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Emerging genetic biomarkers in lung adenocarcinoma

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Abstract

Comprehensive genomic profiling is a next-generation sequencing approach used to detect several known and emerging genomic alterations. Many genomic variants detected by comprehensive genomic profiling have become recognized as significant cancer biomarkers, leading to the development of major clinical trials. Lung adenocarcinoma has become one of the most targeted cancers for genomic profiling with a series of actionable mutations such as EGFR, KRAS, HER2, BRAF, FGFR, MET, ALK, and many others. The importance of these mutations lies in establishing targeted therapies that significantly change the outcome in lung adenocarcinoma besides the prognostic value of some mutations. This review sheds light on the development of the comprehensive genomic profiling field, mainly lung adenocarcinoma, and discusses the role of a group of mutations in this disease.

Keywords

Comprehensive genomic profiling, next-generation sequencing, lung cancer, targeted therapy, actionable mutations

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Introduction

Comprehensive genomic profiling (CGP) is a next-generation sequencing (NGS) approach that performs simultaneous sequencing of millions of DNA fragments and detects new genomic alterations, providing an in-depth view of the complete genomic content of each tumor. Knowledge and interpretation of the different genomic findings can serve in guidance, diagnosis, prognosis, and treatment decisions, by matching cancer patients to corresponding targeted therapies, known as precision medicine.[1,2]

CGP can detect all four classes of genomic aberrations present in cancers, namely, base pair substitutions, insertions and deletions, copy number variations, and rearrangements.

Other important findings relevant to cancer genomics include microsatellites, highly preserved, repetitive DNA sequences. Microsatellite instability (MSI) refers to a hypermutable genome due to a defective DNA mismatch repair (MMR) system. Four genes involved in the MMR process and determination of the mutational status of these genes can be used as a biomarker to predict the tumor response to immunotherapy.

Another important cancer genomic term is the tumor mutational burden (TMB), which refers to the number of genetic mutations present in a cancer cell. A higher number of mutations were associated with a better likelihood of response to immune checkpoint inhibitor treatment.

The recent rise in molecular profiling of tumors has led to the development of clinical trials driven by biomarkers. The Initiative for Molecular Profiling and Advanced Cancer Therapy (IMPACT) trial at MD Anderson in 2007 demonstrated the clinical importance of matching patients with therapies tailored to their cancer genomics compared with conventional therapy. Results showed a significant survival advantage in patients treated with matched targeted therapy in terms of median progression-free survival (PFS) and overall survival (OS), emphasizing the need for rapid and

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specific genomic profiling and its implementation in treatment decisions.[3,4] The NCI-Molecular Analysis for Therapy Choice (NCI-MATCH) is another trial looking into the efficacy of targeted drugs specific to tumor genomic alterations. Using NGS profiling with a panel of more than 100 genomic alterations and corresponding targeted therapies, a large network trial was created.[5] Interestingly, recent results published in 2020 also included the discovery of many genomic variants in advanced less-known cancers, potentially opening the door for better management of these tumors.[6]

The Targeted Agent and Profiling Utilization Registry (TAPUR) trial joined the rise by looking into the efficacy and safety of the off-label use of anticancer drugs based on tumor genomic profiling.[7] Published data showed different results with different tumor types and treatments, shedding light on combinations with promising results.[8,9]

Main CGP kits and companies

A current race between pharmaceutical companies is happening in the CGP field, which has led to the creation of a diversity of different kits.

One of the most utilized kits is the FoundationOne CDx, which tests 324 different genes. It is a DNA sequencing modality that can show MSI and TMB. Ten slides with at least 20% of tumor specimens are required to be able to conduct the test. This kit does not cover any RNA sequence analysis.[10]

In contrast, Caris CDx fills this gap by analyzing DNA, RNA, and proteins in the sample. It sequences over 592 genes and has the same tissue requirement as FoundationOne CDx. It can also show mRNA variants.[11] The Tempus XT sequencing platform sequences both the DNA and RNA components of the specimen being assessed. It covers over 596 different genes and only requires 10 slides of tissue (no percentage of tumor needed).[11] When available, the sequencing of the samples can be matched with normal blood samples or saliva. TruSight Oncology 500 is another platform that can assess over 523 DNA genes and 55 RNA genes for mutations and variants.[12] This assay can also measure TMB and MIS.

