Biomarker testing in non-small cell lung cancer (NSCLC)
Lung cancer is the second most common cancer diagnosed annually and the leading cause of mortality in the US.\textsuperscript{2}

\textbf{228,820}

Estimated newly diagnosed cases in 2020\textsuperscript{1}

- 20.5% 5-year survival rate\textsuperscript{1}
- 57% Advanced or metastatic at diagnosis\textsuperscript{1}
- 5.8% 5-year relative survival with distant disease\textsuperscript{1}
- 80-85% NSCLC\textsuperscript{2}

\textbf{135,720}

Estimated deaths in 2020\textsuperscript{1}


NSCLC is both histologically and genetically diverse

NSCLC distribution by histology

- Adenocarcinoma: 40%
- Squamous cell carcinoma: 25%
- Large cell carcinoma: 10%
- Other: 25%

Prevalence of genetic alterations in NSCLC

- ALK, anaplastic lymphoma kinase
- BRAF, V-raf murine sarcoma homolog B gene
- DDR2, discoidin domain receptor tyrosine kinase 2 gene
- EGFR, epidermal growth factor receptor gene
- FGFR1, fibroblast growth factor receptor 1 gene
- HER2, human epidermal receptor 2 gene
- KRAS, Kirsten rat sarcoma viral oncogene homolog
- MET, MNNG HOS transforming gene
- NFE1, neurofibromin 1 gene
- NTRK1, neurotrophic receptor tyrosine kinase 1 gene
- PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha gene
- PTEN, phosphatase and tensin homolog gene
- RIT1, Ras like without CAAX 1 gene
- ROS1, ROS proto-oncogene 1.6
- NRG1, neuregulin 1 gene
- Other or wild type: 55%

Assessment of genetic alterations in NSCLC

• Numerous gene alterations have been identified in NSCLC, therefore testing lung cancer specimens for these alterations is important¹

• College of American Pathologists and the International Association for the Study of Lung Cancer (CAP–IASLC) and National Comprehensive Cancer Network (NCCN) Guidelines provide evidence-based recommendations for molecular testing to identify predictive and prognostic biomarkers¹,²

CAP; College of American Pathologists; IASLC, International Association for the Study of Lung Cancer; NSCLC, non-small cell lung cancer.
## CAP-IASLC molecular testing guidelines

### “Must-test” biomarkers

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EGFR</strong></td>
<td>Base substitutions (e.g. p.T790M and p.L858R) and INDELS (e.g. deletion of exon 19) are observed in NSCLC tumor specimens.</td>
</tr>
<tr>
<td><strong>ALK</strong></td>
<td>Gene rearrangements are observed in NSCLC tumor specimens, which can generate several different oncogenic fusions (e.g. EML4).</td>
</tr>
<tr>
<td><strong>ROS1</strong></td>
<td>Gene rearrangements are observed in NSCLC tumor specimens, which can generate several different oncogenic fusions (e.g. CD74, SLC34A2, CCDC6 and FIG).</td>
</tr>
</tbody>
</table>

### “Should-test” biomarkers

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BRAF</strong></td>
<td>Base substitutions (e.g. p.V600E) are observed in NSCLC tumor specimens. Activating alterations in BRAF may lead to unregulated signaling through the ERK pathway.</td>
</tr>
<tr>
<td><strong>ERBB2/HER2</strong></td>
<td>Base substitutions (e.g. L755F) and gene amplification are observed in NSCLC tumor specimens.</td>
</tr>
<tr>
<td><strong>RET</strong></td>
<td>Gene rearrangements are observed in NSCLC tumor specimens.</td>
</tr>
<tr>
<td><strong>KRAS</strong></td>
<td>Base substitutions (e.g. p.G12C and p.Q61H) are observed in NSCLC tumor specimens. Activating alterations in KRAS may lead to unregulated signaling through the ERK pathway.</td>
</tr>
<tr>
<td><strong>MET</strong></td>
<td>Exon 14 skipping alterations and gene amplification are observed in NSCLC tumor specimens.</td>
</tr>
</tbody>
</table>

