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Lung Cancer Biomarkers

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Synopsis

The molecular characterization of lung cancer has considerably changed the classification and treatment of these tumors, becoming an essential component of pathologic diagnosis and oncologic therapy decisions. Through the recognition of novel biomarkers, such as epidermal growth factor receptor mutations and anaplastic lymphoma kinase translocations, it has been possible to identify subsets of patients who benefit from targeted molecular therapies. The success of targeted anticancer therapies and new immunotherapy approaches has created a new paradigm of personalized therapy and has also led to accelerated development of new drugs for lung cancer treatment. This review focuses on clinically relevant cancer biomarkers as targets for therapy, as well as potential new targets for drug development.

Keywords

lung cancer; genotyping; biomarkers; molecular targets

1. Introduction

Lung cancer has shown a decrease in incidence and mortality in recent decades; however, it remains one of the cancers with the highest incidence and ranks first in cancer-related deaths in the United States (1). An estimated 221,200 new cases and 158,040 deaths are expected to occur in 2015, representing approximately 13% of all cancers diagnosed and 27% of all cancer deaths (2). Despite advances in early detection and standard treatment, most patients are diagnosed at an advanced stage and have a poor prognosis, with an overall 5-year

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survival rate of 10% to 15% (3). Lung cancer is a heterogeneous disease comprising several subtypes with pathologic and clinical relevance (4). The recognition of histologic subtypes of non-small cell lung carcinoma (NSCLC), namely adenocarcinoma, squamous cell carcinoma, and large cell lung carcinoma as the most frequent subtypes, has become important as a determinant of therapy in this disease (5). In addition, in recent years, the identification of molecular abnormalities in a large proportion of patients with lung cancer has allowed the emergence of personalized targeted therapies and has opened new horizons and created new expectations for these patients (6). The use of predictive biomarkers to identify tumors that could respond to targeted therapies has meant a change in the paradigm of lung cancer diagnosis (5).

This paradigm change affects all stakeholders in the fight against lung cancer including pathologists. Currently, several multiplex genotyping platforms for the detection of oncogene mutations, gene amplifications, and rearrangement are moving to the clinical setting. Genome-wide molecular investigations using next-generation sequencing (NGS) technologies have been evaluated in the research setting, with promising results. Further investigations in NSCLC are required for a better understanding of the implications of intratumor heterogeneity and the roles of tumor suppressor genes and epigenetic events with no known driver mutations. NGS in the clinical setting will provide comprehensive information cheaper and faster by using small amounts of tissue. Pathologists should be able to precisely handle tissue adequacy in terms of quantity and quality and maintaining tumor cells for detection of molecular alterations. The recent clinical successes of immunotherapy approaches to lung cancer have posed additional challenges to the scientific community and pathologists to develop predictive biomarkers of response to these therapies and have highlighted the need for proper procurement and processing of tissue specimens from patients with lung cancer.

In this review, we will focus on the major predictive biomarkers in NSCLC, with special emphasis on their clinical and molecular importance, as well as the current status of molecular testing for these biomarkers.

2. Histologic Subtyping of NSCLC

The advent of molecular profiling and targeted therapy has renewed interest in the classification of NSCLC into major subtypes such as adenocarcinoma, squamous cell carcinoma, and large cell lung carcinoma (7). Other subtypes, including sarcomatoid carcinoma, neuroendocrine large cell carcinoma, and others, represent a very minor proportion of the total NSCLC cases (7). The most recent histologic classification of lung cancer published by the World Health Organization in 2015 incorporates relevant genetics and immunohistochemistry (IHC) aspects of different tumor subtypes (Figure 1) (7). Lung cancers are increasingly diagnosed and staged by transthoracic core needle biopsy and fine-needle aspiration (FNA), transbronchial needle aspiration, endobronchial ultrasound-guided transbronchial needle aspiration, and endoscopic ultrasound-guided FNA. It is well established that poorly differentiated adenocarcinoma and squamous cell carcinoma of the lung can appear indistinguishable by routine microscopy, particularly in small biopsy and cytology specimens. In these small specimens, particularly in poorly differentiated tumors,

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we need to integrate morphology with IHC analysis to make a precise diagnosis. This includes the examination of IHC expression of thyroid transcription factor (TTF1) and the novel aspartic proteinase of the pepsin family A (napsin A) for adenocarcinoma and p40 and cytokeratin 5/6 for squamous cell carcinoma (8). In addition, histochemical staining of mucin is useful for the diagnosis of adenocarcinoma histology. The correct histologic diagnosis of these specimens is important, but it is also imperative to exercise judicial use of the tissue to maximize the yield for molecular testing (Table 1).

