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Molecular In My Pocket...

ONCOLOGY: *Molecular Biomarkers of Lung Cancer*

What to test:

Tumor Stage: Advanced-stage (stages IIIb and IV), metastatic or recurrent lung cancer. Consideration of testing early-stage patients (based on institutional policy); in particular, *EGFR* mutation testing on diagnostic biopsy or post-surgical resection specimens for use in making adjuvant treatment decisions in stage IB to IIIA non-small cell lung cancer (NSCLC).

Histologic subtypes: Adenocarcinomas, large cell, NSCLC not otherwise specified (NOS); consideration of molecular testing for squamous cell carcinoma.

Specimens: Formalin-fixed paraffin-embedded tissue (FFPE); fresh, frozen, or alcohol-fixed tissue; any type of cytology specimen with adequate cellularity and appropriate validation. Macro/microdissection encouraged for tumor enrichment*. Peripheral blood (plasma circulating tumor DNA) can be a surrogate sample.

Notes: In general, the mutations/alterations described below are seen in a non-overlapping fashion, although between 1%–3% of NSCLC may harbor concurrent alterations.

* Some clinicopathologic features - such as smoking status, ethnicity, and histology - are associated with the presence of an EGFR, ALK, ROS1, ERBB2 alterations; however, these features should not be utilized in selecting patients for testing.

* For any patient with progression on targeted therapy, histologic transformation (such as small cell) is a possible mechanism of resistance. Tissue biopsy of a progressing lesion should be considered to evaluate morphology and biomarker analysis.

* Testing in the setting of a limited number of pulmonary nodules can aid in distinguishing separate primary lung carcinoma versus intrapulmonary metastatic disease.

Biomarker	Specific Alterations	Indications	Result Interpretation Significance	Assays Techniques*
Must Test (Broad Molecular Profiling Recommended) **				
EGFR	Exons 18-21 (exon 19 deletions, p.L858R point mutation in exon 21)	Therapy with EGFR-targeted tyrosine kinase inhibitors (TKIs)	Responsiveness to EGFR-targeted TKIs (e.g., afatinib, erlotinib, osimertinib)	NGS, PCR-based assays NSCLC stage IB–IIIA and stage IIIB
	Exon 20 in-frame duplication or insertion	Therapy with EGFR-targeted TKIs	Primary resistance to traditional EGFR-targeted TKI therapy; responsiveness to EGFR-targeted TKIs specific for exon 20 insertion	
	T790M	Arises in response to and as a mechanism of resistance to first- and second-generation EGFR TKIs	Third generation TKIs are typically efficacious. If identified in the absence of prior EGFR TKI therapy, genetic counseling and possible germline genetic testing are warranted. Identification of germline *EGFR* p.T790M confers a high risk for lung cancer regardless of smoking status.	
ALK	*R*earrangements: The most common fusion partner is *EML4*	Therapy with targeted inhibitors	Predicts response to oral ALK TKIs (e.g., alectinib, brigatinib, lorlatinib,ceritinib, crizotinib)	FISH, IHC, NGS, RT-PCR††
ROS1	Rearrangements; common fusion partners: *CD74*, *SLC34A2*, *CCDC6*, *GOPC* (FIG)	Therapy with targeted inhibitors	Predicts responsiveness to oral ROS1 TKIs (e.g., ceritinib, crizotinib)	FISH†, RT-PCR††, NGS†††; IHC as a screening with FISH or molecular confirmation of positive IHC results
BRAF	Point mutations Most common p.V600E	Therapy with targeted inhibitors	Predicts response to BRAF/MEK inhibitors (e.g., dabrafenib-trametinib, vemurafenib)	NGS, Sanger sequencing, PCR-based assays, IHC after extensive validation
KRAS***	Point mutations Codon 12, 13, 61, 146	Therapy with targeted inhibitors	Predicts response to sotorasib (*KRAS* G12C); diminished likelihood of another targetable oncogenic alteration; prognostic of poor survival when compared to patients with tumors without *KRAS* mutation	NGS, PCR-based assays
MET	Exon 14 skipping alterations	Therapy with targeted inhibitors	Predicts response to oral MET TKIs (e.g., capmatinib, crizotinib)	NGS†††
RET	Rearrangements Common fusion partners: *KIF5B*, *NCOA4*, *CCDC6*	Therapy with targeted inhibitors	Predicts response to oral RET TKIs (e.g., selpercatinib, pralsetinib,cabozantinib, vandetanib)	FISH†, RT-PCR††, NGS†††
***ERBB2* (HER2)**	Mutations (insertion/duplications in exon 20, substitutions at codon S310, amplifications)	Therapy with targeted inhibitors	Predicts response to fam-trastuzumab deruxtecan-nxki (alternative ado-trastuzumab emtansine)	NGS, PCR-based methods
NTRK1/2/3	Rearrangements * To date, no specific clinicopathologic	Therapy with targeted inhibitors	Predicts response to oral TRK inhibitors (e.g., larotrectinib, entrectinib)	FISH, IHC, RT-PCR††, NGS†††

	features, other than absence of other driver alterations, have been identified in association with these fusions.			
Emerging Biomarkers				
MET	High-level amplification * For NGS-based results, a copy number > 10 is consistent with high-level amplification	Consideration for a clinical trial with MET targeted therapy	Predicts response to capmatinib, tepotinib, crizotinib Secondary resistance to EGFR-targeted TKIs	FISH, NGS

Plasma Cell-Free/Circulating Tumor DNA Testing ("Liquid Biopsy"):

Considerations: Cell-free tumor DNA testing should not be used *in lieu* of a histologic tissue diagnosis. Cell-free DNA testing may have very high specificity, but low sensitivity (up to 30% false-negative rate).

When to Use: When a patient is unfit for invasive tissue biopsy or diagnostic biopsy is insufficient for molecular analysis. Follow-up tissue analysis should be planned for all patients in which an oncogenic driver is not found.

Assay Techniques: NGS, PCR

Abbreviations:

NGS: next-generation sequencing; IHC: immunohistochemistry; FISH: fluorescent *in situ* hybridization; TKI: tyrosine kinase inhibitor; RT–PCR: reverse transcription–polymerase chain reaction

*Analytic methods should be able to detect mutation in a sample with 20% or more malignant cell content.

**When feasible, testing should be performed by broad, panel-based approach (NGS). If identifiable driver oncogenes are not identified, consider RNA-based NGS, if not already performed, to maximize fusion detection.

*** Single-gene *KRAS* test may be performed to exclude patients with *KRAS*-mutant cancer from expanded panel in sequential testing algorithm.

†FISH may under-detect some fusions, such as *FIG-ROS1* variant.

††RT-PCR assays show reduced sensitivity in detecting novel fusion partners and breakpoints.

†††RNA-based NGS panels have higher sensitivity than DNA-based panels for some *ROS1*, *RET*, and *NTRK1/2/3* rearrangements, as well as *MET* exon 14 skipping alterations.

Where to test: Testing should be performed in the laboratories that are certified under clinical laboratory improvement amendments of 1988 (CLIA-88) as qualified to perform high complexity molecular pathology testing.

***References*:**

1. Lindeman, N. I., *et al*. (2018). Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors: Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. https://jmd.amjpathol.org/article/S1525-1578(17)30590-1/fulltext

2. National Comprehensive Cancer Network. Clinical practice Guidelines in Oncology. Non-Small Cell Lung Cancer. Version 3.2023; NCCN.org. Accessed 7/7/2023

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