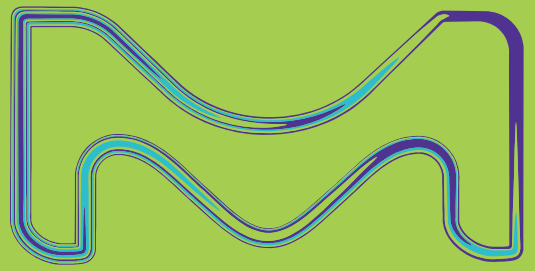
The biopharma business of Merck KGaA, Darmstadt, Germany operates as EMD Serono in the U.S. and Canada.

Biomarker testing in non-small cell lung cancer (NSCLC)



 US/TEP/1119/0018(1)

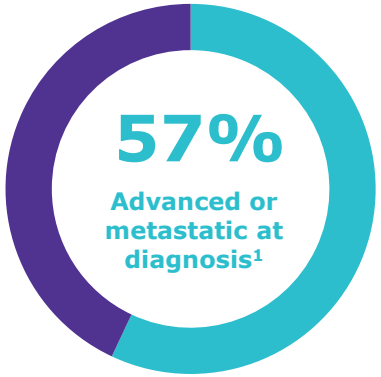
EMD SERONO

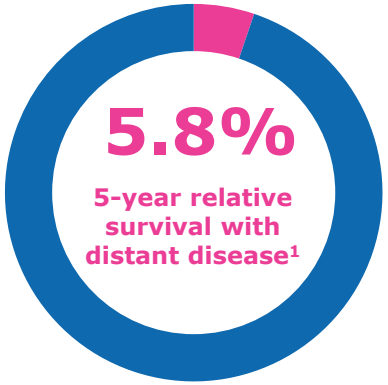
Lung cancer in the US: Incidence, mortality, and survival

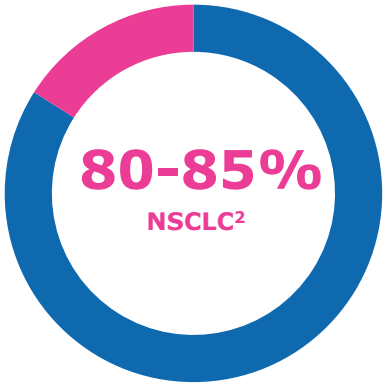
Lung cancer is the second most common cancer diagnosed annually and the leading cause of mortality in the US.[2]

228,820

Estimated newly diagnosed cases in 2020[1]







135,720

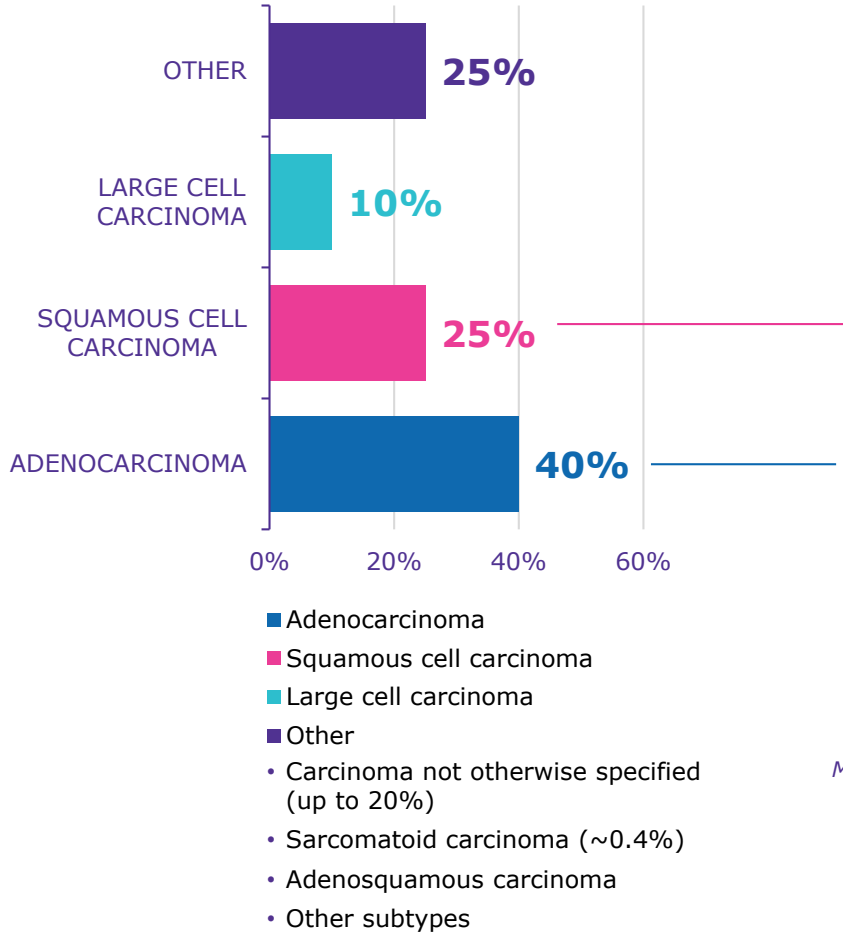
Estimated deaths in 2020[1]

NSCLC, non-small cell lung cancer; US, United States.