3. Genomic Biomarkers in NSCLC

Advances in elucidating the molecular biology of lung cancer have led to the identification of a number of potential biomarkers that could be relevant in the clinical management of patients with NSCLC.

3.1. Epidermal growth factor receptor (*EGFR*)

The epidermal growth factor receptor (EGFR) is a tyrosine kinase receptor member of the ERBB family. The *EGFR* gene is located on the short arm of chromosome 7 at position 12 (9). When the extracellular ligand binds to EGFR, it generates homo- or heterodimerization of the receptor, leading to phosphorylation of sites in the cytoplasmic tyrosine kinase and activation of various intracellular pathways, including the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) and RAS/RAF/mitogen-activated protein kinase (MAPK) pathways, which lead to cell proliferation, metastasis, and prevention of apoptosis (10). EGFR is overexpressed in 62% of NSCLCs, and its expression has been associated with poor prognosis (11). Approximately 10% of patients with adenocarcinoma of the lung in the United States and 30% to 50% in East Asia have lung tumors associated with *EGFR* mutations (11). These mutations occur within exons 18-21, which encode for a portion of the EGFR kinase domain (10). Approximately 90% of *EGFR* mutations occur as in-frame deletions in exon 19 or as missense mutations in exon 21 (44% and 41% of all mutations, respectively) (4). Activating mutations in the kinase domain of EGFR trigger ligand-independent tyrosine kinase activation, leading to hyperactivation of downstream antiapoptotic signaling pathways (10). *EGFR* mutations are found more often in adenocarcinomas with lepidic features from female never smokers (10). The high response rates (55%-78%) to treatment with tyrosine kinase inhibitors (TKIs), such as gefitinib, erlotinib and afatinib, in patients with *EGFR*-mutant tumors, and the significantly greater progression-free survival (PFS) of these patients, have made EGFR TKIs the standard treatment for patients with these mutations (6). However, most of these patients develop resistance and relapse in a short time, owing to the occurrence of a new mutation (T790M) in exon 20 of the EGFR kinase domain (50%), amplification of the *MET* oncogene (21%), or mutations of *PI3KCA* (4).

EGFR mutations are identified mostly with the use of gene sequencing methodologies and real-time polymerase chain reaction (PCR)-based assays. Both methods have been reported to have high performance and sensitivity in the detection of these mutations in formalin-fixed and paraffin-embedded tissues (4). Detection of *EGFR* mutations by an IHC-based

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approach with specific antibodies against mutant proteins has been attempted but showed variable sensitivity and significant variability between studies (12).

3.2. Anaplastic lymphoma kinase (*ALK*)

Anaplastic lymphoma kinase (ALK) is a tyrosine kinase receptor member of the insulin receptor superfamily. The *ALK* gene is located on the short arm of chromosome 2 at position 23 (13). *ALK* gene rearrangement was originally identified in anaplastic large cell lymphoma (14) and was subsequently described in a subset of NSCLC tumors harboring a fusion of *ALK* and echinoderm microtubule-associated protein-like 4 (*EML4*) genes (4). This rearrangement encodes for a chimeric protein with constitutive kinase activity, which promotes malignant growth and proliferation (14). The *EML4-ALK* fusion has been detected in 3.7% to 7% of NSCLCs (10, 14), usually in adenocarcinomas with signet-ring cells or cribriform histology features, and is more common in young patients who have never smoked (14). There are several *EML4-ALK* rearrangement variants and also *ALK* fusion with other less frequent partners, such as kinesin family member 5B (*KIF5B*), TRK-fused gene (*TFG*), kinesin light chain 1 (*KLC1*), and huntingtin-interacting protein 1 (*HIP1*) genes, resulting in oncogenic transformation (13, 15). It has been shown that *EGFR*, Kirsten rat sarcoma viral oncogene homolog gene (*KRAS*), and *ALK* molecular alterations are mutually exclusive events (4); nevertheless, they have been described in up to 2.7% of lung adenocarcinoma cases with concurrent molecular alterations (16). The *ALK* fusion defines a distinct subpopulation of patients with lung adenocarcinoma who are highly responsive (57%-74%) to ALK inhibitors such as crizotinib. Patients treated with crizotinib demonstrated significantly better median PFS and response rate compared to patients who received chemotherapy (17). As a result, testing for *ALK* rearrangements in patients with advanced lung adenocarcinoma is recommended in current clinical practice guidelines (5, 10). However, despite initial responses, a fraction of the patients develop acquired resistance to crizotinib, owing to secondary mutations within the kinase domain of EML4-ALK; these include L1196M, C1156Y, and F1174L, among others (18, 19). Several second-generation ALK inhibitors that target ALK-positive NSCLC, such as alectinib, ceritinib, and AP26133, have been developed and are currently under evaluation in clinical trials (20).

