Exploring Key Biomarkers and Actionable Genomic Alterations in NSCLC

First-generation EGFR tyrosine kinase inhibitors (TKIs) were first approved by the FDA 20 years ago; their efficacy was demonstrated in the treatment of non–small cell lung cancer (NSCLC) with sensitizing EGFR gene mutations. This marked the beginning of biomarker-driven management of NSCLC with targeted therapies.1,2 The discovery of other actionable mutations and better understanding of the molecular basis of the disease have continued the development of targeted therapies in the treatment landscape.1 Also, beginning in 2012, clinical guidelines strongly recommended that every patient with newly diagnosed, advanced-stage NSCLC—and some with resectable early-stage NSCLC—is tested for predictive biomarkers.3,4 These changes and the introduction of targeted treatments resulted in improved overall survival (OS) and a rapid decline in NSCLC mortality starting in 2006 and accelerating in 2013.4

Still, NSCLC represents 76% to 90% of all diagnosed lung cancer tumors; more than half of patients present with advanced stage disease, which has an estimated 5-year survival rate of 8.2%.5,6 The prevalence of predictive biomarkers among patients with NSCLC and the reduction in mortality associated with targeted therapies underscore a critical need for personalized therapies.

The Table summarizes the estimated frequencies for key predictive and emerging biomarkers in NSCLC and lists associated guideline-recommended testing technologies and therapies.3,8-10

The Role of Biomarkers

EGFR Mutations

Common EGFR mutations, including exon 19 deletions and the exon 21 L858R point mutation, account for 85% to 90% of all EGFR mutations in NSCLC. These mutations are strong predictors of a positive clinical response to EGFR TKIs (EGFRi).11-13 They enable first-generation EGFRi to outcompete adenosine triphosphate (ATP) binding to the mutated receptor, preventing its activation and leading a higher response rate and extended progression-free survival (PFS).12,13 However, the EGFR T790M mutation increases ATP affinity, leading to the activation of the receptor despite the presence of inhibitors.13 Second-generation EGFRi were developed to bind irreversibly to EGFR and overcome resistance. Their efficacy has been limited by poor selectivity and toxicities. Third-generation EGFRi, like osimertinib, have shown greater selectivity for EGFR T790M mutations versus the wild-type EGFR. This has led to their successful use in treating patients with NSCLC with EGFR T790M mutations.12,13

Uncommon EGFR mutations, including those within exons 18 to 25, comprise the remaining 10% to 15% of EGFR mutations in NSCLC; these are associated with poorer responses to EGFRi.13,14 These mutations (including exon 18 deletions and substitutions in EGFR E709 and EGFR G719X, exon 19 insertions, exon 20 insertions, the EGFR S768I and exon 21 L861Q substitutions, EGFR kinase domain duplications, and complex mutations) exhibit varying sensitivities to EGFRi compared to common mutations. For instance, exon 20 insertion mutations induce structural changes that lead to activation of the kinase domain.13 However, these mutations often show resistance to first- and second-generation EGFRi. Clinical trials have revealed differences in EGFRi sensitivity for distinct types of exon 20 insertions, demonstrating the heterogeneity of these mutations.13

