



IASLC ATLAS OF MOLECULAR TESTING FOR TARGETED THERAPY IN LUNG CANCER



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Foreword

When the International Association for the Study of Lung Cancer (IASLC) was founded 50 years ago, the vision was to create an organization to bring together people focused on understanding and treating lung cancer. IASLC has grown into a strong global multidisciplinary organization that facilitates collaborations between members and provides opportunities to advance the field. One of the strongest and most active committees of IASLC is the pathology committee, known for its incredible work in establishing the staging criteria for lung cancer and other thoracic malignancies and for publishing standards on key pathologic topics such as comprehensive testing guidelines for ALK positivity in the setting of lung cancer and comparing modalities to standardize PD-L1 testing. More recently, the *2020 IASLC Atlas of Diagnostic Immunohistochemistry* summarized state-of-the-art developments in immunohistochemistry (IHC). This new atlas expands into the area of molecular diagnostics with a truly international editorial board of globally recognized experts who have guided the work of an incredible author list.

As IASLC has grown over the years to include prominent voices from the patient advocate community, it is fitting that this atlas starts with a chapter on perspectives from the patient advocates, helping us to remember how knowledge in this atlas directly provides benefit to patients living with lung cancer.

At the heart, this book is a practical guide for physicians working in the world of lung cancer. One cannot discuss the evaluation of pathologic specimens without a review of best techniques for specimen acquisition, and thus the second chapter is of wide utility with perspectives from pulmonologists, surgeons, interventional radiologists, and pathologists. This atlas also provides a significant chapter on liquid biopsy, which is increasingly critical in optimal management of patients with non-small cell lung carcinoma (NSCLC), particularly when tissue is scarce and difficult to obtain. The authors explore global perspectives on molecular testing with reference back to other published guidelines. The majority of the atlas is broken down by particular genes of interest including *EGFR*, *ALK*, *ROS1*, *KRAS*, and others and emerging targets such as *NRG1*. The authors end with a focus on molecular-testing algorithms and future perspectives.

This *IASLC Atlas of Molecular Testing* in NSCLC is an incredible reference, putting the latest developments in molecular testing at the fingertips of readers. Optimal therapy of lung cancer requires up-to-date knowledge of the latest advances in the molecular underpinnings of lung cancer, as well as how to understand those alterations and their clinical relevance

so that information can be used to provide the best possible care for patients. This atlas is a concise and practical guide for all pathologists, pulmonologists, surgeons, interventional radiologists, medical oncologists, allied health professionals, advocates, and others working to provide optimal care for patients living with lung cancer.

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Abbreviations

The following abbreviations are used in the text:

ACMG: American College of Medical Genetics and Genomics

ADC: antibody-drug conjugate

AHRQ: US Agency for Healthcare Research and Quality

ALK: anaplastic lymphoma kinase

ALKi: ALK tyrosine kinase inhibitor

AMP: anchored multiplex polymerase chain reaction

AMP: Association for Molecular Pathology

APC: adenomatous polyposis coli

AR: acquired resistance

AREG: amphiregulin

ARMS: allele-refractory mutation system

ASCO: American Society of Clinical Oncology

ATP: adenosine triphosphate

BRAF: V-raf murine sarcoma viral oncogene homolog B

B-TMB: blood-based tumor mutational burden

CAP: College of American Pathologists

CAR T-cell: chimeric antigen receptor T cell

CDx: companion diagnostic

CEP: chromosome enumeration probe

cfDNA: cell-free DNA

CHIP: clonal hematopoiesis of indeterminate potential

CI: confidence interval

CML: chronic myeloid leukemia

CNB: core needle biopsy

CNS: central nervous system

CPI: checkpoint inhibitor

CPK: creatinine phosphokinase

CT: computed tomography

ctDNA: circulating tumor DNA

DAPI: 4',6-diamidino-2-phenylindole

DCR: disease control rate

ddPCR: droplet digital polymerase chain reaction

del: deletion

DFG: aspartic acid, phenylalanine, glycine
dNTP: deoxynucleotide triphosphate
DOR: duration of response
EBM: evidence-based medicine
EBUS: endobronchial ultrasound
ECD: extracellular domain
EDTA: ethylenediaminetetraacetic acid
EGF: epidermal growth factor
EGFR: epidermal growth factor receptor
ELCC: European Lung Cancer Congress
ELISA: enzyme-linked immunosorbent assay
EMA: European Medicines Agency
EML4: echinoderm microtubule-associated protein-like 4
EMN: electromagnetic navigation
EQA: external quality assessment
ERBB2: erb-b2 receptor tyrosine kinase 2
ERK: extracellular signal-regulated kinase
ESMO: European Society for Medical Oncology
EU: European Union
18 FDG-PET: fluorine-18-fluorodeoxyglucose positron emission tomography
FDA: US Food and Drug Administration
FFPE: formalin-fixed paraffin-embedded
FGFR-1: fibroblast growth factor receptor-1
FISH: fluorescence in situ hybridization
FNA: fine-needle aspiration
GAP: GTPase-activating protein
GCN: gene copy number
GDNF: glial-derived neurotrophic factor
GDP: guanosine diphosphate
GEF: guanine nucleotide exchange factor
GEJ: gastroesophageal junction
GFRAL: GDNF family receptor α -like
GI: gastrointestinal
GLIDES: GuideLines Into Decision Support
GRADE: Grading of Recommendations Assessment, Development and Evaluation
GTP: guanosine triphosphate
GTPase: guanosine-triphosphate hydrolase or guanosine triphosphatase
H&E: hematoxylin and eosin
HER2: human epidermal growth factor receptor 2
HGF: hepatocyte growth factor
HR: hazard ratio
HSP: heat shock protein
IASLC: International Association for the Study of Lung Cancer
ICI: immune checkpoint inhibitor
IGFR-1: insulin-like growth factor receptor-1
IHC: immunohistochemistry
ILD: interstitial lung disease
IMA: invasive mucinous adenocarcinoma

indel: insertion and/or deletion
ins: insertion
IOM: Institute of Medicine
IPASS: International Post-Authorization Surveillance Study
ISH: in situ hybridization
IVCS: intravenous conscious sedation
IVD: in vitro diagnostic
JAK: Janus kinase
JLCS: Japanese Lung Cancer Society
JMD: juxtamembrane domain
KD: kinase domain
kDa: kilodalton
KDD: kinase domain duplication
KRAS: Kristen rat sarcoma viral oncogene
LDCT: low-dose computed tomography
LSI: locus-specific identification
LuCE: Lung Cancer Europe
MAPK: mitogen-activated protein kinase
MDT: multidisciplinary team
MEK: mitogen-activated protein kinase (MAPK) kinase
MEKi: MEK1/2 inhibitor
MEN: multiple endocrine neoplasia
MET: MET protooncogene receptor tyrosine kinase
mIF: multiplex immunofluorescence
mOS: median overall survival
mPFS: median progression-free survival
MRD: minimal residual disease
MRFF: Medical Research Future Fund
mRNA: messenger RNA
MTB: molecular tumor board
MTC: medullary thyroid carcinoma
mTOR: mechanistic target of rapamycin kinase
NCCN: National Comprehensive Cancer Network
NGS: next-generation sequencing
NHMRC: National Health and Medical Research Council
NIR: near-infrared
NK cell: natural killer cell
NLST: National Lung Screening Trial
NOS: not otherwise specified
NRG1: neuregulin-1
NSCLC: non-small cell lung carcinoma
NTRK: neurotrophic tropomyosin receptor kinase
ORR: overall response rate
OS: overall survival
PA: aortopulmonary
PCR: polymerase chain reaction
PD-L1: programmed death–ligand 1
PFS: progression-free survival

PI3K: phosphoinositide 3-kinase
PKC: protein kinase C
PLC γ : phospholipase C-gamma
PSI: plexin-semaphorin-integrin
PTEN: phosphatase and tensin homolog
RAS: rat sarcoma viral oncogene homolog
RATS: robotic-assisted thoracoscopic surgery
RET: rearranged during transfection
RNAseq: RNA sequencing
ROS1: c-ros oncogene 1
ROSE: rapid onsite evaluation
RR: response rate
RTK: receptor tyrosine kinase
RT-PCR: reverse transcription polymerase chain reaction
SCLC: small cell lung carcinoma
SNV: single nucleotide variant
SOC: standard of care
STAT: signal transducer and activator of transcription
SV: structural variant
TAT: turnaround time
TBNA: transbronchial needle aspiration
TGF α : transforming growth factor α
TIL: tumor infiltrating lymphocyte
TKD: tyrosine kinase domain
TKI: tyrosine kinase inhibitor
TM: transmembrane
TMB: tumor mutational burden
TMD: transmembrane domain
TPS: tumor proportion score
TRK: tropomyosin receptor kinase
TROP2: trophoblast cell surface antigen 2
TTF1: thyroid transcription factor 1
TTNB: transthoracic needle biopsy
US: United States
VATS: video-assisted thoracoscopic surgery
VBN: virtual bronchoscopic navigation
VEGF: vascular endothelial growth factor
VEGFR2: vascular endothelial growth factor receptor 2
VUS: variant(s) of unknown significance or variant(s) of uncertain clinical significance
WBC: white blood cell
WES: whole exome sequencing
WGS: whole genome sequencing

Introduction

1

By Lynette M. Sholl, Wendy A. Cooper, Keith M. Kerr, Daniel SW Tan, Ming-Sound Tsao, and James Chih-Hsin Yang

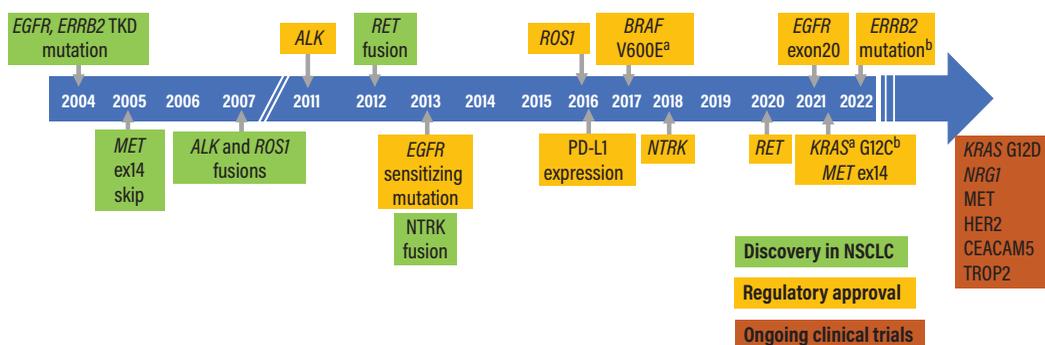
Over the past 2 decades, biomarker testing for lung cancer patients has gone from nonexistent and of limited clinical relevance to widespread and essential for routine oncologic management. What has prompted this evolution from one-size-fits-all chemotherapy to highly tailored precision therapy? A number of insights and technical advances have fueled this progress, starting with the recognition that some cancers harbor alterations leading to uncontrolled signaling through protein kinases. What followed was the revolutionary and initially controversial concept of targeting tyrosine kinases with small-molecule tyrosine kinase inhibitors (TKIs) to halt the growth and proliferation of neoplastic cells.

The first TKI therapy in clinical use was the ABL inhibitor imatinib in patients with chronic myeloid leukemia (CML), a relatively genomically homogenous neoplasm driven by the *BCR::ABL1* fusion event. This first targeted therapy was a spectacular success, with many patients achieving rapid symptomatic relief and long-term remission (and possibly even cure) with minimal drug toxicity. From here, the race was on to identify other examples of tumors with kinase dependencies and the therapies that could specifically interfere with these key kinase signaling pathways.

One factor driving the astonishing success of imatinib in patients with CML was the robust understanding of the structure and function of ABL, as well as the role of the oncogenic fusion event that drove its activity in the neoplastic cells. Importantly, imatinib did not work in just any patient with leukemia, but only in those patients whose tumors harbored an *ABL* fusion or some other kinase-driven oncogenic event with sensitivity to this particular inhibitor. Thus, the field of rational, or biomarker-driven, cancer care was born. This demanded not only an accurate histopathologic diagnosis, but precise genomic characterization, thus pushing molecular diagnosis from its initial focus on diagnostic biomarkers to a growing focus on predictive testing to direct treatment decisions.

With these principles in mind, this atlas reflects 2 decades of extraordinary progress in lung cancer biomarker and drug discovery, beginning with the recognition that *EGFR*

kinase domain mutations predict responsiveness to EGFR TKIs. Today, literally dozens of rational therapies targeting mostly tyrosine kinases and mitogenic pathway members are available for lung cancer patients whose tumors harbor certain well-defined oncogenic alterations (Figure 1-1; Table 1-1). This diversification of personalized, targeted treatment has been accompanied by demonstrable improvement in outcomes for patients receiving the appropriate drugs. The development of these targeted therapies was made possible in large part by the tremendous advances in tumor genome characterization resulting from massively parallel sequencing technologies. These technologies of scale—borrowing from the principles of semiconductor evolution—have driven down the cost of genomic sequencing by several orders of magnitude since the 1990s and permitted institutional, national, and



^a Dates of discovery are not shown on the timeline; *KRAS* mutations first observed in NSCLC in 1984; *BRAF* mutations in 2002.

^b Indicates approval in the second-line setting only.

For clinical trials supporting the first- (or second-) line regulatory approval, see references.¹⁻⁸

Biomarker discovery reports are listed in the references.⁹⁻¹⁷

Figure 1-1. The timeline of biomarker-dependent US Food and Drug Administration (FDA) drug approvals in the first-line setting for patients with advanced NSCLC. Abbreviations: NSCLC = non-small cell lung carcinoma; TKD = tyrosine kinase domain.

Table 1-1. Biomarker-Driven Therapies Significantly Improve Survival of Advanced NSCLC Patients

Target	Drug approval by FDA	Line of therapy	Median OS (m)
No biomarker	Chemotherapy ¹⁸	1L	8-10
EGFR (1G) ^{19,20}	Erlotinib, gefitinib	1L	21.6-35.5 ^a
EGFR (2G) ^{21,22}	Afatinib, dacomitinib	1L	31.4-34.1
EGFR (3G) ²⁰	Osimertinib	1L	38.6
ALK fusion ²³⁻²⁶	Crizotinib, ceritinib, alectinib, brigatinib, ^b lorlatinib ^b	1L	>51
ROS1 fusion ^{23,27}	Crizotinib, entrectinib ^c	≥1L	51.4; 47.8 ^b
BRAF V600E ²⁸	Dabrafenib + trametinib	1L	17.3
TRK fusion ^{29,30}	Larotrectinib, entrectinib	≥2L	40.7; NE
RET fusion ^{31,32}	Selpercatinib, pralsetinib	1L	b
PD1/PD-L1 ³³	Nivolumab, pembrolizumab, atezolizumab	1L	26.3 ^d

Abbreviations: 1L = first line; 2L = second line; FDA = US Food and Drug Administration; NSCLC = non-small cell lung carcinoma; OS = overall survival; TKI = tyrosine kinase inhibitor.

^aPatients may have received third-generation EGFR TKI upon progression.

^bImmature OS data.

^cEntrectinib analysis performed on ROS1 TKI-naïve cohort.

^dPembrolizumab with PD-L1 ≥50%.

multinational efforts such as The Cancer Genome Atlas to systematically define the genomic and transcriptomic alterations underlying most common tumor types. At the same time, commercialization of both focused and comprehensive sequencing technologies has made tumor genomic profiling relatively accessible to cancer patients in most developed countries.

Challenges remain, however, in providing timely, accurate, and clinically relevant biomarker testing for patients with lung cancer. The sheer diversity of mechanisms by which a tumor can hijack mitogenic pathways to drive growth and proliferation requires that laboratories develop and validate a host of strategies targeting DNA, RNA, and/or protein to ensure complete biomarker testing.

This diversity is exemplified by genes that undergo rearrangement (*ALK*, *ROS1*, *RET*, *NTRK1-3*, *NRG1*, and others) or acquire intragenic “fusions” driven by aberrant splicing (*MET*). At the DNA level, rearrangements typically result from breakages in the intronic DNA. These “breakpoints” are unpredictable and occur across a broad region of the genome, confounding detection in the DNA. In contrast, the oncogenic products of these rearrangements give rise to highly reproducible fusion transcripts, facilitating their detection from tumor RNA. While the diversity of mutagenesis may be more limited in those genes prone to single nucleotide variants or small insertion deletions (*EGFR*, *KRAS*, *BRAF*, *ERBB2*), novel or rare mechanisms of oncogenic activation continue to be discovered, requiring expert molecular genetic annotation. The “real-time” discovery that comes with routine sequencing of patient samples requires an integrated strategy for reporting that today often takes the form of multidisciplinary meetings, where the pathologist, oncologist, proceduralist, and entire patient care team can leverage biomarker data to guide management (see Figure 2-2).

Despite evidence that treatment with most targeted therapies leads to the best clinical outcomes when given in the first line, many patients still receive chemotherapy and/or immunotherapy before targeted therapy, even with approvals for use of the latter in the first-line setting. The failure to employ targeted therapies can be attributed in part to lack of biomarker testing and/or delays in receipt of timely biomarker results. Biomarker testing from tissue is frequently complicated by limited tissue quality or quantity; while this challenge can be overcome in part by active communication between proceduralist and pathologist during tissue acquisition, this requires a measure of coordination and expertise that is not available in all environments.

The increasing reliability and sensitivity of circulating tumor DNA testing derived from patient plasma has both improved access to and decreased time to biomarker results. This testing is not available in all regions of the world, however, and can be difficult to access because of economic constraints even when technically available. Furthermore, this approach does not, alone, reliably cover all of the required biomarkers and has sensitivity that is limited by the extent of tumor DNA shed into the circulation. This points to the critical role of advocacy to demand better access to reliable testing and targeted therapies across the globe.

Last but not least, in this *IASLC Atlas of Molecular Testing for Targeted Therapy in Lung Cancer*, we have used the terms “molecular,” “biomarker,” “molecular biomarker,” and “predictive biomarker” somewhat interchangeably in the context of targeted therapy in lung cancer. Despite the fact that each of these terms may have more specific and slightly different meanings, and notwithstanding a recent proposal to harmonize the terminologies to “biomarker testing,”³⁴ they are frequently used in a synonymous fashion. The term “mutation

testing” has more specific meaning than biomarker/molecular testing, as it tends to be used for tests at the gene level, while biomarkers or molecules may include genes and/or proteins. Ideally, a consensus will be reached in the future on terms that are ubiquitously accepted and understood by all stakeholders who may benefit from this atlas.

It is our hope that this atlas, authored by international experts in lung cancer diagnostics and treatment, will help a broad audience understand the foundations of molecular abnormalities in lung cancer that help direct targeted therapy used in patients with non-small cell lung carcinoma today.

References

1. Paez JG, Jänne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science*. 2004;304(5676):1497-1500. doi:10.1126/science.1099314.
2. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med*. 2004;350(21):2129-2139. doi:10.1056/NEJMoa040938.
3. Stephens P, Hunter C, Bignell G, et al. Lung cancer: intragenic ERBB2 kinase mutations in tumours. *Nature*. 2004;431(7008):525-526. doi:10.1038/431525b.
4. Ma PC, Jagadeeswaran R, Jagadeesh S, et al. Functional expression and mutations of c-Met and its therapeutic inhibition with SU11274 and small interfering RNA in non-small cell lung cancer. *Cancer Res*. 2005;65(4):1479-1488. doi:10.1158/0008-5472.CAN-04-2650.
5. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature*. 2007;448(7153):561-566. doi:10.1038/nature05945.
6. Rikova K, Guo A, Zeng Q, et al. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. *Cell*. 2007;131(6):1190-1203. doi:10.1016/j.cell.2007.11.025.
7. Wang R, Hu H, Pan Y, et al. RET fusions define a unique molecular and clinicopathologic subtype of non-small-cell lung cancer. *J Clin Oncol*. 2012;30(35):4352-4359. doi:10.1200/JCO.2012.44.1477.
8. Vaishnavi A, Capelletti M, Le AT, et al. Oncogenic and drug-sensitive NTRK1 rearrangements in lung cancer. *Nat Med*. 2013;19(11):1469-1472. doi:10.1038/nm.3352.
9. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol*. 2012;13(3):239-246. doi:10.1016/S1470-2045(11)70393-X.
10. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer [published correction appears in *N Engl J Med*. 2011 Feb 10;364(6):588]. *N Engl J Med*. 2010;363(18):1693-1703. doi:10.1056/NEJMoa1006448.
11. Shaw AT, Ou SH, Bang YJ, et al. Crizotinib in ROS1-rearranged non-small-cell lung cancer. *N Engl J Med*. 2014;371(21):1963-1971. doi:10.1056/NEJMoa1406766.
12. Planchard D, Besse B, Groen HJM, et al. Dabrafenib plus trametinib in patients with previously treated BRAF(V600E)-mutant metastatic non-small cell lung cancer: an open-label, multicentre phase 2 trial. *Lancet Oncol*. 2016;17(7):984-993. doi:10.1016/S1470-2045(16)30146-2.
13. Drilon A, Laetsch TW, Kummar S, et al. Efficacy of larotrectinib in TRK fusion-positive cancers in adults and children. *N Engl J Med*. 2018;378(8):731-739. doi:10.1056/NEJMoa1714448.
14. Wolf J, Seto T, Han JY, et al. Capmatinib in MET exon 14-mutated or MET-amplified non-small-cell lung cancer. *N Engl J Med*. 2020;383(10):944-957. doi:10.1056/NEJMoa2002787.
15. Paik PK, Felip E, Veillon R, et al. Tepotinib in non-small-cell lung cancer with MET exon 14 skipping mutations. *N Engl J Med*. 2020;383(10):931-943. doi:10.1056/NEJMoa2004407.
16. Skoulidis F, Li BT, Dy GK, et al. Sotorasib for lung cancers with KRAS p.G12C mutation. *N Engl J Med*. 2021;384(25):2371-2381. doi:10.1056/NEJMoa2103695.

17. Li BT, Smit EF, Goto Y, et al. Trastuzumab deruxtecan in HER2-mutant non-small-cell lung cancer. *N Engl J Med.* 2022;386(3):241-251. doi:10.1056/NEJMoa2112431.
18. Schiller JH, Harrington D, Belani CP, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N Engl J Med.* 2002;346(2):92-98. doi:10.1056/NEJMoa011954.
19. Lee CK, Davies L, Wu YL, et al. Gefitinib or erlotinib vs chemotherapy for EGFR mutation-positive lung cancer: individual patient data meta-analysis of overall survival. *J Natl Cancer Inst.* 2017;109(6):10.1093/jnci/djw279. doi:10.1093/jnci/djw279.
20. Ramalingam SS, Vansteenkiste J, Planchard D, et al. Overall survival with osimertinib in untreated, EGFR-mutated advanced NSCLC. *N Engl J Med.* 2020;382(1):41-50. doi:10.1056/NEJMoa1913662.
21. Yang JC, Wu YL, Schuler M, et al. Afatinib versus cisplatin-based chemotherapy for EGFR mutation-positive lung adenocarcinoma (LUX-Lung 3 and LUX-Lung 6): analysis of overall survival data from two randomised, phase 3 trials. *Lancet Oncol.* 2015;16(2):141-151. doi:10.1016/S1470-2045(14)71173-8.
22. Mok TS, Cheng Y, Zhou X, et al. Updated overall survival in a randomized study comparing dacomitinib with gefitinib as first-line treatment in patients with advanced non-small-cell lung cancer and EGFR-activating mutations. *Drugs.* 2021;81(2):257-266. doi:10.1007/s40265-020-01441-6.
23. Drilon A, Chiu CH, Fan Y, et al. Long-term efficacy and safety of entrectinib in ROS1 fusion-positive NSCLC. *JTO Clin Res Rep.* 2022;3(6):100332. doi:10.1016/j.jtocrr.2022.100332.
24. Nishio M, Felip E, Orlov S, et al. Final overall survival and other efficacy and safety results from ASCEND-3: phase II study of ceritinib in ALKi-naive patients with ALK-rearranged NSCLC. *J Thorac Oncol.* 2020;15(4):609-617. doi:10.1016/j.jtho.2019.11.006.
25. Camidge DR, Kim HR, Ahn MJ, et al. Brigatinib versus crizotinib in ALK inhibitor-naive advanced ALK-positive NSCLC: final results of phase 3 ALTA-1L trial [published correction appears in *J Thorac Oncol.* 2022 Oct 14]. *J Thorac Oncol.* 2021;16(12):2091-2108. doi:10.1016/j.jtho.2021.07.035.
26. Solomon BJ, Bauer TM, Mok TSK, et al. Efficacy and safety of first-line lorlatinib versus crizotinib in patients with advanced, ALK-positive non-small-cell lung cancer: updated analysis of data from the phase 3, randomised, open-label CROWN study. *Lancet Respir Med.* 2022;S2213-2600(22)00437-4. doi:10.1016/S2213-2600(22)00437-4.
27. Shaw AT, Riely GJ, Bang YJ, et al. Crizotinib in ROS1-rearranged advanced non-small-cell lung cancer (NSCLC): updated results, including overall survival, from PROFILE 1001. *Ann Oncol.* 2019;30(7):1121-1126. doi:10.1093/annonc/mdz131.
28. Planchard D, Besse B, Groen HJM, et al. Phase 2 study of dabrafenib plus trametinib in patients with BRAF V600E-mutant metastatic NSCLC: updated 5-year survival rates and genomic analysis. *J Thorac Oncol.* 2022;17(1):103-115. doi:10.1016/j.jtho.2021.08.011.
29. Drilon A, Tan DSW, Lassen UN, et al. Efficacy and safety of larotrectinib in patients with tropomyosin receptor kinase fusion-positive lung cancers. *JCO Precis Oncol.* 2022;6:e2100418. doi:10.1200/PO.21.00418.
30. Demetri GD, De Braud F, Drilon A, et al. Updated integrated analysis of the efficacy and safety of entrectinib in patients with NTRK fusion-positive solid tumors [published correction appears in *Clin Cancer Res.* 2022;28(10):2196]. *Clin Cancer Res.* 2022;28(7):1302-1312. doi:10.1158/1078-0432.CCR-21-3597.
31. Drilon A, Subbiah V, Gautschi O, et al. Selpercatinib in patients with RET fusion-positive non-small-cell lung cancer: updated safety and efficacy from the registrational LIBRETTO-001 phase I/II trial. *J Clin Oncol.* 2023;41(2):385-394. doi:10.1200/JCO.22.00393.
32. Griesinger F, Curigliano G, Thomas M, et al. Safety and efficacy of pralsetinib in RET fusion-positive non-small-cell lung cancer including as first-line therapy: update from the ARROW trial. *Ann Oncol.* 2022;33(11):1168-1178. doi:10.1016/j.annonc.2022.08.002.
33. Reck M, Rodríguez-Abreu D, Robinson AG, et al. Five-year outcomes with pembrolizumab versus chemotherapy for metastatic non-small-cell lung cancer with PD-L1 tumor proportion score ≥ 50 . *J Clin Oncol.* 2021;39(21):2339-2349. doi:10.1200/JCO.21.00174.
34. Martin NA, Tepper JE, Giri VN, et al. Adopting consensus terms for testing in precision medicine. *JCO Precis Oncol.* 2021;5:PO.21.00027. doi:10.1200/PO.21.00027.

Clinical Relevance of Biomarker Testing in Lung Cancer

2

By Benjamin J. Solomon, Paul J. Hesketh, Lyudmila Bazhenova, Shani Shilo, and Lecia V. Sequist

Concept of Personalized Therapy for Lung Cancer

Why is accurate, timely, and complete biomarker testing so critical for the optimal care of patients with non-squamous non-small cell lung carcinoma (NSCLC)? In short, this is because evidence-based recommendations for treatment at nearly every stage and situation in this disease rely heavily on tumor biomarker knowledge to prioritize and personalize the myriad treatments available to improve outcomes for patients with NSCLC. Over the last 15 years, the paradigm of testing an NSCLC for driver mutations at the time of initial diagnosis has evolved from an experimental to a standard requirement for patients with metastatic disease and is becoming standard care for all disease stages. Simultaneously, the number of genes that must be tested to optimize treatment recommendations has expanded from mutations in a single gene to a large and ever-expanding list, and multiple platforms and technologies have been developed for testing both tumor tissue and plasma for circulating tumor DNA (ctDNA).

Since the early 2000s, the identification of oncogenic genomic alterations in NSCLC and the development of targeted therapies designed to block the oncogenic driver has enabled individualized treatment and transformed outcomes. Although their frequency varies between Asian and Western populations, potentially actionable molecular targets can be identified in most lung adenocarcinomas (Figure 2-1). Effective therapies now exist to treat tumors with specific mutations in *EGFR*, *MET*, *BRAF*, *ERBB2 (HER2)*, and *KRAS* as well as oncogenic fusions involving *ALK*, *ROS1*, *RET*, and *NTRK1-3* (Figure 2-2; Table 2-1). Comprehensive mutational testing and biomarker-directed therapy have enabled delivery of personalized medicine for NSCLC and improvements in clinical outcome.

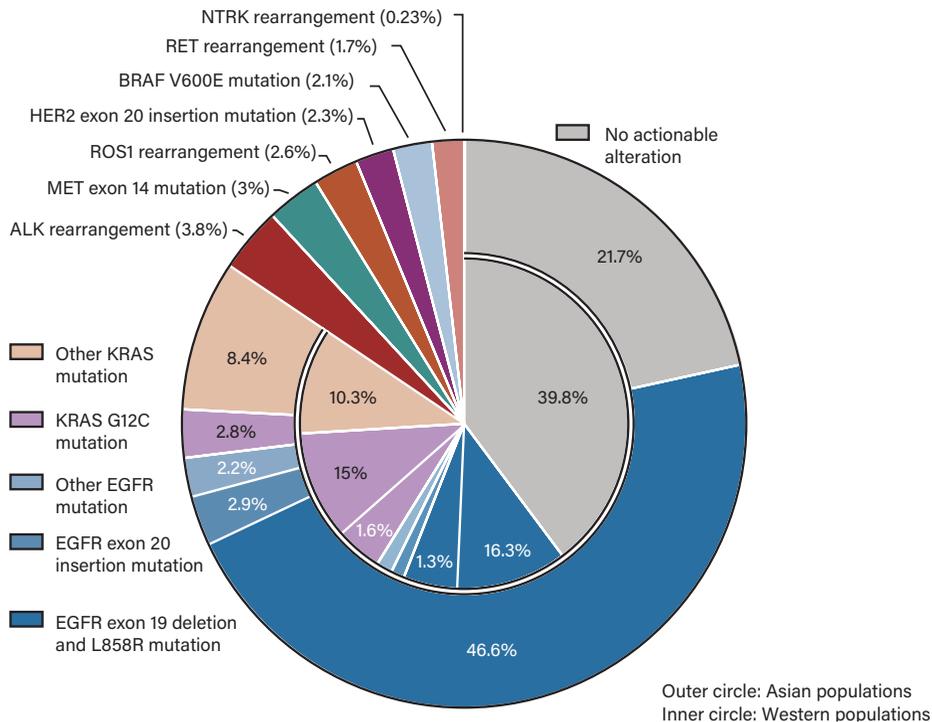


Figure 2-1. Frequency of oncogenic drivers in NSCLC (Source: Tan and Tan 2022,¹ copyright © 2022, by the American Society of Clinical Oncology. Published by Wolters Kluwer. All rights reserved.)

Development of Targeted Therapy for NSCLC: Historical Perspective

The first breakthrough was the discovery of *EGFR* mutations in lung cancer in 2004. At that time, testing of novel *EGFR*-blocking tyrosine kinase inhibitors (TKIs) revealed that although most lung cancer patients did not benefit from this therapy, a few had dramatic benefit with rapid and durable responses.²⁻⁵ Further study of the tumors from the extraordinary responders uncovered mutations in the *EGFR* tyrosine kinase domain that conferred an “oncogene-addicted” biology in which the survival of the cancer was uniquely dependent on signaling from the *EGFR* pathway and could therefore be uniquely corrupted by therapeutic inhibition of this pathway.^{6,7} The IPASS study demonstrated that the best outcomes resulted from selecting patients based on detection of *EGFR* mutations in their tumors, rather than on the basis of clinical characteristics, and starting therapy with *EGFR* TKIs in the first-line setting (Table 2-1).^{8,9} These findings have been subsequently confirmed in multiple other studies.¹⁰⁻¹³

These studies demonstrated improvements in response rate and progression-free survival, as well as survival improvement compared to historical controls.¹⁴ Overall survival advantage has been more challenging to demonstrate in frontline trials of gefitinib or erlotinib versus chemotherapy in *EGFR*-mutated NSCLC, likely due to crossover.¹⁵ Second-generation compounds have been developed including afatinib¹⁶ and dacomitinib¹⁷ that show improved efficacy and survival advantage over first-generation TKIs in some populations, albeit with additional toxicity. The third-generation TKI osimertinib has demonstrated increased efficacy, including improved survival with reduced toxicity compared to first-generation TKIs,^{20,21} establishing a new standard of care for first-line treatment of newly diagnosed patients with *EGFR* mutations. Most recently, osimertinib became the first targeted therapy

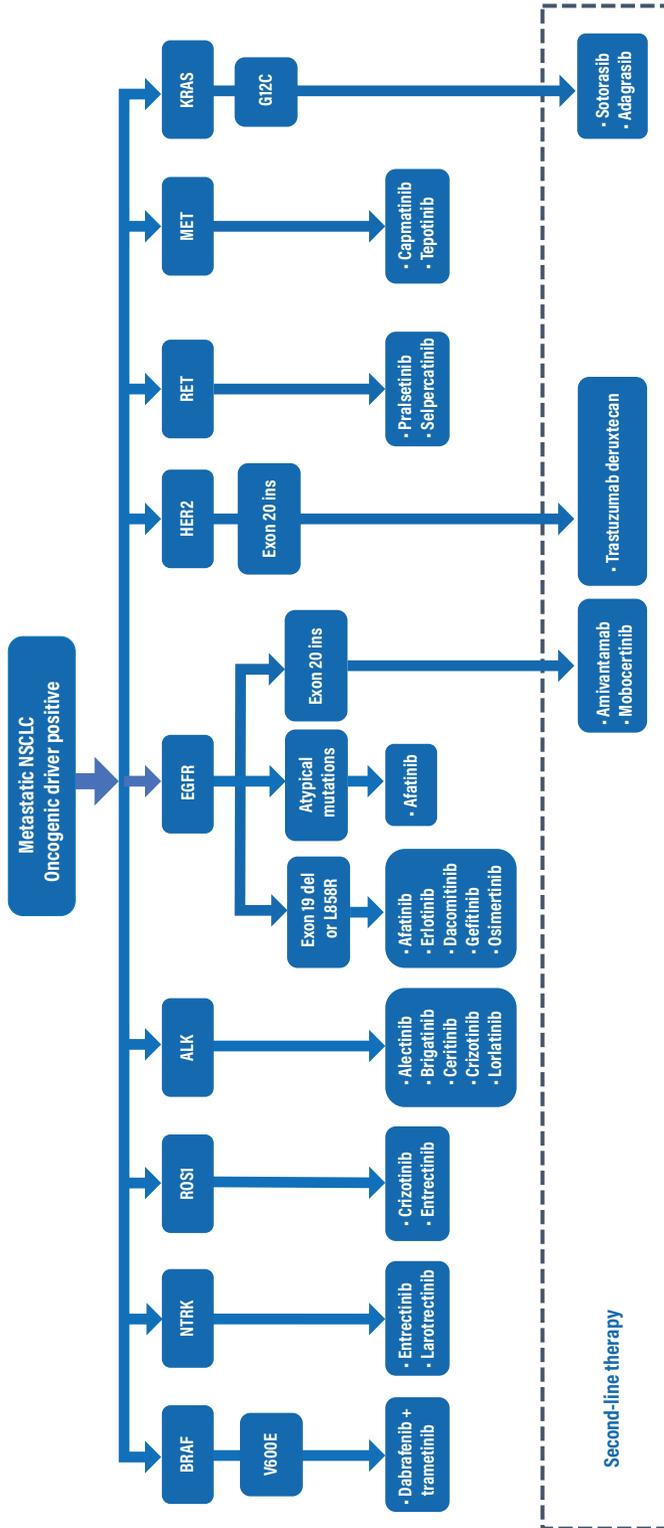


Figure 2-2. Biomarker-driven algorithm for use of targeted therapy in NSCLC (see also updated guidelines from the National Comprehensive Cancer Network [NCCN]¹⁸ and European Society for Medical Oncology [ESMO]¹⁹)

Table 2-1. Efficacy and Toxicity Profiles of FDA-Approved Targeted Agents

Inhibitor	Study	Drug	Study design	N	Objective response rate (%)	Median duration of response (months)	Median progression free survival (months)	Median overall survival (months)	Major treatment-related adverse events					
EGFR	IPASS ^{8,9}	Gefitinib	Gefitinib vs chemotherapy	1217, overall population	43.0 vs 32.2	NS	5.7 vs 5.8; HR, 0.74	18.8 vs 17.4; HR, 0.90	Rash, diarrhea					
										261 with EGFR mutations	71.2 with gefitinib vs 47.3	NS	HR, 0.48	21.6 vs 21.9; HR, 1.00
										176 without mutations	1.1 vs 23.5	NS	HR, 2.85	11.2 vs 12.7; HR, 1.18
	EURTAC ¹¹	Erlotinib	Erlotinib vs chemotherapy	174	64 vs 1	NS	9.7 v 5.2; HR, 0.37	19.3 vs 19.5	Rash, diarrhea					
										345	56 vs 23	11.1 vs 5.5	11.1 vs 6.9; HR, 0.58	28 vs 28 (survival benefit in patients with exon 19 deletions, 33 vs 21.1)
										452	76 vs 70	14.8 vs 8.3	14.7 vs 9.2; HR, 0.59	34 vs 27; HR, 0.78
ALK	Archer 1050 ¹⁷	Dacomitinib	Dacomitinib vs gefitinib	452	76 vs 70	14.8 vs 8.3	14.7 vs 9.2; HR, 0.59	34 vs 27; HR, 0.78	Dermatitis, diarrhea, elevated transaminases					
										556	80 vs 76	17.2 vs 8.5	18.9 vs 10.2; HR, 0.46	38.6 vs 31.8; HR, 0.80 (0.64-0.997)
										343	74 vs 45	11.3 vs 5.3	10.9 vs 7.0; HR, 0.45	NR vs 47.5 (at 4-year landmark, 56.6% crizotinib vs 49.1% chemotherapy)
ALK	FLAURA ^{20,21}	Osimertinib	Osimertinib vs gefitinib or erlotinib	556	80 vs 76	17.2 vs 8.5	18.9 vs 10.2; HR, 0.46	38.6 vs 31.8; HR, 0.80 (0.64-0.997)	Rash/acne, diarrhea, dry skin, paronychia, stomatitis					
										376	73 vs 50	23.9 vs 11.1	16.6 vs 8.1; HR, 0.55	NR vs 26.2 (at 2-year landmark, 70.6% ceritinib vs 58.2% chemotherapy)
										303	83 vs 76	NR vs 11.1	34.8 vs 10.9; HR, 0.43	NR vs 57.4 (at 5 years, OS rate 62.5% vs 45.5%)
ALK	ASCEND-4 ²³	Ceritinib	Phase 3—ceritinib vs chemotherapy	376	73 vs 50	23.9 vs 11.1	16.6 vs 8.1; HR, 0.55	NR vs 26.2 (at 2-year landmark, 70.6% ceritinib vs 58.2% chemotherapy)	Diarrhea, nausea, vomiting, and an increase in alanine aminotransferase					
										303	83 vs 76	NR vs 11.1	34.8 vs 10.9; HR, 0.43	NR vs 57.4 (at 5 years, OS rate 62.5% vs 45.5%)
ALK	ALEX ^{24,25}	Alectinib	Phase 3— alectinib vs chemotherapy	303	83 vs 76	NR vs 11.1	34.8 vs 10.9; HR, 0.43	NR vs 57.4 (at 5 years, OS rate 62.5% vs 45.5%)	Anemia, myalgia, increased bilirubin, increased weight, musculoskeletal pain, and photosensitivity reaction					

(continues)

Table 2-1. Efficacy and Toxicity Profiles of FDA-Approved Targeted Agents (Continued)

Inhibitor	Study	Drug	Study design	N	Objective response rate (%)	Median duration of response (months)	Median progression free survival (months)	Median overall survival (months)	Major treatment-related adverse events
ALK	ALTA-1L ²⁶	Brigatinib	Phase 3—brigatinib vs crizotinib	275	74 vs 62	NR vs 13.9	24.0 vs 11.1; HR, 0.48	NR	GI events (diarrhea, nausea, vomiting) increased blood CPK, cough, and increased aminotransferases
	CROWN ²⁷	Lorlatinib	Phase 3—lorlatinib vs crizotinib	296	76 vs 58	NR vs 11.1	NR vs 9.3; HR, 0.28	NR	Hyperlipidemia, edema, increased weight, peripheral neuropathy, and cognitive effects
ROS1	Dziedziszko et al ²⁸	Entrectinib	Mostly platinum pretreated	107	67.1	15.7	15.7	NE	Dysgeusia, dizziness, constipation, fatigue, diarrhea, weight gain, paresthesia
	Shaw et al ²⁹	Crizotinib	13% treatment naïve	53	72	24.7	19.3	51.4	Vision disorder, nausea, edema, diarrhea, vomiting, elevated transaminases, and constipation
RET	Drlon et al ³⁰	Selpercatinib	Platinum pretreated	105	61	28.6	24.6	NE	Dry mouth, diarrhea, increased aspartate aminotransferase, increased alanine aminotransferase, hypertension
			Treatment naïve	39	84	20.2	22	NE	
	Griesinger et al ³¹	Pralsetinib	Platinum pretreated	136	59	22.3	16.5	NR	Neutropenia, leukopenia, increased aspartate aminotransferase, anemia, increased alanine aminotransferase, constipation, fatigue, increased blood creatine phosphokinase
NTRK	Demetri et al ³²	Entrectinib	Treatment naïve	75	72	Not reached	13	NR	
			Mainly pretreated	22	63.6	19.9	14.9	NE (20.8-NE)	Dysgeusia, diarrhea, fatigue, and weight increase.
	Drlon et al ³³	Larotrectinib	Mainly pretreated	15	73	33.3	35.4	40.7	Myalgias, dizziness, nausea, increased alanine aminotransferase

(continues)

Table 2-1. Efficacy and Toxicity Profiles of FDA-Approved Targeted Agents (Continued)

Inhibitor	Study	Drug	Study design	N	Objective response rate (%)	Median duration of response (months)	Median progression free survival (months)	Median overall survival (months)	Major treatment-related adverse events
HER2	Li et al ³⁴	Trastuzumab deruxtecan 6.4 mg/kg	Platinum pretreated	91	55	9.2	8.2	17.8	Nausea, fatigue, alopecia, vomiting, neutropenia, anemia, diarrhea
	Goto et al ³⁵	Trastuzumab deruxtecan 6.4 mg/kg	Platinum pretreated	28	42.9	5.9	NR	NR	Drug-related ILD 14%
		Trastuzumab deruxtecan 5.4 mg/kg	Platinum pretreated	52	53.8	NE	NR	NR	Drug-related ILD 5.9%
MET	Wolf et al ³⁶	Capmatinib	Treatment naïve	28	68	12.6	12.4	20.8	Peripheral edema, nausea, vomiting, blood creatinine increased, fatigue, diarrhea
	Paik et al ³⁷	Tepotinib	Platinum pretreated	69	41	9.7	5.4	13.3	
			43% treatment naïve	99	46	11.1	8.5	17.1	Peripheral edema, nausea, diarrhea, blood creatinine increased, hyponatremia
BRAF	Planchard et al ³⁸	Dabrafenib plus trametinib	Treatment naïve	36	64	10.2	10.8	17.3	Pyrexia, nausea, vomiting, dry skin, peripheral edema, diarrhea, decreased appetite, and cough
			Platinum pretreated	57	68.4	9.8	10.2	18.2	
KRAS	Skoulidis et al ³⁹	Sotorasib	Platinum pretreated	124	37.1	11.1	6.8	12.5	Diarrhea, nausea, increased alanine aminotransferase, increased AST, fatigue
	Jänne et al ⁴⁰	Adagrasib	Platinum pretreated	116	42.9	8.5	6.5	12.6	Diarrhea, nausea, vomiting, fatigue, increased alanine aminotransferase or AST, increased creatinine
EGFR exon 20	Park et al ⁴¹	Amivantamab	Platinum pretreated	81	40	11.1	8.3	22.8	Infusion reactions, rash, paronychia
	Zhou et al ⁴²	Mobocertinib	Platinum pretreated	114	28	17.5	7.3	24	Diarrhea, rash

Abbreviations: AST = aspartate aminotransferase; CPK = creatinine phosphokinase; GI = gastrointestinal; HR = hazard ratio; ILD = interstitial lung disease; NE = not estimable; NR = not reported; NS = not significant; OS = overall survival.

approved by the US Food and Drug Administration (FDA) in the adjuvant setting given its demonstrated improvement in disease-free survival.⁴³

This concept of tumor-informed therapy received further validation in NSCLC drug development and clinical practice after the second oncogenic driver—*ALK* gene fusion—was described in lung cancer in 2007.⁴⁴ Serendipitously, a phase 1 first-in-human trial of crizotinib, a novel multikinase TKI that was developed to inhibit MET but known also to inhibit ALK and ROS1 had just begun.⁴⁵ The trial design rapidly pivoted focus from MET to identifying and enrolling patients whose tumors harbored *ALK* rearrangements by fluorescence in situ hybridization (FISH), and this trial and subsequent phase 3 trials showed significant efficacy and improvements in outcomes compared to standard chemotherapy.^{22,45,46} Within 4 years, crizotinib received FDA approval as the first drug to be studied and approved in a genotype-specific manner. The rapid and successful development of crizotinib set the standard for targeted drug development that continues today.

Newer-generation ALK TKIs have been developed including ceritinib, alectinib, brigatinib, ensartinib, and lorlatinib with increased efficacy and better central nervous system (CNS) penetration.^{23,24,26,27,47} Four of these newer-generation compounds (alectinib, brigatinib, ensartinib, and lorlatinib) have shown improved efficacy over crizotinib in phase 3 clinical trials, raising the bar for first-line therapy for *ALK*-rearranged NSCLC. These targeted therapies have improved outcomes compared with historical controls⁴⁸ and resulted in unprecedented survival rates with median survival in excess of 5 years.²⁵

Subsequently, there has been rapid development of kinase inhibitors directed at *BRAF* V600E mutations,³⁸ *MET* exon 14 skipping mutations,^{36,37} as well as *ROS1*,^{28,29} *RET*,^{30,31} and *NTRK1-3*^{32,33} gene rearrangements. Approvals for these therapies have largely been based on results of single-arm phase 1 or 2 trials, demonstrating high response rates in tumors with the specific oncogenic target, without accompanying phase 3 trials, largely because of the limitations of conducting large randomized clinical trials in rare populations with these uncommon targets. Utilization of novel targeting strategies, such as bispecific antibodies and antibody drug conjugates, has extended the spectrum of actionable oncogenes to include *EGFR* exon 20 mutations^{41,42} and *HER2* mutation.³⁴ And though mutations in the *KRAS* guanosine-triphosphate hydrolase (GTPase) have been historically challenging to target,⁴⁹ the *KRAS* G12C inhibitor, sotorasib, was approved by FDA in May 2021,³⁹ and subsequently, adagrasib was approved in this indication in December 2022 on the basis of response rates in previously treated NSCLC patients.⁴⁰

Resistance to Targeted Therapy

Acquired resistance will ultimately develop and limit the efficacy of targeted therapies. Common themes in targeted therapy resistance are on-target aberrations in the inhibited pathway and off-target resistance via acquisition of bypass tracks or histologic transformation to small cell or squamous cell carcinoma. Postprogression biopsies can help understand mechanisms of resistance and guide postprogression therapy in some instances. Mechanisms of resistance in the context of different oncogenic drivers is discussed in greater detail in the gene-specific chapters in this atlas.

Outcomes of Biomarker-Directed Therapy in Lung Cancer Patients

The availability and utilization of targeted therapies has improved outcomes for patients with advanced NSCLC. Reductions in US population-level mortality and improvements in survival after NSCLC diagnosis between 2013 and 2016 coincided with approvals for EGFR and ALK TKIs.⁵⁰ Large-scale regional and national molecular testing programs in the United States⁵¹ and Europe^{52,53} demonstrated the feasibility of testing for multiple molecular drivers in large populations and the value of directing patients identified as having molecular alterations to the appropriate targeted therapy. The US Lung Cancer Mutation Consortium analyzed tumors from 733 patients for 10 genes and was able to identify a potentially targetable driver mutation in 64% of cases. Outcomes were improved for patients with an oncogenic driver who received a targeted agent (n = 260, median survival 3.5 years) compared to patients with a driver who did not receive targeted therapy (n = 318, median survival 2.4 years).⁵¹ A French nationwide program identified molecular alterations in about half of 17,664 NSCLC patients tested over a 1-year period with a 6-gene panel,⁵² and improvements in outcomes were noted, including response rates, progression-free survival, and overall survival in patients found to have actionable genomic alterations. Similarly, a German study involving 5145 patients demonstrated genotyping of tumors was feasible and associated with improved survival outcomes for patients with *EGFR* mutations or *ALK* rearrangements who received the appropriate targeted therapy.⁵³ A real-world analysis of 4064 US NSCLC patients using the Flatiron electronic database linked with standard-of-care genomic profiling found that patients whose tumors harbored a driver alteration and were treated with an appropriate targeted therapy (n = 575) had improved overall survival compared to those who did not have a driver alteration (n = 560) (median, 18.6 months vs 11.4 months; $P < 0.001$).⁵⁴

Barriers to Biomarker Testing

Several barriers to clinically appropriate biomarker testing have been identified.⁵⁵⁻⁵⁹ Although the relative impact of each barrier varies by geographic locale (developed vs developing countries or rural vs metropolitan) and care setting (community vs academic), there has been a remarkable consistency across settings in the key factors. One of the most comprehensive surveys assessing barriers to testing in a global setting was conducted by the International Association for the Study of Lung Cancer.⁵⁹ Five factors were identified common to all locales. Most frequent in every geographic region was cost. Tissue quality (sample inadequacy, suboptimal handling) was also a major limiting factor. Additional factors included access to testing (single gene vs single broader panel), prolonged turnaround times, and awareness (familiarity with guidelines and difficulties in interpretation of results). In addition, in developing and middle-income countries, lack of access to various targeted agents provides an important disincentive to pursue biomarker testing.⁶⁰

Potential solutions to address these issues include the use of reflex testing protocols; optimizing the interaction between providers obtaining the tumor samples, pathologists, and medical oncologists; case review by multidisciplinary tumor boards; and improving turnaround times by the increasing utilization of plasma-based ctDNA testing. Given the rapid evolution of multiple new potentially treatable molecular targets, enhanced provider education efforts, particularly directed at community and non-thoracic-focused providers, could potentially optimize molecular testing efforts.⁵⁶ A survey conducted by the American

Society of Clinical Oncology showed that generalists ordered biomarker testing significantly more frequently for their patients with squamous NSCLC compared to thoracic specialists despite the relative dearth of targetable oncogenes in this tumor type. Further, they were less likely to defer treatment with nontargeted therapies while waiting for biomarker results when compared to specialist oncologists despite the demonstrated benefits of using targeted therapies in the first-line setting.⁵⁶

Impact of Not Testing

The value of performing timely and precise biomarker testing to guide treatment decisions in advanced NSCLC is well recognized. The 5-year survival rates for patients receiving targeted or immunotherapies range between 15% and 50% depending on the biomarker, compared to a historical rate of approximately 6% with cytotoxic chemotherapy.⁶² Recently, 2 studies have reinforced the impact of adherence to biomarker testing guidelines on outcomes in NSCLC.^{62,63} John et al retrospectively studied a cohort of 28,784 patients with advanced NSCLC drawn from 280 US cancer clinics.⁶² Two-thirds of these patients had National Comprehensive Cancer Network (NCCN)-recommended biomarker testing (adherent group). Improved clinical outcomes, including lower risk of mortality and longer median survival was observed in the adherent group compared to the patients' not undergoing biomarker testing (nonadherent group). The impact of a molecular tumor board (MTB) in helping to overcome the many barriers to obtaining and applying appropriate biomarker testing was demonstrated in a study conducted at the University of Kentucky,⁶³ where cases reviewed by the MTB had better survival outcomes than propensity-matched controls without MTB review (hazard ratio [HR], 8.61; $P < 0.0001$).

While the role of biomarker testing in earlier stages of disease has been less well defined, the approval of adjuvant osimertinib in resected EGFR mutation-positive NSCLC⁴³ and the efficacy of neoadjuvant and adjuvant immune-oncology drugs in patients whose tumors are negative for EGFR and ALK has established the essential role of biomarker testing in this setting as well.

Patient Advocate's Perspective

The new era of personalized medicine has provided renewed hope and optimism to lung cancer patients and their family caregivers. Biomarker testing is crucial for determining the optimal personalized treatment to achieve the best possible outcomes. From the patient or care provider point of view, there are several important considerations.

Access, Cost, and Equity

Although, in many countries, biomarker testing is routine and covered by the health systems upon diagnosis, this is not universally the case. There are significant differences between Western/Northern and Eastern Europe, for example, as described in the Lung Cancer Europe (LuCE) position paper⁶⁴ and major disparities within countries according to race.⁶⁵ Moreover, repeated biomarker testing to examine mechanisms of acquired resistance to targeted therapy may not be reimbursed by health systems, forcing patients to incur out-of-pocket expenses to access subsequent targeted options.

Education and Hope

The impact of identifying an actionable driver mutation may be tremendous—sometimes this literally represents the difference between life and death. However, many lung cancers do not carry an oncogenic driver, and for some, the genetic alterations found on testing do not yet have corresponding available targeted therapies. It is critical that patients and their families receive counseling at the time of molecular testing that covers the range of possible results, the significance of potential findings and the limitations of testing. Managing patients' and families' expectations is crucial. In addition, interpretation of molecular pathology reports can be challenging, and support should be readily available. Understandable educational material available online or in paper format from patient advocacy, professional, and educational organizations plays an important role, especially in helping those who may not have been offered molecular testing by their provider to advocate for the necessary tests. Furthermore, there are digital social communities where patients and families share experiences and learn from each other, providing support and hope. Medical providers can help by directing newly diagnosed lung cancer patients toward these resources.

Conclusion

Current diagnosis and treatment of NSCLC relies heavily on timely, well-integrated, and thorough tumor biomarker testing as well as appropriate utilization of available therapies. This atlas is a comprehensive reference to enable readers to provide the optimal personalized therapeutic approaches for patients with lung cancer.

References

1. Tan AC, Tan DSW. Targeted therapies for lung cancer patients with oncogenic driver molecular alterations. *J Clin Oncol*. 2022;40(6):611-625.
2. Gatzemeier U, Pluzanska A, Szczesna A, et al. Phase III study of erlotinib in combination with cisplatin and gemcitabine in advanced non-small-cell lung cancer: the Tarceva Lung Cancer Investigation Trial. *J Clin Oncol*. 2007;25:1545-1552.
3. Herbst RS, Prager D, Hermann R, et al. TRIBUTE: a phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced non-small-cell lung cancer. *J Clin Oncol*. 2005;23:5892-5899.
4. Herbst RS, Giaccone G, Schiller JH, et al. Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: a phase III trial—INTACT 2. *J Clin Oncol*. 2004;22:785-794.
5. Giaccone G, Herbst RS, Manegold C, et al. Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase III trial—INTACT 1. *J Clin Oncol*. 2004;22:777-784.
6. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science*. 2004;304:1497-1500.
7. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med*. 2004;350:2129-2139.
8. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med*. 2009;361:947-957.
9. Fukuoka M, Wu YL, Thongprasert S, et al. Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). *J Clin Oncol*. 2011;29:2866-2874.
10. Sequist LV, Yang JC, Yamamoto N, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. *J Clin Oncol*. 2013;31:3327-3334.

11. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol.* 2012;13:239-246.
12. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med.* 2010;362:2380-2388.
13. Mitsudomi T, Morita S, Yatabe Y, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. *Lancet Oncol.* 2010;11:121-128.
14. Takano T, Fukui T, Ohe Y, et al. EGFR mutations predict survival benefit from gefitinib in patients with advanced lung adenocarcinoma: a historical comparison of patients treated before and after gefitinib approval in Japan. *J Clin Oncol.* 2008;26:5589-5595.
15. Lee CK, Davies L, Wu YL, et al. Gefitinib or erlotinib vs chemotherapy for EGFR mutation-positive lung cancer: individual patient data meta-analysis of overall survival. *J Natl Cancer Inst.* 2017;109.
16. Yang JC, Wu YL, Schuler M, et al. Afatinib versus cisplatin-based chemotherapy for EGFR mutation-positive lung adenocarcinoma (LUX-Lung 3 and LUX-Lung 6): analysis of overall survival data from two randomised, phase 3 trials. *Lancet Oncol.* 2015;16:141-151.
17. Mok TS, Cheng Y, Zhou X, et al. Improvement in overall survival in a randomized study that compared dacomitinib with gefitinib in patients with advanced non-small-cell lung cancer and EGFR-activating mutations. *J Clin Oncol.* 2018;36:2244-2250.
18. NCCN Guidelines Committee. *Clinical Practice Guidelines in Oncology: Non-Small Cell Lung Cancer*, version 5.2022. https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf.
19. ESMO Guidelines Committee. *Clinical Practice Guidelines—Metastatic Non-Small-Cell Lung Cancer: ESMO Clinical Practice Guidelines for Diagnosis, Treatment and Follow-Up*. Updated September 15, 2020. <https://www.esmo.org/guidelines/guidelines-by-topic/lung-and-chest-tumours/clinical-practice-living-guidelines-metastatic-non-small-cell-lung-cancer>.
20. Ramalingam SS, Vansteenkiste J, Planchard D, et al. Overall survival with osimertinib in untreated, EGFR-mutated advanced NSCLC. *N Engl J Med.* 2020;382:41-50.
21. Soria JC, Ramalingam SS. Osimertinib in EGFR mutation-positive advanced NSCLC. *N Engl J Med.* 2018;378:1262-1263.
22. Solomon BJ, Mok T, Kim DW, et al. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. *N Engl J Med.* 2014;371:2167-2177.
23. Soria JC, Tan DSW, Chiari R, et al. First-line ceritinib versus platinum-based chemotherapy in advanced ALK-rearranged non-small-cell lung cancer (ASCEND-4): a randomised, open-label, phase 3 study. *Lancet.* 2017;389:917-929.
24. Peters S, Camidge DR, Shaw AT, et al. Alectinib versus crizotinib in untreated ALK-positive non-small-cell lung cancer. *N Engl J Med.* 2017;377:829-838.
25. Mok T, Camidge DR, Gadgeel SM, et al. Updated overall survival and final progression-free survival data for patients with treatment-naïve advanced ALK-positive non-small-cell lung cancer in the ALEX study. *Ann Oncol.* 2020;31:1056-1064.
26. Camidge DR, Kim HR, Ahn MJ, et al. Brigatinib versus crizotinib in ALK-positive non-small-cell lung cancer. *N Engl J Med.* 2018;379:2027-2039.
27. Shaw AT, Bauer TM, de Marinis F, et al. First-line lorlatinib or crizotinib in advanced ALK-positive lung cancer. *N Engl J Med.* 2020;383:2018-2029.
28. Dziedziszko R, Krebs MG, De Braud F, et al. Updated integrated analysis of the efficacy and safety of entrectinib in locally advanced or metastatic ROS1 fusion-positive non-small-cell lung cancer. *J Clin Oncol.* 2021;39:1253-1263.
29. Shaw AT, Riely GJ, Bang YJ, et al. Crizotinib in ROS1-rearranged advanced non-small-cell lung cancer (NSCLC): updated results, including overall survival, from PROFILE 1001. *Ann Oncol.* 2019;30:1121-1126.
30. Drilon A, Subbiah V, Gautschi O, et al. Selpercatinib in patients with RET fusion-positive non-small-cell lung cancer: updated safety and efficacy from the Registrational LIBRETTO-001 phase I/II trial. *J Clin Oncol.* 2022;JCO2200393.
31. Griesinger F, Curigliano G, Thomas M, et al. Safety and efficacy of pralsetinib in RET fusion-positive non-small-cell lung cancer including as first-line therapy: update from the ARROW trial. *Ann Oncol.* 2022;33(11):1168-1178.

32. Demetri GD, De Braud F, Drilon A, et al. Updated integrated analysis of the efficacy and safety of entrectinib in patients with NTRK fusion-positive solid tumors. *Clin Cancer Res*. 2022;28:1302-1312.
33. Drilon A, Tan DSW, Lassen UN, et al. Efficacy and safety of larotrectinib in patients with tropomyosin receptor kinase fusion-positive lung cancers. *JCO Precis Oncol*. 2022;6:e2100418.
34. Li BT, Smit EF, Goto Y, et al. Trastuzumab deruxtecan in HER2-mutant non-small-cell lung cancer. *N Engl J Med*. 2022;386:241-251.
35. Goto K, Sang-We K, Kubo T, et al. Trastuzumab deruxtecan (T-DXd) in patients (Pts) with HER2-mutant metastatic non-small cell lung cancer (NSCLC): interim results from the phase 2 DESTINY-Lung02 trial. *Ann Oncol*. 2022;33:S808-S869.
36. Wolf J, Garon EB, Groen HJM, et al. Capmatinib in MET exon 14-mutated, advanced NSCLC: updated results from the GEOMETRY mono-1 study. *J Clin Oncol*. 2021;39:9020.
37. Paik PK, Felip E, Veillon R, et al. Tepotinib in non-small-cell lung cancer with MET exon 14 skipping mutations. *N Engl J Med*. 2020;383:931-943.
38. Planchard D, Besse B, Groen HJM, et al. Phase 2 study of dabrafenib plus trametinib in patients with BRAF V600E-mutant metastatic NSCLC: updated 5-year survival rates and genomic analysis. *J Thorac Oncol*. 2022;17:103-115.
39. Skoulidis F, Li BT, Dy GK, et al. Sotorasib for lung cancers with KRAS p.G12C mutation. *N Engl J Med*. 2021;384:2371-2381.
40. Jänne PA, Riely GJ, Gadgeel SM, et al. Adagrasib in non-small-cell lung cancer harboring a KRASG12C mutation. *N Engl J Med*. 2022;387(2):120-131. doi:10.1056/NEJMoa2204619.
41. Park K, Haura EB, Leighl NB, et al. Amivantamab in EGFR exon 20 insertion-mutated non-small-cell lung cancer progressing on platinum chemotherapy: initial results from the CHRYSALIS phase I study. *J Clin Oncol*. 2021;39:3391-3402.
42. Zhou C, Ramalingam SS, Kim TM, et al. Treatment outcomes and safety of mobocertinib in platinum-pretreated patients with EGFR exon 20 insertion-positive metastatic non-small cell lung cancer: a phase 1/2 open-label nonrandomized clinical trial. *JAMA Oncol*. 2021;7:e214761.
43. Wu YL, Tsuboi M, He J, et al. Osimertinib in resected EGFR-mutated non-small-cell lung cancer. *N Engl J Med*. 2020;383:1711-1723.
44. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature*. 2007;448:561-566.
45. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med*. 2010;363:1693-1703.
46. Shaw AT, Kim DW, Nakagawa K, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *N Engl J Med*. 2013;368:2385-2394.
47. Horn L, Wang Z, Wu G, et al. Ensartinib vs crizotinib for patients with anaplastic lymphoma kinase-positive non-small cell lung cancer: a randomized clinical trial. *JAMA Oncol*. 2021;7:1617-1625.
48. Shaw AT, Yeap BY, Solomon BJ, et al. Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harbouring ALK gene rearrangement: a retrospective analysis. *Lancet Oncol*. 2011;12:1004-1012.
49. Uprety D, Adjei AA. KRAS: From undruggable to a druggable cancer target. *Cancer Treat Rev*. 2020;89:102070.
50. Howlader N, Forjaz G, Mooradian MJ, et al. The effect of advances in lung-cancer treatment on population mortality. *N Engl J Med*. 2020;383:640-649.
51. Kris MG, Johnson BE, Berry LD, et al. Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs. *JAMA*. 2014;311:1998-2006.
52. Barlesi F, Mazieres J, Merlio JP, et al. Routine molecular profiling of patients with advanced non-small-cell lung cancer: results of a 1-year nationwide programme of the French Cooperative Thoracic Intergroup (IFCT). *Lancet*. 2016;387:1415-1426.
53. Clinical Lung Cancer Genome Project (CLCGP); Network Genomic Medicine (NGM). A genomics-based classification of human lung tumors. *Sci Transl Med*. 2013;5:209ra153.
54. Singal G, Miller PG, Agarwala V, et al. Association of patient characteristics and tumor genomics with clinical outcomes among patients with non-small cell lung cancer using a clinicogenomic database. *JAMA*. 2019;321:1391-1399.
55. Smeltzer MP, Wynes MW, Lantuejoul S, et al. The International Association for the Study of Lung Cancer Global Survey on Molecular Testing in Lung Cancer. *J Thorac Oncol*. 2020;15:1434-1448.

56. Mileham KF, Schenkel C, Bruinooge SS, et al. Defining comprehensive biomarker-related testing and treatment practices for advanced non-small-cell lung cancer: results of a survey of U.S. oncologists. *Cancer Med.* 2022;11:530-538.
57. Pennell NA, Arcila ME, Gandara DR, et al. Biomarker testing for patients with advanced non-small cell lung cancer: real-world issues and tough choices. *Am Soc Clin Oncol Educ Book.* 2019;39:531-542.
58. Rivera MP, Charlot M, Durham DD, et al. Molecular biomarker and programmed death-ligand 1 expression testing in patients with advanced stage non-small cell lung cancer across North Carolina community hospitals. *Chest.* 2021;160:1121-1130.
59. Dalurzo ML, Aviles-Salas A, Soares FA, et al. Testing for EGFR mutations and ALK rearrangements in advanced non-small-cell lung cancer: considerations for countries in emerging markets. *Onco Targets Ther.* 2021;14:4671-4692.
60. Hirsch FR, Zaric B, Rabea A, et al. Biomarker testing for personalized therapy in lung cancer in low- and middle-income countries. *Am Soc Clin Oncol Educ Book.* 2017;37:403-408.
61. Ettinger DS, Wood DE, Aisner DL, et al. NCCN Guidelines Insights: Non-Small Cell Lung Cancer, version 2.2021. *J Natl Compr Canc Netw.* 2021;19:254-266.
62. John A, Yang B, Shah R. Clinical impact of adherence to NCCN guidelines for biomarker testing and first-line treatment in advanced non-small cell lung cancer (aNSCLC) using real-world electronic health record data. *Adv Ther.* 2021;38:1552-1566.
63. Huang B, Chen Q, Allison D, et al. Molecular tumor board review and improved overall survival in non-small-cell lung cancer. *JCO Precis Oncol.* 2021;5:PO.21.00210.
64. *Position Paper: Disparities and Challenges in Access to Lung Cancer Diagnostics and Treatment across Europe.* Lung Cancer Europe (LuCE); 2020.
65. Harrison S, Judd J, Chin S, et al. Disparities in lung cancer treatment. *Curr Oncol Rep.* 2022;24:241-248.

Specimen Acquisition and Pre-Analytical Considerations

3

Patients with lung cancer can present in a variety of ways and may have early or advanced-stage disease involving different sites. Regardless of their presentation, optimal treatment requires tissue acquisition for diagnosis and, in many instances, molecular characterization to direct optimal therapy. This chapter reviews the complementary roles of pulmonary physicians, radiologists, and surgeons in tissue acquisition and the pre-analytic variables that impact optimal pathologic assessment and molecular characterization of a patient's tumor.

Pulmonologist's Perspective

By Emily Stone and Kwun M. Fong

The time when pulmonologists only needed to obtain diagnostic specimens to confirm malignancy and distinguish small cell lung carcinoma (SCLC) from non-small cell lung carcinoma (NSCLC) is long gone. Procuring adequate diagnostic tissue for molecular characterization in the safest possible way is now a priority, particularly for NSCLC.¹ Every sampling procedure should maximize yield, as tissue truly remains the issue for both histopathologic diagnosis and ancillary molecular characterization notwithstanding the utility of liquid biopsy in some settings.²⁻⁸

Optimal tissue acquisition is a crucial pre-analytic factor for biomarker analysis whether single gene testing, small panel, or comprehensive next-generation sequencing (NGS) genomic profiling is utilized.⁹⁻¹¹ Best practice aims to obtain sufficient tissue for relevant histopathologic and molecular testing in the safest, most effective, and affordable way, noting global barriers for biomarker testing.¹²⁻¹⁴

This can be challenging in lung cancer, particularly with smaller and/or inaccessible lesions; with patient comorbidities, such as severe lung disease; or in the setting of advanced-stage presentation contraindicating surgical resection specimens, which provide more tissue than minimally invasive bronchoscopic biopsies, needle aspiration, and core biopsies.¹⁵

Table 3-1. Commonly Used Bronchoscopic Approaches and Sampling Tools for Lung Cancer Tissue Acquisition

Tumor characteristics	Modality	Sampling tool
Central or endobronchial tumor	Bronchoscopy	Endobronchial biopsy Cryobiopsy Brushing Washes/bronchoalveolar lavage
Peripheral tumor	Ultrathin bronchoscopy Radial EBUS	Transbronchial biopsy Transbronchial cryobiopsy TBNA Transbronchial brushing Washes/(targeted) bronchoalveolar lavage
Mediastinal tumor or extrinsic tumor/lymph node adjacent to airway	Linear EBUS	TBNA

Abbreviations: EBUS = endobronchial ultrasound; TBNA = transbronchial needle aspiration.

Flexible Bronchoscopy

Conventional flexible bronchoscopy has a high diagnostic yield for central lesions and a low yield for smaller, peripheral lesions. Newer technologies, including navigation bronchoscopy, endobronchial ultrasound (EBUS), and ultrathin and robotic bronchoscopy, enhance bronchoscopic reach together with modern sampling tools, for example, flexible and core needles, along with tunneling tools that are changing this paradigm.¹⁶⁻²² The traditional use of bronchoscopy for central lesions and transthoracic approaches for peripheral lesions is being challenged as these advanced bronchoscopy techniques improve diagnostic yield for central and peripheral lesions with the additional ability to sample nodes in the same procedure with lower risk of pneumothorax (Table 3-1).²³

For endobronchial and transbronchial approaches, 5 endobronchial/transbronchial forceps biopsies have been recommended with an additional 5 forceps biopsies or 2 cryobiopsies considered to maximize tissue acquisition, with the latter reported to have a higher diagnostic yield but with a higher complication risk, particularly bleeding.^{11,24}

Lesions visible either bronchoscopically or imaging-wise (EBUS, fluoroscopy, cone beam computed tomography [CT]) can also be sampled with a conical bristle-brush of the lesion; smeared onto a slide for cytology and shaken vigorously in saline to dislodge cells that are pelleted by centrifugation into a cell block.²⁵ One study suggested brushings showed similar adequacy to biopsies for immunohistochemistry (PD-L1, *ALK*, *ROS1*) and NGS (*EGFR*, *KRAS*, *BRAF*).²⁶

Bronchial washes provide cytology specimens and cell blocks, albeit any shed tumor cells are diluted by other cell types and debris. Washes can be used to detect somatic mutations, aberrant methylation, and PD-L1 staining but are relatively untargeted and limited by a low proportion of tumor cells in many instances. More targeted bronchoalveolar lavage requires a guide sheath navigated through the bronchoscope to the lesion.²⁷⁻²⁹

Transbronchial needle aspiration (TBNA) can be performed without EBUS. Modern EBUS-guided TBNA significantly improves the diagnostic yield for sampling of central lesions and mediastinal and hilar lymph nodes, and radial EBUS is increasingly implemented for sampling peripheral lesions.^{14,25,30,31} TBNA samples can be smeared on a slide and made into a cell block suitable for histopathologic and molecular analyses.³² Larger

19-gauge TBNA needles may procure “cores,” although they often consist of clotted blood with entrapped tissue fragments.³³ Careful coordinated multidisciplinary stewardship of all of the precious samples is vital for optimal diagnosis and molecular profiling.³⁴

Rapid onsite evaluation (ROSE) is recommended to ensure that TBNAs and other EBUS samples are adequate for molecular testing. ROSE, however, requires additional resources of a cytopathologist/technologist in the procedural room to confirm the presence of tumor cells and sampling adequacy/yield.^{33,35-37} When undertaking EBUS TBNA without ROSE, a minimum of 3 and up to 5 passes has been suggested.¹¹

Navigation Bronchoscopy

Technological advances have led to novel ways to guide the bronchoscope to the target. Navigation systems, which include virtual bronchoscopic navigation (VBN) or electromagnetic navigation (EMN), use 3-dimensional fly-through images of the anatomic bronchial route to the target and are mainly for peripheral lung lesions.^{18,19,25,38-40}

Ultrathin Bronchoscopy

Ultrathin bronchoscopes can reach more distal airways (fifth- vs fourth-generation airway) than conventional bronchoscopes. They provide a greater diagnostic yield for peripheral lesions because of a disproportionately large working channel of 1.7 mm, allowing a small radial EBUS probe and multi-tool sampling.^{25,41,42}

Robotic Bronchoscopy

Another innovation is robotic bronchoscopy for both tissue acquisition and potential therapy. Two commercial systems are currently available. Advantages include navigation, ability to hold position, improved articulation, and maneuverability; however, high cost may be a barrier to its widespread adoption.^{21,43,44}

Sample Preservation and Transfer

Bronchoscopic specimens are typically collected in normal saline or formalin for interdepartmental transfer, consistently practiced globally. Nonetheless, alternatives are emerging to overcome the negative impact of formalin fixation on nucleic acid quality, for example, non-cross-linking fixatives.⁴⁵ As RNA-based testing is increasingly used, RNA-preserving reagents may help address the challenge of RNA degradation.⁴⁶

Conclusion

Personalized medicine critically relies on tumor tissue acquisition of sufficient quantity and quality for molecular testing, which is particularly challenging in lung cancer where diagnostic samples are mostly small. For ancillary testing, procuring biopsies or cell blocks where possible will increase the chance of sufficient tumor cells. Pulmonologists need to optimize current techniques and learn new bronchoscopy skills to ensure that samples provide the requisite quality and quantity of tumor cells for histopathologic diagnoses and molecular testing.^{30,47} Informative multisociety and evidence-based guidance will need regular updates to address the rapidly evolving needs. Nonetheless, there is parallel progress in advanced bronchoscopy practice,^{48,49} such that the dilemma of testing “more with less” can evolve to testing “more with more.”⁵⁰

Interventional Radiologist's Perspective

By Ritu R. Gill and Apoorva Gogna

Image-guided percutaneous transthoracic needle biopsy (TTNB) forms the standard of care in the diagnostic evaluation of lung nodules and masses, with a high diagnostic accuracy for both benign and malignant etiologies, with a pooled sensitivity and specificity of 0.95 and 0.99, respectively.^{51,52} CT is the preferred image guidance method for image-guided biopsies and allows safe sampling of superficial and deep lesions while minimizing morbidity by limiting needle passage through aerated lung and helping to avoid vessels, fissures, and bullae.⁵³ Ultrasound can be used for lesions without lung interface as well as chest wall- and pleural-based lesions.^{54,55} Fluoroscopy and intraoperative C-arm can also be used for image-guided biopsies^{56,57} but play a limited role in tissue procurement for diagnosis and molecular profiling. Both fine-needle aspiration (FNA) and core needle biopsy (CNB) are used to obtain specimens for diagnosis and molecular characterization.^{53,58} The accuracy for diagnosis and molecular profiling of lung cancer using image-guided biopsies ranges from 75% to 92%.^{52,59-64} As many patients with lung cancer are not surgical candidates, it is important that sufficient material is obtained for both diagnostic purposes and molecular profiling.