Current diagnostic approaches to detect *ALK* fusion genes and their results include break-apart fluorescence in situ hybridization (FISH), IHC, and reverse-transcription PCR (RT-PCR) (21). Break-apart FISH has been established in clinical trials as the standard method for confirmation of *ALK* status (22). FISH and IHC have shown high concordance in several reports, especially with the development of IHC antibodies (clones 5A4 and D5F3) with better sensitivities and specificities (83%-100%) for the detection of ALK rearrangement (23, 24). As a result, IHC detection of the ALK protein is being considered as an adequate screening tool to test NSCLC samples for ALK rearrangements or as a tool to evaluate cases that are not interpretable by FISH (25, 26). Nevertheless, other studies reveal major discordances, suggesting the need to combine both tests to optimize detection (22). Other methods, such as RT-PCR and NGS, are in use but have not been examined systematically compared to FISH as a predictor of response to ALK inhibitors (27, 28).

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3.3. Kirsten rat sarcoma viral oncogene homolog (*KRAS*)

KRAS is an oncogene located on the long arm of chromosome 12 at position 12.1 (29). It is a member of the RAS family of membrane-associated G proteins and encodes for a protein with intrinsic GTPase activity, which is involved in a variety of cellular responses including proliferation, cytoskeletal reorganization, and survival (30). KRAS acts downstream of a number of tyrosine kinases receptors, including EGFR, and is associated with activation of the RAS/RAF/MAP kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) and RAS/MAPK signaling pathways (10). *KRAS* mutations occur in 25% to 35% of patients with NSCLC, principally adenocarcinomas with a solid pattern (31), and are found more often in white patients compared to Asians, in former or current smokers, but without sex predilection (32). Mutations in the form of single-nucleotide missense variants are found in codons 12 and 13 in approximately 95% of cases (4, 32). In never smokers, the most common *KRAS* mutations are G12D and G12V, whereas G12C is the most common mutation associated with smoking (31, 32). The presence of *KRAS* mutation may be associated with unfavorable outcome (33) and could be a negative predictor of responsiveness to chemotherapy (34). In addition, it is associated with an increased likelihood of having a second primary tumor (35) and is a predictor of resistance to targeted therapy with EGFR-TKIs, such as gefitinib or erlotinib, in patients with NSCLC (36).

Because *KRAS*, *EGFR*, and *ALK* molecular alterations are mutually exclusive, it has been suggested that *KRAS* testing could be a surrogate assay to exclude *EGFR*- and *ALK*-positive cases (10); however, this approach is not currently recommended. Although there are no targeted therapies approved for patients with lung cancer and *KRAS* mutation, several clinical trials aimed at downstream signaling targets are under way. Different phase 2 trials have reported improvements in both PFS and response rate with the combination of selumetinib (MEK1/MEK2 inhibitor) and docetaxel compared to docetaxel alone (37) and promising results with sorafenib (RAS/RAF pathway inhibitor), with a disease control rate of approximately 50% (38). Conversely, trametinib (MEK1/MEK2 inhibitor) did not show advantages over docetaxel in patients with NSCLC (39).

3.4. ROS proto-oncogene 1, receptor tyrosine kinase (*ROS1*)

ROS proto-oncogene 1, receptor tyrosine kinase (ROS1) is a tyrosine kinase receptor member of the insulin receptor family and is located on the long arm of chromosome 6 at position 22. ROS1 plays a role in epithelial cell differentiation during the development of a variety of organs, but no ligand for this receptor has been identified (40). *ROS1* rearrangements were originally described in glioblastoma and have also been reported in cholangiocarcinoma and ovarian cancer (41). Approximately 1% to 2% of NSCLCs harbor *ROS1* rearrangements (41), and several fusion partners, including *CD74*, solute carrier family 34, member 2 (*SLC34A2*), leucine-rich repeats and immunoglobulin-like domains 3 (*LRIG3*), ezrin (*EZR*), syndecan 4 (*SDC4*), tropomyosin 3 (*TPM3*), and *FIG*, have been reported in these tumors. All of these fusions result in a chimeric protein that has been reported to be oncogenic (40, 41). *ROS1*-rearranged NSCLC typically occurs in young, female, never smokers with a histologic diagnosis of adenocarcinoma (40, 41) and is usually mutually exclusive with other oncogenic drivers (*EGFR*, *KRAS, ALK*) (41). Clinical trials have reported that patients with advanced NSCLC harboring *ROS1* rearrangement have

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benefited from crizotinib treatment, showing response rates up to 80% (40, 42). Ongoing phase 1 and 2 studies are investigating the activity of crizotinib and ceritinib (ALK inhibitor) in *ROS1*-rearranged NSCLC (43, 44). ROS1 testing is indispensable for identifying patients who could benefit from crizotinib treatment. The National Comprehensive Cancer Network 2014 guidelines recommend that all patients with advanced triple-negative (*EGFR*, *ALK*, and *KRAS*) lung adenocarcinoma be tested for other molecular markers including *ROS1* (5).