Actionable mutations in lung cancers

EGFR in non-small cell lung cancer

Mechanism of action. The EGFR pathway is a key target in the treatment of non-small cell lung cancer (NSCLC). It is a member of the ErbB receptor tyrosine kinase (RTK) family. The EGF receptor is a transmembrane protein consisting of an extracellular binding domain, a hydrophobic transmembrane segment, and a cytoplasmic tyrosine kinase domain.[13] Upon ligand binding, EGFR is activated via dimerization-leading to autophosphorylation of the tyrosine kinase domain. Phosphorylation activates downstream signaling pathways, which include the MAPK pathway, PI3K/AKT, and STAT signaling pathways.[14] The activation of the EGFR receptor leading to cell growth and proliferation is seen in many solid tumors, notably in NSCLC. Approximately, a 19% incidence of EGFR mutation frequency in Western patients and a 48% incidence in Asian patients.[15]

Relevant mutations. Mutation carriers are most commonly women, nonsmokers, and young patients.[16] EGFR kinase domain mutations target four exons (18–25), which encode parts of the tyrosine kinase domain, clustered around the ATP-binding pocket of the enzyme. The most common mutations are frame deletions in exon 19, and a point mutation in exon 21 (L858R). Combined, these classical EGFR mutations represent 85%–90% of EGFR mutations in lung cancer.[17] As for the remaining 15% of EGFR mutations, these mainly include point mutations, deletions, and insertions within exons 18–25 of the EFGR gene. Exons 19 and 21 are sensitive to tyrosine kinase inhibitors (TKIs) such as Erlotinib, Gefitinib, and Afatinib. In contrast, exon 18 and 20 mutations are less sensitive or completely resistant to TKIs.[18] New studies have shown that exon 20 insertions are nowadays reported in up to 10% of all observed EGFR mutations and seem to be the next common EGFR mutation in NSCLC, after exons 19 and 21.[19]

Therapies. TKIs are used for the treatment of NSCLC and have served as excellent targeted drugs.[20]

Many agents such as Gefitinib, Erlotinib, Cetuximab, and Panitumumab are being used.

Both Gefitinib and Erlotinib are reversible competitive inhibitors of the tyrosine kinase domain, leading to blockade of the downstream pathway involved in cellular signaling and growth. As mentioned previously, EGFR mutations in exons 19 and 21 confer sensitivity to TKI.

These agents improved PFS compared to chemotherapy as first-line therapy with acceptable toxicity as compared to standard chemotherapy.[21] New reports have reported partial responses to Erlotinib in patients with one exon 20 insertional mutation (A763_Y764insFQEA insertion), with similar sensitivity to EFGR TKI as exons 19 and 21.[19]

Other EGFR TKIs, such as Poziotinib and Tarloxotinib, have shown promising results in patients with exon 20 insertional mutations.[19]

Although target therapy in NSCLC offers disease control, tumors start to inexorably develop drug resistance secondary to different mutations.

For instance, studies have shown that some patients develop drug resistance to first-generation EGFR-TKI after 10–16 months of therapy.[22]

Mechanisms of drug resistance to first-generation EGFR-TKI in NSCLC include mutations in the TK domain mutation (T790M), MET amplification, and RAS mutation.[23]

The most commonly acquired mutation in NSCLC patients is the T790M mutation: 60% of patients are

found to have a substitution of methionine at amino acid position 790 with threonine (p.Thre790Met point mutation) in the gene encoding EGFR.[24,25] Some patients with NSCLC have the T790 mutation even though they have not undergone EGFR-TKI treatment, suggesting an intrinsic EGFR TKI resistance.[22] These findings underline the need for newer drugs and therapies to overcome drug resistance.[26]

Osimertinib has been developed as a third-generation EGFR-TKI which can act against both sensitive and resistant T790M EGFR mutations.[27] Studies have shown that NSCLC patients treated with Osimertinib had a longer median PFS compared with patients treated with first-generation EGFR-TKIs (Gefitinib and Erlotinib). Patients with EGFR T790M advanced NSCLC had a higher objective response rate (ORR), disease control rate, longer duration of response, and PFS with oral Osimertinib.[28]

A recent study has revealed that drug resistance to Osimertinib has started to emerge. Different mutations found include EGFR C797S mutations, mutations in PIK3CA, KRAS, BRAF, and MET amplification.[29–31]

Moreover, studies showed that aiming at MET amplification could increase therapeutic efficacy: MET inhibitors (Crizotinib or SGX532) could increase the sensitivity of NSCLC to Gefitinib.[32] KRAS has also been identified as a mechanism of EGFR-TKI resistance in NSCLC.[33] Targeting KRAS mutation can potentially improve therapeutic efficacy and overcome drug resistance.[34]

KRAS in lung adenocarcinoma

Mechanism of action. KRAS, which codes for a small GTPase membrane-bound protein, is responsible for signal transduction by interacting with different downstream effectors. They act to integrate signals from external growth factors with a variety of downstream effectors such as members of the RAF-MEK-ERK and PI3K-AKT-mTOR.[35] RAS protein exists in two different states: active and inactive, during which they are bound to GTP and GDP, respectively. GTP-bound activated RAS involves multiple effector molecules belonging to multiple signal transduction cascades, which control cell proliferation and survival, apoptosis, cell cycling, motility, and endocytosis.[36] The constitutively activated RAS oncoprotein initiates intracellular cascades without the existence of extracellular signals, leading to oncogenesis.[37]

Relevant mutations. KRAS mutations are commonly found in lung adenocarcinomas but can be infrequently seen in squamous cell carcinomas.[38]

KRAS mutations tend to be mutually exclusive to other known lung cancer drivers such as EGFR or ALK fusions, although there are some exceptions to the rule. There is significant heterogeneity in the specific type of point mutation that is present in KRAS. In an analysis of data from the Catalogue of Somatic Mutations in Cancer (COSMIC), the most common mutation was G12C (42%), followed by G12V (21%) and G12D (17%).[39] Transition point mutations are commonly seen in never-smokers, whereas transversion point mutations are more common in former or current smokers, with KRAS G12C being the most common transversion.[40]

Activating mutations lead to constitutive signaling in both adenocarcinomas and squamous cell carcinomas. These mutations are associated with a poorer prognosis, resistance to chemotherapy, and resistance to EGFR-TKI treatment.