Pre-Procedure Assessment

Patients referred for image-guided biopsy originate from many services including thoracic surgery, pulmonology, oncology, and general practice, including patients with indeterminate lesions and those with known lung cancer. Any request for a lung biopsy should prompt the radiologist to review the available imaging, usually a CT with contrast; plan the approach; and schedule the patient for the procedure. Fluorine-18-fluorodeoxyglucose positron emission tomography (18 FDG-PET) CT can be helpful in lesion selection especially in the event of a previous nondiagnostic biopsy but is not necessary prior to the procedure. The eligibility criteria for biopsy include a lesion preferably greater than 1 cm and a trajectory accessible by image guidance. Hypervascular lesions or presence of unavoidable vascular structures in the trajectory are a contraindication to image-guided biopsy (Figure 3-1). Centrally located lesions, uncorrectable coagulopathy, and presence of severe pulmonary hypertension are also exclusion criteria.

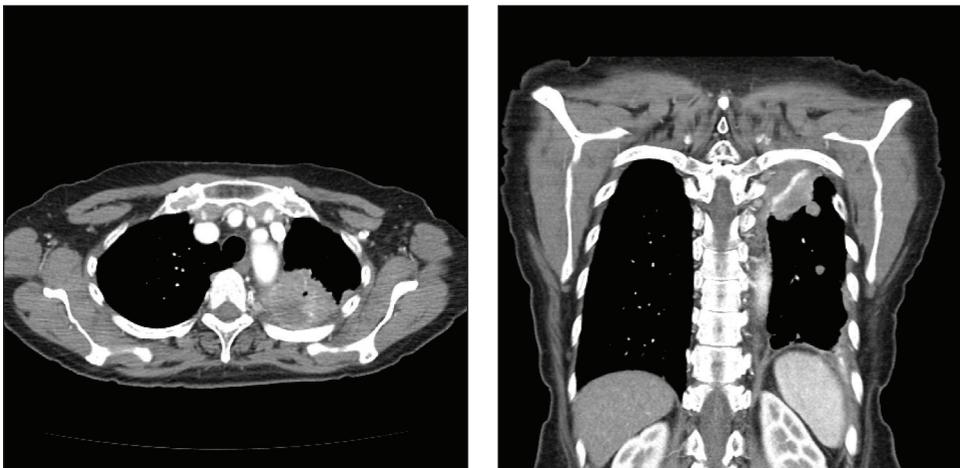


Figure 3-1. Large vessels within the lesion are a relative contraindication for biopsy.

Biopsy Procedure

TTNB is usually an outpatient procedure, with most patients discharged within a few hours of the biopsy. The procedure may be performed under only local anesthetic or under intravenous conscious sedation, and appropriate arrangements are required (fasting, care postprocedure, etc).

The standard operating procedures of TTNBs include patient positioning in the supine, prone, or lateral decubitus position based on the preprocedural evaluation; placement of a superficial marking grid; and limited CT scan of the area of interest to plan the shortest biopsy route. Intravenous conscious sedation (IVCS), using titrated incremental doses of fentanyl and midazolam, is administered according to the physician and patient preference. After the chosen entry site is prepared and draped in a sterile fashion, local anesthesia is administered. The biopsy needle is then inserted into the target lesion using intermittent, operator-controlled CT fluoroscopy to confirm needle position before obtaining tissue samples.

The decision whether to perform FNA and/or CNB, the size of the CNB needle used, and whether to use a coaxial introducer system are determined by the performing radiologist based on individual patient and lesion factors (Figure 3-2), as well as the indication for biopsy (pathologic diagnosis vs genomics vs trial protocol). FNA samples are generally obtained with a 22- to 24-gauge 15/9-cm needle, and core needle specimens can be obtained with either an 18- or 20-gauge automated cutting biopsy needle with needle lengths ranging from 6 to 20 cm and core lengths of 1 or 2 cm. The size and model of the CNB used can be chosen by the performing radiologist based on the individual case and physician preference. The presence of an onsite cytologist can help determine adequacy of the specimen using rapid onsite evaluation. Three or more fine needle passes typically yield enough tissue for diagnosis and molecular profiling. Similarly, at least 3 core biopsy specimens are generally recommended to enable diagnosis and molecular profiling, although not all specimens will have sufficient material. Bone lesions require special consideration as described in the “Tissue and Cytology Samples—Pathologist’s Perspective” section. Other than steps required in the pathology laboratory, sampling of any soft tissue components and collection of washings from the bone sampling instruments is recommended (Figure 3-3).

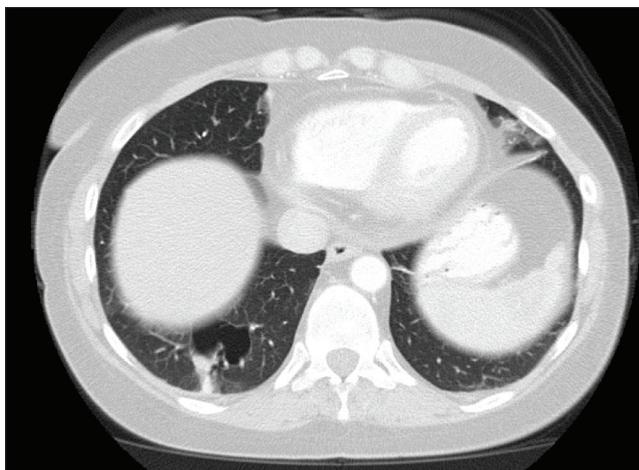


Figure 3-2. Fine-needle aspiration biopsy is more suitable than core needle biopsy if the lesion is small and close to major vessels or cysts as seen in these computed tomography (CT) images.

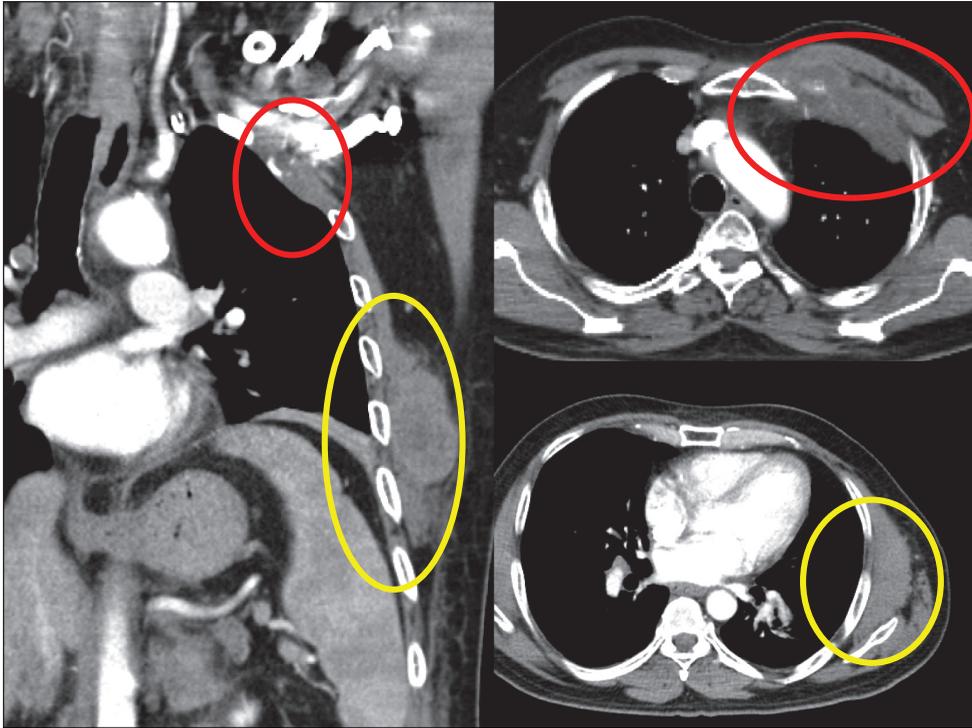


Figure 3-3. The left lateral chest wall lesion (yellow circle) is more suitable for genomic profiling than the left anterior rib metastases (red circle). Decalcification of bone metastases, if required, renders the tissue suboptimal for genetic evaluation.

Immediately after the procedure, a chest radiograph or CT thorax is performed to assess for complications including pneumothorax and hemorrhage. Outpatients are then monitored for at least 2 hours in an observation unit with a chest x-ray obtained before discharge to assess for delayed pneumothorax and/or hemorrhage. Inpatients are returned to the ward for monitoring, with a post-procedure x-ray to be obtained at 2 hours. Patients with significant complications, for example, a pneumothorax requiring chest tube placement, are hospitalized for further management and observation, as deemed clinically appropriate by the interventional team.

Biopsy Complications

Most frequently reported complications in TTNB are pneumothorax and hemorrhage, with reported pneumothorax and chest tube placement rates of 12% to 45% and 2% to 15%, respectively^{52,64,65} (Figure 3-4). Degree of emphysema, longer needle path, needle gauge, dwell times, and crossing a fissure increase the odds of pneumothorax by a factor of 3.7.⁶⁶ The reported rates of pulmonary hemorrhage post-TTNB vary from 8% to 65%,⁶⁷ and severe cases of bleeding may require embolization or surgical intervention. Rare complications, such as air embolism and death, have been reported.⁶⁸

Biopsies may be nondiagnostic, and all cases of insufficient specimens should be reviewed and rebiopsy considered only after re-review of images (Figure 3-5). The need for an alternate

strategy, additional imaging, or an alternative approach (such as transbronchial) should be considered on a case-by-case basis.

Conclusion

Image-guided biopsies are a safe and effective method for acquiring tissue for lung cancer diagnosis and molecular profiling with a low complication rate. CT guidance is the mainstay for image-guided biopsies and for patients undergoing rebiopsy. A multidisciplinary approach is key to optimal patient management as lesions that are more appropriate for endobronchial or transbronchial biopsies should be referred to interventional pulmonology. Effective communication and labeling of specimens are also needed to ensure that clinically relevant information is relayed to the reporting pathologist to enable appropriate tissue management for diagnosis and molecular testing (Table 3-2).



Figure 3-4. (A) Computed tomography (CT)-guided transthoracic needle biopsy (B) complicated by left pneumothorax post biopsy. (C) Pigtail catheter placement to treat pneumothorax.

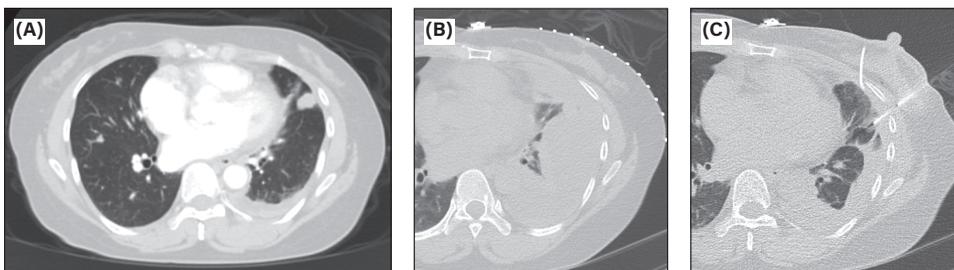


Figure 3-5. (A) Pre-procedure left upper lobe lesion for re-biopsy. (B) On the day of the procedure, a large pleural effusion obscures the lesion. (C) Following drainage of the effusion and submission for cytology, a core biopsy of the lesion is also taken to ensure adequate material for diagnosis and molecular profiling.

Table 3-2. Key Considerations in Radiologically Guided Specimen Acquisition

- Transthoracic needle biopsy (TTNB) is a safe and effective method of obtaining sufficient tissue for genomic profiling.
- Standardized institution-based protocols detailing the workflow from a review of the images to acquiring the specimen, clear labeling and transport to pathology, and closed-loop communication are vital in improving the procedure yield for molecular profiling.
- Core biopsies are generally preferred for molecular profiling; however, if only fine-needle aspiration (FNA) is possible, 3 or more passes should be attempted.
- Malignant pleural fluid, if aspirated at the time of biopsy, should also be submitted for analyses.

Thoracic Surgeon's Perspective

By Jessica S. Donington and Paul E. Van Schil

Introduction

Ideally, biopsies are minimally invasive and, in a single procedure, establish diagnosis and stage, while providing adequate tissue for molecular analysis. For this reason, the lung is rarely the ideal biopsy site for locally advanced or metastatic NSCLC. Percutaneous and endoscopic needle biopsies are the most common source of diagnostic tissue in NSCLC, but not all lesions are amenable to these approaches. When a thoracic surgical biopsy is required, minimally invasive procedures are preferred, and thoracotomy for tissue acquisition is no longer appropriate in most circumstances. Thoracic surgery biopsies for NSCLC fall into 3 broad categories: lymph node, pleural, and parenchymal biopsies. Tissue obtained from anatomic resections of early stage disease can also be used for molecular testing to direct adjuvant treatment or treatment upon disease progression.

Lymph Node Biopsies

Tissue acquisition from mediastinal or hilar nodes is typically performed by EBUS. This has largely replaced mediastinoscopy because of its minimally invasive features and ability to reach lymph nodes within the lung hilum.⁶⁹ Mediastinoscopy still has some indications, primarily for diagnosing lymphoma and following a nondiagnostic EBUS. Biopsies obtained at mediastinoscopy are larger than those obtained at EBUS, and therefore adequacy of tissue for molecular analysis is rarely an issue, but communication with the pathologist is essential to assure tissue is reserved for molecular analysis in cases where frozen section is requested. Classically, mediastinoscopy can access lymph nodes at stations 4R, 4L, and 7, but hilar nodes and aortopulmonary (PA) window nodes are not routinely reached.

Isolated station 5 or 6 lymph node involvement from left upper lobe tumors is within the purview of the thoracic surgeon. EBUS biopsy at this location requires a needle traversing the pulmonary artery, and while reported, has not been widely adopted. Similarly, mediastinoscopy can only reach these stations through an extended procedure described by Ginsberg et al in 1987, but never broadly adopted.⁷⁰ Surgical access to the PA window can be via anterior mediastinotomy (Chamberlain) procedure or left minimally invasive surgery, using video-assisted thoracoscopic surgery (VATS) or robotic-assisted thoracoscopic surgery (RATS). The advantages of the Chamberlain procedure are that it does not require single lung ventilation and is typically performed without chest tube and as an outpatient procedure. But the incision is painful and unsightly and visualization within the chest is limited. Chamberlain procedures have therefore largely been replaced by a left-sided VATS or RATS approach to PA window biopsies. These typically require single lung ventilation but can be performed as outpatient procedures and permit excellent intrapleural visualization and full assessment of the pleural space for disease spread.

Pleural Biopsy

Pleural dissemination is a pattern of spread in NSCLC, typically resulting in pleural effusion. Cytologic analysis provides a diagnosis in up to 60% of malignant effusions, but diagnosing squamous cell cancers can be challenging by cytology alone.⁷¹ Direct visualization of the pleural space for diagnosis and biopsy is required in approximately 20% of malignant

effusions, and VATS has largely replaced closed pleural biopsy.⁷² Single-port procedures are recommended and can be performed under local or general anesthesia; lung isolation is not mandatory. Surface inspection is performed following the drainage of fluid and directed biopsies of parietal pleura performed. Frozen section may be appropriate to ensure diagnostic material, and visceral pleural biopsies are discouraged. Diagnostic accuracy from VATS pleural biopsy is above 90% and complications are rare.⁷³ Palliative interventions, either pleurodesis or tunneled pleural catheter, should be performed simultaneously, but adequacy of biopsy for molecular analysis and appropriate tissue stewardship need to remain front of mind.

Parenchymal Biopsies

In NSCLC, tissue confirmation is typically required prior to treatment; an exception represents clinical stage IA disease. Some surgeons and patients prefer pathologic confirmation in advance of an anatomic resection, pretreatment biopsy may not be required especially in the context of a high risk for malignancy, low risk for mediastinal disease, and no indication for induction therapy. A surgical wedge biopsy can be the initial step of a planned anatomic resection, and frozen section is used to determine the need to proceed or not. Communication with pathologists is critical to ensure that they are aware that the resected specimen likely represents the full extent of the patient's disease and to avoid sampling the entire lesion in frozen section if possible. Surgical wedge biopsies are also frequently undertaken in the setting of oligometastatic or oligoprogressive disease for therapeutic and diagnostic benefit.

Surgical lung biopsies for NSCLC, if performed as part of a larger planned resection or as a stand-alone procedure, should be performed by VATS or RATS whenever possible and should preserve parenchyma while assuring complete excision of the lesion without bisecting it. Localization of small, nonsolid, or deep nodules can be challenging during minimally invasive surgery. Technologies for nodule localization fall into 4 categories: (1) intraoperative imaging adjuncts, such as thoracoscopic ultrasound; (2) physical markers, such as hook wires, microcoils, and fiducials; (3) parenchymal dyes and “tattoos” used with or without near-infrared (NIR) imaging;⁷⁴ and (4) molecular targets.⁷⁵ Ideally, localization and resection are performed under a single anesthetic. This is facilitated by thoracic hybrid operating rooms that include cone beam CT scanners, electromagnetic navigation, fluoroscopy, and RATS and/or VATS equipment included in a single operating theater.⁷⁶

Conclusion

Procurement of tissue for molecular analysis will continue to increase in importance in the multimodality treatment of NSCLC. Surgeons rarely have significant issues with the acquisition of adequately sized biopsies for molecular analysis but need to be cognizant of their central role in appropriate handling and communication of the biopsy specimens in the era of molecular oncology. This will be ever more so as more neoadjuvant and adjuvant targeted therapies become standards of care.

Tissue and Cytology Samples—Pathologist's Perspective

By Mary Beth Beasley, Lukas Bubendorf, and Deepali Jain

Regardless of whether specimens have been obtained surgically, percutaneously, or endoscopically, pre-analytic variables have the potential to impact pathologic diagnosis and the

efficacy of molecular testing. Such factors include specimen volume as well as handling and processing factors (Table 3-3). Prolonged cold ischemia time may result in DNA and RNA degradation, which may impact testing results. Optimally, cold ischemia time should be less than 1 hour, but placement of the tissue in formalin should occur as quickly as possible. Specimens should be promptly fixed in 10% neutral buffered formalin, and mercury-containing or acidic fixatives should be avoided. Ideally, the recommended fixation time is 6 to 48 hours with an optimal range of 8 to 18 hours for larger surgical specimens.^{49,77} It is important to remember that tissue samples can only be fixed and processed once. These steps must be permissive of *all* the diagnostic techniques that the sample may require, including standard morphology, immunohistochemistry (IHC), and sequencing. While formalin fixation and paraffin embedding is used in laboratories worldwide as a standard method for preserving tissue for diagnosis, formalin does have the potential to modify nucleotides (C to T and G to A changes). Additionally, both underfixation and overfixation may compromise DNA and RNA quality, with overfixation additionally resulting in potential DNA fragmentation and sequence alteration. Overfixation, in particular, may additionally result in cross-linkage

Table 3-3. Pre-Analytic Variables That Can Impact Molecular Testing of Lung Cancer Specimens

All specimens
Cold ischemia time
<ul style="list-style-type: none"> – Less than 1 hour is optimal.
<ul style="list-style-type: none"> • Type of fixative <ul style="list-style-type: none"> – Preference is for 10% neutral buffered formalin. – Acidic or mercury-based fixatives and harsh decalcifying agents should be avoided. – Ethylenediaminetetraacetic acid (EDTA)-based decalcification protocols may be used if necessary.
<ul style="list-style-type: none"> • For adequacy of fixation, 6 to 48 hours is recommended.
<ul style="list-style-type: none"> • Selection of tissue block and sections <ul style="list-style-type: none"> – Select the maximal amount of tumor relative to background stromal and/or inflammatory cells. – Optimal selection is generally 20% or more of tumor cells. – Precautions should be taken when sectioning to avoid potential cross-contamination. – Tumor percentage may be enriched by macro- or microdissection or coring.
Cytology/small biopsy specimens
<ul style="list-style-type: none"> • Needle gauge <ul style="list-style-type: none"> – 14 to 20 gauge for core needle biopsies – 20 to 25 gauge for transthoracic fine-needle aspiration (FNA) – 19, 21, or 22 gauge for endobronchial ultrasound (EBUS) transbronchial FNAs
<ul style="list-style-type: none"> • Number of biopsies/passes <ul style="list-style-type: none"> – Aim to maximize amount of tissue obtained where possible. – Suggested recommendations <ul style="list-style-type: none"> – Minimum of 3 core needle biopsies – With EBUS transbronchial needle aspiration (TBNA), 3 to 5 passes – Multiple passes for transthoracic FNA with focus on material for cell block preparation
<ul style="list-style-type: none"> • Use of rapid onsite evaluation (ROSE) when possible
<ul style="list-style-type: none"> • Management of small specimens by the laboratory <ul style="list-style-type: none"> – Separating core and bronchial biopsy specimens into separate blocks when possible – Optimizing laboratory cutting protocols to minimize tissue waste – Limiting investigation at the initial diagnostic phase only to essential steps (judicious use of immunohistochemistry)

between proteins and nucleic acids impeding analysis.⁷⁷ While resection specimens are not immune to pre-analytical variables (see later text), the influence of most pre-analytic variables tends to impact cytology and small biopsy specimens to a greater extent.

Given that many patients with NSCLC are diagnosed by either a cytology specimen, such as FNA, or a small tissue biopsy, such as a bronchial or core biopsy, and such a sample may be the only material available for molecular testing, appropriate management of specimens is critical in attempting to maximize the amount of diagnostic, prognostic, and predictive information obtained.⁷⁸ Most current NGS testing systems encompassing DNA and RNA sequencing typically require 100 to 200 ng of DNA or RNA for optimal results, although smaller quantities (down to 10-20 ng) may be sufficient for targeted NGS panels using amplicon-based methods, less comprehensive rapid multiplex reverse transcription polymerase chain reaction (RT-PCR)-based testing platforms or single gene analysis. As such, the success rate of both small biopsies and cytology specimens is generally high; however, careful handling can help ensure a greater chance of success.⁹

While the number of tumor cells is important, at least 200 as a general rule for DNA-based panels, more importantly, the sample must have sufficient proportion of tumor cells to provide an adequate amount of DNA and/or RNA that will not be diluted by that present in the nucleated cells in the remainder of the sample. Ideally, the tissue block or smear selected for genomic analysis should have the highest available percentage of tumor nucleated cells, optimally greater than 20% of the total nucleated cells in a specimen. Macro- or microdissection of samples and coring of high-tumor cellularity area prior to nucleic acid extraction can help further increase the ratio of tumor cells to background nucleated cells in the sample (tumor enrichment).^{78,79} Although a tumor surface area of greater than 25 mm² has been shown to improve molecular testing success rates,⁹ this amount of tumor cannot always be obtained in small biopsy specimens, even by increasing the number of sections used.

To overcome the risk of failure caused by an inadequate specimen, tissue sample size should be maximized when feasible, which involves input from the clinician performing the diagnostic procedure. A guideline document on the collection and handling of thoracic small biopsy and cytology specimens from the College of American Pathologists (see also the preceding section) recommends at least 3 core biopsies, if possible, using a 14- to 20-gauge needle, and multiple biopsies should be taken if possible during bronchoscopic biopsies.⁴⁹ Similarly, for transthoracic FNA, multiple passes obtained using a 20- to 25-gauge needle is recommended. For EBUS TBNAs, it is recommended that a 19-, 21-, or 22-gauge needle be used to perform 3 to 5 passes when feasible, particularly when onsite evaluation is not available.

The use of ROSE, although not available in all practice situations, can assist with ensuring adequacy of material and triaging for ancillary testing. For all cytology specimens, an attempt should be made to collect adequate material for preparation of a cell block.⁴⁹ Formalin-fixed cell block material is the most common and widely used cytology preparation utilized for performance of molecular studies; however, smear slides or other methods, such as liquid-based cytology, may be used if appropriately validated.^{80,81} Although the preceding recommendations apply to needle aspiration specimens, pleural fluid cytology specimens or, less commonly, other specimens, such as bronchioloalveolar lavages/bronchial washings or brushings, can also be successfully utilized for genomic testing providing there is a sufficiently high proportion of tumor cells.^{82,83} Because cytology specimens, including

cell block preparations ultimately fixed in formalin, are often initially exposed to varying amounts of ethanol or other fixatives, rigorous validation should be performed prior to use for ancillary studies; however, most cytology preparations provide excellent results provided sufficient tumor material is present.

For core or bronchial biopsy specimens, touch imprint cytology may be used to evaluate for specimen adequacy; however, care should be taken as this process may compromise the tissue specimen.⁸⁴ Separate core needle or bronchial biopsies should ideally be distributed over separate blocks, as opposed to submitting entirely in a single block, so that 1 block can be used for diagnostic stains and the remaining block(s) preserved for molecular testing. This approach, however, would require that all fragments contain tumor.

Tissue sectioning procedures should also be designed to minimize potential waste of tissue. Laboratories must devise their own optimal protocol based on available capabilities; however, a variety of approaches, from single-slide superficial cutting to a range of strategies involving precutting of multiple unstained slides, often with hematoxylin and eosin (H&E) evaluation of levels preceding and following those used for DNA and RNA extraction, have been suggested. Precautions to avoid cross contamination should be taken at all points of processing and cutting; this may involve using a dedicated microtome and a new, unused blade each time sections are cut for DNA/RNA extraction.

Additionally, while biopsy of a bone lesion provides an opportunity to potentially diagnose and stage a tumor simultaneously and may be the most accessible site of disease, careful consideration must be taken into account with the avoidance of decalcifying agents if possible.⁸⁴ If decalcification cannot be avoided, an ethylenediaminetetraacetic acid (EDTA)-based protocol should be followed.⁹ Interventional radiologists taking biopsies from bone metastases should be advised to rinse the biopsy needle or collect extra FNA specimens in order to avoid delaying the diagnostic process by decalcification time and provide additional material for molecular testing.

Surgically resected specimens generally pose less of an issue in regard to tumor volume considerations; however, other issues may impact the results of molecular testing. Cold ischemia time may be influenced by transport time to the laboratory and time taken for any frozen section analysis and/or collection of tissue for biobanking and/or research purposes. Additionally, larger specimens may experience improper fixation as formalin poorly penetrates the visceral pleura. Resection specimens require inflation or injection with formalin or, in some instances, sectioning prior to immersion in formalin to ensure adequate fixation. Resection specimens following neoadjuvant therapy may require special handling to facilitate residual tumor assessment.⁸⁵ Tumor heterogeneity is not generally an issue with genomic profiling of driver mutations, but, as was described with small biopsy specimens, the block selected for genetic testing should contain the highest ratio of tumor cells relative to background stromal or inflammatory cells.⁷⁹

Conclusion

The way in which specimens are handled in the pathology laboratory is crucial in determining success, or failure, in any required molecular biomarker testing. Pre-analytical variables relating to tissue fixation and processing, while essential to permit microscopy, can harm DNA, RNA, and antigen/epitope integrity. Standardized procedures should be developed

to facilitate *all* steps of the diagnostic process. It is essential to get this right the first time as samples can only be fixed and processed once, and these samples are often very small and contain little tumor. Paraffin blocks should be cut as few times as possible; reflex cutting of blank sections and minimal use of IHC for diagnosis are recommended. DNA and RNA yield can be improved by microdissection. Cytology-type samples are perfectly adequate for molecular biomarker testing provided they are processed correctly and contain sufficient tumor. Although surgical resection specimens generally have abundant tumor, issues with cold ischemia and poor fixation should be anticipated and avoided.

References

1. Kerr KM. Personalized medicine for lung cancer: new challenges for pathology. *Histopathology*. 2012;60(4):531-546.
2. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med*. 2009;361(10):947-957.
3. Mitsudomi T, Morita S, Yatabe Y, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. *Lancet Oncol*. 2010;11(2):121-128.
4. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med*. 2010;362(25):2380-2388.
5. Skoulidis F, Heymach JV. Co-occurring genomic alterations in non-small-cell lung cancer biology and therapy. *Nat Rev Cancer*. 2019;19(9):495-509.
6. Nicholson AG, Tsao MS, Beasley MB, et al. The 2021 WHO classification of lung tumors: impact of advances since 2015. *J Thorac Oncol*. 2022;17(3):362-387.
7. Lindeman NI, Cagle PT, Aisner DL, et al. 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. *J Thorac Oncol*. 2018;13(3):323-358.
8. Hirsch FR, Wynes MW, Gandara DR, Bunn PA Jr. The tissue is the issue: personalized medicine for non-small cell lung cancer. *Clin Cancer Res*. 2010;16(20):4909-4911.
9. Mata DA, Harries L, Williams EA, et al. Method of tissue acquisition affects success of comprehensive genomic profiling in lung cancer. *Arch Pathol Lab Med*. 2022. doi:10.5858/arpa.2021-0313-OA.
10. Penault-Llorca F, Kerr KM, Garrido P, et al. Expert opinion on NSCLC small specimen biomarker testing—part 2: analysis, reporting, and quality assessment. *Virchows Arch*. 2022;481(3):351-366.
11. Penault-Llorca F, Kerr KM, Garrido P, et al. Expert opinion on NSCLC small specimen biomarker testing—part 1: tissue collection and management. *Virchows Arch*. 2022;481(3):335-350.
12. Mino-Kenudson M, Le Stang N, Daigneault JB, et al. The International Association for the Study of Lung Cancer global survey on programmed death-ligand 1 testing for NSCLC. *J Thorac Oncol*. 2021;16(4):686-696.
13. Smeltzer MP, Wynes MW, Lantuejoul S, et al. The International Association for the Study of Lung Cancer global survey on molecular testing in lung cancer. *J Thorac Oncol*. 2020;15(9):1434-1448.
14. Dietel M, Bubendorf L, Dingemans AM, et al. Diagnostic procedures for non-small-cell lung cancer (NSCLC): recommendations of the European Expert Group. *Thorax*. 2016;71(2):177-184.
15. Gan Q, Roy-Chowdhuri S. Small but powerful: the promising role of small specimens for biomarker testing. *J Am Soc Cytopathol*. 2020;9(5):450-460.
16. Harzheim D, Sterman D, Shah PL, Eberhardt R, Herth FJ. Bronchoscopic transparenchymal nodule access: feasibility and safety in an endoscopic unit. *Respiration*. 2016;91(4):302-306.
17. Rivera MP, Mehta AC, Wahidi MM. Establishing the diagnosis of lung cancer: diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest*. 2013;143(5 suppl):e142S-e65S.
18. Leong S, Ju H, Marshall H, et al. Electromagnetic navigation bronchoscopy: a descriptive analysis. *J Thorac Dis*. 2012;4(2):173-185.

19. Wang Memoli JS, Nietert PJ, Silvestri GA. Meta-analysis of guided bronchoscopy for the evaluation of the pulmonary nodule. *Chest*. 2012;142(2):385-393.
20. Steinfort DP, Vincent J, Heinze S, Antippa P, Irving LB. Comparative effectiveness of radial probe endobronchial ultrasound versus CT-guided needle biopsy for evaluation of peripheral pulmonary lesions: a randomized pragmatic trial. *Respir Med*. 2011;105(11):1704-1711.
21. Fielding DIK, Bashirzadeh F, Son JH, et al. First human use of a new robotic-assisted fiber optic sensing navigation system for small peripheral pulmonary nodules. *Respiration*. 2019;98(2):142-150.
22. Oki M, Saka H. Diagnostic value of ultrathin bronchoscopy in peripheral pulmonary lesions: a narrative review. *J Thorac Dis*. 2020;12(12):7675-7682.
23. Fielding DI, Chia C, Nguyen P, et al. Prospective randomised trial of endobronchial ultrasound-guide sheath versus computed tomography-guided percutaneous core biopsies for peripheral lung lesions. *Intern Med J*. 2012;42(8):894-900.
24. Haentschel M, Boeckeler M, Ehab A, et al. Cryobiopsy increases the EGFR detection rate in non-small cell lung cancer. *Lung Cancer*. 2020;141:56-63.
25. Shinagawa N. A review of existing and new methods of bronchoscopic diagnosis of lung cancer. *Respir Investig*. 2019;57(1):3-8.
26. Botticella MA, De Summa S, Cisternino L, et al. The role of rapid on site evaluation on touch imprint cytology and brushing during conventional bronchoscopy. *Diagn Cytopathol*. 2021;49(7):832-837.
27. Um SW, Kim Y, Lee BB, et al. Genome-wide analysis of DNA methylation in bronchial washings. *Clin Epigenetics*. 2018;10:65.
28. Roncarati R, Lupini L, Miotto E, et al. Molecular testing on bronchial washings for the diagnosis and predictive assessment of lung cancer. *Mol Oncol*. 2020;14(9):2163-2175.
29. Bubendorf L, Conde E, Cappuzzo F, et al. A noninterventional, multinational study to assess PD-L1 expression in cytological and histological lung cancer specimens. *Cancer Cytopathol*. 2020;128(12):928-938.
30. Fielding D, Kurimoto N. Endobronchial ultrasound-guided transbronchial needle aspiration for diagnosis and staging of lung cancer. *Clin Chest Med*. 2018;39(1):111-123.
31. Fox AH, Jett JR, Roy UB, et al. Knowledge and practice patterns among pulmonologists for molecular biomarker testing in advanced non-small cell lung cancer. *Chest*. 2021;160(6):2293-303.
32. Stone E, Leong TL. Contemporary concise review 2021: pulmonary nodules from detection to intervention. *Respirology*. 2022;27(9):776-785.
33. Sung S, Heymann JJ, Crapanzano JP, et al. Lung cancer cytology and small biopsy specimens: diagnosis, predictive biomarker testing, acquisition, triage, and management. *J Am Soc Cytopathol*. 2020;9(5):332-345.
34. Parente P, Carbonelli C, Biancofiore G, et al. Handling and standardization of EBUS needle aspiration in NSCLC patients: the value of the cell block, a monoinstitutional experience. *Thorac Cancer*. 2022;13(17):2480-2488.
35. Jain D, Allen TC, Aisner DL, et al. Rapid on-site evaluation of endobronchial ultrasound-guided transbronchial needle aspirations for the diagnosis of lung cancer: a perspective from members of the Pulmonary Pathology Society. *Arch Pathol Lab Med*. 2018;142(2):253-262.
36. van der Heijden EH, Casal RF, Trisolini R, et al. Guideline for the acquisition and preparation of conventional and endobronchial ultrasound-guided transbronchial needle aspiration specimens for the diagnosis and molecular testing of patients with known or suspected lung cancer. *Respiration*. 2014;88(6):500-517.
37. Fassina A, Corradin M, Zardo D, et al. Role and accuracy of rapid on-site evaluation of CT-guided fine needle aspiration cytology of lung nodules. *Cytopathology*. 2011;22(5):306-312.
38. Asano F, Matsuno Y, Shinagawa N, et al. A virtual bronchoscopic navigation system for pulmonary peripheral lesions. *Chest*. 2006;130(2):559-566.
39. Asano F, Shinagawa N, Ishida T, et al. Virtual bronchoscopic navigation combined with ultrathin bronchoscopy. A randomized clinical trial. *Am J Respir Crit Care Med*. 2013;188(3):327-333.
40. Ishida T, Asano F, Yamazaki K, et al. Virtual bronchoscopic navigation combined with endobronchial ultrasound to diagnose small peripheral pulmonary lesions: a randomised trial. *Thorax*. 2011;66(12):1072-7.
41. Oki M, Saka H, Ando M, et al. Ultrathin bronchoscopy with multimodal devices for peripheral pulmonary lesions. A randomized trial. *Am J Respir Crit Care Med*. 2015;192(4):468-476.

42. Oki M, Saka H, Asano F, et al. Use of an ultra-thin vs thin bronchoscope for peripheral pulmonary lesions: a randomized trial. *Chest*. 2019;156(5):954-964.
43. Rojas-Solano JR, Ugalde-Gamboa L, Machuzak M. Robotic bronchoscopy for diagnosis of suspected lung cancer: a feasibility study. *J Bronchology Interv Pulmonol*. 2018;25(3):168-175.
44. Murgu SD. Robotic assisted-bronchoscopy: technical tips and lessons learned from the initial experience with sampling peripheral lung lesions. *BMC Pulm Med*. 2019;19(1):89.
45. Southwood M, Krenz T, Cant N, et al. Systematic evaluation of PAXgene(R) tissue fixation for the histopathological and molecular study of lung cancer. *J Pathol Clin Res*. 2020;6(1):40-54.
46. Dotson T, Bellinger C, Su J, et al. Feasibility of lung cancer RNA acquisition from a single transbronchial or transthoracic needle pass (FASTT trial). *Lung Cancer*. 2019;127:6-11.
47. Fielding D, Oki M. Technologies for targeting the peripheral pulmonary nodule including robotics. *Respirology*. 2020;25(9):914-923.
48. Gilbert CR, Wahidi MM, Yarmus LB, Roy-Chowdhuri S, Pastis NJ 2nd. Key highlights for the College of American Pathology statement on collection and handling of thoracic small biopsy and cytology specimens for ancillary studies. *Chest*. 2020;158(6):2282-2284.
49. Roy-Chowdhuri S, Dacic S, Ghofrani M, et al. Collection and handling of thoracic small biopsy and cytology specimens for ancillary studies: guideline from the College of American Pathologists in Collaboration with the American College of Chest Physicians, Association for Molecular Pathology, American Society of Cytopathology, American Thoracic Society, Pulmonary Pathology Society, Papanicolaou Society of Cytopathology, Society of Interventional Radiology, and Society of Thoracic Radiology. *Arch Pathol Lab Med*. 2020. doi:10.5858/arpa.2020-0119-CP.
50. Hirsch FR, Kerr KM, Bunn PA Jr, et al. Molecular and immune biomarker testing in squamous-cell lung cancer: effect of current and future therapies and technologies. *Clin Lung Cancer*. 2018;19(4):331-339.
51. Zhang HF, Zeng XT, Xing F, Fan N, Liao MY. The diagnostic accuracy of CT-guided percutaneous core needle biopsy and fine needle aspiration in pulmonary lesions: a meta-analysis. *Clin Radiol*. 2016;71(1):e1-10.
52. Takeshita J, Masago K, Kato R, et al. CT-guided fine-needle aspiration and core needle biopsies of pulmonary lesions: a single-center experience with 750 biopsies in Japan. *AJR Am J Roentgenol*. 2015;204(1):29-34.
53. Lee C, Guichet PL, Abtin F. Percutaneous lung biopsy in the molecular profiling era: a survey of current practices. *J Thorac Imaging*. 2017;32(1):63-67.
54. Diacon AH, Schuurmans MM, Theron J, et al. Safety and yield of ultrasound-assisted transthoracic biopsy performed by pulmonologists. *Respiration*. 2004;71(5):519-522.
55. Koh DM, Burke S, Davies N, Padley SP. Transthoracic US of the chest: clinical uses and applications. *Radiographics*. 2002;22(1):e1.
56. Gill RR, Barlow J, Jaklitsch MT, et al. Image-guided video-assisted thoracoscopic resection (iVATS): translation to clinical practice-real-world experience. *J Surg Oncol*. 2020;121(8):1225-1232.
57. Kang DY, Kim HK, Kim YK, et al. Needlescopy-assisted resection of pulmonary nodule after dual localisation. *Eur Respir J*. 2011;37(1):13-17.
58. Gill RR, Murphy DJ, Kravets S, et al. Success of genomic profiling of non-small cell lung cancer biopsies obtained by trans-thoracic percutaneous needle biopsy. *J Surg Oncol*. 2018;118(7):1170-1177.
59. Cardarella S, Johnson BE. The impact of genomic changes on treatment of lung cancer. *Am J Respir Crit Care Med*. 2013;188(7):770-775.
60. Kim L, Tsao MS. Tumour tissue sampling for lung cancer management in the era of personalised therapy: what is good enough for molecular testing? *Eur Respir J*. 2014;44(4):1011-1122.
61. Lizotte PH, Jones RE, Keogh L, et al. Fine needle aspirate flow cytometric phenotyping characterizes immunosuppressive nature of the mesothelioma microenvironment. *Sci Rep*. 2016;6:31745.
62. Loh SE, Wu DD, Venkatesh SK, et al. CT-guided thoracic biopsy: evaluating diagnostic yield and complications. *Ann Acad Med Singap*. 2013;42(6):285-290.
63. Schneider F, Smith MA, Lane MC, et al. Adequacy of core needle biopsy specimens and fine-needle aspirates for molecular testing of lung adenocarcinomas. *Am J Clin Pathol*. 2015;143(2):193-200; quiz 306.
64. Tian P, Wang Y, Li L, et al. CT-guided transthoracic core needle biopsy for small pulmonary lesions: diagnostic performance and adequacy for molecular testing. *J Thorac Dis*. 2017;9(2):333-343.

65. Heerink WJ, de Bock GH, de Jonge GJ, et al. Complication rates of CT-guided transthoracic lung biopsy: meta-analysis. *Eur Radiol.* 2017;27(1):138-148.
66. Nour-Eldin NE, Alsubhi M, Emam A, et al. Pneumothorax complicating coaxial and non-coaxial CT-guided lung biopsy: comparative analysis of determining risk factors and management of pneumothorax in a retrospective review of 650 patients. *Cardiovasc Intervent Radiol.* 2016;39(2):261-270.
67. Tai R, Dunne RM, Trotman-Dickenson B, et al. Frequency and severity of pulmonary hemorrhage in patients undergoing percutaneous CT-guided transthoracic lung biopsy: single-institution experience of 1175 cases. *Radiology.* 2016;279(1):287-296.
68. Freund MC, Petersen J, Goder KC, et al. Systemic air embolism during percutaneous core needle biopsy of the lung: frequency and risk factors. *BMC Pulm Med.* 2012;12:2.
69. Silvestri GA, Gonzalez AV, Jantz MA, et al. Methods for staging non-small cell lung cancer: diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest.* 2013;143(5 suppl):e211S-e250S.
70. Ginsberg RJ, Rice TW, Goldberg M, Waters PF, Schmock BJ. Extended cervical mediastinoscopy. A single staging procedure for bronchogenic carcinoma of the left upper lobe. *J Thorac Cardiovasc Surg.* 1987;94(5):673-678.
71. Hooper C, Lee YC, Maskell N, Group BTS Pleural Guideline Group. Investigation of a unilateral pleural effusion in adults: British Thoracic Society Pleural Disease Guideline 2010. *Thorax.* 2010;65(suppl 2):ii4-ii17.
72. Ferrer J, Roldan J, Teixidor J, et al. Predictors of pleural malignancy in patients with pleural effusion undergoing thoracoscopy. *Chest.* 2005;127(3):1017-1022.
73. Menzies R, Charbonneau M. Thoracoscopy for the diagnosis of pleural disease. *Ann Intern Med.* 1991;114(4):271-276.
74. Hachey KJ, Digesu CS, Armstrong KW, et al. A novel technique for tumor localization and targeted lymphatic mapping in early-stage lung cancer. *J Thorac Cardiovasc Surg.* 2017;154(3):1110-1118.
75. Predina JD, Okusanya O, Newton AD, Low P, Singhal S. Standardization and optimization of intraoperative molecular imaging for identifying primary pulmonary adenocarcinomas. *Mol Imaging Biol.* 2018;20(1):131-138.
76. Zhao ZR, Lau RWH, Ng CSH. Hybrid theater and uniportal video-assisted thoracic surgery: the perfect match for lung nodule localization. *Thorac Surg Clin.* 2017;27(4):347-355.
77. Compton CC, Robb JA, Anderson MW, et al. Preanalytics and precision pathology: pathology practices to ensure molecular integrity of cancer patient biospecimens for precision medicine. *Arch Pathol Lab Med.* 2019;143(11):1346-1363.
78. Bubendorf L, Lantuejoul S, de Langen AJ, Thunnissen E. Nonsmall cell lung carcinoma: diagnostic difficulties in small biopsies and cytological specimens: number 2 in the series "Pathology for the clinician," ed. Peter Dorfmueller and Alberto Cavazza. *Eur Respir Rev.* 2017;26(144):170007.
79. Aggarwal C, Bubendorf L, Cooper WA, et al. Molecular testing in stage I-III non-small cell lung cancer: approaches and challenges. *Lung Cancer.* 2021;162:42-53.
80. Baum JE, Zhang P, Hoda RS, et al. Accuracy of next-generation sequencing for the identification of clinically relevant variants in cytology smears in lung adenocarcinoma. *Cancer Cytopathol.* 2017;125(6):398-406.
81. Mohan AA, Vallonthaiel AG, Nambiar A, et al. EGFR analysis on scrapings from cytology smears in lung carcinoma, an effective alternative to testing on trucut biopsies. *Diagn Cytopathol.* 2021;49(10):1155-1163.
82. Dalvi SD, Chau K, Sajjan S, et al. Adequacy of pleural fluid cytology for comprehensive molecular analysis of lung adenocarcinoma: experience of a large health-care system. *Cytojournal.* 2022;19:7.
83. Lindeman NI, Cagle PT, Aisner DL, et al. 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. *Arch Pathol Lab Med.* 2018;142(3):321-346.
84. Vanderlaan PA, Yamaguchi N, Folch E, et al. Success and failure rates of tumor genotyping techniques in routine pathological samples with non-small-cell lung cancer. *Lung Cancer.* 2014;84(1):39-44.
85. Travis WD, Dacic S, Wistuba I, et al. IASLC multidisciplinary recommendations for pathologic assessment of lung cancer resection specimens after neoadjuvant therapy. *J Thorac Oncol.* 2020;15(5):709-740.

Liquid Biopsy: Specimen Acquisition, Testing Strategies, and Clinical Roles

4

Specimen Handling, Processing, and Testing Strategies

By Fernando Lopez-Rios, Maria E. Arcila, and Christian Rolfo

Broadly defined, liquid biopsies encompass a wide range of components (circulating tumor cells, extracellular vesicles, cell-free nucleic acids, and various metabolites, among others). However, circulating tumor DNA (ctDNA) has attracted the most attention in the clinical space and is the main focus of this chapter. In contrast to other sources of tumor DNA, the biology and technical aspects of ctDNA are unique and more complex, with several confounding factors that remain responsible for the limitations on widespread implementation in routine clinical diagnostics.

Cell-free DNA (cfDNA) represents the sum of short fragments of DNA released from cells both *in vivo* and *in vitro*, including both tumor and normal-cell derived. Evidence demonstrates that pre-analytical variables across sample collection, processing, and storage may have profound and adverse effects on the total yield of cfDNA, degree of genomic DNA contamination, overall integrity of the sample, and nucleic acid fragment lengths. The type of collection tube, anticoagulants used, stabilization media, transport conditions, timing of the plasma separation from blood, tube agitation, centrifugation speeds and protocols, plasma storage/duration conditions, number of freeze-thaw cycles, cfDNA extraction, and nucleic acid quantification methods can all directly impact subsequent downstream analysis.¹⁻⁷ Highlights of the principal pre-analytic steps influencing performance are included in Figure 4-1.

In blood samples, immediate pre-analytical considerations focus on the stabilization of ctDNA and the prevention of contamination with genomic DNA from hematopoietic cells, which can render ctDNA nondetectable. *In vivo*, the half-life of ctDNA is generally estimated at around 2 hours. In the absence of active physiologic elimination systems, the *in vitro* half-life is longer, but its isolation may be compromised by contamination with genomic DNA released from white blood cells (WBCs) during the clotting process or by cellular lysis during

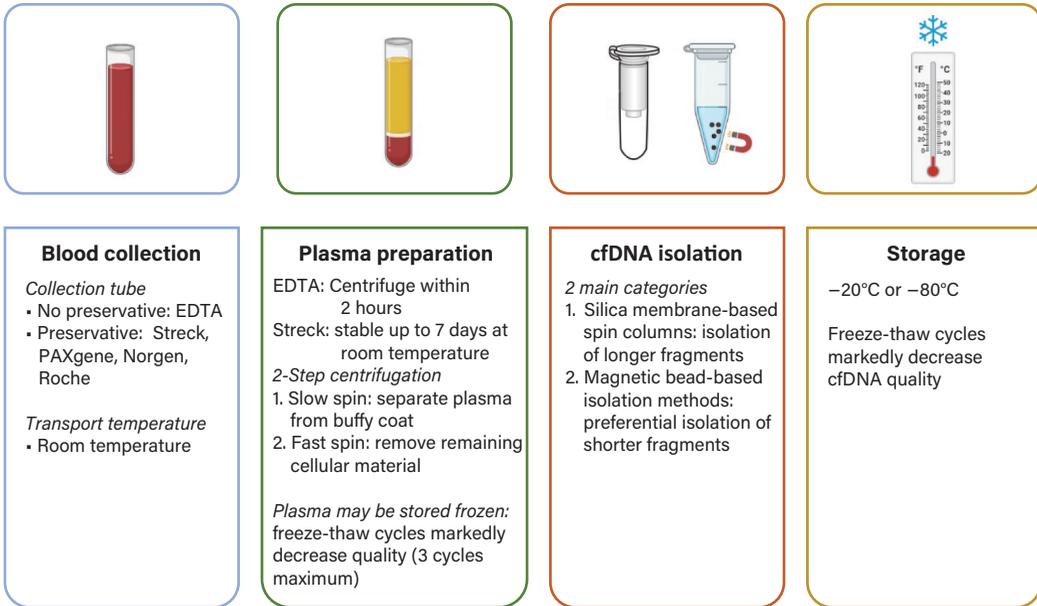


Figure 4-1. Pre-analytical factors influencing cfDNA. Abbreviations: cfDNA = cell-free DNA; EDTA = ethylenediaminetetraacetic acid.

storage. Several stabilizing collection tube options are commercially available (eg, Streck, PAXgene [BD Biosciences], Norgen Biotek, Roche Diagnostics) and exhibit variable capacities for preservation and stabilization of WBCs. In contrast to ethylenediaminetetraacetic acid (EDTA) collection tubes, these allow a wider processing window between blood draw and plasma separation and enable batching of samples collected at different times.¹⁻⁷ A 2-step centrifugation protocol generally follows, consisting of an initial low-speed centrifugation step to separate the plasma and a subsequent high-speed cycle to eliminate any remaining cellular material.⁸

There is wide variability in the extraction methods and the amount of plasma used for cfDNA isolation.⁹ Common isolation methods and commercially available kits fall into 2 main categories: silica membrane-based spin columns and magnetic bead-based isolation methods.¹⁰ The yield, integrity, and fragment size are affected by the method. Magnetic bead systems are reported to preferentially isolate shorter cfDNA fragments¹¹ compared to membrane-based methods. Precipitation-based methods are associated with higher DNA integrity in several studies.¹²⁻¹⁴ cfDNA may be stored for several months at subzero temperatures of –20°C or –80°C for later use. Significant lowering of cfDNA integrity is reported after 3 freeze-thaw cycles.^{1,15}

Several methodologies for cfDNA analysis have been implemented in clinical practice and for research purposes, including polymerase chain reaction (PCR)-based assays and next-generation sequencing (NGS)-based technologies.¹⁶ PCR-based approaches intrinsically allow more restricted gene analyses (a single target or a few) but may deliver higher sensitivity for the target gene(s). NGS approaches can provide more comprehensive tumor genotyping, identifying not only somatic mutations, but also copy number alterations, as well as gene rearrangements.¹⁷ The growing demand for biomarkers to guide appropriate

patient management and the challenge of limited tissue availability have led to a dramatic change in the potential uses of liquid biopsy in advanced non-small cell lung carcinoma (NSCLC), as outlined in the following “Clinical Application” section. This dramatic shift has been made possible thanks to major technologic advancements over the last few years, with several plasma NGS platforms now available in clinical practice whose utility are supported by robust clinical data.¹⁸⁻²⁰ Currently, 2 commercially available cfDNA assays have gained US Food and Drug Administration (FDA) approval for tumor genotyping in advanced NSCLC, and several recently approved targeted therapies have liquid biopsy-based companion diagnostics tests.

Clonal hematopoiesis of indeterminate potential (CHIP) needs to be taken into consideration when interpreting cfDNA results in clinical practice, particularly in patients who are more than 70 years old. CHIP can be defined as the somatic variations that hematopoietic stem cells acquire with age. For example, genes frequently involved include *ASXL1*, *ATM*, *CBL*, *CHEK2*, *DNMT3A*, *JAK2*, *KMT2D (MLL2)*, *KRAS*, *BRAF*, *NRAS*, *MPL*, *MYD88*, *SF3B1*, *TET2*, *TP53*, and *U2AF1*. This source of noise can be filtered out by sequencing patient-matched peripheral blood mononuclear cells or mitigated by using dedicated bioinformatics algorithms.²¹

Liquid biopsy testing strategies in patients with treatment-naïve advanced NSCLC are reviewed in detail in the following “Clinical Application” section. Although tissue-based testing still remains the gold standard for tumor genotyping for many cancer patients, because of technical and biologic limitations of ctDNA analysis, cfDNA analysis can be used either sequentially, when tumor tissue is insufficient/inadequate for testing, or concurrently, when tissue is scant or of uncertain adequacy for genotyping.^{16,22,23}

In addition, a plasma-first approach in advanced NSCLC has been recently proposed based on promising results of prospective²⁴⁻²⁷ and retrospective²⁸⁻³⁰ studies showing that plasma NGS might provide more complete tumor genotyping with lower turnaround time as compared with standard-of-care tissue genotyping at comparable sensitivity and specificity for clinically relevant driver oncogenes. The major expected limitation of this approach is the hypothesized increased costs compared with tissue genotyping alone, albeit a recent cost-effectiveness analysis in a Canadian population³¹ showed that a concurrent approach of tissue plus plasma NGS analysis in clinically selected patients with advanced NSCLC does not increase system costs and raises the proportion of patients receiving appropriate targeted therapy.

As plasma NGS increases detection rates of clinically relevant biomarkers in patients with limited tissue results but does not increase detection in patients with tissue NGS results available,²⁷ a concurrent plasma and tissue testing strategy may be sensible in the context of small tissue biopsies with uncertain adequacy for NGS analyses, increasing the chances of obtaining a comprehensive tumor genotype for all the recommended oncogenic drivers. Furthermore, recent studies showed that incorporation of cfDNA analysis in the initial diagnostic work-up of patients with suspected advanced NSCLC can lead to faster molecular results and shortened time to treatment.^{32,33} Further prospective studies with larger patient populations are needed to better clarify the clinical utility and cost-efficacy of this approach.

Clinical Application

By **Natasha B. Leigh, Myung-Ju Ahn, and David R. Gandara**

The clinical application of liquid biopsy in diagnostics and therapeutic decision-making for NSCLC continues to rapidly evolve. Figure 4-2 displays the NSCLC continuum of care, from screening and early diagnosis to determination of minimal residual disease (MRD) in the postsurgical setting of early stage disease, to genomic and immunodiagnosics of advanced-stage disease.^{16,34} It is notable that while tumor tissue profiling is applicable in patients with stage IV NSCLC, liquid biopsy is uniquely suited to have the potential to address screening of at-risk individuals, determination of MRD, and treatment response monitoring.²²

Screening and Early Detection of Lung Cancer

Early detection of lung cancer is essential to reduce morbidity and mortality, especially in high-risk individuals. The National Lung Screening Trial (NLST) demonstrated a reduction of 20% in lung cancer–specific mortality rate with low-dose computed tomography (LDCT) among high-risk individuals.³⁵ The European NELSON trial showed a lung cancer mortality reduction of 25% at 10 years.³⁶ However, the rate of false positivity, overdiagnosis, and unnecessary invasive procedures still remain as challenges.³⁷

Liquid biopsy has an emerging role in the screening and early detection of lung cancer using circulating tumor cells, circulating cfDNA (genomics or epigenomics), circulating micro-RNAs, tumor-derived exosomes, and tumor-educated platelets (Figure 4-3).³⁸ Table 4-1 summarizes the advantages and limitations of these analytes.³⁸ Although these

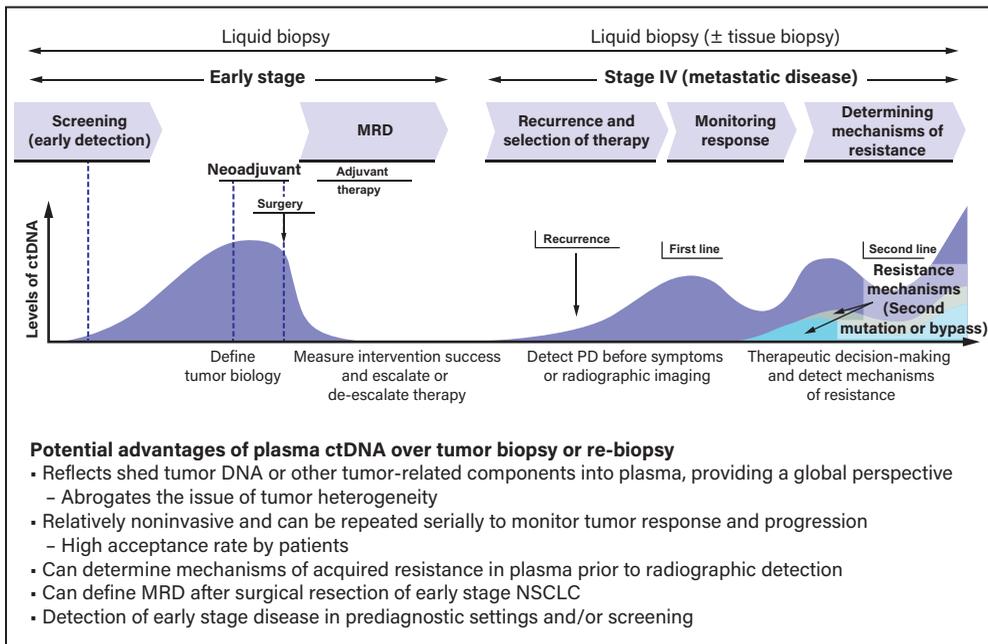


Figure 4-2. Liquid biopsy across the cancer care continuum in individual patients. Abbreviations: ctDNA = circulating tumor DNA; MRD = minimal residual disease; NSCLC = non-small cell lung carcinoma; PD = progressive disease. (Adapted from Wan et al.³⁴)

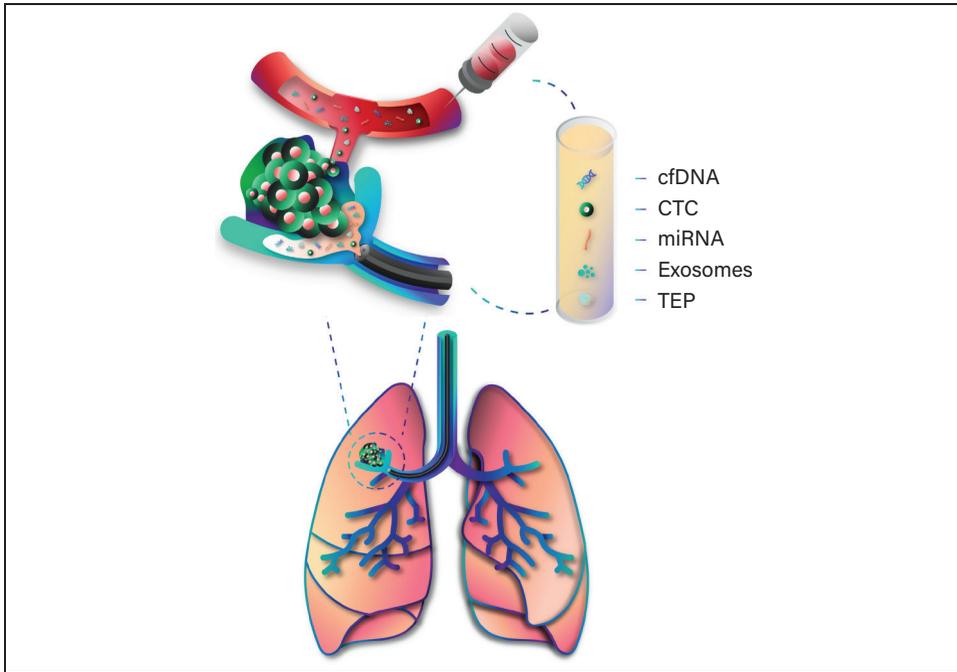


Figure 4-3. Components of liquid biopsy. Abbreviations: cfDNA = cell-free DNA; CTC = circulating tumor cells; miRNA = micro-RNA; TEP = tumor-educated platelets. (Source: Freitas et al.³⁸)

biomarkers are promising in the early detection of lung cancer, their clinical significance is still limited because of the significant proportion of falsely negative results and lack of standardization. Recently, several plasma cfDNA genotyping assays received FDA breakthrough device designation (Table 4-2).³⁹ Development of more sensitive and specific assays and further larger and robust studies combining liquid biopsies with imaging will be required before implementation in clinical practice for the screening or early diagnosis of lung cancer.

Minimal Residual Disease in NSCLC

One of the most important issues in oncology in general, and NSCLC in particular, is improved identification of patients at high risk for recurrence following definitive local therapy such as surgical resection.⁴⁰ At present, adjuvant therapy is delivered based primarily on stage, a clinical variable that does not consistently identify those patients who have achieved surgical cure versus those who require additional treatment. MRD determination by plasma liquid biopsy, if sufficiently sensitive and specific, offers the potential to better select those patients who need either therapy intensification or, conversely, in which adjuvant therapy could be omitted.

At present, multiple MRD assays are under evaluation in NSCLC, some based on ctDNA alone, others in combination with epigenetic components, and either “tissue informed” or “tissue agnostic.”^{41,42} Each of these approaches offers potential advantages and disadvantages (Table 4-3). Recently completed studies suggest that regardless of methodology, further refinement of MRD assays will be required before widespread clinical application. For example, in a preliminary retrospective analysis of MRD in the IMpower-010 trial of adjuvant atezolizumab, a tissue-informed assay based on a single plasma specimen at approximately 8 weeks following surgery was insufficient to alter an all-comer approach to adjuvant therapy.

Table 4-1. Summary of Advantages and Disadvantages in Lung Cancer Diagnosis According to Liquid Biopsy-Based Biomarker

Biomarkers	Advantages	Disadvantages
cfDNA	<ul style="list-style-type: none"> • Increased in cancer patients • Genetic and epigenetic alterations reflect those of original tumor • Representation of tumor heterogeneity and dynamics • Highly sensitive assays available (PCR, NGS) 	<ul style="list-style-type: none"> • Markedly diluted compared to germline circulation DNA • Positively correlates with tumor size and staging • Increased in some benign or premalignant conditions • High costs
CTC	<ul style="list-style-type: none"> • Allows morphologic analysis and tumor molecular characterization • Correlates with prognosis • Emerging enrichment and characterization techniques 	<ul style="list-style-type: none"> • No validated assay • Rare in bloodstream in non-small cell lung cancer • Epithelial to mesenchymal transition with loss of epithelial-specific markers • Role in cancer spreading still to be clarified
miRNA	<ul style="list-style-type: none"> • Different profiles among early stage cancer patients • Stable in most types of body fluids • Released by several structures (eg, exosome, TEPs) • Commercial kits available 	<ul style="list-style-type: none"> • High variability, according to patients and technologies, requiring normalization methods • Quantification and detection methods need to be validated • Unspecific for a cancer type
Exosomes	<ul style="list-style-type: none"> • Contains several types of biomarkers such as proteins and nucleic acids • Increased in lung cancer patients • Stable and accessible in most types of body fluid • Commercial kits available 	<ul style="list-style-type: none"> • Extraction approach, detection, and characterization methods are challenging and require standardization • High costs
TEP	<ul style="list-style-type: none"> • Platelet mRNA profiles are distinct in cancer patients • Abundant • Easily isolated • Acquire specific RNA from tumor cells reflecting its genetic alterations • Dynamic mRNA repertoire due to short life-span 	<ul style="list-style-type: none"> • No validated assay nor standardized approach • Reproducibility • Detection techniques not widely available • Time consuming and requires extensive computational resources

Abbreviations: cfDNA = cell-free DNA; CTC = circulating tumor cell; mRNA = messenger RNA; miRNA = micro-RNA; NGS = next-generation sequencing; PCR = polymerase chain reaction; TEP = tumor-educated platelet.

Source: Freitas et al.³⁸

Although outcomes were clearly different in MRD-positive and MRD-negative populations, both groups showed continuous relapse patterns.^{43,44} The results of ongoing prospective trials are highly awaited.

First-Line Treatment Selection in Advanced NSCLC

The selection of optimal therapy for patients with advanced and, increasingly, early stage NSCLC, requires both histopathologic and genomic assessment. Routine testing of nonsquamous NSCLC samples for actionable genomic alterations and PD-L1 is guideline-recommended.⁴⁵ Testing may also benefit those with other pathologic subtypes in selected scenarios (eg, younger patients, never-smokers, or light smokers). Patients with targetable tumor alterations (eg, in *EGFR*, *ALK*, *ROS1*, *BRAF*, *MET*, *RET*, *NTRK1-3*) have better outcomes with first-line targeted therapy for advanced disease. Others may be candidates for

Table 4-2. NSCLC Plasma cfDNA Genotyping Assays That Have Received Breakthrough Device Designation as Multicancer Early Detection Tests

Kit or test	Company	Technology and application	FDA status
Multicancer early detection test	GRAIL	NGS blood test analyzing ctDNA methylation patterns for detecting multiple cancer types	FDA breakthrough device designation (May 2019)
CancerSEEK	Thrive Earlier Detection	Multianalyte test that combines multiplexed PCR detection of mutations in ctDNA at 1933 loci with measurements of validated protein biomarkers to diagnose 8 common cancer types including breast, ovarian, and liver cancer	FDA breakthrough device designation (August 2018)
Ivy-Gene CORE test; Ivy-Gene Dx liver test	Laboratory for Advanced Medicine	Analyzes presence of hypermethylated ctDNA from multiple gene targets to confirm the presence of breast, colon, liver, and lung cancers as early as stage I	FDA breakthrough device designation (September 2019)
CASCADE-LUNG cancer screening assay	Delfi Diagnostics	Machine learning-driven method that analyzes patterns of cfDNA fragmentation to detect the presence of cancer	FDA breakthrough device designation (March 2021)

Abbreviations: cfDNA = cell-free DNA; ctDNA = circulating tumor DNA; FDA = US Food and Drug Administration; NGS = next-generation sequencing; PCR = polymerase chain reaction.

Source: Gray et al.³⁹

Table 4-3. Liquid Biopsy Approaches to Minimal Residual Disease

Parameter	Tissue naïve	Tissue informed
Adequacy of tumor tissue sample	Not required	Practical limitation
Sensitivity	MRD-specific assays improve	Lower LOD
Specificity	CHIP requires filtering algorithm; improved by baseline ctDNA	Tumor specific
Emergent variants	Detects	Unable to assess
Resistance variants	Detects	Unable to assess
Turnaround time	Much shorter	Longer

Abbreviations: CHIP = clonal hematopoiesis of indeterminate potential; ctDNA = circulating tumor DNA; LOD = limit of detection; MRD = minimal residual disease.

second-line targeted agents or emerging therapies (eg, *KRAS* G12C, *EGFR* exon 20, *ERBB2*, *NRG1* alterations). However, obtaining sufficient tissue for genomic assessment can be challenging in patients with advanced lung cancer.

Patients are often diagnosed using small biopsy or cytology samples that may be inadequate for biomarker analysis. In some situations, the patient cannot wait for repeat biopsies or delayed genomic results.⁴⁶ The use of plasma cfDNA testing is recommended to ensure complete genotyping for patients with insufficient or unavailable tissue for genotyping and is playing an increasing role as a complementary testing strategy even when tissue testing is available (Figure 4-4),¹⁶ enabling more patients to access targeted therapy. Liquid biopsy results are highly concordant with tissue, yield similar treatment outcomes, offer faster turnaround time, and may even be cost saving.^{24,25,28,30,31} Earlier implementation of liquid biopsy in the diagnostic pathway has been shown to accelerate first-line treatment initiation in advanced disease, which may further improve patient outcomes.^{29,47,48}

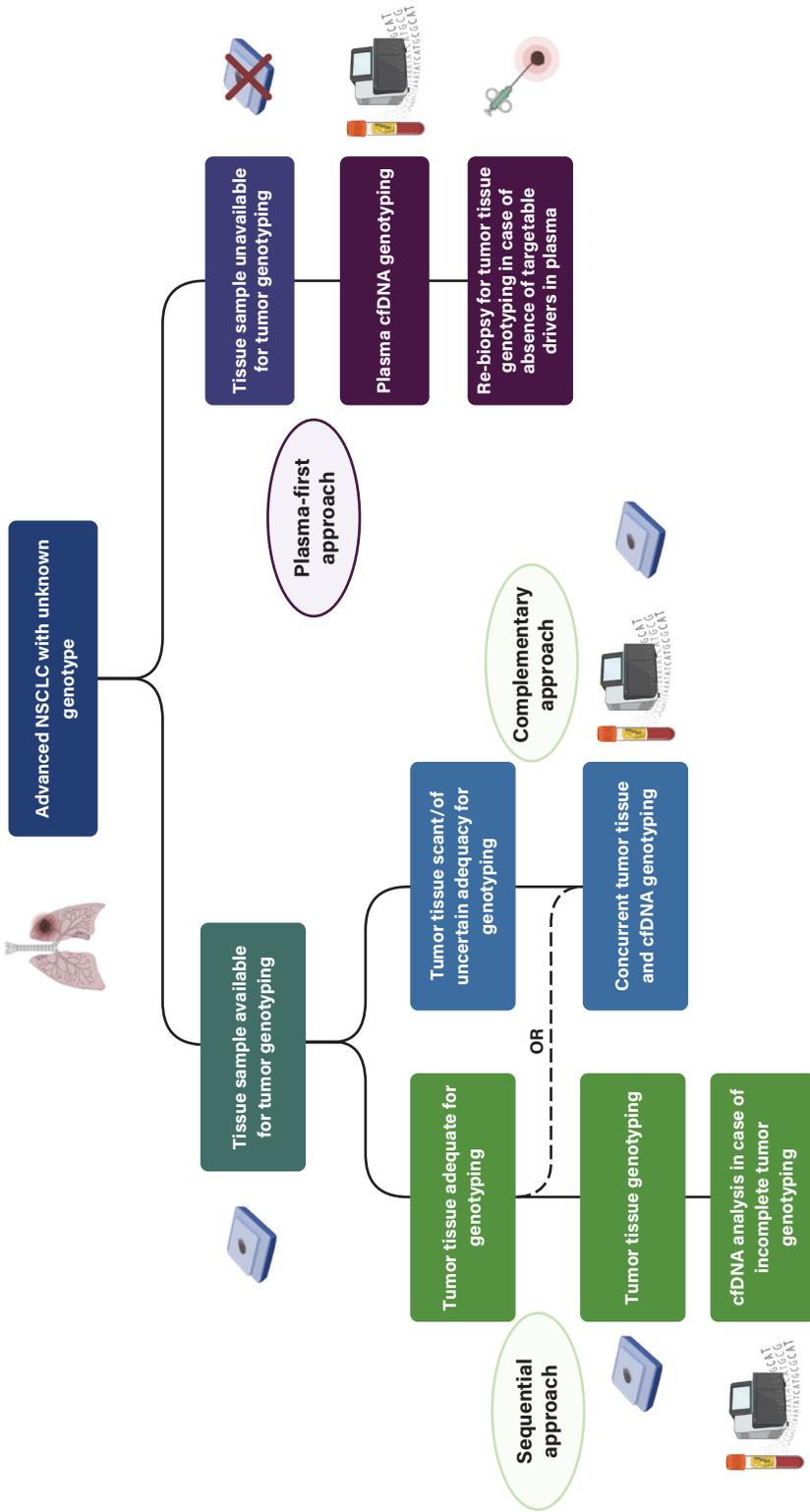
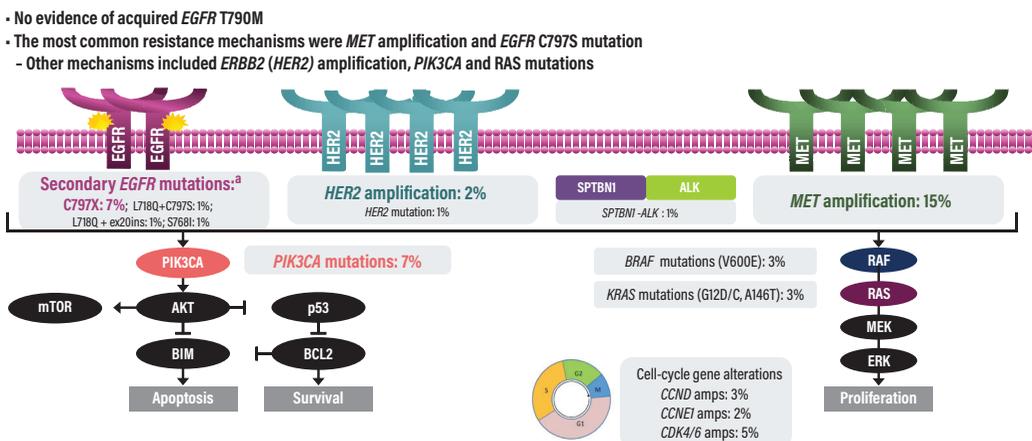


Figure 4-4. Diagnostic algorithm for liquid biopsy use in treatment-naïve advanced non-small cell lung cancer (NSCLC) (Adapted from Rolfo et al.¹⁶)

Acquired Resistance Genomics in Advanced-Stage NSCLC

Molecular targeted agents are associated with significant improvements in clinical outcomes for NSCLC patients with oncogenic drivers; however, acquired resistance to these agents remains an unsolved fundamental challenge. To determine the resistance mechanism, comprehensive molecular testing is essential and can help to guide subsequent therapy. Although tissue genotyping is considered the gold standard for molecular profiling,⁴⁵ repeat biopsies in the context of relapsed disease are not always feasible.²⁸ Liquid biopsy has notable advantages over tissue genotyping in terms of non-invasiveness, short turnaround time, and its ability to capture heterogeneous variants arising at different sites of disease. However, liquid biopsy has limitations, including the risk of false-negative results when ctDNA is not detectable in the blood and its inability to capture histologic information such as transformation to small cell lung cancer. Several FDA-approved assays for plasma cfDNA genotyping in NSCLC are available, which include the PCR-based Cobas EGFR mutation test (Roche Diagnostics) and NGS-based Guardant 360 companion diagnostic (CDx) and FoundationOne liquid CDx (Foundation Medicine).