ALK Rearrangements

ALK gene rearrangements are present in about 2% to 8% of NSCLC. These rearrangements, primarily occurring in adenocarcinomas, are more prevalent in females and never- or light smokers.15 Compared to chemotherapy, use of the first-generation ALK TKI crizot-
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<td><strong>EGFR mutations</strong>a</td>
<td>Common EGFR mutations, 10.0% Less common EGFR mutations, ≤ 10%</td>
<td>Real-time PCR, Sanger sequencing (ideally paired with tumor enrichment), and NGS</td>
<td>First-line therapy: afatinib, dacomitinib, erlotinib, gefitinib, osimertinib, erlotinib/ramucirumab, erlotinib/bevacizumab Subsequent therapy: amivantamab-vmjw, osimertinib</td>
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<td>KRAS G12C mutations</td>
<td>25.0%</td>
<td>Real-time PCR, Sanger sequencing (ideally paired with tumor enrichment), and NGS</td>
<td>Subsequent therapy: adagrasib, sotorasib</td>
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<td><strong>ALK rearrangements</strong>b</td>
<td>5.0%</td>
<td>FISH, IHC, NGS, and real-time PCR</td>
<td>First-line therapy: alectinib, brigatinib, ceritinib, crizotinib, lorlatinib Subsequent therapy: alectinib, brigatinib, ceritinib, lorlatinib</td>
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<td><strong>ROS1 rearrangements</strong>b</td>
<td>1.0%-2.0%</td>
<td>FISH, IHC, NGS, and real-time PCR</td>
<td>First-line therapy: ceritinib, crizotinib, entrectinib Subsequent therapy: entrectinib, lorlatinib</td>
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<td><strong>BRAF V600E mutations</strong>c</td>
<td>1.0%-2.0%</td>
<td>Real-time PCR, Sanger sequencing (ideally paired with tumor enrichment), and NGS</td>
<td>First-line therapy: dabrafenib/trametinib, encorafenib/binimetinib, dabrafenib, vemurafenib Subsequent therapy: dabrafenib/trametinib, encorafenib/binimetinib</td>
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<td><strong>NTRK1/2/3 gene fusions</strong>b</td>
<td>&gt; 1.0%-3.0%</td>
<td>FISH, IHC, PCR, and NGS</td>
<td>First-line/subsequent therapy: larotrectinib, entrectinib</td>
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<td><strong>MET exon 14 skipping mutation</strong></td>
<td>3.0%-4.0%</td>
<td>NGS</td>
<td>First-line/subsequent therapy: capmatinib, crizotinib, tepotinib</td>
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<td><strong>RET rearrangements</strong></td>
<td>1.0%-2.0%</td>
<td>FISH, real-time reverse-transcriptase PCR, and NGS</td>
<td>First-line/subsequent therapy: selpercatinib, pralsetinib, cabozantinib</td>
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<td><strong>ERBB2 (HER2) mutations</strong></td>
<td>3%</td>
<td>NGS, Sanger sequencing, and PCR</td>
<td>Subsequent therapy: fam-trastuzumab deruxtecan-nxki, ado-trastuzumab emtansine Immune checkpoint inhibitors (pembrolizumab, nivolumab, atezolizumab, cemiplimab-rwlc, ipilimumab, tremelimumab-actl, durvalumab) alone or in combination with each other and/or with chemotherapy</td>
</tr>
<tr>
<td><strong>PD-L1 expression levels</strong>d</td>
<td>TPS ≥ 50.0%, 33%; TPS = 1.0%-49.0%, 30.0%; TPS &lt; 1.0%, 37.0%</td>
<td>IHC</td>
<td></td>
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<td>Data not available</td>
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FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NCCN, National Comprehensive Cancer Network; NGS, next-generation sequencing; NSCLC, non–small cell lung cancer; PCR, polymerase chain reaction; TPS, tumor proportion score.

*aNo specific driver known in over one-third of cases
*bPredicts response to targeted therapy with kinase inhibitors
*cPredicts response to BRAF with/without MEK inhibitors
*dPredicts response to immunotherapy
*eUnder investigation as predictive biomarkers with the goal of identifying appropriate therapies for patients

The mission of the Biomarker Consortium is to bring stakeholders together to provide accurate and relevant information about the importance of testing and biomarker identification, and how to utilize biomarker status to inform treatment decisions.
tinib demonstrated superior activity and improved overall response rates (ORRs) and PFS.\textsuperscript{15-17} This led to the development of newer ALK TKIs (eg, ceritinib, alectinib, brigatinib, lorlatinib) with greater efficacy, particularly in terms of central nervous system activity.\textsuperscript{15-17} Despite demonstrated ORRs of up to 80%, resistance to ALK TKIs evolves.\textsuperscript{15} This includes on-target alterations (eg, ALK mutations/gene amplification) and off-target bypass signaling pathways changes.\textsuperscript{16} The treatment landscape for ALK-rearranged NSCLC has shifted toward using second- and third-genera
tion ALK TKIs as first-line treatment.\textsuperscript{15-17}

**BRAF Mutations**

*BRAF* mutations, specifically the V600E mutation, play a pivotal role in the development and progression of NSCLC. Occurring in 1% to 2% of lung adenocarcinomas, *BRAF* mutations, a part of the MAPK/ERK signaling pathway, are commonly found in patients with a history of smoking. These mutations contribute to the growth of cancer cells through enhanced signaling pathways.\textsuperscript{18,19} Targeted therapies, specifically BRAF-directed TKIs, are the standard treatment for patients with advanced NSCLC with *BRAF* mutations.\textsuperscript{3} However, affected patients often develop resistance to BRAF-targeted therapy due to intrinsic or extrinsic mechanisms.\textsuperscript{19}

