THE ROLE OF BIOMARKER TESTING IN ADVANCED NSCLC
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OVERVIEW OF BIOMARKERS IN NSCLC

Known biomarkers and use of biomarker testing for patient care
NSCLC is a heterogenous group of diseases with distinct histological subtypes and numerous oncogenic drivers

**Oncogenic drivers in adenocarcinoma**

- HER2 1.9%
- RET 0.7%
- NTRK1 1.7%
- ROS1 1.7%
- RIT1 2.2%
- DDR2 2.9%
- NRG1 3.2%
- METexon14 4.2%
- BRAF 6.9%
- KRAS 25.5%
- NFI 8.1%
- EGFR 16.1%
- Wild type 20.8%

**Oncogenic drivers in squamous cell carcinoma**

- DDR2 3%
- PIK3CA 12%
- FGFR1 20%
- PTEN 10%
- Other/wild type 55%

Up to 60% of patients with adenocarcinoma have ≥1 known oncogenic driver.

50% to 80% of patients with squamous cell carcinoma have ≥1 known oncogenic driver.

NSCLC includes 3 main histological subtypes:

- Adenocarcinoma (49.7%)
- Squamous cell carcinoma (22.7%)
- Large cell carcinoma (1.4%)

Known oncogenic drivers differ in commonality between these subgroups.

- Actionable oncogenic drivers that occur in adenocarcinoma also occur in squamous-cell carcinoma, but at lower frequencies.

Oncogenic drivers may serve as prognostic or predictive biomarkers to help guide patient management.

Importance of biomarker testing in NSCLC\textsuperscript{1-3}

- NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines\textsuperscript{®}) recommend biomarker testing in all appropriate patients with metastatic NSCLC based on data showing clinical benefit for patients receiving appropriate targeted therapy or immunotherapy as opposed to chemotherapy options\textsuperscript{1}

  - **Predictive biomarkers** are indicative of therapeutic efficacy because there is an interaction between the biomarker and therapy on patient outcome
  
  - **Prognostic biomarkers** are indicative of patient survival independent of the treatment received

- Molecular testing to detect actionable targets as part of a diagnostic work-up can help personalize care

- Longitudinal biomarker testing can provide insights into tumor evolution, heterogeneity, and resistance
Current actionable biomarkers in NSCLC according to NCCN Guidelines®

- Numerous gene alterations have been identified that impact therapy selection in NSCLC
- Testing for these alterations not only helps identify potentially efficacious targeted therapies, but also those therapies unlikely to provide clinical benefit

<table>
<thead>
<tr>
<th>Predictive biomarkers associated with responsiveness to targeted therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EGFR</strong> mutations such as exon 19 indels, exon 20 mutations (eg, p.T790M) or exon 21 mutations (eg, p.L858R)</td>
</tr>
<tr>
<td>Fusion between <strong>ALK</strong> and other genes</td>
</tr>
<tr>
<td><strong>ROS1</strong> gene fusions</td>
</tr>
<tr>
<td><strong>KRAS</strong> mutations¹²</td>
</tr>
<tr>
<td><strong>BRAF</strong> V600E point mutations</td>
</tr>
<tr>
<td><strong>MET</strong> exon 14 skipping mutations</td>
</tr>
<tr>
<td><strong>RET</strong> gene rearrangements</td>
</tr>
<tr>
<td><strong>NTRK1,2,3</strong> gene fusions</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Predictive biomarkers associated with responsiveness to immunotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PD-L1</strong> protein expression level</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Emerging biomarkers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High-level MET</strong> amplification</td>
</tr>
<tr>
<td><strong>ERBB2 (HER2)</strong> mutations</td>
</tr>
</tbody>
</table>

- Numerous other mutations are under investigation for biomarker use

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¹The NCCN Guidelines for NSCLC provide recommendations for certain individual biomarkers that should be tested and recommend testing techniques but do not endorse any specific commercially available biomarker assays or commercial laboratories. ¹Considered must test biomarkers by CAP-IASLC molecular testing guidelines. ²**KRAS** mutations are a prognostic biomarker in the NCCN Guidelines. ¹¹Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for NSCLC V.5.2021. © National Comprehensive Cancer Network, Inc. 2021. All rights reserved. Accessed June 15, 2021. To view the most recent and complete version of the guidelines, go to NCCN.org. 2. Bernicker E, et al. J Thorac Dis. 2019;11(Suppl 1): S81–S88.
Despite the identification of actionable biomarkers and known patient benefit, biomarker testing may be limited