---

**Gene abbreviations:** ALK, anaplastic lymphoma kinase gene; BRAF, V-raf murine sarcoma homolog B gene; C, cysteine; CAP, College of American Pathologists; CCDC6, coiled-coil domain containing 6; CD74, HLA class II histocompatibility antigen gamma chain; EGRF, epidermal growth factor receptor gene; EML4, echinoderm microtubule-associated protein-like 4; ERBB2, erb-b2 receptor tyrosine kinase 2 gene; ERK, extracellular receptor kinase; F, phenylalanine; FIG, fused in glioblastoma; G, glycine; H, histidine; HER2, human epidermal receptor 2 gene; IASLC, International Association for the Study of Lung Cancer; INDELS, insertions and deletions; KRAS, Kirsten rat sarcoma viral oncogene homolog; L, leucine; MET, MNNG HOS transforming gene; M, methionine; NSCLC, non-small cell lung cancer; p, protein; Q, glutamine; R, arginine; RET, RET proto-oncogene; ROS1, ROS proto-oncogene 1; SLC34A2, solute carrier family 34 (sodium phosphate), member 2; T, threonine; V, valine.

NCCN guidelines for molecular testing of NSCLC

Broad molecular profiling is a key component to the improvement of care of patients with NSCLC.

The NCCN NSCLC Guidelines Panel strongly advises broad molecular profiling to identify rare oncogenic driver alterations.

NCCN Guidelines recommend molecular testing to assess the following biomarkers:

- **EGFR, BRAF, and MET exon 14 skipping** alterations
- **ALK, ROS1, and RET** rearrangements
- **NTRK** gene fusions (as part of broad molecular profiling)
- **PD-L1** expression levels

Emerging Biomarkers identified by the Guidelines Panel include:

- High-level **MET** amplification
- **ERBB2 (HER2)** alterations
- **Tumor mutational burden (TMB)**

---

*TMB is an evolving biomarker that may be helpful in selecting patients for immunotherapy; however, there is no consensus on how to measure TMB.*

ALK, anaplastic lymphoma kinase gene; BRAF, V-raf murine sarcoma homolog B gene; EGFR, epidermal growth factor receptor gene; ERBB2, erb-b2 receptor tyrosine kinase 2 gene; HER2, human epidermal receptor 2 gene; MET, MNNG HOS transforming gene; NCCN, National Comprehensive Cancer Network; NSCLC, non-small cell lung cancer; NTRK, neurotrophic receptor tyrosine kinase gene; PD-L1, programmed-death ligand 1; RET, RET proto-oncogene; ROS1, ROS proto-oncogene 1; TMB, tumor mutational burden.

Next-generation sequencing (NGS) can provide a comprehensive profile of oncogenic alterations

Numerous studies have demonstrated the excellent sensitivity of NGS methods relative to single-gene targeted assays, particularly for single-nucleotide substitution alterations.1

NGS methods:
- Require less input DNA1
- Can accommodate smaller samples with lower concentrations of malignant cells1
- Can often be performed more rapidly than sequential multiple single-gene assays (though typically slower than one single-gene assay)1
- Can use samples obtained through either a tissue or liquid biopsy1,3

Retesting of tissue samples with NGS following a negative result revealed2:
- Genomic alterations with a corresponding targeted therapy in 26% of retested samples*
- A targeted agent in a clinical trial was available for 39% of retested samples

*Based on NCCN guidelines.
DNA, deoxyribonucleic acid; NCCN, National Comprehensive Cancer Network; NGS, next-generation sequencing.
**Molecular analysis using liquid biopsies**

Liquid biopsy is recommended in cases with insufficient tumor tissue specimens or where specimens were not obtained.\(^1\) Specimens are isolated from peripheral blood and can include\(^1\):

- Circulating tumor DNA (ctDNA)
- Circulating tumor cells (CTCs)
- Circulating exosomes
- Platelet RNA
- Circulating tumor RNA (ctRNA)

**IASLC recommendation**: These approaches have significant potential to improve patient care, and immediate implementation in the clinic is justified in a number of therapeutic settings relevant to NSCLC.\(^1\)

**Advantages of liquid biopsy\(^1\):**

- Enables molecular tumor analysis in patients unable to undergo a biopsy due to suboptimal clinical condition
- Avoids complications associated with computed tomography-guided transthoracic lung biopsies
- Saves tissue biopsy specimens for other analyses
- Decreases cost and sample processing time
- More reflective of overall systemic tumor burden

---

CTC, circulating tumor cells; ctDNA, circulating tumor DNA; ctRNA, circulating tumor RNA; DNA, deoxyribonucleic acid; IASLC, International Association for the Study of Lung Cancer; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; RNA, ribonucleic acid.