EGFR T790M mutation is the most common resistance mechanism to first- or second-generation EGFR tyrosine kinase inhibitors (TKIs), and T790M testing is crucial for starting osimertinib as subsequent therapy. In practices where first- or second-generation EGFR TKIs are used in the first-line treatment setting, liquid biopsy may be used at progression in lieu of tissue biopsy to detect T790M-driven resistance; however, if plasma testing is negative, tissue biopsy is recommended.⁴⁹ Osimertinib is recommended as preferred first-line therapy based on the results of the FLAURA trial;⁵⁰ heterogeneous resistance mechanisms to osimertinib detected at progression following first-line therapy have been characterized using liquid biopsy (Figure 4-5).^{51,52} Although the detection of resistance mechanisms following treatment with targeted agents against other oncogenic drivers is not mandatory, comprehensive testing using cfDNA can be used to guide subsequent treatment and will likely play a larger management role in the future. Figure 4-6 shows a flowchart recommended by the International Association for the Study of Lung Cancer for the use of the liquid biopsy in patients progressing during TKI treatment.⁵³



^a Two patients had de novo T790M mutations at baseline of whom one acquired C797S at progression.

Figure 4-5. Candidate-acquired resistance mechanisms with osimertinib in FLAURA study (n = 91). The reported resistance mechanism may overlap with another. (Source: Ramalingam et al.⁵² Copyright © 2018, Elsevier.)

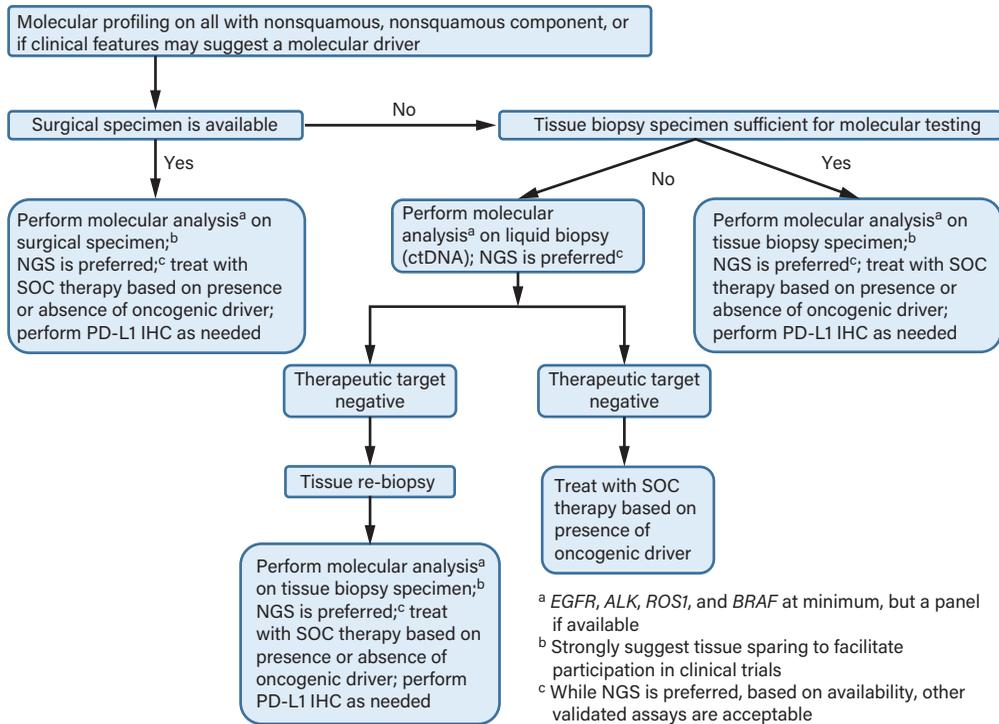


Figure 4-6. Patient with NSCLC progressive or recurrent disease during treatment with TKI. Abbreviations: ctDNA = circulating tumor DNA; IHC = immunohistochemistry; NGS = next-generation sequencing; NSCLC = non-small cell lung carcinoma; SOC = standard of care; TKI = tyrosine kinase inhibitor. (Source: Rolfo et al.⁵³)

Treatment Monitoring in Lung Cancer

Levels of plasma ctDNA have emerged as a potential surrogate of prognosis and treatment response. Several studies have confirmed that the absence of plasma ctDNA in advanced lung cancer patients prior to starting therapy is a good prognostic factor associated with improved outcomes irrespective of treatment.⁵⁴ Clearance or reduction of plasma ctDNA has been associated with response to TKIs, immunotherapy, and chemotherapy.⁵⁴⁻⁵⁹ In locally advanced disease, the persistence of ctDNA after chemoradiation may identify a population at higher risk of relapse, identifying potential candidates for more intensified consolidation therapy.⁶⁰ In patients receiving long-term checkpoint inhibitor (CPI) therapy, detectable-plasma ctDNA may identify patients at risk of relapse, whereas absence may identify those who could stop maintenance CPI and remain progression-free.⁶¹ However, the clinical utility of liquid biopsy monitoring to inform treatment decisions, such as the intensification or safe de-escalation of therapy, remains to be proven. The risk of false-positive or negative results in serial testing remains an important technical limitation. Multiple studies are exploring the monitoring of ctDNA levels and treatment with EGFR kinase inhibitors (NCT02856893, NCT04410796), as well as immunotherapy and chemotherapy (NCT04093167, NCT04966676; Figure 4-7). Although the current use of liquid biopsy monitoring to inform treatment decisions is not ready for routine practice, it is an important potential surrogate endpoint under active investigation.

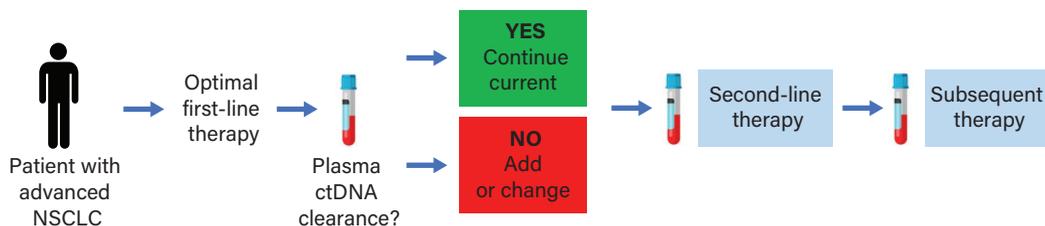


Figure 4-7. Future integration of liquid biopsy monitoring into treatment decision-making. Abbreviations: ctDNA = circulating tumor DNA; NSCLC = non-small cell lung carcinoma.

Predictive Biomarkers for Checkpoint Immunotherapy

A large number of biomarkers are under development for CPI, reflecting the complexity of tumor-stroma microenvironment interactions as well as the continuous nature of the output for most of these assays (eg, PD-L1 and tumor mutational burdens [TMBs]), in contrast to discrete biomarkers (eg, mutant vs wild-type gene status).¹⁶ Of these, currently, the most pertinent to liquid biopsy is TMB. While tissue-assessed TMB is a pan-tumor FDA-approved biomarker in the United States, mixed results and variations in assay methodology, cut-point definition, and other parameters have clouded its clinical application. Blood-based assays (B-TMB) have been analytically and clinically validated and assessed in both retrospective and prospective studies.^{62,63} Mostly recently, the phase 3 cohort of BFAST has been reported, in which patients with treatment-naïve advanced NSCLC and high B-TMB (≥ 16 mt/Mb) were randomized to either atezolizumab or platinum-based chemotherapy.⁶⁴ While the primary endpoint of progression-free survival trended toward the atezolizumab group (hazard ratio [HR] 0.77; 0.59-1.00, $p = 0.054$), differences were not statistically significant. Of note, results favored those with squamous histology, while there was no benefit in those in the nonsquamous cohort. Thus the use of B-TMB is not currently indicated for treatment selection in patients with advanced lung cancer in routine practice. While a wide variety of other plasma-based assays are in development, including measurement of PD-L1 by messenger RNA or extracellular vesicles, they remain investigational at the present time.^{65,66}

References

- Chan KC, Yeung SW, Lui WB, Rainer TH, Lo YM. Effects of preanalytical factors on the molecular size of cell-free DNA in blood. *Clin Chem*. 2005;51(4):781-784.
- Henaó Diaz E, Yachnin J, Grönberg H, Lindberg J. The in vitro stability of circulating tumour DNA. *PLoS One*. 2016;11(12):e0168153.
- Medina Diaz I, Nocon A, Mehnert DH, et al. Performance of Streck cfDNA blood collection tubes for liquid biopsy testing. *PLoS One*. 2016;11(11):e0166354.
- Norton SE, Luna KK, Lechner JM, Qin J, Fernando MR. A new blood collection device minimizes cellular DNA release during sample storage and shipping when compared to a standard device. *J Clin Lab Anal*. 2013;27(4):305-311.
- Parpart-Li S, Bartlett B, Popoli M, et al. The effect of preservative and temperature on the analysis of circulating tumor DNA. *Clin Cancer Res*. 2017;23(10):2471-2477.
- Toro PV, Erlanger B, Beaver JA, et al. Comparison of cell stabilizing blood collection tubes for circulating plasma tumor DNA. *Clin Biochem*. 2015;48(15):993-998.
- Wong D, Moturi S, Angkachatchai V, et al. Optimizing blood collection, transport and storage conditions for cell free DNA increases access to prenatal testing. *Clin Biochem*. 2013;46(12):1099-1104.
- El Messaoudi S, Rolet F, Moulriere F, Thierry AR. Circulating cell free DNA: preanalytical considerations. *Clin Chim Acta*. 2013;424:222-230.
- Haselmann V, Ahmad-Nejad P, Geilenkeuser WJ, et al. Results of the first external quality assessment scheme (EQA) for isolation and

- analysis of circulating tumour DNA (ctDNA). *Clin Chem Lab Med*. 2018;56(2):220-228.
10. Lu JL, Liang ZY. Circulating free DNA in the era of precision oncology: pre- and post-analytical concerns. *Chronic Dis Transl Med*. 2016;2(4):223-230.
 11. Klotten V, Rüchel N, Brüchle NO, et al. Liquid biopsy in colon cancer: comparison of different circulating DNA extraction systems following absolute quantification of KRAS mutations using Intplex allele-specific PCR. *Oncotarget*. 2017;8(49):86253-86263.
 12. Fleischhacker M, Schmidt B, Weickmann S, et al. Methods for isolation of cell-free plasma DNA strongly affect DNA yield. *Clin Chim Acta*. 2011;412(23-24):2085-2088.
 13. Malentacchi F, Pizzamiglio S, Verderio P, et al. Influence of storage conditions and extraction methods on the quantity and quality of circulating cell-free DNA (ccfDNA): the SPIDIA-DNAplas External Quality Assessment experience. *Clin Chem Lab Med*. 2015;53(12):1935-1942.
 14. Yuan H, Zhu ZZ, Lu Y, et al. A modified extraction method of circulating free DNA for epidermal growth factor receptor mutation analysis. *Yonsei Med J*. 2012;53(1):132-137.
 15. Lee TH, Montalvo L, Chrebtov V, Busch MP. Quantitation of genomic DNA in plasma and serum samples: higher concentrations of genomic DNA found in serum than in plasma. *Transfusion*. 2001;41(2):276-282.
 16. Rolfo C, Mack P, Scagliotti GV, et al. Liquid biopsy for advanced NSCLC: a consensus statement from the International Association for the Study of Lung Cancer. *J Thorac Oncol*. 2021;16(10):1647-1662.
 17. Russo A, De Miguel Perez D, Gunasekaran M, et al. Liquid biopsy tracking of lung tumor evolutions over time. *Expert Rev Mol Diagn*. 2019;19(12):1099-1108.
 18. Odegaard JI, Vincent JJ, Mortimer S, et al. Validation of a plasma-based comprehensive cancer genotyping assay utilizing orthogonal tissue- and plasma-based methodologies. *Clin Cancer Res*. 2018;24(15):3539-3549.
 19. Pritchett MA, Camidge DR, Patel M, et al. Prospective clinical validation of the InVisionFirst-Lung Circulating Tumor DNA assay for molecular profiling of patients with advanced nonsquamous non-small-cell lung cancer. *JCO Precis Oncol*. 2019;3:PO.18.00299. doi:10.1200/PO.18.00299.
 20. Schrock AB, Welsh A, Chung JH, et al. Hybrid capture-based genomic profiling of circulating tumor DNA from patients with advanced non-small cell lung cancer. *J Thorac Oncol*. 2019;14(2):255-264.
 21. Krebs MG, Malapelle U, André F, et al. Practical considerations for the use of circulating tumor DNA in the treatment of patients with cancer: a narrative review. *JAMA Oncol*. 2022;8(12):1830-1839. doi:10.1001/jamaoncol.2022.4457.
 22. Aggarwal C, Rolfo CD, Oxnard GR, et al. Strategies for the successful implementation of plasma-based NSCLC genotyping in clinical practice. *Nat Rev Clin Oncol*. 2021;18(1):56-62.
 23. Pascual J, Attard G, Bidard FC, et al. ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group. *Ann Oncol*. 2022;33(8):750-768.
 24. Leighl NB, Page RD, Raymond VM, et al. Clinical utility of comprehensive cell-free DNA analysis to identify genomic biomarkers in patients with newly diagnosed metastatic non-small cell lung cancer. *Clin Cancer Res*. 2019;25(15):4691-4700.
 25. Page RD, Drusbosky LM, Dada H, et al. Clinical outcomes for plasma-based comprehensive genomic profiling versus standard-of-care tissue testing in advanced non-small cell lung cancer. *Clin Lung Cancer*. 2022;23(1):72-81.
 26. Palmero R, Taus A, Viteri S, et al. Biomarker discovery and outcomes for comprehensive cell-free circulating tumor DNA versus standard-of-care tissue testing in advanced non-small-cell lung cancer. *JCO Precis Oncol*. 2021;5:93-102.
 27. Schwartzberg LS, Li G, Tolba K, et al. Complementary roles for tissue- and blood-based comprehensive genomic profiling for detection of actionable driver alterations in advanced NSCLC. *JTO Clin Res Rep*. 2022;3(9):100386.
 28. Aggarwal C, Thompson JC, Black TA, et al. Clinical implications of plasma-based genotyping with the delivery of personalized therapy in metastatic non-small cell lung cancer. *JAMA Oncol*. 2019;5(2):173-180.
 29. Cui W, Milner-Watts C, O'Sullivan H, et al. Up-front cell-free DNA next generation sequencing improves target identification in UK first line advanced non-small cell lung cancer (NSCLC) patients. *Eur J Cancer*. 2022;171:44-54.
 30. Mack PC, Banks KC, Espenschied CR, et al. Spectrum of driver mutations and clinical impact of circulating tumor DNA analysis in

- non-small cell lung cancer: analysis of over 8000 cases. *Cancer*. 2020;126(14):3219-3228.
31. Ezeife DA, Spackman E, Juergens RA, et al. The economic value of liquid biopsy for genomic profiling in advanced non-small cell lung cancer. *Ther Adv Med Oncol*. 2022;14:17588359221112696.
 32. Cui W, Milner-Watts C, McVeigh TP, et al. A pilot of blood-first diagnostic cell free DNA (cfDNA) next generation sequencing (NGS) in patients with suspected advanced lung cancer. *Lung Cancer*. 2022;165:34-42.
 33. Garcia-Pardo M, Czarnecka K, Law JH, et al. Plasma-first: accelerating lung cancer diagnosis and molecular profiling through liquid biopsy. *Ther Adv Med Oncol*. 2022;14:17588359221126151.
 34. Wan JCM, Massie C, Garcia-Corbacho J, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. *Nat Rev Cancer*. 2017;17(4):223-238. doi:10.1038/nrc.2017.7.
 35. Aberle DR, Adams AM, Berg CD, et al; National Lung Screening Trial Research Team. Reduced lung-cancer mortality with low-dose computed tomographic screening. *N Engl J Med*. 2011;365:395-409.
 36. De Koning HJ, Van Der Aalst CM, De Jong PA, et al. Reduced lung-cancer mortality with volume CT screening in a randomized trial. *N Engl J Med*. 2020;382:503-513.
 37. Patz EF Jr, Pinsky P, Gatsonis C, et al. Overdiagnosis in low-dose computed tomography screening for lung cancer [published correction appears in *JAMA Intern Med*. 2014;174(5):828]. *JAMA Intern Med*. 2014;174(2):269-274. doi:10.1001/jamainternmed.2013.12738.
 38. Freitas C, Sousa C, Machado F et al. The role of liquid biopsy in early diagnosis of lung cancer. *Front Oncol*. 2021;11:634316.
 39. Gray J, Thompson JC, Carpenter EL, et al. Plasma cell-free DNA genotyping: from an emerging concept to a standard-of-care in metastatic non-small cell lung cancer. *Oncologist*. 2021;26:e1812-1821.
 40. Malapelle U, Pisapia P, Addeo A, et al. Liquid biopsy from research to clinical practice: focus on non-small cell lung cancer. *Expert Rev Mol Diagn*. 2021;21(11):1165-1178. doi:10.1080/14737159.2021.1985468.
 41. Moding EJ, Nabet BY, Alizadeh AA, Diehn M. Detecting liquid remnants of solid tumors: circulating tumor DNA minimal residual disease. *Cancer Discov*. 2021;11(12):2968-2986. doi:10.1158/2159-8290.CD-21-0634.
 42. Pellini B, Chaudhuri AA. Circulating tumor DNA minimal residual disease detection of non-small-cell lung cancer treated with curative intent. *J Clin Oncol*. 2022;40(6):567-575. doi:10.1200/JCO.21.01929.
 43. Zhou C, Das Thakur M, Srivastava MK, et al. IMPower010: biomarkers of disease-free survival (DFS) in a phase 3 study of atezolizumab (atezo) vs best supportive care (BSC) after adjuvant chemotherapy in stage IB-IIIa NSCLC. ESMO Immuno-Oncology Congress 2021, Abstract 2O. *Ann Oncol*. 2021;32(suppl 7):S1373-S1391.
 44. Carbone D, Lee J, Kris M, et al. Clinical/biomarker data for neoadjuvant atezolizumab in resectable stage IB-IIIb NSCLC: primary analysis in the LCMC3 study. *J Thorac Oncol*. 2021;16(3 suppl):S115-S116. doi:10.1016/j.jtho.2021.01.294.
 45. Lindeman NI, Cagle PT, Aisner DL, et al. 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. *Arch Pathol Lab Med*. 2018;142:321-346.
 46. Lim C, Tsao MS, Le LW, et al. Biomarker testing and time to treatment decision in patients with advanced nonsmall-cell lung cancer. *Ann Oncol*. 2015;26(7):1415-1421. doi:10.1093/annonc/mdv208.
 47. Garcia-Pardo M, Czarnecka K, Law JH, et al. Plasma first: Accelerating lung cancer diagnosis through liquid biopsy. Abstract 3039. *J Clin Oncol*. 2022;40(16 suppl):3039. doi:10.1200/JCO.2022.40.16_suppl.3039.
 48. Thompson JC, Aggarwal C, Wong J, et al. Plasma genotyping at the time of diagnostic tissue biopsy decreases time-to-treatment in patients with advanced NSCLC: results from a prospective pilot study. *JTO Clin Res Rep*. 2022;3(4):100301. doi:10.1016/j.jtocr.2022.100301.
 49. Oxnard GR, Thress KS, Alden RS, et al. Association between plasma genotyping and outcomes of treatment with osimertinib (AZD9291) in advanced non-small-cell lung cancer. *J Clin Oncol*. 2016;34:3375-3382.
 50. Soria JC, Ohe Y, Vansteenkiste J et al. Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. *N Engl J Med*. 2018;378:113-125.

51. Romero A, Serna-Blasco R, Alfaro C, et al. ctDNA analysis reveals different molecular patterns upon disease progression in patients treated with osimertinib. *Transl Lung Cancer Res.* 2020;9(3):532-540. doi:10.21037/tlcr.2020.04.01.
52. Ramalingam SS, Cheng Y, Zhou C, et al. Mechanisms of acquired resistance to first-line osimertinib: preliminary data from the phase III FLAURA study. *Ann Oncol.* 2018;29:viii740.
53. Rolfo C, Mack PC, Scagliotti GV et al. Liquid biopsy for advanced non-small cell lung cancer (NSCLC): a statement paper from the IASLC. *J Thorac Oncol.* 2018;13:1248-1268.
54. Gray JE, Okamoto I, Sriuranpong V, et al. Tissue and plasma EGFR mutation analysis in the FLAURA trial: osimertinib versus comparator EGFR tyrosine kinase inhibitor as first-line treatment in patients with EGFR-mutated advanced non-small cell lung cancer. *Clin Cancer Res.* 2019;25(22):6644-6652. doi:10.1158/1078-0432.CCR-19-1126.
55. Anagnostou V, Forde PM, White JR, et al. Dynamics of tumor and immune responses during immune checkpoint blockade in non-small cell lung cancer. *Cancer Res.* 2019;79(6):1214-1225. doi:10.1158/0008-5472.CAN-18-1127.
56. Horn L, Whisenant JG, Wakelee H, et al. Monitoring therapeutic response and resistance: analysis of circulating tumor DNA in patients with ALK+ lung cancer. *J Thorac Oncol.* 2019;14(11):1901-1911. doi:10.1016/j.jtho.2019.08.003.
57. Iijima Y, Hirotsu Y, Amemiya K, et al. Very early response of circulating tumour-derived DNA in plasma predicts efficacy of nivolumab treatment in patients with non-small cell lung cancer. *Eur J Cancer.* 2017;86:349-357. doi:10.1016/j.ejca.2017.09.004.
58. Song Y, Hu C, Xie Z, et al; on behalf of AME Lung Cancer Collaborative Group. Circulating tumor DNA clearance predicts prognosis across treatment regimen in a large real-world longitudinally monitored advanced non-small cell lung cancer cohort. *Transl Lung Cancer Res.* 2020;9(2):269-279. doi: 10.21037/tlcr.2020.03.17.
59. Zulato E, Attili I, Pavan A, et al. Early assessment of KRAS mutation in cfDNA correlates with risk of progression and death in advanced non-small-cell lung cancer. *Br J Cancer.* 2020;123(1):81-91. doi:10.1038/s41416-020-0833-7.
60. Moding EJ, Liu Y, Nabet BY, et al. Circulating tumor DNA dynamics predict benefit from consolidation immunotherapy in locally advanced non-small cell lung cancer. *Nat Cancer.* 2020;1(2):176-183. doi:10.1038/s43018-019-0011-0.
61. Hellmann MD, Nabet BY, Rizvi H, et al. Circulating tumor DNA analysis to assess risk of progression after long-term response to PD-(L)1 blockade in NSCLC. *Clin Cancer Res.* 2020;26(12):2849-2858. doi:10.1158/1078-0432.CCR-19-3418.
62. Gandara DR, Paul SM, Kowanetz M, et al. Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab. *Nat Med.* 2018;24(9):1441-1448. doi:10.1038/s41591-018-0134-3.
63. Drusbosky L, Bilen MA, Azzi G, et al. Blood-based tumor mutational burden from circulating tumor DNA (ctDNA) across advanced solid malignancies using a commercially available liquid biopsy assay. Abstract. *J Clin Oncol.* 2021;39(15 suppl):3040.
64. Peters S, Dziadziuszko R, Morabito A, et al. Atezolizumab versus chemotherapy in advanced or metastatic NSCLC with high blood-based tumor mutational burden: primary analysis of BFAST cohort C randomized phase 3 trial. *Nat Med.* 2022;28(9):1831-1839. doi:10.1038/s41591-022-01933-w.
65. Raez LE, Danenberg K, Sumarriva D, et al. Using cfRNA as a tool to evaluate clinical treatment outcomes in patients with metastatic lung cancers and other tumors. *Cancer Drug Resist.* 2021;4(4):1061-1071. doi:10.20517/cdr.2021.78.
66. Rodriguez-Martinez A, Simon-Saez I, Perales S, et al. Exchange of cellular components between platelets and tumor cells: impact on tumor cells behavior. *Theranostics.* 2022;12(5):2150-2161. doi:10.7150/thno.64252.

Technologies for Detection of Biomarkers

5

By Yasushi Yatabe, Lynette M. Sholl, Sanja Dacic, Keith M. Kerr, Erik Thunnissen, and Anders Skanderup

There are multiple technologies for biomarker detection, and they have differing abilities to detect genetic variants and protein changes. There are currently 5 major genetic and protein abnormalities relevant to cancer clinical practice (Table 5-1):

1. **Single nucleotide variant (SNV):** One nucleotide is replaced by another nucleotide when compared to the reference sequence. Pathogenic SNVs are non-synonymous and lead to an amino acid change that may result in either oncogenic gain of function (eg, *EGFR* L858R, *KRAS* G12C, and *BRAF* V600E) or loss of function in the case of tumor suppressor genes (eg, *TP53* and *RB1*). In the case of mutations that cause stop codons (TAA, TAG, and TGA), the protein is not generated from the mutation (nonsense mutation). SNVs do not always result in amino acid changes (synonymous nucleotide changes), and these are typically nonpathogenic although some synonymous SNVs can induce functional changes due to altered transcript splicing.¹
2. **Insertion and/or deletion (indel):** Indel is defined as an insertion or deletion of 1 or more nucleotides that may alter the amino acid sequence and potentially lead to a shift in the reading frame. The indels may confer either oncogenic activity or functional impairment. In-frame *EGFR* exon 19 deletions and *HER2* exon 20 insertions are examples of oncogenic mutations, whereas most indels in tumor suppressor genes, such as *TP53*, *APC*, and *RB1*, are more commonly out-of-frame and result in functional deterioration of the mutated allele.
3. **Gene amplification, copy number variation:** Gene amplification describes increases in the copy number of a specific DNA segment that can lead to protein overexpression. Copy number variation is a more general term used to include increased or decreased gene copies, for example, 4 copies of *MET*, which may not be oncogenic.

Table 5-1. Clinically Relevant Gene Alterations in Lung Cancer

Gene alterations	Major genes involved in lung cancer	Detection methods
SNV, indel	<i>EGFR, KRAS, BRAF, HER2</i>	PCR direct sequencing, mutation-specific PCR, NGS-amplicon, NGS-hybrid capture, ddPCR
Gene amplification	<i>FGFR, MET</i>	FISH, NGS-hybrid capture, ddPCR
Gene rearrangement	<i>ALK, ROS1, RET, NTRK, NRG</i>	RT-PCR direct sequencing, NGS (DNA- or RNA-based), FISH, IHC (ALK)
Protein expression/overexpression	ALK, PD-L1, HER2	IHC, ELISA, ^a mass spectrometry ^a

Abbreviations: ddPCR = droplet digital polymerase chain reaction; ELISA = enzyme-linked immunosorbent assay; FISH = fluorescence in situ hybridization; IHC = immunohistochemistry; indel = insertion and/or deletion; NGS = next-generation sequencing; RT = reverse transcription; SNV = single nucleotide variant.

^a Not used in routine clinical practice.

- Gene rearrangement:** Chromosomal rearrangements (also known as *translocations*) occur when a chromosomal segment breaks off and attaches to another chromosome or to a different site on the same chromosome. Inversions occur when the segment reattaches to the same chromosome in the reverse direction. Rearrangements can result in oncogenic gene fusions (eg, *EML4::ALK*, *CD74::ROS1*, and *KIF5B::RET*).
- Protein expression/overexpression:** Gene amplification and rearrangement may be translated to aberrant protein overexpression. HER2 overexpression can be caused by gene amplification. Gene rearrangements can lead to alterations of protein expression (eg, *ALK* gene fusion results in abnormal expression of the ALK kinase domain). Abnormal expression of a protein can be pathogenic but may not directly relate to a genetic alteration (eg, lung cancers overexpressing PD-L1 do not demonstrate alterations in the *CD274/PD-L1* gene except in rare instances).

To detect abnormalities, various methods have been developed for different assays, ranging from in situ detection of protein or genetic alterations in formalin-fixed paraffin-embedded (FFPE) sections to simultaneous detection of multiple gene alterations in extracted DNA or RNA. Each assay has advantages and disadvantages relating to types of samples that can be tested, alterations detected, cost, and time to report (turnaround time [TAT]). Understanding the assay properties is important for optimal assay selection and is critical to avoid pitfalls.

Turnaround Time

TAT is defined as the time from ordering a test to receiving a report. In clinical practice, TAT is a crucial factor and relates to the specific assay as well as other factors, such as transfer of specimens between laboratories, laboratory staffing, and batching of cases. Optimal laboratory working days for various tests (discussed below) are as follows: less than 2 working days for immunohistochemistry (IHC), 2 to 3 days for fluorescence in situ hybridization (FISH), 3 to 4 days for polymerase chain reaction (PCR)/reverse transcription (RT)-PCR direct sequencing, 4 to 5 days for amplicon-based sequencing, and 5 to 14 days for hybrid capture-based next-generation sequencing (NGS).

In the case of hybrid DNA/RNA analysis, either DNA or RNA result may not be obtained, either because of quantitative or qualitative sample inadequacy. (See Chapter 3, “Specimen Acquisition and Pre-Analytical Considerations.”) The overall time to patient treatment decision should be considered, not just a nominal working time of the laboratory

test. Importantly, if all results are returned as being analysis failures, the clinical status will be back to the beginning.

Immunohistochemistry

IHC is a powerful diagnostic tool, and protocols have become robust in the detection of proteins that are relevant to pathology tumor classification and identification of some targetable molecular alterations. A simplified graphical display for direct and indirect IHC is shown in Figure 5-1. The basic technique has been described in the *IASLC Atlas of Diagnostic Immunohistochemistry*. The method itself is more qualitative than quantitative.² However, semiquantitative assessment can be achieved through careful protocol construction and validation along a short range (factor of 2) of protein concentration.³

Basic Principles

The basic procedure resulting in microscopic visible color in situ contains the following steps: epitope retrieval, incubation with primary antibody, washing away unbound antibodies, incubation with labeled secondary antibody that binds to the heavy chain of the primary antibody, washing away unbound antibodies, and subsequent visualization with a signal enhancement step.

Validation of IHC for Predictive Assays

Methods of validation differ slightly in diagnostic compared to predictive markers. For diagnostic purposes, a minimum validation set of 20 samples, containing clear positive and negative controls, is usually required in the United States.⁵ For predictive marker testing, the threshold of a test plays a crucial role. As not all PD-L1 assays are equal,^{6,7} analytical and indirect clinical validation seems to be the best possible approximation.⁷

Fluorescence Microscopy

With fluorescence labeling (immunofluorescence), a higher sensitivity is achieved than with an absorption substrate. This implies fluorescence microscopy may be needed to detect signals with a lower epitope concentration.³ To visualize fluorescent images, 2 filters are used. The first is positioned before the light reaches the tissue section, and it allows the light to pass at a relatively low wavelength but blocks the light at a higher wavelength. In the section, the light will reach the fluorescent dye and causes excitation of the dye at a higher wavelength. The second filter blocks light of the lower wavelength and allows light of a higher wavelength to pass. A camera system, instead of the eyes, may be used for capturing a digital image. When multiple fluorophores are used, the wavelength that will pass through these filters may be narrowed (with band pass filters), where 1 of the fluorophores is excited at a lower wavelength and the other emits an essentially higher wavelength after excitation. While immunofluorescence is a highly sensitive technique for protein detection, it is not currently used in routine clinical practice for lung cancer.

Mutation-Specific IHC

Because an acquired pathogenic *EGFR* mutation will lead to a change in the 3-dimensional protein structure, an epitope may be present that is unique for this mutation and differs from

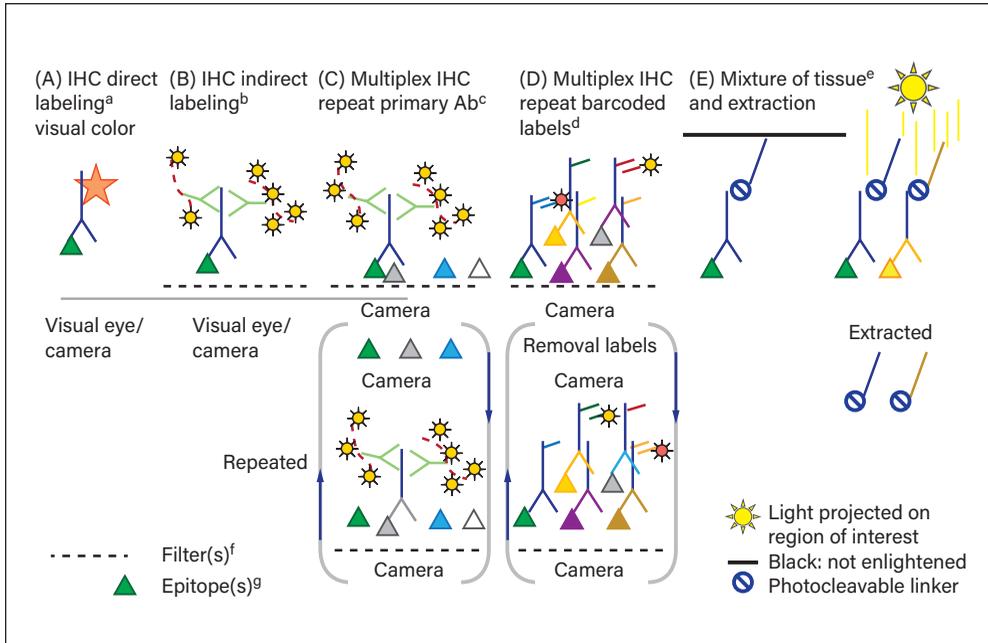


Figure 5-1. Simplified graphical display of different technologies using spatial tissue protein analysis.

^a High epitope concentration required for visual signal absorption color or fluorescent label. For the latter, a slightly lower epitope concentration is required than for a chromogen.

^b Signal enhancement through (1) binding with several secondary antibodies (eg, mouse anti-rabbit; goat anti-human, etc) labeled with (2) enzyme for generation of insoluble chromogen (eg, brown diaminobenzidine); (3) initial amplification with streptavidin-biotin (SAB) complex and later with polymers (factor 5-20× more than SAB complex).

^c After the first round of capturing image, indirect fluorescent labeling and capturing digital image, dye inactivation/removal is required with a subsequent capturing of digital image of background/autofluorescence. The procedure can be repeated on the same section with different primary antibodies. The image before incubation of the primary antibody (with autofluorescence) is subtracted from the image after labeling. Single or multiple markers may be projected (superimposed) on the same section.

^d Each primary antibody is conjugated with unique barcode (DNA). Multiple primary antibodies are incubated at once. Labels with fluorophore and complementary strand to a barcode bind to primary antibody with a maximum of 3 fluorophores at a time (in the schematic representation, 2 different fluorophores are shown). After imaging and removal of the fluorophore labels and image capture, a next set (usually up to 3) of unique complementary labels (each with a different fluorophore) may be incubated. This procedure may be repeated. All markers may be examined individually or any combination superimposed on the section.

^e With unique oligonucleotide tags and photocleavable linker-labeled antibodies with high-affinity binding on an epitope in histologic section may in a region of interest selectively receive light, causing release of photocleavable oligonucleotides, which then are extracted and used for readout with, eg, next-generation sequencing, flow cytometry, or mass spectrometry. The readout may digitally be superimposed on the digital image of the histologic section.⁴

^f The filters imply 1 filter before the light passes the tissue/cells and 1 between tissue and eye/camera. The first blocks light from higher wavelength, and the second of the lower wavelength. Only light with an emitted wavelength will pass the second filter.

^g For graphic display, the epitopes are shown as a triangle. In vivo, the essential characteristic is a 3-dimensional (3-D) structure, where the variable domain of the antibody stereologically binds with high affinity. Different epitopes have different 3-D structures.

In all digital approaches, the region of interest (may also be one cell) needs to be determined. This will require segmentation of the region/object. To this end, the number of pixels in the charge-coupled device (CCD) camera needs to match the resolution of the microscope objective lens. To keep the error rate of segmentation low, high-quality CCD cameras need to be used.

the wild-type protein. In addition, some *EGFR* mutations occur at a high frequency, especially L858R in exon 21 and deletions in exon 19. This has led to the development of *EGFR* mutation-specific antibodies.⁹⁻¹³ These may be applied in cases of limited sample size inadequate for DNA-based testing. A positive IHC reaction is likely associated with the mutation. However, the antibodies may have cross-reactions to *EGFR* exon 20 insertion mutations that are insensitive for first-generation *EGFR* tyrosine kinase inhibitors (TKIs), and the detection rate of the exon 19 deletions may vary. Exon 20 insertions—A769 insertion ASV and D770 insertion SVD—were positive for the antibodies in 1 study,¹⁰ and exon 19 deletion, other than the deletion of 5 amino acids (ELREA), may not be detected.¹¹ Importantly, negative staining does not exclude the presence of a clinically actionable *EGFR* mutation.

Mutation-specific antibodies against BRAF V600E mutation have also been developed^{14,15} and are highly sensitive. In contrast to *EGFR* mutation-specific antibodies, BRAF V600E mutation-specific IHC has no cross-reactions to non-V600E mutations.¹⁶

The high sensitivity with mutation-specific antibodies suggests that this IHC approach may be used as a screening tool for their respective specific mutations. Nevertheless, in IHC negative cases, the presence of other mutations in the same gene with possible actionable mutations remains possible. Furthermore, confirmation by molecular assay may be warranted.

IHC to Detect or Screen for Gene Rearrangements

Predictive IHC testing with wild-type antibodies, such as for ALK protein, is highly sensitive and specific for *ALK* alterations as the ALK protein is not expressed in normal lung tissue.¹⁷⁻¹⁹ In contrast, IHC for ROS1 and NTRK has lower specificity, so an orthogonal technique (eg, FISH or NGS) is required to demonstrate the presence of a rearrangement.^{20,21} While the sensitivity for ROS1 IHC is high, making it suitable for screening, the sensitivity for NTRK IHC is approximately 80%,^{22,23} implying that most *NTRK* fusions may be detected, but not all.

Fluorescent In Situ Hybridization

FISH assays have been developed to detect a range of genomic alterations including amplifications, rearrangements/translocations, gains, and losses of either the entire chromosomes or specific chromosomal regions. Several basic probes are used for FISH, including the chromosome enumeration probes (CEPs) and gene/locus-specific identification (LSI) probes. CEPs are often used to distinguish polysomy from true gene amplification as CEPs reflect the chromosomal number.

Detection of Rearrangements

Two types of FISH probes have been designed to detect chromosomal rearrangements: fusion and break-apart (Figure 5-2). Fusion probe sets consist of differentially labeled probes to 2 distinct loci, where the 2 fused genes are not normally located close to each other. False-positive signals may occur in cells where the 2 probes lie near each other. Therefore, each laboratory should test negative control cases and develop cutoff criteria for the percentage of nuclei containing a fusion pattern. Break-apart probes consist of 2 differentially labeled probes that hybridize to the same gene but are specific for regions that lie on opposite sides of the translocation breakpoint. This break-apart strategy is especially valuable for detecting translocations

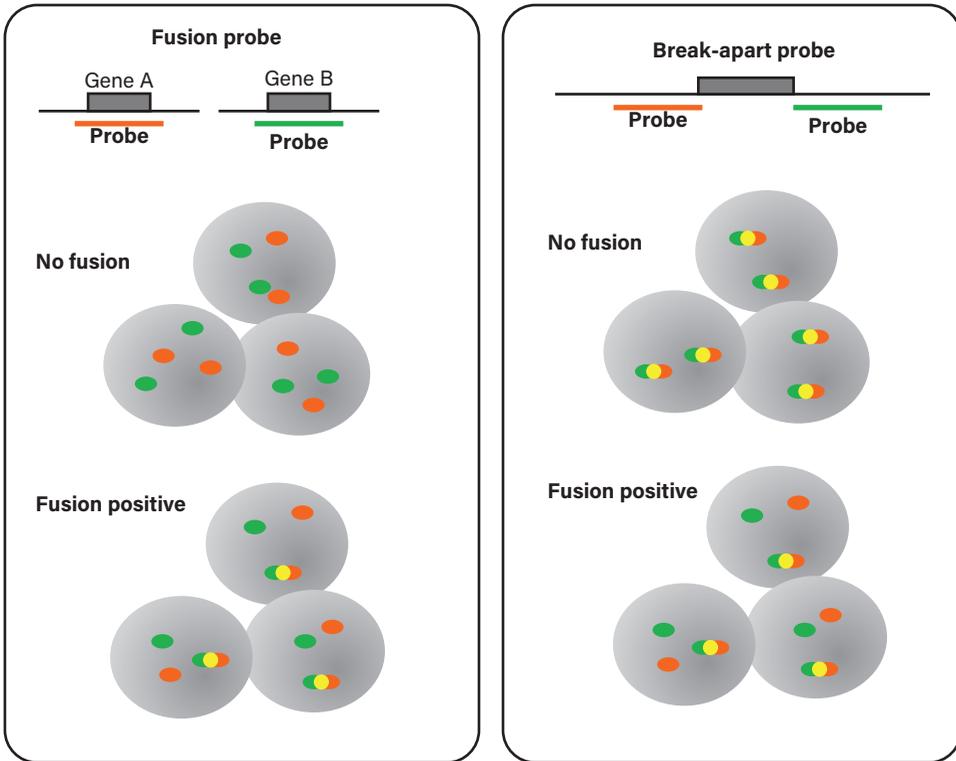


Figure 5-2. Fluorescence in situ hybridization using fusion and break-apart probes for translocation detection.

involving the so-called promiscuous genes (ie, *ALK*), where fusions may occur with multiple partner genes. A distance between the 2 signals and minimum percentage (15% for *ALK*) of nuclei showing the separated signals are usually defined to diagnose the presence of gene translocation.²⁴

Detection of Gene Amplification

Assays for gene amplification employ a probe specific for the target gene of interest and are often combined with a differentially labeled probe for the corresponding centromere. Multiple target gene signals are observed in the amplification setting, and by calculating the ratio of target gene signals to centromere signals, even low amplification levels can be distinguished from polysomy for the entire chromosome.

Samples for FISH Analysis

A successful analysis of tissue or cytology samples using any FISH protocol requires appropriate fixation and, for the archival paraffin blocks, storage conditions.^{25,26} Tissues processed in strong acid solutions are not suitable for FISH assay, whereas mild decalcification with ethylenediaminetetraacetic acid (EDTA) or formic acid does not usually impact the test performance. The recommendations for pre-analytical steps outlined in the pathology practice guidelines from the College of American Pathologists (CAP), Association for Molecular Pathology (AMP), International Association for the Study of Lung Cancer (IASLC), and others should be followed.^{27,28} TAT is determined by different FISH protocols. Overall, simple

and automated protocols are better than manual protocols. In general, the basic steps are similar to those of IHC, that is, a 2-day assay requiring approximately 3 to 4 hours on day 1 and 30 minutes on day 2. Depending on the laboratory workflow, an additional day may be needed for interpretation.

Selection of FISH Assays

Although US Food and Drug Administration (FDA)-approved FISH assays are currently commercially available, some laboratories may choose to develop their own “homemade” probes, which are usually more cost-effective. Cloning vectors, such as bacterial artificial chromosomes, are excellent sources of homemade FISH probes. It is essential to verify that the correct clone was obtained either by screening for the DNA sequence of interest using PCR or by performing metaphase FISH. Whether the FDA-approved commercial assay is used or not, the probe set used for clinical testing should be characterized in detail, and cut-off values should show reproducible performance with normal controls and known abnormal patient specimens and/or cell lines for accurate and precise results.²⁷ The specificity and sensitivity should be equivalent to its commercially available counterpart. For laboratories that opt to use laboratory-developed probes, attention should also be given to batch variability of the clones, DNA-labeling enzymes, and other reagents.²⁷

PCR, RT-PCR, and ddPCR

Polymerase Chain Reaction

PCR is a method of amplifying DNA commonly used to detect SNV/indel-type mutations. Because of the extremely small amount of DNA remaining in biopsied tissues for direct analyses, tissue DNA must be amplified by PCR. Furthermore, oncogenic mutations, such as in *EGFR*, occur in 1 of the 2 alleles, and clinical specimens consistently contain a mixture of nonneoplastic tissues; thus, various approaches to detect mutant alleles have been developed and established as clinically feasible assays. The following assays are commonly used and approved in *EGFR* mutation detection.

Allele-Specific Real-Time PCR Assays

The key allele-specific real-time PCR assay is a probe that complements the mutated sequence and identifies the mutant allele with a particular technique. Therascreen (Qiagen) uses a Scorpion probe,²⁹ whereas mutations are detected by displacement and cleavage of the reporter dye and quencher-labeled probes based on the 5'-3' exonuclease activity when hybridized to the complementary sequence in the Cobas system (Roche Diagnostics). Amoy Diagnostics (AmoyDx) uses a TaqMan probe, of which the detection system is similar to the Cobas using Taq polymerase. In these systems, mutant signals can be detected as increased fluorescent levels.

Single-Base Primer Extension Assays

In single-base primer extension assays, targeted regions, including mutation sites, are amplified by PCR. The probe adjacent to the mutation site is annealed, and a single-base extension is performed using a distinguishable dideoxynucleoside. The difference of mutated and

wild-type alleles in incorporated bases is detected by fluorescent dye (SNaPshot [ThermoFisher Scientific]) or mass spectrometry (MassArray [Agena Bioscience]) (Figure 5-3).

Reverse Transcription-Polymerase Chain Reaction

RT-PCR is commonly used to detect fusion genes, which are difficult to detect by DNA PCR due to long intron sequences. To cover various fusion patterns, multiplex PCR with multiple primer sets is commonly performed. For example, 6 primer sets have been developed for multiplex PCR to cover different *EML4-ALK* fusions.³⁰

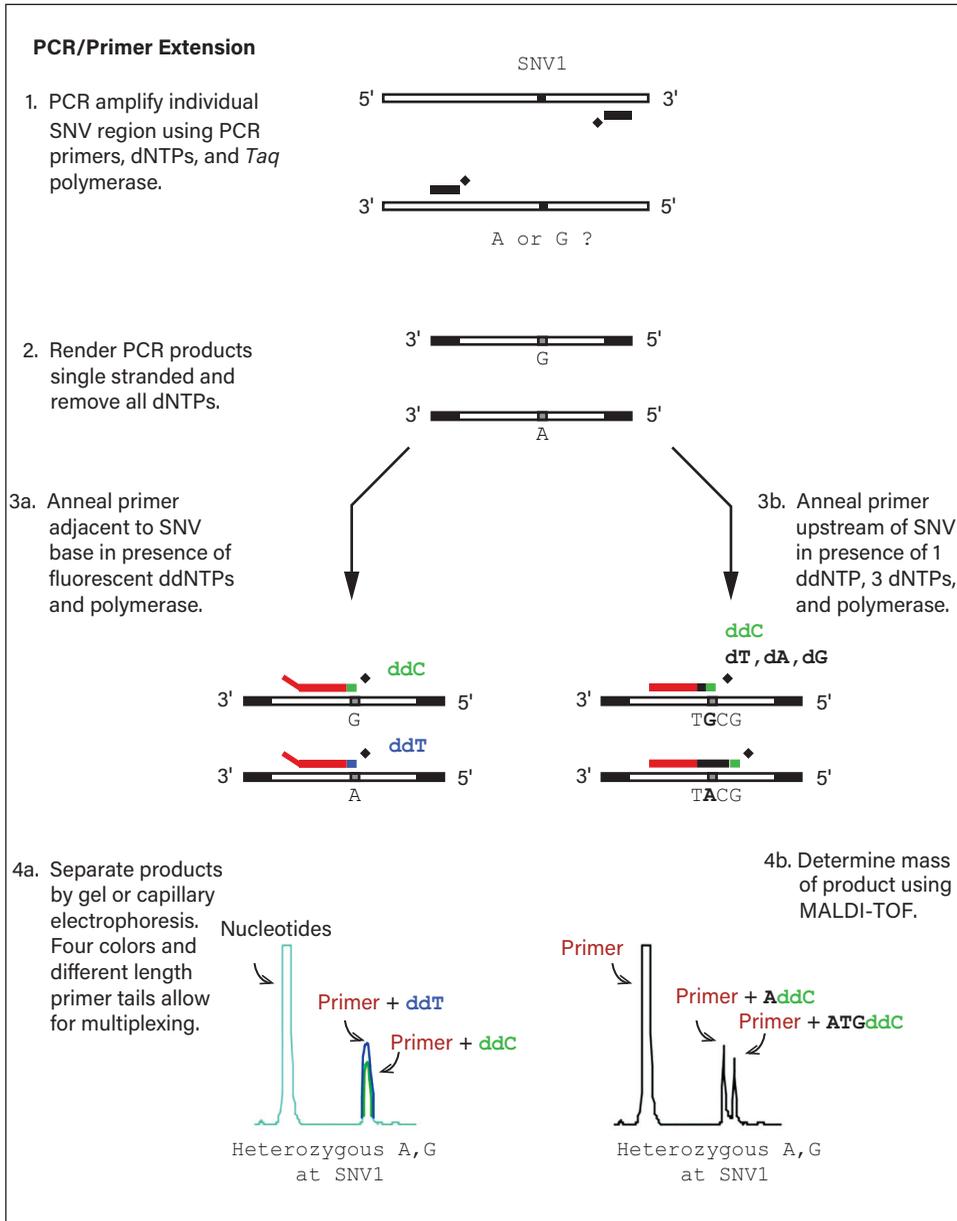


Figure 5-3. Generalized scheme of single nucleotide extension assay.³¹ Abbreviations: dNTP = deoxynucleotide triphosphate; ddNTP = dideoxynucleotide triphosphate MALDI-TOF = matrix-assisted laser desorption/ionization time-of-flight [mass spectrometry]; PCR = polymerase chain reaction; SNV = single nucleotide variant.

Droplet Digital Polymerase Chain Reaction

With droplet digital PCR (ddPCR), sample DNA undergoes limiting dilution into small-volume compartments. Optimally, only a single target molecule is contained within each compartment. Positive amplification with PCR in the compartment indicates the presence of target molecules. Based on the distribution of the presence or absence in micro-reaction units, the copy number/density of the target molecule is estimated with Poisson distribution (digital PCR).³¹ To create micro-reaction units, water-oil emulsion droplet technology provides a high-throughput, cost-effective assay for ddPCR. Because ddPCR is advantageous due to its high sensitivity and accurate quantification, it is a preferred technique for circulating tumor DNA analysis from liquid biopsy (Figure 5-4).

Next-Generation Sequencing

Basic Principles

NGS offers a high-throughput parallel sequencing approach to characterize the molecular landscape of a tumor.

Targeted Whole Genome Sequencing/Whole Exome Sequencing

While whole genome sequencing (WGS)/whole exome sequencing (WES) is frequently applied across large tumor cohorts to discover and characterize new disease drivers and biomarkers,³⁴ targeted NGS sequencing is more commonly used in clinical practice. The sequencing by synthesis principle is the core of the technology.³⁵ Briefly, DNA fragments isolated from a tumor sample are attached to an array and sequenced in parallel by sequentially synthesizing the reverse-complementary fragments (reads). NGS instruments can perform this process in a massively parallel fashion allowing for sequencing of up to 10 billion reads in 1 day.

Sequencing Technology

There are 2 major platforms that are based on different principles for the parallel sequencing (Figure 5-5). In the Ion Torrent PGM system (ThermoFisher Scientific), DNA fragments with specific adapter sequences are diluted to a single molecule of the fragment, and emulsion PCR is performed, resulting in amplified products that are linked on the surface of the beads (Figure 5-5A). Then, the beads are loaded into proton-sensing wells, and as sequencing proceeds, one deoxynucleotide triphosphate (dNTP) is incorporated sequentially from adapter sites, with releasing protons. Differences in the number of releasing protons according to incorporated nucleotides is converted to nucleotide sequences. With this semiconductor sequencer, somewhat less data per run is generated, but run times are shorter with longer reads (~400 bp).

In contrast, Illumina HiSeq system uses a different method, using 2 steps: bridge PCR and subsequent sequencing by synthesis (Figure 5-5B). After end-ligation of 2 different adapter sequences, denatured target DNA are hybridized with the 5'-end and 3'-end adapter sequences pre-fixed on the flow cell (bridge formation). Under this state, DNA polymerase performs a DNA elongation reaction and denatures the DNA resulting in 2 single-stranded DNA fragments. Subsequently, by repeating the bridge-joining, elongation, and denaturation reactions, a large number of single-stranded DNA fragments can be locally amplified

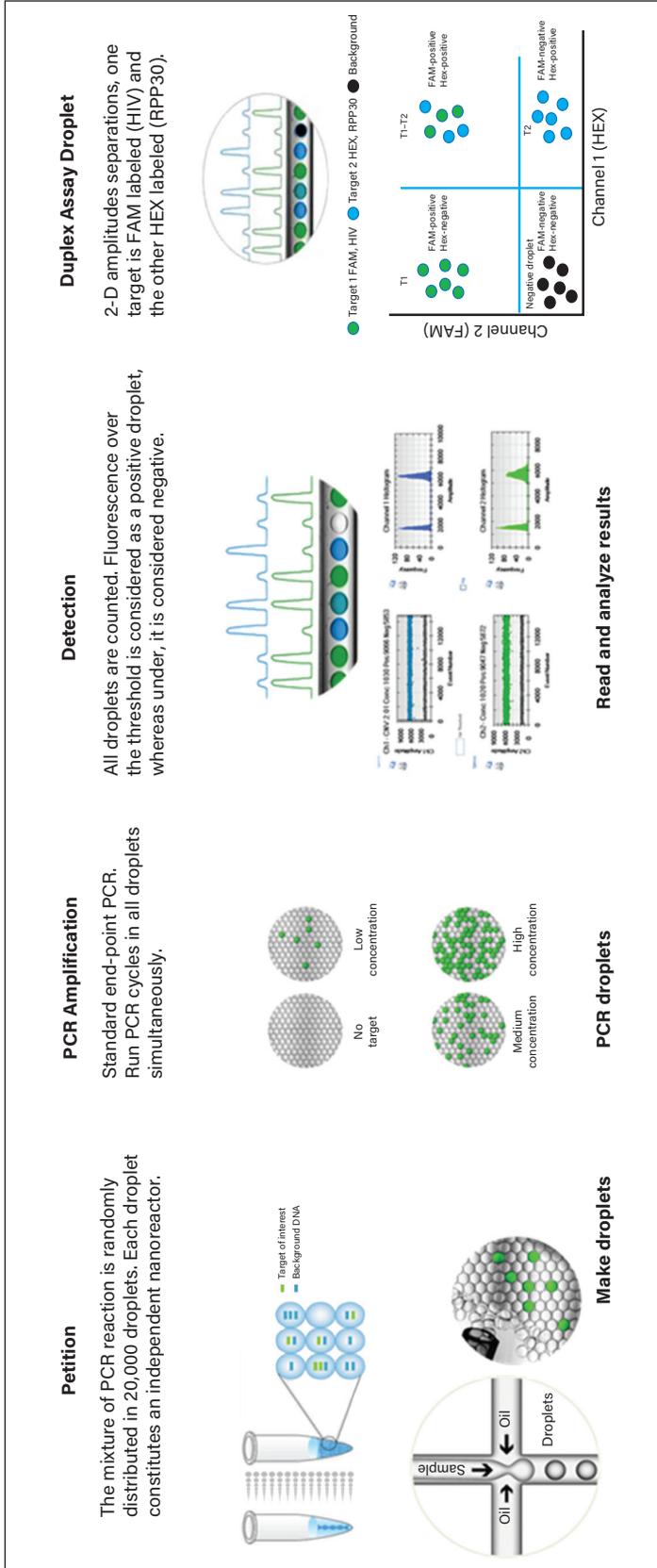


Figure 5-4. Workflow of droplet digital polymerase chain reaction (ddPCR) assay using Bio-Rad ddPCR system.³³

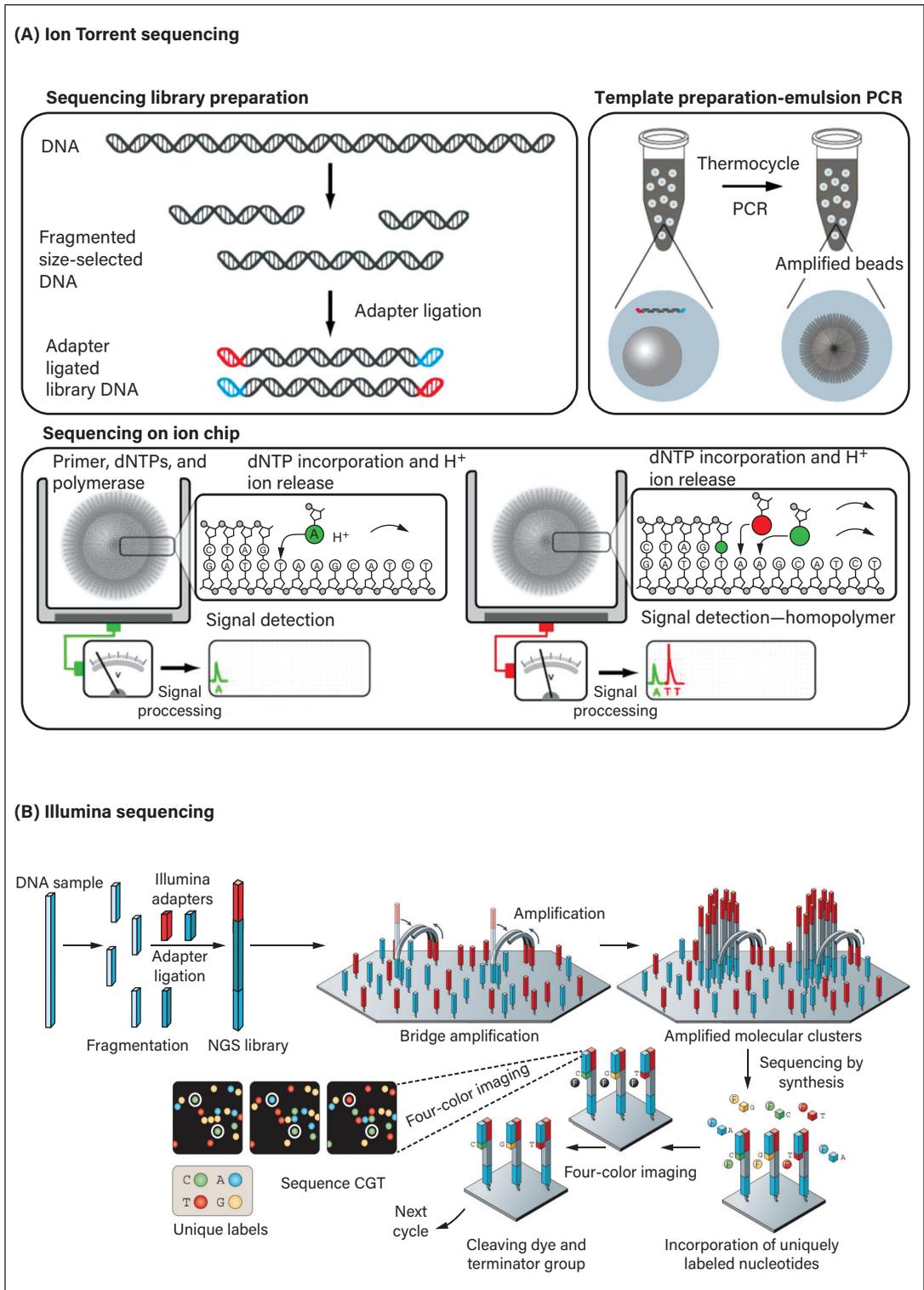


Figure 5-5. The sequencing platforms of **(A)** ThermoFisher Scientific Ion Torrent and **(B)** Illumina system.³⁸ Abbreviations: dNTP = deoxynucleotide triphosphate; NGS = next-generation sequencing; PCR = polymerase chain reaction.

and fixed as clusters on the flow cell (bridge PCR). For sequencing, a single-base elongation of 3'-end blocking fluorescent dNTPs is performed by DNA polymerase. Then, the fluorescent dye bound to the base is excited by a laser beam, and the emission is recorded as a photograph. Four photographs are taken (A, C, G, T) to determine the 4 different bases in individual clusters. This process is repeated until the entire length is sequenced.

A key decision when applying NGS to a tumor sample is the selection of regions that should be targeted for sequencing. Typical targeted NGS assays focus on 100 to 500 genes with relevance for cancer. Conversely, assays that sequence all human genes or the entire genome are typically referred to as WES and WGS, respectively.³⁶ A clear trade-off occurs between targeted and broad (WES/WGS) assays. A targeted assay typically provides a higher depth of sequencing and sensitivity to detect mutations in selected genes. In contrast, WES/WGS has a lower depth of sequencing but greater potential to discover novel biomarker mutations and structural variants. These broad assays typically also require more sequencing data, have higher computational requirements, and have longer TATs, often leading to higher costs compared to targeted assays.³⁷

Amplicon and Hybrid Capture

When performing targeting sequencing, another key decision point is the technology used to select and enrich DNA fragments in the genes of interest. Two commonly used techniques are amplicon sequencing and hybrid capture (Figure 5-6),³⁶ both of which are supported by most platforms (eg, AmpliSeq Focus Panel and TruSight Oncology 500 as amplicon and capture enrichment technologies in the Illumina platform, respectively).

Hybrid capture uses complementary DNA probes to bind and capture DNA fragments from the selected genes. Amplicon sequencing selects and enriches DNA fragments from genes of interest using primers and multiplexed PCR amplification. Amplicon sequencing is

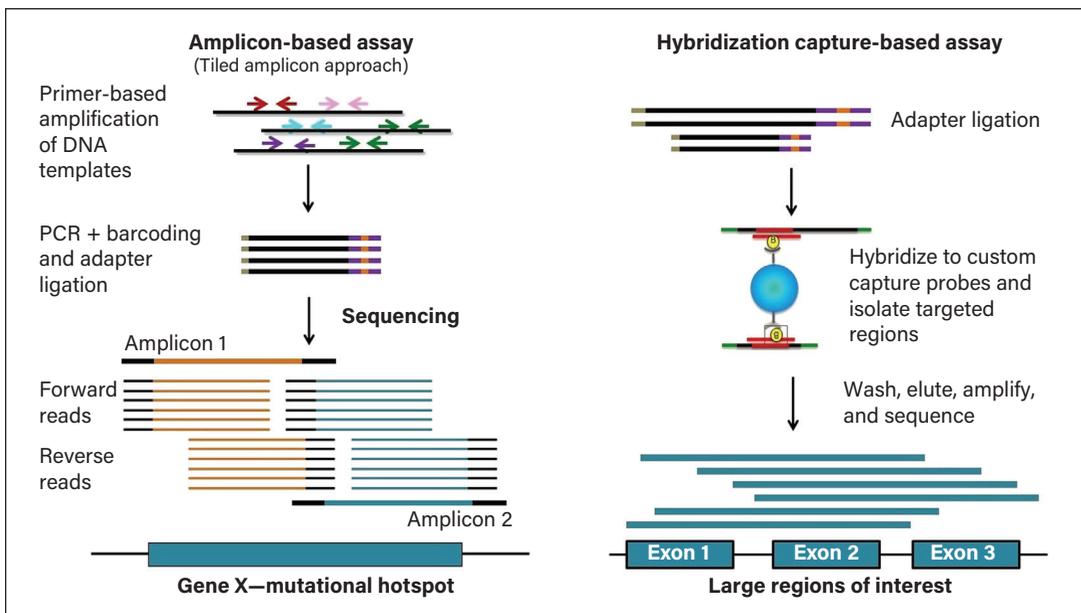


Figure 5-6. Comparison between amplicon-based and hybrid capture-based assays. Abbreviation: PCR = polymerase chain reaction. (Source: Jennings et al 2017.³⁹)

potentially advantageous because it can work from lower amounts of input DNA; however, it is still associated with risks of introducing allele-specific bias in the data from PCR amplification. Moreover, amplicon sequencing imposes a limit on the number of genes/regions that can be sequenced, usually restricting it to small gene panels or hotspot regions. Conversely, hybrid capture has no such limit and can scale up to the whole exome.³⁷ Furthermore, the probe-based selection of DNA fragments with hybrid capture is also better suited for the detection of fusion proteins.³⁶

Tumor Cell Content

The tumor cell content (also known as *tumor cellularity* or *tumor purity*) of the sample is a key consideration for performing NGS. Some tumor specimens may have low numbers and low proportion of tumor cells because of the mixture of nonneoplastic tissue, such as stromal and immune cells. DNA from nontumor cells will dilute mutation signals and negatively impact the process of calling somatic mutations with NGS, resulting in reduced sensitivity of mutation and copy number variation detection in samples with low tumor cell content.³⁶ This issue can partially be alleviated by performing deeper sequencing; however, when feasible, prioritizing samples with high tumor-cell content for NGS analysis may be a more cost-effective strategy.³⁷ As a standard value, most NGS panels are designed for 20% or more tumor cell content.

Estimation of Tumor Mutational Burden

Tumor mutational burden (TMB) has emerged as a biomarker of response to immune checkpoint inhibition⁴⁰ and can be assessed using the same assay used for identifying targetable genetic alterations. TMB is defined as the number of somatic mutations per megabase of interrogated genomic sequence, and NGS allows parallel and unbiased detection of somatic mutations across a large number of genes, which enables quantitative estimation of the TMB in a sample. However, if the NGS gene panel is too small, the TMB may not be reliably estimated.⁴⁰ Additionally, since the tumor cell content may impact the mutation detection sensitivity of the NGS assay, tumor cellularity should also be considered as a potential confounding variable for TMB estimation.⁴¹

Commercially Available Panel Tests

Table 5-2 shows current, commonly used commercially available panel tests. Individual panels are characterized by the following factors.

Targeted Nucleotide (DNA-Based, RNA-Based, or Both)

Because DNA is more stable than RNA, DNA-based panels have been established ahead of RNA-based panels. However, there are some limitations with DNA panels.⁴² Rearrangement of particular genes with large and/or repetitive introns or deletions, such as *NTRK*, may not be detected with DNA-based analysis. Therefore, other approaches can be utilized such as DNA/RNA hybrid panels or sequential panel testing (RNA panel sequencing if negative results obtained in the DNA panel).

Table 5-2. Examples of Currently Available NGS Panel Tests

Biopsy type	Tissue biopsy			Both			Liquid biopsy			
	DNA	RNA	Both	DNA	RNA	Both	DNA	DNA	DNA	DNA
Panel name	FoundationOne CDx (Foundation Medicine)	TruSight Tumor 26 assay (Illumina)	Archer FusionPlex Lung (Invitae)	Oncomine Dx Target Test (ThermoFisher Scientific)	TruSight Oncology Comprehensive (Illumina)	FoundationOne Liquid (Foundation Medicine)	Archer LiquidPlex (Invitae)	Guardant 360 (Guardant Health)	PlasmaSELECT (Personal Genome Diagnostics)	
Material read	Tumor DNA	Tumor DNA	Tumor RNA	Tumor DNA or RNA	Tumor DNA or RNA	Circulating tumor DNA	Circulating free DNA	Circulating free DNA	Circulating tumor DNA	
Enrichment	Hybrid capture	Amplicon	Hybrid capture	Amplicon	Hybrid capture	Hybrid capture	Hybrid capture	Hybrid capture	Hybrid capture	
Sample required	FFPE tissue; 50-1000 ng DNA	FFPE tissue; 30-300 ng	FFPE tissue; 10 ng RNA	FFPE tissue; 10 ng DNA, 10 ng RNA	FFPE tissue; $\geq 2.0 \text{ mm}^3$ or 40 ng DNA and 40 ng RNA	2 x 8.5 mL blood samples	5-10 ng DNA	10 mL blood sample for 5-30 ng DNA	2 x 10 mL blood samples	
Number of genes	324 genes and 36 fusions	26 genes	17 genes and 16 fusions	23 genes and 21 fusions	523 genes and 55 fusions	70 genes	28 genes	73 genes	64 genes	
Turnaround time	<2 weeks	2-3 days	2 days	3 days	~1 week	<2 weeks	2-3 days	7 days	Not reported	

Abbreviations: FFPE = formalin-fixed paraffin-embedded; NGS = next-generation sequencing.

Enrichment Method (Hybrid Capture vs Amplicon)

Small biopsy tissues or the need for a fast TAT may benefit from amplicon sequencing, whereas comprehensive genomic analysis using hybrid capture sequencing enables assessment of further alterations for possible clinical trials.

Gene Coverage

Based on the nature of a targeted panel, the gene coverage of the panels is not identical, even after including major alterations. In addition, the detection performance of larger indels, even with 12 bp or more, is different among panels because of a choice of bioinformatics tools.

Required DNA/RNA Input

Individual assays require different DNA/RNA inputs. Even using tissue of recommended size, yields of the extracted DNA/RNA may differ, possibly relating to factors such as tissue fixation and storage of blocks and unstained sections. Particularly, decalcified specimens, inappropriately fixed tissues, and aged blocks (≥ 5 years) may have lower yields compared with standard FFPE samples.

Conclusion

This chapter has provided a broad overview of the various technologies currently available for clinical detection of molecular biomarkers in lung cancer, including their advantages and disadvantages and ability to identify various genetic variants and protein changes. As no single technology is suitable for all biomarkers and given the rapid advances in this area, an understanding of current and emerging technologies is essential to delivering efficient optimal clinical care of lung cancer patients.