Although biomarker testing rates have increased in the last few years, challenges to biomarker testing in NSCLC remain\(^1\)-\(^3\)

**Biomarker testing rates, 2017-2019 (% of patients tested; N=1203)**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>2017</th>
<th>2018</th>
<th>2019</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td>54%</td>
<td>51%</td>
<td>43%</td>
</tr>
<tr>
<td>ALK</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ROS1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRAF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All 4 genes</td>
<td></td>
<td></td>
<td>22%</td>
</tr>
</tbody>
</table>

**Treatment of biomarker-positive patients**

- **45%** On targeted therapy
- **55%** No targeted therapy

Less than half of patients in community practices with actionable mutations received targeted therapy despite being biomarker positive\(^2\)

Current challenges to biomarker testing include\(^3\),\(^4\):  
- Tissue sample adequacy  
- Selecting the appropriate biomarker test  
- Interpretation of biomarker test results  
- Financial considerations  
- Turnaround time for some results

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ALK, anaplastic lymphoma kinase gene; BRAF, B-Raf proto-oncogene; EGFR, epidermal growth factor receptor gene; NSCLC, non-small cell lung cancer; ROS1, ROS proto-oncogene 1.

Recent Data on Testing Rate

Real world biomarker testing rates in US oncology network community practices

- Retrospective observational chart review of mNSCLC patients initiating 1L therapy between (4/2018-3/2020): N=3,474
  - Assessed testing rates for ALK, BRAF, EGFR, PD-L1, ROS1:
    - 90% of patients received ≥1 biomarker test
    - 46% received all 5 biomarker tests
    - NGS testing increased from 33% to 44% (p<0.0001)
  - Median (IQR) time from dx to 1L therapy: 35 (22, 55) days
    - Median (IQR) turn around time (TAT) from biomarker testing orders to results: 10 (6, 17) - 15 (10, 22) days
    - Median (IQR) time from mNSCLC dx to biomarker results: 14 (7, 26) to 21 (12, 36) days

CHALLENGES IN BIOMARKER TESTING

NSCLC tissue biopsy size is often small and may not be sufficient to test the increasing number of actionable biomarkers

A core lung biopsy* will give 200µm of material\(^1\)

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Requirements</th>
<th>Total Waste</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NGS</strong></td>
<td>10 x 5µm for NGS testing = 50µm for tests + wastage</td>
<td>Total=60µm</td>
<td></td>
</tr>
<tr>
<td><strong>ALK ROS1 PD-L1</strong></td>
<td>5 x 4µm for ALK and ROS1 FISH/IHC and PD-L1 IHC = 20µm for tests + wastage</td>
<td>Total=30µm</td>
<td></td>
</tr>
<tr>
<td><strong>EGFR</strong></td>
<td>6 x 10µm for EGFR testing = 60µm for tests + wastage</td>
<td>Total=70µm</td>
<td></td>
</tr>
<tr>
<td><strong>H&amp;E IHC</strong></td>
<td>1 x 4µm H+E 4 x 4µm additional Ab 2 x 4µm controls = 28µm for tests + wastage</td>
<td>Total=38µm</td>
<td></td>
</tr>
</tbody>
</table>

\(*Core needle biopsies provide more intact material than fine needle aspiration\(^2\)

*Core needle biopsies provide more intact material than fine needle aspiration\(^2\)*

Efficient use of tissue is important so that critical molecular testing can be performed\(^3\):
- On adequate tissue
- In a timely fashion

Simultaneous detection of multiple biomarkers (eg, through multiplex arrays) may allow for increased efficiency with small tissue samples\(^3\).
NSCLC tissue biopsy size is often limited – NILE study¹