**MET alterations in the NSCLC landscape**

- Alterations in the MET gene have been identified as primary oncogenic drivers in NSCLC and are associated with poor prognosis:
  - MET exon 14 (METex14) skipping alterations are observed in 3-4% of patients with lung adenocarcinomas.
  - Amplification of the MET gene is observed in 1-7% of patients with NSCLC, depending on the assay and cut point used.
  - MET amplification has been reported in ~5% of cases of acquired resistance to first- and second-generation EGFR TKIs and in 15-19% of patients who failed a third-generation TKI.
  - MET amplification is the second most common cause of acquired (secondary) resistance to EGFR TKI therapy (after EGFR p.T790M).
  - Concurrent MET amplification is found to occur in ~20% of patients with METex14 skipping alterations.
  - Strong MET expression is observed in 61% of NSCLC.

---

EGFR, epidermal growth factor receptor; M, methionine; MET, MNNG HOS transforming gene; MET, mesenchymal-epithelial transition factor; METex14, MET exon 14; NSCLC, non-small cell lung cancer; T, threonine; TKI, tyrosine kinase inhibitor.
MET is a receptor tyrosine kinase encoded by the MET gene. It is activated by HGF, its only known high affinity ligand.\textsuperscript{1,3}

MET signaling is critical for\textsuperscript{1,2}:
- Embryonic and organ development
- Liver regeneration
- Tissue repair
- Wound healing

MET drives cell proliferation, motility, and apoptosis through activation of downstream signaling pathways, including\textsuperscript{1,2}:
- RAS/ERK/MAPK
- PI3K/AKT
- JAK/STAT
- Wnt/β-catenin

How do METex14 skipping alterations and MET gene amplification lead to oncogenesis?

MET signaling can drive tumor growth and progression\textsuperscript{1,2}

HGF/MET pathway is frequently deregulated in human cancer\textsuperscript{1}, leading to dependency on MET signaling, known as “oncogenic addiction.”

**Deregulation can occur via\textsuperscript{1,2}:**

- MET and/or HGF overexpression
- MET gene amplification
- Activating MET alterations, including MET\textsuperscript{ex14} skipping alterations

\textbf{HGF, hepatocyte growth factor; MET, MNNG HOS transforming gene; MET, mesenchymal-epithelial transition factor; MET\textsuperscript{ex14}, MET exon 14; P, phosphorylated; PI3K, phosphoinositide 3-kinase; RAS, rat sarcoma GTPase; STAT, signal transducers and activator of transcription.}

Based on preclinical studies, METex14 skipping alterations may protect MET receptors from ubiquitination and degradation¹

- **MET**ex14 encodes part of the juxtamembrane domain of the MET protein, which contains the c-Cbl E3 ubiquitin ligase-binding site at tyrosine 1003 (Y1003)
  - c-Cbl transfers ubiquitin onto the MET receptor at Y1003, which acts as a flag for degradation
- **MET**ex14 skipping results in MET protein missing the c-Cbl binding site, preventing it from being targeted for degradation
- This can increase the number of MET receptors on the cell surface, driving cancer cell survival, proliferation and invasiveness

---

AKT, protein kinase B; c-Cbl, Casitas B-lineage lymphoma; E, enzyme; ERK, extracellular receptor kinase; HGF, hepatocyte growth factor; JAK, Janus kinase; MET, MNNG HOS transforming gene; MET, mesenchymal-epithelial transition factor; METex14, MET exon 14; PI3K, phosphoinositide 3-kinase; RAS, rat sarcoma; STAT, signal transducers and activators of transcription; Y, tyrosine.

