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REVIEW

Mechanisms of Resistance in Non-Small Cell Lung Cancer

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Abstract

Objectives: Treatment of patients with non-small cell lung cancer continues to evolve and the discovery and approval of tyrosine kinase inhibitors in patients with NSCLC and targetable alterations brought an important contribution in treating these patients.
Material and Methods: This review aims to describe the main alterations that can affect the signaling pathways involved in NSCLC, present the therapy that can target these alterations and describe the mechanisms that can determine resistance to targeted therapy. Literature research was performed on PubMed and Google Scholar and 60 relevant articles were selected for the purpose of our review.
Discussion: The data of the present research were structured in nine paragraphs, each one presenting the main alterations that can affect the signaling pathways in patients diagnosed with NSCLC, targeted therapy used in managing these cases and mechanisms of resistance to targeted therapy.
Conclusions: Testing for molecular biomarkers should be mandatory for every newly diagnosed NSCLC patient in order to offer the most appropriate treatment.

Keywords: NSCLC, signaling pathways, genetic alteration, TKIs, mechanisms of resistance.

Rezumat

Obiective: Tratamentul pacienților cu neoplasm pulmonar non-microcelular este într-o continuă evoluție, iar descoperirea și aprobarea inhibitorilor de tirozin kinază pentru pacienții cu neoplasm pulmonar non-microcelular și alterări moleculare targetabile au adus o importantă contribuție în ceea ce privește tratamentul.
Material și metodă: Această lucrarea are ca scop descrierea principalelor alterări care pot afecta căile de semnalizare implicate în cancerul pulmonar non-microcelular, prezentarea terapiilor țintite care pot fi utilizate în aceste cazuri și descrierea mecanismelor de acțiune care pot conduce la dezvoltarea rezistenței la tratamentul țintit. În urma unei cercetări atente asupra datelor din literatură, publicate pe PubMed și Google Scholar, 60 de publicații relevante au fost selectate pentru îndeplinirea scopului lucrării noastre.
Discuții: Datele prezentate în lucrarea de față au fost structurate în nouă paragrafe, reprezentând fiecare în parte principalele modificări care pot afecta căile de semnalizare în cazul pacienților diagnosticați cu neoplasm pulmonar non-microcelular, terapiile țintite utilizate în cazul acestor pacienți și mecanismele de rezistență la terapiile țintite utilizate.
Concluzii: Testarea biomarkerilor moleculari ar trebui să fie obligatorie la fiecare pacient diagnosticat cu neoplasm pulmonar non-microcelular pentru a putea oferi tratamentul adecvat.

Cuvinte cheie: NSCLC, cai de semnalizare, alterări genetice, TKIs, mecanisme de rezistență.

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INTRODUCTION

Cancer is one of the most important health issues worldwide and one of the main causes of death. According to World Health Organization, lung cancer is responsible for the majority of cancer-related deaths despite the important progress in diagnosis and treatment[1].

Non-small cell lung cancer (NSCLC) occurs in 85 percent of lung cancers[2]. It can be classified into two major histological subtypes: squamous cell carcinoma on the one hand and adenocarcinoma, large-cell carcinoma and other subtypes on the other hand.

In the last decades, the prognosis and quality of life in lung cancer patients were improved due to chemotherapy and the development of targeted therapy. It is known now that almost two-thirds of NSCLC patients could carry an oncogenic alteration that could be targeted with specific drugs, so testing for molecular biomarkers is mandatory for the management of these patients. The molecular biomarkers testing panel should include: epidermal growth factor receptor (EGFR) mutations, anaplastic lymphoma kinase (ALK) rearrangements, V-raf murine sarcoma viral oncogene homolog B (BRAF) mutations, receptor tyrosine kinase ROS oncogene 1 (ROS1) gene rearrangements, mesenchymal-epithelial transition (MET) factor exon 14 skipping mutations and MET amplifications, Kirsten Rat Sarcoma virus (KRAS) mutations, human epidermal growth factor receptor 2 (HER2) mutations, neurotrophic tyrosine receptor kinase 1/2/3 (NTRK 1/2/3) gene fusion and rearranged during transfection (RET) rearrangements[3].

The aim of testing for molecular biomarkers is to assess the presence of oncogenic alteration that could be targeted with specific therapy.

The National Comprehensive Cancer Network (NCCN) Guidelines Version 3.2023 recommends genomic profiling in patients with metastatic NCSLC, in eligible patients with locally advanced or resectable early-stage NSCLC, but also in patients with progressive disease after treatment with targeted therapy[3].

The purpose of this review is to discuss the most frequent oncogenic alterations in patients with non-small cell lung cancer, the targeted therapy used in these patients and the biggest challenge in managing these cases – the mechanisms of resistance mutations.

MATERIALS AND METHODS

In order to write this paper, we performed research on PubMed and Google Scholar using keywords and a combination of keywords such as "NSCLC", "targeted therapy", "mechanisms of resistance", "signaling pathways" and so on. Randomized clinical trials, clinical cases and literature reviews written in the English language were considered eligible for the purpose of our work. When choosing the literature, the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) criteria were respected[4]. A number of 60 relevant articles were selected for the aim of our study.

DISCUSSION

The articles and trials considered relevant for our study were analyzed and the collected data were structured and detailed in the following paragraphs:

EGFR signaling pathway. EGFR gene alterations. Targeted therapy and mechanisms of resistance to TKIs

Approximately one in four non-small cell lung cancers have EGFR gene mutations. The overall prevalence of EGFR mutations in NSCLC varies by ancestry, ranging from 10% to 20% in white populations and as high as 30% in Asian populations; higher frequencies are also observed in women and people who have never smoked[5,6].