- Sequential biomarker testing using a tissue biopsy occurred in 84.8% of patients
- Of the patients with complete genotyping using a tissue sample:
  - 68.6% had comprehensive NGS genotyping
  - 31.3% had sequential testing of all eight biomarkers

With cfDNA available, all eight guideline-recommended biomarkers were fully assessed in 95% of patients

If all currently recommended tests are performed sequentially, there may not be sufficient sample to test all biomarkers

* Did not have a guideline-recommended biomarker identified and were not assessed for all guideline-recommended biomarkers
Payer coverage is one of the barriers to access to molecular testing in NSCLC¹

- In 2020, a study among 246 cases (01/2017-04/2018) in New York showed majority of tests denied payer coverage (77%, n = 190) and only 10.75% of the total NGS service charge was reimbursed²

- In 2018, centers for Medicare & Medicaid Services (CMS) released a National Coverage Determination (NCD) for NGS testing for Medicare beneficiaries with advanced cancer³
  - Limited to patients who have not been previously tested using the same NGS test for the same primary cancer diagnosis
  - Coverage through private payers may be variable

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TESTING FOR BIOMARKERS IN NSCLC

Technical approaches, testing needs, and clinical guideline recommendations
Tissue biopsy is well established and sensitive, but has significant challenges.

**Tissue biopsy**

1. Selection of tumor tissue block
2. Manual microdissection
3. DNA isolation and purification
4. Biomarker testing and treatment decision

**Strengths and Limitations**

- Invasive and repeat samples may be needed to capture progression or treatment response, resistance, etc. **Tissue may not be accessible**
- May not capture **tumor heterogeneity**
- Samples often isolated from archival tissue; may not represent the tumor in its current form
- Highly **sensitive**, requires a low number of tumor cells
- Tissue biopsy is the **gold standard for molecular analysis**

References:

Liquid biopsy makes repeat sampling and detecting tumor heterogeneity easier, but may have limited sensitivity.

**Strengths and Limitations**

- **Minimally invasive**, repeat sampling to monitor acquired resistance mutations is easier\(^2\)
  - Captures tumor heterogeneity\(^1,2\)

- **Faster preparation time** than tissue biopsy, more likely to represent current tumor environment\(^1,3\)

- cfDNA breaks down rapidly, and therefore can be a real-time biomarker of tumor stage and other biological features\(^1\)

- cfDNA and CTC shedding varies by tumor type and stage; **low concentrations** of cfDNA and CTCs may be **difficult to detect**\(^1-3\)

- Can detect cancers **earlier**, before disease progression\(^1,4\)

- **Clinical significance of early mutations** and the percentage of mutations detected are **not yet clear**\(^2\)
  - Not all techniques available; cost and limited availability\(^1,2\)

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The use of plasma cfDNA/ctDNA testing (plasma testing) can be considered in specific clinical circumstances:

- If a patient is medically unfit for invasive tissue sampling
- In the initial diagnostic setting following pathologic confirmation of NSCLC if there is insufficient material for molecular analysis and if follow-up tissue-based analysis is planned for patients without oncogenic drivers

Cell free tumor DNA testing:

- Should not be used in lieu of a histologic tissue diagnosis
- Has very high specificity, but significantly compromised sensitivity (up to 30% false-negative rate)
- Does not have established standards/guidelines for analytical performance characteristics
- Can identify alterations that are unrelated to a lesion of interest

### Overview of assessment techniques

<table>
<thead>
<tr>
<th>Method</th>
<th>Used to assess/detect:</th>
<th>Sensitivity (%)</th>
<th>Turnaround time</th>
<th>Biopsy method</th>
<th>Point mutations</th>
<th>Small indels</th>
<th>CNAs</th>
<th>Rearrangements</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR and Sanger Sequencing¹,²</td>
<td>DNA changes, including point mutations, insertions, or deletions</td>
<td>20–50</td>
<td>3 to 4 days</td>
<td>Liquid</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>RT-PCR¹⁻³</td>
<td>RNA expression, including fusion transcripts</td>
<td>0.00001</td>
<td>2 to 3 days</td>
<td>Liquid</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>FISH²⁻⁶</td>
<td>Gene rearrangements including deletions, amplifications, translocations, and fusions</td>
<td>&lt;1</td>
<td>2 to 3 days</td>
<td>Tissue</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>NGS: targeted approach¹,⁴</td>
<td>Genetic changes in multiple genes simultaneously</td>
<td>1–10</td>
<td>7–20 days</td>
<td>Liquid</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>May not reliably detect fusions</td>
</tr>
<tr>
<td>NGS: WES/ WGS¹,⁴</td>
<td></td>
<td>Variable</td>
<td>Weeks</td>
<td>Liquid</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>(As long as in design)</td>
</tr>
<tr>
<td>IHC⁴,⁵,⁷,⁸</td>
<td>Protein expression, localization or specific alterations, including fusions</td>
<td>Variable</td>
<td>1 to 2 days</td>
<td>Tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