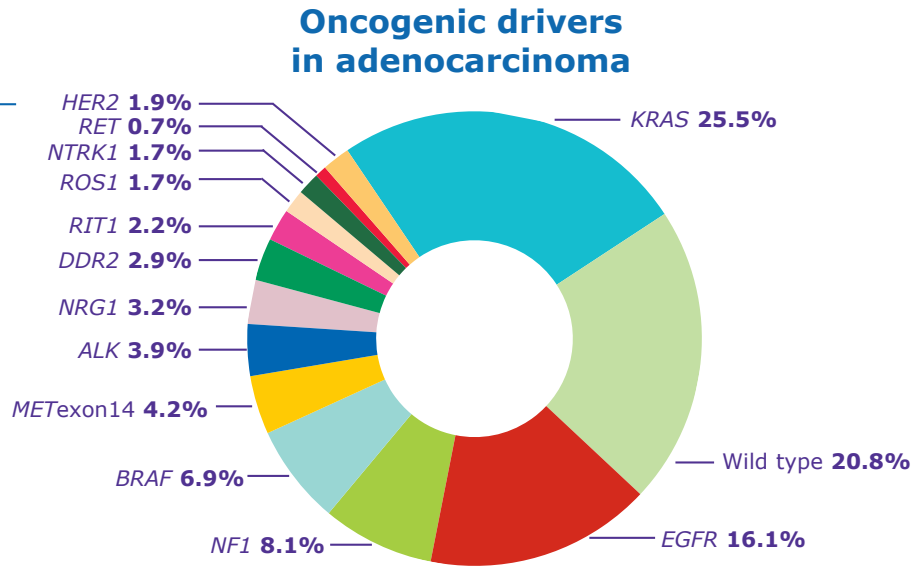
1. National Institutes of Health (NIH), National Cancer Institute. Cancer Stat Facts: Lung and Bronchus Cancer website. www.seer.cancer.gov/statfacts/html/lungb.html. Accessed May 20, 2020. 2. American Cancer Society. What is Lung Cancer? website. https://www.cancer.org/cancer/non-small-cell-lung-cancer/about/what-is-non-small-cell-lung-cancer.html. Accessed May 20, 2020.

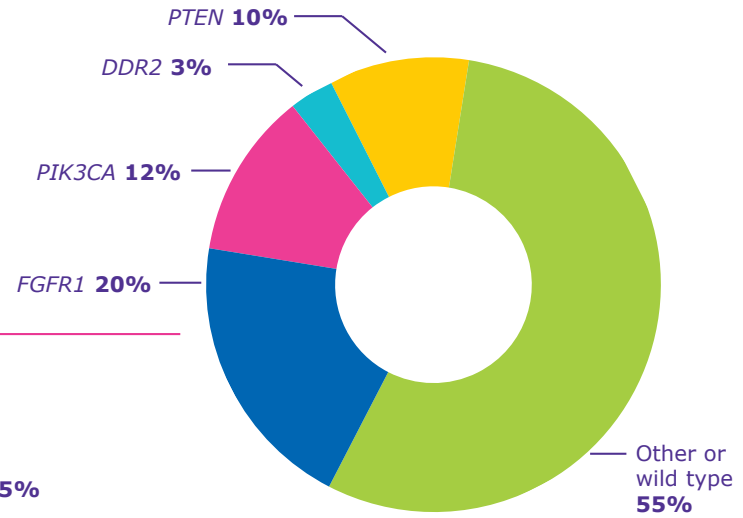
NSCLC is both histologically and genetically diverse

NSCLC distribution by histology[1-3]



Prevalence of genetic alterations in NSCLC[4]





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; *NF1*, neurofibromin 1 gene; *NRG1*, neuregulin 1 gene; NSCLC, non-small cell lung cancer; *NTRK1*, neurotrophic receptor tyrosine kinase 1 gene; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha gene; *PTEN*, phosphatase and tensin homolog gene; *RET*, RET proto-oncogene; *RIT1*, Ras like without CAAX 1 gene; *ROS1*, ROS proto-oncogene 1.

1. American Cancer Society. What is Lung Cancer? website. https://www.cancer.org/cancer/non-small-cell-lung-cancer/about/what-is-non-small-cell-lung-cancer.html. Accessed November 20, 2019. 2. Ou SA, Zell JA. *J Thorac Oncol*. 2009;4:1202–1211. 3. Schrock AB, et al. *J Thorac Oncol.* 2017;12:931–942. 4. Rosell R, Karachaliou N. *Lancet.* 2016;387(10026):1354–1356.

Assessment of genetic alterations in NSCLC

- Numerous gene alterations have been identified in NSCLC, therefore testing lung cancer specimens for these alterations is important[1]

- 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 biomarkers[1,2]

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

1. NCCN Clinical Practice Guidelines in Oncology, NSCLC, v4.2020. Accessed May 5, 2020. 2. Drilon A, et al. *Clin Cancer Res*. 2015;21(16):3831–3639.

CAP-IASLC molecular testing guidelines

"Must-test" biomarkers[1-2]

EGFR

Base substitutions (e.g. p.T790M and p.L858R) and INDELS (e.g. deletion of exon 19) are observed in NSCLC tumor specimens.

ALK

Gene rearrangements are observed in NSCLC tumor specimens, which can generate several different oncogenic fusions (e.g. EML4).

ROS1

Gene rearrangements are observed in NSCLC tumor specimens, which can generate several different oncogenic fusions (e.g. CD74, SLC34A2, CCDC6 and FIG).

"Should-test" biomarkers[1-2]

BRAF

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.

KRAS

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.

ERBB2/HER2

Base substitutions (e.g. L755F) and gene amplification are observed in NSCLC tumor specimens.

RET

Gene rearrangements are observed in NSCLC tumor specimens.

MET

Exon 14 skipping alterations and gene amplification are observed in NSCLC tumor specimens.

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; *EGFR*, 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.