The largest group of EGFR mutations, also known as "classical" or "typical" mutations, are represented by deletions in exon 19 and mutations in exon 21 (mostly L858R). They are gain-of-function mutations and they are found near the ATP-binding pocket, leading to a decreased receptor's affinity for ATP. Thus, ligand-independent receptor activation occurs, which will determine an abnormal cell migration, proliferation and apoptotic resistance[7].

Gefitinib and Erlotinib were the first agents used in non-small cell lung cancer patients harboring EGFR mutations. They selectively inhibit EGFR-tyrosine kinase by competing with ATP for its binding site on the intracellular domain of the receptor, inhibiting phosphorylation and activation of the tyrosine kinase with subsequent inhibition of downstream EGFR pathway, cell cycle arrest and inhibition of angiogenesis and metastasis[7].

Despite excellent initial responses, acquired resistance to first-generation EGFR inhibitors develops, usually within a year. The most frequent mechanism of resistance is the gate-keeper point mutation p.T790M, which lowers the affinity of first-line tyrosine kinase inhibitors (TKIs) for the ATP binding pocket [8]. The activation of by-pass tyrosine kinases, such as ERBB2 and MET, represents less frequent resistance mechanisms. Infrequently, mutations within the genes encoding the downstream signaling molecules such as BRAF, KRAS, PIK3CA and CTNNB1 are observed. The histological transformation into a small cell or sarcomatoid lung cancer was also described as a mechanism of acquired resistance. Compound resistance by multiple mechanisms in the same or in different tumor locations has been encountered[9].

The second-generation inhibitors, including Afatinib and Dacomitinib, are irreversible inhibitors which covalently bind to EGFR tyrosine kinase. Several phase 3 studies have shown radiological response and statistically significant improved progression-free survival in patients treated with first or second-generation TKIs compared to platinum-based chemotherapy[10,11,12].

Osimertinib is a third-generation TKI designed to target both EGFR TKI-sensitizing mutations and T790M mutation while sparing wild-type EGFR. It binds to the EGFR kinase domain by targeting the cysteine-797 residue in the ATP binding site through covalent bond formation[8,9].

Third-generation TKIs have been developed to target the T790M mutation alongside the L858R point mutation or exon 19 deletion. The FLAURA trial has indicated the superiority, including an OS benefit, of Osimertinib over Gefitinib or Erlotinib as a first-line treatment in patients with classical EGFR mutations. Osimertinib is usually the preferred TKI for these patients due to its efficacy and ability to penetrate the central nervous system (CNS). Recently, Osimertinib was also approved in the adjuvant therapy setting for patients with stage IB-IIIA NSCLC whose tumors bear classical EGFR mutations[8,9,13].

Primary disease progression under TKI treatment was observed in 4–10% of newly diagnosed patients, indicating primary rather than acquired TKI resistance. The mechanisms of primary resistance can be: BIM polymorphism, exon 20 insertions and co-occurring mutations in other pathways[13,14].

BIM is a pro-apoptotic member of the BCL-2 family of proteins, which is required for apoptosis. In vitro, inhibition of BIM expression has been found to induce intrinsic resistance to EGFR TKI. In the EURTAC study (Erlotinib versus chemotherapy as first-line treatment for EGFR-mutant NSCLC patients), patients treated with Erlotinib who had low or intermediate levels of expression of BIM mRNA had a worse median PFS than those with high levels of BIM mRNA (7.2 versus 12.9 months), whereas BIM expression levels had no impact on PFS in chemotherapy-treated patients[13,14].

Exon 20 insertions account for 5–10% of all EGFR mutations. In vitro, exon 20 insertions have been found to activate EGFR by promoting the active conformation of the receptor. EGFR harboring an exon 20 insertion does not show an increased affinity for EGFR TKI, thus resulting in intrinsic resistance. Specific inhibitors of the EGFR ins20 are represented by Amivantanab and Mobocertinib[13,15].

Mobocertinib is an oral, irreversible TKI targeting EGFR ex20ins which has a structure related to Osimertinib. It was designed to exploit a small pocket of the ATP-bing site, which any other TKI cannot target. Mobocertinib was approved by the FDA in 2021 for the treatment of patients with EGFR ex20ins-positive NSCLC[15].

Amivantanab is a fully human Fc-active immunoglobulin G1 (IgG1) bispecific antibody targeting epidermal growth factor receptor (EGFR) and mesenchymal-epithelial transition factor (MET). Amivantamab targets the extracellular domains of EGFR and MET, inhibiting both pathways independent of their intracellular cancer-driving or treatment-acquired mutation. In preclinical studies, Amivantinib determined antitumor response by immune cell-directing activity, ligand blocking and receptor degradation. It was approved for the treatment of patients with advanced or metastatic NSCLC with EGFR exon 20 insertion mutations whose disease has progressed on or after platinum-based chemotherapy[16,17].

ALK signaling pathway. ALK gene alterations. Targeted therapy and mechanisms of resistance to TKIs

The incidence of ALK rearrangements is 3–7% in NSCLC. Patients with ALK-positive lung cancer are relatively younger and have a history of never or light smoking. A poor differentiation with acinar-predominant structure and mucin/signet-ring cell pattern can be observed on histological analysis. In non-small cell lung

cancer (NSCLC), chromosomal rearrangements involving the ALK gene loci on chromosome 2 are found in approximately 3 to 5 percent of NSCLC tumors. The most common ALK rearrangement in NSCLC juxtaposes the 5' end of the echinoderm microtubule-associated protein-like 4 (EML4) gene with the 3' end of the ALK gene, resulting in the novel fusion oncogene EML4-ALK[8,9,18].

