with metastatic NSCLC harbouring activated EGFR mutations, treated with an EGFR TKIs (gefatinib, erlotinib, afatinib), will progress after 9 to 12-13 months of treatment[1,19], but if patients do not have multiple systemic symptomatic lesions, the TKI therapy may be continued until they become symptomatic[1].

In this situation, it is mandatory to look for an explanation.

The development of **acquired resistance to first-generation and second-generation EGFR TKIs** which limits the long-term efficacy of these molecules is the main cause of the progressive disease.

There are several mechanisms responsible for the acquired resistance to EGFR TKIs:

- The most frequent mechanism (in about 50-60% of patients with disease progression after the initial response to EGFR TKIs therapy) is the acquisition of a mutation in exon 20 of the EGFR gene, known as T790 mutation, which increases the affinity of tyrosine kinase for ATP to the detriment of EGFR TKIs[1,19]. The approved FDA therapy for patients with metastatic EGFR T790M-positive NSCLC and progression on/after EGFR TKIs is osimertinib, a third-generation, irreversible EGFR TKI, which inhibits both EGFR T790 resistance mutation and EGFR sensitizing mutation. National Comprehensive Cancer Network (NCCN) and the European Society for Medical Oncology (ESMO) guidelines recommend osimertinib in patients with metastatic NSCLC who have developed EGFR T790 resistance mutation and who have progressed on erlotinib, gefetinib or afatinib therapy[1,19].
- The second mechanism (5-20% of cases) for acquired resistance to EGFR TKIs is MET gene amplification and the activation of downstream PI3K/AKT/mTOR pathway responsible for cell proliferation, survival, anti-apoptosis and TKIs resistance. It is correlated with a poor clinical outcome[20].
- Other acquired resistance mechanisms are[20]:
  - ✔ HER2 mutation in about 2% of NSCLC patients, especially in never-smokers, adenocarcinoma, females and oriental ethnicity.
  - ✔ PIK3CA mutation in about 5% of patients with acquired TKIs resistance.
  - ✔ Small cell lung cancer (SCLC) transformation (14%) – the mechanism could be: pre-existing cells which are growing up while sensitive EGFR clones are inhibited by TKIs, or are developed from the multipotent stem cells or transdifferentiated from the adenocarcinoma cells.
  - ✔ Epithelial to mesenchymal transition (EMT) plays a role in the 5% of acquired resistance of NSCLC to TKIs; also, it must be noted that *in vitro* NSCLCs with lower degree of EMT are more sensitive to EGFR TKIs even without EGFR activating mutation.
  - ✔ Amplified EGFR wild-type allele confers resistance to third-generation EGFR TKIs[21].

Primary or intrinsic resistance to EGFR TKI therapy occurs in 20-30% of patients with NSCLC harbouring EGFR mutation, but the mechanisms are currently not very well understood.

The intratumor heterogeneity, tumoral microenvironment, genetic (e.g., mutation in exon 20 of the EGFR) and epigenetic alterations could be responsible for primary resistance to EGFR TKIs[22].

Patients who progress after EGFR TKI therapy **should undergo a rebiopsy** to analyse the molecular pattern, especially looking for EGFR T790 mutation or other oncogenic alteration, SCLC transformation, for guiding future treatment according to the NSCLC guidelines.

Liquid biopsy (cell-free circulating tumor DNA; ctDNA) could be an alternative method for searching resistance mutations of the entire tumor genome of both primary and metastatic lesions compared to the single site sample during conventional tumor tissue biopsy. It was accepted (by latest versions of NCCN and ESMO guidelines) for detecting EGFR T790M mutation in order to identify patients eligible for third generation TKI therapy (osimertinib)[23].

The anaplastic lymphoma kinase (ALK) gene rearrangement is another genomic alteration of NSCLC which benefits of targeted therapy with an improvement in progression-free survival (PFS) and quality of life when compared to standard chemotherapy.

The molecular mechanism consist in a paracentric inversion occurring on the short arm of chromosome 2, between the anaplastic lymphoma kinase (ALK) gene and the echinoderm microtubule-associated protein-like 4 (EML4) gene, resulting the fusion oncogene EML4-ALK with aberrant expression of the ALK fusion protein in the cytoplasm[24].

The percentage of ALK rearrangement in patients with NSCLC varies from 2% to 7%.

The patients have similar clinical characteristics to those with EGFR mutation: never- or light-smokers, adenocarcinoma (especially acinus forms of adenocarcinoma in Asian patients and signet-ring cell adenocarcinoma in Caucasian patients), but they may be younger. So, in selected populations (non-smokers and young patients) the frequency of an ALK rearrangement can be between 17% and 30%[1,24].

The presence of an ALK rearrangement generally excludes the presence of sensitizing EGFR or KRAS mutation.

Screening for ALK rearrangements in NSCLC is important because ALK positive tumors are sensitive to ALK tyrosine kinase inhibitor therapy.