There is not a gold standard method, but currently available diagnostic methods include FISH, RT-PCR, and IHC (45). FISH is the only method approved by the US Food and Drug Administration (FDA) to detect *ALK*-rearranged NSCLC and has been used in clinical trials as the standard method for confirmation of *ROS1* rearrangement; nevertheless, it is an expensive and laborious technique. Because *ROS1*-rearranged lung cancer is rare, assessment of ROS1 protein expression by IHC may be used as a screening tool for the identification of candidates suitable for ROS1-targeted therapy. In fact, studies have found that ROS1 IHC (D4D6 clone) has a high sensitivity (100%) and specificity (92%-97%) for ROS1 rearrangements compared to FISH (45).

3.5. Human epidermal growth factor receptor 2 (*HER2*)

The human epidermal growth factor receptor 2 gene *HER2* (*ERBB2*) is a proto-oncogene located on chromosome 17 at position 12 (46). It encodes for a tyrosine kinase receptor member of the ERBB receptor family (47). HER2 lacks a specific ligand. Nevertheless, it can be combined with other ERBB receptors to form a heterodimer (48). This allows for the activation of important signal transduction pathways, including the MAPK and PI3K pathways, involved in cell proliferation, differentiation, and migration (47). HER2 expression and/or amplification is found in many cancers including breast and gastric cancer (48). Overexpression of HER2 has been reported in 7% to 34.9% of NSCLCs and has been associated with poor prognosis in patients with these tumors (47). Activating mutations of *HER2* have been found in 1.6% to 4% of lung cancers (47, 49). These mutations occur in the 4 exons of the tyrosine kinase domain (exons 18-21) and are found more often in adenocarcinomas in female, Asian, never or light smokers. *HER2* mutations are almost always mutually exclusive with other driver oncogene alterations in lung cancer described above (49). Different studies reinforce the importance of screening lung adenocarcinomas for *HER2* mutation as a method to select patients who could benefit from *HER2*-targeted therapies (afatinib and trastuzumab), which have shown response rates of approximately 50% (50). Several clinical trials of targeted agents, such as trastuzumab, neratinib and pyrotinib, among others, are being conducted in patients with *HER2* mutation (47). HER2 mutations are usually assessed via sequencing approaches.

3.6. *RET* proto-oncogene

The *RET* proto-oncogene is located on the long arm of chromosome 10 at position 11.2. It encodes for a tyrosine kinase receptor for the glial cell line–derived neurotrophic factor family of ligands and is involved in cell proliferation, migration, and differentiation, as well as neuronal navigation (51). *RET* chromosomal rearrangements were originally described in papillary thyroid carcinoma (51). Approximately 1% to 2% of NSCLCs harbor *RET* fusions, and several fusion partners, including kinesin family member 5B (*KIF5B*) (90%), coiled-coil

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domain containing 6 (*CCDC6*), nuclear receptor coactivator 4 (*NCOA4*), and tripartite motif-containing 33 (*TRIM33*), have been described (52, 53). *RET*-rearranged NSCLC typically occurs in adenocarcinomas with more poorly differentiated solid features in young never smokers, and it is mutually exclusive with known driver oncogenes (52, 54). In vitro studies showed that *RET* fusions lead to oncogenic transformation, which can be inhibited by multitargeted kinase inhibitors such as vandetanib, sorafenib, and sunitinib (54). Preliminary studies with cabozantinib (MET and vascular endothelial growth factor receptor 2 inhibitor) in *RET*-rearranged lung adenocarcinoma are promising (53).

FISH is currently the standard diagnostic assay for detection of *RET* chromosomal rearrangements. RT-PCR is usually insufficient for the detection of new partners or isoforms, and *RET* IHC has shown low sensitivity and specificity for *RET* rearrangements (52, 54). Sequencing approaches, including NGS methodologies, are also frequently used to detect *RET* translocations.