Screening for this fusion gene in NSCLC is essential, as ALK-positive tumors are highly sensitive to therapy with ALK-targeted inhibitors. ALK-positive targeted therapies are represented by Crizotinib, Ceritinib, Alectinib and Lorlatinib[9].

Crizotinib is a multitargeted small molecule tyrosine kinase inhibitor, which was first developed as an inhibitor of the mesenchymal-epithelial transition growth factor (c-MET). It is also a potent inhibitor of ALK phosphorylation. This inhibition is associated with G1-S phase cell cycle arrest and induction of apoptosis in positive cells. Crizotinib also inhibits the related ROS1 receptor tyrosine kinase. This agent had substantial efficacy in patients with advanced-stage ALK-rearranged NSCLC in phase I/II trials, with ORRs of ~60%, which led to FDA approval for this indication in 2011. Subsequent randomized phase III trials demonstrated Crizotinib superior to chemotherapy for patients with ALK-rearranged NSCLC (median PFS of 9.5 months vs. three months). Although molecular-targeted compounds have elicited a good response in tumors expressing the specific target, acquired resistance to Crizotinib was common, typically emerging within a year of starting treatment[8,18.]

Resistance to ALK inhibitors may be mediated by acquired secondary mutations in the ALK kinase domain, such as F1174 L, F1174C, L1196 M, I1171T, G1202R, S1206Y, G1269S, and G1269A mutations[8].

More potent second-generation ALK TKIs have been developed, including Ceritinib, Alectinib and Brigatinib, with the initial goal of overcoming crizotinib resistance. These ALK TKIs had improved efficacy on CNS metastases due to improved CNS penetration. Second-generation ALK TKIs were then preferred as standard first-line therapy for patients with advanced ALK-rearranged NSCLC, with superior efficacy demonstrated in phase III trials comparing Alectinib, Brigatinib, or Ensartinib with Crizotinib (PFS at 12 months). Typically, ~65% vs. ~45%, median PFS by independent blinded review ranged from 24-26 months)[8,19].

To date, two ALK secondary mutations that seem to be refractory to Ceritinib and/or Alectinib: G1202R mutation, which is insensitive to Ceritinib and Alectinib, and F1174C mutation, which is insensitive to Ceritinib[9].

In addition to ALK gene alterations, other mechanisms of resistance to Crizotinib include the activation of tyrosine kinase receptors such as EGFR, KRAS, or Ckit and ALK gene amplification[8,9].

Although Alectinib is one of the most potent ALK inhibitors, patients with ALK-positive NSCLC harbouring G1202R or I1171X mutations do not respond to Alectinib treatment, so Lorlatinib was developed. Lorlatinib is a third-generation, macrocyclic, highly potent and selective ALK/ROS1 TKI developed to address acquired resistance to earlier-generation ALK TKIs, with potency against a broad spectrum of ALK kinase domain resistance mutations[8,9].

Tabel 1 – EGFR and ALK gene alterations in NSCLC. Targeted therapy and mechanisms of resistance

	Gene alteration	Targeted therapy	Mechanism of resistance
EGFR	mutations in exon 21 (mostly L858R)[7,8,9] deletions in exon 19[7,8,9] T790M mutation[8,9] exon 20 insertions[13,15]	Gefitinib Erlotinib Afatinib Dacomitinib Osimertinib Amivantanab Mobicertinib	Mutations within the genes encoding the downstream signalling molecules[8,9] Histological transformation[9] BIM polymorphism[13] Co-occurring mutations in other signalling pathways[8,9]
ALK	fusion oncogene EML4-ALK[8,9,18]	Crizotinib Brigatinib Ceritinib Alectinib Lorlatinib	Secondary ALK mutations (F1174 L, F1174C, L1196 M, I1171T, G1202R, S1206Y, G1269S, and G1269A)[8,9] ALK gene amplifications[8] Activation of tyrosine kinase receptors such as EGFR, KRAS, or Ckit[9] Histological transformation[9] Activation of bypass and/or downstream signalling pathways[8,9]

ALK: anaplastic lymphoma kinase; EGFR: epidermal growth factor receptor; KRAS: Kirsten Rat Sarcoma virus gene.

BRAF signalling pathway. BRAF mutations. Targeted therapy and mechanisms of resistance to TKIs

V-Raf murine sarcoma viral oncogene homolog B (BRAF) gene encodes a serine/threonine kinase which belongs to the RAS-RAF-MEK-ERK axis and that plays an important role in cellular signaling, cellular growth, proliferation, differentiation and survival. In healthy tissues, BRAF kinase action is modulated by a negative feedback response received when the next point of the downstream signaling is activated. Mutations in the BRAF gene can lead to abnormal activation of BRAF kinase with consequent activation of the signalling cascade, promoting uncontrolled cell proliferation[19].

Mutations in the BRAF gene occur in 8% of malignant diseases. In non-small-cell lung cancers, BRAF mutations were reported in 3-5 percent of cases, 1-2% in adenocarcinoma. Patients are generally women and former smokers [20]. There are three classes of BRAF mutations. Class I is represented by V600E, V600K, V600D and V600R mutants. They act as monomers and determine a strong activation of BRAF kinase and the constitutive activation of the MAPK pathway. This class of mutants is known to have a high response to BRAF and MEK inhibitors[21]. Class II mutants act as dimers and also determine kinase activation. The mutations classified as Class III are kinase-impaired and they amplify ERK signaling when an upstream activation occurs or when there is an increase in RAS activity. Class II and III of BRAF mutants are non-V600 mutations[22]

The current treatment of patients with advanced non-small-cell lung cancer and BRAF mutation is represented by BRAF-targeted therapy.