1. Drilon A, et al. *Clin Cancer Res*. 2015;21(16):3831–3639. 2. Lindeman NI, et al. *Journal Thorac Oncol*. 2018;13(3):323–358.

NCCN guidelines for molecular testing of NSCLC[1]

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.
1. NCCN Clinical Practice Guidelines in Oncology, NSCLC, v4.2020. Accessed May 15, 2020.

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 DNA[1]
- Can accommodate smaller samples with lower concentrations of malignant cells[1]
- 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 biopsy[1,3]

Retesting of tissue samples with NGS following a negative result revealed[2]:

- 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.
1. Lindeman NI, et al. *J Thorac Oncol*. 2018;13(3):323–358. 2. Drilon A, et al. *Clin Cancer Res*. 2015;21(16):3831–3639 3. Rolfo C, et al. *J Thorac Oncol*. 2019;13(9):1248–1268.

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)

Advantages of liquid biopsy[1]:

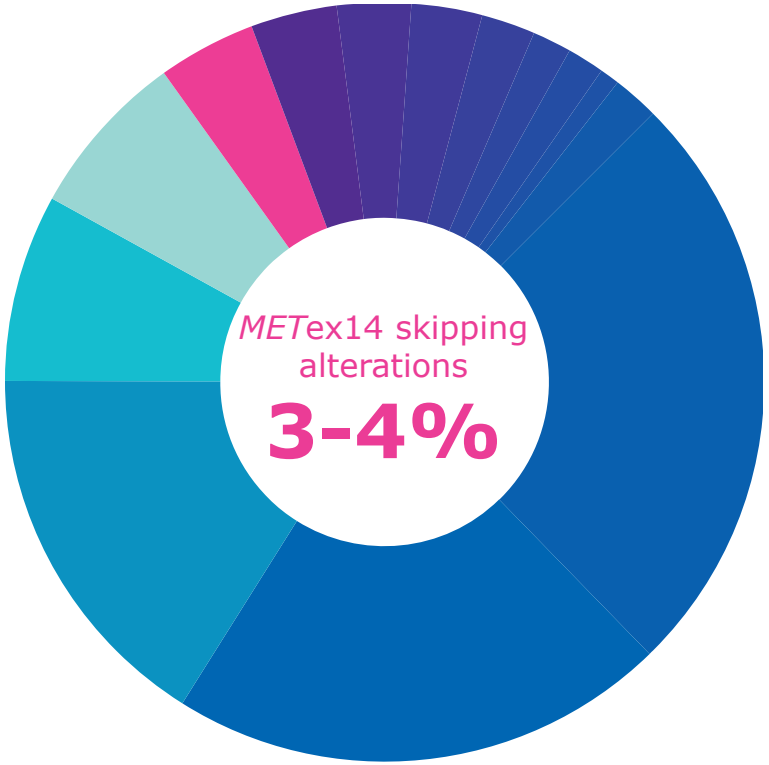
- Enables molecular tumor analysis in patients unable to undergo a biopsy due to suboptimal clinical condition
- Avoids complications associated with computed tomography-guided transthoracic lung biopsies
- Saves tissue biopsy specimens for other analyses
- Decreases cost and sample processing time
-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

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]

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.

1. Rolfo C, et al. *J Thorac Oncol.* 2019;13(9):1248–1268.

MET alterations in the NSCLC landscape



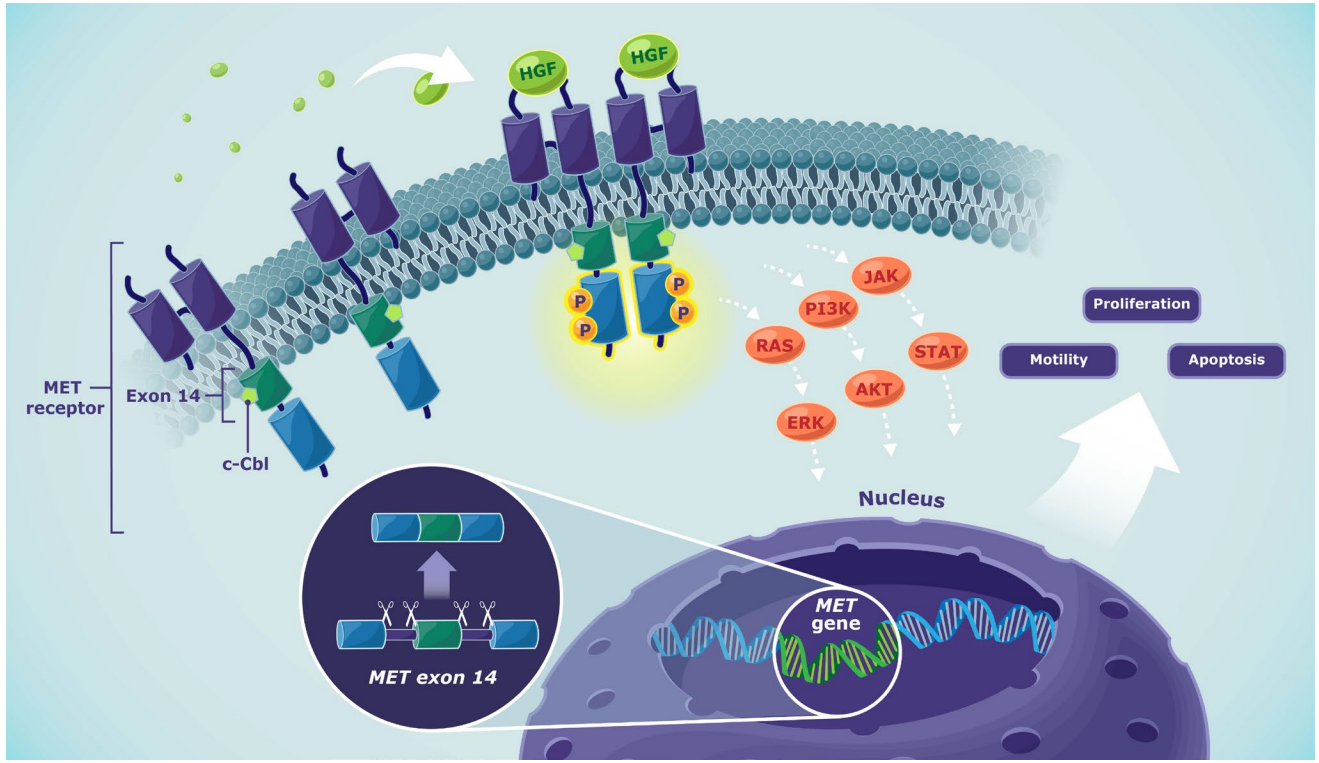
Oncogenic drivers in lung adenocarcinoma[1]

