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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) 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).

Histology – Adenocarcinomas, large cell, or NSCLC not otherwise specified: Consideration of testing for squamous cell carcinoma.

Materials – 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*

Biomarker	Specific Alterations	Indications	Result Interpretation Significance	Assays Techniques*
Must Test (Broad Molecular Profiling Recommended) **				
EGFR	Exons 18-21 (p. L858R; exon 19 deletions)	Consideration of therapy with EGFR-targeted tyrosine kinase inhibitors (TKI)	Responsiveness to EGFR-targeted TKIs	NGS, PCR-based assays
	Exon 20 insertions	Consideration of therapy with EGFR-targeted TKIs	Primary resistance to EGFR-targeted TKI therapy (with some exceptions)	
	T790M	Progression after treatment with early generation EGFR-targeted TKIs	Consideration of third-generation EGFR-targeted therapy (osimertinib)	
ALK	*ALK* rearrangements	Consideration of therapy with targeted inhibitors	Predicts response to alectinib, brigatinib, lorlatinib (also ceritinib, crizotinib)	FISH, IHC, NGS, RT-PCR††
ROS1	*ROS1* rearrangements	Consideration of therapy with targeted inhibitors	Predicts response to ceritinib, crizotinib	FISH†, RT-PCR††, NGS†††; IHC as a screening with FISH or molecular confirmation of positive IHC results
BRAF	p.V600E	Consideration of therapy with targeted inhibitors	Predicts response to BRAF/MEK inhibitors (dabrafenib-trametinib)	NGS, Sanger sequencing, PCR-based assays, IHC after extensive validation
KRAS**	Codon 12, 13, 61, and 146	Consideration of therapy with targeted inhibitors	Predicts response to sotorasib (*KRAS* G12C); Diminished likelihood of another targetable oncogenic alteration	NGS, PCR-based assays
MET	Exon 14 skipping variants	Consideration of therapy with targeted inhibitors	Predicts response to capmatinib, crizotinib	NGS†††
RET	*RET* rearrangements	Consideration of therapy with targeted inhibitors	Predicts response to selpercatinib, pralsetinib (also cabozantinib, vandetanib)	FISH†, RT-PCR††, NGS†††
NTRK1/2/3	*NTRK* rearrangements	Consideration of therapy with targeted inhibitors	Predicts response to larotrectinib, entrectinib	FISH, IHC, RT-PCR††, NGS†††

Emerging Biomarkers				
***ERBB2* (HER2)**	*ERBB2* (HER2) mutations (in frame insertions in exon 20, substitutions at codon S310, & amplification)	Consideration for a clinical trial with ERBB2 (HER2)-targeted therapy	Predicts response to ERBB2-targeted therapy (afatanib, TDM1)	NGS, PCR-based methods
MET	High- level amplification	Consideration for a clinical trial with MET targeted therapy	Predicts response to crizotinib Secondary resistance to EGRF-targeted TKIs	FISH, NGS

Cell-Free Plasma DNA (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 higher sensitivity than DNA-based panels for some *ROS1*, *RET*, and *NTRK1/2/3* rearrangements, as well as *MET* exon 14 skipping events.

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 2.2021 – 12/15/20; NCCN.org. Accessed 1/7/21

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Revised 7/2021