Dabrafenib is an oral adenosine triphosphate-competitive inhibitor of BRAF-kinase, selective for the V600E BRAF kinase. It was first approved for treating patients with advanced unresectable or metastatic melanoma with BRAF V600 mutation. The benefit of Dabrafenib was also studied in patients with advanced non-small-cell lung cancer harboring BRAF V600E mutation. In a multicenter, phase 2 trial where were enrolled 84 previously treated or untreated patients with advanced NSCLC and BRAF V600E mutation, Dabrafenib showed an important antitumor activity. The ORR was 33% and the median progression-free survival was 5.5 months in the previously treated patients, while in the previously untreated group of patients the longest PFS was 16.6 months[23].

Vemurafenib is another oral selective inhibitor of the BRAF V600 kinase. In a phase 2 „basket" study, Vemurafenib was tested in nonmelanoma malignancies with BRAF V600 mutation. In NSCLC patients, the ORR was 42% and the PFS was 7.3 months[24].

The combination regimen of Dabrafenib plus Trametinib is now recommended as first-line therapy in non-small-cell lung cancer with BRAF V600E mutant, but it is also recommended as subsequent therapy. The dual blockade of the BRAF pathway with a BRAF inhibitor and a MEK inhibitor is supposed to produce a stronger response in these patients. In an open-label phase 2 trial, Dabrafenib plus Trametinib was studied in previously untreated patients. The results showed an overall rate response of 64% and progression-free survival of 14.6 months [25]. The benefit of using these two drugs was also studied in an open-label phase 2 study where were included patients who previously received at least one line of therapy for advanced NSCLC and who tested positive for BRAF V600E mutation. The result was similar, with an ORR of 63.2% and a median PFS of 10 months[26].

Despite promising responses to BRAF and BRAF-MEK inhibitors in patients with BRAF mutant NSCLC, the disease will progress sooner or later due to the development of resistance to these drugs. The main resistance mechanisms to BRAF inhibitors are represented by the reactivation of the MEK/ERK pathway and the activation of alternative bypass signaling pathways.

The reactivation of the MAPK pathway can be determined by mutations in genes that are involved in the MEK/ERK signaling pathway, such as MEK1/2 mutations or NRAS/KRAS mutations, which can activate the MEK/ERK signaling despite BRAF inhibition[19]. Another way for MAPK pathway restoration is represented by an upregulation or overexpression of EGFR, which will stimulate RAS and turn on CRAF-MEK-ERK signaling[9]. It is also known that a high amount of RAF isoforms such as ARAF and CRAF and the MAP3K8/COT could activate the MEK/ERK pathway independent of BRAF[9].

The activation of bypass pathways is another mechanism of resistance to BRAF inhibitors. The activation of the PI3K/AKT pathway due to overexpression of some tyrosine kinase receptors or alterations that determine the uncontrolled activation of AKT could lead to MCL-1 activation. MCL-1 is a pro-survival factor and its activity inhibits apoptosis[19,9]. The inactivation of

PTEN, a tumor suppressor, was described as responsible for targeted therapy resistance in patients with BRAF mutations due to inhibition of apoptosis mediated by BIM[9].

Lin et al. found two molecular mechanisms for BRAF-inhibitor resistance in patients with non-small cell lung cancer. The first is represented by the loss of the full length of BRAF V600E and expressing an abnormal BRAF p61VE. The second mechanism of acquired resistance refers to the activation of the EGFR pathway due to the upregulation and activation of its ligands mediated by c-Jung signalling[27].

ROS-1 signaling pathway. ROS-1 rearrangements. Targeted therapy and mechanisms of resistance to TKIs

The ROS-1 gene encodes a transmembrane protein, which contains a tyrosine-kinase domain. Its physiological role is not well known yet but it is supposed to be involved in the epithelial tissue differentiation during embryonic development [28]. ROS-1 is involved in some signaling pathways with a role in cell growth and differentiation, proliferation and survival[29].

ROS-1 rearrangements determine the fusion of a portion of ROS-1, including the tyrosine kinase domain, with other protein partners. As a result, ROS-1 kinase is constitutively activated and leads to upregulation of MAPK/ERK, PI3K/AKT and JAK/STAT signaling pathways, which will promote cell growth, proliferation and survival[30]. In NSCLC, ROS-1 rearrangements occur in 1-2% of cases, more often in young ages, women and light or never smokers. The most frequent histological subtype is adenocarcinoma. In these patients, brain metastases are common.

ROS1-positive non-small cell lung cancer treatment is based on tyrosine kinase inhibitors.

Crizotinib is a small molecule tyrosine kinase inhibitor acting against targets such as ROS1, MET and ALK. It binds to the protein kinase domain and inhibits ATP-dependent cellular function [30]. Based on the results of PROFILE 1001, a multicentric, single-arm, phase I clinical trial, Crizotinib was the first TKI approved in NSCLC with ROS-1 rearrangements[31]. In this clinical trial, 50 patients with advanced ROS-1 NSCLC were enrolled. The ORR was 72% and the median PFS was 19.2 months[32].

Ceritinib is a second-generation ALK and ROS1 TKI. In preclinical studies, Ceritinib showed a twenty times greater potency and it also has a greater blood-to-brain penetration ratio than Crizotinib[30]. In an open-label, multicenter, phase II study where were enrolled 32 Crizotinib naive and Crizotinib pretreated patients with advanced NSCLC and ROS1 rearrangements, the ORR was 62%. In the Crizotinib naive group, the PFS was 19.3 months and the median PFS for all patients enrolled was 9.3 months. Of the patients with brain metastases, intracranial disease control was reported in 63% of patients[33].