References

1. Supek F, Miñana B, Valcárcel J, Gabaldón T, Lehner B. Synonymous mutations frequently act as driver mutations in human cancers. *Cell*. 2014;156(6):1324-1335. doi:10.1016/j.cell.2014.01.051.
2. Yatabe Y, Borczuk AC, Cooper WA, et al, eds. *IASLC Atlas of Diagnostic Immunohistochemistry*. International Association for the Study of Lung Cancer;2020.
3. Prinsen CF, Klaassen CH, Thunnissen FB. Microarray as a model for quantitative visualization chemistry. *Appl Immunohistochem Mol Morphol*. 2003;11(2):168-173. doi:10.1097/00129039-200306000-00014.
4. Merritt CR, Ong GT, Church SE, et al. Multiplex digital spatial profiling of proteins and RNA in fixed tissue. *Nat Biotechnol*. 2020;38:586-599.
5. Fitzgibbons PL, Bradley LA, Fatheree LA, et al. Principles of analytic validation of immunohistochemical assays: Guideline from the College of American Pathologists Pathology and Laboratory Quality Center. *Arch Pathol Lab Med*. 2014;138(11):1432-1443. doi:10.5858/arpa.2013-0610-CP.
6. Tsao MS, Kerr KM, Kockx M, et al. PD-L1 immunohistochemistry comparability study in real-life clinical samples: results of blueprint phase 2 project. *J Thorac Oncol*. 2018;13(9):1302-1311. doi:10.1016/j.jtho.2018.05.013.
7. Dodson A, Parry S, Lissenberg-Witte B, et al. External quality assessment demonstrates that PD-L1 22C3 and SP263 assays are systematically different. *J Pathol Clin Res*. 2020;6(2):138-145. doi:10.1002/cjp2.153.
8. Thunnissen E. How to validate predictive immunohistochemistry testing in pathology? A practical approach exploiting the heterogeneity of programmed death ligand-1 present in non-small cell lung cancer. *Arch Pathol Lab Med*. 2019;143(1):11-12. doi:10.5858/arpa.2018-0410-ED.
9. Kawahara A, Yamamoto C, Nakashima K, et al. Molecular diagnosis of activating EGFR

- mutations in non-small cell lung cancer using mutation-specific antibodies for immunohistochemical analysis. *Clin Cancer Res.* 2010;16(12):3163-3170. doi:10.1158/1078-0432.CCR-09-3239.
10. Kitamura A, Hosoda W, Sasaki E, Mitsudomi T, Yatabe Y. Immunohistochemical detection of EGFR mutation using mutation-specific antibodies in lung cancer. *Clin Cancer Res.* 2010;16(13):3349-3355. doi:10.1158/1078-0432.CCR-10-0129.
 11. Simonetti S, Molina MA, Queralt C, et al. Detection of EGFR mutations with mutation-specific antibodies in stage IV non-small-cell lung cancer. *J Transl Med.* 2010;8:135. doi:10.1186/1479-5876-8-135.
 12. Brevet M, Arcila M, Ladanyi M. Assessment of EGFR mutation status in lung adenocarcinoma by immunohistochemistry using antibodies specific to the two major forms of mutant EGFR. *J Mol Diagn.* 2010;12(2):169-176. doi:10.2353/jmoldx.2010.090140.
 13. Jiang G, Fan C, Zhang X, et al. Ascertain an appropriate diagnostic algorithm using EGFR mutation-specific antibodies to detect EGFR status in non-small-cell lung cancer. *PLoS One.* 2013;8(3):e59183. doi:10.1371/journal.pone.0059183.
 14. Sasaki H, Shimizu S, Tani Y, et al. Usefulness of immunohistochemistry for the detection of the BRAF V600E mutation in Japanese lung adenocarcinoma. *Lung Cancer.* 2013;82(1):51-54. doi:10.1016/j.lungcan.2013.06.014.
 15. Ilie M, Long E, Hofman V, et al. Diagnostic value of immunohistochemistry for the detection of the BRAFV600E mutation in primary lung adenocarcinoma Caucasian patients. *Ann Oncol.* 2013;24(3):742-748.
 16. Seto K, Haneda M, Masago K, et al. Negative reactions of BRAF mutation-specific immunohistochemistry to non-V600E mutations of BRAF. *Pathol Int.* 2020;70(5):253-261. doi:10.1111/pin.12903.
 17. Lindeman NI, Cagle PT, Beasley MB, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology [published correction appears in *J Thorac Oncol.* 2013;8(10):1343]. *J Thorac Oncol.* 2013;8(7):823-859. doi:10.1097/JTO.0b013e318290868f.
 18. Lindeman NI, Cagle PT, Aisner DL, et al. 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. *Arch Pathol Lab Med.* 2018;142(3):321-346. doi:10.5858/arpa.2017-0388-CP.
 19. Cutz JC, Craddock KJ, Torlakovic E, et al. Canadian anaplastic lymphoma kinase study: a model for multicenter standardization and optimization of ALK testing in lung cancer. *J Thorac Oncol.* 2014;9(9):1255-1263. doi:10.1097/JTO.0000000000000239.
 20. Thunnissen E, Allen TC, Adam J, et al. Immunohistochemistry of pulmonary biomarkers: a perspective from members of the Pulmonary Pathology Society. *Arch Pathol Lab Med.* 2018;142(3):408-419. doi:10.5858/arpa.2017-0106-SA.
 21. Penault-Llorca F, Rudzinski ER, Sepulveda AR. Testing algorithm for identification of patients with TRK fusion cancer. *J Clin Pathol.* 2019;72(7):460-467. doi:10.1136/jclinpath-2018-205679.
 22. Gatalica Z, Xiu J, Swensen J, Vranic S. Molecular characterization of cancers with NTRK gene fusions. *Mod Pathol.* 2019;32(1):147-153. doi:10.1038/s41379-018-0118-3.
 23. Hsiao SJ, Zehir A, Sireci AN, Aisner DL. Detection of tumor NTRK gene fusions to identify patients who may benefit from tyrosine kinase (TRK) inhibitor therapy. *J Mol Diagn.* 2019;21(4):553-571. doi:10.1016/j.jmoldx.2019.03.008.
 24. Rodig SJ, Mino-Kenudson M, Dacic S, et al. Unique clinicopathologic features characterize ALK-rearranged lung adenocarcinoma in the Western population [published correction appears in *Clin Cancer Res.* 2009;15(22):7110]. *Clin Cancer Res.* 2009;15(16):5216-5223. doi:10.1158/1078-0432.CCR-09-0802.
 25. Cook JR. Paraffin section interphase fluorescence in situ hybridization in the diagnosis and classification of non-Hodgkin lymphomas. *Diagn Mol Pathol.* 2004;13(4):197-206. doi:10.1097/01.pdm.0000135286.05198.89.
 26. Hunt JL. Molecular pathology in anatomic pathology practice: a review of basic principles. *Arch Pathol Lab Med.* 2008;132(2):248-260. doi:10.5858/2008-132-248-MPIAPP.
 27. Lindeman NI, Cagle PT, Aisner DL, et al. 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. *J Thorac Oncol*. 2018;13(3):323-358. doi:10.1016/j.jtho.2017.12.001.
28. Roy-Chowdhuri S, Dacic S, Ghofrani M, et al. Collection and handling of thoracic small biopsy and cytology specimens for ancillary studies: guideline from the College of American Pathologists in collaboration with the American College of Chest Physicians, Association for Molecular Pathology, American Society of Cytopathology, American Thoracic Society, Pulmonary Pathology Society, Papanicolaou Society of Cytopathology, Society of Interventional Radiology, and Society of Thoracic Radiology. *Arch Pathol Lab Med*. 2020;10.5858/arpa.2020-0119-CP. doi:10.5858/arpa.2020-0119-CP.
 29. Whitcombe D, Theaker J, Guy SP, Brown T, Little S. Detection of PCR products using self-probing amplicons and fluorescence. *Nat Biotechnol*. 1999;17(8):804-807. doi:10.1038/11751.
 30. Takeuchi K, Choi YL, Soda M, et al. Multiplex reverse transcription-PCR screening for EML4-ALK fusion transcripts. *Clin Cancer Res*. 2008;14(20):6618-6624. doi:10.1158/1078-0432.CCR-08-1018.
 31. Vogelstein B, Kinzler KW. Digital PCR. *Proc Natl Acad Sci U S A*. 1999;96(16):9236-9241. doi:10.1073/pnas.96.16.9236.
 32. Kirk BW, Feinsod M, Favis R, Kliman RM, Barany F. Single nucleotide polymorphism seeking long term association with complex disease. *Nucleic Acids Res*. 2002;30(15):3295-3311. doi:10.1093/nar/gkf466.
 33. Chung HK, Hattler JB, Narola J, et al. Development of droplet digital PCR-based assays to quantify HIV proviral and integrated DNA in brain tissues from viremic individuals with encephalitis and virally suppressed aviremic individuals. *Microbiol Spectr*. 2022;10(1):e0085321. doi:10.1128/spectrum.00853-21.
 34. Garraway LA, Lander ES. Lessons from the cancer genome. *Cell*. 2013;153(1):17-37. doi:10.1016/j.cell.2013.03.002.
 35. Shendure J, Balasubramanian S, Church GM, et al. DNA sequencing at 40: past, present and future [published correction appears in *Nature*. 2019;568(7752):E11]. *Nature*. 2017;550(7676):345-353. doi:10.1038/nature24286.
 36. Gagan J, Van Allen EM. Next-generation sequencing to guide cancer therapy. *Genome Med*. 2015;7(1):80. doi:10.1186/s13073-015-0203-x.
 37. Berger MF, Mardis ER. The emerging clinical relevance of genomics in cancer medicine. *Nat Rev Clin Oncol*. 2018;15(6):353-365. doi:10.1038/s41571-018-0002-6.
 38. Anandhakumar C, Kizaki S, Bando T, Pandian GN, Sugiyama H. Advancing small-molecule-based chemical biology with next-generation sequencing technologies. *Chembiochem*. 2015;16(1):20-38. doi:10.1002/cbic.201402556.
 39. Jennings LJ, Arcila ME, Corless C, et al. Guidelines for validation of next-generation sequencing-based oncology panels: a joint consensus recommendation of the Association for Molecular Pathology and College of American Pathologists. *J Mol Diagn*. 2017;19(3):341-365.
 40. Budczies J, Allgäuer M, Litchfield K, et al. Optimizing panel-based tumor mutational burden (TMB) measurement. *Ann Oncol*. 2019;30(9):1496-1506. doi:10.1093/annonc/mdz205.
 41. Anagnostou V, Niknafs N, Marrone K, et al. Multimodal genomic features predict outcome of immune checkpoint blockade in non-small-cell lung cancer. *Nat Cancer*. 2020;1(1):99-111. doi:10.1038/s43018-019-0008-8.
 42. Benayed R, Offin M, Mullaney K, et al. High yield of RNA sequencing for targetable kinase fusions in lung adenocarcinomas with no mitogenic driver alteration detected by DNA sequencing and low tumor mutation burden. *Clin Cancer Res*. 2019;25(15):4712-4722. doi:10.1158/1078-0432.CCR-19-0225.

A Global Perspective on Molecular Testing Guidelines and Practices

6

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In the last one and a half decades, the management of lung cancer, especially non-small cell lung carcinoma (NSCLC), has increasingly shifted to incorporate targeted therapies.^{1,2} Molecular testing during or following the diagnostic work-up of lung cancer has become an essential tool to identify predictive biomarkers for the selection of patients for personalized therapy.^{3,4} Most of these biomarkers are detected by modern molecular techniques such as polymerase chain reaction (PCR) or sequencing-based methods, which provide fast and accurate results. Further, it is strongly encouraged that genetic testing required to support patient management is performed in laboratories that are compliant with specific national and local standards (eg, Clinical Laboratory Improvement Amendments regulations in the United States). In addition, multiple guidelines issued by different international groups (College of American Pathologists [CAP], International Association for the Study of Lung Cancer [IASLC], Association for Molecular Pathology [AMP], American Society of Clinical Oncology [ASCO], European Society for Medical Oncology [ESMO], National Comprehensive Cancer Network [NCCN]) provide detailed recommendations for the selection of lung cancer patients for testing and treatment, sample requirements, and molecular testing methods and platforms.^{3,5-7} The purpose of this chapter is to provide an overview of the implementation of these and other molecular testing guidelines for patients with lung cancer at the global level.

Guideline Development and Guidelines

In the past, clinical decision-making relied largely on the experience and skill of the physician in charge. However, once evidence-based medicine (EBM) was proposed by Guyatt in Canada in 1991,⁸ the concept spread to various clinical fields along with a growing social awareness of the need for quality medical care. The current practice guidelines are based on

a 2011 report by the Institute of Medicine (IOM) 2011 report, *Clinical Practice Guidelines We Can Trust*,⁹ which is derived from the concept of EBM. This report provides the definition of a practice guideline, as well as some methods for guideline development. Grading of Recommendations Assessment, Development and Evaluation (GRADE) is the most widely used system in the world.¹⁰ GuideLines Into Decision Support (GLIDES) is a system resulting from a project under contract to the US Agency for Healthcare Research and Quality (AHRQ) from 2008 to 2013.¹¹ Both systems determine the evidence level through systematic reviews. Recent CAP/IASLC/AMP molecular testing guidelines have been developed using this system.¹²

CAP/IASLC/AMP, ASCO, and NCCN

Certain guidelines, such as from the CAP/IASLC/AMP molecular testing panel, have focused on the laboratory community and empower the pathologist and/or molecular diagnostician to direct the use of resource-intensive molecular assays and ensure appropriate test selection apropos to available targeted therapies. The original CAP/IASLC/AMP guideline was published in 2013 and has served as a model for subsequent national and international efforts.¹³ The original guideline and its update in 2018 prompted endorsements from ASCO and serve as key references for the NCCN guidelines.^{5,6,14} These documents diverge in their testing and treatment recommendations as a result of their timing relative to the fast pace of targeted therapy approvals in the last decade. The CAP/IASLC/AMP guidelines are currently under revision with an updated publication expected in 2024.

European Society for Medical Oncology

ESMO regularly publishes guidelines for the management of patients with lung cancer,⁷ including recommendations on testing a wide range of targets for which the European Medicines Agency (EMA) has approved therapies.⁷

EMA approval is permissive but does not guarantee access to drugs in individual countries. At least in the context of national public health systems, individual countries may approve drugs and, to a variable degree, facilitate testing. Thus, the main determining factor driving testing is the availability of relevant targeted therapies; this leads to innumerable national or even regional guidelines reflecting variance in local practice. The recommended test targets are not consistent across countries; however, *EGFR*, *ALK*, *ROS1*, and PD-L1 are recommended in all guidelines and *BRAF* and *NTRK* in most, but *KRAS*, *MET*, *RET*, and *ERBB2* (*HER2*) testing is not addressed in many.¹⁵ Delivery of testing then depends on access to laboratory facilities and arrangements for test reimbursement.¹⁵ Although next-generation sequencing (NGS) is embedded as a recommendation in ESMO and many national guidelines, access to NGS is far from universal.¹⁶ Initiatives within the European Union are aimed at addressing this disparity. According to the 2023 ESMO guidelines on oncogene-addicted metastatic NSCLC, the recommended biomarkers for testing are *EGFR*, *ALK*, *ROS1*, *BRAF*, *RET*, *MET* (exon 14 skipping as well as amplification), *NTRK*, *ERBB2* (*HER2*) mutation, *EGFR* exon 20 insertion mutation, and *KRAS* G12C mutation.⁷ A summary of US and European guidelines are provided in Table 6-1.

Table 6-1. Summary of US and European Guidelines

Guideline	Population	Target	Method of testing ^a
CAP/IASLC/AMP 2018	Newly diagnosed patients	<i>EGFR</i>	PCR/NGS
		<i>ALK</i>	IHC ± FISH
		<i>ROS1</i>	IHC (screening) and FISH/PCR/NGS
		<i>RET, MET, ERBB2 (HER2), KRAS, BRAF</i>	Part of multiplex panel of NGS; either initially or when <i>EGFR, ALK, ROS1</i> negative
	Relapsed patients on targeted therapy	<i>EGFR</i> T790M (cfDNA/tissue DNA)	5% analytic sensitivity (PCR-based/NGS)
ESMO 2023 ^b	Newly diagnosed patients	<i>EGFR</i>	Any validated method to cover mutations in exon 18-21 (DNA NGS preferred)
		<i>ALK</i>	RNA NGS; IHC ± molecular confirmation (NGS, FISH)
		<i>ROS1</i>	RNA NGS; IHC screening, molecular confirmation essential (NGS, FISH)
		<i>RET, MET, NTRK, ERBB2 (HER2), KRAS, BRAF</i>	DNA/RNA NGS panel testing
		PD-L1	IHC
		<i>EGFR</i> T790M, <i>MET</i> (as appropriate) (cfDNA/tissue DNA)	PCR/NGS/ISH
	Relapsed patients on targeted therapy	<i>EGFR</i> (category 1)	Broad molecular profiling (NGS) ^c
NCCN 2022	Newly diagnosed patients	<i>ALK</i> (category 1)	Broad molecular profiling (NGS)
		<i>KRAS, ROS1, BRAF, NTRK1/2/3, MET</i> exon 14 skipping, <i>RET</i>	
		PD-L1	
	Relapsed patients on targeted therapy	<i>EGFR</i> T790M and other genomic resistance mutations (cfDNA/tissue DNA)	Broad molecular profiling (NGS)

Abbreviations: AMP = Association for Molecular Pathology; ASCO = American Society of Clinical Oncology; CAP = College of American Pathologists; cfDNA = cell-free DNA; ESMO = European Society for Medical Oncology; FISH = fluorescence in situ hybridization; IASLC = International Association for the Study of Lung Cancer; IHC = immunohistochemistry; ISH = in situ hybridization; NCCN = National Comprehensive Cancer Network; NGS = next generation sequencing; PCR = polymerase chain reaction.

^aMultiplexed sequencing panels are preferred over single gene tests. ASCO endorsed these guidelines in addition to inclusion the of *BRAF* mutation.⁵

^bESMO 2023 guidelines.⁷

^cAlso aim to detect emerging biomarkers such as high-level *MET* amplification and *ERBB2 (HER2)* mutation.

Region-Specific Biomarker Testing Practices

The selection of biomarkers varies by country as per their specific needs and availability of resources. For many countries, the implementation of these biomarker testing practices for molecular screening to identify predictive biomarkers is challenging because of operational and logistic constraints.^{15,17,18} Therefore, most countries follow their own biomarker testing practices for molecular testing of lung cancer in their region, which not only include lower- or middle-income countries but also some developed nations. Every country has its

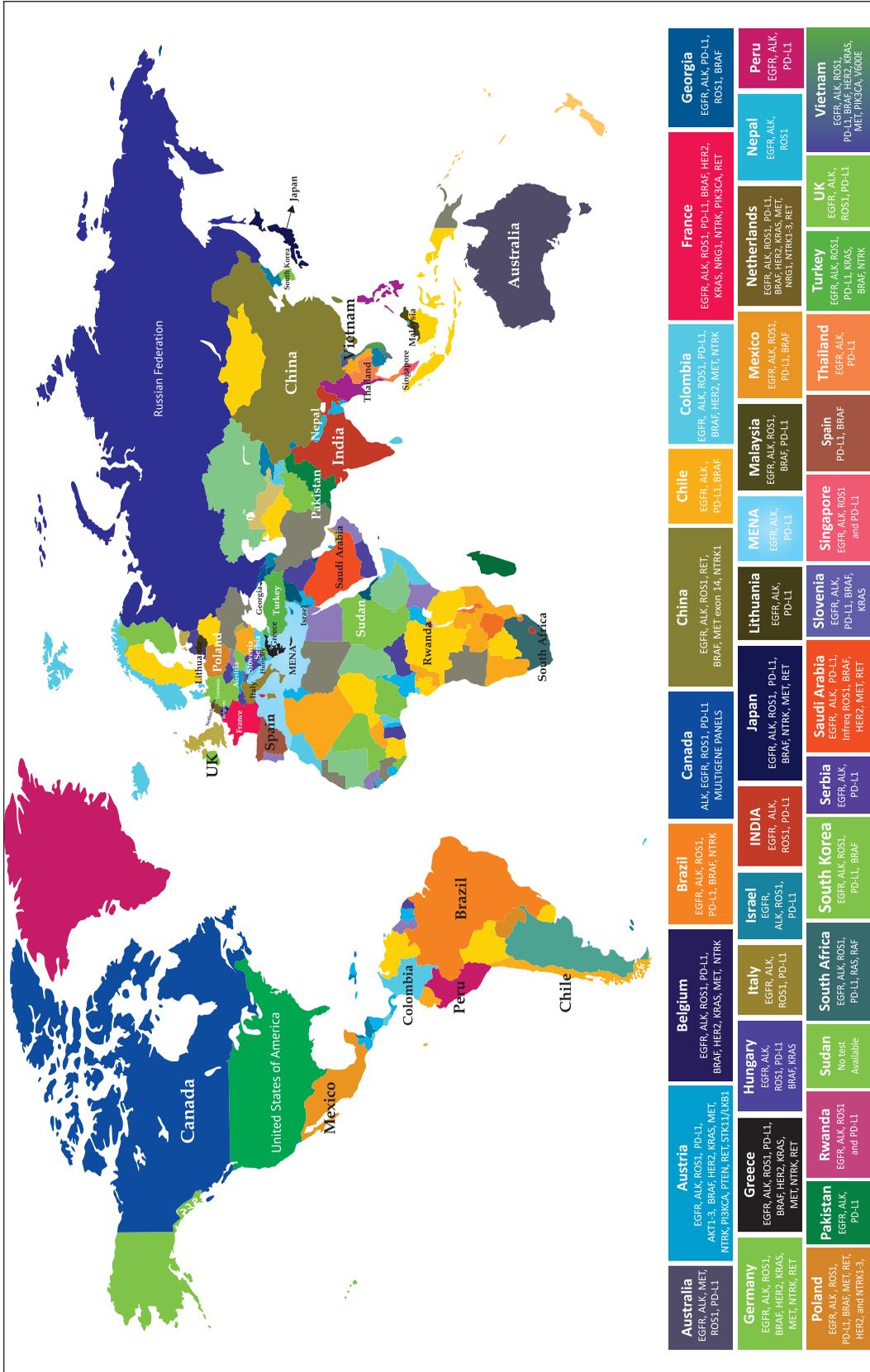


Figure 6-1. Predictive biomarkers tested in different countries on a world map. Abbreviation: MENA = Middle East and North Africa. (Data from *Journal of Thoracic Oncology* series.²¹)

own constraints, which include cost of molecular testing, availability of testing infrastructure, access to the technical and interpretive expertise, government policies regarding health insurance and reimbursement, and requirements for testing approval from local authorities. In some countries, targeted treatments are not available or are financially inaccessible to patients despite the availability of the molecular test.^{15,19,20} Figure 6-1 provides biomarker testing practices in different countries. While most countries perform *EGFR*, *ALK*, *ROS1*, and PD-L1, there are a few where either *EGFR* or only *EGFR* and *ALK* are offered. Some country-specific examples are provided next.

United States

In the United States, the NCCN guidelines are widely used to guide treatment decisions and as a benchmark for payers (predominantly private insurance companies).⁶ These guidelines represent expert opinions based on current clinical evidence and are heavily influenced by regulatory (US Food and Drug Administration [FDA]) drug approvals. Because the NCCN guidelines can be updated several times a year to reflect new approvals, they tend to reflect the most up-to-date testing and treatment environment. In contrast, systematic guidelines, such as those from CAP/IASLC/AMP, depend on a rigorous and time-intensive review of peer-reviewed, published evidence¹² and for practical reasons are only updated every few years.

Despite the recognized role and value of available guidelines, adherence to their recommendations remains suboptimal. A survey based on real-world data collected from community oncology practices between 2018 and 2020 showed that although 91% of the patients with metastatic non-squamous NSCLC had at least 1 single gene analysis performed, only 49% had 5 or more therapeutically relevant genes evaluated, and NGS was performed in only 39%.²² Examination of broad, setting-agnostic health care databases has also uncovered significant racial disparities regarding NGS testing among NSCLC patients in the United States, with approximately 55% of White patients receiving NGS at any point in their care versus approximately 44% of Black patients.²³ In light of recognized challenges of sample insufficiency and prolonged turnaround times for tissue-based testing, cell-free DNA (cfDNA) analysis (liquid biopsy) has gained a significant foothold in US practice. While cfDNA-specific testing guidelines are lacking in the United States, the updated CAP/IASLC/AMP guidelines recognized their utility in patients lacking sufficient tumor tissue for biomarker testing, or in those needing molecular characterization following relapse on targeted therapy.¹² IASLC published recommendations in 2021,²⁴ albeit based on expert opinion, not on a systematic review of the literature. (See Chapter 4.)

Canada

In Canada, the health care system is almost fully government funded, thus the scope of routinely available molecular testing for patients with cancer is determined by the health care authorities. As provincial governments have jurisdiction over health care, the type and system of testing available to patients may vary between provinces. Testing is mainly conducted at molecular diagnostic laboratories, most of which are established within the laboratory medicine or pathology departments of major hospitals. In general, most provincial cancer care authorities would initiate a consideration of the funding for biomarker testing when a

new drug that has been approved by Health Canada is being considered for public funding. Major issues that are considered in such deliberations include the cost effectiveness of the test and testing methods/assays being proposed, and their impact on the delivery of patient care for the relevant cancers.²⁵⁻²⁸ While funding generally follows guidelines that are accepted internationally (eg, CAP/IASLC/AMP, ASCO, ESMO), periodic consensus or review publications from groups of Canadian key-opinion leaders in medical oncology, pathology, and molecular pathology may provide recommendations in Canadian context.²⁹⁻³⁵ At present for lung cancer, molecular testing is recommended for all targetable alterations, including *EGFR*, *ALK*, *ROS1*, *BRAF V600E*, *NTRK*, *KRAS G12C*, and *EGFR T790M* mutations in patients with *EGFR*-mutant NSCLC who have progressed on first- and/or second-generation *EGFR* kinase inhibitors.³⁵ New markers will be included as the corresponding targeted drugs are approved in Canada. Testing for circulating *EGFR T790M* mutation in plasma samples (liquid biopsy) is also recommended as an alternative (preferred) or complementary method. While the choice of testing method is determined by the laboratory, based on multidisciplinary input, laboratories are required to follow the acceptable guidelines to validate individual assays before clinical implementation. This is crucial, as unlike in the United States, Health Canada commonly associates specific drug approvals to a “validated assay,” instead of a specific companion diagnostic assay. This approach has prompted the conduct of several Canadian multicenter studies to optimize and standardize lung biomarker tests, such as for *EGFR*, *ALK*, *ROS1*, and PD-L1.³⁶⁻³⁹

Europe

According to a 2019 survey,⁴⁰ European physicians are aware of (inter)national guidelines on NSCLC biomarker testing. Selection criteria for testing are similar across countries: PD-L1 for all patients with metastatic NSCLC and molecular testing for oncogenic drivers in those with non-squamous NSCLC or squamous cell histology but without a smoking history. The status of molecular testing across Europe is very heterogeneous. In around two-thirds of European countries, “reflex testing” (pathologist driven) is common; in the others, testing is on demand. Testing is usually carried out in house or in a regional laboratory. In around three-quarters of countries, NGS is a common practice; in the others, real-time PCR is used.^{15,40}

European countries have a diverse range of health care systems and health economies, all of which impact the delivery of tumor molecular profiling.^{41,42} In all countries, *EGFR*, *ALK*, and PD-L1 testing is available, but reimbursement practice varies between full coverage by national or private health insurance or funding by pharmaceutical companies, to no funding or even no testing for certain biomarkers.⁴⁰ In countries such as France, structured programs supported by the government exist to achieve centralized systematic molecular testing in a limited number of centers.⁴³

Although awareness regarding testing criteria exists, not all patients have access to testing, and even in countries with access, not all tumors fulfilling testing criteria are tested, although testing rates are increasing over time.^{15,44-47} Importantly, patient awareness of their biomarker test results seems low, as in a European survey, 23% of patients knowing their tumors were tested did not know the results.⁴⁸

Asia-Pacific Region

The Asia-Pacific region is highly diverse and includes countries like Japan where NGS testing is reimbursed, as well as other countries such as Indonesia where access to oncologic care is suboptimal. In Asia, there is less emphasis on companion diagnostics, and the cost and reimbursement of NGS is very much determined by individual countries. Many adopt a pragmatic approach where biomarker testing is predicated on access to novel therapies, taking into account that local drug approvals can often lag FDA/EMA approvals by up to 2 years. This is the case even in Hong Kong, Taiwan, and Singapore.

Australia

In Australia, testing for *EGFR*, *ALK*, *ROS1*, and *MET* alterations, as well as PD-L1 immunohistochemistry (IHC), is routinely performed and is government funded. Although NGS panel testing is undertaken in larger centers, this is not universally available. For *ALK* and *ROS1*, IHC screening is undertaken and confirmation of a rearrangement by fluorescence in situ hybridization (FISH) testing is currently required to access targeted agents. Testing for less common fusions, such as *RET* and *NTRK1-3*, is less widely available. Molecular testing using liquid biopsies is uncommonly performed in Australia currently and is not government funded.⁴⁹

China

The approved therapeutic targets in Mainland China include *EGFR* and *BRAF* mutations, *ALK*, *ROS1*, *RET*, and *NTRK* fusions, and *MET* exon 14 skipping mutations. Therefore, Chinese guidelines recommend the preceding genes must be tested. Drugs targeting *KRAS* and *ERBB2* (*HER2*) gene mutation, *MET* gene amplification, and other genes are still in clinical trials, and these targets are recommended as components of expanded testing.^{50,51}

PCR-based methods and NGS are recommended testing methods for mutations whereas gene fusions are detected by FISH, allele-refractory mutation system (ARMS) polymerase chain reaction (PCR), NGS (RNA-based), and IHC. *ALK* IHC (D5F3) is recommended as a companion diagnostic test, while *ROS1* and neurotrophic tyrosine receptor kinase (*NTRK*/pan-*TRK*) IHC are recommended as screening tests.^{50,52,53} Molecular testing is routinely performed on surgical samples of NSCLC with non-squamous histology at stage IB or above. For advanced NSCLC, tissue biopsies are preferred. Liquid biopsy can also be considered if tissue samples are not available. In addition, PD-L1 detection is equally important and should be performed at the same time.^{54,55}

India

Indian guidelines recommend sequential or simultaneous testing as per the discretion of the oncologist.⁵⁶ Presently, most of the therapies for novel gene targets (*BRAF*, *MET*, *RET*, *HER2*, *KRAS*) are not available in India. Therefore, these genes are not included in routine molecular testing in all patients with advanced NSCLC but may be tested in patients who progress or develop resistance to first-line therapy. Expert panels recommend *EGFR*, *ALK*, and *ROS1* genes, and PD-L1 protein, as first-line biomarkers for molecular testing.⁵⁶

Depending on the oncologist's judgment and the patient's financial means, both single gene testing and NGS are advised. ARMS-PCR, digital PCR, and NGS are the preferred methods for testing of *EGFR* mutation in NSCLC. IHC is recommended for the ALK rearrangement and PD-L1 expression whereas *ROS1* rearrangement is screened by IHC followed by FISH for confirmation.

Because of limited availability of osimertinib in first-line settings, patients are often tested for *EGFR* T790M mutation on liquid biopsy at relapse. However, they are advised to undergo a tumor biopsy if liquid biopsy results are negative.

Japan

Biomarker testing in Japan is regulated by the health care policy of the government, and most predictive biomarker testing is covered by health insurance. Patients with advanced lung cancer are commonly examined with focused multiplex testing after the diagnosis, and comprehensive genomic profiling test is applied in some patients who progress after standard therapy.^{57,58} In terms of treatment guidelines, the Japanese Lung Cancer Society (JLCS) releases guidelines for lung cancer treatment, which are developed by the GRADE system and updated every year; a partial summary has been reported in the international literature.⁵⁹ For individual predictive biomarkers, the biomarker committee of the JLCS facilitates appropriate implementation of molecular testing with guidance for *EGFR*, *ALK*, *ROS1*, *BRAF*, *MET*, *KRAS*, and multiplex testing. The guidance for *ALK*,⁶⁰ *MET*,⁶¹ and multiplex testing⁵⁸ are available in English.

Africa and Middle East

Biomarker testing for NSCLC patients in Africa and the Middle East is heterogeneous, as the region includes high- and low-to-middle-income countries. Unlike in the high-income countries of the Middle East, availability of lung cancer biomarker testing is very limited in sub-Saharan countries.^{62,63} Local testing expertise is also scarce. Testing sites may not even exist in the country or are centrally located, resulting in extended turnaround times. Several countries rely on outsourcing to European laboratories for molecular testing. In most of the cases, the patient bears the cost of the test. In countries such as South Africa, access to biomarker testing and targeted therapies is dictated in part by the patient's access to private versus public sector health care services.⁶⁴ This applies also to diagnostic tools, as histopathology laboratory tests may not be available nationwide, and routine techniques in other settings, such as immunohistochemistry, can be hard to find.⁶⁵ This means that in certain countries, even basic histologic typing of NSCLC using immunohistochemistry is not available, let alone molecular testing.¹⁹ Moreover, access to targeted therapies is very limited and the cost of therapy is generally not covered by most public health systems. This major gap results in a delayed care for NSCLC and hinders efforts to provide the best possible care to these patients.

Conclusion

Global implementation of standard biomarker testing guidelines in lung cancer depends on country-specific local and regional factors that include availability of resources and infrastructure, affordability, reimbursement policies by government or private parties, access to testing assays, and drug approval mechanisms. Although the recommendations on the

specific tests are not consistent across countries, testing for *EGFR*, *ALK*, *ROS1*, and PD-L1 is widely implemented. With increased availability of novel targeted therapies, we anticipate this list to expand, necessitating a shift toward multiplexed testing.

References

- Alexander M, Kim SY, Cheng H. Update 2020: management of non-small cell lung cancer. *Lung*. 2020;198(6):897-907.
- Morgensztern D, Campo MJ, Dahlberg SE, et al. Molecularly targeted therapies in non-small-cell lung cancer annual update 2014. *J Thorac Oncol*. 2015;10(1 suppl 1):S1-63.
- Lindeman NI, Cagle PT, Aisner DL, et al. 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. *Arch Pathol Lab Med*. 2018;142(3):321-346.
- Nooreldeen R, Bach H. Current and future development in lung cancer diagnosis. *Int J Mol Sci*. 2021;22(16):8661.
- Kalemkerian GP, Narula N, Kennedy EB, et al. Molecular testing guideline for the selection of patients with lung cancer for treatment with targeted tyrosine kinase inhibitors: American Society of Clinical Oncology endorsement of the College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology clinical practice guideline update. *J Clin Oncol Off J Am Soc Clin Oncol*. 2018;36(9):911-919.
- Ettinger DS, Wood DE, Aisner DL, et al. Non-small cell lung cancer, version 3.2022, NCCN clinical practice guidelines in oncology. *J Natl Compr Cancer Netw*. 2022;20(5):497-530.
- Hendriks LE, Kerr K, Menis J, et al. Oncogene-addicted metastatic non-small-cell lung cancer: ESMO clinical practice guideline for diagnosis, treatment and follow-up. *Ann Oncol*. 2023. [https://www.annalsofncology.org/article/S0923-7534\(22\)04781-0/fulltext](https://www.annalsofncology.org/article/S0923-7534(22)04781-0/fulltext).
- Guyatt GH. Evidence-based medicine. *ACP J Club*. 1991;114(2):A16.
- Institute of Medicine. *Clinical Practice Guidelines We Can Trust*. National Academies Press; 2011.
- Welcome to the GRADE Working Group. GRADE. 2004-2002. Updated January 21, 2021. <https://www.gradeworkinggroup.org/>.
- GuideLines Into DEcision Support (GLIDES). Yale School of Medicine. Updated January 21, 2021. <https://medicine.yale.edu/cmi/glides/>.
- Lindeman NI, Cagle PT, Aisner DL, et al. 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. *J Thorac Oncol*. 2018;13(3):323-358.
- Lindeman NI, Cagle PT, Beasley MB, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. *J Thorac Oncol*. 2013;8(7):823-859.
- Leighl NB, Rekhtman N, Biermann WA, et al. Molecular testing for selection of patients with lung cancer for epidermal growth factor receptor and anaplastic lymphoma kinase tyrosine kinase inhibitors: American Society of Clinical Oncology endorsement of the College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology guideline. *J Clin Oncol*. 2014;32(32):3673-3679.
- Kerr KM, Bibeau F, Thunnissen E, et al. The evolving landscape of biomarker testing for non-small cell lung cancer in Europe. *Lung Cancer Amst Neth*. 2021;154:161-175.
- Horgan D, Curigliano G, Rieß O, et al. Identifying the steps required to effectively implement next-generation sequencing in oncology at a national level in Europe. *J Pers Med*. 2022;12(1):72.
- Barta JA, Powell CA, Wisnivesky JP. Global epidemiology of lung cancer. *Ann Glob Health*. 2019;85(1):8.
- Smeltzer MP, Wynes MW, Lantuejoul S, et al. The International Association for the Study of Lung Cancer global survey on molecular testing in lung cancer. *J Thorac Oncol*. 2020;15(9):1434-1448.

19. Elhassan MMA, Mohamedani AA, Mohamed SAH, et al. Lung cancer in Sudan. *J Thorac Oncol*. 2022;17(4):489-498.
20. Sheikh HS, Munawar K, Sheikh F, Qamar MFU. Lung cancer in Pakistan. *J Thorac Oncol*. 2022;17(5):602-607.
21. Lung cancer worldwide. Editorial series. *J Thorac Oncol*. 2019-2022.
22. Robert NJ, Espirito JL, Chen L, et al. Biomarker testing and tissue journey among patients with metastatic non-small cell lung cancer receiving first-line therapy in the US Oncology Network. *Lung Cancer Amst Neth*. 2022;166:197-204.
23. Bruno DS, Hess LM, Li X, Su EW, Patel M. Disparities in biomarker testing and clinical trial enrollment among patients with lung, breast, or colorectal cancers in the United States. *JCO Precis Oncol*. 2022;6:e2100427.
24. Rolfo C, Mack P, Scagliotti GV, et al. Liquid biopsy for advanced NSCLC: a consensus statement from the International Association for the Study of Lung Cancer. *J Thorac Oncol*. 2021;16(10):1647-1662.
25. Djalalov S, Beca J, Hoch JS, et al. Cost effectiveness of EML4-ALK fusion testing and first-line crizotinib treatment for patients with advanced ALK-positive non-small-cell lung cancer. *J Clin Oncol Off J Am Soc Clin Oncol*. 2014;32(10):1012-1019.
26. Ontario Health (Quality). Cell-free circulating tumour DNA blood testing to detect EGFR T790M mutation in people with advanced non-small cell lung cancer: a health technology assessment. *Ont Health Technol Assess Ser*. 2020;20(5):1-176.
27. Makarem M, Ezeife DA, Smith AC, et al. Reflex ROS1 IHC screening with FISH confirmation for advanced non-small cell lung cancer—a cost-efficient strategy in a public healthcare system. *Curr Oncol Tor Ont*. 2021;28(5):3268-3279.
28. Ezeife DA, Spackman E, Juergens RA, et al. The economic value of liquid biopsy for genomic profiling in advanced non-small cell lung cancer. *Ther Adv Med Oncol*. 2022;14:17588359221112696.
29. Ellis PM, Blais N, Soulieres D, et al. A systematic review and Canadian consensus recommendations on the use of biomarkers in the treatment of non-small cell lung cancer. *J Thorac Oncol*. 2011;6(8):1379-1391.
30. Melosky B, Blais N, Cheema P, et al. Standardizing biomarker testing for Canadian patients with advanced lung cancer. *Curr Oncol Tor Ont*. 2018;25(1):73-82.
31. Stockley T, Souza CA, Cheema PK, et al. Evidence-based best practices for EGFR T790M testing in lung cancer in Canada. *Curr Oncol Tor Ont*. 2018;25(2):163-169.
32. Melosky B, Cheema P, Agulnik J, et al. Canadian perspectives: update on inhibition of ALK-positive tumours in advanced non-small-cell lung cancer. *Curr Oncol Tor Ont*. 2018 Oct;25(5):317-328.
33. Cheema PK, Gomes M, Banerji S, et al. Consensus recommendations for optimizing biomarker testing to identify and treat advanced EGFR-mutated non-small-cell lung cancer. *Curr Oncol Tor Ont*. 2020;27(6):321-329.
34. Bebb DG, Banerji S, Blais N, et al. Canadian consensus for biomarker testing and treatment of TRK fusion cancer in adults. *Curr Oncol Tor Ont*. 2021;28(1):523-548.
35. Ionescu DN, Stockley TL, Banerji S, et al. Consensus recommendations to optimize testing for new targetable alterations in non-small cell lung cancer. *Curr Oncol Tor Ont*. 2022;29(7):4981-4997.
36. Cutz JC, Craddock KJ, Torlakovic E, et al. Canadian anaplastic lymphoma kinase study: a model for multicenter standardization and optimization of ALK testing in lung cancer. *J Thorac Oncol*. 2014;9(9):1255-1263.
37. Torlakovic E, Albadine R, Bigras G, et al. Canadian multicenter project on standardization of programmed death-ligand 1 immunohistochemistry 22C3 laboratory-developed tests for pembrolizumab therapy in NSCLC. *J Thorac Oncol*. 2020;15(8):1328-1337.
38. Cheung CC, Smith AC, Albadine R, et al. Canadian ROS proto-oncogene 1 study (CROS) for multi-institutional implementation of ROS1 testing in non-small cell lung cancer. *Lung Cancer Amst Neth*. 2021;160:127-135.
39. Leighl NB, Kamel-Reid S, Cheema PK, et al. Multicenter validation study to implement plasma epidermal growth factor receptor T790M testing in clinical laboratories. *JCO Precis Oncol*. 2020;4:520-533.
40. Thunnissen E, Weynand B, Udovicic-Gagula D, et al. Lung cancer biomarker testing: perspective from Europe. *Transl Lung Cancer Res*. 2020;9(3):887-897.
41. Horgan D, Ciliberto G, Conte P, et al. Bringing greater accuracy to Europe's healthcare systems: the unexploited potential of biomarker testing in oncology. *Biomed Hub*. 2020;5(3):182-223.

42. Horgan D, Ciliberto G, Conte P, et al. Bringing onco-innovation to Europe's healthcare systems: the potential of biomarker testing, real world evidence, tumour agnostic therapies to empower personalised medicine. *Cancers*. 2021;13(3):583.
43. Hofman P, Rouleau E, Sabourin JC, et al. Predictive molecular pathology in non-small cell lung cancer in France: the past, the present and the perspectives. *Cancer Cytopathol*. 2020;128(9):601-610.
44. Griesinger F, Eberhardt W, Nusch A, et al. Biomarker testing in non-small cell lung cancer in routine care: analysis of the first 3,717 patients in the German prospective, observational, nation-wide CRISP registry (AIO-TRK-0315). *Lung Cancer Amst Neth*. 2021;152:174-184.
45. Salas C, Martín-López J, Martínez-Pozo A, et al. Real-world biomarker testing rate and positivity rate in NSCLC in Spain: Prospective Central Lung Cancer Biomarker Testing Registry (LungPath) from the Spanish Society of Pathology (SEAP). *J Clin Pathol*. 2022;75(3):193-200.
46. Provencio M, Cobo M, Rodriguez-Abreu D, et al. Determination of essential biomarkers in lung cancer: a real-world data study in Spain with demographic, clinical, epidemiological and pathological characteristics. *BMC Cancer*. 2022;22(1):732.
47. Steeghs EMP, Groen HJM, Schuurung E, et al. Mutation-tailored treatment selection in non-small cell lung cancer patients in daily clinical practice. *Lung Cancer Amst Neth*. 2022;167:87-97.
48. Tufman A, Redmond K, Giannopoulou A, et al. Patient perception of burden of disease and treatment preferences in non-small cell lung cancer: results from a European survey. *Lung Cancer Amst Neth*. 2022;168:59-66.
49. John T, Cooper WA, Wright G, et al. Lung cancer in Australia. *J Thorac Oncol*. 2020;15(12):1809-1814.
50. Chinese Society of Pathology, Pathology Quality Control Center; Chinese Medical Association Chinese Society of Oncology; China Anti-Cancer Association Chinese Society of Lung Cancer; Chinese Thoracic Oncology Group. Guidelines on clinical practice of molecular tests in non-small cell lung cancer in China. Article in Chinese. *Zhonghua Bing Li Xue Za Zhi*. 2021;50(4):323-332.
51. Hong DS, DuBois SG, Kummar S, et al. Larotrectinib in patients with TRK fusion-positive solid tumours: a pooled analysis of three phase 1/2 clinical trials. *Lancet Oncol*. 2020;21(4):531-540.
52. Davies KD, Lomboy A, Lawrence CA, et al. DNA-based versus RNA-based detection of MET exon 14 skipping events in lung cancer. *J Thorac Oncol*. 2019;14(4):737-741.
53. Wu YL, Tsuboi M, He J, et al. Osimertinib in resected EGFR-mutated non-small-cell lung cancer. *N Engl J Med*. 2020;383(18):1711-1723.
54. Chinese Anti-Cancer Association, Lung Cancer Study Group of Committee of Oncopathology; Chinese Society of Lung Cancer; Expert Group on PD-L1 Testing Consensus. Chinese expert consensus on standards of PD-L1 immunohistochemistry testing for non-small cell lung cancer. Article in Chinese. *Zhongguo Fei Ai Za Zhi*. 2020;23(9):733-740.
55. Felip E, Altorki N, Zhou C, et al. Adjuvant atezolizumab after adjuvant chemotherapy in resected stage IB-IIIa non-small-cell lung cancer (IMpower010): a randomised, multicentre, open-label, phase 3 trial. *Lancet*. 2021;398(10308):1344-1357.
56. Prabhash K, Advani SH, Batra U, et al. Biomarkers in non-small cell lung cancers: Indian consensus guidelines for molecular testing. *Adv Ther*. 2019;36(4):766-785.
57. Motoi N, Yatabe Y. Lung cancer biomarker tests: the history and perspective in Japan. *Transl Lung Cancer Res*. 2020;9(3):879-886.
58. Yatabe Y, Sunami K, Goto K, et al. Multiplex gene-panel testing for lung cancer patients. *Pathol Int*. 2020;70(12):921-931.
59. Ninomiya K, Teraoka S, Zenke Y, et al. Japanese Lung Cancer Society guidelines for stage IV NSCLC with EGFR mutations. *JTO Clin Res Rep*. 2021;2(1):100107.
60. Mitsudomi T, Yatabe Y, Akita H, et al.; Biomarker Committee, the Japan Lung Cancer Society. *Guidance for ALK Gene Testing in Lung Cancer Patients*. Version 1.2. Japan Lung Cancer Society;2011. <https://www.haigan.gr.jp/uploads/files/photos/641.pdf>.
61. Yatabe Y, Goto K, Matsumoto S, et al. METex14 skipping testing guidance for lung cancer patients: the guidance from the Biomarker Committee, the Japan Lung Cancer Society. *Haigan*. 2021;61(5):361-370. <https://doi.org/10.2482/haigan.61.361>.

62. Slavik T, Asselah F, Fakhruddin N, et al. Diagnosis and predictive molecular analysis of non-small-cell lung cancer in the Africa-Middle East region: challenges and strategies for improvement. *Clin Lung Cancer*. 2014;15(6):398-404.
63. Niyibizi BA, Muhizi E, Ndoli DA, et al. Lung cancer in Rwanda. *J Thorac Oncol*. 2022;17(9):1074-1077.
64. van Eeden R, Tunmer M, Geldenhuys A, Nayler S, Rapoport BL. Lung cancer in South Africa. *J Thorac Oncol*. 2020;15(1):22-28.
65. Patel K, Strother RM, Ndiangui F, et al. Development of immunohistochemistry services for cancer care in western Kenya: implications for low- and middle-income countries. *Afr J Lab Med*. 2016;5(1):187.

Gene Structure and Function

The epidermal growth factor receptor (EGFR/ErbB1), with ErbB2 (HER2), ErbB3 (HER3), and ErbB4 (HER4), is a member of the *ERBB* receptor family. These receptor family members signal as homo- and heterodimers upon ligand binding, with the exception of HER2, which participates in dimerization in absence of a known ligand.¹ The EGFR protein is a transmembrane receptor tyrosine kinase that comprises an extracellular domain (ECD), transmembrane domain (TMD), juxtamembrane domain (JMD), tyrosine kinase domain (TKD), and C-terminal tail.²

Upon binding to the various ligands, including epidermal growth factor (EGF), transforming growth factor α (TGF α), amphiregulin (AREG), and so forth,² conformational changes in the ECD permit receptor dimerization.³ This dimerization induces asymmetric interaction between the C- and N-lobes of the TKDs, resulting in autophosphorylation of the receiver tyrosine kinase and of phosphorylation sites on the C-terminal tail. The JMD has been demonstrated to stabilize this interaction via a juxtamembrane-latching mechanism (Figure 7-1).^{4,5} Within the TKD, this phosphorylation induces conformational changes in the positioning of the α C-helix and, consequently, the positioning of the activation loop, which adopts an open conformation permissive of substrate binding.⁶ Within the C-terminal tail, phosphorylated docking sites bind additional mediators of downstream signaling. The activation of EGFR ultimately drives the activation of multiple downstream signaling pathways, including the mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)/AKT, and Janus kinase (JAK)/signal transducers and activators of transcription (STAT) pathways, which promote cell growth and survival.⁷

Epidemiologic, Clinical, and Histologic Characteristics

The global incidence of *EGFR* mutations varies, ranging from approximately 12% of lung adenocarcinoma in European populations, to 15% in North America to 49% in Asian

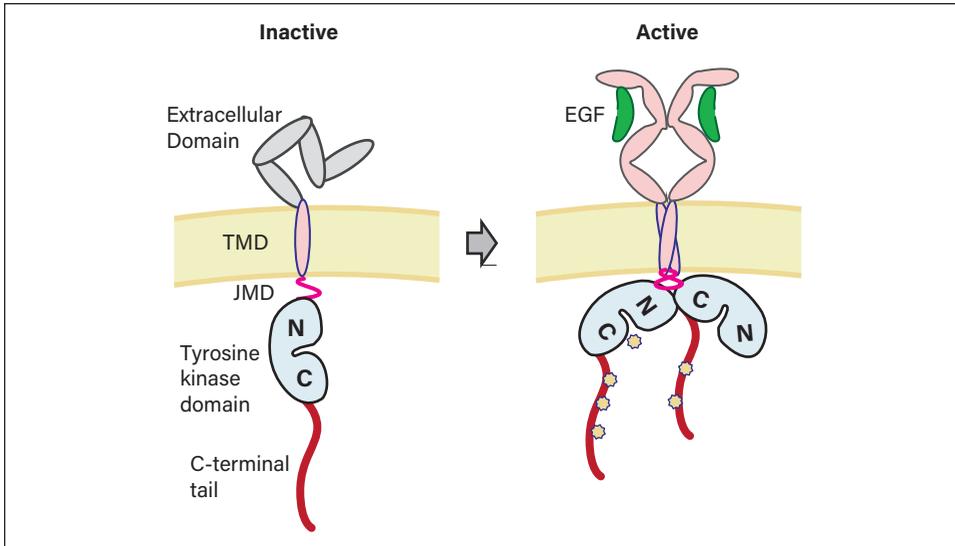


Figure 7-1. EGFR protein structure in the inactive and active conformations. Binding of the ligand EGF induces conformational changes permitting protein dimerization. Interaction between the tyrosine kinase domains permits kinase catalytic activation as well as docking of mediators of downstream signaling at the C-terminal tail. Abbreviations: EGF = epidermal growth factor; C = C-lobe; JMD = juxtamembrane domain; N = N-lobe; TMD = transmembrane domain. (Adapted from Jura et al.⁴)

populations.⁸ Prevalence is highest in women, those without history of tobacco use, and in younger patients.⁹ Testing remains important among all patients with adenocarcinoma, as *EGFR*-activating mutations are detectable among all lung cancer patient populations. For example, in 1 case series while 52% of patients with no prior history of tobacco use were found to have *EGFR*-activating mutations, *EGFR* mutations were additionally present in 21% of those with former tobacco use and 6% of those with ongoing tobacco use.¹⁰ Similarly, while *EGFR* mutations are uncommon in non-small cell lung carcinoma (NSCLC) with squamous histology, they are still detected with low prevalence, particularly in those with other classic demographic features (eg, never-smokers) or with mixed adenosquamous histology.¹¹

For the time being, the molecular epidemiology of *EGFR* mutations should be interpreted with great caution, as the frequencies are dependent on the different testing strategies, which need to take into account the geographic heterogeneity of *EGFR* mutations. For example, using real-time polymerase chain reaction (PCR) might be a sensible approach in high-incidence areas, even if some uncommon and compound mutations will be missed.¹² Conversely, the use of next-generation sequencing (NGS) may change our understanding of the frequency of uncommon mutations.¹³

Testing Strategies

Everyone involved in lung cancer biomarker testing needs to be aware of the performance metrics of the different techniques that are commonly used to identify *EGFR* mutations (Table 7-1).^{14,15} The following section discusses each of the types of testing available to detect *EGFR* mutations. Direct (or Sanger) sequencing and rapid PCR approaches were mainly used when *EGFR* mutations were initially discovered.^{16,17} Over the past 2 decades, initially, commercial real-time PCR assays were developed, followed by the clinical application of NGS panels.¹⁸⁻²⁰ Accordingly, those are the 2 most used techniques for detecting *EGFR* mutations, and

Table 7-1. Methods for Detecting *EGFR* Mutations

Technique	Analytical sensitivity	Diagnostic sensitivity	Precise annotation of variants	Allele frequency reported	Input DNA	Cost	Turn-around time
PCR and direct sequencing	Lowest	Excellent	Yes	No	High	Lowest	3-4 days
PCR and pyrosequencing	Variable	Intermediate	Sometimes	No	High	Low	3-4 days
Real-time PCR	High	Intermediate	Sometimes	No	Low	Low	Hours to 1-2 days
Digital PCR	Highest	Low	Yes	No	Lowest	Low	Hours to 1-2 days
NGS-targeted amplicon based	Variable (high)	Variable (high)	Yes	Yes	Low	Intermediate	1-2 to 10 days
NGS-targeted hybridization capture	Variable (high)	Variable (high)	Yes	Yes	High	Intermediate	15-20 days
NGS-whole exome	Variable	Excellent	Yes	Yes	High	High	Weeks
NGS-whole genome	Variable	Excellent	Yes	Yes	High	Highest	Weeks

Abbreviations: NGS = next-generation sequencing; PCR = polymerase chain reaction. Adapted from Pao et al¹⁴; Pennell et al.¹⁵

there are several US Food and Drug Administration (FDA)-approved real-time PCR assays and NGS panels. Most national and international guidelines recommend comprehensive biomarker testing, and therefore there is increased adoption of NGS-based *EGFR* testing.^{21,22}

The analytical sensitivity (often referred to as just *sensitivity*) is the limit of detection, the smallest percentage of tumor cells able to be detected in a given sample. The diagnostic sensitivity relates to the comprehensiveness of the assay or the percentage of all mutations described for the gene detectable by the given assay.¹⁵ Along those lines, some of these assays cannot discriminate between variants (ie, the results are presented as “mutation detected” vs “mutation not detected” or “exon 19 deletion, not otherwise specified”), and only NGS panels offer a reliable estimate of the allele fraction. Success rates are often related to the amount of DNA present in the specimen, as hybridization-capture NGS panels require much more input DNA than amplicon-based NGS or real-time PCR assays. Finally, it is worth emphasizing that ultrarapid real-time PCR kits or even fully automated NGS workflows, with minimal hands-on time, can provide results in 1 to 2 days.²³⁻²⁵

Type of *EGFR* Alterations and Clinical Implications

To better understand testing strategies and clinical implications of *EGFR* mutations in patients with NSCLCs, it is useful to divide them into the following 5 categories.

1. Common Mutations

The most common activating *EGFR* mutations are in-frame deletions of exon 19 (amino acid residues 747-750) and L858R point mutations of exon 21, representing 80% to 85% of all *EGFR* mutations.²¹ NSCLC patients with these mutations respond well to *EGFR* tyrosine kinase inhibitors (TKIs), and *EGFR* TKIs are the current standard of care in the first-line treatment

of advanced/metastatic disease. First-generation EGFR TKIs, including gefitinib and erlotinib, as well as second-generation EGFR TKIs, such as afatinib or dacomitinib, showed statistically significant longer progression-free survival (PFS) compared with platinum-based chemotherapy in the first-line setting.²⁶⁻³⁰ In a phase 2 trial, afatinib reported a longer PFS compared with gefitinib, but without difference in the overall survival (OS) rate.^{31,32} Similarly, in a phase 3 trial, dacomitinib, another second-generation EGFR TKI, reported a significant improvement in PFS but not in OS (p value required 0.025, p value achieved 0.04) compared with gefitinib. However, in contrast to the afatinib trial, in the dacomitinib-trial, patients with brain metastases were not allowed.³³⁻³⁵ Although second-generation EGFR TKI reported better outcome, these EGFR TKIs are associated with higher incidence of toxicities, including skin rash and diarrhea, compared with gefitinib.^{31,33}

Osimertinib, a third-generation EGFR TKI, was initially developed for patients with acquired resistant T790M mutation after failure on prior first- or second-generation EGFR TKI in the phase 1 AURA and the phase 3 AURA3 study.³⁶⁻³⁸ With the encouraging efficacy in the treatment-naïve patient cohort in the AURA study,³⁹ osimertinib was evaluated for use in the first-line setting in the FLAURA study. In this study, treatment-naïve patients with advanced/metastatic *EGFR* exon 19 deletion and exon 21 L858R mutation were randomized to osimertinib versus erlotinib or gefitinib. The study showed that osimertinib significantly improved the PFS, the primary endpoint, and follow-up OS analysis also showed benefit of osimertinib.^{40,41} In addition, osimertinib showed improved central nervous system (CNS) activity compared with first-generation EGFR TKI.⁴² Currently, osimertinib is the preferred treatment option in the first-line treatment of advanced/metastatic disease. However, third-generation EGFR TKIs aumolertinib,⁴³ furmonertinib,⁴⁴ and lazertinib,⁴⁵ have already reported longer PFS rates than gefitinib in first-line settings in patients with *EGFR*-mutant advanced NSCLC. In addition, in the first-line setting, the MARIPOSA trial (NCT04487080) evaluates lazertinib plus amivantamab (an EGFR-MET bispecific monoclonal antibody) combination; the FLAURA2 trial (NCT04035486) evaluates the benefit of adding platinum-based chemotherapy to osimertinib; and the ECOG-ACRIN 5182 phase 3 study (NCT04181060) evaluates osimertinib with or without bevacizumab. Recently, an adjuvant clinical trial with osimertinib showed markedly improved disease-free survival compared with placebo in patients with surgically resected stage IB to IIIA NSCLC with *EGFR* mutation-positive disease.⁴⁶

2. Uncommon (or Atypical) Mutations

Uncommon or atypical mutations include all mutations except exon 19 deletions, L858R, and T790M mutations. The most frequently identified are G719X in exon 18 (0.9%-4.8% of all *EGFR* mutations), L861X in exon 21 (0.5%-3.5% of all *EGFR* mutations), and S768I in exon 20 (0.5%-2.5% of all *EGFR* mutations).⁴⁷ These are rare and not included in some more limited panels, so NGS may be required to identify them; it is important to look for these uncommon mutations because they can be sensitive to EGFR TKIs. These atypical *EGFR* mutations show clinical response to gefitinib or erlotinib; however, the overall response rate (ORR) and the PFS rate is lower than in common *EGFR* mutations.^{48,49} Afatinib showed activity for G719X, L861Q, and S768I mutations and has received FDA approval.^{50,51} Osimertinib also showed

clinically meaningful activity for uncommon mutations in a single-arm phase 2 study,⁵² as well as in a real-life retrospective study.⁵³

Less common alterations in *EGFR* that can carry clinical implications include kinase domain duplications (KDDs) and oncogenic *EGFR* fusions. Patients with *EGFR*-KDD showed clinical response to EGFR TKIs with various durations of response.⁵⁴⁻⁵⁶ KDDs are a rare activating genomic alteration found across the *ERBB* family members. *EGFR*-KDD result from the tandem in-frame duplication of the *EGFR* TKD (exons 18-25). These occur in up to 1.4% of NSCLC cases and are additionally found in other solid tumors, most commonly glioma and gastrointestinal (GI) malignancies. *EGFR*-KDD produce a protein that forms constitutively active intramolecular TKD dimers absent the need for EGF ligand binding.⁵⁷ The oncogenic *EGFR* fusions are another rare driver alteration reported in 0.5% to 0.13% of NSCLC cases with variable reported *EGFR* fusion partners, the most common of which is the *EGFR*-*RAD51* fusion.⁵⁸ These fusions are able to drive dimerization and oncogenic signaling and may impact EGFR protein degradation.⁵⁹ Several case series have demonstrated activity of EGFR TKI therapy against activating EGFR fusions.^{59,60}

Historically, *EGFR* amplification and overexpression have been evaluated as biomarkers for response to *EGFR*-targeted therapies. While EGFR overexpression has not offered a reliable biomarker for activity of EGFR TKI therapies,⁶¹ the acquisition of *EGFR* amplification or overexpression at acquired resistance (AR) to EGFR TKI therapy for management of the classically actionable *EGFR* driver mutations remains an area of active clinical investigation.^{62,63}

3. *EGFR* Exon 20 Insertion

EGFR exon 20 insertions are the largest subset of atypical mutations (0.8%-4.2% of all *EGFR* mutations)^{12,13,47} that are now actionable but very heterogenous, from a biological and clinical point of view, thus NGS is required to detect the full range of exon 20 insertions and provide a precise annotation of the variant.²¹ Patients with *EGFR* exon 20 insertion do not respond well to conventional EGFR TKIs except perhaps the FQEA subtype.^{50,64} Poziotinib is an irreversible EGFR TKI and showed clinical activity in patients with *EGFR* exon 20 insertion, but the high rate of toxicities, such as skin rash and diarrhea, resulted in frequent dose interruption and reduction leading to relatively short PFS duration.^{65,66} Recently, in platinum-refractory NSCLC, a novel EGFR TKI mobocertinib⁶⁷ and amivantamab⁶⁸ showed improved ORR and PFS rate compared with historical control. Therefore, FDA has granted accelerated approval to mobocertinib and amivantamab for patients with metastatic NSCLC and *EGFR* exon 20 insertion mutations, whose disease progressed on or after platinum-based chemotherapy. Other drugs, such as sunvozertinib, CLN081, and furmonertinib, are being tested in this population with initial promising results.⁶⁹⁻⁷¹

4. Compound Mutations

Compound mutations have also been referred as *double*, *complex*, or *multiple*, because by definition these are multiple independent *EGFR* mutations in the same sample, accounting for 4% to 26% of all *EGFR* mutations. Any type of combination can be found: common and common (10%-20%), common and uncommon (30%-50%), 2 uncommon (25%-40%), and also the presence of a common or uncommon mutation with a de novo T790M mutation (10%-50%).⁷²

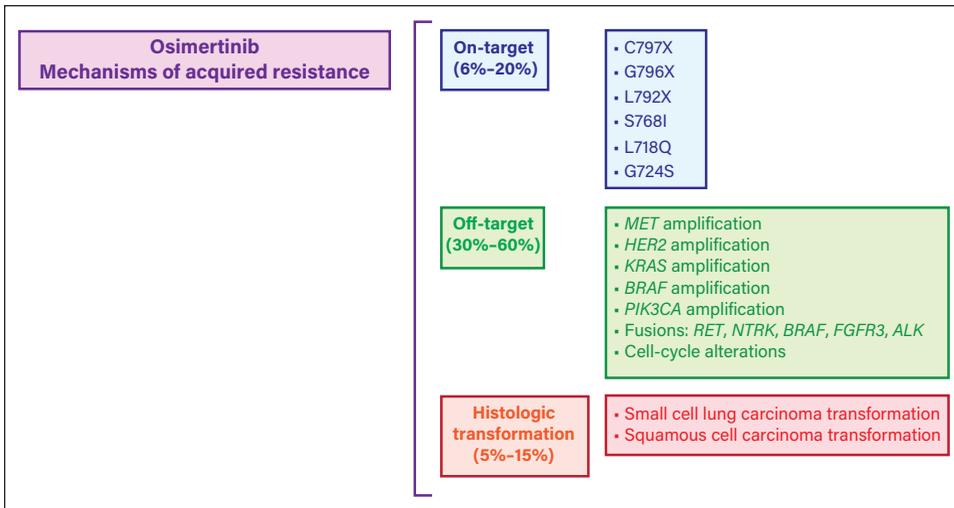


Figure 7-2. Mechanisms of acquired resistance to osimertinib

5. Resistance Mutations

For patients with *EGFR*-mutated lung cancer initially treated with first- and second-generation *EGFR* TKIs, 50% to 60% of cases may develop the AR *EGFR* mutation T790M in exon 20 that confers sensitivity to osimertinib efficacy in this setting.³⁷

For those patients initially treated with osimertinib in the first-line setting, the mechanisms of AR to osimertinib are heterogeneous and complex⁷³ and can be divided into 3 major categories (Figure 7-2): on-target resistance (10%-20%), off-target resistance through activation of bypass oncogenic pathways (30%-60%), and histologic transformation (5%-15%). Characterization of these mechanisms may reveal actionable insights for selecting subsequent treatments or enrolling patients in clinical trials. Therefore, re-biopsy (liquid vs tissue vs both) is recommended at disease progression.²¹ Of note, liquid biopsy will not capture histologic transformation as an AR mechanism. However, in up to half of patients, the mechanisms of AR on osimertinib remains unknown, and it could be related to suboptimal TKI plasma concentrations.⁷⁴ In a recent prospective phase 2 cohort (ELIOS study), only 39% of patients had paired tissue biopsies at the time of progression on osimertinib. In this study, on-target resistances were reported in 15% of cases, and a bypass mechanism occurred in 17% of cases. These data highlight the challenges of obtaining post-progression tissue biopsies and the need for more comprehensive noninvasive testing methods.⁷⁵

Post-Osimertinib On-Target Resistance

Osimertinib selectively blocks mutated *EGFR* by irreversibly binding to its C797 residue in exon 20. Mutations in this spot are the most common *EGFR*-dependent mechanisms of AR, usually a substitution to serine, leading to the C797S mutation. The incidence of C797X in the clinical setting differs depending on the treatment line of osimertinib (7% in first-line and 15%-22% in second-line settings).⁷⁶⁻⁷⁸ In second-line settings, to overcome osimertinib-resistant NSCLC with T790M-positive and acquired C797X-mutation in *cis* cases (same allele, 66% of cases), fourth-generation *EGFR* TKIs have been developed, such as EAI045, JBJ-04-1252, BBT-176, and BLU-945, which have reported preclinical activity.⁷⁹⁻⁸¹ In the phase 1 SYMPHONY study (NCT04862780), BLU-945 plus osimertinib has reported dose-dependent clinical activity in

11 patients with EGFR/T790M/C797S-positive osimertinib-resistant NSCLC. Similarly, in second-line settings for those patients with acquired C797X mutation and T790M mutation in *trans* cases (different allele, 34% of cases), preclinical data and some case reports support the combination of first- or second-generation EGFR TKI plus osimertinib.^{73,82} Finally, the use of first- or second-generation EGFR TKI after osimertinib failure in the first-line setting in tumors with C797X mutation in the absence of coexisting T790M mutation could be an option.⁷³ In this setting, preclinical models suggest that OBX02-011 and BLU-701 may be a promising new EGFR TKI to overcome C797S-mediated resistance in NSCLC.⁸³ Indeed, the BLU-701 reported to be a brain-penetrant drug ($K_{p_{uu}} > 0.9$).⁸³ However, clinical evidence of all the preceding strategies remains limited, and platinum-based chemotherapy with or without immune checkpoint inhibitors and bevacizumab are a standard second-line treatment option.^{83,84} However, the role of chemotherapy plus immunotherapy in this setting remains controversial after the results of the CheckMate 722 trial, which did not report PFS and OS with this strategy compared with chemotherapy alone.⁸⁶ Recently, antibody-drug conjugated (ADC) agents, such as the anti-HER3 patritumab deruxtecian⁸⁷ and the anti-trophoblast cell surface antigen 2 (TROP2) datopotamab deruxtecian,⁸⁸ demonstrated clinical activity spanning known and unknown EGFR TKI resistance mechanisms, suggesting these drugs as potential agnostic treatment strategies when biomarker-driven approaches are not feasible or in absence of specific AR mechanism.

Off-Target Resistance

MET amplification is the most common off-target mechanism of AR to osimertinib (15% and 20% in first- and second-line osimertinib, respectively, both detected in liquid biopsies, which could underestimate the real incidence).^{76,77} Of note, different diagnostic tools and inconsistent definitions of *MET* amplification used across various clinical trials has confounded the use of *MET* inhibitors in clinical practice. Currently, the most widely adopted definition of *MET* amplification is the presence of a *MET* gene copy number of 5 or more or a *MET/CEP7* ratio of 2 or more.⁸⁹ The *MET* amplification resistance triggers EGFR-independent phosphorylation of ERBB3 and downstream activation of PI3K/AKT pathway, providing a bypass track even in the presence of an EGFR inhibitor.⁹⁰

Traditionally, this mechanism of AR has been clinically addressed through the addition of a *MET* TKI to the EGFR TKI, and the efficacy of this combination is firmly established in several phase 1 and 2 clinical trials⁹¹⁻⁹⁴ and recently confirmed in the INSIGHT2 and SAVANNAH trials.^{95,96} Indeed, initial data suggests that this personalized strategy improves the outcome compared with standard chemotherapy⁸⁹; however, it must be confirmed in randomized phase 3 clinical trials ongoing: GEOMETRY-E (NCT04816214), SAFFRON (NCT05261399), and MARIPOSA-2 (NCT04988295). Similarly, amivantamab plus lazertinib reported clinical activity in osimertinib-relapsed NSCLC. The benefit was similar regardless of previous treatment with chemotherapy, and responses occurred in patients with and without identified EGFR/*MET* resistance, but the outcome was of special interest in patients with a positive immunohistochemistry (IHC) score (EGFR + *MET* score ≥ 400).^{63,97} Finally for osimertinib-relapsed NSCLC, the combination of osimertinib and telisotuzuamb (an ADC anti-*MET*) reported activity in tumors with *MET* overexpression ($\geq 25\%$ tumor cells at 3+ intensity).⁹⁸ Although *MET* expression is an easy biomarker, patient classification by IHC could vary in up to 40% among different tumor areas.⁹⁹

The efficacy of osimertinib with specific TKIs according to the identified pattern of resistance has been also explored with other mechanisms of bypass resistance, such as *RET*-fusion^{100,101} and *BRAF V600E* mutation,^{102,103} although the evidence is still limited. The ORCHARD trial (NCT03944772) prospectively assesses the role of the biomarker-driven strategy at progression with different combinational approaches along with osimertinib. Of note, patritumab deruxtecxa⁸⁷ reported efficacy across various EGFR TKI AR mechanisms, including bypass mechanisms. Finally, other potential agnostic treatment options in T790M-negative tumors are the combination of osimertinib either with necitumumab (a monoclonal antibody anti-EGFR)¹⁰⁴ or selumetinib (MAPK kinase/extracellular signal-regulated kinase [MEK/ERK] inhibitor).¹⁰⁵

Histologic Transformation

The histologic transformation mechanism of AR is associated with poor clinical outcomes. Tumors with baseline *TP53* or *Rb* mutation and hypermutated APOBEC signatures have a greater risk of small cell lung carcinoma (SCLC) transformation,^{106,107} as well as that with acquired *TERT* amplification.¹⁰⁸ Tumors with SCLC-transformation retain *EGFR* mutation,¹⁰⁹ which suggests that these were not independent de novo cancers. Although platinum-etoposide chemotherapy is the standard treatment, the efficacy is limited.¹¹⁰ Squamous cell carcinoma transformation has also been reported in 15% of osimertinib-relapsed tumors regardless of line of treatment.⁶² Tumors with squamous transformation exhibited considerable genomic complexity, and it remains unknown whether the best treatment approach should be chemotherapy with or without immune checkpoint inhibitors.

Role of Liquid Biopsy

In daily practice, because of the location or size of the progressive disease, not all patients who start treatment with first-, second-, or third-generation EGFR TKIs are suitable candidates for new tissue biopsies at the time of progression, which can delay treatment initiation. Moreover, tumor heterogeneity is a well-recognized issue that makes a single tissue biopsy in a metastatic site not representative of the entire genomic landscape of the tumor at progression.¹¹¹⁻¹¹³ Therefore, liquid biopsies have been established as good tools for genomic profiling at baseline as well as the time of AR. Currently, most of the mechanisms of AR to first-line osimertinib have been reported based on liquid biopsy assessment.⁷⁶ (See also Chapter 4.)

References

1. Du Z, Lovly CM. Mechanisms of receptor tyrosine kinase activation in cancer. *Mol Cancer*. 2018;17(1):58. doi:10.1186/s12943-018-0782-4.
2. Purba ER, Saita EI, Maruyama IN. Activation of the EGF receptor by ligand binding and oncogenic mutations: the “rotation model.” *Cells*. 2017;6(2):13. doi:10.3390/cells6020013.
3. Burgess AW, Cho HS, Eigenbrot C, et al. An open-and-shut case? recent insights into the activation of EGF/ErbB receptors. *Mol Cell*. 2003;12(3):541-552. doi:10.1016/S1097-2765(03)00350-2.
4. Jura N, Endres NF, Engel K, et al. Mechanism for activation of the EGF receptor catalytic domain by the juxtamembrane segment [published correction appears in *Cell*. 2009;138(3):604]. *Cell*. 2009;137(7):1293-1307. doi:10.1016/j.cell.2009.04.025.
5. Red Brewer M, Choi SH, Alvarado D, et al. The juxtamembrane region of the EGF receptor functions as an activation domain. *Mol Cell*. 2009;34(6):641-651. doi:10.1016/j.molcel.2009.04.034.