Therapies. Many clinical trials are currently investigating a wide range of novel therapeutic strategies to target KRAS-mutant NSCLC. Attempts to target KRAS directly have been difficult. This is probably caused by the high heterogeneity within KRAS mutant lung tumors. Multiple coexisting genetic events and mutant KRAS allele copy number represent distinct metabolic profiles and tumor microenvironments both contributing to differential drug sensitivities in seemingly similar tumors. Alternatively, molecular stratification along with KRAS aberrations may change the course of treatment of KRAS-positive NSCLC.

A striking breakthrough has been achieved with covalent inhibitors such as MRTX849 and AMG 510, as well as with LC-2, which is a degrader molecule against the endogenous protein in patients with KRAS G12C lung tumors.[41]

Monoclonal antibodies targeting PD-1 and its main ligand PD-L1 are being incorporated as first-line treatment in patients with advanced-stage NSCLC.[42] PD-1/PD-L1 inhibition is one of the most promising new therapies in KRAS-mutant NSCLC. Moreover, recent data suggest that single-agent anti-PD1/PD-L1 therapy is effective and might achieve tumor regression in a subset of patients with KRAS-TP53 co-mutant tumors. Patients with KRAS mutations and co-occurring STK11/LKB1 genomic alterations demonstrate, in contrast, resistance to PD-L1 axis inhibitors in KRAS-mutant lung tumors.[43]

This highlights the need to individualize treatment and the need for genomic profiling, which may enhance or improve treatment in patients with NSCLC.

HER2 in lung cancer

Mechanism of action. Human epidermal growth factor 2 (HER2 erbB-2/neu), found to be overexpressed in NSCLC, is regarded as a new therapeutic target for treating NSCLC. HER2, a member of the erbB RTK family, has no known ligand and when triggered, it leads to the constitutive activation of the receptor and downstream PI3K/AKT and MEK/ERK pathways.[44] The activation of these two pathways occurs following the activation of HER2 via homodimerization with other members of the erbB family, leading to phosphorylation of intracellular tyrosine residues which facilitate cell growth and migration.[45]

Relevant mutations. HER2 was shown to be overexpressed in 13%–20% of NSCLC. HER2 gene amplification assessed via fluorescent in situ hybridization (FISH) was found to be relatively uncommon, seen in 2%–4% of predominantly adenocarcinoma-type NSCLCs.[44] HER2 amplifications were identified as a potential mechanism of resistance to EGFR TKI therapy. T790M (acquired EGFR mutation) and HER2 amplification were found to be mutually exclusive.[46] HER2 mutations mainly consist of exon 20 in-frame insertions. These mutations lead to constitutive activation of the receptor and initiate downstream pathways. HER2 mutations can occur in the extracellular, transmembrane/juxtamembrane or kinase domain. The location of the mutation appears to be associated with the tumor histology.[47]

Therapies. The combination of anti-HER2 therapies, particularly the monoclonal antibody Trastuzumab, with cytotoxic agents is considered the standard first-line regimen for HER2-positive breast and gastric cancers. However, it is still unclear whether the use of anti-HER2 therapies, standard chemotherapy, TKIs, or any combination of these treatments provides additional benefit in patients with NSCLC.[45] Despite the presence of several effective anti-HER2 agents, including Trastuzumab, Lapatinib, Pertuzumab, and Trastuzumab-emtansine, the study of these agents in NSCLC has been slowed down after the negative results of the first clinical trials of Trastuzumab in combination with chemotherapy in advanced NSCLC.[48] Lapatinib, an oral reversible dual TKI of EGFR and HER2, has been tested in a phase II trial that included 75 patients with recurrent or metastatic NSCLC with no responses detected.[49] In addition, Afatinib, a potent irreversible ErbB receptor family blocker was studied in an exploratory phase II study. The study involved five patients with HER2 mutated advanced adenocarcinoma treated with Afatinib, three of which were evaluable for response, and an objective response was observed in all three.[50] Pertuzumab, a first-in-class HER2 dimerization inhibitor, is a humanized monoclonal anti-HER2 antibody that was studied in a phase II trial as monotherapy in 43 patients with recurrent NSCLC and showed no response.[51] Dacomitinib, an irreversible pan-HER TKI tested in a phase II cohort of patients with HER2-mutant or amplified lung cancers, demonstrated an overall 13% response rate in the 26 HER2-mutant patients. Neratinib, another irreversible pan-ErbB-receptor family blocker, is still being evaluated as monotherapy and in combination with temsirolimus in patients with HER2-mutant NSCLC in phase II trial.[44]