It is important to choose the technique that ensures accurate and reliable detection of the selected biomarker(s); some techniques require the knowledge of a specific change in DNA/RNA or protein for biomarker detection¹,⁹

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### Advantages and disadvantages of assessment techniques

<table>
<thead>
<tr>
<th>DNA and RNA</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGS(^1)</td>
<td>RT-PCR(^2)</td>
</tr>
<tr>
<td>- Large throughput</td>
<td></td>
</tr>
<tr>
<td>- High accuracy</td>
<td></td>
</tr>
<tr>
<td>- Rich content information</td>
<td></td>
</tr>
<tr>
<td>- Multiple types of genetic alterations</td>
<td></td>
</tr>
<tr>
<td>- Highly sensitive</td>
<td></td>
</tr>
<tr>
<td>- Detects fusion transcripts at the RNA level</td>
<td></td>
</tr>
<tr>
<td>- Allows for rapid testing</td>
<td></td>
</tr>
<tr>
<td>- Knowledge of fusion partner not required</td>
<td></td>
</tr>
<tr>
<td>- Rearrangements can be discriminated from polysomy/amplifications</td>
<td></td>
</tr>
<tr>
<td>- Sensitive</td>
<td></td>
</tr>
<tr>
<td>- Familiar</td>
<td></td>
</tr>
<tr>
<td>- Time saving and easily automatable</td>
<td></td>
</tr>
<tr>
<td>- Cost-friendly</td>
<td></td>
</tr>
<tr>
<td>- Many validated antibodies available</td>
<td></td>
</tr>
<tr>
<td>- Turnaround time</td>
<td></td>
</tr>
<tr>
<td>- Tissue sample needs</td>
<td></td>
</tr>
<tr>
<td>- Reports can be hard to interpret</td>
<td></td>
</tr>
<tr>
<td>- Wide variety of NGS assay platforms</td>
<td></td>
</tr>
<tr>
<td>- Poor quality of FFPE RNA samples</td>
<td></td>
</tr>
<tr>
<td>- Limited number of variants tested at once</td>
<td></td>
</tr>
<tr>
<td>- Only test 1 gene at a time</td>
<td></td>
</tr>
<tr>
<td>- Requires high tumor enrichment</td>
<td></td>
</tr>
<tr>
<td>- Not all rearrangements produce an expressed fusion transcript</td>
<td></td>
</tr>
<tr>
<td>- May miss unknown variants</td>
<td></td>
</tr>
<tr>
<td>- May require confirmatory test</td>
<td></td>
</tr>
<tr>
<td>- Accuracy can vary by fixative and background</td>
<td></td>
</tr>
<tr>
<td>- Insufficient tumor content of tissue</td>
<td></td>
</tr>
<tr>
<td>- Skilled pathologist required</td>
<td></td>
</tr>
</tbody>
</table>

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### NCCN recommended use of assessment techniques*1

<table>
<thead>
<tr>
<th></th>
<th>DNA &amp; RNA</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NGS</td>
<td>RT-PCR</td>
</tr>
<tr>
<td><strong>EGFR</strong></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td><strong>ALK</strong></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td><strong>ROS1</strong></td>
<td>✓</td>
<td>(Unlikely to detect fusions with novel partners)</td>
</tr>
<tr>
<td><strong>BRAF</strong></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td><strong>MET exon 14 skipping</strong></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td><strong>RET</strong></td>
<td>✓</td>
<td>(Unlikely to detect fusions with novel partners)</td>
</tr>
<tr>
<td><strong>NTRK 1/2/3</strong></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td><strong>PD-L1</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The NCCN Guidelines for NSCLC provide recommendations for certain individual biomarkers that should be tested and recommend testing techniques but do not endorse any specific commercially available biomarker assays or commercial laboratories.