6. Zhang X, Gureasko J, Shen K, Cole PA, Kuriyan J. An allosteric mechanism for activation of the kinase domain of epidermal growth factor receptor. *Cell*. 2006;125(6):1137-1149. doi:10.1016/j.cell.2006.05.013.
7. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol*. 2001;2(2):127-137. doi:10.1038/35052073.
8. Melosky B, Kambartel K, Häntschel M, et al. Worldwide prevalence of epidermal growth factor receptor mutations in non-small cell lung cancer: a meta-analysis. *Mol Diagn Ther*. 2022;26(1):7-18. doi:10.1007/s40291-021-00563-1.
9. Zhang YL, Yuan JQ, Wang KF, et al. The prevalence of EGFR mutation in patients with non-small cell lung cancer: a systematic review and meta-analysis. *Oncotarget*. 2016; 7(48):78985-78993. doi:10.18632/oncotarget.12587.
10. D'Angelo SP, Pietanza MC, Johnson ML, et al. Incidence of EGFR exon 19 deletions and L858R in tumor specimens from men and cigarette smokers with lung adenocarcinomas. *J Clin Oncol*. 2011;29(15):2066-2070. doi:10.1200/JCO.2010.32.6181.
11. Ho HL, Kao HL, Yeh YC, Chou TY. The importance of EGFR mutation testing in squamous cell carcinoma or non-small cell carcinoma favor squamous cell carcinoma diagnosed from small lung biopsies. *Diagn Pathol*. 2019;14(1):59. doi:10.1186/s13000-019-0840-2.
12. Lin HM, Yin Y, Crossland V, Wu Y, Ou SI. EGFR testing patterns and detection of EGFR exon 20 insertions in the United States. *JTO Clin Res Rep*. 2022;3(3):100285. doi:10.1016/j.jtocrr.2022.100285.
13. Passaro A, Mok T, Peters S, Popat S, Ahn MJ, de Marinis F. Recent advances on the role of EGFR tyrosine kinase inhibitors in the management of NSCLC with uncommon, non exon 20 insertions, EGFR mutations. *J Thorac Oncol*. 2021;16(5):764-773. doi:10.1016/j.jtho.2020.12.002.
14. Pao W, Ladanyi M. Epidermal growth factor receptor mutation testing in lung cancer: searching for the ideal method. *Clin Cancer Res*. 2007;13(17):4954-4955. doi:10.1158/1078-0432.CCR-07-1387.
15. Pennell NA, Arcila ME, Gandara DR, West H. Biomarker testing for patients with advanced non-small cell lung cancer: real-world issues and tough choices. *Am Soc Clin Oncol Educ Book*. 2019;39:531-542. doi:10.1200/EDBK_237863.
16. Pan Q, Pao W, Ladanyi M. Rapid polymerase chain reaction-based detection of epidermal growth factor receptor gene mutations in lung adenocarcinomas. *J Mol Diagn*. 2005;7(3):396-403. doi:10.1016/S1525-1578(10)60569-7.
17. Conde E, Angulo B, Tang M, et al. Molecular context of the EGFR mutations: evidence for the activation of mTOR/S6K signaling. *Clin Cancer Res*. 2006;12(3 pt 1):710-717. doi:10.1158/1078-0432.CCR-05-1362.
18. Angulo B, Conde E, Suárez-Gauthier A, et al. A comparison of EGFR mutation testing methods in lung carcinoma: direct sequencing, real-time PCR and immunohistochemistry. *PLoS One*. 2012;7(8):e43842. doi:10.1371/journal.pone.0043842.
19. Lopez-Rios F, Angulo B, Gomez B, et al. Comparison of molecular testing methods for the detection of EGFR mutations in formalin-fixed paraffin-embedded tissue specimens of non-small cell lung cancer. *J Clin Pathol*. 2013;66(5):381-385. doi:10.1136/jclinpath-2012-201240.
20. Cheng DT, Mitchell TN, Zehir A, et al. Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): a hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. *J Mol Diagn*. 2015;17(3):251-264. doi:10.1016/j.jmoldx.2014.12.006.
21. Passaro A, Leigh N, Blackhall F, et al. ESMO expert consensus statements on the management of EGFR mutant non-small-cell lung cancer. *Ann Oncol*. 2022;33(5):466-487. doi:10.1016/j.annonc.2022.02.003.
22. Hess LM, Krein PM, Haldane D, Han Y, Sireci AN. Biomarker testing for patients with advanced/metastatic nonsquamous NSCLC in the United States of America, 2015 to 2021. *JTO Clin Res Rep*. 2022;3(6):100336. doi:10.1016/j.jtocrr.2022.100336.
23. Momeni-Boroujeni A, Salazar P, Zheng T, et al. Rapid EGFR mutation detection using the Idylla platform: single-institution experience of 1200 cases analyzed by an in-house developed pipeline and comparison with concurrent next-generation sequencing results. *J Mol Diagn*. 2021;23(3):310-322. doi:10.1016/j.jmoldx.2020.11.009.
24. Bormann Chung C, Lee J, Barritault M, et al. Evaluating targeted next-generation sequencing assays and reference materials for NTRK fusion detection. *J Mol Diagn*. 2022;24(1):18-32. doi:10.1016/j.jmoldx.2021.09.008.

25. Zehir A, Benayed R, Shah RH, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients [published correction appears in *Nat Med*. 2017 Aug 4;23 (8):1004]. *Nat Med*. 2017;23(6):703-713. doi:10.1038/nm.4333.
26. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med*. 2009;361(10):947-957. doi:10.1056/NEJMoa0810699.
27. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med*. 2010;362(25):2380-2388. doi:10.1056/NEJMoa0909530.
28. Zhou C, Wu YL, Chen G, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. *Lancet Oncol*. 2011;12(8):735-742. doi:10.1016/S1470-2045(11)70184-X.
29. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol*. 2012;13(3):239-246. doi:10.1016/S1470-2045(11)70393-X.
30. Yang JCH, Wu YL, Schuler M, et al. Afatinib versus cisplatin-based chemotherapy for EGFR mutation-positive lung adenocarcinoma (LUX-Lung 3 and LUX-Lung 6): analysis of overall survival data from two randomised, phase 3 trials. *Lancet Oncol*. 2015;16(2):141-151. doi:10.1016/S1470-2045(14)71173-8.
31. Park K, Tan EH, O'Byrne K, et al. Afatinib versus gefitinib as first-line treatment of patients with EGFR mutation-positive non-small-cell lung cancer (LUX-Lung 7): a phase 2B, open-label, randomised controlled trial. *Lancet Oncol*. 2016;17(5):577-589. doi:10.1016/S1470-2045(16)30033-X.
32. Paz-Ares L, Tan EH, O'Byrne K, et al. Afatinib versus gefitinib in patients with EGFR mutation-positive advanced non-small-cell lung cancer: overall survival data from the phase IIB LUX-Lung 7 trial. *Ann Oncol*. 2017;28(2):270-277. doi:10.1093/annonc/mdw611.
33. Wu YL, Cheng Y, Zhou X, et al. Dacomitinib versus gefitinib as first-line treatment for patients with EGFR-mutation-positive non-small-cell lung cancer (ARCHER 1050): a randomised, open-label, phase 3 trial. *Lancet Oncol*. 2017;18(11):1454-1466. doi:10.1016/S1470-2045(17)30608-3.
34. Mok TS, Cheng Y, Zhou X, et al. Improvement in overall survival in a randomized study that compared dacomitinib with gefitinib in patients with advanced non-small-cell lung cancer and EGFR-activating mutations. *J Clin Oncol*. 2018;36(22):2244-2250. doi:10.1200/JCO.2018.78.7994.
35. Mok TS, Cheng Y, Zhou X, et al. Updated overall survival in a randomized study comparing dacomitinib with gefitinib as first-line treatment in patients with advanced non-small-cell lung cancer and EGFR-activating mutations. *Drugs*. 2021;81(2):257-266. doi:10.1007/s40265-020-01441-6.
36. Jänne PA, Yang JCH, Kim DW, et al. AZD9291 in EGFR inhibitor-resistant non-small-cell lung cancer. *N Engl J Med*. 2015;372(18):1689-1699. doi:10.1056/NEJMoa1411817.
37. Mok TS, Wu YL, Ahn MJ, et al. Osimertinib or platinum-pemetrexed in EGFR T790M-positive lung cancer. *N Engl J Med*. 2017;376(7):629-640. doi:10.1056/NEJMoa1612674.
38. Papadimitrakopoulou VA, Mok TS, Han JY, et al. Osimertinib versus platinum-pemetrexed for patients with EGFR T790M advanced NSCLC and progression on a prior EGFR-tyrosine kinase inhibitor: AURA3 overall survival analysis. *Ann Oncol*. 2020;31(11):1536-1544. doi:10.1016/j.annonc.2020.08.2100.
39. Ramalingam SS, Yang JCH, Lee CK, et al. Osimertinib as first-line treatment of EGFR mutation-positive advanced non-small-cell lung cancer. *J Clin Oncol*. 2018;36(9):841-849. doi:10.1200/JCO.2017.74.7576.
40. Soria JC, Ohe Y, Vansteenkiste J, et al. Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. *N Engl J Med*. 2018;378(2):113-125. doi:10.1056/NEJMoa1713137.
41. Ramalingam SS, Vansteenkiste J, Planchard D, et al. Overall survival with osimertinib in untreated, EGFR-mutated advanced NSCLC. *N Engl J Med*. 2020;382(1):41-50. doi:10.1056/NEJMoa1913662.
42. Reungwetwattana T, Nakagawa K, Cho BC, et al. CNS response to osimertinib versus standard epidermal growth factor receptor tyrosine kinase inhibitors in patients with untreated EGFR-mutated advanced non-small-cell lung cancer. *J Clin Oncol*. 2018;JCO2018783118. doi:10.1200/JCO.2018.78.3118.
43. Lu S, Dong X, Jian H, et al. AENEAS: a randomized phase III trial of aumolertinib

- versus gefitinib as first-line therapy for locally advanced or metastatic non-small-cell lung cancer with *EGFR* exon 19 deletion or L858R mutations. *J Clin Oncol*. 2022;40(27):3162-3171. doi:10.1200/JCO.21.02641.
44. Shi Y, Chen G, Wang X, et al. Furmonertinib (AST2818) versus gefitinib as first-line therapy for Chinese patients with locally advanced or metastatic EGFR mutation-positive non-small-cell lung cancer (FURLONG): a multicentre, double-blind, randomised phase 3 study. *Lancet Respir Med*. 2022;10(11):1019-1028. doi:10.1016/S2213-2600(22)00168-0.
 45. Cho BC, Ahn M-J, Kang JH, et al. LBA7: a randomized, double-blind, multinational phase III study to assess the efficacy and safety of lazertinib versus gefitinib in the first-line treatment of patients with EGFR mutation (EGFRm), advanced NSCLC (LASER301; NCT04248829). *Ann Oncol*. 2022;33:S1560. <https://doi.org/10.1016/j.annonc.2022.10.349>.
 46. Wu YL, Tsuboi M, He J, et al. Osimertinib in resected EGFR-mutated non-small-cell lung cancer. *N Engl J Med*. 2020;383(18):1711-1723. doi:10.1056/NEJMoa2027071.
 47. John T, Taylor A, Wang H, et al. Uncommon EGFR mutations in non-small-cell lung cancer: a systematic literature review of prevalence and clinical outcomes. *Cancer Epidemiol*. 2022;76:102080. doi:10.1016/j.canep.2021.102080.
 48. Keam B, Kim DW, Park JH, et al. Rare and complex mutations of epidermal growth factor receptor, and efficacy of tyrosine kinase inhibitor in patients with non-small cell lung cancer. *Int J Clin Oncol*. 2014;19(4):594-600. doi:10.1007/s10147-013-0602-1.
 49. Janning M, Süptitz J, Albers-Leischner C, et al. Treatment outcome of atypical EGFR mutations in the German National Network Genomic Medicine Lung Cancer (nNGM). *Ann Oncol*. 2022;33(6):602-615. doi:10.1016/j.annonc.2022.02.225.
 50. Yang JCH, Sequist LV, Geater SL, et al. Clinical activity of afatinib in patients with advanced non-small-cell lung cancer harbouring uncommon EGFR mutations: a combined post-hoc analysis of LUX-Lung 2, LUX-Lung 3, and LUX-Lung 6. *Lancet Oncol*. 2015;16(7):830-838. doi:10.1016/S1470-2045(15)00026-1.
 51. Yang JC, Schuler M, Popat S, et al. Afatinib for the treatment of NSCLC harboring uncommon EGFR mutations: a database of 693 cases. *J Thorac Oncol*. 2020;15(5):803-815. doi:10.1016/j.jtho.2019.12.126.
 52. Cho JH, Lim SH, An HJ, et al. Osimertinib for patients with non-small-cell lung cancer harboring uncommon EGFR mutations: a multicenter, open-label, phase II trial (KCSG-LU15-09). *J Clin Oncol*. 2020;38(5):488-495. doi:10.1200/JCO.19.00931.
 53. Bar J, Peled N, Schokrpur S, et al. Uncommon EGFR mutations: international case series on efficacy of osimertinib in real-life practice in first line setting (UNICORN). *J Thorac Oncol*. 2022;S1556-0864(22)01854-8. doi:10.1016/j.jtho.2022.10.004.
 54. Gallant JN, Sheehan JH, Shaver TM, et al. EGFR kinase domain duplication (EGFR-KDD) is a novel oncogenic driver in lung cancer that is clinically responsive to afatinib. *Cancer Discov*. 2015;5(11):1155-1163. doi:10.1158/2159-8290.CD-15-0654.
 55. Wang J, Li X, Xue X, et al. Clinical outcomes of EGFR kinase domain duplication to targeted therapies in NSCLC. *Int J Cancer*. 2019;144(11):2677-2682. doi:10.1002/ijc.31895.
 56. Lee C, Kim M, Kim DW, et al. Acquired resistance mechanism of EGFR kinase domain duplication to EGFR TKIs in non-small cell lung cancer. *Cancer Res Treat*. 2022;54(1):140-149. doi:10.4143/crt.2021.385.
 57. Naidoo J, Sima CS, Rodriguez K, et al. Epidermal growth factor receptor exon 20 insertions in advanced lung adenocarcinomas: clinical outcomes and response to erlotinib. *Cancer*. 2015;121(18):3212-3220. doi:10.1002/cncr.29493.
 58. Robichaux JP, Elamin YY, Tan Z, et al. Mechanisms and clinical activity of an EGFR and HER2 exon 20-selective kinase inhibitor in non-small cell lung cancer. *Nat Med*. 2018;24(5):638-646. doi:10.1038/s41591-018-0007-9.
 59. Prelaj A, Bottiglieri A, Proto C, et al. Pozitotinib for EGFR and HER2 exon 20 insertion mutation in advanced NSCLC: results from the expanded access program. *Eur J Cancer*. 2021;149:235-248. doi:10.1016/j.ejca.2021.02.038.
 60. Zhou C, Ramalingam SS, Kim TM, et al. Treatment outcomes and safety of mobocertinib in platinum-pretreated patients with EGFR exon 20 insertion-positive metastatic non-small cell lung cancer: a phase 1/2 open-label nonrandomized clinical trial. *JAMA Oncol*. 2021;7(12):e214761. doi:10.1001/jamaoncol.2021.4761.
 61. Park K, Haura EB, Leighl NB, et al. Amivantamab in EGFR exon 20 insertion-mutated non-small-cell lung cancer progressing on platinum chemotherapy: initial results

- from the CHRYSALIS phase I study. *J Clin Oncol.* 2021;39(30):3391-3402. doi:10.1200/JCO.21.00662.
62. Wang M, Yang JCH, Mitchell PL, et al. Sunvozertinib, a selective EGFR inhibitor for previously treated non-small cell lung cancer with EGFR exon 20 insertion mutations. *Cancer Discov.* 2022;12(7):1676-1689. doi:10.1158/2159-8290.CD-21-1615.
 63. Yu HA, Tan DSW, Smit EF, et al. Phase (Ph) 1/2a study of CLN-081 in patients (pts) with NSCLC with EGFR exon 20 insertion mutations (Ins20). *J Clin Oncol.* 2022;40(16 suppl):9007. doi:10.1200/JCO.2022.40.16_suppl.9007.
 64. Lin Y, Hu Z, Zhang Y, et al. Preliminary clinical investigations of high-dose furmonertinib in NSCLC with EGFR exon 20 insertions (20ins). *J Clin Oncol.* 2022;40(16 suppl):e21029. doi:10.1200/JCO.2022.40.16_suppl.e21029.
 65. Attili I, Passaro A, Pisapia P, Malapelle U, de Marinis F. Uncommon EGFR compound mutations in non-small cell lung cancer (NSCLC): a systematic review of available evidence. *Curr Oncol.* 2022;29(1):255-266. doi:10.3390/curroncol29010024.
 66. Passaro A, Jänne PA, Mok T, Peters S. Overcoming therapy resistance in EGFR-mutant lung cancer. *Nat Cancer.* 2021;2(4):377-391. doi:10.1038/s43018-021-00195-8.
 67. Du Z, Brown BP, Kim S, et al. Structure-function analysis of oncogenic EGFR kinase domain duplication reveals insights into activation and a potential approach for therapeutic targeting. *Nat Commun.* 2021;12(1):1382. doi:10.1038/s41467-021-21613-6.
 68. Raez LE, Pinto JA, Schrock AB, Ali SM. EGFR-RAD51 fusion: a targetable partnership originated from the tumor evolution? *J Thorac Oncol.* 2018;13(3):e33-e34. doi:10.1016/j.jtho.2017.10.005.
 69. Konduri K, Gallant JN, Chae YK, et al. EGFR fusions as novel therapeutic targets in lung cancer. *Cancer Discov.* 2016;6(6):601-611. doi:10.1158/2159-8290.CD-16-0075.
 70. Copia Sperandio R, Luiza Teixeira Tostes F, Vidal Campregher P, et al. EGFR-RAD51 fusion in lung adenocarcinoma with systemic and intracranial response to osimertinib: a case report and review of the literature. *Lung Cancer.* 2022;166:94-97. doi:10.1016/j.lungcan.2022.02.006.
 71. Fukuoka M, Wu YL, Thongprasert S, et al. Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). *J Clin Oncol.* 2011;29(21):2866-2874. doi:10.1200/JCO.2010.33.4235.
 72. Schoenfeld AJ, Chan JM, Kubota D, et al. Tumor analyses reveal squamous transformation and off-target alterations as early resistance mechanisms to first-line osimertinib in EGFR-mutant lung cancer. *Clin Cancer Res.* 2020;26(11):2654-2663. doi:10.1158/1078-0432.CCR-19-3563.
 73. Bauml J, Cho BC, Park K, et al. Amivantamab in combination with lazertinib for the treatment of osimertinib-relapsed, chemotherapy-naïve EGFR mutant (EGFRm) non-small cell lung cancer (NSCLC) and potential biomarkers for response. *J Clin Oncol.* 2021;39(15 suppl):9006. doi:10.1200/JCO.2021.39.15_suppl.9006.
 74. Geraud A, Mezquita L, Auclin E, et al. Chronic plasma exposure to kinase inhibitors in patients with oncogene-addicted non-small cell lung cancer. *Cancers (Basel).* 2020;12(12):E3758. doi:10.3390/cancers12123758.
 75. Piotrowska Z, Ahn MJ, Pang YK, et al. LBA53 ELIOS: a multicentre, molecular profiling study of patients (pts) with epidermal growth factor receptor-mutated (EGFRm) advanced NSCLC treated with first-line (1L) osimertinib. *Ann Oncol.* 2022;33:S1420-S1421. doi:10.1016/j.annonc.2022.08.055.
 76. Ramalingam SS, Cheng Y, Zhou C, et al. Mechanisms of acquired resistance to first-line osimertinib: preliminary data from the phase III FLAURA study. *Ann Oncol.* 2018;29:viii740. doi:10.1093/annonc/mdy424.063.
 77. Papadimitrakopoulou VA, Wu YL, Han JY, et al. Analysis of resistance mechanisms to osimertinib in patients with EGFR T790M advanced NSCLC from the AURA3 study. *Ann Oncol.* 2018;29:viii741. doi:10.1093/annonc/mdy424.064.
 78. Oxnard GR, Hu Y, Mileham KF, et al. Assessment of resistance mechanisms and clinical implications in patients with EGFR T790M-positive lung cancer and acquired resistance to osimertinib. *JAMA Oncol.* 2018;4(11):1527-1534. doi:10.1001/jamaoncol.2018.2969.
 79. Wang S, Song Y, Liu D. EAI045: the fourth-generation EGFR inhibitor overcoming T790M and C797S resistance. *Cancer Lett.* 2017;385:51-54. doi:10.1016/j.canlet.2016.11.008.
 80. To C, Jang J, Chen T, et al. Single and dual targeting of mutant EGFR with an allosteric inhibitor. *Cancer Discov.* 2019;9(7):926-943. doi:10.1158/2159-8290.CD-18-0903.

81. Schalm SS, Dineen T, Lim SM, et al. BLU-945, a highly potent and selective 4th generation EGFR TKI for the treatment of EGFR T790M/C797S resistant NSCLC. *Ann Oncol.* 2020;31:S1391. doi:10.1016/j.annonc.2020.10.378.
82. Niederst MJ, Hu H, Mulvey HE, et al. the allelic context of the C797S mutation acquired upon treatment with third-generation EGFR inhibitors impacts sensitivity to subsequent treatment strategies. *Clin Cancer Res.* 2015;21(17):3924-3933. doi:10.1158/1078-0432.CCR-15-0560.
83. Conti C, Campbell J, Woessner R, et al. Abstract 1262: BLU-701 is a highly potent, brain-penetrant and WT-sparing next-generation EGFR TKI for the treatment of sensitizing (ex19del, L858R) and C797S resistance mutations in metastatic NSCLC. *Cancer Res.* 2021;81(13 suppl):1262-1262. doi:10.1158/1538-7445.AM2021-1262.
84. Nogami N, Barlesi F, Socinski MA, et al. IMPower150 final exploratory analyses for atezolizumab plus bevacizumab and chemotherapy in key NSCLC patient subgroups with EGFR mutations or metastases in the liver or brain. *J Thorac Oncol.* 2022;17(2):309-323. doi:10.1016/j.jtho.2021.09.014.
85. Lu S, Wu L, Jian H, et al. ORIENT-31: phase III study of sintilimab with or without IBI305 plus chemotherapy in patients with EGFR mutated nonsquamous NSCLC who progressed after EGFR-TKI therapy. *Ann Oncol.* 2022;33(1):112-113. doi:10.1016/j.annonc.2021.10.007.
86. Mok TSK, Nakagawa K, Park K, et al. LBA8: nivolumab (NIVO) + chemotherapy (chemo) vs chemo in patients (pts) with EGFR-mutated metastatic non-small cell lung cancer (mNSCLC) with disease progression after EGFR tyrosine kinase inhibitors (TKIs) in CheckMate 722. *Ann Oncol.* 2022;33:S1561-2. <https://doi.org/10.1016/j.annonc.2022.10.350>.
87. Jänne PA, Baik C, Su WC, et al. Efficacy and safety of patritumab deruxtecan (HER3-DXd) in EGFR inhibitor-resistant, EGFR-mutated non-small cell lung cancer. *Cancer Discov.* 2022;12(1):74-89. doi:10.1158/2159-8290.CD-21-0715.
88. Garon EB, Johnson ML, Lisberg AE, et al. Efficacy of datopotamab deruxtecan (Dato-DXd) in patients (pts) with advanced/metastatic (adv/met) non-small cell lung cancer (NSCLC) and actionable genomic alterations (AGAs): preliminary results from the phase I TROPION-PanTumor01 study. *Ann Oncol.* 2021;32:S1326-S1327. doi:10.1016/j.annonc.2021.08.2128.
89. Wu YL, Cheng Y, Zhou J, et al. Tepotinib plus gefitinib in patients with EGFR-mutant non-small-cell lung cancer with MET over-expression or MET amplification and acquired resistance to previous EGFR inhibitor (INSIGHT study): an open-label, phase 1b/2, multicentre, randomised trial. *Lancet Respir Med.* 2020;8(11):1132-1143. doi:10.1016/S2213-2600(20)30154-5.
90. Engelman JA, Zejnullahu K, Mitsudomi T, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science.* 2007;316(5827):1039-1043. doi:10.1126/science.1141478.
91. Wu YL, Zhang L, Kim DW, et al. Phase Ib/II study of capmatinib (INC280) plus gefitinib after failure of epidermal growth factor receptor (EGFR) inhibitor therapy in patients with EGFR-mutated, MET factor-dysregulated non-small-cell lung cancer. *J Clin Oncol.* 2018;36(31):3101-3109. doi:10.1200/JCO.2018.77.7326.
92. Yang JJ, Fang J, Shu YQ, et al. A phase Ib study of the highly selective MET-TKI savolitinib plus gefitinib in patients with EGFR-mutated, MET-amplified advanced non-small-cell lung cancer. *Invest New Drugs.* 2021;39(2):477-487. doi:10.1007/s10637-020-01010-4.
93. Sequist LV, Han JY, Ahn MJ, et al. Osimertinib plus savolitinib in patients with EGFR mutation-positive, MET-amplified, non-small-cell lung cancer after progression on EGFR tyrosine kinase inhibitors: interim results from a multicentre, open-label, phase 1b study. *Lancet Oncol.* 2020;21(3):373-386. doi:10.1016/S1470-2045(19)30785-5.
94. Yu HA, Ambrose H, Baik C, et al. ORCHARD osimertinib + savolitinib interim analysis: a biomarker-directed phase II platform study in patients (pts) with advanced non-small cell lung cancer (NSCLC) whose disease has progressed on first-line (1L) osimertinib. *Ann Oncol.* 2021;32:S978-S979. doi:10.1016/j.annonc.2021.08.1844.
95. Ahn M, De Marinis F, Bonanno L, et al. EP08.02-140: MET biomarker-based preliminary efficacy analysis in SAVANNAH: savolitinib+osimertinib in EGFRm NSCLC post-osimertinib. *J Thorac Oncol.* 2022;17:S469-70. <https://doi.org/10.1016/j.jtho.2022.07.823>.
96. Mazieres J, Kim TM, Lim BK, et al. LBA52 Tepotinib + osimertinib for EGFRm NSCLC with MET amplification (METamp) after progression on first-line (1L) osimertinib: initial results from the INSIGHT 2 study. *Ann Oncol.* 2022;33:S1419-S1420. doi:10.1016/j.annonc.2022.08.054.

97. Shu CA, Goto K, Ohe Y, et al. Amivantamab and lazertinib in patients with EGFR-mutant non-small cell lung (NSCLC) after progression on osimertinib and platinum-based chemotherapy: updated results from CHRYSALIS-2. *J Clin Oncol*. 2022;40(16 suppl):9006. doi:10.1200/JCO.2022.40.16_suppl.9006.
98. Goldman JW, Horinouchi H, Cho BC, et al. Phase 1/1b study of telisotuzumab vedotin (Teliso-V) + osimertinib (Osi), after failure on prior Osi, in patients with advanced, c-Met overexpressing, EGFR-mutated non-small cell lung cancer (NSCLC). *J Clin Oncol*. 2022;40(16 suppl):9013. doi:10.1200/JCO.2022.40.16_suppl.9013.
99. Casadevall D, Gimeno J, Clavé S, et al. MET expression and copy number heterogeneity in nonsquamous non-small cell lung cancer (nsNSCLC). *Oncotarget*. 2015;6(18):16215-16226. doi:10.18632/oncotarget.3976.
100. Piotrowska Z, Isozaki H, Lennerz JK, et al. Landscape of acquired resistance to osimertinib in EGFR-mutant NSCLC and clinical validation of combined EGFR and RET inhibition with osimertinib and BLU-667 for acquired RET fusion. *Cancer Discov*. 2018;8(12):1529-1539. doi:10.1158/2159-8290.CD-18-1022.
101. Rotow J, Patel J, Hanley M, et al. Combination osimertinib plus selpercatinib for EGFR-mutant non-small cell lung cancer (NSCLC) with acquired RET fusions. *J Thorac Oncol*. 2021;16(3):S230. doi:10.1016/j.jtho.2021.01.150.
102. Meng P, Koopman B, Kok K, et al. Combined osimertinib, dabrafenib and trametinib treatment for advanced non-small-cell lung cancer patients with an osimertinib-induced BRAF V600E mutation. *Lung Cancer*. 2020;146:358-361. doi:10.1016/j.lungcan.2020.05.036.
103. Valet O, Swalduz A, Boussageon M, et al. Response to the combination of osimertinib, dabrafenib, and trametinib in leptomeningitis from EGFR-mutant NSCLC with acquired BRAF V600E mutation: a case report. *JTO Clin Res Rep*. 2021;2(6):100192. doi:10.1016/j.jtocrr.2021.100192.
104. Riess JW, Krailo MD, Padua SK, et al. Osimertinib plus necitumumab in EGFR-mutant NSCLC: final results from an ETCTN California Cancer Consortium phase I study. *J Clin Oncol*. 2022;40(16 suppl):9014. doi:10.1200/JCO.2022.40.16_suppl.9014.
105. Yang JCH, Ohe Y, Chiu CH, et al. Osimertinib plus selumetinib in EGFR-mutated, non-small cell lung cancer after progression on EGFR-TKIs: a phase 1b, open-label, multi-center trial (TATTON Part B). *Clin Cancer Res*. 2022;clinres.4329.2022-1-26 08:36:37.517. doi:10.1158/1078-0432.CCR-21-4329.
106. Lee JK, Lee J, Kim S, et al. Clonal history and genetic predictors of transformation into small-cell carcinomas from lung adenocarcinomas. *J Clin Oncol*. 2017;35(26):3065-3074. doi:10.1200/JCO.2016.71.9096.
107. Offin M, Chan JM, Tenet M, et al. Concurrent RB1 and TP53 alterations define a subset of EGFR-mutant lung cancers at risk for histologic transformation and inferior clinical outcomes. *J Thorac Oncol*. 2019;14(10):1784-1793. doi:10.1016/j.jtho.2019.06.002.
108. McLeer A, Foll M, Brevet M, et al. Detection of acquired TERT amplification in addition to predisposing p53 and Rb pathways alterations in EGFR-mutant lung adenocarcinomas transformed into small-cell lung cancers. *Lung Cancer*. 2022;167:98-106. doi:10.1016/j.lungcan.2022.01.008.
109. Sequist LV, Waltman BA, Dias-Santagata D, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. *Sci Transl Med*. 2011;3(75):75ra26. doi:10.1126/scitranslmed.3002003.
110. Marcoux N, Gettinger SN, O'Kane G, et al. EGFR-mutant adenocarcinomas that transform to small-cell lung cancer and other neuroendocrine carcinomas: clinical outcomes. *J Clin Oncol*. 2019;37(4):278-285. doi:10.1200/JCO.18.01585.
111. Lim C, Sung M, Shepherd FA, et al. Patients with advanced non-small cell lung cancer: are research biopsies a barrier to participation in clinical trials? *J Thorac Oncol*. 2016;11(1):79-84. doi:10.1016/j.jtho.2015.09.006.
112. Piotrowska Z, Niederst MJ, Mino-Kenudson M, et al. Variation in mechanisms of acquired resistance among EGFR-mutant NSCLC patients with more than 1 postresistant biopsy: metastatic non-small cell lung cancer. *Int J Radiat Oncol Biol Phys*. 2014;90(5):S6-S7. doi:10.1016/j.ijrobp.2014.08.032.
113. Hata A, Katakami N, Yoshioka H, et al. Spatiotemporal T790M heterogeneity in individual patients with EGFR-mutant non-small-cell lung cancer after acquired resistance to EGFR-TKI. *J Thorac Oncol*. 2015;10(11):1553-1559. doi:10.1097/JTO.0000000000000647.

ALK Gene Structure and Function

The *anaplastic lymphoma kinase (ALK)* gene is located on the short arm of chromosome 2 (2p23) and encodes a receptor tyrosine kinase that belongs to the insulin receptor superfamily.^{1,2} It encodes a 1620 amino acid protein that forms a single-chain transmembrane receptor comprising an extracellular ligand-binding domain, a transmembrane region, and an intracellular kinase catalytic domain (Figure 8-1).^{1,2} The protein has high homology with the intracellular domains of leucocyte tyrosine kinase, as well as c-ROS and insulin-like growth factor-1 receptor kinase.^{1,2} Upon binding ligand, the ALK receptor undergoes homodimerization, tyrosine phosphorylation in the kinase domain, and subsequent activation of multiple downstream signaling pathways, including phosphoinositide 3-kinase (PI3K)/Akt, PI3K/phospholipase C-gamma, Janus kinase/signal transducer and activator of transcription (JAK/STAT), and RAS/mitogen-activated protein kinase (MAPK), with roles in cell growth, differentiation, and survival.³⁻⁵ Both pleiotrophin and midkine have been proposed as physiologic ligands for ALK, although there is some uncertainty with others unable to substantiate the findings.⁴⁻⁸ Mammalian signaling through ALK is involved in development of the nervous system and cell survival.^{1,2,9} Protein expression occurs in the nervous system during development and reduces postnatally with expression seen in some nerves in adults.^{1,2,9}

Type of Alterations and Role in Oncogenesis

Like other receptor tyrosine kinases, the oncogenic potential of ALK results from activating genetic variants, including rearrangements, mutations, or amplification. It was originally described in anaplastic large cell lymphomas where it was found to form an oncogenic fusion gene with nucleophosmin (*NPM1::ALK*) caused by a translocation between chromosomes 2 and 5 (2;5)(p23;q35).^{1,2} Oncogenic activating translocations have subsequently been described in a variety of malignancies including non-small cell lung carcinoma (NSCLC),

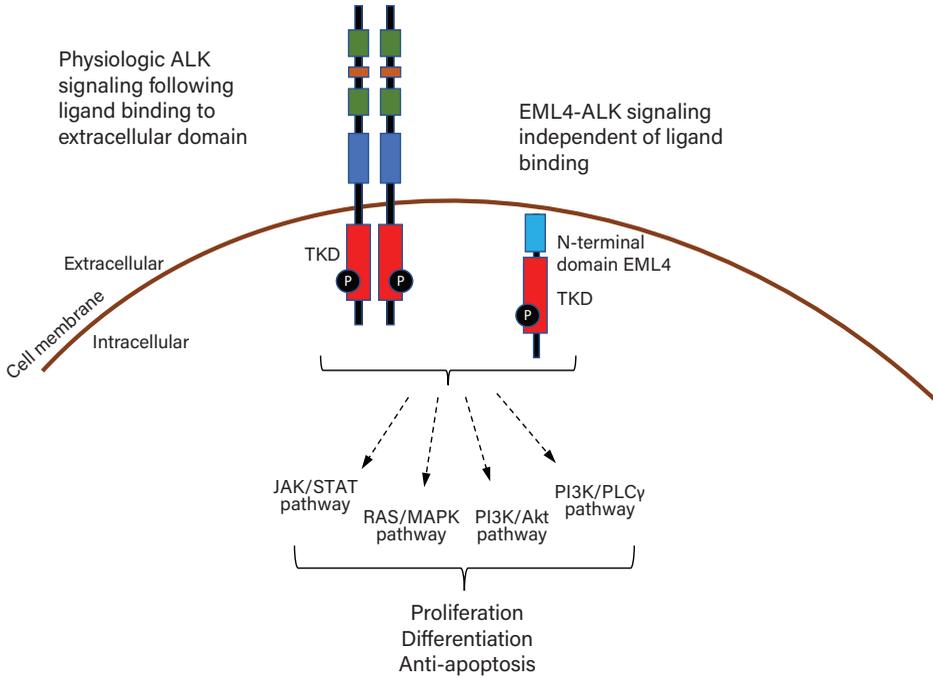


Figure 8-1. Physiologic ALK signaling following ligand binding to the extracellular domain (*left*) and constitutive EML4-ALK signaling without ligand binding (*right*). Abbreviations: JAK = Janus kinase; MAPK = mitogen-activated protein kinase; PI3K = phosphoinositide 3-kinase; PLCγ = phospholipase C-gamma; STAT = signal transducer and activator of transcription; TKD = tyrosine kinase domain.

inflammatory myofibroblastic tumors, and less commonly in melanomas, mesotheliomas, and a variety of carcinomas, including colorectal carcinoma and breast carcinoma.^{4,10,11}

In a subset of lung adenocarcinomas, oncogenic translocations involving *ALK* may occur, most commonly because of a small inversion in chromosome 2 fusing the 3' end of *ALK*, containing the cytoplasmic kinase domain (exons 20-29) with the N-terminal end of echinoderm microtubule-associated protein-like 4 (*EML4*), which encodes a 120 kDa protein crucial for microtubule formation (Inv[2] [p21;p23]).¹²⁻¹⁴ At least 13 *EML4::ALK*-fusion variants have been described to date, all incorporating the same portion of *ALK* encoding the intracellular tyrosine kinase domain (TKD) and variable lengths of *EML4* that include the coiled-coil domain (Table 8-1).^{13,15-19}

Table 8-1. Commonest *EML4::ALK* Variant Fusions

Variants	<i>EML4::ALK</i> -fusion types	Frequency (%)
1	E13;A20	55
2	E20;A20	10
3a/b	E6a/b;A20	30
4	E14;ins11del49A20	3
5a/b	E2:A20 (5a), E2ins117A20 (5b)	1
6	E3;ins69A20	1
7	E14;del12A20	<1
8a/b	E17;ins30A20 (8a), E17ins30;ins65A20 (8b)	<1

Adapted from Choi et al and Li et al.^{18,19}

In addition to *EML4*, which accounts for approximately 95% of *ALK*-fusion partners in NSCLC, at least 90 different novel partners have been identified to date, with increasing use of next-generation sequencing (NGS) accelerating identification of novel partners.¹² Fusion partners are most commonly located on the short arm of chromosome 2 but may also be on other chromosomes and include *KIF5B*, *TFG*, *KLC1*, *PTPN3*, *HIP1*, *STRN*, *TPR*, *DCTN1*, *SQSTM1*, *NPM1*, *BCL11A*, and *BIRC6*.^{10-12,15,20} The oncogenic fusion genes encode aberrant oncoproteins, mostly with constitutive kinase activity, leading to oncogenic properties including uncontrolled proliferation and survival.^{3,15} Aberrant fusion genes characteristically lead to overexpression of ALK protein. Increased *ALK* gene copy number has also been described in several tumors including NSCLC.¹⁰ *ALK* translocations are mutually exclusive with other oncogenic alterations, apart from rare case reports in the literature. Secondary acquired point mutations may occur in NSCLC following *ALK* tyrosine kinase inhibitor (ALKi) therapy as a mechanism of acquired resistance.¹⁰

Epidemiologic, Clinical, and Histologic Characteristics

ALK-rearranged lung adenocarcinoma constitutes 4% to 5% of lung adenocarcinoma, with a similar incidence reported in Asian and non-Asian populations.^{13,21} *ALK* fusions occur more commonly in never-smokers or light smokers where the incidence is 12%, but they are less strongly associated with female sex than *EGFR* mutations.²² The median age of patients with *ALK*-rearranged lung cancer is about 10 years younger than other NSCLC patients, and a higher proportion of people present with advanced-stage disease.²¹ *ALK* rearrangement in lung cancer is strongly associated with adenocarcinoma histology, in particular with acinar and/or solid growth pattern, or with cellular features of signet-ring cell carcinoma.²¹

Testing Strategies

Immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), reverse transcription polymerase chain reaction (RT-PCR), or NGS assays can be used to identify *ALK*-rearranged NSCLC.

Fluorescence In Situ Hybridization

FISH was the first companion diagnostic test used to detect *ALK* fusions in NSCLC. Break-apart probes, rather than dual-fusion signal probes, were adopted for detecting a rearrangement regardless of the fusion partner (Figure 8-2). In the most common *EML4::ALK* fusion, split FISH signals are often observed in close proximity because of the small inversion on chromosome 2. A positive cell by FISH is defined by split signals with separation at least twice the diameter of the largest signal or loss of the 5' signal in at least 15% of cells with a minimum of 50 cells counted (Figure 8-3).

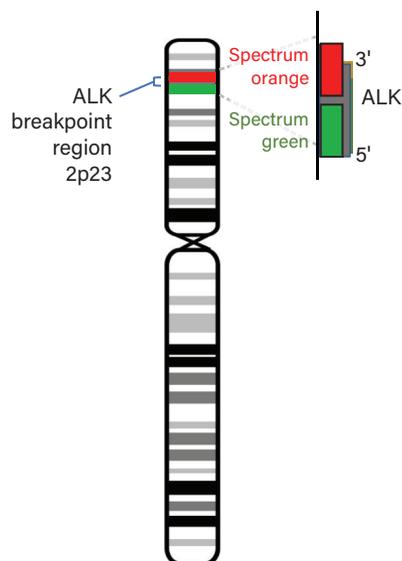


Figure 8-2. Schematic of *ALK* break-apart fluorescence in situ hybridization (FISH) probe design with orange-labeled probe binding to the 3' end of *ALK* and green-labeled probe binding to the 5' end of *ALK*, with each probe on either side of the breakpoint.

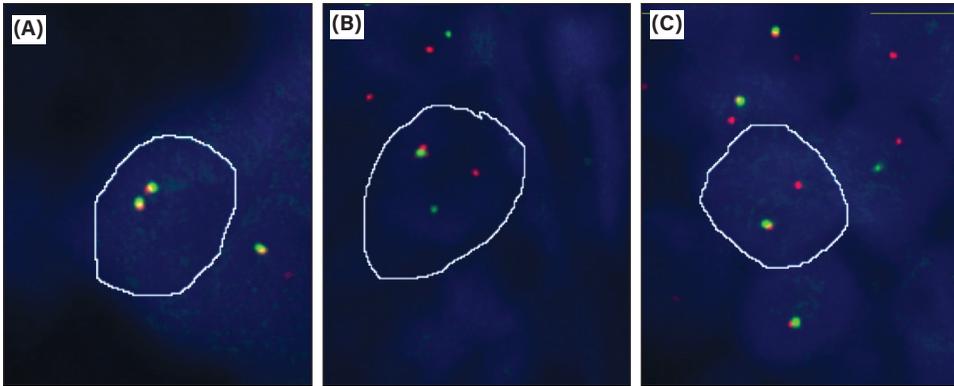


Figure 8-3. *ALK* fluorescence in situ hybridization (FISH) using a break-apart probe. **(A)** Two fused signals indicating a cell lacking *ALK* rearrangement. **(B)** One normal pair of fused signals and a split red 3' and green 5' signals indicating a cell with an *ALK* rearrangement, regardless of the fusion partner. **(C)** One normal pair of fused signals and a single red 3' signal with the *ALK*-kinase domain also indicates a cell positive for *ALK* rearrangement.

Immunohistochemistry

ALK IHC using high-sensitivity clones, such as D5F3 or 5A4, can be used reliably as a surrogate for functional *ALK* rearrangement in NSCLC (Figure 8-4).²³ Early studies found the expression of fused *ALK* protein was lower in NSCLC than in lymphoma, and high-affinity clones using more sensitive detection methods are required for *ALK* IHC in NSCLC. *ALK* IHC assays are now a clinically standard tool, and commercial IHC assays, such as Ventana *ALK* D5F3, have been approved as companion diagnostics for selection of the anti-*ALK* inhibitor alectinib in some countries. Potential pitfalls when interpreting *ALK* IHC include false-negative interpretation because of cytoplasmic intracellular mucin and positive staining in some neuroendocrine carcinomas despite lack of *ALK* fusions.²⁴ While *ALK* IHC using appropriate clones is highly sensitive for *ALK* rearrangements, there have been numerous reports on discrepant results between FISH and IHC, and there is some data suggesting *ALK* IHC-positive/FISH-negative cases are less responsive to treatment, even in the context of a proven fusion event by NGS.^{25,26} The basis for this discrepancy and apparently adverse impact on clinical outcomes is unclear.

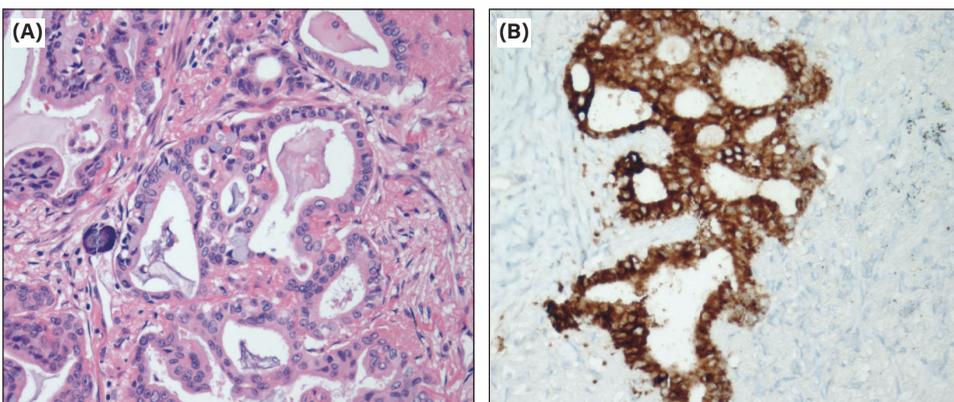


Figure 8-4. **(A)** *ALK*-positive adenocarcinoma with cribriform architecture and a few signet-ring cells. **(B)** Immunohistochemistry for *ALK* (D5F3 clone) shows positive cytoplasmic staining, most likely indicating an *ALK* rearrangement.

Reverse Transcription Polymerase Chain Reaction

RT-PCR was initially not recommended for routine clinical identification of *ALK* fusions because of difficulty obtaining high-quality RNA from formalin-fixed paraffin-embedded (FFPE) tissue and the presence of multiple fusion patterns/partner genes and subsequent risk of false-negative results.²⁷ However, RNA may be relatively well preserved in routine FFPE specimens handled with standardized pre-analytic methods and improved extraction techniques, and several RT-PCR assays using a melting curve are now commercially available. The main limitation of RT-PCR is the inability to identify novel fusions not included in the assay design. Furthermore, RNA-based NGS assays, with improved clinical sensitivity, also confirmed reliable detection of *ALK* fusions using FFPE-derived RNA.

Next-Generation Sequencing Assays

ALK fusions can be detected by NGS using DNA- or RNA-based assays from FFPE and enable detection of known variants (amplicon-based assays) or both known and unknown fusion variants. NGS assays have the advantage of identifying the fusion partner/variant as well as the ability to concurrently test for alterations in other genes in addition to *ALK*. Limitations of NGS assays in identifying *ALK*-rearranged NSCLC include:

- Requirement of relatively large amounts of tissue: Small biopsy or scant cytology samples may not be suitable for hybrid capture-based NGS assays given relatively high nucleic acid requirements. Amplicon-based hot-spot panels and anchored multiplex PCR (AMP)/NGS have the advantage of lower DNA/RNA requirements. AMP/NGS is a form of targeted NGS that allows detection of oncogenic fusions without prior knowledge of fusion partners.
- False negatives in DNA-based NGS assays: Genomic breakpoints in DNA are more diverse compared to fusion patterns on RNA. Therefore, some *ALK* fusions may potentially be missed by DNA NGS and may require RNA sequencing for identification.²⁸
- Suboptimal sensitivity for specimens with low tumor cell content: As NGS is based on allele frequencies of interest, detection of fusions in specimens with low proportions of tumor cells can be difficult although this is not unique to assessment of *ALK*. Molecular barcoding and deep sequencing are useful in the attempt to overcome this challenge.

Clinical Implications

ALK-rearranged NSCLCs are exquisitely sensitive to ALKi therapies. Landmark phase 3 clinical trial evidence first supported the ALKi crizotinib as a superior standard of care over platinum doublet chemotherapy in 2014 with a marked progression-free survival (PFS) and tolerability advantage, before overall survival (OS) was confirmed superior with 57% of patients alive at 4 years with first-line crizotinib.^{29,30}

As experience evolved, it was reported that 70% treated with crizotinib encountered central nervous system (CNS) progression³¹; inherently, *ALK*-rearranged NSCLC carries a propensity to metastasize to the brain, with approximately 25% having CNS disease at diagnosis.²⁹

Next-generation ALKi therapies were consequently developed first to be active at crizotinib failure in overcoming mechanisms of drug resistance, to be highly brain penetrable, and to deliver greater “on-target” affinity to ALK.³²⁻³⁷

With safety and efficacy post crizotinib demonstrated, second-generation ALKi therapies—ceritinib, alectinib, brigatinib, and ensartinib—rapidly moved to frontline phase 3 investigation. All demonstrated superiority to their comparator arm, which was chemotherapy with ceritinib and crizotinib for the remainder as standard of care at trial design.³⁸⁻⁴¹ Given inferior numerical median survival with ceritinib compared to alternative second-generation ALKi therapies, and poorer tolerability, ceritinib's place in the empiric treatment paradigm for ALK has diminished. Cross-trial comparison of alectinib, brigatinib, and ensartinib, however, conclude comparable PFS, with a signal for greater CNS efficacy with alectinib and brigatinib over ensartinib. The longest median PFS to date has been seen with alectinib at 35 months (investigator-assessed) as well as mature OS rate phase 3 data, with 63% alive at 5 years, and median OS rate all-comers expected to exceed 8 years.⁴²

Toxicity profiles among ALKi therapies carry some consistent class effects and potential adverse effects unique among ALKi therapies, which may become clinically relevant in selecting an alternative ALKi in the circumstance of unacceptable toxicity.

Third-generation single-arm phase 1 and 2 data with highly active and brain-penetrant ALKi lorlatinib have now established efficacy in pretreated individuals after 1 to multiple prior lines of ALKi.^{36,43} Lorlatinib is effective in the context of common mutations conferring resistance to prior-generation ALKi therapies.⁴⁴ First-line phase 3 investigation with lorlatinib (vs crizotinib) has now delivered interim positive data, with median PFS not yet reached; however, landmark data demonstrate 64% are progression free at 3 years, and 50% in those with baseline CNS metastases. These are the most compelling immature first-line survival data to date.^{45,46}

In 2022, fourth-generation ALKi therapies have entered early phase investigation, demonstrating preclinical activity in *ALK*-compound resistance mutations, including those pan-resistant to prior ALKi therapies.^{47,48} Novel combination therapy prospective clinical trials are underway, including ALKi therapies with an additional, often targeted therapy, investigating whether this strategy may prevent or overcome the emergence of drug resistance, improve survival, and preserve tolerability.

At present, there are no additional molecular biomarkers that routinely guide the selection of a specific ALKi in routine clinical practice. The presence of baseline brain metastases or intent to protect the brain may assist in selecting the optimal ALKi therapy. Most individuals will receive each line of therapy in an empiric manner based on drug availability and limited sequencing data.

Resistance Mechanisms

Despite superior efficacy and tolerability of ALKi in advanced disease, drug resistance remains inevitable. The mechanism behind this is in part explained by these therapies harboring greater cytostatic than cytotoxic properties, giving rise to the phenomenon of clonal selection.

The mechanisms of drug resistance were first described with crizotinib and broadly categorized into primary and acquired (secondary) resistance and in “acquired” to “ALK-dependent” or “ALK-independent” mechanisms.^{49,50} Primary resistance is rare and may be caused by false-positive genotyping or a molecularly or histologically diverse phenotype. In secondary resistance, histologic transformation may rarely occur, including small

cell, squamous, or neuroendocrine, and is probably more frequent with latter-generation ALKi therapies, which are more potent against ALK.^{51,52}

ALK-dependent mechanisms commonly involve the emergence of an *ALK*-resistance mutation, frequently occurring in the intracellular drug-binding pocket of the TKD. These have been described in 25% to more than 50% of cases treated with first- and second-generation ALKi therapies and may involve 1 or multiple mutations evolving in the same tumor cells or in differing tumor cell clones.⁵³ To determine the mechanism of resistance, either a tissue and/or liquid biopsy is required utilizing a broad molecular gene sequencing panel, capturing both clinically relevant non-*ALK* genes and the ALK-kinase domain as comprehensively as possible.

For crizotinib, the most common TKD is the gatekeeper L1196M mutation, directly blocking drug binding, whereas with second-generation ALKi therapies, the most common is the solvent-front G1202R mutation, which repels drug binding via steric hindrance.⁴⁴ There have been more than 50 *ALK*-resistance mutations described, and preclinical and clinical data are establishing the sensitivity of ALKi therapies in the presence of an array of emerging resistance mutations that often differ between ALKi exposures, given their unique molecular structures and properties. When multiple TKD mutations occur, these are more likely to be resistant to early generation ALKi therapies, and if co-occurring with G1202R, to be also resistant to lorlatinib.⁵⁴

The individual *ALK*-fusion variant may further predispose ALKi durable efficacy and give rise to a unique resistance profile because of differing oncoprotein stabilities and genetic vulnerabilities. There have been reports preclinically and clinically to suggest the particular fusion variant may predict ALKi performance, with the most common *EML4::ALK* variant 1 suggesting superior PFS with first- and second-generation inhibitors; however, *EML4::ALK* variant 3, as compared to variant 1, shows superior outcomes with lorlatinib.⁵⁵⁻⁵⁹ Intriguingly, the variant harbored may also predispose to differing resistance manifestations, with more ALK-dependence in variant 3 and ALK-independence in variant 1.⁵⁷ Outside *ALK*-resistance mutations, another mechanism of *ALK*-dependent resistance may be *ALK* gene copy number gain and heightened expression of the ALK oncoprotein; these features are not routinely tested, nor are the optimal techniques and thresholds to define clinically relevant gene copy or protein-level gain established.

ALK-independent resistance mechanisms (activation of bypass-pathway-mediated mechanisms) may occur in isolation or, more commonly, in concert with ALK-dependent resistance variants and can be demonstrated in over 50% of patients treated with next-generation ALKi (Figure 8-5). ALK-independent resistance increases in frequency with lines of therapy received and later-generation ALKi exposure. ALK-independent resistance mechanisms include aberrant pathway activation secondary to alterations in *KRAS*, *ERBB2*, *BRAF*, *EGFR*, *MET*, *MEK*, *KIT*, and more.^{49,50,60-63} Certain non-*ALK* co-mutations in the context of *ALK* rearrangement predict for an attenuated response to ALKi monotherapy, the most established being *TP53* mutation.⁵⁸ In the circumstance of ALK-independent resistance, the best therapeutic strategies remain to be determined. Preclinical data and few case reports describe effective targeted therapy combinations; however, access to these combination strategies in practice may be limited.^{63,65-67} Clinical trials are exploring all-comer combination therapeutic strategies not in a biomarker-informed personalized manner. Such trials listed

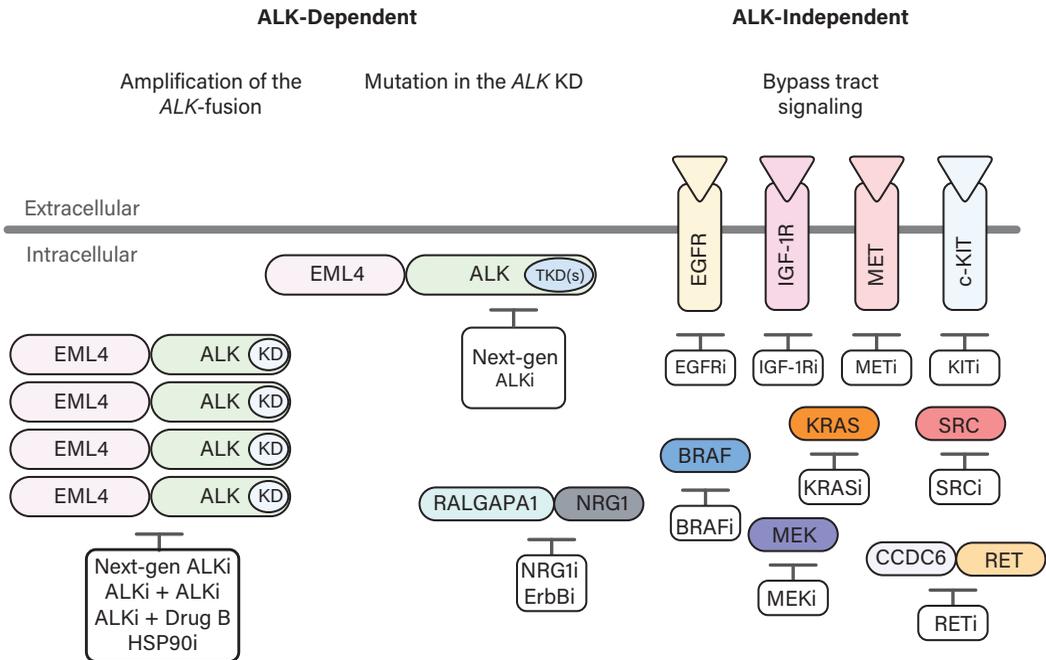


Figure 8-5. Mechanisms of resistance to ALK inhibitors. Resistance mechanisms may be ALK-dependent or ALK-independent and are heterogeneous within and between patients. Abbreviations: HSP = heat shock protein; i = inhibitor; KD = kinase domain; TKD = tyrosine kinase domain. (Adapted from Lovly.⁶⁴)

on ClinicalTrials.gov include administering ALKi therapies with a vascular endothelial growth factor (VEGF), SHP2, or MAPK inhibitor; a second ALKi; or with chemotherapy or radiotherapy.

References

- Iwahara T, Fujimoto J, Wen D, et al., Molecular characterization of ALK, a receptor tyrosine kinase expressed specifically in the nervous system. *Oncogene*. 1997;14(4):439-449.
- Morris SW, Naeve C, Mathew P, et al. ALK, the chromosome 2 gene locus altered by the t(2;5) in non-Hodgkin's lymphoma, encodes a novel neural receptor tyrosine kinase that is highly related to leukocyte tyrosine kinase (LTK) [published correction appears in *Oncogene* 1997 Dec 4;15(23):2883]. *Oncogene*. 1997;14(18):2175-2188.
- Lee CC, Jia Y, Li N, et al. Crystal structure of the ALK (anaplastic lymphoma kinase) catalytic domain. *Biochem J*. 2010;430(3):425-437.
- Palmer RH, Vernersson E, Grabbe C, Hallberg B. Anaplastic lymphoma kinase: signaling in development and disease. *Biochem J*. 2009;420(3):345-361.
- Stoica GE, Kuo A, Aigner A, et al. Identification of anaplastic lymphoma kinase as a receptor for the growth factor pleiotrophin. *J Biol Chem*. 2001;276(20):16772-16779.
- Guan J, Umapathy G, Yamazaki Y, et al. FAM150A and FAM150B are activating ligands for anaplastic lymphoma kinase. *Elife*. 2015;4:e09811.
- Perez-Pinera P, Zhang W, Chang Y, Vega JA, Deuel TF. Anaplastic lymphoma kinase is activated through the pleiotrophin/receptor protein-tyrosine phosphatase beta/zeta signaling pathway: an alternative mechanism of receptor tyrosine kinase activation. *J Biol Chem*. 2007;282(39):28683-28690.
- Stoica GE, Kuo A, Powers C, et al. Midkine binds to anaplastic lymphoma kinase (ALK) and acts as a growth factor for different cell types. *J Biol Chem*. 2002;277(39):35990-35998.
- Vernersson E, Khoo NK, Henriksson ML, et al. Characterization of the expression of the ALK receptor tyrosine kinase in mice. *Gene Expr Patterns*. 2006;6(5):448-461.

10. Du X, Shao Y, Qin HF, Tai YH, Gao HJ. ALK-rearrangement in non-small-cell lung cancer (NSCLC). *Thorac Cancer*. 2018;9(4):423-430.
11. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. [published correction appears in *N Engl J Med*. 2011 Feb 10;364(6):588]. *N Engl J Med*. 2010;363(18):1693-1703.
12. Ou SI, Zhu VW, Nagasaka M. Catalog of 5' fusion partners in ALK-positive NSCLC circa 2020. *JTO Clin Res Rep*. 2020;1(1):100015.
13. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature*. 2007;448(7153):561-566.
14. Solomon B, Varella-Garcia M, Camidge DR. ALK gene rearrangements: a new therapeutic target in a molecularly defined subset of non-small cell lung cancer. *J Thorac Oncol*. 2009;4(12):1450-1454.
15. Della Corte CM, Viscardi G, Di Liello R, et al. Role and targeting of anaplastic lymphoma kinase in cancer. *Mol Cancer*. 2018;17(1):30.
16. Heuckmann JM, Balke-Want H, Malchers F, et al. Differential protein stability and ALK inhibitor sensitivity of EML4-ALK fusion variants. *Clin Cancer Res*. 2012;18(17):4682-4690.
17. Takeuchi K, Choi YL, Soda M, et al. Multiplex reverse transcription-PCR screening for EML4-ALK fusion transcripts. *Clin Cancer Res*. 2008;14(20):6618-6624.
18. Choi YL, Takeuchi K, Soda M, et al. Identification of novel isoforms of the EML4-ALK transforming gene in non-small cell lung cancer. *Cancer Res*. 2008;68(13):4971-4976.
19. Li T, Maus MK, Desai SJ, et al. Large-scale screening and molecular characterization of EML4-ALK fusion variants in archival non-small-cell lung cancer tumor specimens using quantitative reverse transcription polymerase chain reaction assays. *J Thorac Oncol*. 2014;9(1):18-25.
20. Kang J, Deng QM, Peng KC, et al. Clinicopathological features and resistance mechanisms in HIP1-ALK-rearranged lung cancer: a multicenter study. *Genes Chromosomes Cancer*. 2022;61(4):177-186.
21. Fan L, Feng Y, Wan H, Shi G, Niu W. Clinicopathological and demographical characteristics of non-small cell lung cancer patients with ALK rearrangements: a systematic review and meta-analysis. *PLoS One*. 2014;9(6):e100866.
22. Paik PK, Johnson ML, D'Angelo SP, et al. Driver mutations determine survival in smokers and never-smokers with stage IIIB/IV lung adenocarcinomas. *Cancer*. 2012;118(23):5840-5847.
23. Selinger CI, Rogers TM, Russell PA, et al. Testing for ALK rearrangement in lung adenocarcinoma: a multicenter comparison of immunohistochemistry and fluorescent in situ hybridization. *Mod Pathol*. 2013;26(12):1545-1553.
24. Akhoundova D, Haberecker M, Fritsch R, et al. Targeting ALK in neuroendocrine tumors of the lung. *Front Oncol*. 2022;12:911294.
25. Blackhall FH, Peters S, Bubendorf L, et al. Prevalence and clinical outcomes for patients with ALK-positive resected stage I to III adenocarcinoma: results from the European Thoracic Oncology Platform Lungscape project. *J Clin Oncol*. 2014;32(25):2780-2787.
26. Mok T, Peters S, Camidge DR, et al. Outcomes according to ALK status determined by central immunohistochemistry or fluorescence in situ hybridization in patients with ALK-positive NSCLC enrolled in the phase 3 ALEX study. *J Thorac Oncol*. 2021;16(2):259-268.
27. Lindeman NI, Cagle PT, Beasley MB, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology [published correction appears in *J Thorac Oncol*. 2013 Oct;8(10):1343]. *J Thorac Oncol*. 2013;8(7):823-859.
28. Benayed R, Offin M, Mullaney K, et al. High yield of RNA sequencing for targetable kinase fusions in lung adenocarcinomas with no mitogenic driver alteration detected by DNA sequencing and low tumor mutation burden. *Clin Cancer Res*. 2019;25(15):4712-4722.
29. Solomon BJ, Mok T, Kim DW, et al. First-line crizotinib versus chemotherapy in ALK-positive lung cancer [published correction appears in *N Engl J Med*. 2015 Oct 15;373(16):1582]. *N Engl J Med*. 2014;371(23):2167-2177.
30. Solomon BJ, Kim DW, Wu YL, et al. Final overall survival analysis from a study comparing first-line crizotinib versus chemotherapy in ALK-mutation-positive non-small-cell lung cancer. *J Clin Oncol*. 2018;36(22):2251-2258.
31. Costa DB, Shaw AT, Ou SH, et al. Clinical experience with crizotinib in patients with advanced ALK-rearranged non-small-cell lung cancer and brain metastases. *J Clin Oncol*. 2015;33(17):1881-1888.

32. Shaw AT, Kim TM, Crinò L, et al. Ceritinib versus chemotherapy in patients with ALK-rearranged non-small-cell lung cancer previously given chemotherapy and crizotinib (ASCEND-5): a randomised, controlled, open-label, phase 3 trial. *Lancet Oncol.* 2017;18(7):874-886.
33. Novello S, Mazières J, Oh IJ, et al. Alectinib versus chemotherapy in crizotinib-pretreated anaplastic lymphoma kinase (ALK)-positive non-small-cell lung cancer: results from the phase III ALUR study. *Ann Oncol.* 2018;29(6):1409-1416.
34. Gettinger SN, Bazhenova LA, Langer CJ, et al. Activity and safety of brigatinib in ALK-rearranged non-small-cell lung cancer and other malignancies: a single-arm, open-label, phase 1/2 trial. *Lancet Oncol.* 2016;17(12):1683-1696.
35. Popat S, Liu G, Lu S, et al. Brigatinib vs alectinib in crizotinib-resistant advanced anaplastic lymphoma kinase-positive non-small-cell lung cancer (ALTA-3). *Future Oncol.* 2021;17(32):4237-4247.
36. Solomon BJ, Besse B, Bauer TM, et al. Lorlatinib in patients with ALK-positive non-small-cell lung cancer: results from a global phase 2 study [published correction appears in *Lancet Oncol.* 2019 Jan;20(1):e10]. *Lancet Oncol.* 2018;19(12):1654-1667.
37. Horn L, Infante JR, Reckamp KL, et al. Ensartinib (X-396) in ALK-positive non-small cell lung cancer: results from a first-in-human phase I/II, multicenter study. *Clin Cancer Res.* 2018;24(12):2771-2779.
38. Soria JC, Tan DSW, Chiari R, et al. First-line ceritinib versus platinum-based chemotherapy in advanced ALK-rearranged non-small-cell lung cancer (ASCEND-4): a randomised, open-label, phase 3 study. *Lancet.* 2017;389(10072):917-929.
39. Peters S, Camidge DR, Shaw AT, et al. Alectinib versus crizotinib in untreated ALK-positive non-small-cell lung cancer. *N Engl J Med.* 2017;377(9):829-838.
40. Camidge DR, Kim HR, Ahn MJ, et al. Brigatinib versus crizotinib in ALK-positive non-small-cell lung cancer. *N Engl J Med.* 2018;379(21):2027-2039.
41. Horn L, Wang Z, Wu G, et al. Ensartinib vs crizotinib for patients with anaplastic lymphoma kinase-positive non-small cell lung cancer: a randomized clinical trial. *JAMA Oncol.* 2021;7(11):1617-1625.
42. Mok T, Camidge DR, Gadgeel SM, et al. Updated overall survival and final progression-free survival data for patients with treatment-naive advanced ALK-positive non-small-cell lung cancer in the ALEX study. *Ann Oncol.* 2020;31(8):1056-1064.
43. Shaw AT, Felip E, Bauer TM, et al. Lorlatinib in non-small-cell lung cancer with ALK or ROS1 rearrangement: an international, multicentre, open-label, single-arm first-in-man phase 1 trial. *Lancet Oncol.* 2017;18(12):1590-1599.
44. Gainor JF, Dardaei L, Yoda S, et al. Molecular mechanisms of resistance to first- and second-generation ALK inhibitors in ALK-rearranged lung cancer. *Cancer Discov.* 2016;6(10):1118-1133.
45. Shaw AT, Bauer TM, de Marinis F, et al. First-line lorlatinib or crizotinib in advanced ALK-positive lung cancer. *N Engl J Med.* 2020;383(21):2018-2029.
46. Solomon B, Bauer T, Mok T, et al. Updated efficacy and safety from the phase 3 CROWN study of first-line lorlatinib vs crizotinib in advanced anaplastic lymphoma kinase (ALK)-positive non-small cell lung cancer (NSCLC) [AACR conference abstract CT223]. *Cancer Res.* 2022;82(12 suppl):CT223.
47. Murray BW, Zhai D, Deng W, et al. TPX-0131, a potent CNS-penetrant, next-generation inhibitor of wild-type ALK and ALK-resistant mutations. *Mol Cancer Ther.* 2021;20(9):1499-1507.
48. Pelish HE, Tangpeerachaikul A, Kohl NE, et al. NUV-655 (NVL-655) is a selective, brain-penetrant ALK inhibitor with antitumor activity against the lorlatinib-resistant G1202R/L1196M compound mutation. Abstract 1468. *Cancer Res.* 2021;81(13 suppl):1468.
49. Katayama R, Shaw AT, Khan TM, et al. Mechanisms of acquired crizotinib resistance in ALK-rearranged lung cancers. *Sci Transl Med.* 2012;4(120):120ra17.
50. Doebele RC, Pilling AB, Aisner DL, et al. Mechanisms of resistance to crizotinib in patients with ALK gene rearranged non-small cell lung cancer. *Clin Cancer Res.* 2012;18(5):1472-1482.
51. Balla A, Khan F, Hampel KJ, Aisner DL, Sidiropoulos N. Small-cell transformation of ALK-rearranged non-small-cell adenocarcinoma of the lung. *Cold Spring Harb Mol Case Stud.* 2018;4(2):a002394.
52. Fares AF, Lok BH, Zhang T, et al. ALK-rearranged lung adenocarcinoma transformation into high-grade large cell neuroendocrine carcinoma: clinical and molecular description of two cases. *Lung Cancer.* 2020;146:350-354.

53. Gainor J. Next generation ALK inhibitors and mechanisms of resistance to therapy. *Ann Oncol.* 2015;26(suppl 2):iii14.
54. Yoda S, Lin JJ, Lawrence MS, et al. Sequential ALK inhibitors can select for lorlatinib-resistant compound ALK mutations in ALK-positive lung cancer. *Cancer Discov.* 2018;8(6):714-729.
55. Ruano-Ravina A, Provencio-Pulla M. Differential crizotinib response duration among ALK fusion variants in ALK-positive non-small-cell lung cancer. *Transl Cancer Res.* 2017;S54-S56.
56. Camidge DR, Dziadziuszko R, Peters S, et al. Updated efficacy and safety data and impact of the EML4-ALK fusion variant on the efficacy of alectinib in untreated ALK-positive advanced non-small cell lung cancer in the global phase III ALEX study [published correction appears in *J Thorac Oncol.* 2019 Nov;14(11):2023]. *J Thorac Oncol.* 2019;14(7):1233-1243.
57. Lin JJ, Zhu VW, Yoda S, et al. Impact of EML4-ALK variant on resistance mechanisms and clinical outcomes in ALK-positive lung cancer. *J Clin Oncol.* 2018;36(12):1199-1206.
58. Camidge R, Niu H, Kim HY, et al. Correlation of baseline molecular and clinical variables with ALK inhibitor efficacy in ALTA-1L. *J Clin Oncol.* 2020;38(15 suppl):9517-9517.
59. Childress MA, Himmelberg SM, Chen H, et al., ALK fusion partners impact response to ALK inhibition: differential effects on sensitivity, cellular phenotypes, and biochemical properties. *Mol Cancer Res.* 2018;16(11):1724-1736.
60. Crystal AS, Shaw AT, Sequist LV, et al. Patient-derived models of acquired resistance can identify effective drug combinations for cancer. *Science.* 2014;346(6216):1480-1486.
61. Lovly CM, Shaw AT. Molecular pathways: resistance to kinase inhibitors and implications for therapeutic strategies. *Clin Cancer Res.* 2014;20(9):2249-2256.
62. Friboulet L, Li N, Katayama R, et al. The ALK inhibitor ceritinib overcomes crizotinib resistance in non-small cell lung cancer. *Cancer Discov.* 2014;4(6):662-673.
63. Dagogo-Jack I, Yoda S, Lennerz JK, et al. MET alterations are a recurring and actionable resistance mechanism in ALK-positive lung cancer. *Clin Cancer Res.* 2020;26(11):2535-2545.
64. Lovly CM. Combating acquired resistance to tyrosine kinase inhibitors in lung cancer. *Am Soc Clin Oncol Educ Book.* 2015;e165-e173. doi:10.14694/EdBook_AM.2015.35.e165.
65. Miyawaki M, Yasuda H, Tani T, et al. Overcoming EGFR bypass signal-induced acquired resistance to ALK tyrosine kinase inhibitors in ALK-translocated lung cancer. *Mol Cancer Res.* 2017;15(1):106-114.
66. Tanizaki J, Okamoto I, Okabe T, et al. Activation of HER family signaling as a mechanism of acquired resistance to ALK inhibitors in EML4-ALK-positive non-small cell lung cancer. *Clin Cancer Res.* 2012;18(22):6219-6226.
67. Tanizaki J, Okamoto I, Takezawa K, et al. Combined effect of ALK and MEK inhibitors in EML4-ALK-positive non-small-cell lung cancer cells. *Br J Cancer.* 2012;106(4):763-767.

ROS1

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Gene Structure and Function

ROS proto-oncogene 1 (ROS1) is a receptor tyrosine kinase (RTK) that belongs to the insulin receptor family and is evolutionarily close to the ALK family. The *ROS1* gene is located on chromosome 6 (region 6q22.1) and encodes a transmembrane receptor protein with unique features. The extracellular N-terminal domain spans exons 1 through 32, which makes it one of the largest extracellular domains among all the human RTK family. The C-terminal portion of *ROS1* encodes a kinase domain (KD) and a single transmembrane (TM) domain (Figure 9-1). Very little is known about the physiologic role of wild-type ROS1 protein, and its ligands have not been identified in humans.^{1,2}

Type of Alterations and Role in Oncogenesis

Oncogenic *ROS1* gene rearrangements occur at the 5' end of exons 32, 34, 35, or 36, or introns 31 or 33.^{3,4} The extracellular coil-coil domain is not included in *ROS1* fusions, in contrast with the intracytoplasmic KD, which fuses with the N-terminal end of gene partners. The main partner genes identified are *CD74* (38%-54%), *EZR* (13%-24%), *SDC4* (9%-13%), *SLC34A2* (5%-10%), *TPM3* (3%-15%), and *FIG* or *GOPC* (2%-3%), with up to 28 other partners also reported to date but accounting for less than 1% each.⁵ The ROS1 fusion protein is an activated

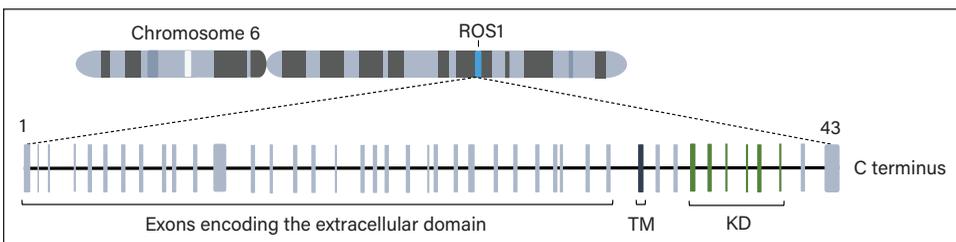


Figure 9-1. Schematic of *ROS1* gene on chromosome 6. Abbreviations: KD = kinase domain; TM = transmembrane.

kinase with oncogenic properties, which can activate different signaling pathways through phosphorylation such as the phosphoinositide 3-kinase (PI3K)-AKT-mechanistic target of rapamycin kinase (mTOR) pathway, which is involved in cell differentiation, proliferation, growth, and survival. Oncogenic *ROS1* fusions can occur in a large variety of cancers other than non-small cell lung carcinoma (NSCLC), including glioblastoma, cholangiocarcinoma, and inflammatory myofibroblastic tumor. No activating *ROS1* gene mutations or amplification have been reported in NSCLC, and *ROS1* rearrangements occurring with oncogenic co-mutations are extremely rare, mainly represented by *EGFR* or *KRAS* mutations.⁶

Epidemiologic, Clinical, and Histologic Characteristics

ROS1 fusions are very uncommon in lung cancer with a frequency ranging from 0.9% to 2.9%. These fusions are more prevalent in young patients, women, and never-smokers.^{7,8} They are usually diagnosed in patients with advanced-stage thyroid transcription factor-1 (TTF1)-positive adenocarcinomas^{1,9} and are only very rarely found in squamous cell or large cell carcinomas.¹⁰ *ROS1*-rearranged tumors usually have a solid architecture often with cribriform features, a psammoma-rich stroma, and signet-ring tumor cells.¹⁰ Patients may have an increased risk of thromboembolic events and disseminated intravascular coagulation.^{11,12}

Testing Strategies

Fluorescence in situ hybridization (FISH) is the gold standard for *ROS1* fusion detection, and most laboratories use dual-color break-apart probes (eg, *ROS1* Dual Color Break Apart Probe [CytoCell], ZytoLight SPEC *ROS1* Dual Color Break Apart Probe [ZytoVision/Zytomed], or Vysis *ROS1* Break Apart FISH Probe [Abbott]). The principle is to label the 3' (centromeric) part of the fusion breakpoint with 1 fluorochrome and the 5' (telomeric) part with another fluorochrome. Two *ROS1*-rearrangement patterns are considered diagnostic. One is the classic break-apart pattern, with 1 fusion signal (native *ROS1*) and 2 separated 3' and 5' signals. The other one, called *atypical*, takes into account an isolated 3' (green) signal, with 1 fusion signal (native *ROS1*) and no 5' signal.^{2,13,14} At least 50 tumor cells, ideally 100, have to be analyzed, and the threshold of positivity is 15% or more of positive cells. A count of 5 to 25 positive cells is considered as equivocal and needs a second analysis by another observer.