Diversity of METex14 skipping alterations: Comprehensive diagnostic testing could be a challenge\textsuperscript{1,2}

Alterations that result in METex14 skipping are highly diverse at the DNA level and include\textsuperscript{1}:

- Insertions/deletions (INDELs)
- Base substitutions
- Splice site alterations

RNA-based testing might provide a means of getting around the underlying variety of DNA-based changes as it focuses on the more uniform splice-altered message\textsuperscript{1}

Alterations disrupt regions important for intron splicing, including the branch point, polypyrimidine tract, 3’ splice site of intron 13, and 5’ intron splice site of intron 14\textsuperscript{1}

---

<table>
<thead>
<tr>
<th>#</th>
<th>Genetic alteration</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>c.2888-35_2888-17del</td>
</tr>
<tr>
<td>3</td>
<td>c.2888-35_2888del</td>
</tr>
<tr>
<td>20</td>
<td>c.2888-28_2888-14del</td>
</tr>
<tr>
<td>16</td>
<td>c.2888-22_2888-2del</td>
</tr>
<tr>
<td>7</td>
<td>c.2888-21_2888-5del</td>
</tr>
<tr>
<td>13</td>
<td>c.2888-20_2888-1del</td>
</tr>
<tr>
<td>25</td>
<td>c.2888-20_2939-1del</td>
</tr>
<tr>
<td>5</td>
<td>c.2888-19_2888-3del</td>
</tr>
<tr>
<td>8</td>
<td>c.2888-7_2920del</td>
</tr>
<tr>
<td>14</td>
<td>c.2888-2_2915del</td>
</tr>
<tr>
<td>21</td>
<td>c.2903_3028+67del</td>
</tr>
<tr>
<td>6</td>
<td>c.2905_2940del</td>
</tr>
<tr>
<td>28</td>
<td>c.3008A&gt;G (p.Y1003C)</td>
</tr>
<tr>
<td>18</td>
<td>c.3010_3028+8del</td>
</tr>
<tr>
<td>1</td>
<td>c.3018_3028+8del</td>
</tr>
<tr>
<td>24</td>
<td>c.3020_3028+24del</td>
</tr>
<tr>
<td>10</td>
<td>c.3025_3027GAA&gt;G fs</td>
</tr>
<tr>
<td>17</td>
<td>c.3028G&gt;A</td>
</tr>
<tr>
<td>15</td>
<td>c.3028G&gt;A</td>
</tr>
<tr>
<td>2</td>
<td>c.1702-2A&gt;T + c.3028G&gt;A</td>
</tr>
<tr>
<td>4</td>
<td>c.3028+1G&gt;T</td>
</tr>
<tr>
<td>11</td>
<td>c.3028+1G&gt;T</td>
</tr>
<tr>
<td>27</td>
<td>c.3028+1G&gt;T</td>
</tr>
<tr>
<td>19</td>
<td>c.3028+1_3028+9del</td>
</tr>
<tr>
<td>12</td>
<td>c.3028+2T&gt;C</td>
</tr>
<tr>
<td>9</td>
<td>c.3028+2T&gt;C</td>
</tr>
<tr>
<td>23</td>
<td>c.3028+2T&gt;C</td>
</tr>
<tr>
<td>26</td>
<td>c.3028+2T&gt;C</td>
</tr>
</tbody>
</table>

A, adenine; C, cytosine; c., coding DNA reference sequence; C, cysteine; del, deletion; DNA, deoxyribonucleic acid; fs, frameshift; G, guanine; INDELs, insertions or deletions; IPT, immunoglobulin-plexin-transcription domain; MET, MNNG HOS transforming gene; METex14, MET exon 14; p., protein; PSI, cysteine rich domain found in plexins; RNA, ribonucleic acid; Sema, semaphorin-like domain; SP, signal peptide; T, thymine; TKD, tyrosine kinase domain; TM, transmembrane domain; Y, tyrosine.