There are still no approved HER2-targeted therapies to date despite promising results with some anti-HER2 agents.[52]

BRAF in NSCLC

Mechanism of action. BRAF is short for V-raf murine sarcoma viral oncogene homolog B. This gene mutation is found in approximately 4% of NSCLCs.[53] However, in a recent study on CGP of BRAF mutations in more than 3000 lung cancer patients, its prevalence was found to be 0% in SCLCs (ESMO reference). This gene codes for a serine/threonine kinase, a part of the MAPK pathway. It oversees the regulation of gene transcription and cell proliferation and survival. RAS activates BRAF, which in turn phosphorylates the MAPK1/2 protein, also called MEK1/2. This sequence of events causes the activation of ERK1/2, which is responsible for all the regulatory cellular functions.[53]

Relevant mutations. Of the mutations of BRAF described, BRAF V600E remains the most relevant one. In a study published in the *Journal of Thoracic Diseases*, the prevalence of BRAF V600E was 56.7% in NSCLCs while BRAF non-V600E represented 43.3%, among which the most common ones were G469 and D594 (0.8% and 0.6% of lung adenocarcinomas, respectively) (ESMO reference).[54] These alterations in the BRAF gene cause the whole pathway to be constantly activated, thus promoting unregulated cell growth and proliferation.

Therapies. Dabrafenib, a selective inhibitor of mutated BRAF kinase, was FDA approved for usage in combination with Trametinib for the treatment of BRAF V600E NSCLC.[55,56] The combination therapy showed a response rate of 66% compared to a response rate of 33% on Dabrafenib only.[57]

PI3K/Akt/mTOR in lung cancer

Mechanism of action. The PI3K/Akt/mTOR pathway is also a potential target for NSCLC as it has been implicated in both tumorigenesis and disease progression. It is a family of intracellular lipid kinases which phosphorylate the 3′-hydroxyl group of phosphoinositides.[58]

The PI3K/AKT/mTOR pathway was shown to activate the upstream receptors (EGFR and PDGR). It is mutated in multiple cancers, such as breast cancer, gastric cancer, and NSCLC. It is suggested to be a predictor of poor prognosis for lung adenocarcinoma.[59]

Mutations. Loss of PTEN is the most common genetic alteration in the PI3K pathway in NSCLC.[60] PIK3CA mutations are found in about 4% of NSCLC tumors that express PTEN protein, which normally inhibits the PI3K/AKT/mTOR pathways.[61]

This mutation was also detected to be commonly concurrent with EGFR mutations. Patients with EGFR/PIK3CA had a shorter PFS than those with a single EGFR mutation. Specifically, loss of the PTEN gene (a protein product which functions downstream of the PI3K/AKT/mTOR pathway) has been shown to play an important role in Erlotinib and Gefitinib resistance in EGFR-mutated NSCLC.[60]

Therapies. There have been multiple attempts to target the PI3 K/AKT/mTOR pathways in NSCLC. LY294002, a PI3K,

inhibitor has been reported to potentiate the effect of chemotherapy and radiation on NSCLC.[62] Temsirolimus (CCI-770) is another mTOR inhibitor which targets molecules downstream of the PI3K pathways. It has been shown in phase I trials for NSCLC to have promising antitumor activity.[63] Other PI3 K/AKT/mTOR inhibitors such as Everolimus are also in preclinical stages.[29] However, no data on the clinical application of PI3K/Akt/mTOR inhibitors in lung cancer exist yet, and this challenging area is the future for researchers in lung cancer treatment.[64]

MET in NSCLC

Mechanism of action. The mesenchymal to epithelial transition (MET) factor, also called Hepatocyte Growth Factor Receptor (HGFR), is a proto-oncogene that regulates cell proliferation, apoptosis, and cell migration. Once HGF binds to MET, receptor dimerization occurs, and tyrosine residues are phosphorylated.[65] This chain reaction can increase the activity of different pathways such as MAPK, nuclear factor-κB PI3K/AKT, signal transducers, and transcription proteins. These pathways are crucial in the embryonic development of various structures, liver regeneration, and wound healing.[66]

Relevant mutations. Cancer cells have developed various processes to increase the activity of the MET proto-oncogene through altering its turnover, increasing its expression and the levels of its ligand (HGF), and decreasing its need for ligand activation. Among NSCLC patients, overexpression of MET happens in 35%–72%.[67] The most important mutations in the MET gene are the MET exon 14 skipping mutations and the MET amplification which comprise 3%–4% and 1%–6% of NSCLCs, respectively.[68] MET's exon 14 is responsible for coding the JM domain and Y1003 residue, which control MET's downregulation. They are the binding sites for casitas B-lineage lymphoma (CBL), an E3 ubiquitin ligase. This splicing error causes a decrease in the ubiquitination of MET and subsequently delays its degradation.[67]

Another significant MET mutation process is gene amplification, which occurs in 5%–22% of NSCLC patients who develop resistance to first-generation EGFR-TKIs (Erlotinib/Gefitinib).[66] By amplifying MET gene expression, the cancerous cells circumvent the blockade and can activate the PI3K/AKT pathway.[65] Hence, MET exon 14 skipping mutations and MET amplification became potential predictive biomarkers in NSCLC.