As *ROS1* rearrangements are rare, and FISH is time-consuming and not implemented in all pathology laboratories, immunohistochemistry (IHC) can be used as a screening technique.¹⁵ Three commercialized anti-*ROS1* antibodies are available: clone D4D6 (Cell Signaling Technology), used in clinical studies; clone SP384 (Roche Tissue Diagnostics, formerly Ventana); and clone 1A1 (Origene). They all have a high sensitivity (90%-100%) compared to FISH and next-generation sequencing (NGS), but with lower specificity, ranging from 70% to 90%, and FISH or other molecular techniques are required to confirm *ROS1* rearrangement in IHC-positive tumors.² The use of a positive external control, such as a *ROS1*-rearranged tumor sample or cell line, is highly recommended, as well as participation in external quality control programs. While there is no universally accepted scoring system, diffuse strong cytoplasmic staining is more likely to indicate a *ROS1*-rearranged tumor than patchy staining.¹⁶ While staining is usually cytoplasmic and granular, Golgi body or membrane staining have been reported in some variants (*CD74* and *EZR*).¹⁴ Of note, normal type II pneumocytes and macrophages are often stained, as well as one-third of *EGFR*-, *ALK*-, or

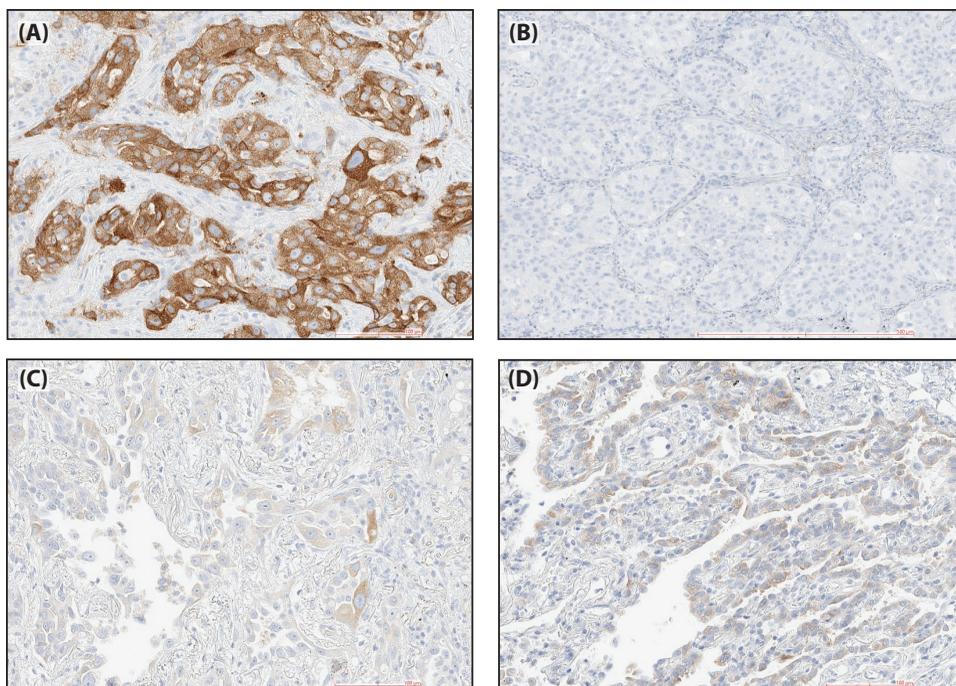


Figure 9-2. (A) ROS1 immunohistochemistry (IHC) (clone D4D6) in a lung adenocarcinoma with *CD74::ROS1* fusion; strong and diffuse cytoplasmic staining. (B) ROS1 IHC (clone D4D6) in a lung adenocarcinoma with exon 21 p.L858R *EGFR* gene mutation; no staining. (C) ROS1 IHC (clone D4D6) in a lung adenocarcinoma with exon 21 p.L858R *EGFR* gene mutation; faint and focal cytoplasmic staining. (D) ROS1 IHC (clone D4D6) in a lung adenocarcinoma with *KIF5B::RET* fusion; faint and focal cytoplasmic staining.

MET-driven NSCLC^{13,17} (Figure 9-2). Confirmation of *ROS1* fusion in IHC-positive tumors is required and can be undertaken by FISH or molecular techniques. The latter include multiplex reverse transcription polymerase chain reaction (RT-PCR) and NGS.¹⁵ Hybrid DNA-RNA or RNA-based NGS designed for detecting rearrangements in intron regions are favored.¹⁸ Interestingly, comprehensive genomic profiling technologies are now able to detect any fusion in up to 92% of circulating tumor DNA (ctDNA) samples and 10 of 13 *ROS1* fusions.¹⁹

Clinical Implications: Treatment of *ROS1* Tumors in NSCLC Patients

Crizotinib and entrectinib are the 2 agents that the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) approved for treating *ROS1*-rearranged NSCLC,²⁰⁻²³ although other agents have also been developed (Table 9-1).

Crizotinib has activity against *ALK*- and *ROS1*-rearranged tumors and was approved based on the PROFILE 1001 trial in metastatic *ROS1*-rearranged NSCLC, with an overall response rate (ORR) of 72%, a disease control rate (DCR) of 90%, a median duration of response (DOR) of 24.7 months, and a median progression-free survival (PFS) and overall survival (OS) rate at 19.3 and 51.4 months, respectively.²⁴ The phase 3 AcSé trial found an ORR of 47.2%, with median PFS and OS, respectively, at 5.5 and 17.2 months.²⁵ EUCROSS and METROS studies showed ORR of 70% and 65%, respectively, with median PFS at 20 and 22.8 months.^{26,27} The most common side effects, all of grade 1 and 2, were vision disturbances (82%), diarrhea (44%), and nausea (40%). Because crizotinib has low central nervous

Table 9-1. ROS1 Inhibitors (Approved and in Development)

TKI	Phase (Clinical Study)	N	ORR (%)	mPFS (months)	mOS (months)
Crizotinib	1 and 2 (Profile 1001)	53	72	19	51
	1 and 2 (AcSé)	36	47	6	17
	2 (EUCROSS)	34	70	20	NR
	2 (METROS)	26	65	23	NR
Ceritinib	2	32	62	19	24
Entrectinib	2 (STARTRK 1/2, ALKA 372)	172	69	18	NR
Lorlatinib	1 and 2	69	62 ^a	21	NA
			35 ^b	9	NA
Cabozantinib	2	6	33	NA	NA
Taletrectinib	2	40	90 ^c	NA	NA
			47 ^d	NA	NA
Repotrectinib	2	55	86	30.9	NA
Ensartinib	2	59	27	NA	NA
TQ-B3101	2	111	78	15	NA

Abbreviations: mOS = median overall survival; mPFS = median progression-free survival; NA = not applicable; NR = not reported; ORR = overall response rate; TKI = tyrosine kinase inhibitor.

^a First-line setting.

^b Second-line setting and beyond.

^c Crizotinib naïve.

^d Crizotinib resistance.

system (CNS) penetration, brain metastasis developed in up to 47% of patients on top of the 36% of patients with *ROS1*-rearranged NSCLC who had brain metastases at diagnosis.^{9,28}

Ceritinib has activity against *ALK*- and *ROS1*-rearranged tumors²⁹ and in a phase 2 study, 32 *ROS1*-rearranged NSCLCs, most in first-line (1L) settings, achieved an ORR of 62% and a median PFS of 9.3 months³⁰; 8 patients with brain metastases had an ORR of 63%. The toxicity profile was acceptable with 37% grade-3 adverse events.

Entrectinib targets *ROS1*, *ALK*, and *NTRK* fusions in NSCLC.²³ The phase 2 study STARTRK-2 confirmed entrectinib efficacy in 172 patients with *ROS1*-rearranged NSCLCs,^{31,32} with an ORR of 69%, a median PFS of 17.7 months, and a median 1-year OS of 81%. Because of its CNS penetration, the ORR was 79.2% for patients with brain metastases. Most frequent adverse events were dysgeusia (41.4%), fatigue (27.9%), vertigo (25.4%), and constipation (23.7%).

Lorlatinib has activity against *ALK* and *ROS1*, with an ORR in 1L patients of 62%, a median PFS of 21 months, and brain ORR of 64%.³³⁻³⁵ In patients already treated with crizotinib, ORR, median PFS, and intracerebral ORR were of 35%, 8.5 months, and 50%, respectively. The most common adverse events of grades 3 or 4 occurred in 43% and 6% of the patients, respectively, the most frequent being hypercholesterolemia (65%), hypertriglyceridemia (42%), peripheral edema (39%), and peripheral neuropathies (35%).

Repotrectinib can target *ROS1*, *NTRK*, or *ALK* and has CNS activity. Preclinical models showed antitumor activity against brain metastases, treatment-naïve tumors, ceritinib-resistant tumors, and tumors with 1G2032R-resistant mutation.³⁶ A clinical phase 1 and 2 trial is ongoing.

Ensartinib (X-396) is a tyrosine kinase inhibitor (TKI) with proven efficacy against ALK,³⁷ and a phase 2 trial of ROS1-positive NSCLCs showed low efficacy with an ORR of 27% but CNS activity in three-fourths of patients.³⁸

Resistance Mechanisms

On-target resistance mechanisms include acquired ROS1 mutations,³⁹⁻⁴⁵ with G2032R as the most common followed by D2033N, and both are solvent-front mutations. Importantly, L2086F is emerging as an important resistance mutation as it confers resistance to all type I ROS1 TKIs (crizotinib, lorlatinib, taletrectinib).⁴³⁻⁴⁵ Other rarer acquired resistance mutations include gatekeeper mutations L2026M, G2032K, S1986F/Y, L2000V, and F2004V.³⁹⁻⁴⁵

Lorlatinib has efficacy against the K1991E- or S1986F-resistant mutations but is more limited against the G2032R mutation^{33,46} and after failure on entrectinib.⁴⁷ Sequential use of crizotinib and lorlatinib has led to compound mutations of G2032R/L2086F, but fortunately, cabozantinib is expected to overcome these compound mutations.^{44,48} It selectively targets MET, VEGFR2, RET, ROS1, and AXL, with good brain penetration. It can be used to overcome the resistance against crizotinib, ceritinib, and entrectinib despite the presence of resistance mutations, such as D2033N or G2032R.^{30,49,50} Brigatinib, another ROS1 inhibitor, has demonstrated antitumor activity against several crizotinib-resistance mechanisms,⁵¹ including L2026M mutation but not against the G2032R mutation.⁵¹⁻⁵³ Taletrectinib is a ROS1 and NTRK inhibitor targeting resistant ROS1-rearranged NSCLCs,⁴⁸ with in vitro activity against G2032R, L1951R, S1986F, and L2026M mutations, but less against D2033N mutation.

Off-target resistance mechanisms involve MET amplification, KRAS mutations, and small cell lung transformation.^{46,54,55} Finally, like all RTK fusion-positive NSCLC, progression in the CNS is always a resistance mechanism,⁵⁶ and ROS1 TKIs that confer CNS activity plus overcome many of the predicted acquired ROS1 mutations in ROS1-rearranged NSCLC should be the treatment of choice in this setting.

Recently, data from TQ-B3101, a novel small-molecule RTK inhibitor, which targets to ALK, ROS1, and MET was released during the European Lung Cancer Congress (ELCC) 2022 showing that in 111 patients with ROS1-rearranged NSCLC, it can achieve ORR 78.4% and DCR of 87.4%. Median PFS 15.6 months and median DOR was 20.3. Median OS has not been reached and the 12- and 24-month OS rate were 98.1% and 88.1%, respectively. No data has been released regarding resistance yet.⁵⁷

References

1. Drilon A, Jenkins C, Iyer S, et al. ROS1-dependent cancers: biology, diagnostics and therapeutics. *Nat Rev Clin Oncol*. 2021;18(1):35-55.
2. Bubendorf L, Büttner R, Al-Dayel F, et al. Testing for ROS1 in non-small cell lung cancer: a review with recommendations. *Virchows Arch*. 2016;469(5):489-503.
3. Davies KD, Doebele RC. Molecular pathways: ROS1 fusion proteins in cancer. *Clin Cancer Res*. 2013;19(15):4040-4045.
4. Cui M, Han Y, Li P, et al. Molecular and clinicopathological characteristics of ROS1-rearranged non-small-cell lung cancers identified by next-generation sequencing. *Mol Oncol*. 2020;14(11):2787-2795.
5. Gendarme S, Bylicki O, Chouaid C, Guisier F. ROS-1 fusions in non-small-cell lung cancer: evidence to date. *Curr Oncol*. 2022;29(2):641-658.
6. Lambros L, Guibourg B, Uguen A. ROS1-rearranged non-small cell lung cancers with concomitant oncogenic driver alterations:

- about some rare therapeutic dilemmas. *Clin Lung Cancer*. 2018;19(1):e73-74.
7. Zhang Q, Wu C, Ding W, et al. Prevalence of ROS1 fusion in Chinese patients with non-small cell lung cancer. *Thorac Cancer*. 2019;10(1):47-53.
 8. Cai W, Li X, Su C, et al. ROS1 fusions in Chinese patients with non-small-cell lung cancer. *Ann Oncol*. 2013;24(7):1822-1827.
 9. Patil T, Smith DE, Bunn PA, et al. The incidence of brain metastases in stage IV ROS1-rearranged non-small cell lung cancer and rate of central nervous system progression on crizotinib. *J Thorac Oncol*. 2018;13(11):1717-1726.
 10. Park E, Choi YL, Ahn MJ, Han J. Histopathologic characteristics of advanced-stage ROS1-rearranged non-small cell lung cancers. *Pathol Res Pract*. 2019;215(7):152441.
 11. Zhu VW, Zhao JJ, Gao Y, et al. Thromboembolism in ALK+ and ROS1+ NSCLC patients: a systematic review and meta-analysis. *Lung Cancer*. 2021;157:147-155.
 12. Woodford R, Lu M, Beydoun N, et al. Disseminated intravascular coagulation complicating diagnosis of ROS1-mutant non-small cell lung cancer: a case report and literature review. *Thorac Cancer*. 2021;12(17):2400-2403.
 13. Mescam-Mancini L, Lantuéjoul S, Moro-Sibilot D, et al. On the relevance of a testing algorithm for the detection of ROS1-rearranged lung adenocarcinomas. *Lung Cancer*. 2014;83(2):168-173.
 14. Yoshida A, Tsuta K, Wakai S, et al. Immunohistochemical detection of ROS1 is useful for identifying ROS1 rearrangements in lung cancers. *Mod Pathol*. 2014;27(5):711-720.
 15. Kalemkerian GP, Narula N, Kennedy EB, et al. Molecular testing guideline for the selection of patients with lung cancer for treatment with targeted tyrosine kinase inhibitors: American Society of Clinical Oncology Endorsement of the College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology Clinical Practice Guideline Update. *J Clin Oncol*. 2018;36(9):911-919.
 16. Fielder T, Butler J, Tierney G, et al. ROS1 rearrangements in lung adenocarcinomas are defined by diffuse strong immunohistochemical expression of ROS1. *Pathology*. 2022;54(4):399-403.
 17. Mitiushkina NV, Romanko AA, Preobrazhenskaya EV, et al. Comprehensive evaluation of the test for 5'-/3'-end mRNA unbalanced expression as a screening tool for ALK and ROS1 fusions in lung cancer. *Cancer Med*. 2022;11(17):3226-3237.
 18. Paz-Ares L, Gonds A, Saldana D, et al. Genomic testing among patients with newly diagnosed advanced non-small cell lung cancer in the United States: a contemporary clinical practice patterns study. *Lung Cancer*. 2022;167:41-48.
 19. Lee JK, Hazar-Rethinam M, Decker B, et al. The pan-tumor landscape of targetable kinase fusions in circulating tumor DNA. *Clin Cancer Res*. 2022;28(4):728-737.
 20. FDA approves entrectinib for NTRK solid tumors and ROS-1 NSCLC [news release]. US Food and Drug Administration, August 16, 2019. <https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-entrectinib-ntrk-solid-tumors-and-ros-1-nsclc>.
 21. FDA approves crizotinib capsules [news release]. US Food and Drug Administration, March 11, 2016. <https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-crizotinib-capsules>.
 22. Meeting highlights from the Committee for Medicinal Products for Human Use (CHMP) October 19-22, 2015. European Medicines Agency, October 23, 2015. <https://www.ema.europa.eu/en/news/meeting-highlights-committee-medicinal-products-human-use-chmp-19-22-october-2015>.
 23. Fan Y, Drilon A, Chiu C-H, et al. Entrectinib in patients with ROS1 fusion-positive (ROS1-fp) NSCLC: updated efficacy and safety analysis. *J Thorac Oncol*. 2022;17(9 suppl):S89-S90.
 24. Shaw AT, Ou SHI, Bang YJ, et al. Crizotinib in ROS1-rearranged non-small-cell lung cancer. *N Engl J Med*. 2014;371(21):1963-1971.
 25. Moro-Sibilot D, Cozic N, Pérol M, et al. Crizotinib in c-MET- or ROS1-positive NSCLC: results of the AcSé phase II trial. *Ann Oncol*. 2019;30(12):1985-1991.
 26. Michels S, Massutí B, Schildhaus HU, et al. Safety and efficacy of crizotinib in patients with advanced or metastatic ROS1-rearranged lung cancer (EUCROSS): a European phase II clinical trial. *J Thorac Oncol*. 2019;14(7):1266-1276.
 27. Chiari R, Ricciuti B, Landi L, et al. ROS1-rearranged non-small-cell lung cancer is associated with a high rate of venous thromboembolism: analysis from a phase II, prospective, multicenter, two-arms trial (METROS). *Clin Lung Cancer*. 2020;21(1):15-20.

28. Wu YL, Yang JCH, Kim DW, et al. Phase II study of crizotinib in east Asian patients with ROS1-positive advanced non-small-cell lung cancer. *J Clin Oncol.* 2018;36(14):1405-1411.
29. Shaw AT, Kim TM, Crinò L, et al. Ceritinib versus chemotherapy in patients with ALK-rearranged non-small-cell lung cancer previously given chemotherapy and crizotinib (ASCEND-5): a randomised, controlled, open-label, phase 3 trial. *Lancet Oncol.* 2017;18(7):874-886.
30. Drilon A, Somwar R, Wagner JP, et al. A novel crizotinib-resistant solvent-front mutation responsive to cabozantinib therapy in a patient with ROS1-rearranged lung cancer. *Clin Cancer Res.* 2016;22(10):2351-2358.
31. Drilon A, Siena S, Dziadziuszko R, et al. Entrectinib in ROS1 fusion-positive non-small-cell lung cancer: integrated analysis of three phase 1-2 trials. *Lancet Oncol.* 2020;21(2):261-270.
32. Dziadziuszko R, Krebs MG, De Braud F, et al. Updated integrated analysis of the efficacy and safety of entrectinib in locally advanced or metastatic ROS1 fusion-positive non-small-cell lung cancer. *J Clin Oncol.* 2021;39(11):1253-1263.
33. Shaw AT, Felip E, Bauer TM, et al. Lorlatinib in non-small-cell lung cancer with ALK or ROS1 rearrangement: an international, multicentre, open-label, single-arm first-in-man phase 1 trial. *Lancet Oncol.* 2017;18(12):1590-1599.
34. Shaw AT, Solomon BJ, Chiari R, et al. Lorlatinib in advanced ROS1-positive non-small-cell lung cancer: a multicentre, open-label, single-arm, phase 1-2 trial. *Lancet Oncol.* 2019;20(12):1691-1701.
35. Solomon BJ, Besse B, Bauer TM, et al. Lorlatinib in patients with ALK-positive non-small-cell lung cancer: results from a global phase 2 study. *Lancet Oncol.* 2018;19(12):1654-1667.
36. Yun MR, Kim DH, Kim SY, et al. Repotrectinib exhibits potent antitumor activity in treatment-naïve and solvent-front-mutant ROS1-rearranged non-small cell lung cancer. *Clin Cancer Res.* 2020;26(13):3287-3295.
37. Horn L, Infante JR, Reckamp KL, et al. Ensartinib (X-396) in ALK-positive non-small cell lung cancer: results from a first-in-human phase I/II, multicenter study. *Clin Cancer Res.* 2018;24(12):2771-2779.
38. Ai X, Wang Q, Cheng Y, et al. Safety but limited efficacy of ensartinib in ROS1-positive NSCLC: a single-arm, multicenter phase 2 study. *J Thorac Oncol.* 2021;16(11):1959-1963.
39. Awad MM, Katayama R, McTigue M, et al. Acquired resistance to crizotinib from a mutation in CD74-ROS1. *N Engl J Med.* 2013;368(25):2395-2401.
40. Gainor JF, Tseng D, Yoda S, et al. Patterns of metastatic spread and mechanisms of resistance to crizotinib in ROS1-positive non-small-cell lung cancer. *JCO Precis Oncol.* 2017;2017:PO.17.00063.
41. Dagoogo-Jack I, Rooney M, Nagy RJ, et al. Molecular analysis of plasma from patients with ROS1-positive NSCLC. *J Thorac Oncol.* 2019;14(5):816-824.
42. Dimou A, Ou SHI, Doebele RC. Dramatic response to lorlatinib in a patient with CD74-ROS1-positive lung adenocarcinoma with acquired F2004V mutation. *JCO Precis Oncol.* 2019;3:PO.19.00013.
43. Zhang Y, Huang Z, Zeng L, et al. Disease progression patterns and molecular resistance mechanisms to crizotinib of lung adenocarcinoma harboring ROS1 rearrangements. *NPJ Precis Oncol.* 2022;6(1):20.
44. Lin JJ, Choudhury NJ, Yoda S, et al. Spectrum of mechanisms of resistance to crizotinib and lorlatinib in ROS1 fusion-positive lung cancer. *Clin Cancer Res.* 2021;27(10):2899-2909.
45. Papadopoulos KP, Borazanci E, Shaw AT, et al. U.S. Phase I first-in-human study of taletrectinib (DS-6051b/AB-106), a ROS1/TRK inhibitor, in patients with advanced solid tumors. *Clin Cancer Res.* 2020;26(18):4785-4794.
46. Wu X, Wang Y, Wan S, Zhang J. Investigation on the binding mechanism of lorlatinib with the c-ros oncogene 1 (ROS1) receptor tyrosine kinase via molecular dynamics simulation and binding free energy calculations. *J Biomol Struct Dyn.* 2018;36(12):3106-3113.
47. Facchinetti F, Levy A, Ammari S, et al. Meningeal "Lazarus response" to lorlatinib in a ROS1-positive NSCLC patient progressing to entrectinib. *Cancer Manag Res.* 2021;13:2805-2810.
48. Li W, Yang N, Ma H-W, et al. The efficacy and safety of taletrectinib in patients with TKI-naïve or crizotinib-pretreated ROS1-positive non-small cell lung cancer (NSCLC) [abstract 8572]. *J Clin Oncol.* 2022;40(suppl 16):8572. doi:10.1200/JCO.2022.40.16_suppl.8572.
49. Sun TY, Niu X, Chakraborty A, Neal JW, Wakelee HA. Lengthy progression-free survival and intracranial activity of cabozantinib in patients with crizotinib and ceritinib-resistant ROS1-positive non-small cell lung cancer. *J Thorac Oncol.* 2019;14(2):e21-24.

50. Katayama R, Kobayashi Y, Friboulet L, et al. Cabozantinib overcomes crizotinib resistance in ROS1 fusion-positive cancer. *Clin Cancer Res.* 2015;21(1):166-174.
51. Zhang S, Anjum R, Squillace R, et al. The potent ALK inhibitor brigatinib (AP26113) overcomes mechanisms of resistance to first- and second-generation ALK inhibitors in preclinical models. *Clin Cancer Res.* 2016;22(22):5527-5538.
52. Hegde A, Hong DS, Behrang A, et al. Activity of brigatinib in crizotinib and ceritinib-resistant ROS1-rearranged non-small-cell lung cancer. *JCO Precis Oncol.* 2019;3:PO.18.000267.
53. Camidge DR, Kim DW, Tiseo M, et al. Exploratory analysis of brigatinib activity in patients with anaplastic lymphoma kinase-positive non-small-cell lung cancer and brain metastases in two clinical trials. *J Clin Oncol.* 2018;36(26):2693-2701.
54. McCoach CE, Le AT, Gowan K, et al. Resistance mechanisms to targeted therapies in ROS1+ and ALK+ non-small cell lung cancer. *Clin Cancer Res.* 2018;24(14):3334-3347.
55. Lin JJ, Langenbucher A, Gupta P, et al. Small cell transformation of ROS1 fusion-positive lung cancer resistant to ROS1 inhibition. *NPJ Precis Oncol.* 2020;4:21.
56. Ou SHI, Zhu VW. CNS metastasis in ROS1+ NSCLC: an urgent call to action, to understand, and to overcome. *Lung Cancer.* 2019;130:201-207.
57. Lu S, Pan H, Wu L, et al. The efficacy and safety of TQ-B3101 monotherapy in the first-line treatment in patients with ROS1-positive non-small cell lung cancer. *Ann Oncol.* 2022;33(suppl 2):S27-S70. doi:10.1016/annonc/annonc856.

Gene Structure, Signaling, and Function

V-Raf murine sarcoma viral oncogene homolog B (*BRAF*) gene encodes BRAF kinase, a member of the RAF family of serine/threonine kinases that are components of the RTK-RAS-RAF-MEK-ERK growth factor signaling pathway (RAS/MAPK signaling).¹ The BRAF kinase is an important step in intracellular signaling after activation of EGFR. BRAF is a 95 kDa serine/threonine kinase protein encoded in the long arm of chromosome 7 (7q). Globally, BRAF plays a central role in regulating cell proliferation, division, and death. In normal tissues, the BRAF kinase is generally silenced via negative feedback once the signal has moved on to the next point in the cascade. *BRAF* mutations lead to activation of the RAS-RAF-MEK-ERK pathway resulting in uncontrolled cell growth and proliferation.

Type of Alterations and Role in Oncogenesis

Mutations

Nearly 300 distinct *BRAF* mutations have been identified in various tumors and cancer cell lines including melanoma, colorectal cancer, papillary thyroid cancer, and non-small cell lung carcinoma (NSCLC). Most of these mutations occur in the activation loop near the V600 codon, or in the phosphate-binding loop at residues 464 through 469 and have been classified into 3 classes.² Classes of *BRAF* mutations are reported based on key aspects: (1) kinase activity, (2) status of dimerization, and (3) RAS dependency.

Class I *BRAF* mutations are considered RAS-independent because of their high kinase activity even in their monomeric status. Point mutations in codon 600 of exon 15 (*BRAF* V600) belong to this class, which result in the substitution of valine with other amino acids such as glutamate (V600E), lysine (V600K), aspartate (V600D), methionine (V600M), leucine (V600L), and arginine (V600R). Class I mutants promote constitutive activation of the mitogen-activated protein kinase (MAPK) pathway, causing strong activation of BRAF

kinase. These types of mutations are usually highly sensitive to BRAF and MAPK kinase (MEK) inhibitors.

Class II *BRAF* mutations are characterized by an intermediate kinase activity that allows RAS independency but requires homodimer formation to be fully active. The class II mutants, including *K601*, *L597*, *G464*, and *G469* mutations, are located in the activation segment or P-loop and signal as RAS-independent dimers.

Class III *BRAF* mutations have a compromised kinase activity, thus requiring RAS activation and wild-type CRAF heterodimer formation to be fully functional. These class III mutants occur in the P-loop, catalytic loop, or DFG motif, and have impaired BRAF kinase activity. Class II and III mutations are non-V600 mutations. Approximately 50% of *BRAF* mutations in NSCLC are non-V600 mutations.

Concurrent Mutations in *BRAF*-Mutant NSCLC

Simultaneous genetic alterations may be identified in up to 90% of patients with *BRAF*-mutant NSCLC. *TP53*, *STK11*, *KRAS*, *NF1*, and some tyrosine kinase receptors are the most frequently co-altered genes both in tissue specimens and cell-free DNA (cfDNA). Alterations in *SMAD4* and *PIK3CA* are associated with *BRAF* V600E, whereas alterations in *KEAP1*, *NF1*, *MET*, *RICTOR*, *KRAS*, *MYC*, *STK11*, and *TP53* occur more frequently with non-V600E *BRAF* mutations.

Deletions

BRAF deletion mutations can occur in solid tumors and may serve as a type of resistance mechanism to BRAF inhibitors and MEK inhibitors. Deletion mutations happen adjacent to the α C helix in the kinase domain of *BRAF*, resulting in enhanced kinase activity by suppressing the α C helix in its active conformation.

Fusions

At least 18 different 5' fusion partners have been found across different cancer types including NSCLC. The most common fusion partner is *AGK* in NSCLC. The rate of occurrence of *BRAF* fusions is less than 1% in NSCLC, and all NSCLCs with *BRAF* fusions are adenocarcinomas.³ Most *BRAF* fusion patterns are in-frame with breakpoints in the BRAF kinase domain. Activating *BRAF* fusions occur by truncation of the N-terminal CR1 auto-inhibitory domain, leading to the constitutive activation of the BRAF pathway that resembles class II *BRAF* mutants.

Amplifications

Acquired resistance to MEK1/2 inhibitors (MEKi) arises through amplification of *BRAF* V600E or *KRAS* to reinstate ERK1/2 signaling. *BRAF* V600E amplification and MEKi resistance are reversible following drug withdrawal. Cells with a *BRAF* V600E amplification are addicted to MEKi to maintain a precise level of ERK1/2 signaling that is optimal for cell proliferation and survival and tumor growth in vivo. *BRAF* V600E amplification confers a selective disadvantage after drug withdrawal, validating intermittent dosing to forestall resistance.

Epidemiologic, Clinical, and Histologic Characteristics

BRAF mutations are present in 8% of human cancers, predominantly in hairy cell leukemia (100%), melanoma (40%-50%), thyroid carcinoma (10%-70%, depending on the histologic subtype), colorectal cancer (10%), and NSCLC (3%-8%).

In NSCLC, *BRAF* mutations are detected in 3.7% of male patients and in 4% of female patients.^{4,5} *BRAF* mutations have been associated with a history of smoking and are detected in around 4.7% of former or current smokers and in 3% of never-smokers. Adenocarcinoma represents the most common histologic pattern as *BRAF* mutations are detected in approximately 4% of adenocarcinomas and in 0.6% of non-adenocarcinomas.^{4,5} *BRAF* mutations are detected in approximately 4% of patients with stage I or II disease and in 7.6% of patients with stage III or IV cancers. *BRAF* V600E mutations account for 54% of all the *BRAF* mutations in NSCLC, with a higher prevalence in female patients (71% vs 38% in male *BRAF*-mutant cases) and stage III or IV disease (57% vs 48% for stage I-II *BRAF*-mutant cases). Globally, *BRAF* mutations are more associated with female patients, but non-V600E mutations seem to occur with a higher frequency in male patients.⁵ Most patients with *BRAF*-mutant NSCLC are current or former smokers but with differences in prevalence: 20% to 30% of patients harboring V600E mutations are never-smokers, and almost all patients with non-V600E alterations are heavy smokers.⁵

BRAF-mutated NSCLC has a propensity toward involvement of the central nervous system. Of note, the incidence of brain metastases at diagnosis is significantly lower for patients with class I alterations compared with classes II and III. The prognostic significance of *BRAF* mutations in NSCLC is unclear. In early stages, patients resected for *BRAF* V600E-mutated NSCLC show shorter disease-free survival and overall survival (OS) rates compared with patients with wild-type mutations, but such a difference did not emerge when comparing all patients with *BRAF* mutations (both V600E and non-V600E) with the wild-type population. In the metastatic setting, no differences in progression-free survival and OS between patients with *BRAF*-mutated and wild-type disease have been identified, but a poorer clinical outcome after platinum-based chemotherapy is reported for patients with *BRAF* V600E-mutated tumors compared with non-V600E.

Testing Strategies

Immunohistochemistry

The antibody used for immunohistochemistry (IHC) for the mutant BRAF protein is clone VE1 (Figure 10-1).⁶ The aim of IHC is to identify a qualitative change (ie, the presence or absence of the protein). The limitation of this test is that it can only test for the *BRAF* V600E mutation and not non-V600E mutations.⁷⁻⁹ Data are limited, but the VE1 clone has the potential to stain between 90% and 100% of p.V600E-mutant adenocarcinomas.¹⁰ Currently, no standard recommendation or consensus has been made for the use of BRAF p.V600E IHC testing of NSCLC.

Molecular Testing

BRAF mutations are identified by DNA sequencing techniques such as the allele-specific polymerase chain reaction (PCR) or next-generation sequencing (NGS). These 2 methods

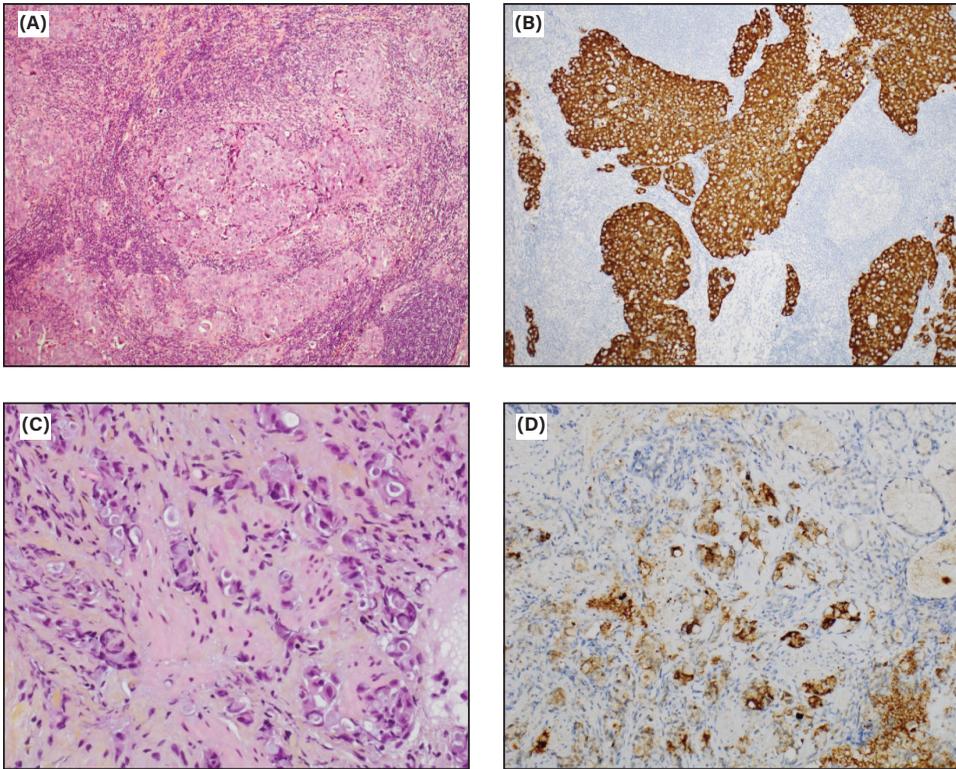


Figure 10-1. (A and B) Solid adenocarcinoma (lung tumor resection, ×40). **(A)** Hematoxylin and eosin (H&E). **(B)** Immunoperoxidase (anti-BRAF V600E; VE1 clone). **(C and D)** Invasive mucinous adenocarcinoma (bronchial biopsy). **(C)** H&E. **(D)** Immunoperoxidase (anti-BRAF V600E; VE1 clone).

possess a comparable sensitivity, specificity, and concordance rate in tissue specimens; however, because of its ability to simultaneously identify multiple oncogenic alterations, NGS is widely considered the preferred test.

Polymerase Chain Reaction

The most commonly used assay is reverse transcription (RT)-PCR. Currently, the Cobas 4800 *BRAF* V600 Mutation Test (Roche Diagnostics) and THxID-*BRAF* kit (Biomérieux) are US Food and Drug Administration (FDA)-approved companion diagnostic tests. Other laboratory-developed tests have also been utilized to test for *BRAF* mutation status, and depending on local approvals, confirmatory tests via other methods may or may not be necessary for reimbursement purposes. The major advantages of RT-PCR are faster turnaround time, reproducibility, specificity, sensitivity, and a lower cost compared with gene sequencing methods. However, some of these methods are merely for the *BRAF* V600E mutation located in exon 15. Therefore, they may lack the ability to detect exon 11 mutations that are also seen in NSCLC.

Next-Generation Sequencing

NGS with a multiple gene panel should be used to evaluate the *BRAF* V600E mutation and non-V600E mutations that can occur in exon 11 and exon 15.¹¹ In addition, with the discovery

of more novel rare driver genes, there is an increased need for multigene testing compared to single-gene approaches. The advantages of NGS include:

- Requires relatively little tumor tissue
- Facilitates testing of multiple biomarkers
- Includes emerging biomarkers for enrollment in clinical trials
- May detect co-mutations occurring with *BRAF* mutations

Generally, it is also more economical than sequential testing. However, because of the amount of data, interpreting NGS reports may be complex, and NGS is not universally available. The turnaround time of NGS is longer than that of RT-PCR and IHC assays.

BRAF Testing with Liquid Biopsies

BRAF V600E mutations can be detected in cfDNA via droplet digital PCR or RT-PCR.¹² *BRAF* V600E and non-V600E mutations can also be detected using NGS in circulating free DNA. Data are lacking as to whether cfDNA could be an alternative analyte for identifying *BRAF* mutations if tissue specimens are unavailable. Matching plasma to tumor formalin-fixed paraffin-embedded samples resulted in a high concordance rate for *BRAF* mutations when using PCR or NGS techniques.¹³

Clinical Implications

The treatment of patients with *BRAF*-mutated NSCLC is mainly divided into 2 types, one for the *BRAF* V600 mutation, the other for *BRAF* non-V600 mutated cases. Current approved targeted drugs were specifically designed around the structure of *BRAF* V600E, while novel therapeutic strategies continue to be explored for class II/III *BRAF* non-V600E in clinical trials.

Targeted Therapy

The identification of *BRAF* mutations and the implication of the MAPK pathway in NSCLC has led to the development of several highly potent and selective adenosine triphosphate (ATP)-competitive MAPK inhibitors, specifically designed to bind to the ATP-binding pocket of the active conformation of *BRAF*, especially *BRAF* V600E, including vemurafenib or dabrafenib.¹⁴ However, despite their effective anti-tumor activity, resistance to *BRAF* inhibitors eventually develops, mostly because of the reactivation of the downstream MEK/REK pathway. The addition of MEK1/2 inhibitors such as trametinib, significantly improved treatment outcomes (overall response rate [ORR] and median progression-free survival [mPFS] of 33% and 5.4 months vs 68% and 10.2 months for dabrafenib or dabrafenib plus trametinib, respectively, in patients with previously treated *BRAF* V600E-mutant metastatic NSCLC).^{15,16} To date, FDA and European Medicines Agency (EMA) have approved dual targeted therapy (dabrafenib and trametinib) for *BRAF* V600E-mutant NSCLC, which is also recommended by the National Comprehensive Cancer Network (NCCN) and European Society for Medical Oncology (ESMO) guidelines.¹⁷⁻²¹

Immunotherapy

Very few data on the benefit of immune checkpoint inhibitors (ICIs) in the *BRAF*-mutant population are available. The results of retrospective studies are generally disappointing. The international IMMUNOTARGET study showed poor outcomes in patients with *BRAF*-mutant disease, with an ORR of 24% and a mPFS of 3.1 months.²² In another retrospective study, ORR to ICI was 9% in class I-altered tumors and 26% in class II/III, with median time on treatment of 1.9 months in both groups.²³ Prospective clinical trials are necessary to identify the optimal use of ICIs with or without *BRAF*-targeted therapy for these patients.²³ Results reinforce the strong place of targeted therapies in patients with *BRAF*-mutant V600E NSCLC.

Chemotherapy

Chemotherapy outcomes are little known in this population. There is no clear evidence that *BRAF* status influences PFS or OS in patients treated with chemotherapy.²⁴

Resistance Mechanisms

As with other targeted therapies for NSCLC, resistance to *BRAF* pathway inhibitors inevitably occurs, leading to disease progression.^{2,25,26} The underlying resistance mechanisms to *BRAF* inhibitors are still poorly understood in the context of NSCLC. Reactivation of extracellular signal-regulated kinase (ERK) signaling through the MAPK pathway represents the main mechanism that involves secondary resistance to *BRAF* inhibitors and can occur either upstream or downstream of *BRAF* kinases. The mechanisms involved include secondary mutations in other genes involved in the MAPK signaling pathway leading to *BRAF*-independent reactivation of ERK signaling, such as *NRAS/KRAS* or *MEK1/2* mutations or *BRAF* splice variants and gene amplifications that increase the level of *BRAF* V600E homodimers. Acquired mutation in the *BRAF* gene has been rarely described as a resistance mechanism. Bypass activation is the main cause of secondary resistance of targeted therapy. Phosphatase and tensin homolog (*PTEN*) lack-of-function alterations may contribute to resistance to combinations of dabrafenib and trametinib in *BRAF* V600E tumors.

References

1. Poulidakos PI, Sullivan RJ, Yaeger R. Molecular pathways and mechanisms of *BRAF* in cancer therapy. *Clin Cancer Res*. 2022;28(21):4618-4628. doi:10.1158/1078-0432.CCR-21-2138.
2. Riudavets M, Cascetta P, Planchard D. Targeting *BRAF*-mutant non-small cell lung cancer: current status and future directions. *Lung Cancer*. 2022;169:102-114. doi:10.1016/j.lungcan.2022.05.014.
3. Kazdal D, Hofman V, Christopoulos P, et al. Fusion-positive non-small cell lung carcinoma: biological principles, clinical practice, and diagnostic implications. *Genes Chromosomes Cancer*. 2022;61(5):244-260. doi:10.1002/gcc.23022.
4. Chen D, Zhang LQ, Huang JF, et al. *BRAF* mutations in patients with non-small cell lung cancer: a systematic review and meta-analysis. *PLoS One*. 2014;9(6):e101354. doi:10.1371/journal.pone.0101354.
5. Marchetti A, Felicioni L, Malatesta S, et al. Clinical features and outcome of patients with non-small-cell lung cancer harboring *BRAF* mutations. *J Clin Oncol*. 2011;29(26):3574-3579. doi:10.1200/JCO.2011.35.9638.
6. Ritterhouse LL, Barletta JA. *BRAF* V600E mutation-specific antibody: a review. *Semin Diagn Pathol*. 2015;32(5):400-408. doi:10.1053/j.semdp.2015.02.010.
7. Ilie M, Long E, Hofman V, et al. Diagnostic value of immunohistochemistry for the detection of the *BRAF*V600E mutation in primary lung adenocarcinoma Caucasian patients. *Ann Oncol*. 2013;24(3):742-748. doi:10.1093/annonc/mds534.

8. Hofman V, Benzaquen J, Heeke S, et al. Real-world assessment of the BRAF status in non-squamous cell lung carcinoma using VE1 immunohistochemistry: a single laboratory experience (LPCE, Nice, France). *Lung Cancer*. 2020;145:58-62. doi:10.1016/j.lungcan.2020.04.024.
9. Sasaki H, Shimizu S, Tani Y, et al. Usefulness of immunohistochemistry for the detection of the BRAF V600E mutation in Japanese lung adenocarcinoma. *Lung Cancer*. 2013;82(1):51-54. doi:10.1016/j.lungcan.2013.06.014.
10. Gow CH, Hsieh MS, Lin YT, Liu YN, Shih JY. Validation of immunohistochemistry for the detection of BRAF V600E-mutated lung adenocarcinomas. *Cancers (Basel)*. 2019;11(6):866. doi:10.3390/cancers11060866.
11. Mosele F, Remon J, Mateo J, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group. *Ann Oncol*. 2020;31(11):1491-1505. doi:10.1016/j.annonc.2020.07.014.
12. Iaccarino A, Pisapia P, Pepe F, et al. Liquid biopsy for BRAF mutations testing in non-small cell lung cancer: a retrospective study. *J Clin Pathol*. 2022;75(1):58-60. doi:10.1136/jclinpath-2020-207107.
13. Leighl NB, Page RD, Raymond VM, et al. Clinical utility of comprehensive cell-free DNA analysis to identify genomic biomarkers in patients with newly diagnosed metastatic non-small cell lung cancer. *Clin Cancer Res*. 2019;25(15):4691-4700. doi:10.1158/1078-0432.CCR-19-0624
14. Tabbò F, Pisano C, Mazieres J, et al; BOLERO Consortium. How far we have come targeting BRAF-mutant non-small cell lung cancer (NSCLC). *Cancer Treat Rev*. 2022;103:102335. doi:10.1016/j.ctrv.2021.102335.
15. Planchard D, Kim TM, Mazieres J, et al. Dabrafenib in patients with BRAF(V600E)-positive advanced non-small-cell lung cancer: a single-arm, multicentre, open-label, phase 2 trial. *Lancet Oncol*. 2016A;17(5):642-50. doi:10.1016/S1470-2045(16)00077-2.
16. Planchard D, Besse B, Groen HJM, et al. Dabrafenib plus trametinib in patients with previously treated BRAF(V600E)-mutant metastatic non-small cell lung cancer: an open-label, multicentre phase 2 trial. *Lancet Oncol*. 2016;17(7):984-993. doi:10.1016/S1470-2045(16)30146-2.
17. Johnson BE, Baik CS, Mazieres J, et al. Clinical outcomes with dabrafenib plus trametinib in a clinical trial versus real-world standard of care in patients with BRAF-mutated advanced NSCLC. *JTO Clin Res Rep*. 2022;3(5):100324. doi:10.1016/j.jtocrr.2022.100324.
18. Leonetti A, Facchinetti F, Rossi G, et al. BRAF in non-small cell lung cancer (NSCLC): pickaxing another brick in the wall. *Cancer Treat Rev*. 2018;66:82-94. doi:10.1016/j.ctrv.2018.04.006.
19. Planchard D, Smit EF, Groen HJM, et al. Dabrafenib plus trametinib in patients with previously untreated BRAFV600E-mutant metastatic non-small-cell lung cancer: an open-label, phase 2 trial. *Lancet Oncol*. 2017;18(10):1307-1316. doi:10.1016/S1470-2045(17)30679-4.
20. Planchard D, Besse B, Groen HJM, et al. Phase 2 study of dabrafenib plus trametinib in patients with BRAF V600E-mutant metastatic NSCLC: updated 5-year survival rates and genomic analysis. *J Thorac Oncol*. 2022;17(1):103-115. doi:10.1016/j.jtho.2021.08.011.
21. Yan N, Guo S, Zhang H, et al. BRAF-mutated non-small cell lung cancer: current treatment status and future perspective. *Front Oncol*. 2022;12:863043. doi:10.3389/fonc.2022.863043.
22. Mazieres J, Drilon A, Lusque A, et al. Immune checkpoint inhibitors for patients with advanced lung cancer and oncogenic driver alterations: results from the IMMUNOTARGET registry. *Ann Oncol*. 2019;30(8):1321-1328. doi:10.1093/annonc/mdz167.
23. Murciano-Goroff YR, Pak T, Mondaca S, et al. Immune biomarkers and response to checkpoint inhibition of BRAFV600 and BRAF non-V600 altered lung cancers. *Br J Cancer*. 2022;126(6):889-898. doi:10.1038/s41416-021-01679-1.
24. Couraud S, Barlesi F, Fontaine-Deraluelle C, et al; Biomarkers France Contributors. Clinical outcomes of non-small-cell lung cancer patients with BRAF mutations: results from the French Cooperative Thoracic Intergroup biomarkers France study. *Eur J Cancer*. 2019;116:86-97. doi:10.1016/j.ejca.2019.04.016.
25. Facchinetti F, Lacroix L, Mezquita L, et al. Molecular mechanisms of resistance to BRAF and MEK inhibitors in BRAFV600E non-small cell lung cancer. *Eur J Cancer*. 2020;132:211-223. doi:10.1016/j.ejca.2020.03.025.
26. Ortiz-Cuaran S, Mezquita L, Swalduz A, et al. Circulating tumor DNA genomics reveal potential mechanisms of resistance to BRAF-targeted therapies in patients with BRAF-mutant metastatic non-small cell lung cancer. *Clin Cancer Res*. 2020;26(23):6242-6253. doi:10.1158/1078-0432.CCR-20-1037.

Gene Structure and Function

The neurotrophic tropomyosin receptor kinase (*NTRK*) genes, *NTRK1* at 1q21-22, *NTRK2* at 9q22.1, and *NTRK3* at 15q25, encode TRKA, TRKB, and TRKC, respectively. These proteins belong to the receptor tyrosine kinase (RTK) family. Like other RTKs, TRK (which hereinafter refers to TRKA, TRKB, and TRKC) has extracellular ligand binding, transmembrane, and intracellular kinase domains. Neurotrophin ligand binding and TRK activation lead to homodimerization of the receptor and downstream signaling including the mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), and/or protein kinase C (PKC) pathways.^{1,2} TRK receptors are involved in central nervous system development, including cell proliferation, migration, differentiation, and apoptosis. In adult tissues, TRK expression is restricted to neuronal tissue and the testes.³

Type of Alterations and Role in Oncogenesis

Activating *NTRK* fusions lead to constitutive overexpression of TRK proteins and are highly predictive of response to TRK inhibitors. In such oncogenic gene fusions, the C-terminal tyrosine kinase domain of *NTRK* fuses with the 5' region of a partner gene, and the chimeric protein leads to constitutive activation of downstream pathways in a ligand-independent manner. Oncogenic *NTRK* fusions can result in strong TRK protein overexpression that is detectable by immunohistochemistry (IHC).^{4,5} More than 80 different *NTRK* fusion partners have been described so far across various tumor types. The specific partners can vary between tumor types. In particular, *ETV6-NTRK3* is prevalent in 90% of secretory breast carcinomas and secretory carcinomas of the salivary glands (previously known as mammary analog secretory carcinoma) for which the fusion serves as both a diagnostic and predictive marker. In lung cancer, at least 16 *NTRK1*, *NTRK2*, or *NTRK3* fusion partners have been identified.^{2,4,6-9}

Epidemiologic, Clinical, and Histologic Characteristics

NTRK fusions are extremely rare and have been found in only 0.1% to 0.3% of non-small cell lung carcinoma (NSCLC).¹⁰ Although data on the distribution of fusions across the 3 *NTRK* genes are still scarce, fusion can involve any of the 3 genes. *NTRK1* and *NTRK3* fusions seem to be more common than *NTRK2* fusions.⁴ *NTRK* fusions are enriched in cancers in the absence of canonical driver mutations.^{7,11} While *NTRK* fusions have mostly been found in lung adenocarcinomas, these can also occur in other histologies such as squamous cell carcinomas and neuroendocrine carcinomas.¹² While *NTRK* fusions are regarded as mutually exclusive with other de novo drivers in NSCLC, they can emerge as resistance mechanisms to tyrosine kinase inhibitor (TKI) therapy such as third-generation EGFR TKIs.¹³ *NTRK* fusions have mostly been reported in middle-aged nonsmokers or never-smokers but can also occur in any NSCLC patients.¹²

Testing Strategies

Several methods are available to detect *NTRK* gene fusions. Each technique has its merits and limitations, and the choice of assays depends on resources and the clinical context.¹⁴ Generally, comprehensive molecular profiling (nucleic acid-based sequencing using appropriate next-generation sequencing [NGS] panels) is the recommended method for *NTRK* testing in tumors where *NTRK* fusions are uncommon such as NSCLC.¹⁴ RNA-based NGS is preferable over DNA-based NGS, as the latter cannot cover large intronic regions of *NTRK3* thus leading to reduced sensitivity. In addition, a lower level of purity is tolerated for RNA-based NGS because of gene fusion overexpression. Conversely, unstable RNA quality can be a concern, especially in aged, archived material. Other RNA-based methods are also available, such as the multiplexed digital color-coded barcode technology (nCounter [NanoString]) on a tissue section.^{15,16} Reverse transcription polymerase chain reaction (RT-PCR) has been used mainly for detecting canonical *ETV6-NTRK3* fusions in tumor types enriched for such alterations.¹⁷

Fluorescence in situ hybridization (FISH) is a well-established method to detect gene fusions. For *NTRK*, separate testing for the *NTRK1*, *NTRK2*, and *NTRK3* is necessary. FISH is used primarily for confirmatory analysis in the high-prevalence setting (ie, for tumors with a high probability of containing an *NTRK* gene fusion), but it is not the first choice for NSCLC.

Pan-TRK protein expression detected by IHC is a surrogate for the presence of an *NTRK* fusion as wild-type TRK is not highly expressed in many nonneoplastic tissues. The commercially available pan-TRK clones are EPR17341 (Abcam) and A7H6R (Cell Signaling Technology). A Ventana in vitro diagnostic (IVD) pan-TRK assay is also available (Roche Tissue Diagnostics). IHC is cost effective, has a fast turnaround time, and can easily be integrated into diagnostic laboratory workflows. Moreover, IHC requires only a relatively small number of tumor cells on tissue sections or cytologic specimens.¹⁸ TRK expression by IHC can show variable intensity and subcellular localization (cytoplasmic, nuclear, or membranous), which might depend on the 5' fusion gene partner.¹⁴ IHC can serve as a useful screening tool for tumor types with a low prevalence, especially when NGS is not available or not routinely performed (Figure 11-1).

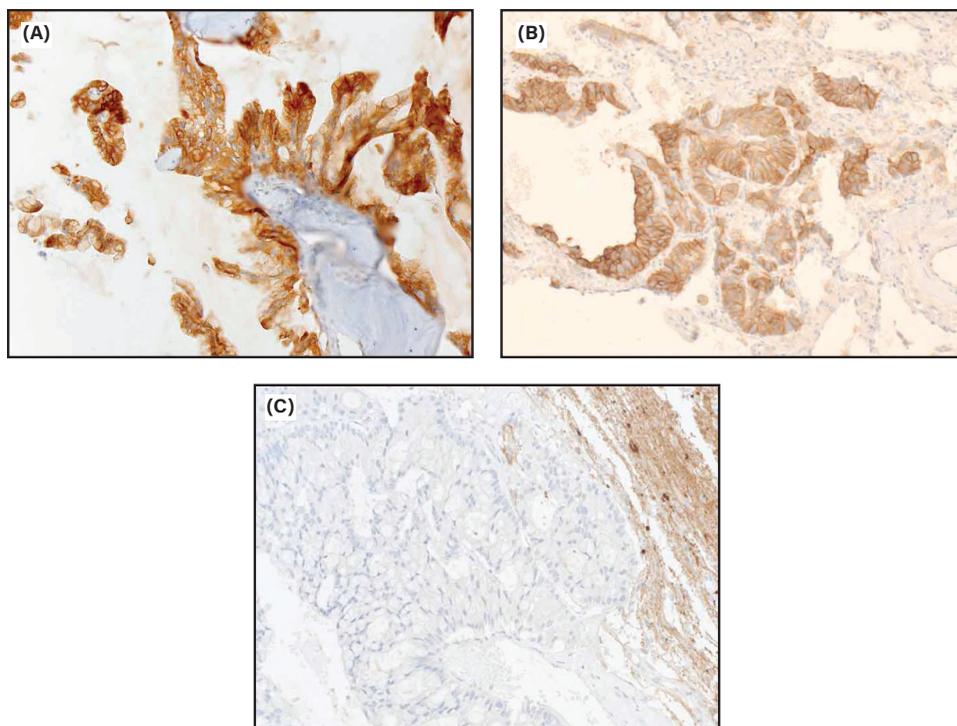


Figure 11-1. Examples of immunohistochemistry (IHC) using the pan-TRK antibody 17341 (Ventana [Roche Tissue Diagnostics]). **(A)** Bone metastasis of lung adenocarcinoma with strong cytoplasmic TRK staining. Confirmed *CLIP1-NTRK1* fusion (RNA-based next-generation sequencing [NGS] by Archer FusionPlex Custom kit [Diagnóstica Longwood]). **(B)** Lung adenocarcinoma with distinct membranous TRK staining. *NTRK* fusion was not confirmed despite using several methods (RNA-based NGS by Archer and OncoPrint [ThermoFisher Scientific] comprehensive, and fluorescence in situ hybridization [FISH] using 3 FISH probes against *NTRK1*, *NTRK2*, and *NTRK3*). **(C)** Pan-TRK IHC-negative brain metastasis of lung adenocarcinoma; adjacent neuronal brain tissue serving as positive internal staining control.

Few studies have been published regarding the staining protocol, sensitivity, and specificity of TRK IHC. The currently reported sensitivity of TRK IHC for *NTRK1* or *NTRK2* fusions is 96% and 100%, respectively, but only 55% to 79% for *NTRK3*.^{4,8} While the specificity of TRK IHC in NSCLC was 100% (24:24) in 1 study, others reported TRK staining in the absence of an *NTRK* fusion in approximately 1.8% (11/617) of cases, corresponding to an estimated positive predictive value of 10%.¹⁵ The prevalence and significance of TRK expression in the absence of an *NTRK* fusion requires further studies. Interestingly, 3% to 4% of NSCLCs harbor *NTRK1* gene amplification according to cBioPortal.¹⁹ It remains to be studied if such amplifications can explain TRK expression in NSCLC. Notably, a partial response to the TRK inhibitor larotrectinib was reported in an *NTRK*-amplified esophageal carcinoma.²⁰

Most studies have used a cutoff of at least 1% tumor cell staining above background.⁵ However, there is currently no consensus on how to best define TRK positivity by IHC. Thus, any expression by IHC requires confirmation by an orthogonal, preferably nucleic acid-based, test.⁴

Figure 11-2 shows a proposed diagnostic testing algorithm for non-squamous NSCLC. This algorithm is in line with European Society for Medical Oncology (ESMO) recommendations¹⁴ and guideline-recommended broad molecular testing for predictive alterations including oncogenic mutations or rearrangements. In most advanced lung squamous cell

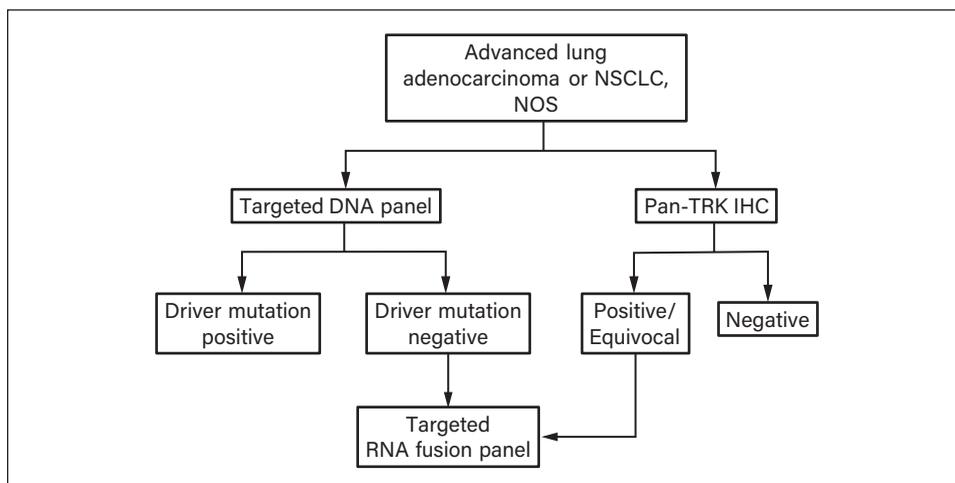


Figure 11-2. Proposed diagnostic testing algorithm for *NTRK* gene fusions in lung adenocarcinoma or NSCLC, NOS. Alternative to sequential testing, simultaneous DNA- and RNA-based NGS testing is an option depending on local practice environment and coverage of extra costs. Abbreviations: IHC = immunohistochemistry; NGS = next-generation sequencing; NSCLC, NOS = non-small cell lung carcinoma, not otherwise specified. (Adapted from Koehne de González et al.²¹)

carcinomas, broad molecular testing is not yet a standard; however, molecular testing including *NTRK* should be considered based on clinical profile (young patient and, never-smoker or light smoker).

Clinical Implications

TRK inhibitors are highly active in patients with *NTRK* fusion-positive cancers. Response is quickly achieved in many patients, and durable disease control is observed. The overall response rate (ORR) to larotrectinib in all *NTRK* fusion-positive cancers is 75%, regardless of the age of the patient or the tumor type.²² In *NTRK* fusion-positive lung cancers, the ORR is 83% with a median duration of response (DOR) and a median progression-free survival (PFS) that have not been reached. The median overall survival is approximately 41 months. In patients with *NTRK* fusion-positive solid tumors, the ORR of entrectinib, a TRK and TROS1 inhibitor is 57%.²³ In *NTRK* fusion-positive lung cancers, the ORR is 69%, the median DOR has not been reached, the median PFS is 15 months, and the median overall survival (OS) is 15 months.

These studies led to the US Food and Drug Administration (FDA)'s and European Medicines Agency (EMA)'s landmark approvals of the first-generation TRK inhibitors, larotrectinib and entrectinib, in patients of various ages with advanced *NTRK* fusion-positive solid tumors, regardless of tumor histology. In fact, the use of a TRK inhibitor in *NTRK* fusion-positive cancers is currently approved or authorized in up to 40 countries around the world.

Resistance Mechanisms

Despite these rapid and durable responses to TRK inhibitors, resistance unfortunately develops in most patients. Known mechanisms of resistance are either on- or off-target. On-target resistance involves *NTRK1/2/3* mutations that lead to amino acid substitutions at different regions: solvent front, gatekeeper, or the DFG motif characterized by 3 sequential amino

acids—aspartic acid (D), phenylalanine (F), and glycine (G)—of the TRK kinase domain.²⁴ Such on-target mutations can be targeted by next-generation TRK inhibitors such as selitrectinib, repotrectinib, and taletrectinib among others.² Off-target mechanisms involve acquired MAPK-pathway mutations or amplifications, such as *KRAS G12D*, *BRAF V600E* or *MEK* mutations, or *MET* amplification.^{2,25,26} Data regarding the spectrum of off-target mechanisms in NSCLC remain to be described, but combination targeted therapies (eg, a TRK inhibitor with a second small molecule directed against bypass resistance) have demonstrated proof of concept activity in selected patients with off-target resistance.²⁶

References

- Cocco E, Scaltriti M, Drilon A. NTRK fusion-positive cancers and TRK inhibitor therapy. *Nat Rev Clin Oncol*. 2018;15(12):731-747.
- Qin H, Patel MR. The challenge and opportunity of NTRK inhibitors in non-small cell lung cancer. *Int J Mol Sci*. 2022;23(6).
- Kheder ES, Hong DS. Emerging targeted therapy for tumors with NTRK fusion proteins. *Clin Cancer Res*. 2018;24(23):5807-5814.
- Solomon JP, Linkov I, Rosado A, et al. NTRK fusion detection across multiple assays and 33,997 cases: diagnostic implications and pitfalls. *Mod Pathol*. 2020;33(1):38-46.
- Hechtman JF, Benayed R, Hyman DM, et al. Pan-TRK immunohistochemistry is an efficient and reliable screen for the detection of NTRK fusions. *Am J Surg Pathol*. 2017;41(11):1547-1551.
- Zhang L, Liu H, Tian Y, Wang H, Yang X. A novel NCOR2-NTRK1 fusion detected in a patient of lung adenocarcinoma and response to larotrectinib: a case report. *BMC Pulm Med*. 2021;21(1):125.
- Vaishnavi A, Capelletti M, Le AT, et al. Oncogenic and drug-sensitive NTRK1 rearrangements in lung cancer. *Nat Med*. 2013;19(11):1469-1472.
- Gatalica Z, Xiu J, Swensen J, Vranic S. Molecular characterization of cancers with NTRK gene fusions. *Mod Pathol*. 2019;32(1):147-153.
- Farago AF, Le LP, Zheng Z, et al. Durable clinical response to entrectinib in NTRK1-rearranged non-small cell lung cancer. *J Thorac Oncol*. 2015;10(12):1670-1664.
- Forsythe A, Zhang W, Phillip Strauss U, et al. A systematic review and meta-analysis of neurotrophic tyrosine receptor kinase gene fusion frequencies in solid tumors. *Ther Adv Med Oncol*. 2020;12:1758835920975613.
- Rosen EY, Goldman DA, Hechtman JF, et al. TRK fusions are enriched in cancers with uncommon histologies and the absence of canonical driver mutations. *Clin Cancer Res*. 2020;26(7):1624-1632.
- Farago AF, Taylor MS, Doebele RC, et al. Clinicopathologic features of non-small-cell lung cancer harboring an NTRK gene fusion. *JCO Precis Oncol*. 2018;2018:PO.18.00037.
- Xia H, Xue X, Ding H, et al. Evidence of NTRK1 fusion as resistance mechanism to EGFR TKI in EGFR+ NSCLC: results from a large-scale survey of NTRK1 fusions in Chinese patients with lung cancer. *Clin Lung Cancer*. 2020;21(3):247-254.
- Marchio C, Scaltriti M, Ladanyi M, et al. ESMO recommendations on the standard methods to detect NTRK fusions in daily practice and clinical research. *Ann Oncol*. 2019;30(9):1417-1427.
- Elfving H, Brostrom E, Moens LNJ, et al. Evaluation of NTRK immunohistochemistry as a screening method for NTRK gene fusion detection in non-small cell lung cancer. *Lung Cancer*. 2021;151:53-59.
- Ali G, Bruno R, Savino M, et al. Analysis of fusion genes by NanoString system: a role in lung cytology? *Arch Pathol Lab Med*. 2018;142(4):480-489.
- Skalova A, Vanecek T, Simpson RH, et al. Mammary analogue secretory carcinoma of salivary glands: molecular analysis of 25 ETV6 gene rearranged tumors with lack of detection of classical ETV6-NTRK3 fusion transcript by standard RT-PCR: report of 4 cases harboring ETV6-X gene fusion. *Am J Surg Pathol*. 2016;40(1):3-13.
- Matter MS, Chijioko O, Savic S, Bubendorf L. Narrative review of molecular pathways of kinase fusions and diagnostic approaches for their detection in non-small cell lung carcinomas. *Transl Lung Cancer Res*. 2020;9(6):2645-2655.
- Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for

- exploring multidimensional cancer genomics data. *Cancer Discov.* 2012;2(5):401-404.
20. Hempel D, Wieland T, Solfrank B, et al. Antitumor activity of larotrectinib in esophageal carcinoma with NTRK gene amplification. *Oncologist.* 2020;25(6):e881-e886.
 21. Koehne de González A, Mansukhani MM, Fernandes H, Hsiao SJ. Pan-tumor screening for NTRK gene fusions using pan-TRK immunohistochemistry and RNA NGS fusion panel testing. *Cancer Genet.* 2022;262-263:47-52.
 22. Drilon A, Laetsch TW, Kummar S, et al. Efficacy of larotrectinib in TRK fusion-positive cancers in adults and children. *N Engl J Med.* 2018;378(8):731-739.
 23. Doebele RC, Drilon A, Paz-Ares L, et al. Entrectinib in patients with advanced or metastatic NTRK fusion-positive solid tumours: integrated analysis of three phase 1-2 trials. *Lancet Oncol.* 2020;21(2):271-282.
 24. Russo M, Misale S, Wei G, et al. Acquired resistance to the TRK inhibitor entrectinib in colorectal cancer. *Cancer Discov.* 2016;6(1):36-44.
 25. Drilon A, Nagasubramanian R, Blake JF, et al. A next-generation TRK kinase inhibitor overcomes acquired resistance to prior TRK kinase inhibition in patients with TRK fusion-positive solid tumors. *Cancer Discov.* 2017;7(9):963-972.
 26. Cocco E, Schram AM, Kulick A, et al. Resistance to TRK inhibition mediated by convergent MAPK pathway activation. *Nat Med.* 2019;25(9):1422-1427.

Gene Structure and Function

The REarranged during Transfection (*RET*) gene on chromosome 10q11.21 was originally identified through a NIH3T3 fibroblast cell line transfection assay with human lymphoma DNA. Its product is a membrane receptor containing extracellular (with 4 cadherin-like repeats, 1 calcium-binding site, and a cysteine-rich site), transmembrane, and intracellular domains (Figure 12-1). The latter contains a juxtamembrane portion, the tyrosine kinase, and 2 *RET* tails (*RET-9* and *RET-51*) generated by alternative splicing, determining 2 *RET* protein isoforms. *RET* ligands include the glial cell line-derived neurotrophic factor (GDNF) family that binds to coreceptor GFR α and GDF15, which binds to coreceptor GDNF family receptor α -like (GFRAL), forming a ternary complex with *RET* extracellular domain and triggering *RET* dimerization and tyrosine phosphorylation and activation. Downstream signal transduction involves the RAS/MAPK, PI3K/AKT/mTOR, and JAK/STAT pathways. Physiologically, *RET* plays a role in development of the kidneys and enteric nervous system.^{1,2}

Type of Alterations and Role in Oncogenesis

RET mutations, fusions, and amplifications were found in 39%, 31%, and 25% of tumors, respectively, according to a large study of more than 4800 diverse cancers.³ Germline mutations of *RET* cause hereditary diseases and tumors, including multiple endocrine neoplasia (MEN) types 2A and 2B. Somatic alterations, including mutations and fusions, occur across malignancies.⁴

RET gain-of-function mutations resulting in aberrant *RET* activation occur in sporadic or hereditary medullary thyroid carcinoma (MTC) and are rarely described in other cancers. These mutations affect the cysteine-rich portion of the extracellular domain in the case of familial MTC and MEN2A or the tyrosine kinase domain in the case of MEN2B and sporadic cases.

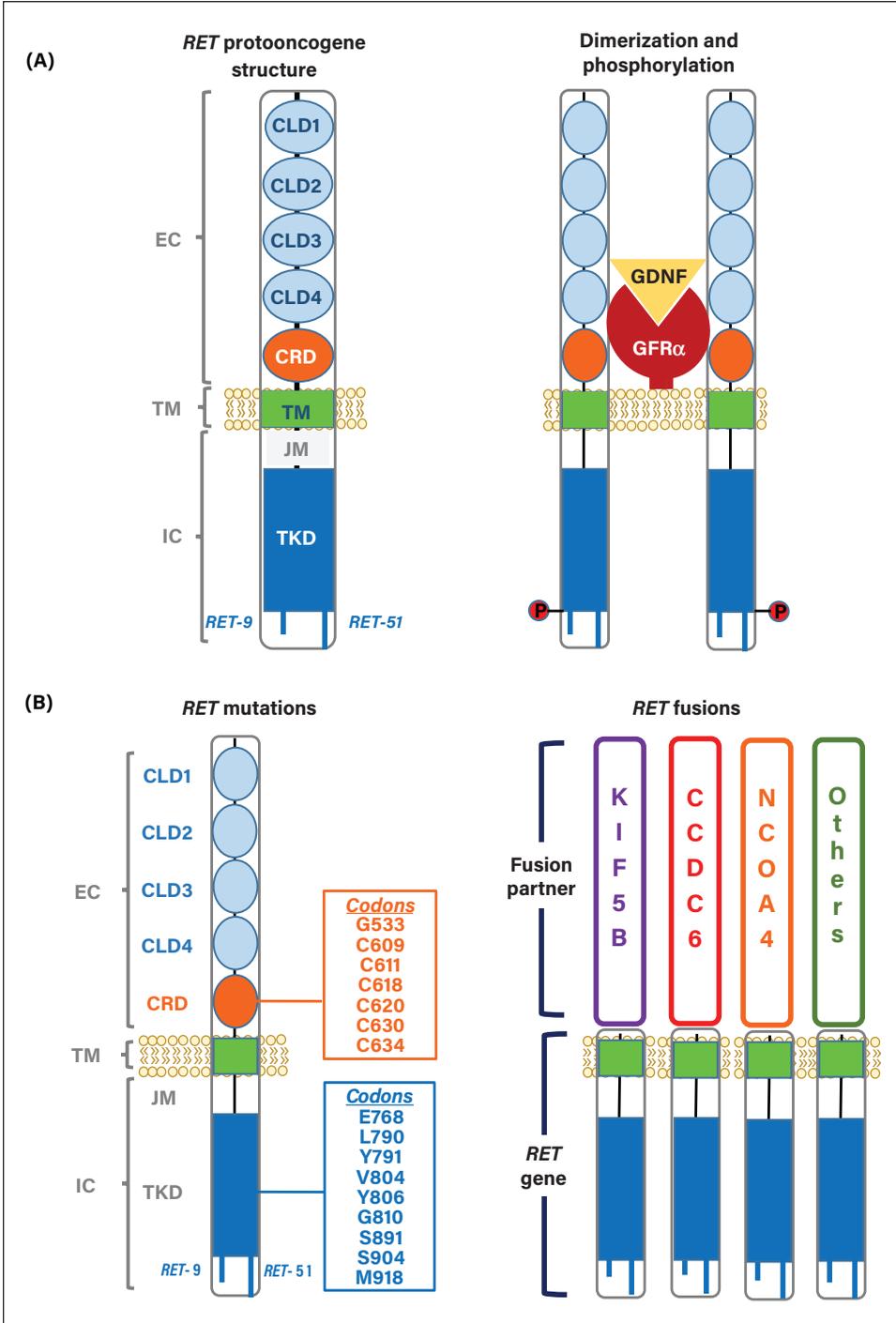


Figure 12-1. (A) RET oncogene structure and ligand-receptor interactions. (B) Genetic alterations include mutations and gene rearrangements with different partners. Abbreviations: CLD = cadherin-like domain; CRD = cysteine-rich domain; EC = extracellular portion; GDNF = glial cell line-derived neurotrophic factor; GFR α = GDNF factor coreceptor alpha 1; IC = intracellular portion; JM = juxtamembrane segment; P = phosphorylation site; TKD = tyrosine kinase domain; TM = transmembrane portion. (Adapted from Ferrara et al and Radonic et al.^{1,5})

RET fusions most commonly involve intrachromosomal rearrangements but rarely can involve interchromosomal rearrangements, resulting in fusion of the *RET* kinase domain-encoding region on the 3' end to the 5'-terminal region of various gene partners.⁴ These fusions result in a constitutively active chimeric fusion oncoprotein. *RET* fusions have been described in papillary thyroid carcinoma and non-small cell lung carcinoma (NSCLC), in addition to other tumor types including colorectal, salivary gland, and ovarian adenocarcinomas. In lung cancer, the most common *RET* fusion partners are *KIF5B* (72%), *CCDC6* (23%), *NCOA4* (2%), *EPHA5* (1%), and *PICALM* (1%).⁶ In contrast, in papillary thyroid cancer, *CCDC6* and *NCOA4* are the more common upstream partner genes.⁷⁻¹⁰

Amplifications of *RET* have been rarely reported in pancreatic and breast carcinomas (among others), with a high but heterogeneous *RET* protein expression even in the absence of a *RET* fusion.¹¹

Epidemiologic, Clinical, and Histologic Characteristics

RET fusions have been identified in 1% to 2% of lung adenocarcinomas.⁶ Patients with *RET* fusion-positive lung cancers tend to be younger (≤ 60 years) with relatively equal gender distribution. Most patients are never-smokers, although incidence in current and former smokers has been reported.¹² No association with therapeutic radiation exposure has been observed in lung adenocarcinomas with *RET* fusions.¹³

Testing Strategies

The screening methods to detect *RET* fusions have not yet been standardized. Immunohistochemistry (IHC), reverse transcription polymerase chain reaction (RT-PCR), fluorescence in situ hybridization (FISH), and DNA/RNA-based next-generation sequencing (NGS) have all been used to identify *RET* fusions. Of note, in contrast to ALK IHC, *RET* IHC has low sensitivity (55%-65%) and variable specificity (40%-85%).^{11,13,14} The sensitivity of IHC may also vary according to the fusion partner.¹¹ Thus, *RET* IHC to screen for *RET* fusions or mutations is not currently recommended in lung adenocarcinoma.¹⁵

RET FISH is generally highly sensitive (100%), albeit with lower sensitivity for the uncommon *NCOA4-RET* fusion (66.7%).¹¹ *RET* FISH specificity has been reported to range from 55% to 100%.^{13,14} As *RET* fusions in lung cancers can involve a variety of partner genes; break-apart probes are preferred to fusion probes. The guidelines for *RET* FISH testing and scoring are similar to those for other fusion gene detection. The assay is positive if more than 15% of the tumor cells show either split signal or single 3' signal.