**HER2 Mutations**

*HER2 (ERBB2)* mutations and amplifications have been identified in 2% to 4% of NSCLC. Patients with *HER2*-mutated disease are more likely to be current or former smokers; they typically exhibit a worse prognosis than do patients with *EGFR* and *ALK* mutations, partially because their disease cannot yet be treated with a highly selective, targeted therapy.\textsuperscript{20,21} *HER2* mutations primarily involve insertion or duplication events in exon 20 and other activating mutations, and they are associated with responsiveness to anti-HER2 targeted therapy.\textsuperscript{20,21} *HER2*-mutated NSCLC demonstrates a propensity for brain metastases during treatment, with subtype *HER2* YVMA insertion showing a particularly higher estimated 12-month brain metastasis incidence when compared to the group not having this insertion.\textsuperscript{20,22} Also, de novo *HER2* mutations are usually mutually exclusive with other driver genes, but they predominantly occur in the kinase.\textsuperscript{20}

**KRAS Mutations**

*KRAS* mutations, present in up to about 35% of NSCLC diagnoses, are often early events in lung tumorigenesis that are associated with smoking, a high tumor mutation burden, and markers of immune evasion.\textsuperscript{23-25} *KRAS* mutations in NSCLC lead to more aggressive clinical phenotypes, underscoring the importance of KRAS-targeted therapy.\textsuperscript{23,25} KRAS was long considered “undruggable” due to the lack of good drug-binding pockets. However, the discovery of the allosteric P2 site on G12C-mutant KRAS enabled the development of covalent inhibitors like sotorasib and adagrasib.\textsuperscript{23,25} These options show initial efficacy, yet intrinsic and acquired resistance eventually emerge via bypass signaling, secondary mutations, and histologic transformation. Combination therapies will likely be required for more durable responses.\textsuperscript{24} Furthermore, the presence of comutations (eg, STK11/LKB1, TP53, CDKN2A/B, and KEAP1) adds to the heterogeneity of KRAS-mutated tumors and influences their biological behavior and response to treatment. Tumors with these comutations show altered immune marker expressions (eg, PD-L1), which correlate with resistance to PD-1 blockade therapy in KRAS-mutated lung adenocarcinoma.\textsuperscript{23,24}

**MET Exon 14 Skipping Mutations**

*MET* exon 14 (METex14) skipping mutations are present in about 3% of NSCLC cases; they can lead to decreased MET receptor degradation and sustained MET signaling, which drive cancer cell proliferation and survival.\textsuperscript{26,27} Patients with METex14 mutations show limited benefit from immunotherapy as compared with chemotherapy. Fortunately, the development of selective MET inhibitors (eg, crizotinib, capmatinib, and tepotinib) has led to improved clinical outcomes.\textsuperscript{3,26} However, these drugs show reduced efficacy over time due to acquired resistance mediated by secondary *MET* mutations or amplification or activation of bypass signaling pathways like *KRAS*.\textsuperscript{27,28} Research is focused on testing methods to identify METex14 mutations, use of combination targeted therapies to overcome resistance, and exploration of new MET inhibitors against resistance mutations.\textsuperscript{26,28}

**NTRK1/2/3 gene fusions**

*NTRK* gene fusions occur in less than 1% of NSCLC cases, but they act as oncogenic drivers by causing ligand-independent activation of TRK kinases and of the downstream signaling pathways MAPK and PI3K that promote cancer cell proliferation and survival.\textsuperscript{29,30} NTRK fusions are mutually exclusive with other driver mutations in NSCLC. Data are limited, yet NTRK fusions do not seem to be associated with high PD-L1 expression or CD8+ T-cell infiltration,
and patients with NSCLC with NTRK fusions show little benefit from immunotherapy as compared to treatment with the TRK inhibitors larotrectinib and entrectinib.\textsuperscript{30} These drugs demonstrate significant clinical activity, including against brain metastases, although acquired resistance typically develops via secondary NTRK mutations or activation of bypass pathways (MAPK or PI3K).\textsuperscript{29,30}