Therapies. MET inhibitors are divided into three types. The type I category (i.e. Crizotinib, Capmatinib, Tepotinib, and Savolitinib) comprises drugs that bind the ATP-pocket of the MET receptor in its active form, while the type II category drugs (i.e. Merestinib, Abozantinib, and Glesatinib) bind the same site but in the inactive state.[69] Type III inhibitors such as Tivantinib do not directly bind the ATP-binding site but alter the function of the RTK by binding to allosteric sites.[69] The majority of these drugs are currently being studied in clinical trials to determine their potential benefit in lung cancers, with many showing promising results. Capmatinib and Tepotinib received FDA approval for use in NSCLC patients with MET mutations.

The FDA has also recently approved Capmatinib for the treatment of NSCLCs with 14 exon-skipping MET mutations. The response rate in a recently published trial of 28 patients with NSCLC who had never received any other anticancer treatment was 68%, with a median duration of response of 12.6 months.[68] However, for the 69 NSCLC patients that had already undergone various treatments prior to taking Capmatinib, the response rate was 41% and the median duration of response was 9.7 months.[68] Moreover, in a cohort done by Wolf et al.,[68] Capmatinib has shown efficacy in tumors with higher levels of gene amplification, with limited activity seen in patients with MET-amplified NSCLC with a gene copy number of less than 10. Of its various side effects, Capmatinib mainly causes peripheral edema (in 51%) and nausea (in 45%).[68]

Tepotinib has also been approved for use in metastatic NSCLC patients with a 14 exon skipping MET mutation. In the VISION trial, 152 patients with advanced or metastatic NSCLCs positive for the MET 14 exon skipping mutation received Tepotinib. A 46% response rate was observed with a median response duration of 11.1 months.[70] New studies on the effect of Tepotinib in NSCLC with MET amplification have shown high and clinically meaningful activity, especially in patients who received Tepotinib in first line, where the ORR was 71%.[71] The most common side effect reported was peripheral edema (7%).[70]

FGFR in squamous cell carcinoma

Mechanism of action. FGFR is a tyrosine-kinase receptor that plays a crucial role in tumor development, maintenance, and signal transduction.[72] It was also found to be involved in several NSCLC cellular processes such as metabolism, survival, differentiation, and migration. FGFR also activates multiple signal transduction pathways, including Rat Sarcoma (RAS) kinase and mitogen-activated protein kinase (MAPK), which are involved in angiogenesis and inflammation.[73] FGFRs are encoded by genes on different chromosomes and are activated via the binding of their ligands, the FGFs. Once the ligand binds to the receptor, conformational changes of the receptor occur, leading to its dimerization and subsequent autophosphorylation of the tyrosine kinase intracellular domains, activating the downstream pathways.[74]

Relevant mutations. FGFR aberrations include mutations, fusions, and gene amplifications and are more frequently observed in lung squamous cell carcinomas (6.8%) than in lung adenocarcinomas (1.3%).[75] CGP of squamous cell lung carcinomas has shown that FGFR2 mutations are present in 3% of the cases, with the extracellular domain mutations

W290C and S320C and the kinase domain mutations K660E and K660N being the most involved.[74] However, in a previous CGP of primary lung adenocarcinomas, the FGFR family of receptors was among the highly dysregulated genes, with aberrations found in 19% of cases.[76]

Therapies. Several novel drugs targeting the FGFR pathway have been developed, including TKIs, selective inhibitors, and monoclonal antibodies such as Lenvatinib and Dovitinib that are approved by the FDA even though most of the drugs remain in preclinical tests or clinical research.[77] Recent data have questioned whether FGFR is a real target in NSCLC, mainly the squamous cell subtype. In addition, the management of specific side effects related to inhibition of the physiological role of FGF needs further investigation.[75] The novel FGFR1 inhibitor Ponatinib was studied on NSCLC cell lines expressing high levels of FGFR1, showing significant inhibition of the growth of primary lung cancer cultures in vitro.[78] Moreover, Erlotinib showed response when combined with Bevacizumab in NSCLC patients with increased expression levels of FGFR2/3.[79] A recent study showed that Erdafitinib induced apoptosis in FGF-dependent human squamous cell carcinoma NCI-H1581 and NCI-H520 cells via inhibition of FGF/FGFR.[80]

ALK in lung adenocarcinoma

Mechanism of action. The ALK (anaplastic lymphoma kinase) gene encodes an orphan tyrosine kinase thought to play a role in the development of the nervous system.[81] It is thought to regulate signaling pathways shared with other RTKs such as RAS-MAPK, PI3K-AKT, and JAK-STAT pathways.[81]