Entrectinib is a multi-kinase inhibitor with activity against ROS1, ALK and TRK. It penetrates the blood-to-brain barrier, achieving substantial concentrations and leading to a good intracranial response[31]. In vitro studies showed it was 40 times more potent than Crizotinib. The antitumor activity and safety of Entrectinib were evaluated in an integrated analysis of three phase 1 and 2 trials. There were included patients with advanced and metastatic non-small-cell lung cancer who tested positive for ROS1 rearrangements. These patients did not previously receive treatment with ROS1 TKIs. The objective rate of response was 41% and the median progression-free survival was 19 months[34].

Lorlatinib, a third-generation brain-penetrant tyrosine kinase inhibitor with action against ROS1 and ALK, was evaluated in advanced ROS-1 non-small-cell lung cancer in a multicentric, open-label, single-arm, phase 1-2 trial. In the trial, 69 patients with ROS-1 positive NSCLC were enrolled. The patients were TKI naive or previously treated with Crizotinib or another ROS-1 TKI. The ORR was 41% among all patients. A higher response to Loratinib was observed in previously untreated patients with an ORR of 62% and a median PFS of 21 months. Intracranial response was achieved in 6 of 11 patients with baseline CNS metastases and TKI naive[35].

Mechanisms of resistance to ROS1 targeted therapy are multiple. The main phenomena that can lead to resistance to ROS1 inhibitors are represented by different mutations in the kinase-binding domain of ROS1 kinase (on-target resistance), activation of other pathways involved in cell proliferation and survival (off-target resistance) and phenotypic transformation[9].

The most frequent mutation is G2032R. It is a punctual mutation that occurs in the ATP binding site and determines mesenchymal-to-epithelial transition, promoting cell migration [29]. Another mutation is ROS1 D2033 mutation, which leads to resistance to ROS1 TKI by altering the electrostatic force necessary

for linking between ROS1 TKIs and ATP-binding site, due to an abnormality in the ATP-binding pocket [29]. Other punctual mutations involved in the appearance of resistance to ROS1 TKIs are less frequent and are represented by S1986Y/F, L2026M, L2155S, L1951R, S1886, L2086F, G2032K[9].

The activation of other signaling pathways represents another mechanism of resistance to ROS1 inhibitors, in other words, a ROS1-extrinsic mechanism of resistance. The suppression of ROS1 activity can determine the upregulation of downstream or parallel signaling pathways such as NRAS, KRAS, EGFR, HER2, MEK, KIT, PI3K and BRAF, which make the therapy with ROS1 tyrosine kinase inhibitors inefficient[34]. TKI resistance due to activation of other signaling pathways can also be possible by newly acquired abnormalities: MET mutations or MET gene amplifications, KRAS mutations or amplifications, NRAS, ALK, BRAF and MAP2K1 mutations etc.[29].

Phenotypic changes as a mechanism of resistance to ROS 1 inhibitors can occur. The transformation into a small cell lung cancer was described in one case and it was associated with loss of ROS1-fusion expression and inactivation of TP53 gene and RB1 gene[29].

MET signaling pathway. MET exon 14 skipping mutations and MET amplifications. Targeted therapy and mechanisms of resistance to TKI

MET is a proto-oncogene that encodes a transmembrane tyrosine kinase receptor with a high affinity for hepatocyte growth factor (HGF). The combination of MET receptor and HGF leads to the activation of the intracellular protein kinase domain by autophosphorylation and the consequent activation of the MAPK, PI3K, SRC and STAT downstream signaling pathways[37].

MET exon 14-skipping mutations occur in 3-4% of lung adenocarcinomas. These alterations determine a decreased degradation of MET receptors and sustain MET overexpression and oncogenesis. Typically, other driver mutations are not detected. Patients are generally 70 or older, smokers and have a poor prognosis[38].

MET amplification is detected in 1-6% of NSCLC and the role in cancer development is thought to be the protein overexpression and constitutive kinase activation[37].

Several ATP-competitive tyrosine kinase inhibitors were studied over time for to manage patients with non-small cell lung cancer and MET abnormalities.

Capmatinib is a selective inhibitor of the MET receptor, which penetrates the blood-to-brain barrier. Its approval is based on the results of the GEOMETRY study, where previously treated and untreated advanced NSCLC patients with MET abnormalities where were enrolled. The ORR in patients who received at least one line of therapy was 41%, while in previously untreated patients, the ORR was 68%. The median PFS was 5.4 months in previously treated patients and 12.4 months in those previously untreated[39].

Tepotinib is a small molecule, highly selective, ATP-competitive MET inhibitor. Its benefit in advanced NSCLC harbouring MET exon-14 skipping mutations was evaluated in the VISION study. The ORR was 48% and the median PFS was 8.5 months in the combined-biopsy group[40].

Antitumor activity of Crizotinib, a multi-tyrosine kinase inhibitor with potent activity against ALK and ROS1 rearrangements, was also evaluated for the treatment of NSCLCs with MET exon14 skipping mutations. In an open-label phase I study, antitumor benefits and safety of Crizotinib were evaluated in 69 patients. The ORR in these patients were 32 percent and a median progression-free survival of 7.3 months[41].

Progression of disease in patients with MET amplification or MET exon 14 skipping mutation after treatment with MET TKsI is frequent. The mechanisms of resistance to TKIs are multiple and not well understood. They may be classified into on-target mechanisms of resistance, off-target mechanisms of resistance and inherent resistance.