3.7. MET proto-oncogene

The *MET* gene is located on the long arm of chromosome 7 at position 31 (55). This oncogene encodes for a tyrosine kinase receptor (hepatocyte growth factor receptor), which activates multiple signaling pathways that play fundamental roles in cell proliferation, survival, motility, and invasion (4). Pathologic activation of *MET* includes mutation, gene amplification, and protein overexpression (56). MET alterations were first reported in patients with renal papillary carcinoma and mutations in the MET kinase domain leading to constitutive activation of the receptor (57). In lung cancer, *MET* mutations are found in the extracellular semaphorin and juxtamembrane domains, occurring in 3% of squamous cell lung cancers and 8% of lung adenocarcinomas (56). *MET* amplifications are found in 4% of lung adenocarcinomas and 1% of squamous cell lung cancers and are associated with sensitivity to MET inhibitors (56). In NSCLC, MET and hepatocyte growth factor protein expression, along with high *MET* gene copy number, have been described as poor prognosis factors (58, 59). Activating point mutations affecting splice sites of exon 14 of the *MET* gene (*MET*ex14), which occur in 4% of lung adenocarcinomas, represent a possible oncogenic driver and identify a subset of patients who may benefit from MET inhibitors such as capmatinib and crizotinib (56). This novel alteration is usually assayed by NGS methodology.

3.8. B-RAF proto-oncogene, serine/threonine kinase (*BRAF*)

The B-RAF proto-oncogene, serine/threonine kinase (*BRAF*) oncogene is located on the long arm of chromosome 7 at position 34. It encodes for a serine/threonine kinase, which is involved in the RAS/RAF/MEK/ERK signaling pathway (60). When activated by oncogenic mutations, BRAF phosphorylates MEK and promotes cell growth, proliferation, and survival (60). The highest incidence of *BRAF* mutation is in malignant melanoma (27%-70%), followed by papillary thyroid cancer, colorectal cancer, and serous ovarian cancer (61). *BRAF* mutations have also been reported in 1% to 3% of NSCLCs (60). In contrast to melanoma, only half of *BRAF* mutations in NSCLC are V600E mutations. Other non-V600E mutations reported in NSCLC include G469A (~35%) and D594G (~10%). All *BRAF* mutations are mutually exclusive with other driver alterations such as those of *EGFR,*

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KRAS, and *ALK* (60, 62). *BRAF*-mutated NSCLC has been reported to be mostly adenocarcinoma, and in contrast to patients with *EGFR* mutations or *ALK* rearrangements who are mostly never smokers, patients with *BRAF* mutations are mostly current or former smokers (62). Nevertheless, patients with NSCLC and *BRAF* V600E mutations have a worse prognosis and lower response to platinum-based chemotherapy than patients with wild-type *BRAF*. These patients have benefited from treatment with BRAF and MEK inhibitors (63). BRAF inhibitors, such as vemurafenib and dabrafenib, have high and selective activity against the V600E-mutant BRAF kinase, with overall responses rates from 33% to 42% (63, 64). BRAF and MEK inhibitors targeting *BRAF* mutation–positive NSCLC, such as trametinib, selumetinib, and dasatinib, among others, are currently under evaluation in clinical trials.

3.9. Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*)

PI3Ks are heterodimeric lipid kinases composed of catalytic and regulatory subunits and are part of several downstream pathways involved in cell growth, transformation, adhesion, apoptosis, survival, and motility (65). The *PIK3CA* gene is located on the long arm of chromosome 3 at position 26.3. It encodes for the catalytic subunit p110 alpha of P13Ks (66). *PKI3CA* amplifications, deletions, and somatic missense mutations have been reported in many tumors including lung cancers. In fact, *PIK3CA* is one of the most commonly mutated oncogenes, along with *KRAS*, in human cancers (67). Mutations are found in 1% to 4% of patients with NSCLC, usually affecting exons 9 and 20 (80%) (4, 65, 67-69). These mutations are not mutually exclusive with other driver alterations and have been reported more frequently in lung squamous cell carcinoma compared to adenocarcinoma (6.5% vs 1.5%) (69). However, *PIK3CA* mutations have not shown association with any clinicopathologic features (65, 68, 69). Squamous cell carcinomas with *PIK3CA* gains are not accompanied by other genetic alterations, suggesting that this gene may play a important role in the pathogenesis of squamous cell cancers (65). Studies have shown that *PIK3CA* mutations in *EGFR*-mutated lung cancer confer resistance to EGFRTKIs and are a negative prognostic predictor in patients with NSCLC treated with EGFR-TKIs (70). *PI3KCA* alterations and their downstream effectors, such as phosphatase and tensin homolog (PTEN), mTOR, and AKT, are potential therapeutic targets for NSCLC therapy and are being evaluated in clinical trials for lung cancer (71). Alterations in *PI3KCA* are detected using sequencing approaches, mostly NGS assays.