Amplification of \textit{MET} gene can also increase \textit{MET} receptor signaling

- \textit{MET} gene amplification can occur through two mechanisms\textsuperscript{1}:
  - Focal amplification of the \textit{MET} gene
  - Polysomy of chromosome 7

- \textit{MET} amplification increases the number of \textit{MET} receptors on the cell surface, even with a functional binding site for c-Cbl\textsuperscript{2}

- \textit{MET} amplification leads to increased \textit{MET} signaling and oncogenesis\textsuperscript{2}
Differentiating MET gene amplification from polysomy of chromosome 7

MET polysomy vs focal amplification

In patients with chromosome 7 polysomy, chromosome 7 (containing the MET gene) is duplicated multiple times:

- With polysomy of chromosome 7, copies of MET and CEP7 would increase together
- With MET amplification, there would be more copies of MET than CEP7

In patients with MET amplification, only the MET gene is amplified:

MET amplification is a true oncogenic driver that can be distinguished from polysomy using the MET/CEP7 ratio:

- With polysomy of chromosome 7, copies of MET and CEP7 would increase together
- With MET amplification, there would be more copies of MET than CEP7

Both polysomy and focal MET amplification result in increased MET expression

CEP7, centromeric portion of chromosome 7; MET, MNNG HOS transforming gene; MET, mesenchymal-epithelial transition factor.

Defining the high-level genomic amplification of MET

- **MET** gene dosage assessment can use the absolute copy number (mean GCN per cell) or the relative copy number (**MET/CEP7** ratio)\(^1\)
- **MET** GCN can be assessed using NGS or FISH\(^1\)
- High-level **MET** amplification is usually defined as **MET/CEP7** ratio ≥2 or GCN ≥5 or 6, although there is no official consensus\(^1\)

Both polysomy and focal **MET** amplification result in increased **MET** expression\(^1\)

---


**CEP7**, centromeric portion of chromosome 7; **FISH**, fluorescence in situ hybridization; **GCN**, gene copy number; **MET**, MNNG HOS transforming gene; **MET**, mesenchymal-epithelial transition factor; **NGS**, next-generation sequencing.
**NSCLC** is both histologically and genetically diverse, requiring a complex therapeutic approach\(^1\)\(^-\)\(^4\)

**CAP–IASLC** updated molecular testing guidelines for alterations in NSCLC tumor specimens include\(^5\):
- **"Must-test"** genes *EGFR*, *ALK* and *ROS1*
- **"Should-test"** genes *BRAF*, *MET*, *RET*, *ERBB2* (*HER2*) and *KRAS*

**NCCN** guidelines recommend molecular testing to assess **MET** exon 14 skipping alterations and identify high-level **MET** amplification as an emerging biomarker to assess in broad molecular profiling\(^6\)

**Both MET** exon 14 skipping alterations and **MET** gene amplification may increase **MET** receptor signaling, potentially driving NSCLC tumorigenesis\(^7\)\(^,\)\(^8\)

**Diagnostic testing** for **MET** exon 14 skipping alterations could be challenging due to the diversity of alterations\(^8\)\(^,\)\(^9\)

---

**Summary**

- **NSCLC** is both histologically and genetically diverse, requiring a complex therapeutic approach\(^1\)\(^-\)\(^4\)
- **CAP–IASLC** updated molecular testing guidelines for alterations in NSCLC tumor specimens include\(^5\):
  - **"Must-test"** genes *EGFR*, *ALK* and *ROS1*
  - **"Should-test"** genes *BRAF*, *MET*, *RET*, *ERBB2* (*HER2*) and *KRAS*
- **NCCN** guidelines recommend molecular testing to assess **MET** exon 14 skipping alterations and identify high-level **MET** amplification as an emerging biomarker to assess in broad molecular profiling\(^6\)
- **Both MET** exon 14 skipping alterations and **MET** gene amplification may increase **MET** receptor signaling, potentially driving NSCLC tumorigenesis\(^7\)\(^,\)\(^8\)
- **Diagnostic testing** for **MET** exon 14 skipping alterations could be challenging due to the diversity of alterations\(^8\)\(^,\)\(^9\)

---

**Keywords:** NSCLC, molecular testing, MET, exon 14, MET amplification, diagnostic testing, biomarker, NSCLC tumorigenesis.