- Alterations in the *MET* gene have been identified as primary oncogenic drivers in NSCLC and are associated with poor prognosis[1-3]:
  - *MET* exon 14 *(MET*ex14) 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 *MET*ex14 skipping alterations
- Strong MET expression is observed in 61% of NSCLC[3]

EGFR, epidermal growth factor receptor; M, methionine; *MET*, MNNG HOS transforming gene; MET, mesenchymal-epithelial transition factor; *MET*ex14, *MET* exon 14; NSCLC, non-small cell lung cancer; T, threonine; TKI, tyrosine kinase inhibitor.

1. Rosell R, Karachaliou N. *Lancet*. 2016;387(10026):1354–1356. 2. Drilon A, et al. *J Thorac Oncol*. 2017;12:15–26. 3. Wu YL, et al. *Cancer Treat Rev*. 2017;61:70–81.

The MET pathway is critical for normal biological processes, based on preclinical studies



MET is a **receptor tyrosine kinase** encoded by the *MET* gene. It is activated by HGF, its only known high affinity ligand.[1,3]

MET signaling is critical for[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[1,2]:

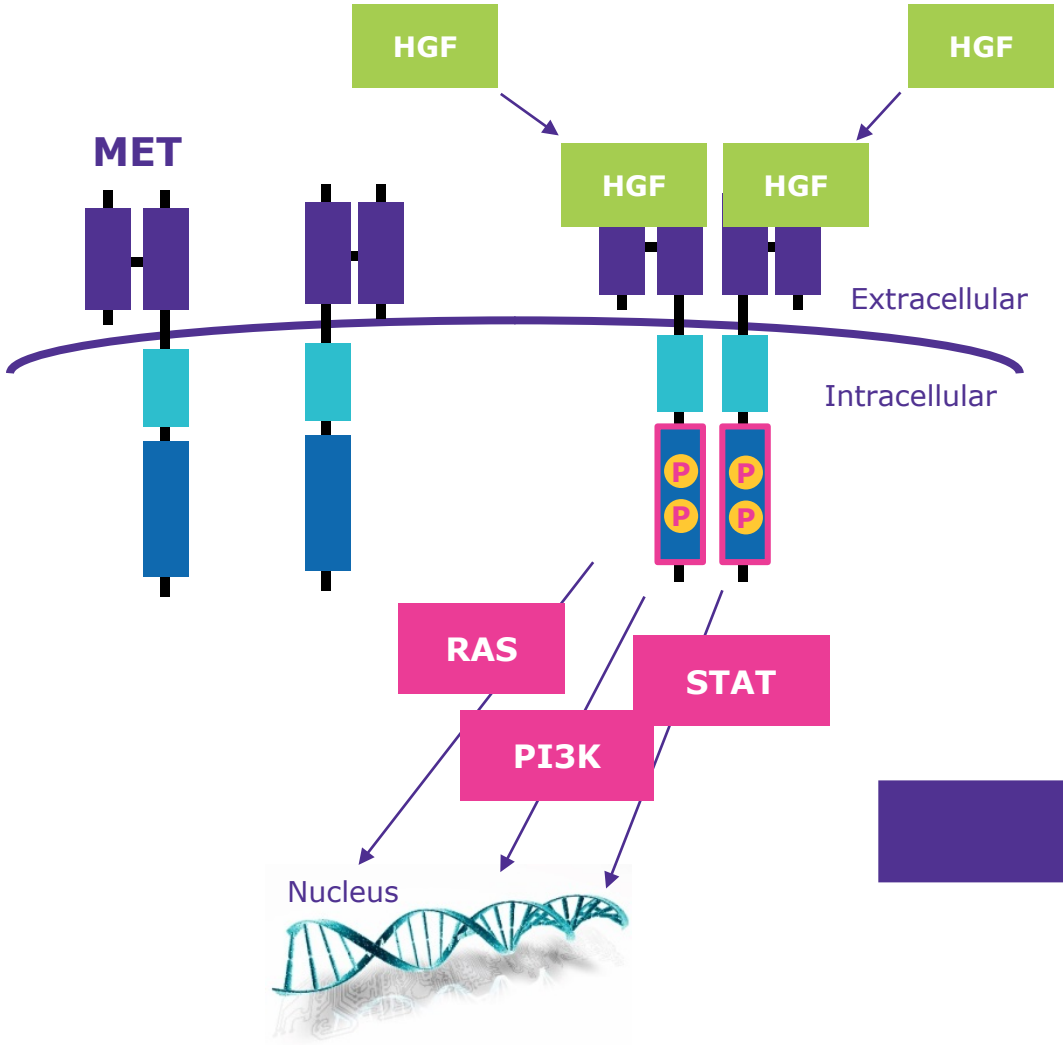
- RAS/ERK/MAPK
- PI3K/AKT
- JAK/STAT
- Wnt/β-catenin

How do *MET*ex14 skipping alterations and *MET* gene amplification lead to oncogenesis?