Relevant mutations. In ALK gene rearrangements, fusion of the intracellular tyrosine kinase domain of ALK to EML4 (echinoderm microtubule-associated protein-like 4) or other partner proteins leads to aberrant expression of ALK fusions in the cytoplasm. The partner protein domains promote dimerization and oligomerization of the fusion proteins, leading to constitutive activation of ALK kinase and its downstream signaling, resulting in uncontrolled cellular proliferation and survival.[82]

ALK gene rearrangement is seen in 3%–7% of patients with lung adenocarcinoma.[82] The most frequent gene mutation involves a small inversion within the short arm of chromosome 2, which fuses the N-terminal end of the gene with the C-terminal domain of ALK, leading to a constitutively active tyrosine kinase. EML4-ALK is the predominant ALK fusion in lung cancer.[82] Epidemiologically, patients with ALK rearrangement are relatively young and nonsmokers or light smokers.

Therapies. ALK-positive patients with NSCLC are therapeutically treated with first-generation (Crizotinib), second-generation (Ceritinib, Alectinib, Brigatinib) and third-generation (Lorlatinib) drugs.[83]

Crizotinib, a tyrosine kinase inhibitor, is considered a first-line therapy option in patients with ALK-positive NSCLC.[84] Alectinib, an ALK inhibitor, demonstrated efficacy and superiority in an independent review facility assessed PFS as compared to Crizotinib.[85]

Patients with ALK translocation often develop resistance to Crizotinib within 1 year of therapy. Resistance develops due to mutations that bypass track activation.[86]

To overcome this resistance, second-generation ALK inhibitors such as Alectinib and Ceritinib have been developed. They have shown high response rates in patients with Crizotinib resistance.[87] In addition, these are used in patients with leptomeningeal carcinomatosis as they have a higher intracranial activity.[83]

There are specific secondary mutations which lead to differential resistance to these inhibitors: some patients develop resistance to Alectinib because of an I1171T mutation. Ceritinib is, however, active against this mutation. Alternatively, Alectinib resistance occurs with G1202R mutations, which confers resistance to most ALK inhibitors except third-generation inhibitors such as Lorlatinib.[88,89]

This differential resistance and response highlight the need for CGP in patients with lung cancer to provide patients with a personalized and more effective approach to treatment.

PD-L1 in small-cell lung cancer

Mechanism of action. Programmed death ligand 1 (PD-L1), another cornerstone protein in NSCLC, is directly linked to immunotherapy. Also known as CD274, it can be expressed on the surface of various cells, including macrophages, APCs, lymphocytes, epithelial, muscle, and endothelial cells.[90] It is considered an immune checkpoint, facilitating anti-tumor suppression of the immune pathway. The expression of PD-L1 is mainly induced by interferon gamma (IFN-γ) released by activated CD8 + T-cells that predominantly express PD-1. PD-L1 and PD1-receptor complex inhibits the immune system through two mechanisms: inhibition of interleukin-2 (IL-2) synthesis and blockage of T-cell receptor that alters the duration of T cell contact with target cells.[90] Elevated levels of PD-L1 were detected on the cell surface of different types of cancer cells, mainly NSCLC, allowing the cancer cells to avoid the immune response.[91]

Relevant mutations. Holmes et al.[92] showed that 29.5% of NSCLC cases had high PD-L1 expression (⩾50%), 43.9% had low expression (1%–49%), and 26.5% had no expression (<1%). Among known EGFR/ALK negative cases, PD-L1 was found to be high, low, and negative in 34.7%, 43.2%, and 22.1% of cases, respectively. The prognostication of PD-L1 is still very debatable with contradictory data differing between stages of the disease. However, PD-L1 expression was a negative prognostic factor for PFS and OS after concurrent chemoradiotherapy in patients with locally advanced NSCLC.[93]

Therapies. Immunotherapy has targeted the PD-1/PD-L1 interaction to interfere with inhibitory signal transduction, allowing T cells to regain their vitality and restart the antitumor immune effect. Multiple clinical trials looked at the relationship between PD-L1 expression on tumor cells and the rate of response to PD-1/PD-L1 inhibitors. Besides the extent of the disease and histology, the level of PD-1 expression affects the choice of treatment in NSCLC that lacks a driver mutation. The high level of PD-L1 expression exhibits a potential benefit to anti-PD-1/PD-L1 treatment in NSCLC.[94] Three PD-1/PD-L1 monoclonal antibodies, Nivolumab, Pembrolizumab, and Atezolizumab, have been approved for different lines of treatment in different stages of NSCLC. KEYNOTE trials studied the effects of Pembrolizumab compared to other treatments in different stages and conditions in patients with NSCLC. These series of clinical trials have shown superior results with Pembrolizumab compared to other treatments. Patients with high PD-L1 expression were typically offered monotherapy with the anti-PD-1 antibody Pembrolizumab, whereas those with PD-L1 of less than 50% were treated with a combination of chemotherapy and Pembrolizumab. Moreover, Pembrolizumab was shown to improve OS in elderly patients diagnosed with PD-L1 positive, advanced NSCLC, and had a more favorable safety profile compared to chemotherapy.[95] Furthermore, the combination of new immune checkpoint inhibitors such as anti-CTLA-4 with anti-PD-1/PD-L1 inhibitors, such as Ipilimumab and Nivolumab, has shown promise in advanced NSCLC among PD-L1 positive patients when compared to platinum-based chemotherapy.[96]

