Best Practices and Recommendations for Molecular Testing in Advanced NSCLC (May 2022)

Evaluation of Advanced NSCLC

- Biomarker assessment is essential for individualizing therapy and predicting treatment response in advanced non-small-cell lung cancer (NSCLC)[1]

Biomarker Testing for NSCLC (Table 1)[1-5]

- Biomarkers to test:
  - **For nonsquamous NSCLC:** test all patients at diagnosis for *EGFR* (including *EGFR* exon 20 insertion mutations), *ALK*, *ROS1*, *BRAF* V600E, *NTRK*, *RET*, *MET*ex14, and *KRAS* G12C
  - **For squamous NSCLC:** consider testing in all patients for same biomarkers as listed for nonsquamous NSCLC
  - **For all histologic subtypes of NSCLC:** test for PD-L1 expression by immunohistochemistry (IHC)
- Choice of assay for biomarker testing depends on institution, resources, and clinical context
  - Suitable specimens are cell blocks or other cytologic preparations[6,7]
  - *Recommended and preferred:* broad next-generation sequencing (NGS) testing[4,8]
    - NGS maximizes the number of mutations detected while minimizing tissue use
    - NGS can identify alterations that are currently actionable as well as those under investigation in clinical trials (eg, *NRG1* fusion, *HER2*)
- Testing should be completed within 10-14 working days of biopsy[4,9]
  - Before acting on PD-L1 results, wait for NGS results
    - While awaiting results, consider 1 cycle of chemotherapy for symptomatic patients

Established Biomarkers Used in NSCLC Management

- Approximately 50% of patients with advanced nonsquamous NSCLC have a driver mutation targetable by an FDA-approved agent[10,11]

Table 1. Molecular Testing for Established Biomarkers Used in Standard-of-Care NSCLC Management[5,12-21]

Biomarker	Incidence, %	Recommended Testing Methodologies	Important Considerations
EGFR mutations (including canonical, atypical, and exon 20 insertions)	20-30	• NGS (preferred) • Real-time PCR • Sanger sequencing, paired with tumor enrichment	• DNA-based NGS is recommended for detection of *EGFR* exon 20 insertions
ALK rearrangements	4-5	• NGS (preferred) • FISH break-apart • IHC • Real-time PCR	• RNA-based NGS is more sensitive than DNA-based NGS for fusions/rearrangements • FDA-approved IHC can be used as a standalone test not requiring confirmation by FISH • Real-time PCR assays are unlikely to detect fusions with novel partners
ROS1 rearrangements	1.54-2.59 in Asian patients; 1.7-2.5 in White patients	• NGS (preferred) • Real-time PCR • Sanger sequencing, paired with tumor enrichment	• RNA-based NGS is more sensitive than DNA-based NGS for fusions/rearrangements • FISH break-apart may underdetect the *FIG-ROS1* variant • IHC for ROS1 fusions has low specificity and follow-up confirmatory testing is necessary • Real-time PCR assays are unlikely to detect fusions with novel partners
BRAF V600E	1-5	• NGS (preferred) • Real-time PCR • Sanger sequencing, paired with tumor enrichment • Anti-BRAF p.V600E-specific monoclonal antibody	• Anti-BRAF p.V600E–specific monoclonal antibody is available but should only be used after extensive validation
KRAS G12C	13.8	• NGS (preferred) • Real-time PCR • Sanger sequencing, paired with tumor enrichment	• Other *KRAS* mutations are not currently actionable
MET exon 14 skipping variants	3-4	• NGS (preferred)	• NGS-based testing is the primary method for detection • RNA-based NGS may have improved detection • IHC is not a method for detection
RET rearrangements	1-2	• NGS (preferred) • FISH break-apart • Real-time reverse-transcriptase PCR assays	• RNA-based NGS is more sensitive than DNA-based NGS for fusions/rearrangements • FISH may underdetect some fusions • Real-time reverse-transcriptase PCR assays are unlikely to detect fusions with novel partners
NTRK1/2/3 fusions	<1	• NGS (preferred) • FISH • IHC • PCR	• RNA-based NGS is more sensitive than DNA-based NGS for fusions/rearrangements • DNA-based NGS may underdetect *NTRK1* and *NTRK3* fusions • False negatives may occur • IHC methods are complicated by baseline expression in some tissues • FISH testing may require at least 3 probe sets for full analysis
PD-L1 expression	PD-L1 ≥1%: 68 PD-L1 ≥50%: 28	• IHC • Cell-free/circulating tumor DNA testing (investigational)	• Various antibody clones have been developed for IHC analysis and some are comparable regarding intensity and proportion of cells stained, but some are not • Some PD-L1 IHC clones are FDA approved for specific indications