AKT, protein kinase B; c-Cbl, Casitas B-lineage lymphoma; ERK, extracellular receptor kinase; HGF, hepatocyte growth factor; JAK, Janus kinase; MAPK, mitogen activated protein kinase; *MET*, MNNG HOS transforming gene MET, mesenchymal-epithelial transition factor; PI3K, phosphoinositide 3-kinase; RAS, rat sarcoma GTPase; STAT, signal transducers and activators of transcription; Wnt, wingless integrated.

1. Wu YL, et al. *Cancer Treat Rev*. 2017;61:70–81. 2. Drilon A, et al. *J Thorac Oncol.* 2017;12:15–26. 3. Parikh PK, Ghate MD. *Eur J Med Chem.* 2018;143:1103–38.

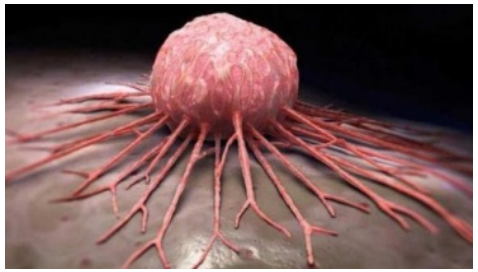
MET signaling can drive tumor growth and progression[1,2]



HGF/MET pathway is frequently deregulated in human cancer[1], leading to dependency on MET signaling, known as "oncogenic addiction."

Deregulation can occur via[1,2]:

- MET and/or HGF overexpression
- *MET* gene amplification
- Activating *MET* alterations, including *MET*ex14 skipping alterations



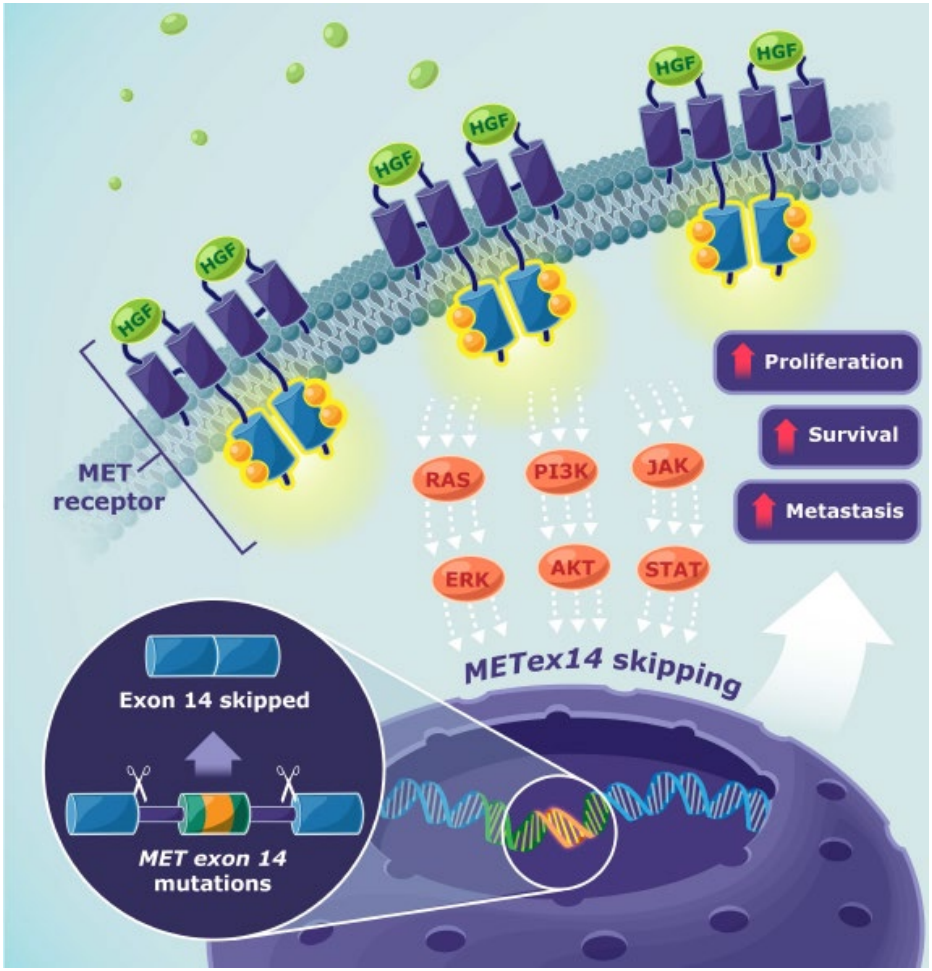
Proliferation | **Survival**

Resistance | **Metastasis**

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

1. Wu YL, et al. *Cancer Treat Rev.* 2017;61:70–81. 2. Drilon A, et al. *J Thorac Oncol.* 2017;12:15–26.

Based on preclinical studies, *MET*ex14 skipping alterations may protect MET receptors from ubiquitination and degradation[1]