HOW TO TEST FOR BIOMARKERS

NCCN Guidelines recommend a broad, panel-based approach (most typically performed by NGS) to test for biomarkers prior to initiating treatment in eligible patient with metastatic NSCLC\(^1\)

NGS can provide a large profile of oncogenic alterations at a point in the patient’s journey without sequential testing, with limited tissue sample and through either tissue or plasma testing (also known as liquid biopsy)\(^2,3\)

Additional benefits of NGS\(^5\):

- More cost effective than single gene testing
- May facilitate an increase in life-years gained in advanced NSCLC, a 10% increase in NGS use compared to single-gene testing resulted in 2630 life-years gained
- Easier to add new biomarker genes in patient assessment
- Can provide value for low frequency biomarkers

Testing tissue samples with NGS following a negative result with non-NGS methods revealed genomic alterations with a corresponding targeted therapy in 26% of retested samples, and a targeted agent in a clinical trial was available for 39% of retested samples\(^6\)

## RECENT DATA ON ECONOMIC IMPACT

**Multiplex gene testing, including NGS, provides an efficient method for identifying predictive biomarkers in patients with NSCLC**

Liquid biopsy was found to cost less and cause fewer complications than tissue biopsy.

<table>
<thead>
<tr>
<th>Study</th>
<th>Descriptions</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yu, 2018¹</td>
<td>Budget impact from a US health care payer perspective was modeled in 2018 to compare NGS to single gene testing.</td>
<td>The overall impact of NGS was expected to be minimally cost additive, this was due to more accurate identification of mutations and more use of target therapy in first line. First-line and maintenance treatment costs increased but were offset by a decrease in second-line and palliative care costs. Over 5 years, the total budget impact was $432,554 ($0.0072 PMPM).</td>
</tr>
<tr>
<td>Steuten, 2019²</td>
<td>Estimated the clinical and cost-effectiveness of multigene panel sequencing (MGPS) relative to single-marker genetic testing (SMGT) in patients diagnosed with aNSCLC using the Flatiron Health database.</td>
<td>The incremental cost-effectiveness ratio of MGPS versus SMGT was $148,478 per LY gained, demonstrating moderate cost effectiveness of MGPS compared with SMGT in patients with NSCLC.</td>
</tr>
<tr>
<td>Pennel, 2019³</td>
<td>Assessed the economic impact of NGS versus single gene testing from perspective of the CMS and US commercial payers.</td>
<td>Upfront NGS testing in patients with metastatic NSCLC was associated with substantial cost savings and shorter time-to-test results for both CMS and commercial payers than alternative testing approaches.</td>
</tr>
<tr>
<td>Arnaud, 2016⁴</td>
<td>Compared the clinical costs and complications of solid biopsies with blood-based biopsies for biomarker testing in NSCLC from a Medicare reimbursement perspective.</td>
<td>The biomarker testing via the blood-based test was both significantly cheaper and patients had fewer complications than both computed tomography (CT)-guided biopsy and navigational bronchoscopy.</td>
</tr>
</tbody>
</table>

DNA-based versus RNA-based NGS assays

NGS assays vary widely in the information they provide in terms of sensitivity, specificity, comprehensiveness, tissue requirements, and turnaround times.