On-target mechanisms of resistance are secondary MET gene alterations that can appear due to mutations in the MET kinase domain, MET exon 14-mutant allele amplification or amplification in the HGF gene. Mutations in D1228 and Y1230 residues of MET kinase were observed after treatment with type I MET TKIs such as Crizotinib and Capmatinib. These abnormalities affect the bounding between the targeted drug and the kinase domain, making therapy inefficient. Mutations in L1195 and F1200 residues of MET kinase were described in patients treated with type II MET TKI. It is important to mention that more than one mutation can be present at the same time due to subclonal selection determined by the treatment[42].

Off-target mechanisms of resistance were also described in patients who underwent treatment with MET tyrosine kinase inhibitors. Acquired resistance due to activation of by-pass signaling pathways in these

patients is determined by alteration in KRAS gene such as KRAS mutations or KRAS amplification, MDM2, PIK3CA, BRAF amplification or amplification of other tyrosine kinase receptors (EGFR, KIT, HER3)[42]. It is not known if these aberrations are present in some clone cells before the treatment or if they appear during therapy with MET TKIs[43].

Inherent resistance, as a mechanism of resistance to MET TKIs, is determined by concurrent gene amplification or mutations in other oncogenes such as KRAS, MDM2, EGFR or loss of expression of PTEN protein. Co-mutations or other alterations in RAS-RAF-MAPK or PI3K/AKT signaling pathways can determine a suboptimal response to MET inhibitors[43].

Tabel 2 – BRAF, ROS-1 and MET gene alterations. Targeted therapy and mechanisms of resistance

	Gene alteration	Targeted therapy	Mechanism of resistance
BRAF	BRAF V600E/D/K/R mutations[21] BRAF non-V600 mutation[22]	Dabrafenib Vemurafenib Dabrafenib+Trametinib	Reactivation of the MAPK pathway[19] Activation of bypass pathways[9,19] Inactivation of PTEN [9] Loss of full length of BRAF V600E[27]
ROS-1	ROS-1 rearrangements[30]	Crizotinib Ceritinib Entrectinib Lorlatinib	Mutations in the kinase binding domain [9][29] Activation of downstream or parallel signaling pathways[9,29] Phenotipic changes[9,29]
MET	MET exon 14 skipping mutation[38] MET amplification[37]	Capmatinib Tepotinib Crizotinib	Mutations in the MET kinase domain[42] MET exon 14-mutant allele amplification[42] Amplification in the HGF gene[42] Activation of bypass signalling pathways[42] Inherent resistance[43]

BRAF: V-raf murine sarcoma viral oncogene homolog B; MET: mesenchymal-epithelial transition factor; ROS1: receptor tyrosine kinase oncogene 1

RET signalling pathway. RET gene fusions and RET rearrangements. Targeted therapy and mechanisms of resistance to TKI

RET gene fusions or rearrangements are the principal aberrations occurring in 1–2% of non-small cell lung cancer (NSCLC)[44]. NSCLC and PTC are the most common cancer types harboring RET fusions. About 2% of patients with NSCLC harbor RET fusions and they tend to occur in relatively younger (<60 years of age), never- or light-smokers, which are similar to those carrying ALK or ROS rearrangements[45].

The RET inhibitors, Selpercatinib and Pralsetinib, are approved by the FDA for adult patients with metastatic NSCLC with a RET fusion-positive NSCLC and it is preferred, according to NCCN, the use of either of these agents in the front-line setting for such patients, rather than immunotherapy and/or chemotherapy. However, it is also acceptable to use these agents in the subsequent-line setting. Selpercatinib is also approved for patients with locally advanced RET-positive NSCLC. Selpercatinib and Pralsetinib have also demonstrated intracranial activity in preclinical models[46].

In the LIBRETTO-001 study regarding Selpercatinib, the median duration of response was 17.5 months (95% CI) and 63% of the responses were ongoing at a median follow-up of 12.1 months. Among 39 previously untreated patients, the percentage with an objective response was 85% (95% CI) and 90% of the responses were ongoing at six months[47].

In the ARROW study regarding Pralsetininb, overall responses were recorded in 53 of 87 patients with previous platinum-based chemotherapy, including five patients with a complete response and 19 of 27 treatment-naive patients, including three with a complete response[48].

The majority of resistance to selective RET inhibition is driven by RET-independent resistance, such as MET amplification. RET TKIs with potency against RET solvent front mutations and combination strategies are needed to overcome resistance[44].

There has been conducted a multi-institutional analysis of repeat tumor or plasma biopsies from a cohort of

patients with RET fusion-positive NSCLC after treatment with Selpercatinib and Pralsetinib. Patients were eligible if they had advanced/metastatic NSCLC with RET fusion identified by local molecular profiling and/or received as any line of systemic therapy Pralsetinib and/or Selpercatinib with subsequent resistant tumor or liquid biopsy analyzed by molecular testing[49]. A RET resistance mutation was thus detected in two cases (10%), both affecting the G810 residue in the RET solvent front.

NTRK signaling pathway. NTRK1, NTRK2, and NTRK3 fusions. Targeted therapy and mechanisms of resistance to TKI

Although NTRK fusions are reported mostly in middle-aged (median age of 47.6 years) and non-smoking history populations, which resembles the clinical profiles of many other fusions, they can also be detected in patients of other age groups or with previous smoking histories, suggesting that NTRK fusions are not related to certain clinical features in NSCLC. Furthermore, most NSCLC patients with positive NTRK fusions have metastasis at diagnosis[50].