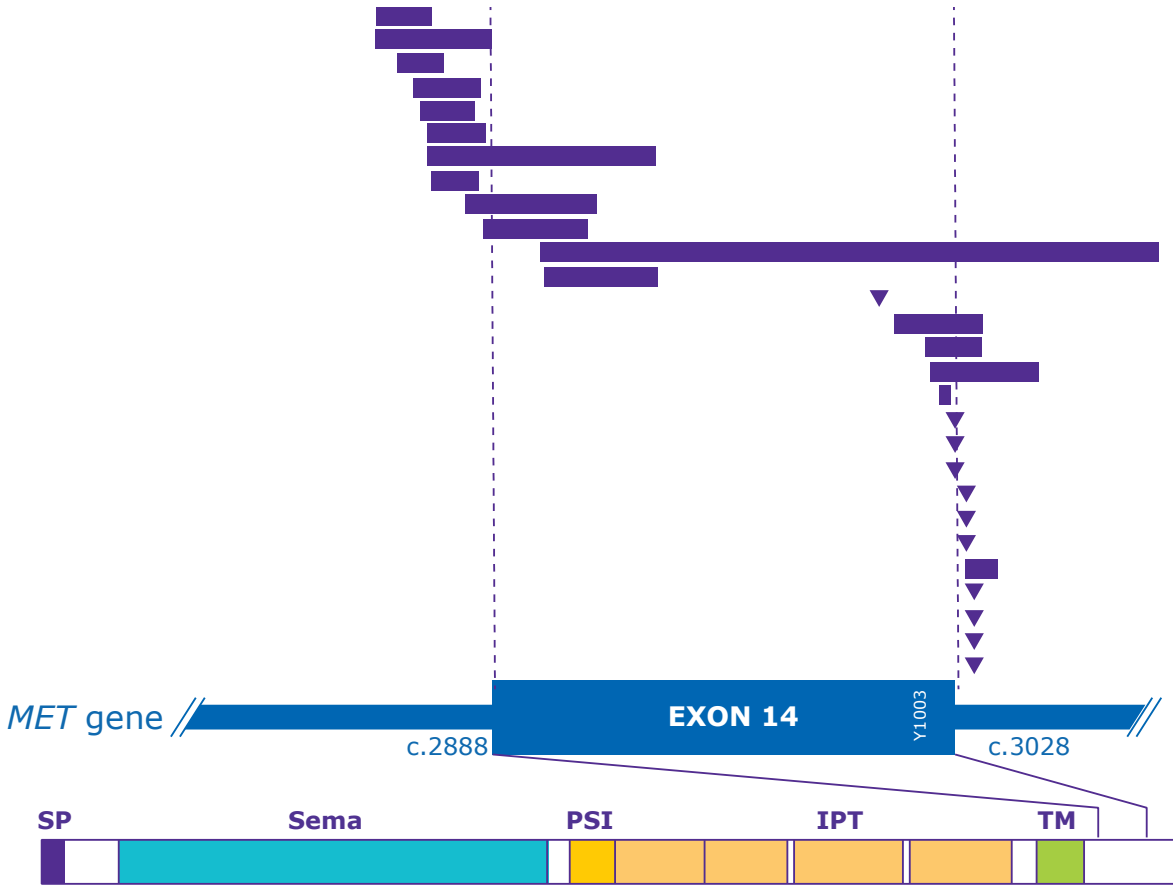


- *MET*ex14 encodes part of the juxtamembrane domain of the MET protein, which contains the c-Cbl E3 ubiquitin ligase-binding site at tyrosine 1003 (Y1003)
    - c-Cbl transfers ubiquitin onto the MET receptor at Y1003, which acts as a flag for degradation
- *MET*ex14 skipping results in MET protein 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; *MET*ex14, *MET* exon 14; PI3K, phosphoinositide 3-kinase; RAS, rat sarcoma; STAT, signal transducers and activators of transcription; Y, tyrosine.

1. Drilon A, et al. *J Thorac Oncol*. 2017;12:15–26.

Diversity of *MET*ex14 skipping alterations: Comprehensive diagnostic testing could be a challenge[1,2]



#	Genetic alteration
22	c.2888-35_288-17del
3	c.2888-35_2888del
20	c.2888-28_2888-14del
16	c.2888-22_2888-2del
7	c.2888-21_2888-5del
13	c.2888-20_2888-1del
25	c.2888-20_2939-1del
5	c.2888-19_2888-3del
8	c.2888-7_2920del
14	c.2888-2_2915del
21	c.2903_3028+67del
6	c.2905_2940del
28	c.3008A>G (p.Y1003C)
18	c.3010_3028+8del
1	c.3018_3028+8del
24	c.3020_3028+24del
10	c.3025_3027GAA>G fs
17	c.3028G>A
15	c.3028G>A
2	c.1702-2A>T + c.3028G>A
4	c.3028+1G>T
11	c.3028+1G>T
27	c.3028+1G>T
19	c.3028+1_3028+9del
12	c.3028+2T>C
9	c.3028+2T>C
23	c.3028+2T>C
26	c.3028+2T>C