3.10. Neurotrophic receptor tyrosine kinase 1(*NTRK1*)

The neurotrophic receptor tyrosine kinase 1 (*NTRK1*) proto-oncogene is located on chromosome 1q21-22 and encodes for a receptor tyrosine kinase, also known as tropomyosin-related kinase (TRK) A, belonging to the TRK superfamily of receptor tyrosine kinases (117). *NTRK1* is involved in the regulation of cell growth and differentiation via activation of several signal transduction pathways including MAPK, PI3K, and phospholipase C-gamma (72). *NTRK1* rearrangements have been found in colon cancer, thyroid cancer, and glioblastoma multiforme (72). In lung cancer, approximately 3% of adenocarcinomas harbor *NTRK1* fusions, and some fusion partners, including myosin phosphatase RHO-interacting protein (*MPRIP*)-*NTRK1* and *CD74-NTRK1*, have been reported (73). All of these fusions result in constitutive TRKA kinase activity, which has

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been reported to be oncogenic (73). In early phase 1 studies, NTRK inhibitors, such as entrectinib and LOXO-101, have shown promising results in patients with solid tumors harboring NTRK fusions (74).

3.11. Fibroblast growth factor receptor (*FGFR*)

The fibroblast growth factor receptor (*FGFR*) gene is located on chromosome 8 at position 12 and encodes for a tyrosine kinase receptor belonging to the FGFR family. The FGFR family includes 4 receptor tyrosine kinases (FGFRs 1-4). When ligand-receptor binding occurs, FGFR dimerizes and phosphorylates FGFR substrate 2-alpha (FRS2α), leading to activation of different pathways, including the RAS/MAPK and PI3K/AKT/mTOR pathways, promoting cell survival, motility, invasiveness, and proliferation (75, 76). In cancer, *FGFR* gene amplifications, somatic missense mutations, and chromosomal translocations are the most frequent mechanisms of activation (76). *FGFR* has been identified as an oncogenic driver in breast, gastric, endometrial, urothelial, and brain tumors, among others (76). In lung cancer, the incidence of *FGFR1* amplification is significantly higher in squamous cell carcinoma (20%) compared to adenocarcinoma (3%) and is more frequent in current smokers compared to former and never smokers. Other specific clinic-demographic features also correlate with *FGFR1* amplification (75, 77). Some studies have recognized *FGFR* amplification as an independent negative prognostic factor in patients with NSCLC (78), whereas other studies have shown the opposite (75). In addition, *FGFR* amplifications may be found in concurrence with other tumor genetic alterations including *TP53* and *PIK3CA* mutation and platelet-derived growth factor receptor A (*PDGFRA*) amplification (77). Somatic *FGFR* mutations in lung tumors usually occur in *FGFR2* and *FGFR3* and have been detected in 6% of lung squamous cell carcinomas (79). Multiple FGFR inhibitors, such as ponatinib, a multitargeted kinase inhibitor that displays potent pan–anti-FGFR activity, are in development, with promising results in cell lines and xenograft models (80). Phase 1 and 2 clinical trials of FGFR inhibitors (dovitinib, nintedanib, ponatinib, and AZD4547, among others) are ongoing in patients with NSCLC (81). *FGFR* gene copy number is usually assayed by FISH; however, members of this family are frequently part of NGS testing panels.

3.12. Discoidin domain receptor tyrosine kinase 2 (*DDR2*)

The discoidin domain receptor tyrosine kinase 2 gene (*DDR2*) is located on the long arm of chromosome 1 at position 23.3 and encodes for a tyrosine kinase receptor that is expressed in mesenchymal tissues and which binds fibrillar collagen as ligand. DDR2 activates important signaling pathways including SRC, SRC homology domain-containing (SHC), Janus kinase (JAK), ERK1/2, and PI3K and promotes cell migration, proliferation, and survival (82). In cancer, *DDR2* mutations have been reported in melanoma as well as uterine, gastric, bladder, and colorectal cancers (83). In lung cancer, *DDR2* mutations occur in 3% to 4% of lung squamous cell carcinomas (84) compared to 0.5% of adenocarcinomas (85) and are only present in smokers (86). No other significant association with clinicopathologic status has been found (84). At least 11 different *DDR2* mutations have been identified (84) distributed throughout the gene and include the extracellular-binding discoidin domain and the cytoplasmic kinase domain (82, 84). *DDR2* mutations have been associated with response to dasatinib (a multitargeted kinase inhibitor) in preclinical models

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and early phase clinical trials. Phase 2 clinical trials of dasatinib in patients with lung squamous cell carcinoma are under way (82, 84).