RET in NSCLC

Mechanism of action. The RET gene, a proto-oncogene found on chromosome 10, is highly involved in embryonal development. It codes for a transmembrane tyrosine kinase that can activate the MAPK, PIK3/AKT, RAS/ERK, and c-Jun N-terminal kinase (JNK) pathways.[97] The ligands of the receptor do not directly bind to the RET protein. An intermediate in the form of a co-receptor is needed for the activation of RET. These co-receptors are the glial-derived neurotrophic factor ligands (GFLs) and the glial-derived neurotrophic factor family receptors (GFRα) which, upon binding together, form the GFLs-GFRα complex, promoting the autophosphorylation of the RTK.[97] This sequence of events activates the various pathways responsible for cellular proliferation, migration, and differentiation.[97]

Relevant mutations. In NSCLCs, RET fusion with other genes has been reported to be the main mutation, representing 2% of the cases.[98] Among the 12 different variants of RET fusion genes that have been described,[99] the most frequent variants involved the fusion of RET with the Kinesin Family 5B gene (KIF5B-RET) and the CCDC6 gene, respectively.[98] These fusions promote the dimerization of the RTK independently of ligand binding, leading to autophosphorylation and a constantly active RET protein, and subsequently uncontrolled proliferation and cell cycle deregulation. A higher brain metastasis risk has been observed with RET fusion mutation.[100]

Therapies. Selpercatinib is the only currently approved therapy for NSCLCs with RET fusion mutations. It selectively inhibits RET kinase by competing for the ATP binding site on the protein, crosses the blood-brain barrier, and is thus very effective in brain metastasis cases. A recent study has shown its effectiveness in NSCLC patients with RET fusion mutation who have undergone platinum-based chemotherapy with an objective response of 64% and a median duration of response of 17.5 months. Moreover, it was also shown to be effective in patients with RET-fusion positive NSCLCs that had never received any treatment, with an ORR of 85%. Among the 11 patients with brain metastasis in the study, 90% had an objective intracranial response to Selpercatinib. The most reported side effects were dry mouth (29%) and diarrhea (21%).[101]

Furthermore, Pralsetinib, a selective RET inhibitor, has been FDA approved in 2020 for use in metastatic NSCLC with RET fusion.[102] In the ongoing ARROW trial, the response rate to Pralsetinib in the previously treated patients was 57%, while it reached 70% in the treatment-naïve group.[102]

Other less selective anti-RET kinase drugs are being studied, such as Alectinib and Cabozantinib, but none of them has been approved yet by the FDA for use in NSCLCs.[101]

ROS1 fusion in lung adenocarcinoma

Mechanism of action. ROS1 is a type I integral membrane protein with a tyrosine kinase activity.

ROS1 fusion genes are present in approximately 1% of lung adenocarcinomas and are often seen in young patients and never-smokers.[103]

Relevant mutation. Gene rearrangements create fusion proteins of ROS1 with different partners, such as CD-74, which will constitutively activate the kinase domain.[103]

Therapy. Crizotinib, a small-molecule tyrosine kinase inhibitor of ALK and MET, was also found to be effective in patients with ROS1 fusions, with an ORR of 72%. The median PFS was found to be 19.2 months. Ceritinib, another tyrosine kinase receptor inhibitor, demonstrated potent clinical activity in a phase II study in patients with ROS1 rearranged NSCLC who were heavily treated with multiple lines of chemotherapy.[104]

NRTK1

Mechanism of action. The neurotrophic receptor tyrosine kinase 1 (NRTK1) gene encodes a tropomyosin-related

kinase A. The tropomyosin-related kinase (Trk) proteins are RTKs encoded by NTRK1, NTRK2, and NTRK3 and function during neuronal development.[105]

Relevant mutations. In all, 0.1% of patients with NSCLC had NTRK1 gene rearrangements. In patients without a known driver mutation (EGFR, KRAS mutations, ALK or ROS1 rearrangement), the frequency of NRTK1 rearrangements increases to 3%.[106] NRTK1 gene rearrangements result in expression of TrkA fusion proteins, leading to oncogenesis in NSCLC by ligand-independent dimerization and activation of the downstream pathway.