While RT-PCR is specific, it is limited to the detection of known fusions. NGS-based testing offers the advantage of identifying *RET* fusions, including with novel fusion partners, and additionally enabling multiplex testing for other actionable targets.¹⁶ DNA-based NGS has limitations in identifying complex fusions, and here, RNA-based NGS can add value. NGS-based testing of cell-free DNA (cfDNA) can also detect *RET* fusions, and a positive result should be considered indicative of the presence of a *RET* fusion.^{17,18}

Therapeutic Targeting of *RET* Fusions in NSCLC

Initial efforts to target *RET* in *RET* fusion-positive NSCLC focused on repurposing multikinase inhibitors with anti-*RET* activity (eg, vandetanib, cabozantinib, lenvatinib).¹⁹⁻²⁴ While

variable degrees of efficacy were reported, responses were typically modest. Furthermore, tolerability was limited by toxicities related to multikinase inhibition such as of vascular endothelial growth factor receptor 2 (VEGFR2).

Selpercatinib (formerly LOXO-292) and pralsetinib (formerly BLU-667) are oral tyrosine kinase inhibitors (TKIs) that were developed to potently and selectively target oncogenic *RET* fusions and mutations. Consistent with their selectivity, selpercatinib and pralsetinib demonstrated favorable safety profiles in phase 1 and 2 trials.^{25,26} In the registrational phase 1 and 2 LIBRETTO-001 trial, selpercatinib demonstrated robust efficacy in advanced *RET* fusion-positive NSCLC, with overall response rates (ORRs) of 85% among treatment-naïve and 64% among platinum-based chemotherapy-pretreated patients.²⁵ In the registrational phase 1 and 2 ARROW trial, pralsetinib similarly had robust efficacy with ORRs of 70% and 61%, respectively, in these populations.²⁶ Of note, both selpercatinib and pralsetinib showed activity against brain metastases. Based on these results, selpercatinib and pralsetinib have received line-agnostic accelerated approvals by the US Food and Drug Administration for the treatment of advanced *RET* fusion-positive NSCLC. These agents have additionally received conditional authorizations by the European Medicines Agency for the immunotherapy- or platinum-based chemotherapy-pretreated indication for selpercatinib and line-agnostic indication for pralsetinib.

Mechanisms of Resistance to Selective RET Inhibitors

Despite their efficacy, the emergence of resistance to RET TKIs remains a major challenge. Both on-target and off-target mechanisms of resistance have been identified. The earliest reported resistance mechanism was the *RET* G810 solvent front mutation, which causes steric interference with TKI binding and has been identified in 7% to 11% of post-progression biopsies.²⁷⁻³⁰ Y806C/N mutations at the hinge region of *RET* also cause resistance to selpercatinib and pralsetinib.^{30,31} L730V/I mutations at the roof region have been identified in pralsetinib-resistant samples but are predicted to remain sensitive to selpercatinib.^{28,32} Novel RET inhibitors are in clinical development and may help to address on-target resistance (eg, LOXO-260 [NCT05241834], TAS0953/HM06 [NCT04683250], TPX-0046 [NCT04161391]).

Off-target mechanisms of resistance to selpercatinib and pralsetinib are also being delineated and thus far appear predominant compared to on-target mechanisms (83%-90% vs 10%-17%, respectively).²⁸⁻³⁰ *MET* amplification has been recurrently identified in patients progressing on selpercatinib and pralsetinib.^{28-30,33} Case reports suggest that dual RET and MET inhibition may represent a viable therapeutic approach in these cases.³³ *KRAS* amplification or mutations, *BRAF* V600E mutation, *FGFR1* amplification, and *NTRK3* fusion have also been identified.^{28-30,34} However, mechanisms of resistance to RET-selective TKIs remain undetermined for a substantial proportion (40%-80%) of cases, underscoring the need for further studies to elucidate the biology and optimal treatment strategies for RET TKI-resistant cancers.

References

1. Ferrara R, Auger N, Auclin E, Besse B. Clinical and translational implications of RET rearrangements in non-small cell lung cancer. *J Thorac Oncol.* 2018;13(1):27-45.
2. Salvatore D, Santoro M, Schlumberger M. The importance of the RET gene in thyroid cancer and therapeutic implications. *Nat Rev Endocrinol.* 2021;17(5):296-306.

3. Kato S, Subbiah V, Marchlik E, et al. RET aberrations in diverse cancers: next-generation sequencing of 4,871 patients. *Clin Cancer Res.* 2017;23(8):1988-1997.
4. Shi M, Wang W, Zhang J, et al. Identification of RET fusions in a Chinese multicancer retrospective analysis by next-generation sequencing. *Cancer Sci.* 2022;113(1):308-318.
5. Radonic T, Geurts-Giele WRR, Samsom KG, et al. RET fluorescence in situ hybridization analysis is a sensitive but highly unspecific screening method for RET fusions in lung cancer. *J Thorac Oncol.* 2021;16(5):798-806. doi:10.1016/j.jtho.2021.01.1619.
6. Gautschi O, Milia J, Filleron T, et al. Targeting RET in patients with RET-rearranged lung cancers: results from the global, multicenter RET registry. *J Clin Oncol.* 2017;35(13):1403-1410.
7. Li F, Feng Y, Fang R, et al. Identification of RET gene fusion by exon array analyses in "pan-negative" lung cancer from never smokers. *Cell Res.* 2012;22(5):928-931.
8. Cai W, Su C, Li X, et al. KIF5B-RET fusions in Chinese patients with non-small cell lung cancer. *Cancer.* 2013;119(8):1486-1494.
9. Takeuchi K, Soda M, Togashi Y, et al. RET, ROS1 and ALK fusions in lung cancer. *Nat Med.* 2012;18(3):378-381.
10. Kohno T, Ichikawa H, Totoki Y, et al. KIF5B-RET fusions in lung adenocarcinoma. *Nat Med.* 2012;18(3):375-377.
11. Yang SR, Aypar U, Rosen EY, et al. A performance comparison of commonly used assays to detect RET fusions. *Clin Cancer Res.* 2021;27(5):1316-1328.
12. Michels S, Scheel AH, Scheffler M, et al. Clinicopathological characteristics of RET rearranged lung cancer in European patients. *J Thorac Oncol.* 2016;11(1):122-127.
13. Wang R, Hu H, Pan Y, et al. RET fusions define a unique molecular and clinicopathologic subtype of non-small-cell lung cancer. *J Clin Oncol.* 2012;30(35):4352-4359.
14. Tsuta K, Kohno T, Yoshida A, et al. RET-rearranged non-small-cell lung carcinoma: a clinicopathological and molecular analysis. *Br J Cancer.* 2014;110(6):1571-1578.
15. Belli C, Penault-Llorca F, Ladanyi M, et al. ESMO recommendations on the standard methods to detect RET fusions and mutations in daily practice and clinical research. *Ann Oncol.* 2021;32(3):337-350.
16. Feng J, Li Y, Wei B, et al. Clinicopathologic characteristics and diagnostic methods of RET rearrangement in Chinese non-small cell lung cancer patients. *Transl Lung Cancer Res.* 2022;11(4):617-631.
17. Mack PC, Banks KC, Espenschied CR, et al. Spectrum of driver mutations and clinical impact of circulating tumor DNA analysis in non-small cell lung cancer: analysis of over 8000 cases. *Cancer.* 2020;126(14):3219-3228.
18. Rich TA, Reckamp KL, Chae YK, et al. Analysis of cell-free DNA from 32,989 advanced cancers reveals novel co-occurring activating RET alterations and oncogenic signaling pathway aberrations. *Clin Cancer Res.* 2019;25(19):5832-5842.
19. Drilon A, Hu ZI, Lai GGY, Tan DSW. Targeting RET-driven cancers: lessons from evolving pre-clinical and clinical landscapes. *Nat Rev Clin Oncol.* 2018;15(3):150.
20. Drilon A, Rekhtman N, Arcila M, et al. Cabozantinib in patients with advanced RET-rearranged non-small-cell lung cancer: an open-label, single-centre, phase 2, single-arm trial. *Lancet Oncol.* 2016;17(12):1653-1660.
21. Lee SH, Lee JK, Ahn MJ, et al. Vandetanib in pretreated patients with advanced non-small cell lung cancer-harboring RET rearrangement: a phase II clinical trial. *Ann Oncol.* 2017;28(2):292-297.
22. Yoh K, Seto T, Satouchi M, et al. Vandetanib in patients with previously treated RET-rearranged advanced non-small-cell lung cancer (LURET): an open-label, multicentre phase 2 trial. *Lancet Respir Med.* 2017;5(1):42-50.
23. Hida T, Velcheti V, Reckamp KL, et al. A phase 2 study of lenvatinib in patients with RET fusion-positive lung adenocarcinoma. *Lung Cancer.* 2019;138:124-130.
24. Horiike A, Takeuchi K, Uenami T, et al. Sorafenib treatment for patients with RET fusion-positive non-small cell lung cancer. *Lung Cancer.* 2016;93:43-46.
25. Drilon A, Oxnard GR, Tan DSW, et al. Efficacy of selpercatinib in RET fusion-positive non-small-cell lung cancer. *N Engl J Med.* 2020;383(9):813-824.
26. Gainor JF, Curigliano G, Kim DW, et al. Pralsetinib for RET fusion-positive non-small-cell lung cancer (ARROW): a multi-cohort, open-label, phase 1/2 study. *Lancet Oncol.* 2021;22(7):959-969.
27. Solomon BJ, Tan L, Lin JJ, et al. RET solvent front mutations mediate acquired resistance to selective RET inhibition in RET-driven malignancies. *J Thorac Oncol.* 2020;15(4):541-549.

28. Gainor JF, Curigliano G, Kim DW, et al. OA05.02 Analysis of resistance mechanisms to pralsetinib in patients with RET fusion-positive non-small cell lung cancer (NSCLC) from the ARROW study. *J Thorac Oncol.* 2021;16(S1):S31-S32.
29. Lin JJ, Liu SV, McCoach CE, et al. Mechanisms of resistance to selective RET tyrosine kinase inhibitors in RET fusion-positive non-small-cell lung cancer. *Ann Oncol.* 2020;31(12):1725-1733.
30. Rosen EY, Won HH, Zheng Y, et al. The evolution of RET inhibitor resistance in RET-driven lung and thyroid cancers. *Nat Commun.* 2022;13(1):1450.
31. Subbiah V, Shen T, Terzyan SS, et al. Structural basis of acquired resistance to selpercatinib and pralsetinib mediated by non-gatekeeper RET mutations. *Ann Oncol.* 2021;32(2):261-268.
32. Shen T, Hu X, Liu X, et al. The L730V/I RET roof mutations display different activities toward pralsetinib and selpercatinib. *NPJ Precis Oncol.* 2021;5(1):48.
33. Rosen EY, Johnson ML, Clifford SE, et al. Overcoming MET-dependent resistance to selective RET inhibition in patients with RET fusion-positive lung cancer by combining selpercatinib with crizotinib. *Clin Cancer Res.* 2021;27(1):34-42.
34. Subbiah V, Shen T, Tetzlaff M, et al. Patient-driven discovery and post-clinical validation of NTRK3 fusion as an acquired resistance mechanism to selpercatinib in RET fusion-positive lung cancer. *Ann Oncol.* 2021;32(6):817-819.

Gene Structure and Function

MET protooncogene receptor tyrosine kinase (*MET*), also known as *hepatocyte growth factor receptor*, encodes for the MET protein, a growth factor receptor expressed predominantly on endothelial and epithelial cells with essential roles in development, regeneration, and homeostasis. Dysregulation of MET is a well-established driver of oncogenesis in many cancer types including lung cancer.^{1,2} Initially cloned from an induced oncogenic fusion in an osteosarcoma cell line, MET and its ligand, hepatocyte growth factor (HGF), subsequently became known for their roles in morphogenesis (tubular branching, including in the liver, kidney, and lung), motility and invasion (carcinoma), and mitogenesis (including hepatocyte regeneration).³⁻⁷

MET is a single-pass transmembrane receptor protein defined by an extracellular ligand-binding (Sema) domain; a plexin-semaphorin-integrin (PSI) domain; an immunoglobulin-like-plexin-transcription factor domain; and transmembrane, intracellular juxtamembrane and tyrosine kinase domains.⁷ Upon binding of its ligand, HGF, MET homodimerizes, leading to phosphorylation of tyrosines 1234/1235 (kinase domain) and 1349/1356 (docking domain), with subsequent downstream activation of mitogen-activated protein kinase (MAPK) and PI3K-mTOR-AKT pathways.⁸ Critically, the regulation of MET protein expression at the cell surface is mediated by the E3 ubiquitin ligase, Cbl, which binds to phosphorylated Y1003 in the receptor's juxtamembrane domain.⁹ Cbl-mediated ubiquitination triggers receptor endocytosis¹⁰ (Figure 13-1); interestingly, transit of MET into the endosomal compartment appears necessary for activation of the full range of downstream signals, including via extracellular signal-regulated kinase-1, -2 (ERK1/2) and signal transducer and activator of transcription-3 (STAT3).¹¹

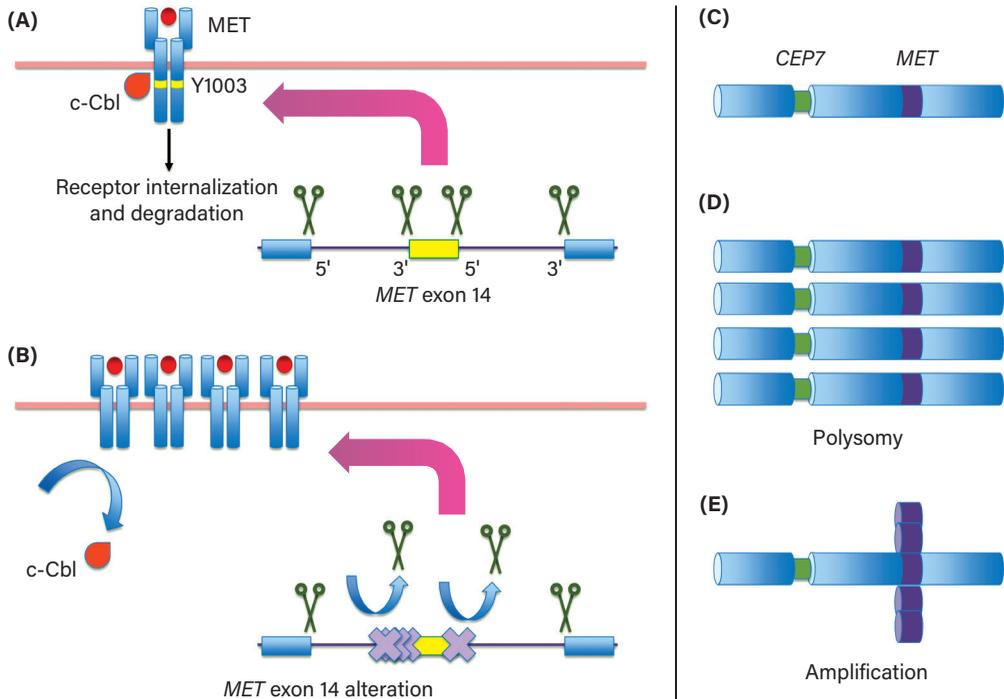


Figure 13-1. A schematic representation of the most common modes of *MET* gene activation. **(A)** Following ligand binding, Met surface expression is normally downregulated by binding of Cbl to the phosphorylated tyrosine at codon 1003 in the Met juxtamembrane domain, leading to internalization and degradation. **(B)** *MET* exon 14 skipping mutations preferentially affect the splice regions flanking exon 14, leading to deletion of the juxtamembrane region containing Y1003 and preventing interaction with Cbl. Alternatively or concomitantly, **(C)** *MET* may undergo relative copy number increase, caused by chromosome 7 **(D)** undergoing one or more rounds of reduplication leading to polysomy or **(E)** caused by focal amplification of *MET*. The latter is more strongly associated with Met activation and oncogenic dependence. (Adapted from Drilon et al.¹²)

Type of Alterations and Role in Oncogenesis

Dysregulation of the *MET* pathway in lung cancer occurs through a variety of molecular mechanisms including gene mutations, amplifications, protein overexpression, and fusions.¹³ Several mutational mechanisms of oncogenesis have been described in *MET*, including at kinase domain hotspots F1200I, Y1230X, and M1250T in papillary renal cell carcinoma and potentially within the extracellular Sema domain in several tumor types.^{14,15} The dominant mechanism in non-small cell lung carcinoma (NSCLC), however, is the introduction of a diversity of missense and deletion-insertion mutations in and around the splice sites flanking exon 14, which lead to “exon 14 skipping” and loss of the regulatory juxtamembrane domain containing the Cbl binding site¹⁶ at Y1003 (Figure 13-2). Persistent oncogenic signaling through the surface-bound *MET* receptor is associated with increased cellular invasion and metastasis.^{16,17}

MET amplifications can be mainly classified into 2 subtypes: de novo and acquired. De novo *MET* amplification occurs in 1% to 5% of NSCLC and is associated with poor prognosis.^{18,19} Notably, the prevalence of *MET* amplifications varies among different studies, depending on the cutoff value and diagnostic assays used. In a subset of cases, exon 14 skipping mutations are accompanied by selective amplification of the mutated allele, an event that likely facilitates the oncogene addiction state.²⁰⁻²²

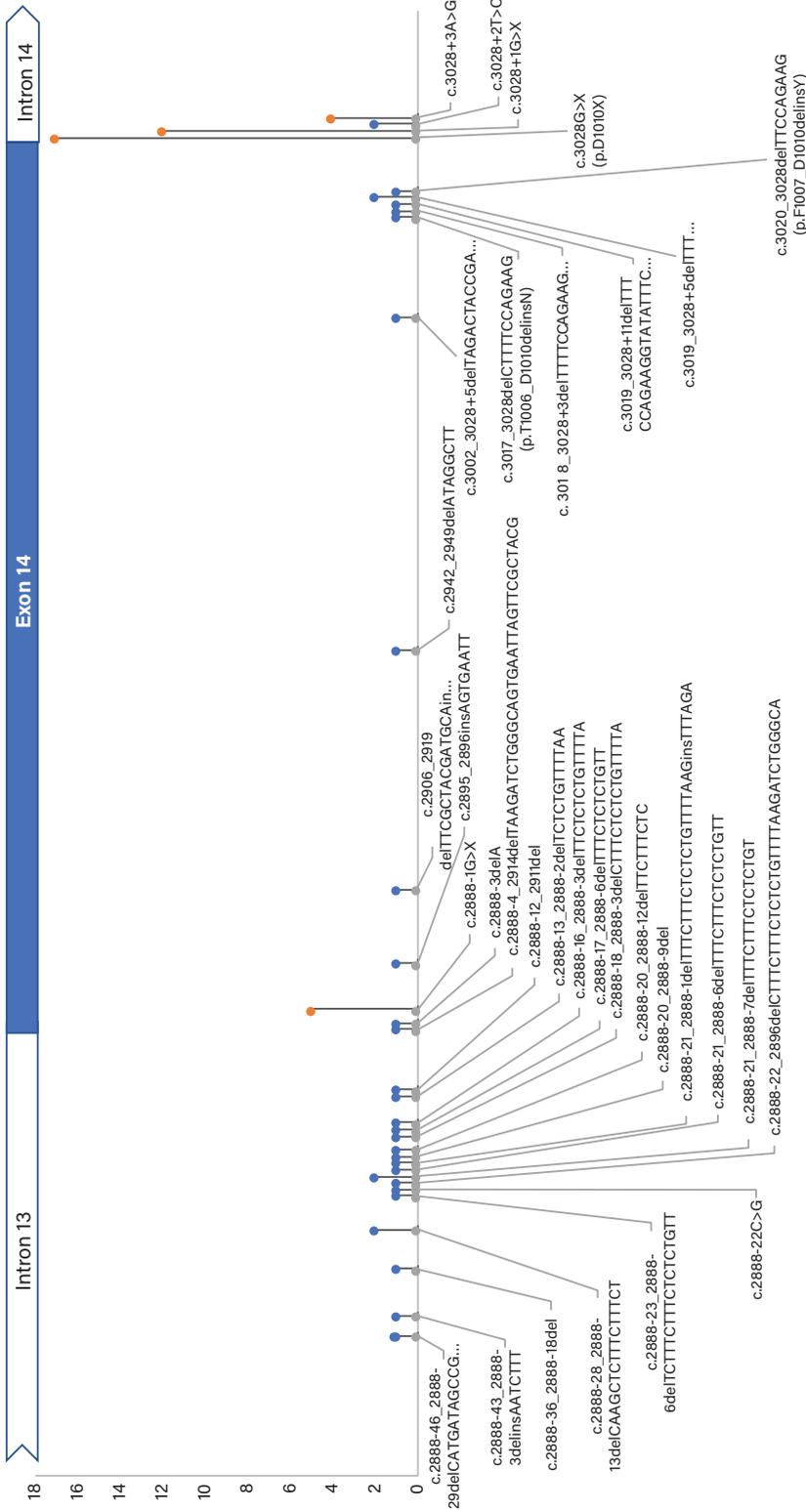


Figure 13-2. Lollipop plot representing the diversity of mutations detected in intron 13, exon 14, and intron 14 of the MET gene that give rise to MET exon 14 skipping and loss of the juxtamembrane regulatory domain including the Cbl binding site at Y1003. Hotspots are designated with orange lollipops. Data from 71 patients is derived from the Dana Farber Cancer Institute’s OncoPanel next-generation sequencing test from 2017 to 2021. (Data are publicly available from AACR Project Genie.²³)

Uncommonly, *de novo* wild-type *MET* amplification is implicated as the sole oncogenic driver; more typically, *MET* amplification is observed with a co-occurring mitogenic driver alteration, including in *EGFR* and *KRAS*.²⁴ Acquired *MET* amplification can be identified in 5% to 20% of patients with epidermal growth factor receptor (EGFR)-mutant NSCLC upon progression with first-, second- and third-generation EGFR-tyrosine kinase inhibitors (TKIs),²⁵⁻²⁷ and is now recognized as a recurrent mechanism of acquired resistance to ALK, RET, and ROS1 targeted therapies.²⁸

Intergenic fusions involving *MET* are rare, but a variety of fusion partners have been identified. Such fusions have largely been described in tumors other than NSCLC, including glioma, papillary renal cell carcinoma, and thyroid.²⁹ The exact frequency of *MET* fusion in NSCLC is poorly defined; best estimates suggest that these occur in 0.5% of otherwise oncogene-negative lung adenocarcinomas.³⁰ The breakpoints within the *MET* gene appear to be enriched in intron 14, and the fusion products juxtapose a 5' partner containing a coiled-coil domain to facilitate homodimerization with an intact Met kinase domain.^{29,30} Evidence regarding the predictive role of *MET* fusions for Met-targeted therapies is largely derived from case reports and small series but suggests that these strategies hold promise.³¹

MET overexpression is common in NSCLC and can be detected in approximately 20% to 48% of patients by immunohistochemistry (IHC).³²⁻³⁵ However, the correlation between *MET* overexpression and *MET* alterations or amplification is weak, and only a subset of *MET* exon 14 skipping mutation-positive tumors demonstrate significant *MET* protein expression (Figure 13-3A-D). Further, high-level expression can be observed in the context of other oncogenic driver alterations^{21,36,37} (Figure 13-4A,B).

Epidemiologic, Clinical, and Histologic Characteristics

MET exon 14 skipping mutations occur in 3% to 4% of patients with NSCLC in White populations, while in China, the frequency is only 0.9%.³⁸⁻⁴⁰ Limited systematic sequencing in other populations precludes confident estimates of *MET* exon 14 mutation frequency. Large-scale sequencing efforts have uncovered possible enrichment of *MET* mutations and amplifications in patients of Ashkenazi Jewish ancestry.⁴¹ *MET* exon 14 skipping mutations are most commonly reported in lung adenocarcinomas, but are also reported in lung squamous cell carcinomas where they may possibly be enriched in patients with a light or never-smoking history.^{42,43} Several studies have identified an apparent enrichment in pulmonary sarcomatoid carcinoma in 5% to 32% of cases.^{21,44,45} The median age of patients with *MET* exon 14 skipping mutations (>70) is high relative to those with other oncogene-driven tumors.^{21,23} Most studies indicate that *MET* mutations occur independent of smoking status.^{23,41} *MET* exon 14 skipping mutations are usually mutually exclusive with other oncogene driver mutations; however, *KRAS* G12X co-mutations have been reported in approximately 4% of cases.^{21,23,38,46}

Testing Strategies

Given the diversity of activating *MET* alterations, comprehensive assessment of actionable *MET* biomarkers requires an integrated diagnostic approach. It is important to consider the suitability and limitations of each assay and to implement a testing strategy that ensures

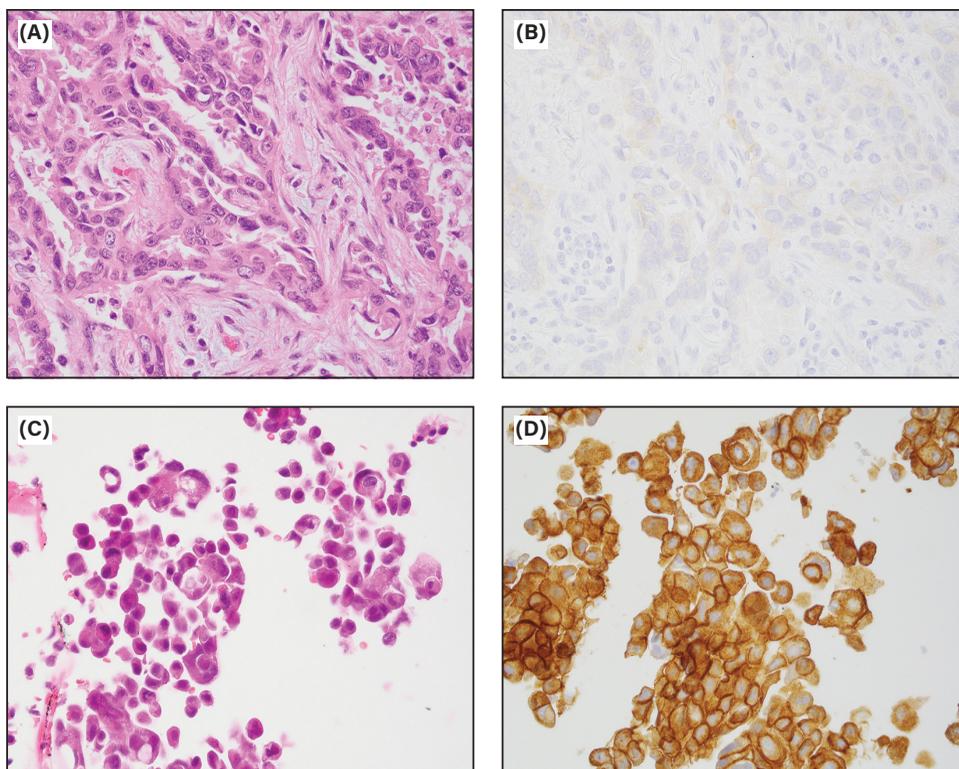


Figure 13-3. Two examples of lung adenocarcinomas with MET exon 14 skipping alterations and highly variable levels of MET protein expression (immunohistochemistry clone SP44 [Spring Bioscience]). **(A)** Lung resection showing acinar-predominant lung adenocarcinoma with **(B)** barely perceptible levels of total MET protein expression. **(C)** Lung core biopsy showing detached clusters of poorly differentiated lung adenocarcinoma with micropapillary features and **(D)** strong, diffuse expression of MET protein.

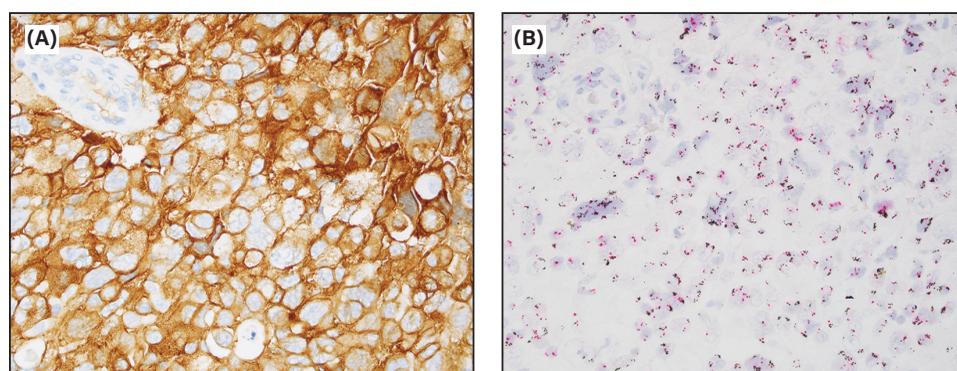


Figure 13-4. Lung adenocarcinoma with KRAS mutation showing strong, diffuse MET protein expression and MET amplification in the form of signal clusters (homogeneously staining regions) by chromogenic in situ hybridization (*black signals* = MET; *red signals* = CEN 7).

broad coverage across different classes of *MET* alterations including amplification, mutations, alternative transcripts, and fusions (Table 13-1).

Important diagnostic concepts for detecting pathogenic *MET* alterations in the clinic and a testing strategy are shown in Figure 13-5. Given the growing adoption of DNA-based next-generation sequencing (NGS) as the standard diagnostic assay for NSCLC and its ability

Table 13-1. Clinical Assays for Detecting *MET* Biomarkers

Assay	Amplification	Exon 14 skipping	Gene fusion	Overexpression
Tissue-based testing	DNA-NGS FISH	DNA-NGS RNA-NGS RT-PCR	DNA-NGS RNA-NGS FISH RT-PCR	IHC
Liquid/plasma-based testing ^a	ctDNA-NGS	ctDNA-NGS	ctDNA-NGS	N/A

Abbreviations: ctDNA = circulating tumor DNA; FISH = fluorescence in situ hybridization; IHC = immunohistochemistry; N/A = not applicable; NGS = next-generation sequencing; RT-PCR = reverse transcriptase polymerase chain reaction.

^aWhile liquid biopsy methods using ctDNA may be used to detect *MET* amplification, exon 14 skipping, and fusions, the limited sensitivity for copy number analysis and fusion detection requires that negative results be confirmed on tissue.

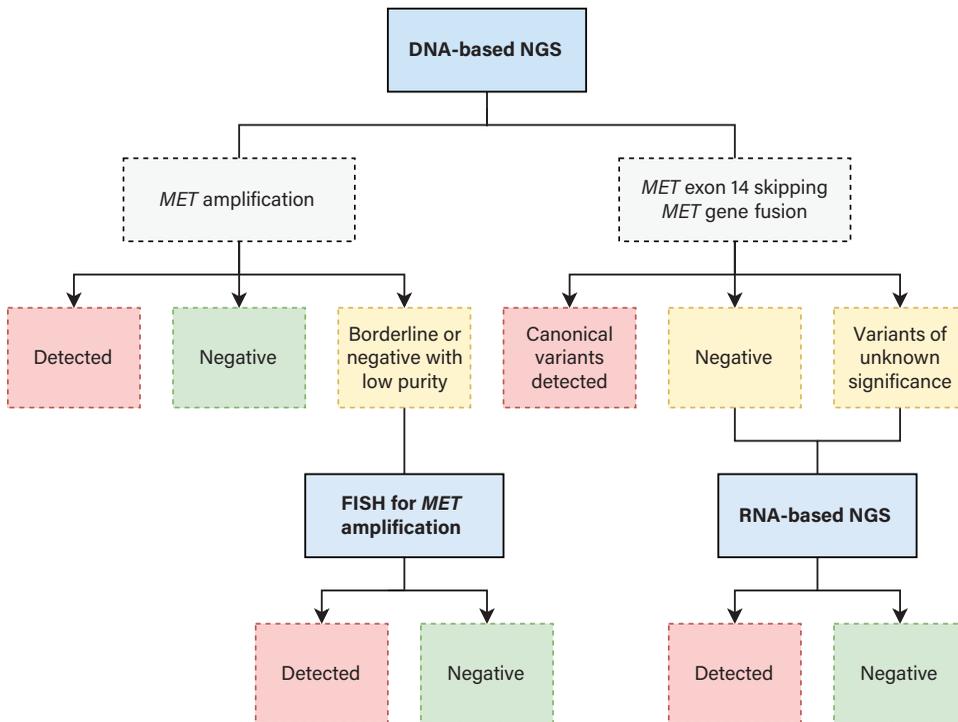


Figure 13-5. Potential testing strategy for detecting *MET* biomarkers. Abbreviations: FISH = fluorescence in situ hybridization; NGS = next-generation sequencing.

to detect multiple types of *MET* alterations, we propose a potential diagnostic workflow using DNA-based NGS as the initial screening assay for *MET* profiling.

When assessing for *MET* amplification, high-level amplification and lack of amplification in the context of sufficient tumor purity represent reliable results that do not require further testing. However, in cases with borderline amplification or lack of amplification in a case with low tumor purity, further testing using fluorescence in situ hybridization (FISH) may provide a more granular analysis and help confirm the NGS results. For *MET* exon 14 skipping and *MET* fusions, detection of canonical variants provides sufficient information for clinical management. However, negative results and variants of unknown significance should be further assessed by RNA-based testing. Alternatively, testing approaches that offer parallel DNA- and RNA-based analysis can circumvent the need to follow a

sequential testing strategy. RNA-based assays optimized for fusion detection can uncover occult fusions missed by DNA-based NGS and provide functional confirmation for variants of uncertain pathogenicity.

MET exon 14 skipping is mediated by pathogenic mutations in donor/acceptor splice sites and large genomic deletions that result in an alternative *MET* transcript lacking exon 14. In most cases, these alterations can be detected by DNA-based NGS if there is sufficient coverage of splice sites and intronic regions flanking exon 14.⁴⁷ Given that there are cryptic splice-altering mutations in deep intronic regions, complete coverage of introns 13 and 14 (in addition to exon 14) may be needed for optimal sensitivity.^{38,48} However, this can be challenging because of the large size of introns and presence of repetitive sequences, both of which can affect the accuracy of DNA-based sequencing. For this reason and others, hybrid capture-based NGS is preferred over amplicon-based NGS because of technical issues including sequencing bias and allelic dropout.^{49,50} However, even with hybrid capture-based NGS, DNA sequencing is known to miss exon 14 skipping alterations and can detect novel, noncanonical variants where their effect on splicing is unclear.⁵¹

In contrast, RNA-based sequencing and reverse transcription polymerase chain reaction (RT-PCR) can circumvent these challenges associated with DNA-level analysis and directly assess for the oncogenic by-product of altered splicing, thus enabling a more sensitive and functional approach⁵² (Figure 13-6). Hence, RNA-based testing may be important to confirm negative results from DNA-based NGS and to further assess noncanonical intronic mutations. However, low-level false-positive exon 14 skipping events have been observed^{53,54}; thus for mutation-negative cases, it may be important to incorporate a validated lower threshold for exon 14 skipping detection at the RNA level to ensure specificity and to correlate these findings with DNA-sequencing data.

MET gene amplification has been traditionally assessed using FISH. Different scoring systems have been proposed including *MET* gene copy number (GCN) and *MET* to CEP7 ratio, with the latter method being able to distinguish amplification from polysomy of chromosome 7.^{55,56} While there are no standardized thresholds for *MET* amplification that are

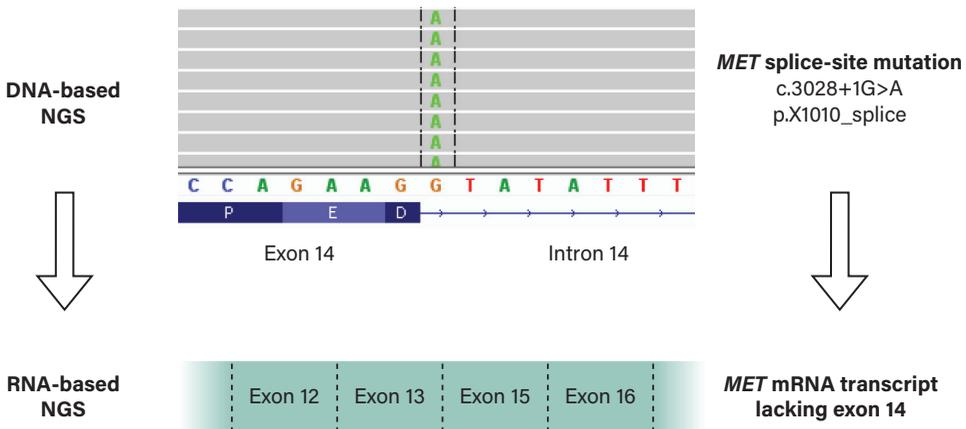


Figure 13-6. DNA- and RNA-based next-generation sequencing (NGS) detects the splice-site mutation and exon-skipping event at the RNA transcript level, respectively. The RNA-based sequencing strategy offers direct evidence of a splicing abnormality, whereas the effects of a mutation at the DNA level can only be inferred based on location within the splice region.

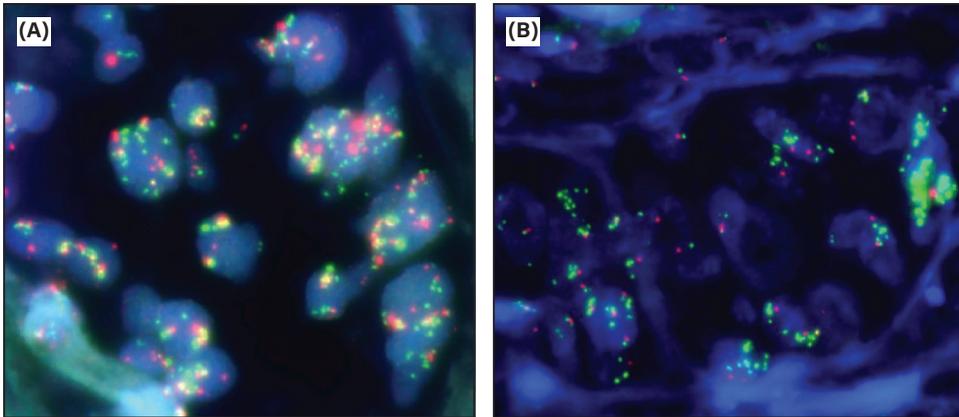


Figure 13-7. (A) Increased signals in *MET* (green) and CEP7 (red) suggesting polysomy of chromosome 7. (B) Heterogeneity of *MET* amplification. Tumor cell in the far right shows high levels of *MET* copies compared to other tumor cells with varying levels of *MET* gain and amplification.

used to guide therapy, studies have shown that high-level *MET* amplification as defined by a *MET*/CEP7 ratio of 4 to 6 or more and/or GCN of 10 or higher may predict response to *MET* inhibition in de novo and acquired resistance settings.⁵⁷⁻⁵⁹

More recently, DNA-based NGS has been increasingly used to screen for *MET* amplification.^{60,61} Similar to FISH, there is no consensus on the methodology and cutoffs for calling *MET* amplification. Advantages of NGS over FISH include the ability to provide (1) a more comprehensive copy number assessment across the entire tumor section, thereby overcoming potential heterogeneity and (2) multiplexed analysis of additional alterations in *MET* and other actionable genes. However, NGS-based copy number analysis is limited by tumor purity requirements for reliable copy number calling (~20% tumor purity).⁶² Negative cases with low tumor content or borderline results from NGS may benefit from additional FISH testing as it provides a higher-resolution analysis at a single-cell level (Figure 13-7A,B).

MET gene fusions can be detected using a variety of DNA- and RNA-based methods. Break-apart FISH represents a rapid and inexpensive method for identifying gene rearrangements involving *MET*. However, this technique does not reveal the fusion partner, the breakpoint, or the location of the kinase domain, which may be important for confirming pathogenicity. Alternatively, DNA-based NGS can be used to detect structural variants (SVs) that are predicted to form pathogenic fusions. As with other gene fusions, the genomic breakpoints underlying *MET* fusions mostly occur in introns, thus limiting the sensitivity of DNA-based sequencing.^{30,63} Furthermore, specificity can be an issue for novel or complex fusions of uncertain significance. In general, these limitations can be resolved by RNA-based methods that directly assess for the oncogenic fusion transcripts. While RT-PCR typically requires the knowledge of the fusion partner and the breakpoint, RNA-based NGS lacks these requirements and can detect fusions with novel partners and breakpoints.

Many studies have shown that *MET* protein overexpression by IHC is a poor surrogate for *MET* amplification and *MET* exon 14 skipping.^{34,37,64} However, there are emerging data suggesting that *MET* overexpression may predict sensitivity to *MET*-targeted therapies in patients with *MET* exon 14 skipping alterations.⁶⁵ While there is currently no role for *MET* IHC as a surrogate biomarker, the therapeutic significance of *MET* expression in patients with activating *MET* alterations may evolve with future data.

Clinical Implications

MET TKIs are divided into 2 groups that are distinguished by binding mechanism.⁵⁰ Type I Met inhibitors (eg, crizotinib, capmatinib, tepotinib, savolitinib) target MET in its active configuration. Among type I TKIs, crizotinib is unique in its reliance on interactions with the G1163 residue, leading to its designation as a type Ia inhibitor to distinguish it from the remaining type I inhibitors, which are considered type Ib agents. In contrast to type I MET inhibitors, type II inhibitors (eg, cabozantinib, merestinib, glesatinib) bind MET in its inactive conformation.

To date, prospective clinical studies evaluating MET inhibitors in *MET*-altered NSCLC have largely explored the efficacy of type I inhibitors. These studies have independently assessed efficacy in patients with NSCLCs harboring either *MET* amplification or *MET* exon 14 skipping alterations. Notably, in the absence of a standardized definition of *MET* amplification, the criteria used to define this genetic event have varied across clinical studies. Efficacy of type I MET inhibitors, including intracranial activity, has been particularly encouraging in the subset of tumors harboring exon 14 skipping alterations.

For example, capmatinib-induced objective responses were reported in 41% of previously treated patients in the phase 1 and 2 GEOMETRY mono-1 study, with a median progression-free survival (PFS) of 5.4 months.⁵⁷ Among patients with untreated NSCLC who received capmatinib, the overall response rate (ORR) and median PFS were 68% and 12.4 months.⁵⁷ In the phase 2 VISION study, an ORR of 46% and median PFS of 8.5 months were observed with tepotinib, with comparable efficacy across lines of previous therapy.⁶⁶ On the basis of these 2 studies, capmatinib and tepotinib gained line-agnostic global regulatory approvals for treatment of NSCLCs harboring *MET* exon 14 skipping. The multikinase type Ia MET TKI crizotinib has also demonstrated promising activity in cohorts of patients with *MET* exon 14 positive (ORR 32%) or *MET*-amplified NSCLC (ORR 28.9%) in the PROFILE 1001 study.^{59,67} In both the PROFILE 1001 and GEOMETRY mono-1 studies, MET TKI activity was greatest for tumors with higher-level *MET* amplification.⁵⁷ For example, capmatinib ORR improved from 12% to 40% and median PFS increased from 2.7 to 4.1 months as the *MET* GCN cutoff was extended from 6 to 9 copies to 10 or more copies.⁵⁷

In addition to MET TKIs, MET antibodies and antibody-drug conjugates have also undergone clinical testing in *MET*-altered NSCLC, including *MET*-overexpressing NSCLC. Historically, anti-MET antibodies have demonstrated limited efficacy.^{68,69} However, investigational MET antibody-drug conjugates and bispecific antibodies with MET activity have shown early promising results in patients with NSCLCs with *MET* overexpression or *MET* exon 14 mutations in ongoing studies.⁷⁰

Resistance Mechanisms

As described earlier, MET TKIs are classified into several groups on the basis of binding mechanics. The 2 approved MET TKIs (capmatinib and tepotinib) bind the receptor in its active conformation in a configuration that relies on interactions with the receptor hinge region and the Y1230 residue in the kinase activation loop. Binding is facilitated by a salt bridge, involving D1228 and K1110 residues, that stabilizes the activation loop. Recurring mutations involving D1228 and Y1230, which are anticipated to destabilize the salt bridge or alter the position of Y1230, have been identified in clinical specimens from patients who have

developed resistance to type I inhibitors, including capmatinib and crizotinib.⁷¹⁻⁷³ As these mutations confer cross-resistance to type I inhibitors, benefit from sequential therapy with existing type I MET TKIs is limited.^{65,74}

In contrast, preclinical studies and case reports suggest that type II MET TKIs (eg, cabozantinib, merestinib, glesatinib, and foretinib) do not rely on interactions with the activation loop and, thus, retain efficacy against *MET* D1228 and Y1230 mutations.^{72,75,76} In addition to these more common mutations, *MET* G1163R has been identified in a crizotinib-resistant specimen.⁷⁷ The solvent front G1163 residue is critical to binding of crizotinib but is not as essential for binding of other MET TKIs.

In addition to acquired on-target alterations, MET-independent resistance mechanisms contribute to both acquired and intrinsic resistance to MET selective inhibitors.⁶⁶ The bypass mechanisms described to date include MAPK pathway reactivation and amplification of *EGFR/ERBB2/ERBB3*.^{46,65,77,78} Identification of *KRAS* G12X mutations in a small subset of NSCLC with *MET* exon 14 skipping in the pretreatment setting highlights *KRAS* activation as a form of primary resistance to MET-targeted therapy.⁴⁶ *KRAS* mutations and amplifications represent approximately one-third of secondary resistance mechanisms to MET-targeted therapies.⁷² Both *KRAS* and *EGFR* amplification have been observed in patients with *MET* exon 14 skipping with progression on crizotinib therapy; interestingly, FISH studies in this context demonstrate that the amplification events occur in independent cell populations, suggesting a role for paracrine signaling between distinct populations of drug-resistant cells.⁷⁹ MET inhibition in the context of *KRAS* pathway activation appears to drive preferential signaling through phosphoinositide 3-kinase (PI3K); PI3K pathway mutations (including in *PIK3CA* and phosphatase and tensin homolog [PTEN]) also represent common mechanisms of acquired resistance to MET-targeted therapies.⁸⁰ The role for combined therapy using MET with *EGFR* or PI3K inhibitors is under investigation.^{80,81}

References

1. Maroun CR, Rowlands T. The Met receptor tyrosine kinase: a key player in oncogenesis and drug resistance. *Pharmacol Ther.* 2014;142(3):316-338.
2. Graveel CR, Tolbert D, Vande Woude GF. MET: a critical player in tumorigenesis and therapeutic target. *Cold Spring Harb Perspect Biol.* 2013;5(7).
3. Peschard P, Park M. From Tpr-Met to Met, tumorigenesis and tubes. *Oncogene.* 2007;26(9):1276-1285.
4. Boccaccio C, Ando M, Tamagnone L, et al. Induction of epithelial tubules by growth factor HGF depends on the STAT pathway. *Nature.* 1998;391(6664):285-288.
5. Ohmichi H, Koshimizu U, Matsumoto K, Nakamura T. Hepatocyte growth factor (HGF) acts as a mesenchyme-derived morphogenic factor during fetal lung development. *Development.* 1998;125(7):1315-1324.
6. Matsumoto K, Nakamura T. Hepatocyte growth factor: molecular structure, roles in liver regeneration, and other biological functions. *Crit Rev Oncog.* 1992;3(1-2):27-54.
7. Matsumoto K, Umitsu M, De Silva DM, Roy A, Bottaro DP. Hepatocyte growth factor/MET in cancer progression and biomarker discovery. *Cancer Sci.* 2017;108(3):296-307.
8. Recondo G, Che J, Janne PA, Awad MM. Targeting MET dysregulation in cancer. *Cancer Discov.* 2020;10(7):922-934.
9. Peschard P, Ishiyama N, Lin T, Lipkowitz S, Park M. A conserved DpYR motif in the juxtamembrane domain of the Met receptor family forms an atypical c-Cbl/Cbl-b tyrosine kinase binding domain binding site required for suppression of oncogenic activation. *J Biol Chem.* 2004;279(28):29565-29571.
10. Petrelli A, Gilestro GF, Lanzardo S, et al. The endophilin-CIN85-Cbl complex mediates

- ligand-dependent downregulation of c-Met. *Nature*. 2002;416(6877):187-190.
11. Barrow-McGee R, Kermorgant S. Met endosomal signalling: in the right place, at the right time. *Int J Biochem Cell Biol*. 2014;49:69-74.
 12. Drilon A, Cappuzzo F, Ou S-HI, Camidge R. Targeting MET in lung cancer: will expectations finally be MET? *J Thorac Oncol*. 2016;12(1):15-26. doi:10.1016/j.jtho.2016.10.014.
 13. Organ SL, Tsao MS. An overview of the c-MET signaling pathway. *Ther Adv Med Oncol*. 2011;3(1 suppl):S7-S19.
 14. Sattler M, Reddy MM, Hasina R, Gangadhar T, Salgia R. The role of the c-Met pathway in lung cancer and the potential for targeted therapy. *Ther Adv Med Oncol*. 2011;3(4):171-184.
 15. Lin J, Lyu Y, Li L, et al. Crizotinib inhibits activation of MET pathway caused by MET extracellular SEMA domain duplication. *Lung Cancer*. 2020;147:64-70.
 16. Kong-Beltran M, Seshagiri S, Zha J, et al. Somatic mutations lead to an oncogenic deletion of met in lung cancer. *Cancer Res*. 2006;66(1):283-289.
 17. Wang F, Liu Y, Qiu W, et al. Functional analysis of MET Exon 14 skipping alteration in cancer invasion and metastatic dissemination. *Cancer Res*. 2022;82(7):1365-1379.
 18. Schildhaus HU, Schultheis AM, Ruschoff J, et al. MET amplification status in therapy-naive adeno- and squamous cell carcinomas of the lung. *Clin Cancer Res*. 2015;21(4):907-915.
 19. Go H, Jeon YK, Park HJ, et al. High MET gene copy number leads to shorter survival in patients with non-small cell lung cancer. *J Thorac Oncol*. 2010;5(3):305-313.
 20. Awad MM, Oxnard GR, Jackman DM, et al. MET exon 14 mutations in non-small-cell lung cancer are associated with advanced age and stage-dependent MET genomic amplification and c-Met overexpression. *J Clin Oncol*. 2016;34(7):721-730.
 21. Lee JK, Madison R, Classon A, et al. Characterization of non-small-cell lung cancers with MET exon 14 skipping alterations detected in tissue or liquid: clinicogenomics and real-world treatment patterns. *JCO Precis Oncol*. 2021;5:PO.21.00122.
 22. Bittoni M, Yang JC, Shih JY, et al. Real-world insights into patients with advanced NSCLC and MET alterations. *Lung Cancer*. 2021;159:96-106.
 23. AACR Project Genie: data. American Association for Cancer Research (AACR); 2022. Updated July 22, 2022. <https://www.aacr.org/professionals/research/aacr-project-genie/aacr-project-genie-data/>.
 24. Aisner DL, Sholl LM, Berry LD, et al. The impact of smoking and TP53 mutations in lung adenocarcinoma patients with targetable mutations—the Lung Cancer Mutation Consortium (LCMC2). *Clin Cancer Res*. 2018;24(5):1038-1047.
 25. Yu HA, Arcila ME, Rekhtman N, et al. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. *Clin Cancer Res*. 2013;19(8):2240-2247.
 26. Oxnard GR, Hu Y, Mileham KF, et al. Assessment of resistance mechanisms and clinical implications in patients with EGFR T790M-positive lung cancer and acquired resistance to osimertinib. *JAMA Oncol*. 2018;4(11):1527-1534.
 27. Schoenfeld AJ, Chan JM, Rizvi H, et al. Tissue-based molecular and histological landscape of acquired resistance to osimertinib given initially or at relapse in patients with EGFR-mutant lung cancers. *J Clin Oncol*. 2019;37(15 suppl):9028-9028.
 28. Coleman N, Hong L, Zhang J, et al. Beyond epidermal growth factor receptor: MET amplification as a general resistance driver to targeted therapy in oncogene-driven non-small-cell lung cancer. *ESMO Open*. 2021;6(6):100319.
 29. Stransky N, Cerami E, Schalm S, Kim JL, Lengauer C. The landscape of kinase fusions in cancer. *Nat Commun*. 2014;5:4846.
 30. Plenker D, Bertrand M, de Langen AJ, et al. Structural alterations of MET trigger response to MET kinase inhibition in lung adenocarcinoma patients. *Clin Cancer Res*. 2018;24(6):1337-1343.
 31. Liu LF, Deng JY, Lizaso A, Lin J, Sun S. Effective response to crizotinib of concurrent KIF5B-MET and MET-CDR2-rearranged non-small cell lung cancer: a case report. *World J Clin Cases*. 2022;10(8):2529-2536.
 32. Li A, Gao HF, Wu YL. Targeting the MET pathway for potential treatment of NSCLC. *Expert Opin Ther Targets*. 2015;19(5):663-674.
 33. Park S, Koh J, Kim DW, et al. MET amplification, protein expression, and mutations in pulmonary adenocarcinoma. *Lung Cancer*. 2015;90(3):381-387.

34. Mignard X, Ruppert AM, Antoine M, et al. c-MET overexpression as a poor predictor of MET amplifications or exon 14 mutations in lung sarcomatoid carcinomas. *J Thorac Oncol.* 2018;13(12):1962-1967.
35. Li A, Niu FY, Han JF, et al. Predictive and prognostic value of de novo MET expression in patients with advanced non-small-cell lung cancer. *Lung Cancer.* 2015;90(3):375-380.
36. Baldacci S, Figeac M, Antoine M, et al. High MET overexpression does not predict the presence of MET exon 14 splice mutations in NSCLC: results from the IFCT PREDICT.amm study. *J Thorac Oncol.* 2020;15(1):120-124.
37. Guo R, Berry LD, Aisner DL, et al. MET IHC is a poor screen for MET amplification or MET exon 14 mutations in lung adenocarcinomas: data from a tri-institutional cohort of the Lung Cancer Mutation Consortium. *J Thorac Oncol.* 2019.
38. Schrock AB, Frampton GM, Suh J, et al. Characterization of 298 patients with lung cancer harboring MET exon 14 skipping alterations. *J Thorac Oncol.* 2016;11(9):1493-1502.
39. Cancer Genome Atlas Research Network. Comprehensive molecular profiling of lung adenocarcinoma. *Nature.* 2014;511(7511):543-550.
40. Liu SY, Gou LY, Li AN, et al. The unique characteristics of MET exon 14 mutation in Chinese patients with NSCLC. *J Thorac Oncol.* 2016;11(9):1503-1510.
41. Adib E, Nassar AH, Abou Alaiwi S, et al. Variation in targetable genomic alterations in non-small cell lung cancer by genetic ancestry, sex, smoking history, and histology. *Genome Med.* 2022;14(1):39.
42. Sands JM, Nguyen T, Shivdasani P, et al. Next-generation sequencing informs diagnosis and identifies unexpected therapeutic targets in lung squamous cell carcinomas. *Lung Cancer.* 2020;140:35-41.
43. Lam VK, Tran HT, Banks KC, et al. Targeted tissue and cell-free tumor DNA sequencing of advanced lung squamous-cell carcinoma reveals clinically significant prevalence of actionable alterations. *Clin Lung Cancer.* 2019;20(1):30-36.e33.
44. Liu X, Jia Y, Stoopler MB, et al. Next-generation sequencing of pulmonary sarcomatoid carcinoma reveals high frequency of actionable MET gene mutations. *J Clin Oncol.* 2016;34(8):794-802.
45. Fujino T, Suda K, Sakai K, et al. Intra-tumor and inter-tumor heterogeneity in MET Exon 14 skipping mutations and co-mutations in pulmonary pleomorphic carcinomas. *Clin Lung Cancer.* 2022;23(3):e185-e195.
46. Suzawa K, Offin M, Lu D, et al. Activation of KRAS mediates resistance to targeted therapy in MET exon 14-mutant non-small cell lung cancer. *Clin Cancer Res.* 2019;25(4):1248-1260.
47. Pruis MA, Geurts-Giele WRR, von der Thüsen JH, et al. Highly accurate DNA-based detection and treatment results of MET exon 14 skipping mutations in lung cancer. *Lung Cancer.* 2020;140:46-54.
48. Frampton GM, Ali SM, Rosenzweig M, et al. Activation of MET via diverse exon 14 splicing alterations occurs in multiple tumor types and confers clinical sensitivity to MET inhibitors. *Cancer Discov.* 2015;5(8):850-859.
49. Poirot B, Doucet L, Benhenda S, et al. MET exon 14 alterations and new resistance mutations to tyrosine kinase inhibitors: risk of inadequate detection with current amplicon-based NGS panels. *J Thorac Oncol.* 2017;12(10):1582-1587.
50. Guo R, Luo J, Chang J, et al. MET-dependent solid tumours—molecular diagnosis and targeted therapy. *Nat Rev Clin Oncol.* 2020;17(9):569-587.
51. Benayed R, Offin M, Mullaney K, et al. High yield of RNA sequencing for targetable kinase fusions in lung adenocarcinomas with no mitogenic driver alteration detected by DNA sequencing and low tumor mutation burden. *Clin Cancer Res.* 2019;25(15):4712-4722.
52. Davies KD, Lomboy A, Lawrence CA, et al. DNA-based versus RNA-based detection of MET exon 14 skipping events in lung cancer. *J Thorac Oncol.* 2019;14(4):737-741.
53. Nosi V, Luca A, Milan M, et al. MET exon 14 skipping: a case study for the detection of genetic variants in cancer driver genes by deep learning. *Int J Mol Sci.* 2021;22(8).
54. Manthei DM, Kleyman-Smith Y, Brown NA, Betz BL. Improved detection of MET exon 14 skipping mutations in lung adenocarcinoma with combined DNA/RNA testing and refined analysis methods [abstract ST009]. *J Mol Diagn.* 2019;19(30391-5):S1525-S1578.
55. Noonan SA, Berry L, Lu X, et al. Identifying the appropriate FISH criteria for defining MET copy number-driven lung adenocarcinoma through oncogene overlap analysis. *J Thorac Oncol.* 2016;11(8):1293-1304.

56. Cappuzzo F, Marchetti A, Skokan M, et al. Increased MET gene copy number negatively affects survival of surgically resected non-small-cell lung cancer patients. *J Clin Oncol.* 2009;27(10):1667-1674.
57. Wolf J, Seto T, Han JY, et al. Capmatinib in MET exon 14-mutated or MET-amplified non-small-cell lung cancer. *N Engl J Med.* 2020;383(10):944-957.
58. Wu YL, Zhang L, Kim DW, et al. Phase Ib/II study of capmatinib (INC280) plus gefitinib after failure of epidermal growth factor receptor (EGFR) inhibitor therapy in patients with EGFR-mutated, MET factor-dysregulated non-small-cell lung cancer. *J Clin Oncol.* 2018;36(31):3101-3109.
59. Camidge DR, Otterson GA, Clark JW, et al. Crizotinib in patients with MET-amplified NSCLC. *J Thorac Oncol.* 2021;16(6):1017-1029.
60. Peng LX, Jie GL, Li AN, et al. MET amplification identified by next-generation sequencing and its clinical relevance for MET inhibitors. *Exp Hematol Oncol.* 2021;10(1):52.
61. Schmitt C, Schulz AA, Winkelmann R, Smith K, Wild PJ, Demes M. Comparison of MET gene amplification analysis by next-generation sequencing and fluorescence in situ hybridization. *Oncotarget.* 2021;12(22):2273-2282.
62. Solomon JP, Yang SR, Choudhury NJ, et al. Bioinformatically-expanded next-generation sequencing analysis optimizes identification of therapeutically relevant MET copy number alterations in >50,000 tumors. *Clin Cancer Res.* 2022.
63. Pan Y, Zhang Y, Ye T, et al. Detection of novel NRG1, EGFR, and MET fusions in lung adenocarcinomas in the Chinese population. *J Thorac Oncol.* 2019;14(11):2003-2008.
64. Bubendorf L, Dafni U, Schobel M, et al. Prevalence and clinical association of MET gene overexpression and amplification in patients with NSCLC: results from the European Thoracic Oncology Platform (ETOP) Lungscape project. *Lung Cancer.* 2017;111:143-149.
65. Guo R, Offin M, Brannon AR, et al. MET exon 14-altered lung cancers and MET inhibitor resistance. *Clin Cancer Res.* 2021;27(3):799-806.
66. Paik PK, Felip E, Veillon R, et al. Tepotinib in non-small-cell lung cancer with MET exon 14 skipping mutations. *N Engl J Med.* 2020;383(10):931-943.
67. Drilon A, Clark JW, Weiss J, et al. Antitumor activity of crizotinib in lung cancers harboring a MET exon 14 alteration. *Nat Med.* 2020;26(1):47-51.
68. Wakelee H, Zvirbule Z, De Braud F, et al. Efficacy and safety of onartuzumab in combination with first-line bevacizumab- or pemetrexed-based chemotherapy regimens in advanced non-squamous non-small-cell lung cancer. *Clin Lung Cancer.* 2017;18(1):50-59.
69. Hirsch FR, Govindan R, Zvirbule Z, et al. Efficacy and safety results from a phase II, placebo-controlled study of onartuzumab plus first-line platinum-doublet chemotherapy for advanced squamous cell non-small-cell lung cancer. *Clin Lung Cancer.* 2017;18(1):43-49.
70. Camidge DR, Bar J, Horinouchi H, et al. Telisotuzumab vedotin (Teliso-V) monotherapy in patients (pts) with previously treated c-Met-overexpressing (OE) advanced non-small cell lung cancer (NSCLC). *J Clin Oncol.* 2022;40(16 suppl):9016-9016.
71. Villaruz LC, Socinski MA, Abberbock S, et al. Clinicopathologic features and outcomes of patients with lung adenocarcinomas harboring BRAF mutations in the Lung Cancer Mutation Consortium. *Cancer.* 2015;121(3):448-456.
72. Recondo G, Bahcall M, Spurr LF, et al. Molecular mechanisms of acquired resistance to MET tyrosine kinase inhibitors in patients with MET exon 14-mutant NSCLC. *Clin Cancer Res.* 2020;26(11):2615-2625.
73. Heist RS, Sequist LV, Borger D, et al. Acquired resistance to crizotinib in NSCLC with MET exon 14 skipping. *J Thorac Oncol.* 2016;11(8):1242-1245.
74. Rotow JK, Gui P, Wu W, et al. Co-occurring alterations in the RAS-MAPK pathway limit response to MET inhibitor treatment in MET exon 14 skipping mutation-positive lung cancer. *Clin Cancer Res.* 2020;26(2):439-449.
75. Fujino T, Suda K, Koga T, et al. Foretinib can overcome common on-target resistance mutations after capmatinib/tepotinib treatment in NSCLCs with MET exon 14 skipping mutation. *J Hematol Oncol.* 2022;15(1):79.
76. Bahcall M, Sim T, Pawletz CP, et al. Acquired METD1228V mutation and resistance to MET inhibition in lung cancer. *Cancer Discov.* 2016;6(12):1334-1341.

77. Dagogo-Jack I, Moonsamy P, Gainor JF, et al. A phase 2 study of capmatinib in patients with MET-altered lung cancer previously treated with a MET inhibitor. *J Thorac Oncol.* 2021;16(5):850-859.
78. Yoda S, Lin JJ, Lawrence MS, et al. Sequential ALK inhibitors can select for lorlatinib-resistant compound ALK mutations in ALK-positive lung cancer. *Cancer Discov.* 2018;8(6):714-729.
79. Bahcall M, Awad MM, Sholl LM, et al. Amplification of wild-type KRAS imparts resistance to crizotinib in MET exon 14 mutant non-small cell lung cancer. *Clin Cancer Res.* 2018;24(23):5963-5976.
80. Jamme P, Fernandes M, Copin MC, et al. Alterations in the PI3K pathway drive resistance to MET inhibitors in NSCLC harboring MET exon 14 skipping mutations. *J Thorac Oncol.* 2020;15(5):741-751.
81. Krebs M, Spira AI, Cho BC, et al. Amivantamab in patients with NSCLC with MET exon 14 skipping mutation: updated results from the CHRYSALIS study. *J Clin Oncol.* 2022;40(16 suppl):9008-9008.

Gene Structure and Function

RAS (rat sarcoma viral oncogene homolog) encodes a membrane-bound protein, initially described by Harvey¹ and Kristen² as a retroviral oncogene involved in cell proliferation, differentiation, and survival.³ RAS belongs to a class of protein called *small guanosine triphosphatase* (GTPase), which is expressed in all mammalian cells.^{4,5} The RAS gene family consists of 3 members (*HRAS*, *NRAS*, and *KRAS*) that code for corresponding isoforms.⁵ However, notably, the *KRAS* (Kristen rat sarcoma viral oncogene) isoform represents approximately 75% to 80% of all *RAS* mutations in cancer, followed by *NRAS* (12%), and, irregularly, *HRAS* (3%).^{6,7} RAS protein is composed of 3 major elements:

1. G-domain is highly conserved between RAS isoforms and contains switch 1 and 2 loops. It is responsible for guanosine triphosphate (GTP)-guanosine diphosphate (GDP) exchange.
2. C-terminal domain, referred to as the *hypervariable region*, shows significant variations among RAS isoforms.
3. C-terminal CaaX box drives post-translational modifications.⁸⁻¹¹

RAS protein acts as a molecular switch, cycling between an active GTP-bound state and an inactive GDP-bound state.^{8,12} The conversion of RAS protein to its active form is facilitated by guanine nucleotide exchange factors (GEFs) while GTPase-activating proteins (GAPs) have a role in maintaining RAS in its inactive state through activating RAS-GTPase and GTP hydrolysis.^{8,12} When *KRAS* is mutated, RAS protein is locked into the GTP-bound active form, which in turn constitutively activates downstream signaling pathways, such as the RAF-MEK-ERK and PI3K-AKT-mTOR pathways, conferring malignant phenotype^{8,12} (Figure 14-1).