4. Immunotherapy Markers in Lung Cancer

Historically, lung cancer has not been considered very immunogenic because of several failed attempts with cytokines and vaccines. Nevertheless, over the past few years, immunotherapy has re-emerged strongly with the development of checkpoint inhibitors as treatments for NSCLC. Immune checkpoints are inhibitory pathways with the functions of maintaining self-tolerance and modulating immune responses (87). Immune checkpoint proteins that have been studied more comprehensively in many types of cancer, including lung cancer, are cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and the programmed death-ligand 1 receptor (PD-1), which are expressed mainly on T cells, and programmed death-ligand 1 (PD-L1), which is expressed on tumor cells and tumor inflammatory infiltrate including macrophages, dendritic cells, and T cells (88). Many other checkpoint molecules, such as T-cell immunoglobulin and mucin domain-containing protein 3 (TIM3), B- and T-lymphocyte-associated protein (BTLA), V-domain Ig suppressor of T-cell activation (VISTA), and lymphocyte-activation gene 3 (LAG3), have been identified and are currently being evaluated as potential targets for cancer immunotherapy (89).

4.1. Cytotoxic T-lymphocyte-associated antigen 4 (*CTLA-4*)

Monoclonal antibodies that inhibit CTLA-4, such as ipilimumab, are available to prevent the binding of CTLA-4 with its ligands (CD80/CD86), leading to reactivation of the antitumor inmune response mediated by specific T cells (88). A phase 2 study of ipilimumab in combination with chemotherapy in patients with advanced NSCLC showed very promising results, with a significant improvement in PFS versus a control group treated with chemotherapy alone (90). A phase 3 trial of ipilimumab in combination with chemotherapy in patients with squamous histology NSCLC is ongoing (91). Currently, there is no biomarker to predict response to CTLA-4 therapy.

4.2. Programmed death-ligand 1 receptor (*PD-1*)

Several monoclonal antibodies targeting the interaction between PD-1 and its ligands PD-L1 and PD-L2 are available. There are different ways to block the PD-1 pathway; one is to use antibodies directed against PD-1 or by blocking its ligand PD-L1 (92). Clinical trials in NSCLC have shown sustained responses in approximately 20% of unselected patients to treatment with monoclonal antibodies against PD-1, such as nivolumab and pembrolizumab, and with antibodies against PD-L1 such as MPDL3280A. The FDA has approved the use of nivolumab in advanced NSCLC on or after platinum-based chemotherapy and pembrolizumab as second-line treatment for NSCLC after chemotherapy (93, 94). A recent study has reported that greater nonsynonymous mutation burden is associated with improved objective response, durable clinical benefit, and PFS in patients with NSCLC treated with pembrolizumab (95). Furthermore, IHC PD-L1 positivity in NSCLC has been identified as a potential predictor of response to anti– PD-1 and anti–PD-L1 monoclonal antibody therapy (96) and also as a prognostic biomarker (97). Other studies reported that PD-L1 overexpression cannot be currently considered a robust predictive biomarker for response to

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immunotherapy or a prognostic biomarker (98). These discrepancies may be due to assay variability and interpretive subjectivity differences for the evaluation of PD-L1 expression, including differences in detection methods, IHC antibody clones, and cut-off values for determining PD-L1 positivity, and heterogeneity in PD-L1 expression and site of PD-L1 expression (tumor cells and tumor inmune cells) (99, 100). Further studies are needed to compare different assays and to clarify and standardize testing protocols to confirm the suitability of PD-L1 expression as a biomarker.

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Key points

- The molecular characterization of lung cancer has changed the classification and treatment of these tumors, becoming an essential component of pathologic diagnosis and therapy decisions.
- The success of targeted therapies and new immunotherapy approaches has created a new paradigm of personalized therapy in lung cancer.
- Pathologists should be able to precisely handle tissue adequacy in terms of quantity and quality and maintaining tumor cells for detection of molecular alterations.
- This review focuses on clinically relevant cancer biomarkers as targets for therapy, as well as potential new targets for drug development.