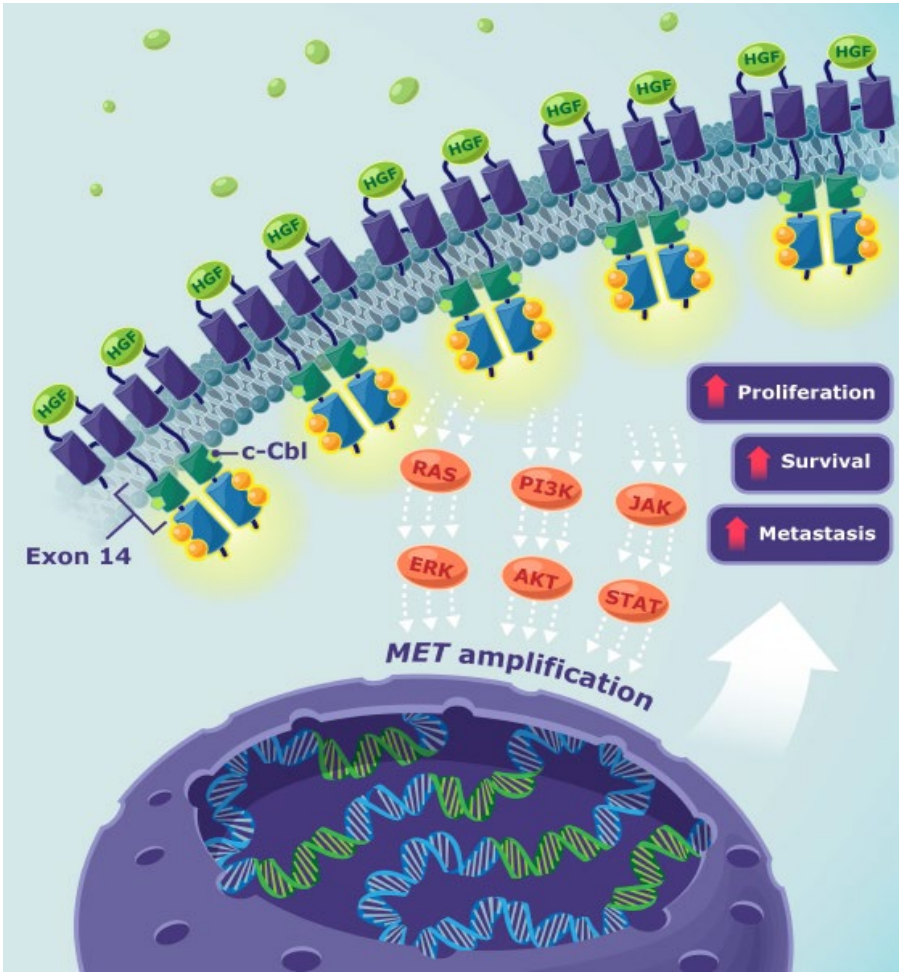
Each rectangle represents a deletion variant and each triangle represents a single nucleotide variant.[2]

- Alterations that result in *MET*ex14 skipping are highly diverse at the DNA level and include[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[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[1]

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; *MET*ex14, 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.

1. Drilon A, et al. *J Thorac Oncol*. 2017;12:15–26. 2. Awad MM, et al. *J Clin Oncol*. 2016;34:721–730.

Amplification of *MET* gene can also increase MET receptor signaling

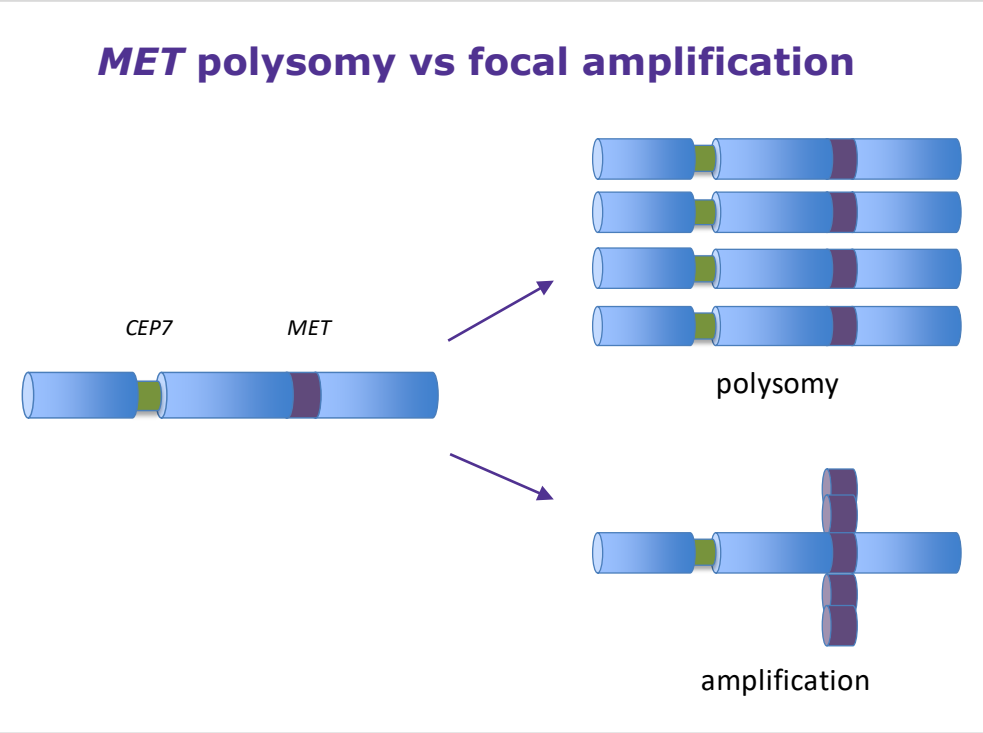


- *MET* gene amplification can occur through two mechanisms[1]:
  - Focal amplification of the *MET* gene
  - Polysomy of chromosome 7
- *MET* amplification increases the number of MET receptors on the cell surface, even with a functional binding site for c-Cbl[2]
- *MET* amplification leads to increased MET signaling and oncogenesis[2]