FISH, fluorescence in situ hybridization; PCR, polymerase chain reaction.

TESTING CONCEPTS

Tissue-Based Testing

- Biomarker testing can be approached by testing multiple genes simultaneously (preferred) or single genes sequentially (**Table 2**)

Table 2. Comparison of Single-Gene vs Multigene Testing Approaches[5,22,23]

	Multigene Testing (eg, by NGS)	Single Gene Testing
Advantages	• Minimizes use of tumor tissue • Facilitates testing of multiple biomarkers, including emerging biomarkers for clinical trial enrollment • Just need to know to test vs which biomarkers to test for • Often less costly than sequential testing	• Potentially routine in practice • Potential for local implementation, rapid turnaround • Higher sensitivity with PCR platforms
Limitations	• Multiple platforms available using different methodology that affect types of alterations detected o RNA-based NGS more sensitive than DNA-based NGS, which may underdetect gene fusions • Complex biomarker reports • Preauthorization requirements • May not be easily accessible in community practice	• Potentially routine in practice • Potential for local implementation, rapid turnaround • Higher sensitivity with PCR platforms

Liquid vs Tissue Biopsy

- A "liquid biopsy"—blood sample—can contain tumor cells and cell-free DNA shed from both primary and metastatic sites[24]
- Liquid and tissue biopsies each have advantages and disadvantages (**Table 3**)

Table 3. Comparison of Tissue-Based vs Blood-Based Testing[25,26]

	Tissue-Based Testing	Blood-Based Testing
Advantages	• Compatible with comprehensive testing platforms with RNA and DNA that better capture fusions • Amenable to novel target discovery • Substantial data supporting treatment selection across multiple tumor types (early and advanced stages)	• Minimally invasive • Safer • Quick turnaround • Captures whole body • May overcome tumor heterogenicity • Serial testing easier
Limitations	• Invasive • Slower turnaround time • Biopsy risks • Serial testing more difficult	• May not capture real mutations • Tumor DNA may not be shed into circulation • Currently available panels are not as comprehensive as those for tissue • RNA degrades in blood

- Data from prospective clinical studies support complementary use of tissue and liquid biopsies for biomarker testing in newly diagnosed advanced NSCLC
  - o The observational NILE study performed concomitant molecular analysis of liquid and tissue biopsies in 282 patients with previously untreated metastatic NSCLC[27]
    - ▪ Tissue genotyping performed per standard of care; blood sample collected pretreatment for comprehensive cell-free DNA analysis
    - ▪ A guideline-recommended biomarker was identified in 21.3% of tissue biopsies vs 27.3% of liquid biopsies (P <.0001)
    - ▪ When cell-free DNA analysis of liquid biopsy was used in addition to tissue genotyping, biomarker detection increased by 48%
  - o A prospective cohort study compared the rates of targetable mutation detection with liquid biopsy only, tissue biopsy only, and both liquid and tissue biopsies collected concurrently in 323 patients with metastatic NSCLC[28]
    - ▪ DNA-based NGS was performed on plasma and tissue samples
    - ▪ Therapeutically targetable mutations were detected in 35% of all patients
    - ▪ In patients with concurrent plasma and tissue testing:
      - Targetable mutations were detected in tissue only among 20.5%
      - Adding plasma testing increased detection rate to 35.8%

Future Directions

- Other uses of liquid biopsy are under investigation[25]
  - Real-time monitoring for disease response to therapy
  - Identifying mechanisms behind resistance to first-line osimertinib, including the development of indirect indicators of transformation to small-cell lung cancer
  - Lung cancer screening

References

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