According to the NCCN guidelines, both Larotrectinib and Entrectinib are recommended as standard therapies for the first-line treatment of NTRK fusion-positive patients with advanced or metastatic NSCLC, as well as progressive patients with previous systemic therapies[51].

Entrectinib and Larotrectinib have not been compared head-to-head. As shown above, response rates in small, nonrandomized trials are similar. In the absence of direct comparisons, either option is appropriate for those with NTRK-positive NSCLC.

The overall response rate in the Larotrectinb study was 75% (95% CI), according to the independent review and 80% (95% CI), according to the investigator's assessment. At one year, 71% of the responses were ongoing and 55% of the patients remained progression-free. The median duration of response and progression-free survival had not been reached. At a median follow-up of 9.4 months, 86% of the patients with a response (38 of 44 patients) were continuing treatment or had undergone surgery that was intended to be curative [52].

Also, in the Entrectinib study, the median follow-up was 12.9 months. 31 of 54 patients had an objective response, of which four (7%) were complete responses and 27 (50%) had partial responses. The median duration of response was 10 months[53].

The mechanisms of acquired resistance include "on-target" mechanisms, secondary mutations occurring at the TRK kinase domain, and "off-target" mechanisms, such as bypass signaling pathways activation[51].

The secondary mutations occurring at the ATP-binding pocket of the TRK kinase domain include the solvent-front, gatekeeper region, and xDFG motif mutations in the activation loop. NTRK1 G595R and NTRK1 G667S mutations were presented in a NSCLC patient. Next-generation TRK inhibitors have already been developed to overcome the on-target resistance mutations during treatment with first-generation TRK inhibitors [54].

This mechanism can develop during TRK inhibitor treatment, which includes genomic alterations of downstream pathway mediators and other receptor tyrosine kinases. BRAF V600E mutation, KRAS G12D mutations, and MET amplifications were also identified as the bypass-mediated resistance mechanisms to TRK inhibitors for patients with NTRK fusions. A dual blockade of TRK and MEK could effectively control tumor growth and delay the emergence of off-target resistance. However, the next-generation TRK inhibitor monotherapy was not effective for resistance mediated by bypass pathway mutations[55].

Second-generation TRK inhibitors include Selirectinib – which showed significant inhibitory cellular activity against NTRK fusions and acquired resistance mutations in vitro, including TRKA G595R, TRKA G667C, and TRKC G623R and Repetrectinib - designed to overcome resistance mutations and potently inhibit wildtype TRK fusions. Highly potent and selective against wildtype ALK, ROS1, and TRK fusion proteins, as well as their solvent-front substitutions in preclinical studies, including TRKA G595R, TRKB G639R and TRKC G623R. Studies have yet managed to show a sustained response from second-generation inhibitors[56].

NRG1 signaling pathway. NRG1 fusions. Targeted therapy and mechanisms of resistance to TKI

Regarding epidemiology, Fernandez-Cuesta et al. found that NRG1 rearrangements are more common in those have never smoked[57].

Retrospective data have suggested poor activity of chemotherapy and immunotherapy in NRG1-fusion cancers. A case series of five who patients who received Afatinib therapy for NRG1-positive lung can-

cer demonstrated four partial radiographic responses in five patients, with three of these lasting over 18 months. Other targeted therapeutics against NRG1 fusions are in clinical trial development, such as Seribantumab[57].

Some case reports have shown primary resistance to Afatinib in patients with NRG1 fusions.

Drilon and colleagues investigated Afatinib treatment in four patients with NRG1-rearranged lung cancers and found progressive disease (PD) shortly after starting treatment. Three of the four patients had CD74-NRG1 fusions and one had an SDC4-NRG1 fusion. One of the patients had previously been treated with the anti-ErbB3 molecule GSK2849330 (no longer in development) and had developed PD after a PR lasting 19 months. These response discrepancies highlight the need for a global data and treatment-sharing platform to gather as much data as possible on these uncommon drivers[58].

In this context, a global multicenter registry of thoracic oncologists was launched to improve our understanding of clinicopathologic characteristics and response to treatment of patients with NRG1 fusion-driven NSCLC.

KRAS signaling pathway. KRAS G12C mutation. Targeted therapy and mechanisms of resistance to TKI

KRAS is the most frequent oncogene in non-small cell lung cancer. The presence of a KRAS mutation is prognostic of poor survival when compared to patients with tumors without KRAS mutation[59].

The focus of targeted therapeutics for patients with KRAS-mutated lung cancer is against downstream effectors of activated KRAS, based on previous supporting preclinical evidence, as well as irreversible inhibitors of KRAS G12C. Multiple agents specifically targeting the KRAS G12C mutation, which comprises almost 50 percent of KRAS mutations in NSCLC, have emerged.

Sotorasib was the first targeted agent with regulatory approval for KRAS G12C-mutated NSCLC. It is approved by the FDA for patients with KRAS G12C-mutated locally advanced or metastatic NSCLC who have received at least one prior systemic therapy. In the subgroup with NSCLC, 32 percent had a confirmed objective response across several doses, and 88 percent had disease control (objective response or stable disease); the median PFS was 6.3 months[60].

Adagrasib is a related molecule that also has FDA approval for patients with KRAS G12C-mutated locally advanced or metastatic NSCLC who have received at least one prior systemic therapy. Adagrasib 600 mg twice daily was associated with a median progression-free survival of 6.5 months, a duration of response of 8.5 months, a response rate of 43 percent and overall survival of 12.6 months[59].

Despite the encouraging clinical results, about half of the patients included in clinical trials with Sotorasib and Adagrasib do not experience significant tumor shrinkage with specific KRAS G12C inhibitors.