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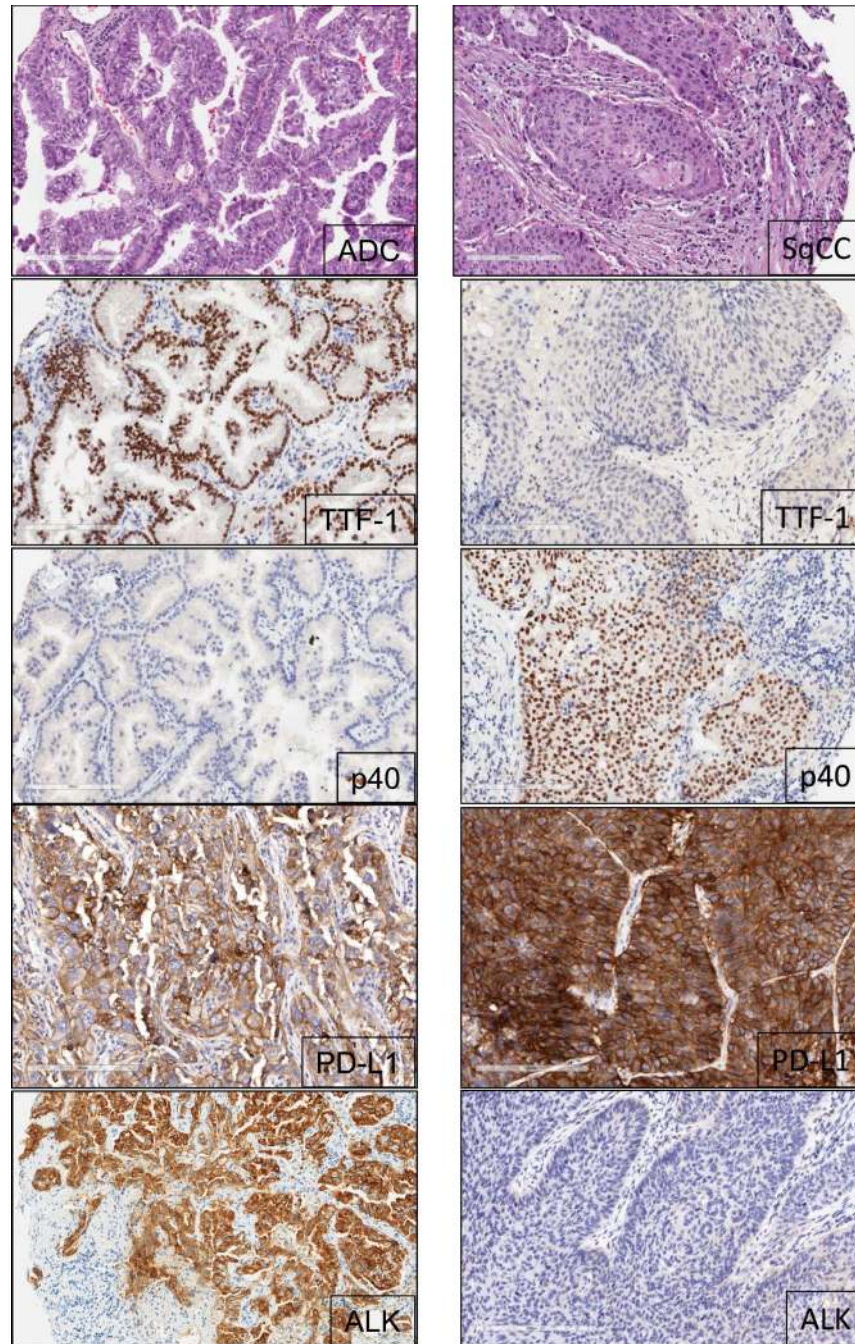

Figure 1.
Representative examples of histology of lung adenocarcinoma (ADC) and squamous cell carcinoma (SqCC) histology and biomarker analysis using IHC. ADC tumor tissue shows positive expression for TTF-1 (*nuclear*), PD-L1 (*membrane and cytoplasm*), and ALK (*cytoplasm*); p40 shows negative expression. SqCC shows positive expression for p40 (*nuclear*) and PD-L1 (*membrane and cytoplasm)*; p40 and ALK expression are negative (20x magnification).

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Table 1

Frequency of the main molecular alterations in lung adenocarcinoma and squamous cell carcinoma

Gene	Alteration	Adenocarcinoma	Squamous Cell Carcinoma
		Frequency	
EGFR	Mutation	10%	3%
ALK	Rearrangement	4-7%	None
ROS	Rearrangement	1-2%	None
KRAS	Mutation	25-35%	5%
MET	Mutation	8%	3%
MET	Amplification	4%	1%
NTRK1	Rearrangement	3%	None
FGFR	Amplification	3%	20%
HER2	Mutation	1.6-4%	None
BRAF	Mutation	1-3%	0.3%
PIK3CA	Mutation	2%	7%
RET	Rearrangement	1-2%	None
DDR2	Mutation	0.5%	3-4%
PTEN	Deletion	-	16%