**RET Rearrangements**

RET rearrangements occur in 1% to 2% of NSCLC cases; they function as potent oncogenic drivers through constitutive activation of the RET tyrosine kinase and the downstream signaling pathways MAPK and PI3K/AKT.\textsuperscript{29,31,32} This leads to increased proliferation of cancer cells and the survival, migration, and invasion of these cells.\textsuperscript{31} RET fusions also enable immune evasion by downregulating major histocompatibility complex class I expression. These tumors tend to have low tumor mutation burden and PD-L1 expression, contributing to poor response to immunotherapy. RET rearrangements confer sensitivity to RET inhibitors (eg, selpercatinib, pralsetinib, cabozantinib), but acquired resistance can develop through secondary RET mutations, alternate pathway activation, or new oncogenic fusions.\textsuperscript{3,31}

**ROS1 Fusions**

ROS1 fusion proteins strongly drive tumorigenesis through constant activation of the ROS1 tyrosine kinase domain and downstream proliferative signaling pathways. Specifically, ROS1 fusions lead to increased signaling through the MAPK, STAT3, and PI3K/AKT pathways.\textsuperscript{33,34} This induces expression of genes involved in cell proliferation, survival, migration, and invasion.\textsuperscript{34} ROS1 fusions occur in 0.9% to 2.6% of NSCLC cases, and their identification as genomic drivers of NSCLC has enabled the development of targeted inhibitors.\textsuperscript{33,34} Crizotinib, ceritinib, and next-generation inhibitors such as lorlatinib and entrectinib are highly active against ROS1 fusion proteins, and their use often leads to dramatic tumor shrinkage.\textsuperscript{3} By specifically blocking the fused ROS1 tyrosine kinase, these drugs can effectively shut down the progrowth signaling pathways. However, resistance to such ROS1 inhibitors inevitably develops after about 1 to 2 years through secondary mutations or activation of bypass signaling pathways.\textsuperscript{34}

**PD-L1 Expression**

PD-L1 is an immunosuppressive checkpoint expressed in immune cells, including in tumor-specific activated T cells, under conditions of chronic antigen exposure. PD-L1 is a PD-1 expressed in tumor cells, tumor-infiltrating cells, and antigen-presenting cells in many cancers, including NSCLC.\textsuperscript{35,36} The expression of these 2 proteins suppresses T-cell function, enabling tumors to evade immune detection; although their expression levels have shown promise as prognostic biomarkers and therapeutic targets for immunotherapies that block the PD-1/PD-L1 pathway, their clinical significance varies across cancer types.\textsuperscript{35} Higher levels of PD-L1 expression are associated with increased tumor proliferation, aggressiveness, and poorer survival.\textsuperscript{36} The advent of immune checkpoint inhibitors that target the PD-1/PD-L1 pathway in patients with advanced NSCLC represents a significant improvement over traditional chemotherapy, especially in patients with high PD-L1 expression. However, challenges remain; these include the occurrence of hyperprogressive disease in some patients treated with these inhibitors, which points to a need for better patient selection and understanding of resistance mechanisms.\textsuperscript{35}

**Biomarker Testing**

The management of NSCLC relies heavily on the detection of NSCLC, with guidelines prioritizing comprehensive genomic testing for diagnosis and monitoring.\textsuperscript{3} Next-generation sequencing (NGS) is crucial to identify targeted therapies and, ultimately, improve patient outcomes.\textsuperscript{37} Fluorescence in situ hybridization, immunohistochemistry, and polymerase chain reaction assays are used to detect biomarkers in NSCLC, with each offering varying degrees of effectiveness.\textsuperscript{3} Tests recommended for each biomarker are summarized in the Table.\textsuperscript{3,8-10} Testing is integral to precise NSCLC diagnosis and individualized treatment.

However, genomic testing patterns indicate that recommended biomarker testing is underused—a significant number of patients with advanced NSCLC do not receive comprehensive genomic testing, and only a minority undergo testing for all recommended biomarkers.\textsuperscript{38,39} The challenges in NSCLC biomarker testing include keeping pace with rapidly evolving guidelines, harvesting of insufficient tissue samples, needing to repeat biopsies, facing technical failures in testing, and dealing with long turnaround times and the complexity of interpreting NGS reports.\textsuperscript{7,39} Addressing these barriers, improving guideline adherence, and enhancing the understanding of complex genomic data among oncologists are crucial unmet needs.\textsuperscript{7,38,40}