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

1. Drilon A, et al. *J Thorac Oncol*. 2017;12:15–26. 2. Wu YL, et al. *Cancer Treat Rev*. 2017;61:70–81.

Differentiating *MET* gene amplification from polysomy of chromosome 7



Both polysomy and focal *MET* amplification result in increased MET expression[1]

In patients with chromosome 7 polysomy, chromosome 7 (containing the *MET* gene) is duplicated multiple times[1]

In patients with *MET* amplification, only the *MET* gene is amplified[1]

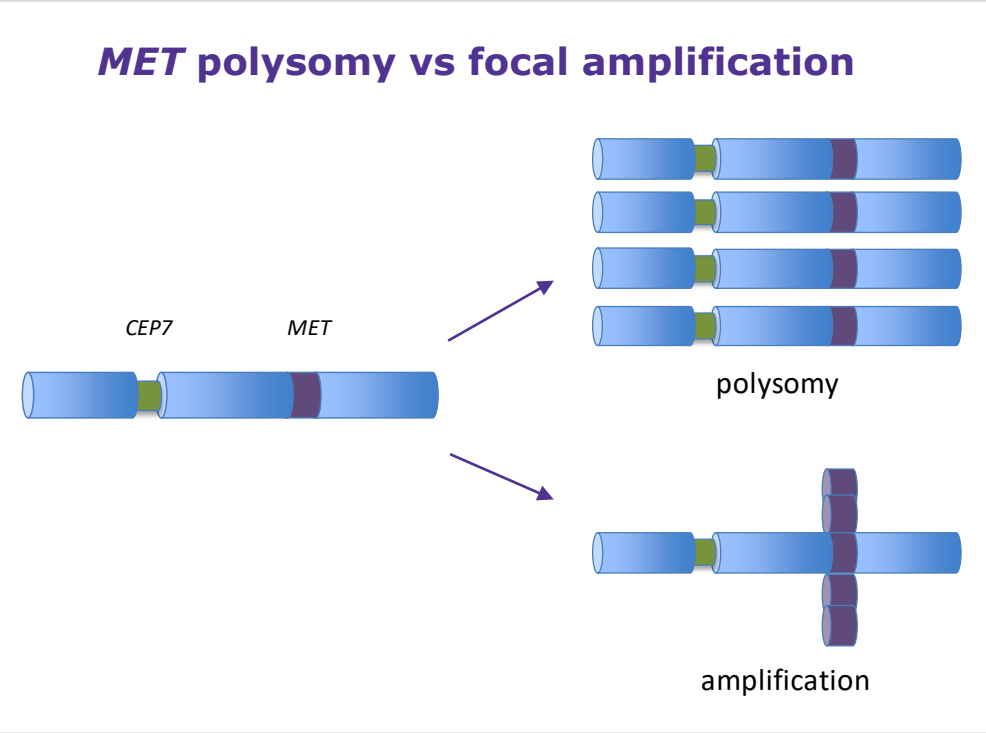
MET amplification is a true oncogenic driver that can be distinguished from polysomy using the *MET/CEP7* ratio[1]:

- 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*

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

1. Drilon A, et al. *J Thorac Oncol*. 2017;12:15–26.

Defining the high-level genomic amplification of *MET*



Both polysomy and focal *MET* amplification result in increased MET expression[1]

- *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]

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.

1. Drilon A, et al. *J Thorac Oncol*. 2017;12:15–26.

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*ex14 skipping alterations and identify high-level *MET* amplification as an emerging biomarker to assess in broad molecular profiling[6]
- Both *MET*ex14 skipping alterations and *MET* gene amplification may increase MET receptor signaling, potentially driving NSCLC tumorigenesis[7,8]
- Diagnostic testing for *MET*ex14 skipping alterations could be challenging due to the diversity of alterations[8,9]

ALK, anaplastic lymphoma kinase gene; *BRAF*, V-raf murine sarcoma homolog B gene; CAP, College of American Pathologists; *EGFR*, epidermal growth factor receptor gene; *ERBB2*, erb-b2 receptor tyrosine kinase 2 gene; *HER2*, human epidermal receptor 2 gene; IALSC, International Association for the Study of Lung Cancer; *KRAS*, kirsten rat sarcoma viral oncogene homolog; *MET*, MNNG HOS transforming gene; MET, mesenchymal-epithelial transition factor; *MET*ex14, *MET* exon 14; NCCN, National Comprehensive Cancer Network; NSCLC, non-small cell lung cancer; *RET*, RET proto-oncogene; *ROS1*, ROS proto-oncogene 1.

1. National Institutes of Health (NIH), National Cancer Institute. Cancer Stat Facts: Lung and Bronchus Cancer website. www.seer.cancer.gov/statfacts/html/lungb.html. Accessed November 20, 2019. 2. Ou SA, Zell JA. *J Thorac Oncol*. 2009;4:1202–1211. 3. Schrock AB, et al. *J Thorac Oncol.* 2017;12:931–942. 4. Rosell R, Karachaliou N. *Lancet.* 2016;387(10026):1354–1356. 5. Lindeman NI, et al. *J Thorac Oncol*. 2018;13(3):323–358. 6. NCCN Clinical Practice Guidelines in Oncology, NSCLC, v4.2020. Accessed May 20, 2020. 7. Wu YL, et al. *Cancer Treat Rev.* 2017;61:70–81. 8. Drilon A, et al. *J Thorac Oncol.* 2017;12:15–26. 9. Awad MM, et al. *J Clin Oncol*. 2016;34:721–730.