**DNA-based NGS assays**¹,²

- Allows the characterization of the exact gene fusion breakpoints and other genetic alterations
- Can detect genetic alterations that lead to aberrant isoforms
- Does not require an additional RNA purification step
- Does not indicate expression of the rearranged locus of some fusion events
- Involves intronic regions

**RNA-based NGS assays**¹,²

- Can be more sensitive, efficient, and functionally definitive
- Fusion gene detection limited to those functionally expressed
- Can discriminate splicing isoforms and quantify fusion transcripts
- Not impacted by intronic regions
- RNA is more complicated to handle
- RNA can be highly degraded in FFPE specimens

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WHEN TO TEST FOR BIOMARKERS

Biomarker testing to guide care of treatment-naïve NSCLC

Molecular profiling on all with non-squamous component, or if clinical features may suggest a molecular driver

- Surgical specimen is available
  - No
  - Yes

Tissue biopsy specimen sufficient for molecular testing

- Yes
  - Perform molecular analysis* on liquid biopsy (ctDNA); NGS is preferred†
  - Therapeutic target negative
  - Tissue re-biopsy
  - Treat with SOC based on presence of oncogenic driver

- No
  - No

Therapeutic target positive

- Molecular analysis* on surgical specimen**
  - NGS is preferred†
  - Treat with SOC based on presence or absence of oncogenic driver
  - PD-L1 IHC as needed

- Perform molecular analysis* on tissue biopsy specimen**
  - NGS is preferred†
  - Treat with SOC based on presence or absence of oncogenic driver
  - PD-L1 IHC as needed

*EGFR, ALK, ROSI, and BRAF at minimum, but a panel if available; **Strongly suggest tissue sparing to facilitate participation in clinical trials; †While NGS is preferred, based on availability, other validated assays are acceptable.

WHEN TO TEST FOR BIOMARKERS

Biomarker testing to guide care of progressive or recurrent NSCLC


Patient with NSCLC progressive or recurrent disease during treatment with TKI

- Perform molecular analysis* on tissue biopsy specimen**
- NGS is preferred†
- Treat with SOC based on presence or absence of oncogenic driver
- PD-L1 IHC as needed

Perform molecular analysis* on liquid biopsy (ctDNA)

Targetable resistance mutation absent

Tissue re-biopsy

Feasible

Not feasible

Targetable resistance mutation present

Treat with SOC based on presence of oncogenic driver

Evaluate the potential benefit of other therapy for marker unknown or BSC

*cobas/ddPCR for EGFR mutation NGS preferred for ALK and ROS1; **Strongly suggest tissue sparing to facilitate participation in clinical trials; †While NGS is preferred, based on availability, other validated assays are acceptable.

Retesting a tumor after progression on targeted therapy can support the appropriate next therapeutic steps
Interpreting biomarker test results

Depending on the testing approach and the facility, testing results may be reported differently, and results may include genes tested, probes used, qualitative data, and quantitative data.¹

However, there have been efforts to standardize reports through templates.¹

**NGS reports may include²:**
- A top-line summary of the key findings
- Clinically relevant biomarkers with an associated FDA-approved therapy
- Biomarkers that are potentially relevant but without a clear consensus
- Negative results that are clinically relevant but have not been identified
- A list of clinical trials for which a patient may be eligible based on the presence of an identified biomarker

**IMPLICATIONS OF TESTING RESULTS FOR PATIENT MANAGEMENT**

**NCCN Guidelines: overview for advanced or metastatic NSCLC**

Validated testing should assess a minimum of:

- **Driver mutation positive**
  - EGFR, ALK, ROS1, BRAF, KRAS, NTRK1/2/3, RET, and MET
  - Targeted therapy (preferred) or chemotherapy
  - 1L therapy

- **Driver mutation negative or unknown**
  - PD-L1 ≥1% with or without contraindications to IO
  - IO or combinations with chemotherapy
  - Chemotherapy doublets with or without IO and single agents depending on PS and histology

- **PD-L1 <1% with or without contraindications to IO**
  - Chemotherapy doublets with or without IO and single agents depending on PS and histology

- **PD-L1 <1% with or without contraindications to IO**
  - IO or chemotherapy
  - IO not recommended following progression on a previous IO

When patients do not have an identifiable driver oncogene, broad panel testing RNA-based NGS should be considered.