Crizotinib is an inhibitor of ALK, ROS and MET tyrosine kinases which is approved by FDA for metastatic NSCLC harbouring ALK rearrangements or ROS rearangements, with a high response rate (>60%), including in patients with brain metastases, the progression-free survival being 7 months to one year. It is recommended as first-line therapy in patients with ALK positive NSCLC[1].

The second generation of ALK tyrosine kinase inhibitors include ceritinib, alectinib and brigatinib, and are recommended by current guidelines for the treatment of patients with ALK positive metastatic NSCLC with progression on crizotinib, or for those intolerant to crizotinib[1].

ROS-1 is a human tyrosine kinase receptor whose structure can be altered in NSCLC by genomic rearrangements, becoming a distinct molecular target in NSCLC.

The ROS-1 gene rearrangements occur in about 1-2% of patients with NSCLC, more frequently in younger, never-smoker women with adenocarcinoma negative for EGFR mutations, ALK rearrangements and KRAS mutation (known as triple negative).

Crizotinib is also effective in patients with ROS-1 positive NSCLC, having 70% response rate, including complete response, with a median duration of response of about 18 months, and for this reason guidelines now recommend testing ROS-1 rearrangements in NSCLC[1].

KRAS (Kirsten rat sarcoma 2 viral oncogene homolog) mutations occur in about 25% of NSCLC – adenocarcinoma, generally associated with a history of smoking, and almost never in the presence of EGFR mutation, ALK or ROS-1 rearrangements.

It has a prognostic value, the patients with KRAS mutation having a shorter survival, resistance to chemotherapy and EGFR TKIs, no targeted therapy being currently available[1].

About 40% of non-squamous NSCLCs have unknown mutation and for the majority of squamous NSCLC there are no efficient targeted therapies, therefore new molecular pathways should be explored and novel therapeutic approaches developed.

Cancer immunotherapy, described as any therapy that interacts with the immune system, with the aim to treat cancer, has become a topic of interest for researchers.

The interactions of tumor cells and the immune system are complex, and tumors must develop the ability to avoid or escape from the immune surveillance. There are several mechanisms of immune escape in lung cancer, but one of the most important is a mechanism based on interaction of membrane-bound ligands and its receptors, known as immune checkpoints, regulating the immune system.

Two major pathways – cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death protein-1 (PD-1) – have become important terapeutic targets in various types of cancer, including NSCLC.

CTLA-4 is expressed exclusively on T-cells and downmodulate the amplitude of T-cell activation by outcompeting CD-28 (for binding of CD-80/CD86), inhibiting IL-2 production and preventing cell proliferation. Physiologically, this inhibitory system maintains a constant level of T-cell activation, but in tumors this pathway is used to avoid immune surveillance[25].

The PD-1 pathway is another mechanism by which tumors become resistant to the immunological attack, and the explanation is the upregulation of the PD-1 receptor on activated T-cells and subsequent binding to PD-L1/PD-L2, usually expressed on tumor cells, resulting the inhibition of T-cell response[25,26].

The immunotherapy in NSCLC is based on several monoclonal antibodies that block the immune checkpoint pathways involving PD-1 and CTLA-4 in order to prevent or reduce tumor-mediated immune supression.

Three immunotherapy agents targeting PD-1 or its ligand PD-L1 have been shown to improve the outcomes in the first-, second- or later-line treatment of metastatic NSCLC, being approved for this indication.

- Nivolumab inhibits PD-1 receptor and improves overall survival rates, with longer duration of response and fewer adverse effects compared to cytotoxic chemotherapy (Check Mate-057 and Check Mate-017 phase 3 randomized trials), and is recommended by guidelines[1,19] as subsequent therapy for patients with metastatic non-squamous or squamous NSCLC who have progressed on or after first-line chemotherapy. Testing for PD-L1 expression in tumor cells is not required for prescribing nivolumab, but it can help the clinician to assess which patients may benefit from the treatment.
- Pembrolizumab, another PD-1 receptor inhibitor, was approved in 2017, based on the results of Keynote-024 trial (comparing pembrolizumab versus platinum-based chemotherapy), as first-line therapy for patients with advanced NSCLC and negative or unknown tests results for EGFR mutation, ALK rearrangements and ROS1 rearrangements, but with high PD-L1 expression in tumor cells (50% or higher). Also, pembrolizumab is recommended by current guidelines[1] as subsequent therapy in squamous and non-squamous metastatic NSCLCs that are PD-L1 positive in tumor cells (>1%).
- Atezolizumab is a PD-L1 inhibitor improving antitumor immunity. It was approved as subsequent therapy for advanced (metastatic) non-squamous or squamous NSCLC, based on the data from the OAK trial (atezolizumab versus docetaxel)[1,25].

In conclusion, significant progress, consisting in the transition from chemotherapy to a more personalized approach based on histology and molecular markers, has been made in the last decade in the treatment of advanced-stage NSCLC, improving the outcome of patients.

Molecular testing for biomarkers will become increasingly important in the management of NSCLC, selecting the patients which are most likely to benefit from targeted therapies or immunotherapy. ■

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