One potential cause of the lack of efficacy of KRAS G12C inhibitors is that not all KRAS mutant cells depend on KRAS activation to maintain their viability. In an attempt to identify a gene expression signature that correlates with the KRAS dependency KRAS mutant cells, Singh et al. found a subgroup of lung cancer-derived cellular lines that maintain their viability despite the ablation of the KRAS mutant protein. Moreover, activation of the two main downstream effectors (ERK and AKT) was not suppressed after KRAS knockdown[61].

A major mechanism of resistance is the adaptive feedback resistance, in which the loss of downstream signaling by the blocked mutant target leads to the reactivation of receptor tyrosine kinase (RTK) mediated signaling through wild-type RAS and RAF. This adaptive feedback resistance was evaluated in KRAS G12C inhibited cell lines using ARS-1610 and AMG-510, finding a rapid and consistent reactivation of signaling by a rebound activation of downstream ERK and RSK in addition to increased levels of active GTP-bound NRAS and HRAS wild type. Also, increased EGFR, HER2, FGFR, and c-MET were observed, suggesting that RTKs upstream activation is one of the critical resistance mechanisms of KRAS G12C-inhibitors[62].

In another study addressing the heterogeneity of initial response in KRAS G12C mutant lung cancer cells, Xue and colleagues described that tumor cells initially adopt a quiescent state, in which some cells will die. Still, other cells will rapidly evade inhibition by the synthesis of new KRAS G12C that is quickly converted to its active state due to upstream stimuli mediated by epithelial growth factor receptor (EGFR) and Aurora Kinase A (AURKA) [63].

Hence, primary resistance relies on that as a canonical and fundamental pathway for cell survival. RAS-RAF-ERK has multiple independent mechanisms that

can maintain signaling in its active state while being selectively targeted.

There are 2 types of acquired resistance mechanisms: "on-target" mechanism, with the acquisition of mutations in key regions within the driver protein that impedes adequate drug binding and target inhibition and "off-target" mechanism due to biological alterations independent of KRAS G12C inhibition[63].

A study from 2021 regarding resistance to KRAS inhibitors identified 38 patients with KRASG12C-mutant cancers who had disease progression while receiving Adagrasib monotherapy. 27 patients had NSCLC, 10 had colorectal cancer, and 1 had appendiceal cancer. At the time of acquired resistance to Adagrasib, tissue was available for analysis for 10 patients, and ctDNA was available for 32 patients; both tissue and ctDNA were obtained from 4 of these patients. In 32 of the 38 patients (84%), the original KRAS G12C mutation was identified at the time of Adagrasib resistance[61].

Putative mechanisms of resistance to Adagrasib were identified in 17 of 38 patients (45%). Novel acquired secondary KRAS mutations within the Adagrasib-binding pocket were found in 4 patients, including a Y96C mutation (NSCLC) and R68S and H95D mutations (NSCLC)[61].

Pathogenic mutations in other receptor tyrosine kinase (RTK)–RAS–MAPK pathway members were also detected in patients with acquired resistance to Adagrasib. Among the 17 of 38 patients with putative resistance mechanisms identified, 9 (53%) had at least one acquired KRAS mutation or amplification. Furthermore, putative resistance mechanisms that did not directly involve KRAS, but were related to the RAS signalling pathway were observed in 12 of the 17 patients (71%). In addition, 7 of the 17 patients (41%) had more than one concurrent potential resistance mechanism identified[62].

Drug-resistance mutations were defined as those that enabled cell survival during treatment with a KRAS G12C inhibitor. Multiple mutations that were identified in the screen at codons 12, 68, 95, and 96 conferred strong resistance to MRTX1257. Additional strong MRTX1257-resistance mutations were detected at codons 8, 9, 64, 99, and 117. All clinically observed Adagrasib-resistance mutations in KRAS G12C scored as strong resistance mutations in the MRTX1257 screen. In the Sotorasib screen, numerous strong resistance mutations were observed at codons 8, 9, 12, 96, and 117[61].

Tabel 3 – RET, NTRK, NRG1 and KRAS gene alterations. Targeted therapy and mechanisms of resistance

	Gene alteration	Targeted therapy	Mechanism of resistance
RET	Gene fusions or rearrangements[45]	Selpercatinib Pralsetinib	RET - independent resistance[44]
NTRK	NTRK1, NTRK2, and NTRK3 fusions[50]	Larotrectinb Entrectinib Second-generation Selirectinib Repretecinib	**on-target mechanisms** secondary mutations occurring at the NTRK kinase domain - NTRK1 G595R and NTRK1 G667S mutations[51,54] **off-target mechanisms** bypass signaling pathways activation[51]
NRG1	NRG1-fusion[57]	Afatinib	CD74-NRG1 fusions[58] SDC4-NRG1 fusion[58]
KRAS	KRAS G12C mutation[59]	Sotorasib Adagrasib	**primary resistance**[63] **acquired resistance** on-target mechanism,with acquisition of mutations in key regions within the driver protein that impedes adequate drug binding and target inhibition[61,63] off-target mechanism due to biological alterations independent of KRAS G12C inhibition[62,63]

KRAS: Kirsten Rat Sarcoma virus gene; NRG1: neuregulin 1 gene; NTRK: neurotrophic tyrosine receptor kinase 1/2/3; RET: rearranged during transfection gene.

CONCLUSION

Lung cancer continues to be an important health issue and a major cause of cancer-related death. The discovery and approval of targeted drugs brought a huge advantage in the treatment of patients harboring a driver mutation, so that, testing for molecular biomarkers should be mandatory for every newly diagnosed NSCLC patient in order to offer the most appropriate treatment.

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