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SUMMARY
NSCLC is both histologically and genetically diverse\(^1\)

Patients should be assessed for biomarker expression at multiple points in the treatment pathway, including at diagnosis and when starting a new therapy\(^2\)

Biomarker testing can help guide patient management and treatment\(^3\)

Current actionable biomarkers according to the NCCN include EGFR, ALK, ROS1, BRAF, KRAS, MET exon 14 skipping mutations, RET, NTRK1/2/3 and PD-L1; NCCN recommends that when feasible, molecular testing be performed via a broad, panel-based approach\(^3\)

Biomarkers can be assessed via well-characterized techniques such as NGS, RT-PCR, PCR, FISH, and IHC, with assay selection depending on biopsy type\(^3,4\)

Broad, panel-based testing can provide a view of the patient’s genetic profile without high tissue demands of sequential testing\(^3,4\)

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Glossary

Ab = antibody
ALK = anaplastic lymphoma kinase
BSC = best supportive care
BRAF = B-Raf proto-oncogene
cfDNA = circulating free DNA
ctDNA = circulating tumor DNA
CTC = circulating tumor cell
CGP = cancer gene panel
CNA = copy number alterations
DDR2 = discoidin domain receptor tyrosine kinase 2 gene
EGFR = epidermal growth factor receptor gene
ERBB2 = erb-b2 receptor tyrosine kinase 2 gene
FDA = Food and Drug Administration
FFPE = formalin-fixed paraffin embedded
FGFR1 = fibroblast growth factor receptor 1 gene
FISH = fluorescence in situ hybridization
H&E = hematoxylin and eosin
HER2 = human epidermal receptor 2 gene
ICI = immune checkpoint inhibitor
IHC = immunohistochemistry
IO = immunotherapy
KRAS = Kirsten rat sarcoma viral oncogene homolog
L = leucine
M = methionine
MET = mesenchymal-epithelial transition proto-oncogene
MET = MET receptor tyrosine kinase
METex14 = MET exon 14
NCCN = National Comprehensive Cancer Network
NF1 = neurofibromin 1 gene
NGS = next generation sequencing
NRG1 = neuregulin 1 gene
NSCLC = non-small cell lung cancer
NTRK = neurotrophic receptor tyrosine kinase gene
PCR = polymerase chain reaction
PD-L1 = programmed-death ligand 1
PIK3CA = phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha gene
PS = performance score
PTEN = phosphatase and tensin homolog gene
R = arginine
RET = RET proto-oncogene
RIT1 = Ras like without CAAAX 1 gene
ROS1 = ROS proto-oncogene 1
RT-PCR = reverse transcription PCR
SOC = standard of care
T = threonine
Trk = tropomyosin receptor kinase
TRS = targeted region sequencing
WES = whole exome sequencing
WGS = whole genome sequencing
# Overview of assessment techniques

<table>
<thead>
<tr>
<th>Overview</th>
<th>DNA and RNA</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overview</strong></td>
<td>NGS&lt;sup&gt;1,2&lt;/sup&gt;</td>
<td>RT-PCR&lt;sup&gt;1-3&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>NGS is a high throughput sequencing technique performed on DNA or RNA, and includes targeted (TRS, CGP) and broad approaches (WES, WGS) which do not need a specific target</td>
<td>RT-PCR converts RNA to DNA for amplification and analysis</td>
</tr>
<tr>
<td></td>
<td>• Used to assess genetic changes in multiple genes simultaneously</td>
<td>• Used to assess RNA expression, including fusion transcripts</td>
</tr>
<tr>
<td><strong>Biopsy method</strong>&lt;sup&gt;6&lt;/sup&gt;</td>
<td>Liquid and tissue biopsy</td>
<td>Liquid and tissue biopsy</td>
</tr>
<tr>
<td><strong>Sensitivity</strong>&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Variable with broader approaches; 1-10% with targeted approaches</td>
<td>0.0001%</td>
</tr>
<tr>
<td><strong>Turnaround time</strong>&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Days to weeks depending on NGS approach</td>
<td>2-3 days</td>
</tr>
<tr>
<td><strong>Variants detected</strong>&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Point mutations, Small indels, CNA*, Rearrangements*</td>
<td>Point mutations, Small indels, Rearrangements</td>
</tr>
</tbody>
</table>

It is important to choose the technique that ensures accurate and reliable detection of the selected biomarker(s); some techniques require the knowledge of a specific change in DNA/RNA or protein for biomarker detection<sup>1,6</sup>.

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<sup>*Excluding amplicon capture</sup>