Therapy. Entrectinib is a pan-tyrosine kinase inhibitor that also acts against ALK and ROS1 fusion proteins. It has been tested in preclinical models and patients with the NTRK translocation and has shown some promising results: treatment with Entrectinib led to rapid and clinically significant improvement of disease, with minimal side effects. It also showed potent CNS penetration and activity in patients with CNS metastases.[107]

Larotrectinib is a first-in-class, highly selective TRK inhibitor that blocks the ATP binding site of the tyrosine kinase receptor. Larotrectinib was found to demonstrate marked antitumor activity in patients of all ages, across all tumor types and irrespective of the NTRK gene or fusion type. It was found to be highly effective and well-tolerated, leading to rapid clinical responses and improvement in the quality of life of patients with TRK fusion cancer.[108]

TMB

An additional parameter tested by CGP is the TMB. TMB reflects the rate of certain mutations in the DNA of cancer cells.[109] The FDA has recently approved the use of Pembrolizumab for cancer patients with TMB $\geqslant$ 10 mutations per megabase (mut/Mb) and whose cancer is unresectable or metastatic.[110] A multicenter trial comprising 102 cancer patients with TMB $\geqslant$ 10 Mut/Mb showed an ORR of 29%.[110] Patients with a high TMB ($\geqslant$10 mut/Mb) had a higher ORR to immunotherapies and a longer PFS than those with a low TMB.[109]

Novice markers

Multiple potential biomarkers are still under research and studies for their role and significance in lung cancer, holding the potential to be used clinically in the future. Heat Shock protein 90 beta, an isoform of HSP90, is a potential biomarker of NSCLC that affects the cell cycle and apoptosis.[111] In addition, Killer Cell Lectin Like Receptor G1 (KLRG1) is a potential marker that is suspected to play a vital role in the proliferation and differentiation of lung cancer. The core-binding factor (CBFA2T3) is also involved in proliferation, which together with KLRG1 are being currently studied for the development of mRNA vaccines in lung adenocarcinoma.[111]

Discussion

There are many barriers that are hindering the integration of CGP universally when caring for cancer patients. These entail valid concerns from both physician and patient perspectives that must be recognized and addressed to allow widespread use.

One of the significant barriers preventing physicians from including genetic sequencing in their patient care is the minimal clinical evidence available to defend it. Even in the presence of promising data, many limitations, including nonrandomization and small sample sizes, made it difficult for physicians to recommend its use.[112,113] Another concern for physicians is the limited number of known actionable mutations and hence limited evidence on potential off-label prescriptions due to diverse cancer genomics.[113] Moreover, most physicians find the interpretation of genomics challenging, adding more uncertainty to the previous points mentioned.[114] In addition, even if physicians endorse the use of these therapies, the publication of the SHIVA study showing no improvement in survival when using these targeted drugs off-label in pretreated cancer patients discourages their use as compared to the typical conventional treatments that physicians have been prescribing for years.[115] However, the SHIVA study has major limitations, including the fact that they used monotherapies for advanced tumors with multiple molecular alterations, which could partly explain study results, which contradict other ongoing trials.[116] There is also hesitancy regarding prescribing anticancer drugs off-label, especially if accompanied by a lack of evidence regarding their efficacy.[113]

Economic factors play an important role in adopting genetic testing, particularly with patients paying out-of-pocket, even if the cost has relatively decreased over the last few years. Even for patients with insurance, the lack of studies proving the clinical utility makes reimbursement less likely,[117,118].[119] This makes it more difficult for the physician to include genetic testing in patient care and for the patient to accept its cost. However, these concerns must be questioned as a study published in 2017 showed that the use of genomic testing and matched targeted therapy was not associated with increased costs as compared with conventional treatment.[120] A second recent study showed that although matched therapy was associated with increased incremental cost, this was largely due to the longer duration of therapy rather than a higher monthly cost, and earlier use of CGP and initiation of matched therapy showed not only better OS but also a smaller increase in costs.[121]

Barriers to the use of genetic testing also include factors from the patient's perspective. One important consideration is the reluctancy to participate in clinical trials. The number of cancer patients enrolled in clinical trials is low, which further limits the feasibility of acquiring higher evidence regarding the use of targeted therapies.[122] Even with a potential increase in trial enrollment, patient recruitment would still be limited due to the lack of availability of local clinical

trials.[123] A study at MD Anderson looking into cancer patients' attitudes toward clinical trials showed that 17% of patients who were willing to travel to distant places to undergo genetic testing for enrollment in a trial ended up not returning, and 13% refused from the start and preferred to be treated in a facility closer to their homes, hence the importance of making trials geographically available.[124] Another important aspect is the patient's knowledge regarding personalized or precision medicine. A survey done in the United States in 2015 showed that 73% of the adults in that population were not familiar with these terms.[125] Interestingly, after explaining precision medicine to them, a positive change was noted in their perception of cancer genomics.

Conclusion

CGP offers significant clinical potential in treating lung cancer despite various barriers to its use. It allows physicians to provide a more personalized approach and predict the response to treatment based on the different mutations detected. Molecular pathology allows us to shift from a traditional approach to a more patient-tailored approach in personalized oncology.

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