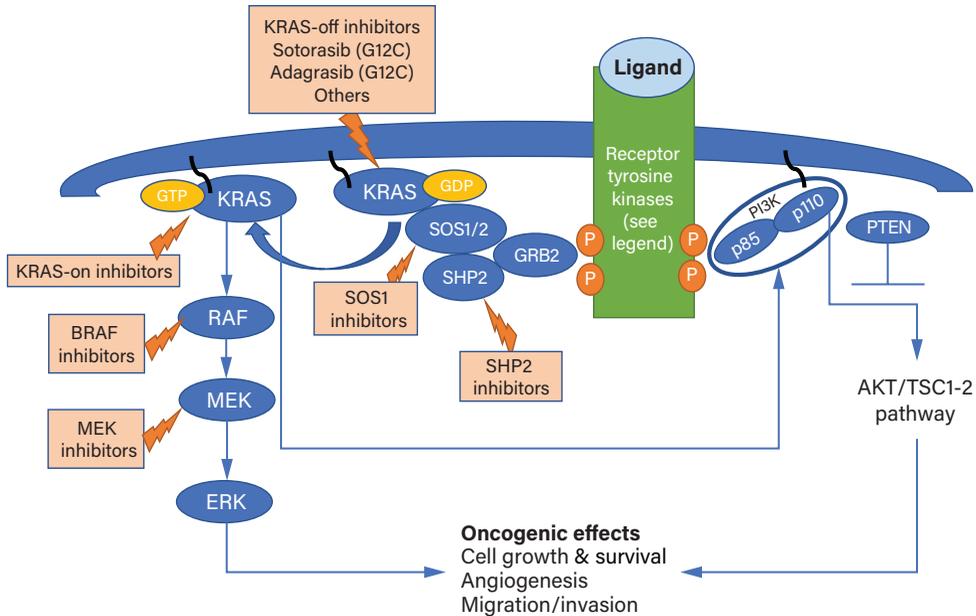


Figure 14-1. A diagrammatic representation of cellular membrane-bound KRAS protein and some of its relationships with other proteins and signaling pathways. (Note: The generic representation of the membrane receptor tyrosine kinase [RTK] is for illustration only.) There are many ligands and RTKs, including the EGFR/HER family, which can form homo- and heterodimers (see relevant chapters). Wild-type KRAS may be activated to its guanosine triphosphate (GTP)-bound state, facilitating downstream signaling via RAF/MEK/ERK and via PI3K/AKT/TSC1-2. This activation is mediated by guanine nucleotide exchange factors (GEFs), a major one of which is SOS1, and related proteins such as GRB2 and SHP2. Mutations in KRAS result in reduction of both hydrolysis of GTP and interaction with guanosine triphosphatase (GTPase)-activating proteins (GAPs) (*not shown in diagram*). These changes maintain KRAS protein in an activated state, driving oncogenesis. Several points in this complex pathway are being therapeutically exploited (see text). The most clinically advanced in this setting are drugs such as sotorasib and adagrasib, which function to maintain KRAS in a GDP-bound inactive state.^{3,13} Abbreviation: PTEN = phosphatase and tensin homolog. (Adapted from Puneekar et al.¹³)

Type of Alterations and Role in Oncogenesis (Mutations, Fusions, Amplification, Protein Expression)

KRAS is the most frequently mutated oncogene in cancer.¹⁴ Similarly, activating mutations in *KRAS* are the most prevalent oncogenic drivers in both early and advanced lung adenocarcinoma, occurring in approximately 25% to 32% of tumors.^{15,16} Most *KRAS* point mutations occur at exons 2 and 3, mainly affecting codons G12, G13, and Q61.⁶ In lung cancer, G12C (ie, mutation from amino acid glycine to cysteine) is the most common (41%), whereas G12V and G12D represent 21% and 17% of *KRAS* mutations, respectively¹⁷ (Figure 14-2). The biologic and clinical significance of *KRAS* mutations varies depending on the mutation subtype in lung cancer. For example, G12C and G12D have significant affinities for binding to RASGDS-Ral-FLIP and PI3K-AKT-mTOR pathways, respectively.¹⁸ In addition, *KRAS* G12C mutations showed higher extracellular signal-regulated kinase-1 and -2 (ERK1/2) phosphorylation than those with the *KRAS* G12D.¹⁹

As seen with other actionable driver mutations in lung adenocarcinoma, *KRAS* mutations do not usually coexist in the context of other oncogene drivers, including *EGFR*, *ALK*, and *ROS*-driven tumors. Although *KRAS* and *EGFR* mutations typically occur in a mutually exclusive fashion in lung cancer,^{17,20} anecdotal reports show evidence of co-occurrence of

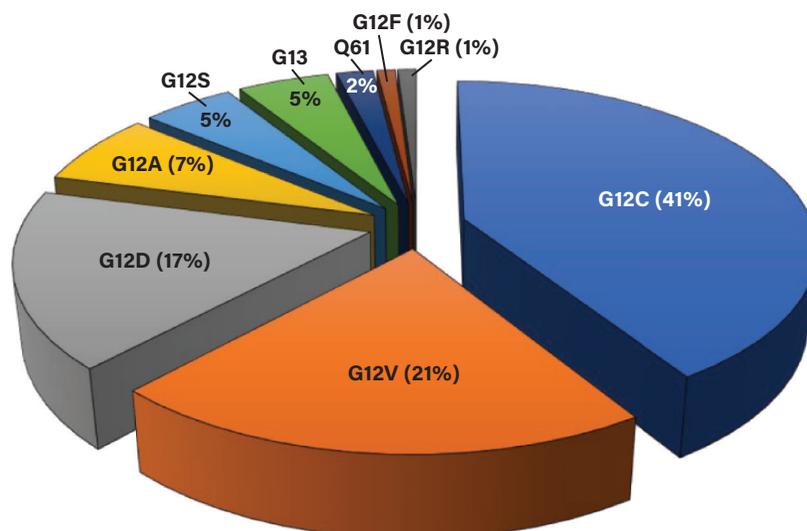


Figure 14-2. Prevalence of the most common *KRAS* mutations in pulmonary adenocarcinoma

KRAS and *EGFR* mutations in lung cancer patients.²¹⁻²³ *KRAS* mutations have been described in *ALK*-translocated lung cancer mostly associated with mechanisms of resistance to *ALK* tyrosine kinase inhibitors (TKIs).²⁴ *KRAS* G12C and other *KRAS* mutations more frequently co-occur with *MET* amplification in both localized²⁵ and metastatic²⁶ treatment-naïve non-small cell lung carcinoma (NSCLC). *ERBB2* amplification ($P = 0.002$) and *ERBB4* mutations ($P = 0.025$) were more frequently found in *KRAS* G12C-mutated tumors when compared to tumors without *KRAS* G12C.^{3,26}

KRAS copy number alterations, including allelic imbalance and increased copy number, have been also reported in NSCLC.^{27,28} *KRAS* amplification was also shown to be a mechanism of resistance to epidermal growth factor receptor (EGFR) TKIs.²⁸ Concomitant mutations in the *KRAS* gene are common as they were observed in about 3.4% of tumors with a *KRAS* mutation and 8% of the *KRAS* G12C mutation. The most frequent co-occurring mutation associated with *KRAS* G12C were *KRAS* G12F and G12V.²⁹ In this study, the presence of co-occurring mutation led to increased resistance to a specific G12C inhibitor. In about half of tumors with *KRAS* mutations, and especially in those occurring in smokers, several other mutations may also be found, so-called co-mutations, which may have biologic significance.^{3,26}

Current data show that co-occurring genetic alterations significantly impact biologic evolution, clinical outcomes, and response to treatments.^{15,30-34} Large-scale sequencing studies have established a census of major *KRAS* co-mutations in lung adenocarcinoma.¹⁵ Co-occurring mutations in *TP53* (~40%), *STK11* (ie, serine/threonine kinase 11; 20%), and *KEAP1* (ie, Kelch-like ECH-associated protein 1; 13%-24%) are the most frequent.^{26,30} These 3 subsets are associated with different biologic properties and therapeutic sensitivities¹⁵ and could act via intrinsic RAS signaling pathways as well as on the tumor microenvironment. In particular, *KRAS/TP53*-mutated NSCLC demonstrated increased levels of inflammatory markers and immune checkpoint effector molecules, while *KRAS/KEAP1* showed lower levels of immune markers, including programmed death-ligand 1 (PD-L1).¹⁵

In addition to promoting tumorigenesis through downstream effectors, mutant *KRAS* cells have been found to interact with the tumor microenvironment. In colorectal cancers, *KRAS* mutations can mediate downregulation of major histocompatibility class 1 molecule expression, an effect of which is to interfere with tumor antigen priming and presentation to T cells.^{3,35}

Similarly, *KRAS* mutations are associated with higher PD-L1 expression in NSCLC, thus contributing to exhausted T cells.^{36,37} Notably, the PD-L1 tumor proportion score (TPS) of 50% or more was reported in a range of 34% to 41% for *KRAS* G12C patients compared with 20% to 26% among patients with *KRAS* wild-type tumors.^{38,39} In NSCLC cell lines with *KRAS* mutations, ectopic expression of PD-L1 was found to be modulated by the MAPK and STAT3 pathways downstream of *KRAS*.^{3,40}

Epidemiologic, Clinical, and Histologic Characteristics

KRAS mutation distribution is usually cancer-type specific, and the frequencies are relatively stable worldwide in various cancer types, with the exception of NSCLC, particularly adenocarcinoma histology.⁷ Geographic differences in *KRAS* prevalence in NSCLC have been observed worldwide with the highest incidence in Europe followed by North America and lowest incidence in East Asia regions and India.^{6,7} This contrasts with *EGFR* prevalence, suggesting the impact of genetics or environment with growing research currently ongoing.⁷ Likewise, it was reported that *KRAS* G12C mutations were more frequent in Black and White patients rather than Asian patients ($P < 0.001$), while in NSCLC, *KRAS* mutations are more frequent in adenocarcinoma histology (32%)^{6,25,41} and very infrequently ($\leq 1\%$) reported in squamous cell carcinoma when rigorous pathology assessment is performed.^{39,42,43}

In adenocarcinoma, higher frequencies of *KRAS* mutations have been described in invasive mucinous carcinomas (61%-71%).^{44,45} *KRAS* mutations in NSCLC are often associated with tobacco history (ie, 7% in never-smokers vs 32% and 37% in former and current smokers, respectively⁴⁶), and the distribution of variant type in NSCLC varies between smokers and never-smokers.³⁹ In the case of *KRAS* G12C, commonly seen in the smoker population (41% of *KRAS* mutations), it has been related to signature 4 (a smoking-related signature), which is associated with C>A transitions.⁴⁷ Moreover, *KRAS* G12C mutation seems to be more frequent in women (ie, 43%; $P = 0.007$) and younger patients (ie, median age 63 years old; $P = 0.0092$) compared with other *KRAS* mutations^{25,42} and *KRAS* wild type. G12D mutations mostly occur in never-smokers at 56% of *KRAS* mutations.⁴¹ Despite some conflicting results,^{38,45,46} *KRAS* mutations in NSCLC seem to be associated with worse prognosis.⁴⁷⁻⁴⁹ It has been reported that patients with G12C-mutated NSCLC have a poorer prognosis when compared to cases with other *KRAS* mutations or *KRAS* wild type.²⁵ In addition, *KRAS* G12C-positive patients present a higher rate of metastasis at diagnosis compared with patients with *KRAS* wild type (ie, 94% vs 88%).³⁸

Testing Strategies

Detection of *KRAS* mutation, including the G12C variant, in lung cancer, from DNA extracted from tumor tissue, cell specimens (cytology), and cell-free DNA (cfDNA) obtained from blood and other fluids (ie, pleural effusion, cerebrospinal fluid) is straightforward. *KRAS* point mutations are harbored in hotspots in codons (12, 13, and 61), which are easily

covered using gene panels probed by both polymerase chain reaction (PCR)-based and next-generation sequencing (NGS) approaches.⁴⁸⁻⁵¹ When used with adequate nucleic acid input (10-100 ng of DNA and 5%-15% tumor content), most of the different commercial platforms, either PCR-based or NGS, showed an excellent overall accuracy and a high level of concordance, although NGS seems to show a slightly superior performance when compared to non-NGS assays.⁵²⁻⁵⁷ As recommended by several guidelines, for tumor tissue diagnosis, NGS panels are a preferred approach. cfDNA could be used as an alternative complementary approach when the tissue specimen is exhausted, the tumor is not accessible for a biopsy, or the yield of tumor or DNA is not enough for proper molecular testing.⁵¹ Molecular analysis could be used to identify genomic-related mechanisms of resistance emerging from KRAS-targeted therapy approaches, such as KRAS G12C inhibitors.

Clinical Implications

Despite thorough preclinical and clinical research, KRAS mutations have long been considered an undruggable target. Recently, however, several covalent KRAS G12C inhibitors, including AMG510 (sotorasib) and MRTX849 (adagrasib), have been developed for KRAS G12C-mutant tumors.⁵⁸⁻⁶⁰ The action of these agents targeting G12C relies on covalent binding to cysteine 12 and the switch-2 binding pocket region when KRAS G12C is in its inactive GDP-state.⁶¹ Several studies have shown that KRAS G12C inhibitors trap KRAS in its inactive GDP-bound state by reducing its susceptibility to nucleotide exchange factors^{62,63} and that efficacy of KRAS G12C inhibition requires intact GTPase activity.^{62,63} Of note, the CodeBreaK100 clinical trial showed a beneficial effect of sotorasib in patients with advanced NSCLC harboring KRAS G12C mutations,⁶⁴ and in May 2021, the US Food and Drug Administration (FDA) approved sotorasib for KRAS G12C-positive NSCLC patients who had received at least 1 prior systemic therapy.

Conversely, KRAS G12C inhibitors do not provide durable responses, and the median progression-free survival (PFS) time of NSCLC patients treated with sotorasib was 6.8 months.⁶⁴ In the phase 3 CodeBreaK 200 trial, sotorasib demonstrated an improvement in PFS versus docetaxel in patients with KRAS G12C-mutated NSCLC who had progressed after prior platinum-based chemotherapy and a checkpoint inhibitor (hazard ratio [HR], 0.66; 95% CI, 0.51-0.86; $P = 0.002$), with also a higher overall response rate (ORR; 28.1% vs 13.2%) with a more favorable safety profile.⁶⁵ No difference was observed, however, in overall survival (OS) between both arms, although the study was not powered for OS, and crossover was permitted (affecting 34% of patients).

Adagrasib is the second irreversible and selective KRAS G12C inhibitor to have entered in clinical trials, showing clinical efficacy (ORR, 42.9%; PFS, 6.5 months), without new safety signals in single-arm clinical trials,⁶⁶ thus leading to FDA approval in December 2022 for KRAS G12C-mutated locally advanced or metastatic NSCLC, as determined by an FDA-approved test, for patients who have received at least one prior system therapy; this drug was optimized to exhibit long half-life and extensive tissue distribution.^{67,68} Several studies are currently ongoing, assessing the efficacy of these KRAS G12C inhibitors versus conventional therapy in phase 3 studies and also other early clinical trials seeking synergies with combinations to other therapies (eg, immunotherapy, chemotherapy, etc). Interestingly, it has been shown that *STK11* and *KEAP1* mutations confer worse clinical outcome to immune

checkpoint inhibition⁶⁹ and chemotherapy⁷⁰ among patients with *KRAS* mutations, including G12C variant.⁷⁰

Resistance Mechanisms

The ORRs obtained for either sotorasib or adagrasib are markedly lower compared to those obtained with EGFR and ALK inhibition in NSCLC,^{71,72} suggesting the presence of intrinsic mechanisms of resistance to *KRAS* G12C inhibitors. Although still under investigation, several intrinsic mechanisms of resistance to both inhibitors have been reported, including adaptive feedback reactivation of RAS signaling pathways such as RAS-MAPK.⁷³ Regarding acquired mechanisms of resistance, both using laboratory models and/or testing tumor specimens from treated patients suggest 3 main mechanisms:

1. On-target mechanisms, represented by the detection of other *KRAS*-activating mutations (ie, sotorasib, G13D, R68M, and A59S; adagrasib, Q99L, Y96D, and R68S0)^{24,74-76}
2. Bypass mechanisms, including the activation of receptor tyrosine kinases and RAS downstream signaling pathways, via MET (including gene amplification) and hepatocyte growth factor (HGF),⁷⁷ insulin-like growth factor receptor-1 (IGFR-1), and fibroblast growth factor receptor-1 (FGFR-1)-related mechanisms⁶⁸
3. Lineage plasticity and acquisition of features of epithelial-to-mesenchymal transition, including initial reports of change from adenocarcinoma to squamous cell carcinoma histology⁷⁶ and the downregulation of E-cadherin and upregulation of vimentin,⁶⁸ respectively.

References

1. Harvey JJ. An unidentified virus which causes the rapid production of tumours in mice. *Nature*. 1964;204:1104-1105.
2. Kirsten WH, Mayer LA. Morphologic responses to a murine erythroblastosis virus. *J Natl Cancer Inst*. 1967;39(2):311-335.
3. Désage A-L, Léonce C, Swalduz A, Ortiz-Cuaran S. Targeting *KRAS* mutant in non-small cell lung cancer: novel insights into therapeutic strategies. *Front Oncol*. 2022;12:796832. doi:10.3389/fonc.2022.796832.
4. Simanshu DK, Nissley DV, McCormick F. RAS proteins and their regulators in human disease. *Cell*. 2017;170(1):17-33.
5. Malumbres M, Barbacid M. RAS oncogenes: the first 30 years. *Nat Rev Cancer*. 2003;3(6):459-465.
6. Prior IA, Hood FE, Hartley JL. The frequency of Ras mutations in cancer. *Cancer Res*. 2020;80(14):2969-2974.
7. Timar J, Kashofer K. Molecular epidemiology and diagnostics of *KRAS* mutations in human cancer. *Cancer Metastasis Rev*. 2020;39(4):1029-1038.
8. Vigil D, Cherfils J, Rossman KL, Der CJ. Ras superfamily GEFs and GAPs: validated and tractable targets for cancer therapy? *Nat Rev Cancer*. 2010;10(12):842-857.
9. Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell*. 2000;103(2):211-225.
10. Gale NW, Kaplan S, Lowenstein EJ, Schlessinger J, Bar-Sagi D. Grb2 mediates the EGF-dependent activation of guanine nucleotide exchange on Ras. *Nature*. 1993;363(6424):88-92.
11. Vetter IR, Wittinghofer A. The guanine nucleotide-binding switch in three dimensions. *Science*. 2001;294(5545):1299-1304.
12. Cherfils J, Zeghouf M. Regulation of small GTPases by GEFs, GAPs, and GDIs. *Physiol Rev*. 2013;93(1):269-309.
13. Punekar SR, Velcheti V, Neel BG, Wong KK. The current state of the art and future trends in RAS-targeted cancer therapies. *Nat Rev Clin Oncol*. 2022;19(10):637-655. doi:10.1038/s41571-022-00671-9.

14. Downward J. Targeting RAS signalling pathways in cancer therapy. *Nat Rev Cancer*. 2003;3(1):11-22.
15. Skoulidis F, Byers LA, Diao L, et al. Co-occurring genomic alterations define major subsets of KRAS-mutant lung adenocarcinoma with distinct biology, immune profiles, and therapeutic vulnerabilities. *Cancer Discov*. 2015;5(8):860-877.
16. Cancer Genome Atlas Research Network. Comprehensive molecular profiling of lung adenocarcinoma. *Nature*. 2014;511(7511):543-550.
17. Soh J, Toyooka S, Matsuo K, et al. Ethnicity affects EGFR and KRAS gene alterations of lung adenocarcinoma. *Oncol Lett*. 2015;10(3):1775-1782.
18. Ihle NT, Byers LA, Kim ES, et al. Effect of KRAS oncogene substitutions on protein behavior: implications for signaling and clinical outcome. *J Natl Cancer Inst*. 2012;104(3):228-239.
19. Li S, Liu S, Deng J, et al. Assessing therapeutic efficacy of MEK inhibition in a KRAS(G12C)-driven mouse model of lung cancer. *Clin Cancer Res*. 2018;24(19):4854-4864.
20. Pao W, Miller VA. Epidermal growth factor receptor mutations, small-molecule kinase inhibitors, and non-small-cell lung cancer: current knowledge and future directions. *J Clin Oncol*. 2005;23(11):2556-2568.
21. Gainor JF, Varghese AM, Ou SH, et al. ALK rearrangements are mutually exclusive with mutations in EGFR or KRAS: an analysis of 1,683 patients with non-small cell lung cancer. *Clin Cancer Res*. 2013;19(15):4273-4281.
22. Choughule A, Sharma R, Trivedi V, et al. Coexistence of KRAS mutation with mutant but not wild-type EGFR predicts response to tyrosine-kinase inhibitors in human lung cancer. *Br J Cancer*. 2014;111(11):2203-2204.
23. Ortiz-Cuaran S, Scheffler M, Plenker D, et al. Heterogeneous mechanisms of primary and acquired resistance to third-generation EGFR inhibitors. *Clin Cancer Res*. 2016;22(19):4837-4847.
24. Camidge DR, Doebele RC. Treating ALK-positive lung cancer—early successes and future challenges. *Nat Rev Clin Oncol*. 2012;9(5):268-277.
25. Finn SP, Addeo A, Dafni U, et al. Prognostic impact of KRAS G12C mutation in patients with NSCLC: results from the European Thoracic Oncology Platform Lungscape Project. *J Thorac Oncol*. 2021;16(6):990-1002.
26. Scheffler M, Ihle MA, Hein R, et al. K-ras mutation subtypes in NSCLC and associated co-occurring mutations in other oncogenic pathways. *J Thorac Oncol*. 2019;14(4):606-616.
27. Soh J, Okumura N, Lockwood WW, et al. Oncogene mutations, copy number gains and mutant allele specific imbalance (MASI) frequently occur together in tumor cells. *PLoS One*. 2009;4(10):e7464.
28. Nakatani K, Yamaoka T, Ohba M, et al. KRAS and EGFR amplifications mediate resistance to rociletinib and osimertinib in acquired afatinib-resistant NSCLC harboring exon 19 deletion/T790M in EGFR. *Mol Cancer Ther*. 2019;18(1):112-126.
29. Vaclova T, Chakraborty A, Sherwood J, et al. Concomitant KRAS mutations attenuate sensitivity of non-small cell lung cancer cells to KRAS G12C inhibition. *Sci Rep*. 2022;12(1):2699.
30. Arbour KC, Jordan E, Kim HR, et al. Effects of co-occurring genomic alterations on outcomes in patients with KRAS-mutant non-small cell lung cancer. *Clin Cancer Res*. 2018;24(2):334-340.
31. Kitajima S, Ivanova E, Guo S, et al. Suppression of STING associated with LKB1 loss in KRAS-driven lung cancer. *Cancer Discov*. 2019;9(1):34-45.
32. Romero R, Sayin VI, Davidson SM, et al. Keap1 loss promotes Kras-driven lung cancer and results in dependence on glutaminolysis. *Nat Med*. 2017;23(11):1362-1368.
33. Wang X, Min S, Liu H, et al. Nf1 loss promotes Kras-driven lung adenocarcinoma and results in Psat1-mediated glutamate dependence. *EMBO Mol Med*. 2019;11(6).
34. Skoulidis F, Goldberg ME, Greenawalt DM, et al. *STK11/LKB1* mutations and PD-1 inhibitor resistance in KRAS-mutant lung adenocarcinoma. *Cancer Discov*. 2018 Jul;8(7):822-835. doi:10.1158/2159-8290.
35. Miura Y, Sunaga N. Role of immunotherapy for oncogene-driven non-small cell lung cancer. *Cancers (Basel)*. 2018;10(8):245.
36. Chen N, Fang W, Lin Z, et al. KRAS mutation-induced upregulation of PD-L1 mediates immune escape in human lung adenocarcinoma. *Cancer Immunol Immunother*. 2017;66(9):1175-1187.
37. Li D, Zhu X, Wang H, Li N. Association between PD-L1 expression and driven gene status in NSCLC: a meta-analysis. *Eur J Surg Oncol*. 2017;43(7):1372-1379.

38. Sebastian M, Eberhardt WEE, Hoffknecht P, et al. KRAS G12C-mutated advanced non-small cell lung cancer: a real-world cohort from the German prospective, observational, nation-wide CRISP registry (AIO-TRK-0315). *Lung Cancer*. 2021;154:51-61.
39. Judd J, Abdel Karim N, Khan H, et al. Characterization of KRAS mutation subtypes in non-small cell lung cancer. *Mol Cancer Ther*. 2021;20(12):2577-2584.
40. Sumimoto H, Takano A, Teramoto K, Daigo Y. RAS-mitogen-activated protein kinase signal is required for enhanced PD-L1 expression in human lung cancers. *PLoS One*. 2016;11(11):e0166626.
41. Dogan S, Shen R, Ang DC, et al. Molecular epidemiology of EGFR and KRAS mutations in 3,026 lung adenocarcinomas: higher susceptibility of women to smoking-related KRAS-mutant cancers. *Clin Cancer Res*. 2012;18(22):6169-6177.
42. Acker F, Stratmann J, Aspacher L, et al. KRAS mutations in squamous cell carcinomas of the lung. *Front Oncol*. 2021;11:788084.
43. Rekhtman N, Paik PK, Arcila ME, et al. Clarifying the spectrum of driver oncogene mutations in biomarker-verified squamous carcinoma of lung: lack of EGFR/KRAS and presence of PIK3CA/AKT1 mutations. *Clin Cancer Res*. 2012;18(4):1167-1176.
44. Hwang DH, Sholl LM, Rojas-Rudilla V, et al. KRAS and NKX2-1 mutations in invasive mucinous adenocarcinoma of the lung. *J Thorac Oncol*. 2016;11(4):496-503.
45. Kadota K, Yeh YC, D'Angelo SP, et al. Associations between mutations and histologic patterns of mucin in lung adenocarcinoma: invasive mucinous pattern and extracellular mucin are associated with KRAS mutation. *Am J Surg Pathol*. 2014;38(8):1118-1127.
46. Wood K, Hensing T, Malik R, Salgia R. Prognostic and predictive value in KRAS in non-small-cell lung cancer: a review. *JAMA Oncol*. 2016;2(6):805-812.
47. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. *Nature*. 2013;500(7463):415-421.
48. Kalemkerian GP, Narula N, Kennedy EB, et al. Molecular testing guideline for the selection of patients with lung cancer for treatment with targeted tyrosine kinase inhibitors: American Society of Clinical Oncology endorsement of the College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology clinical practice guideline update. *J Clin Oncol*. 2018;36(9):911-919.
49. Lindeman NI, Cagle PT, Beasley MB, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. *J Thorac Oncol*. 2013;8(7):823-859.
50. Singh N, Temin S, Baker S Jr, et al. Therapy for stage IV non-small-cell lung cancer without driver alterations: ASCO living guideline. *J Clin Oncol*. 2022;40(28):3323-3343.
51. Lindeman NI, Cagle PT, Aisner DL, et al. 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. *J Mol Diagn*. 2018;20(2):129-159.
52. Gao J, Wu H, Wang L, et al. Validation of targeted next-generation sequencing for RAS mutation detection in FFPE colorectal cancer tissues: comparison with Sanger sequencing and ARMS-scorpion real-time PCR. *BMJ Open*. 2016;6(1):e009532.
53. Gao J, Wu H, Shi X, et al. Comparison of next-generation sequencing, quantitative PCR, and Sanger sequencing for mutation profiling of EGFR, KRAS, PIK3CA and BRAF in clinical lung tumors. *Clin Lab*. 2016;62(4):689-696.
54. Cainap C, Balacescu O, Cainap SS, Pop LA. Next generation sequencing technology in lung cancer diagnosis. *Biology (Basel)*. 2021;10(9):864.
55. Hinrichs JW, van Bloklant WT, Moons MJ, et al. Comparison of next-generation sequencing and mutation-specific platforms in clinical practice. *Am J Clin Pathol*. 2015;143(4):573-578.
56. Merker JD, Devereaux K, Iafrate AJ, et al. Proficiency testing of standardized samples shows very high interlaboratory agreement for clinical next-generation sequencing-based oncology assays. *Arch Pathol Lab Med*. 2019;143(4):463-471.
57. Surrey LF, Oakley FD, Merker JD, et al. Next-generation sequencing (NGS) methods show superior or equivalent performance to non-NGS methods on BRAF, EGFR, and KRAS proficiency testing samples. *Arch Pathol Lab Med*. 2019;143(8):980-984.
58. Canon J, Rex K, Saiki AY, et al. The clinical KRAS(G12C) inhibitor AMG 510 drives

- anti-tumour immunity. *Nature*. 2019;575(7781):217-223.
59. Hallin J, Engstrom LD, Hargis L, et al. The KRAS(G12C) inhibitor MRTX849 provides insight toward therapeutic susceptibility of KRAS-mutant cancers in mouse models and patients. *Cancer Discov*. 2020;10(1):54-71.
 60. Janes MR, Zhang J, Li LS, et al. Targeting KRAS mutant cancers with a covalent G12C-specific inhibitor. *Cell*. 2018;172(3):578-589e17.
 61. Santos E, Martin-Zanca D, Reddy EP, et al. Malignant activation of a K-ras oncogene in lung carcinoma but not in normal tissue of the same patient. *Science*. 1984;223(4637):661-664.
 62. Lito P, Solomon M, Li LS, Hansen R, Rosen N. Allele-specific inhibitors inactivate mutant KRAS G12C by a trapping mechanism. *Science*. 2016;351(6273):604-608.
 63. Patricelli MP, Janes MR, Li LS, et al. Selective inhibition of oncogenic KRAS output with small molecules targeting the inactive state. *Cancer Discov*. 2016;6(3):316-329.
 64. Skoulidis F, Li BT, Dy GK, et al. Sotorasib for lung cancers with KRAS p.G12C mutation. *N Engl J Med*. 2021;384(25):2371-2381.
 65. Johnson M, De Langen J, Waterhouse D, et al., editors. Sotorasib versus docetaxel for previously treated non-small cell lung cancer with KRAS G12C mutation: CodeBreaK 200 phase III study [abstract LBA10]. *Ann Oncol*. 2022;33(suppl 7):S808-S869.
 66. Janne PA, Riely GJ, Gadgeel SM, et al. Adagrasib in non-small-cell lung cancer harboring a KRAS(G12C) mutation. *N Engl J Med*. 2022;387(2):120-131.
 67. Konen JM, Rodriguez BL, Padhye A, et al. Dual inhibition of MEK and AXL targets tumor cell heterogeneity and prevents resistant outgrowth mediated by the epithelial-to-mesenchymal transition in NSCLC. *Cancer Res*. 2021;81(5):1398-1412.
 68. Adachi Y, Ito K, Hayashi Y, et al. Epithelial-to-mesenchymal transition is a cause of both intrinsic and acquired resistance to KRAS G12C inhibitor in KRAS G12C-mutant non-small cell lung cancer. *Clin Cancer Res*. 2020;26(22):5962-5973.
 69. Ricciuti B, Arbour KC, Lin JJ, et al. Diminished efficacy of programmed death-(ligand)1 inhibition in STK11- and KEAP1-mutant lung adenocarcinoma is affected by KRAS mutation status. *J Thorac Oncol*. 2022;17(3):399-410.
 70. Cooper AJ, Muzikansky A, Lennerz J, et al. Clinicopathologic characteristics and outcomes for patients with KRAS G12D-mutant NSCLC. *JTO Clin Res Rep*. 2022;3(9):100390.
 71. Soria JC, Ramalingam SS. Osimertinib in EGFR mutation-positive advanced NSCLC. *N Engl J Med*. 2018;378(13):1262-1263.
 72. Peters S, Camidge DR, Shaw AT, et al. Alectinib versus crizotinib in untreated ALK-positive non-small-cell lung cancer. *N Engl J Med*. 2017;377(9):829-838.
 73. Ryan MB, Fecce de la Cruz F, Phat S, et al. Vertical pathway inhibition overcomes adaptive feedback resistance to KRAS(G12C) inhibition. *Clin Cancer Res*. 2020;26(7):1633-43.
 74. Koga T, Suda K, Fujino T, et al. KRAS secondary mutations that confer acquired resistance to KRAS G12C inhibitors, sotorasib and adagrasib, and overcoming strategies: insights from in vitro experiments. *J Thorac Oncol*. 2021;16(8):1321-1332.
 75. Tanaka N, Lin JJ, Li C, et al. Clinical acquired resistance to KRAS(G12C) inhibition through a novel KRAS switch-II pocket mutation and polyclonal alterations converging on RAS-MAPK reactivation. *Cancer Discov*. 2021;11(8):1913-1922.
 76. Awad MM, Liu S, Rybkin II, et al. Acquired resistance to KRAS^{G12C} inhibition in cancer. *N Engl J Med*. 2021;384(25):2382-2393.
 77. Suzuki S, Yonesaka K, Teramura T, et al. KRAS inhibitor resistance in MET-amplified KRAS (G12C) non-small cell lung cancer induced by RAS- and non-RAS-mediated cell signaling mechanisms. *Clin Cancer Res*. 2021;27(20):5697-5707.

Gene Structure and Function

The human epidermal growth factor receptor 2 (HER2/ERBB2) is a receptor tyrosine kinase of the ERBB family, which is involved in the control of cell growth, survival, differentiation, and migration.^{1,2} Encoded by the *ERBB2* gene (*v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2*) on the long arm of chromosome 17 (17q11.2-q12), this protein shares extensive structural homology with all other members of its family, both along the catalytic intracellular domain and the extracellular putative ligand-binding regions (Figures 15-1 and 15-2). In contrast to other members, however, HER2 has no identified direct ligand and, instead, functions as the preferred dimerization partner for all other ERBB-family receptors.³⁻⁵ Ligand binding to the extracellular domain of EGFR (HER1), HER3, and HER4 results in the formation of catalytically active homo- and heterodimers, that in turn activate several downstream pathways that facilitate cellular proliferation signaling. In addition to the observed superior ability for heterodimerization, HER2 is also less prone to internalization and degradation, conferring longer activated kinase activity and signal transduction.

Types of Alterations and Role in Oncogenesis

Deregulation of the *HER2* gene, through protein overexpression, gene amplification, and mutations, has been well described in non-small cell lung carcinoma (NSCLC).⁶⁻⁸ In contrast to other solid tumors, the biology of HER2 in NSCLC seems more complex, and explicit roles of some alterations remain unclear. The common consequence of oncogenic *HER2* alterations is receptor hyperactivation, leading to uncontrolled cell proliferation. Mutations occur primarily in the intracellular domain within exon 20, with the A775_G776insYVMA being the most common variant. Several other mutations have been identified across the intracellular, transmembrane, and extracellular domains (Figure 15-1; Table 15-1),^{9,10} albeit at far lower frequencies, and many are categorized as variants of unknown significance.

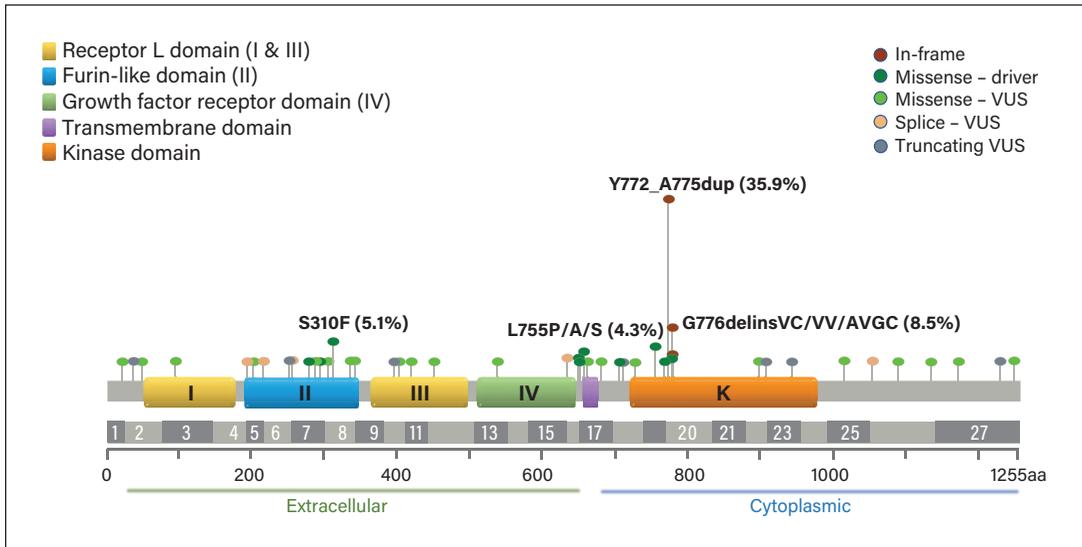


Figure 15-1. Schematic depicts the structure of *HER2* (*ERBB2*), the domain distribution and prevalence of the most common somatic alterations in non-small cell lung carcinoma (NSCLC). The structural domains coding for the extracellular, transmembrane, and cytoplasmic components of the protein are shown. The *HER2* protein is 185 kDa and is composed of 1255 amino acids (aa), which are coded by 27 exons. The extracellular ligand-binding domain (aa 1-652), the transmembrane domain (aa 653-675), and the intracellular domain (aa 676-1255) share significant structural homology with members of the same family. The prevalence of the most common *HER2* mutations in non-squamous NSCLC and location are displayed based on publicly available data from the cBioPortal.¹¹ The most common mutations are insertions in exon 20; the Y772_A775 duplication, also named A775_G776insYVMA is the most common, followed by insertions at codon G776. Missense mutations in exons 8, 17, and 19 are seen at frequencies between 2% and 5%. Rare mutations outside these regions are primarily categorized as variants of unknown significance (VUS).

Epidemiologic, Clinical, and Histologic Characteristics

The incidence rates of both amplification and overexpression of *HER2* vary widely across studies. Amplification has been demonstrated in 2% to 22% of NSCLC cases while overexpression is reported between 2.4% and 38%.¹²⁻¹⁵ This variation may be influenced by the method of evaluation, country or region, as well as the clinical and pathologic setting, and further investigation is warranted. In contrast to other malignancies, most notably breast and gastric/gastroesophageal junction (GEJ) carcinomas, where gene amplification and protein overexpression on the cellular membrane are highly correlated, this association is poor in lung cancer. While some studies document significant associations,¹⁶⁻¹⁸ others report them as unrelated events.^{15,19,20} Similarly, several studies demonstrate a negative association between *HER2* mutations and amplification,^{15,21} with only a minor proportion of reports showing mutations concurrent with amplification.²²⁻²⁴ Overall, this poor concordance suggests that each of the alterations may represent distinct clinicopathologic subsets.

HER2 amplification may be identified as a de novo alteration in approximately 2% to 3% of treatment-naïve NSCLC cases¹⁵ or as a resistance mechanism to first-generation EGFR tyrosine kinase inhibitors (TKIs), where it can be found in up to 13% of patients.²⁵ Overall, no distinct clinicopathologic characteristics are observed in patients with *HER2* amplification or *HER2* expression in the de novo setting^{16,19,22,26} while, in the setting of resistance, characteristics are driven by the original sensitizing driver.

HER2 mutations are detected in less than 2% of all NSCLC and have been primarily associated with adenocarcinoma histology, nonsmoking or never-smoking status, and female sex.

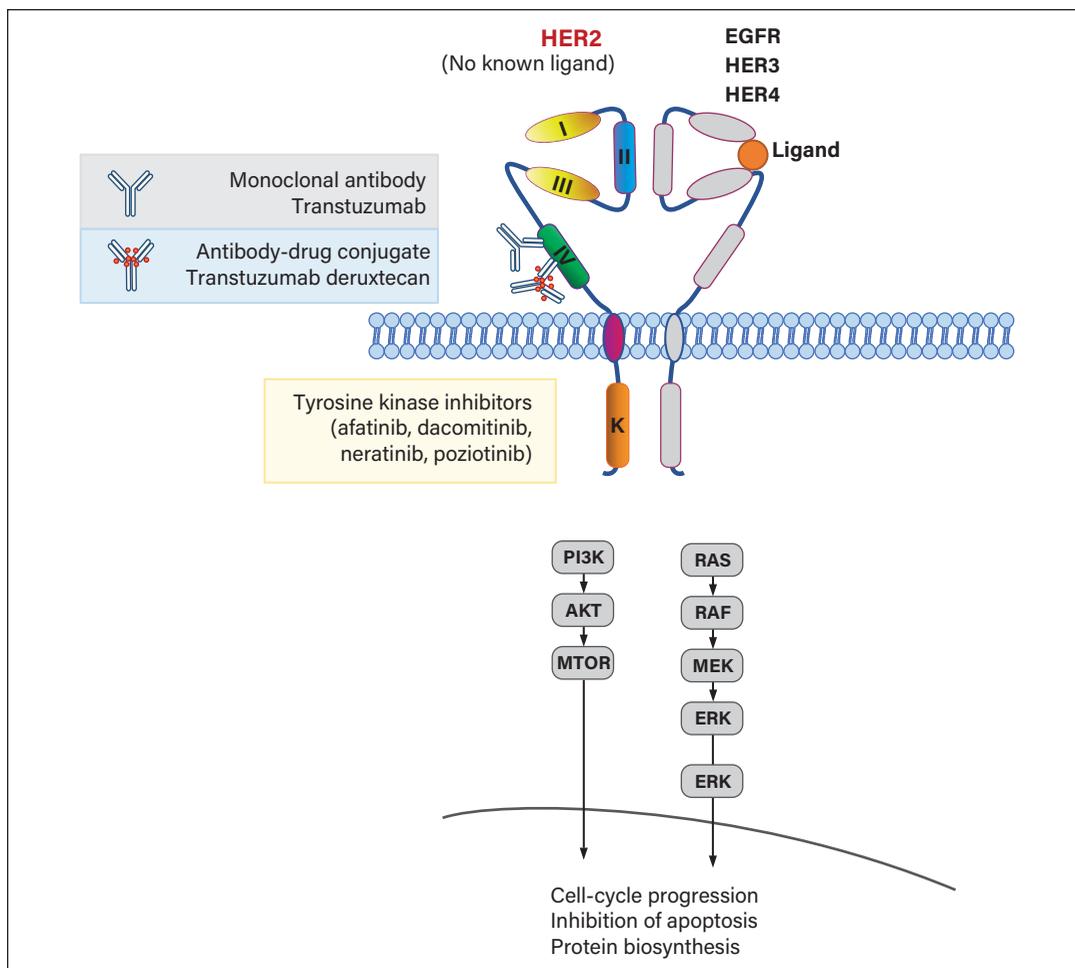


Figure 15-2. Human epidermal growth factor receptor 2 (HER2) structure, signaling, and treatment targets. HER2 is activated by the formation of homodimers or heterodimers with other receptors from the same family. Dimerization results in phosphorylation of specific tyrosine residues in the intracellular domains, which, in turn, leads to the activation of the Ras/Raf/mitogen-activated protein kinase, the phosphoinositide 3-kinase/Akt, and other pathways. Various targeting strategies have emerged for inhibiting HER2: (1) single-epitope monoclonal antibodies that bind to a single extracellular domain, such as trastuzumab (binding to domain IV); (2) antibody-drug conjugates, such as trastuzumab deruxtecan; and (3) small-molecule inhibitors, such as afatinib, dacomitinib, neratinib, poziotinib, designed to bind to the intracellular tyrosine-kinase domain of HER2.

Table 15-1. List of Common Mutations in *HER2* (*ERBB2*)

Location	Type	Mutation
Exon 8	SNV	p.S310F
Exon 19	SNV Indel	p.D769H, p.D769N, p.D769Y, p.I767M/F, p.L755A/M/P/S/W p.L755_E757delinsS, p.L755_T759delLRENT
Exon 20	SNV Indel	p.I767M, p.G776C, p.G776S, p.G776V, p.V777L, p.V777M p.T798I p.A775_G776insV, p.A775_G776insYVMA (p.Y772_A775dup), p.A775_G776insTVMA (p.Y772_V773VMAT), p.G776_V777insL, p.G776_V777insVC, p.G776_V777insVGC (p.G778_S779CVG), p.G776>LC, p.G776delinsVC (p.G776>VC), p.G778dup (p.V777_G778insG), p.G778_P780dup (p.P780_Y781GSP, V777_G778insGSP), p.G778_S779insCPG (p.V777_G778insGCP), p.G778_S779insLPS, p.S779_P780insVGS, p.V777_G778insCG

Abbreviations: indel = insertion and/or deletion; SNV = single nucleotide variant.

Patients with *HER2* mutant tumors often develop brain metastases, reported in up to 47% of cases.²⁷ Mutations are mutually exclusive with other driver molecular alterations and are not commonly associated with *HER2* amplification or overexpression.^{21,28,29} The percentage of patients with concurrent *HER2* mutations and PD-L1 expression (tumor proportion score [TPS] \geq 1%) is lower compared to other oncogene-addicted tumors, with no more than 20% of cases displaying high levels of expression (TPS \geq 50%).³⁰ In contrast to *HER2* amplification, *HER2* mutations or *HER2* overexpression have not been associated with acquired resistance to targeted therapies.

Testing Strategies

Methods used to assess *HER2* alterations vary depending on the alteration type and encompass various polymerase chain reaction (PCR)-based and sequencing techniques (next-generation sequencing [NGS] and non-NGS), immunohistochemistry (IHC), and fluorescence in situ hybridization (FISH).^{25,31-35} A summary of the methodology is presented in Table 15-2, along with current suggested criteria for interpretation of IHC and FISH.³⁶

Table 15-2. Summary of Methods for Detection of *HER2* (*ERBB2*) Alterations and Clinical Implications

Genetic alteration	Mutations	Amplification	Overexpression
Description	Point mutations Insertions	Abnormally high number of <i>HER2</i> (<i>ERBB2</i>) gene copies	Abnormally high expression of the protein receptor on the surface of tumor cells
Testing method	Sequencing: • Sanger • NGS (preferred) Other: • Fragment analysis • ARMS-PCR • dPCR	FISH: preferred and recommended method for testing in NSCLC-related clinical studies <i>HER2</i> amplification criteria by FISH ^a • <i>HER2</i> to <i>CEP17</i> ratio >2.0 or <i>HER2</i> signals connected into clusters: <i>HER2</i> amplification positive • <i>HER2</i> to <i>CEP17</i> ratio <2.0 : <i>HER2</i> copy number ≥ 6.0 : <i>HER2</i> amplification positive • <i>HER2</i> copy number <4.0 : <i>HER2</i> amplification negative • <i>HER2</i> copy number ≥ 4.0 but <6.0 : amplification status cannot be determined Other methods: qPCR, NGS • In current clinical practice, NGS is a commonly used method for detecting <i>HER2</i> amplification in NSCLC • Criteria for interpretation not yet developed	IHC is the standard method and can be scored as for breast carcinoma; however, <i>HER2</i> IHC is not currently indicated in clinical practice in NSCLC
Clinical implication	Oncogenic drivers, mutually exclusive with other drivers Trastuzumab deruxtecan FDA approved for second-line treatment in <i>HER2</i> (<i>ERBB2</i>) mutant advanced NSCLC	Oncogenic driver—de novo or secondary as resistance mechanism Treatment implications under investigation	Amplification and expression are independent, unlike breast carcinoma, and there are currently no clinical implications for assessing <i>HER2</i> expression in NSCLC

Abbreviations: ARMS-PCR = amplification refractory mutations system–polymerase chain reaction; *CEP17* = chromosome 17 centromere; dPCR = digital PCR; FDA = US Food and Drug Administration; FISH = fluorescence in situ hybridization; *HER2* = human epidermal growth factor receptor 2; IHC = immunohistochemistry; NGS = next-generation sequencing; NSCLC = non-small cell lung carcinoma; qPCR = quantitative PCR.

^a Cutoffs for *HER2* FISH not strictly established for NSCLC. Criteria from breast cancer have been adapted.

Tumor tissue is the preferred template for HER2 testing. However, when tissue is unavailable or too limited, cell-free DNA may be used³⁵⁻³⁷ and allows detection of mutations and amplifications, if there is sufficiently high circulating tumor DNA (ctDNA), but precludes the assessment of HER2 expression.

Clinical Implications

The prognostic implications of *HER2* mutations, as a whole, remain controversial as available data is still scant and conflicting. A trend for a negative prognostic effect has been observed in some studies,^{29,38} while others report a possible indolent outcome for mutated cases when compared to an unselected NSCLC population.²⁸ When stratified into subsets, the specific mutation may influence prognosis as reported in a recent study that identified worse survival associated with A775_G776insYVMA compared to less common *HER2* alterations.³⁹ Overall, *HER2* mutated tumors seem less sensitive to pemetrexed,⁴⁰ an agent generally effective in other oncogene-addicted NSCLC. Available data on sensitivity to immunotherapy, largely retrospective, also suggest that *HER2* mutant tumors are less sensitive to checkpoint inhibitors, similar to findings in *EGFR*-mutated or *ALK*-rearranged NSCLC.^{41,42}

Several targeted therapies have shown activity in patients with *HER2* mutant tumors. Three dominant treatment strategies have emerged: TKIs, monoclonal antibodies, and antibody-drug conjugates (ADCs; Figure 15-2). *HER2* TKIs, including afatinib, dacomitinib, neratinib, and poziotinib, have shown modest activity with consistent toxicity.^{13,43,44} More promising results have been observed with monoclonal antibodies, such as trastuzumab, in combination with chemotherapy, demonstrating remarkable activity only in retrospective investigations.^{31,45} Most recently, trastuzumab deruxtecan, a *HER2* ADC, was shown to provide durable anticancer activity in patients with previously treated *HER2*-mutant NSCLC,³² leading to the first drug to be approved by the US Food and Drug Administration (FDA) for treatment of *HER2*-mutant NSCLC. Efficacy was observed in most patients regardless of *HER2* IHC expression or amplification status.^{46,47}

Of note, the differential efficacy of targeted treatment regimens on specific mutation types is an area of active investigation but remains largely undefined. Tumors harboring the *HER2* mutation A775_G776insYVMA have been noted to be least responsive to *HER2*-targeting TKI therapy in previous studies.^{48,49} By contrast, longer overall survival and better response were recently reported for this mutation, compared to less common alterations when using trastuzumab-based therapies in a single-center real-world retrospective cohort.³⁹

Resistance Mechanisms

Historically, *HER2* amplification has been regarded as one of the bypass resistance mechanisms of *EGFR* TKIs, detected in 13% of patients who progressed after treatment.²⁵ At present, the degree to which amplification can confer resistance remains ill-defined as study results are controversial, some of them reporting even better response to *EGFR* TKIs in *HER2*-amplified tumors.⁵⁰

In the setting of de novo *HER2*-altered NSCLC treated with targeted therapies, evidence of secondary resistance mechanisms is currently scant. One in vitro study, investigating an acquired resistance to the *HER2*-inhibitor poziotinib, identified the secondary

HER2 mutation, C805S, at the covalent binding site of the drug as a potential mechanism of acquired resistance, with HSP90 inhibitors presenting a possible therapeutic strategy.⁵¹

With the exception of a few mutations, most *HER2* exon 20 insertions have exhibited primary resistance to available pan-HER TKIs. While mechanisms of resistance remain largely undefined, structural and molecular dynamics analysis suggest that the conformational landscape of the insertion affecting the kinase domain has variable effects in the affinity and sensitivity to the TKI.⁴⁹ The A775_G776insYVMA mutation, for example, leads to a high conformational rigidity that renders the adenosine triphosphate (ATP) binding site less accessible to small-molecule TKIs compared to other mutations.

References

1. Moasser MM. The oncogene *HER2*: its signaling and transforming functions and its role in human cancer pathogenesis. *Oncogene*. 2007;26(45):6469-6487. doi:10.1038/sj.onc.1210477
2. Akiyama T, Sudo C, Ogawara H, Toyoshima K, Yamamoto T. The product of the human *c-erbB-2* gene: a 185-kilodalton glycoprotein with tyrosine kinase activity. *Science*. 1986;232(4758):1644-1646. doi:10.1126/science.3012781
3. Pinkas-Kramarski R, Soussan L, Waterman H, et al. Diversification of Neu differentiation factor and epidermal growth factor signaling by combinatorial receptor interactions. *Embo J*. 1996;15(10):2452-2467.
4. Tzahar E, Waterman H, Chen X, et al. A hierarchical network of interreceptor interactions determines signal transduction by Neu differentiation factor/neuregulin and epidermal growth factor. *Mol Cell Biol*. 1996;16(10):5276-5287. doi:10.1128/mcb.16.10.5276
5. Klapper LN, Glathe S, Vaisman N, et al. The ErbB-2/*HER2* oncoprotein of human carcinomas may function solely as a shared coreceptor for multiple stroma-derived growth factors. *Proc Natl Acad Sci U S A*. 1999;96(9):4995-5000. doi:10.1073/pnas.96.9.4995
6. Shimamura T, Ji H, Minami Y, et al. Non-small-cell lung cancer and Ba/F3 transformed cells harboring the ERBB2 G776insV_G/C mutation are sensitive to the dual-specific epidermal growth factor receptor and ERBB2 inhibitor HKI-272. *Cancer Res*. 2006;66(13):6487-6491. doi:10.1158/0008-5472.Can-06-0971
7. Perera SA, Li D, Shimamura T, et al. *HER2YVMA* drives rapid development of adenocarcinoma lung tumors in mice that are sensitive to BIBW2992 and rapamycin combination therapy. *Proc Natl Acad Sci U S A*. 2009;106(2):474-479. doi:10.1073/pnas.0808930106
8. Cancer Genome Atlas Research Network. Comprehensive molecular profiling of lung adenocarcinoma. *Nature*. 2014;511(7511):543-550. doi:10.1038/nature13385
9. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal*. 2013;6(269):pl1. doi:10.1126/scisignal.2004088
10. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov*. 2012;2(5):401-404. doi:10.1158/2159-8290.Cd-12-0095
11. Most common *HER2* mutations in non-squamous NSCLC, prevalence and location data. cBioPortal for Cancer Genomics;2022. <https://www.cbioportal.org/>.
12. Cappuzzo F, Gregorc V, Rossi E, et al. Gefitinib in pretreated non-small-cell lung cancer (NSCLC): analysis of efficacy and correlation with *HER2* and epidermal growth factor receptor expression in locally advanced or metastatic NSCLC. *J Clin Oncol*. 2003;21(14):2658-2663. doi:10.1200/jco.2003.01.039
13. Hyman DM, Piha-Paul SA, Won H, et al. *HER* kinase inhibition in patients with *HER2*- and *HER3*-mutant cancers. *Nature*. 2018;554(7691):189-194. doi:10.1038/nature25475
14. Kim EK, Kim KA, Lee CY, Shim HS. The frequency and clinical impact of *HER2* alterations in lung adenocarcinoma. *PLoS One*. 2017;12(2):e0171280. doi:10.1371/journal.pone.0171280
15. Li BT, Ross DS, Aisner DL, et al. *HER2* amplification and *HER2* mutation are distinct molecular targets in lung cancers. *J Thorac Oncol*. 2016;11(3):414-419. doi:10.1016/j.jtho.2015.10.025

16. Heinmöller P, Gross C, Beyser K, et al. HER2 status in non-small cell lung cancer: results from patient screening for enrollment to a phase II study of herceptin. *Clin Cancer Res.* 2003;9(14):5238-5243.
17. Cox G, Vyberg M, Melgaard B, et al. Herceptest: HER2 expression and gene amplification in non-small cell lung cancer. *Int J Cancer.* 2001;92(4):480-483. doi:10.1002/ijc.1214
18. Tan D, Deeb G, Wang J, et al. HER-2/neu protein expression and gene alteration in stage I-IIIa non-small-cell lung cancer: a study of 140 cases using a combination of high throughput tissue microarray, immunohistochemistry, and fluorescent in situ hybridization. *Diagn Mol Pathol.* 2003;12(4):201-211. doi:10.1097/00019606-200312000-00004
19. Hirsch FR, Varella-Garcia M, Franklin WA, et al. Evaluation of HER-2/neu gene amplification and protein expression in non-small cell lung carcinomas. *Br J Cancer.* 2002;86(9):1449-1456. doi:10.1038/sj.bjc.6600286
20. Nakamura H, Saji H, Ogata A, et al. Correlation between encoded protein overexpression and copy number of the HER2 gene with survival in non-small cell lung cancer. *Int J Cancer.* 2003;103(1):61-66. doi:10.1002/ijc.10795
21. Arcila ME, Chaft JE, Nafa K, et al. Prevalence, clinicopathologic associations, and molecular spectrum of ERBB2 (HER2) tyrosine kinase mutations in lung adenocarcinomas. *Clin Cancer Res.* 2012;18(18):4910-4918. doi:10.1158/1078-0432.CCR-12-0912
22. Yoshizawa A, Sumiyoshi S, Sonobe M, et al. HER2 status in lung adenocarcinoma: a comparison of immunohistochemistry, fluorescence in situ hybridization (FISH), dual-ISH, and gene mutations. *Lung Cancer.* 2014;85(3):373-378. doi:10.1016/j.lungcan.2014.06.007
23. Suzuki M, Shiraishi K, Yoshida A, et al. HER2 gene mutations in non-small cell lung carcinomas: concurrence with Her2 gene amplification and Her2 protein expression and phosphorylation. *Lung Cancer.* 2015;87(1):14-22. doi:10.1016/j.lungcan.2014.10.014.
24. Meert AP, Martin B, Verdebout JM, et al. Is there a relationship between c-erbB-1 and c-erbB-2 amplification and protein overexpression in NSCLC? *Lung Cancer.* 2005;47(3):325-336. doi:10.1016/j.lungcan.2004.07.047.
25. Yu HA, Arcila ME, Rekhman N, et al. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. *Clin Cancer Res.* 2013;19(8):2240-2247. doi:10.1158/1078-0432.CCR-12-2246.
26. Pellegrini C, Falleni M, Marchetti A, et al. HER-2/Neu alterations in non-small cell lung cancer: a comprehensive evaluation by real time reverse transcription-PCR, fluorescence in situ hybridization, and immunohistochemistry. *Clin Cancer Res.* 2003;9(10, pt 1):3645-3652.
27. Offin M, Feldman D, Ni A, et al. Frequency and outcomes of brain metastases in patients with HER2-mutant lung cancers. *Cancer.* 2019;125(24):4380-4387. doi:10.1002/cncr.32461.
28. Mazières J, Peters S, Lepage B, et al. Lung cancer that harbors an HER2 mutation: Epidemiologic characteristics and therapeutic perspectives. *J Clin Oncol.* 2013;31(16):1997-2003. doi:10.1200/JCO.2012.45.6095.
29. Pillai RN, Behera M, Berry LD, et al. HER2 mutations in lung adenocarcinomas: a report from the Lung Cancer Mutation Consortium. *Cancer.* 2017;123(21):4099-4105. doi:10.1002/cncr.30869.
30. Negrao MV, Skoulidis F, Montesion M, et al. Oncogene-specific differences in tumor mutational burden, PD-L1 expression, and outcomes from immunotherapy in non-small cell lung cancer. *J Immunother Cancer.* 2021;9(8):e002891. doi:10.1136/jitc-2021-002891.
31. Mazieres J, Barlesi F, Filleron T, et al. Lung cancer patients with HER2 mutations treated with chemotherapy and HER2-targeted drugs: results from the European EUHER2 cohort. *Ann Oncol.* 2016;27(2):281-286. doi:10.1093/annonc/mdv573.
32. Li BT, Smit EF, Goto Y, et al. Trastuzumab deruxtecan in HER2-mutant non-small-cell lung cancer. *N Engl J Med.* 2022;386(3):241-251. doi:10.1056/NEJMoa2112431.
33. Ignatius Ou SH, Madison R, Robichaux JP, et al. Characterization of 648 non-small cell lung cancer (NSCLC) cases with 28 unique HER2 exon 20 insertions [abstract]. *J Clin Oncol.* 2019;37(15 suppl):9063. doi:10.1200/JCO.2019.37.15_suppl.9063.
34. Yang S, Wang Y, Zhao C, et al. Exon 20 YVMA insertion is associated with high incidence of brain metastasis and inferior outcome of chemotherapy in advanced non-small cell lung cancer patients with HER2 kinase domain mutations. *Transl Lung Cancer Res.* 2021;10(2):753-765. doi:10.21037/tlcr-20-559.
35. Lee J, Franovic A, Shiotsu Y, et al. Detection of ERBB2 (HER2) gene amplification events in cell-free DNA and response to anti-HER2

- agents in a large Asian cancer patient cohort. *Front Oncol.* 2019;9:212. doi:10.3389/fonc.2019.00212.
36. Ren S, Wang J, Ying J, et al. Consensus for HER2 alterations testing in non-small-cell lung cancer. *ESMO Open.* 2022;7(1):100395. doi:10.1016/j.esmoop.2022.100395.
 37. Li BT, Janku F, Jung B, et al. Ultra-deep next-generation sequencing of plasma cell-free DNA in patients with advanced lung cancers: results from the Actionable Genome Consortium. *Ann Oncol.* 2019;30(4):597-603. doi:10.1093/annonc/mdz046.
 38. Jebbink M, de Langen AJ, Boelens MC, Monkhorst K, Smit EF. The force of HER2—a druggable target in NSCLC? *Cancer Treat Rev.* 2020;86:101996. doi:10.1016/j.ctrv.2020.101996.
 39. Waliyan S, Wakelee H, Ramchandran K, et al. Characterization of ERBB2 (HER2) alterations in metastatic non-small cell lung cancer and comparison of outcomes of different trastuzumab-based regimens. *Clin Lung Cancer.* 2022;23(6):498-509. doi:10.1016/j.clcc.2022.05.015.
 40. Wang Y, Zhang S, Wu F, et al. Outcomes of pemetrexed-based chemotherapies in HER2-mutant lung cancers. *BMC Cancer.* 2018;18(1):326. doi:10.1186/s12885-018-4277-x.
 41. Guisier F, Dubos-Arvis C, Vinas F, et al. Efficacy and safety of Anti-PD-1 immunotherapy in patients with advanced NSCLC with BRAF, HER2, or MET mutations or RET translocation: GFPC 01-2018. *J Thorac Oncol.* 2020;15(4):628-636. doi:10.1016/j.jtho.2019.12.129.
 42. Mazieres J, Drilon A, Lusque A, et al. Immune checkpoint inhibitors for patients with advanced lung cancer and oncogenic driver alterations: results from the IMMUNOTARGET registry. *Ann Oncol.* 2019;30(8):1321-1328. doi:10.1093/annonc/mdz167.
 43. Dziadziuszko R, Smit EF, Dafni U, et al. Afatinib in NSCLC with HER2 mutations: results of the prospective, open-label phase II NICHE trial of European Thoracic Oncology Platform (ETOP). *J Thorac Oncol.* 2019;14(6):1086-1094. doi:10.1016/j.jtho.2019.02.017.
 44. Elamin YY, Robichaux JP, Carter BW, et al. Poziotinib for patients with HER2 exon 20 mutant non-small-cell lung cancer: results from a phase II trial. *J Clin Oncol.* 2022;40(7):702-709. doi:10.1200/JCO.21.01113.
 45. Cappuzzo F, Bemis L, Varella-Garcia M. HER2 mutation and response to trastuzumab therapy in non-small-cell lung cancer. *N Engl J Med.* 2006;354(24):2619-2621. doi:10.1056/NEJMc060020.
 46. Li BT, Smit EF, Goto Y, et al. Trastuzumab deruxtecan in HER2-mutant non-small-cell lung cancer. *N Engl J Med.* 2022;386(3):241-251. doi:10.1056/NEJMoa2112431.
 47. Smit EF, Felip E, Uprety D, et al. Trastuzumab deruxtecan in patients (pts) with HER2-overexpressing (HER2-OE) metastatic non-small cell lung cancer (NSCLC): results from the DESTINY-Lung01 trial. *Ann Oncol.* 2022; 33(suppl 7):S448-S554. doi:10.1016/annonc/annonc1064.
 48. Robichaux JP, Elamin YY, Tan Z, et al. Mechanisms and clinical activity of an EGFR and HER2 exon 20-selective kinase inhibitor in non-small cell lung cancer. *Nat Med.* 2018;24(5):638-646. doi:10.1038/s41591-018-0007-9.
 49. Zhao S, Fang W, Pan H, et al. Conformational landscapes of HER2 exon 20 insertions explain their sensitivity to kinase inhibitors in lung adenocarcinoma. Article. *J Thorac Oncol.* 2020;15(6):962-972. doi:10.1016/j.jtho.2020.01.020.
 50. Zhao J, Xia Y. Targeting HER2 alterations in non-small-cell lung cancer: a comprehensive review. *JCO Precis Oncol.* 2020;4:411-425. doi:10.1200/PO.19.00333.
 51. Koga T, Kobayashi Y, Tomizawa K, et al. Activity of a novel HER2 inhibitor, poziotinib, for HER2 exon 20 mutations in lung cancer and mechanism of acquired resistance: an in vitro study. *Lung Cancer.* 2018;126:72-79. doi:10.1016/j.lungcan.2018.10.019.51.

Gene Structure and Function

The neuregulin-1 (*NRG1*) gene, located on chromosome 8, is approximately 1.4 Mb in length and represents one of the largest human genes.¹ The gene contains large intronic regions, and only 0.3% of the gene encodes protein (neuregulin-1), representing 1 of 4 proteins in the neuregulin family that interact with the ErbB family of receptors.¹ The NRG1 protein is produced in at least 31 different isoforms as a result of alternative splicing; however, all isoforms share an epidermal growth factor (EGF)-like domain, which allows them to bind and activate ErbB3 (HER3), and less commonly, ErbB4 (HER4).²⁻⁴ Upon ligand binding, the ErbB3 receptors form homodimers or heterodimers with ErbB2 (HER2), leading to activation of the PIK3-AKT and MAPK pathways.^{5,6} The various NRG1 isoforms are essential during embryogenesis and play a central role in the proliferation, differentiation, and survival of glial cells, neurons, and cardiomyocytes.¹

Type of Alterations and Role in Oncogenesis

Driver alterations involving *NRG1* occur in the form of fusions. *NRG1* fusions can involve many different partner genes, with *CD74* being the most common partner, accounting for approximately 30% of the cases.⁷⁻⁹ The *NRG1* component contributes the EGF-like domain, which serves as a ligand for ErbB3, while the partner gene typically contributes a transmembrane domain that tethers the chimeric protein to the cell membrane.⁷ The chimeric protein retains its EGF-like domain, enabling it to interact with ErbB3 on the same cell or an adjacent cell and leading to activation of downstream pathways.⁷

Epidemiologic, Clinical, and Histologic Characteristics

NRG1 fusions occur at low frequencies in multiple tumor types, with overall incidence in solid tumors of 0.2%.⁹ Among non-small cell lung carcinomas (NSCLCs), *NRG1* fusions occur with a rate of 0.3%.⁹ *NRG1* fusions occur primarily in light or never-smokers and

Table 16-1. Testing Strategies

Type of assay	Advantages	Drawbacks
FISH ¹⁰	Technique available in most laboratories Short turnaround time (2-3 days)	Single-gene assay Extensive hands-on time Requires expertise in interpretation Does not provide information on fusion partner
Targeted DNA-based NGS ^{7,9}	Multigene assay provides information on other drivers	Low sensitivity for <i>NRG1</i> fusion due to poor coverage/lack of coverage
Targeted RNA-based NGS ^{7,9}	Highest sensitivity Multigene assay provides information on other drivers	Requires adequate RNA quality (older samples are suboptimal due to RNA degradation)

Abbreviations: FISH = fluorescence in situ hybridization; NGS = next-generation sequencing.

tumors with the histotype of invasive mucinous adenocarcinomas (IMAs), formerly known as mucinous bronchioloalveolar carcinomas.^{8,11-13} Among IMAs, *NRG1* fusions occur with a frequency of 7%, and they are mutually exclusive with *KRAS* mutations and other oncogenic drivers. Among *KRAS*-wild type IMAs, *NRG1* fusion frequency is 17% to 25%.^{11,13}

Among IMAs, *NRG1* fusions are associated with more aggressive histologic and clinical features, including a higher rate of distant metastases and worse survival compared to IMAs harboring *KRAS* mutations.¹³

Although rare, *NRG1* fusions also occur in various other types of solid tumors, including pancreatic adenocarcinoma, gallbladder carcinoma, renal cell carcinoma, ovarian carcinoma, breast carcinoma, and bladder carcinoma and sarcoma; the incidence of *NRG1* fusions in these tumors is 0.1% to 0.5%.⁹ Similar to lung adenocarcinoma, *NRG1* fusions in pancreatic adenocarcinoma are mutually exclusive with *KRAS* mutations and are thus highly enriched in *KRAS* wild-type tumors.¹⁴

Table 16-1 outlines the testing strategies for *NRG1* tumors.

Clinical Implications

The presence of an *NRG1* fusion in NSCLC has been associated with poor outcomes to standard therapy. In the eNRGy1 Global Multicenter Registry of more than 100 cases of *NRG1* fusion-positive NSCLC, response to platinum-doublet chemotherapy was only 13% with a median progression-free survival (PFS) of 5.8 months.¹² Median PFS with chemo-immunotherapy was even shorter, at only 3.3 months. There are, however, emerging therapeutic options that target the HER2/HER3 pathway triggered by the *NRG1* fusion protein. Afatinib, a pan-ErbB kinase inhibitor, has shown clear efficacy with a response rate (RR) of 25% in the retrospective eNRGy1 registry.¹² Multiple case reports and series have also demonstrated responses to afatinib in *NRG1* fusion-positive tumors,¹⁵⁻¹⁷ with prospective studies underway. The HER2-HER3 bispecific antibody zenocutuzumab showed early efficacy in *NRG1* fusion-positive tumors including responses in both NSCLC and pancreatic cancer.¹⁸ The ongoing phase 2 eNRGy trial reported an initial RR of 34% (n = 79) with a median duration of response of 9.1 months.¹⁸ The HER3 monoclonal antibody seribantumab is being studied in *NRG1* fusion-positive tumors and has reported an RR of 33% (n = 12).¹⁹ Both zenocutuzumab and seribantumab were awarded fast track designation by the US Food and Drug Administration.

Resistance Mechanisms

Little is known about acquired or intrinsic resistance to any of the preceding investigational agents, and this remains an area of active study.

References

1. Falls DL. Neuregulins: functions, forms, and signaling strategies. *Exp Cell Res*. 2003;284(1):14-30.
2. Meyer D Yamaai T, Garratt A, et al. Isoform-specific expression and function of neuregulin. *Development*. 1997;124(18):3575-3586.
3. Steinhorsdottir V, Stefansson H, Ghosh S, et al. Multiple novel transcription initiation sites for NRG1. *Gene*. 2004;342(1):97-105.
4. Tan W, Wang Y, Gold B, et al. Molecular cloning of a brain-specific, developmentally regulated neuregulin 1 (NRG1) isoform and identification of a functional promoter variant associated with schizophrenia. *J Biol Chem*. 2007;282(33):24343-24351.
5. Kim HG, Lee CK, Cho SM, et al. Neuregulin 1 up-regulates the expression of nicotinic acetylcholine receptors through the ErbB2/ErbB3-PI3K-MAPK signaling cascade in adult autonomic ganglion neurons. *J Neurochem*. 2013;124(4):502-513.
6. Tzabar E, Levkowitz G, Karunakaran D, et al. ErbB-3 and ErbB-4 function as the respective low and high affinity receptors of all Neu differentiation factor/herregulin isoforms. *J Biol Chem*. 1994;269(40):25226-25233.
7. Drilon A, Somwar R, Mangatt BP, et al. Response to ERBB3-directed targeted therapy in NRG1-rearranged cancers. *Cancer Discov*. 2018;8(6):686-695.
8. Fernandez-Cuesta L, Plenker D, Osada H, et al. CD74-NRG1 fusions in lung adenocarcinoma. *Cancer Discov*. 2014;4(4):415-422.
9. Jonna S, Feldman RA, Swensen J, et al. Detection of NRG1 gene fusions in solid tumors. *Clin Cancer Res*. 2019;25(16):4966-4972.
10. Duruisseau M, McLeer-Florin A, Antoine M, et al. NRG1 fusion in a French cohort of invasive mucinous lung adenocarcinoma. *Cancer Med*. 2016;5(12):3579-3585.
11. Chang JC, Offin M, Falcon C, et al. Comprehensive molecular and clinicopathologic analysis of 200 pulmonary invasive mucinous adenocarcinomas identifies distinct characteristics of molecular subtypes. *Clin Cancer Res*. 2021;27(14):4066-4076.
12. Drilon A, Duruisseau M, Han JY, et al. Clinicopathologic features and response to therapy of NRG1 fusion-driven lung cancers: the eNRGy1 Global Multicenter Registry. *J Clin Oncol*. 2021;39(25):2791-2802.
13. Nakaoku T, Tsuta K, Ichikawa H, et al. Druggable oncogene fusions in invasive mucinous lung adenocarcinoma. *Clin Cancer Res*. 2014;20(12):3087-3093.
14. Jones MR, Williamson LM, Topham JT, et al. NRG1 gene fusions are recurrent, clinically actionable gene rearrangements in KRAS wild-type pancreatic ductal adenocarcinoma. *Clin Cancer Res*. 2019;25(15):4674-4681.
15. Cadranel J, Liu SV, Duruisseau M, et al. Therapeutic potential of afatinib in NRG1 fusion-driven solid tumors: a case series. *Oncologist*. 2021;26(1):7-16.
16. Cheema PK, Doherty M, Tsao MS. A case of invasive mucinous pulmonary adenocarcinoma with a CD74-NRG1 fusion protein targeted with afatinib. *J Thorac Oncol*. 2017;12(12):e200-e202.
17. Wu X, Zhang D, Shi M, et al. Successful targeting of the NRG1 fusion reveals durable response to afatinib in lung adenocarcinoma: a case report. *Ann Transl Med*. 2021;9(19):1507.
18. Schram AM, Goto K, Kim D-W, et al. Efficacy and safety of zenocutuzumab, a HER2 x HER3 bispecific antibody, across advanced NRG1 fusion (NRG1+) cancers. *J Clin Oncol*. 2022;40(16 suppl):105.
19. Carrizosa DR, Burkard ME, Elamin YY, et al. CRESTONE: initial efficacy and safety of seribantumab in solid tumors harboring NRG1 fusions. *J Clin Oncol*. 2022;40(16 suppl):3006.

New Targets and Technologies

By Kurt A. Schalper and David M. Hwang

17

Novel and emerging therapeutic avenues to treat patients with lung cancer comprise a wide array of strategies, leveraging molecular tumor characteristics in a variety of ways, including:

- Exploiting the differential expression of proteins in tumor relative to non-tumor cells to direct and locally enrich cytotoxic agents using antibody-drug conjugates (ADCs)
- Using small molecules to target mutant proteins present in cancer cells with oncogenic effect or involved in critical metabolic processes (eg, KRAS [non-G12C], PIK3CA, STK11, KEAP1)
- Providing immunostimulatory therapies that modulate co-stimulatory T-cell receptors or regulatory enzymes, using modified pro-inflammatory cytokines with favorable therapeutic indexes, vaccines targeting tumor-specific neoantigens, *or* selective recognition and elimination of tumor cells expressing specific surface proteins using modified chimeric antigen receptor (CAR) T cells

From these, ADCs have shown prominent clinical activity in multiple tumor types, including lung cancer, bringing with them the potential need for new biomarker tests and diagnostic approaches. In addition, novel molecular analysis platforms allowing for the simultaneous interrogation of numerous alterations with high sensitivity, single-cell resolution, and/or preservation of the spatial context are expanding the arsenal of tools to discover and develop novel biomarkers for patient selection and monitoring.

Antibody-Drug Conjugates

ADCs consist of 3 fundamental components: (1) a humanized monoclonal antibody targeting an antigen that is overexpressed in tumor cells but demonstrates limited expression or is absent in normal cells, (2) a cytotoxic drug payload, and (3) a covalent linker joining the cytotoxic agent to the antibody¹⁻⁴ (Figure 17-1). Presently, the targets of ADCs in clinical development for lung cancer include numerous proteins expressed in tumor cells such as HER2, HER3, MET, TROP2, CEACAM5, DLL3, mesothelin, EGFR, and PTK7. The

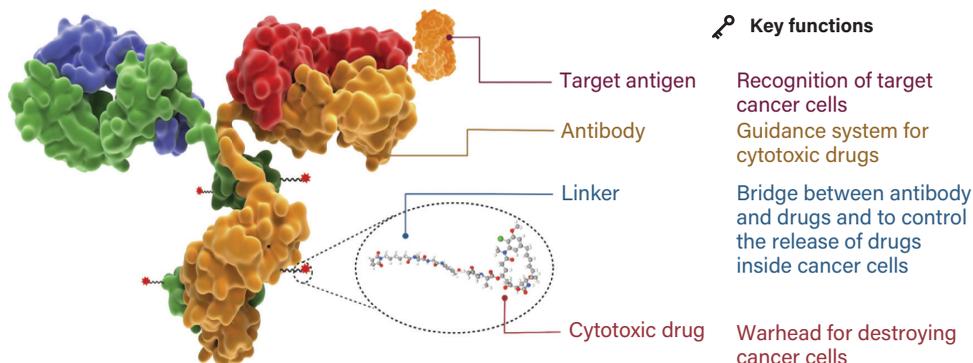


Figure 17-1. Structure and characteristics of antibody-drug conjugates (Source: Fu et al.³)

cytotoxic payload components of ADCs fall broadly into 3 categories based on the mechanism of action: microtubule inhibitors (eg, DM1, the drug payload of emtansine), topoisomerase inhibitors (eg, DXd, the drug payload of deruxtecan), and DNA cleavage agents (eg, calicheamicin, ozogamicin).^{2,4} A growing number of ADCs are under active investigation for the treatment of both non-small cell lung carcinoma (NSCLC) and small cell lung carcinoma (SCLC)⁴ (Figure 17-2), including:

- Human epidermal growth factor receptor 2 (HER2 or ERBB2)-targeted ADCs: Ado-trastuzumab emtansine and trastuzumab deruxtecan have been studied in HER2 protein overexpressing⁵⁻⁷ and/or *HER2* mutant^{5,8,9} tumors, with higher activity demonstrated in *HER2*-mutant tumors, with or without demonstrable HER2 protein overexpression.^{8,9} In August 2022, the US Food and Drug Administration (FDA) granted accelerated approval of trastuzumab deruxtecan for adult patients who had received prior systemic therapy for unresectable or metastatic NSCLC with activating *HER2* (*ERBB2*) mutations, as detected by an FDA-approved test.¹⁰ FDA-approved companion diagnostics for this indication were the Oncomine Dx Target Test (ThermoFisher Scientific) for tissue-based testing and the Guardant360 CDx (Guardant Health) for plasma-based testing.
- Human epidermal growth factor receptor 3 (HER3 or ERBB3)-targeted ADCs: While HER3 demonstrates aberrant expression in multiple cancer types, it has been of interest as an ADC target primarily in the setting of *EGFR*-mutant lung cancers that have developed resistance to EGFR tyrosine kinase inhibitors (TKIs).¹¹ Patritumab deruxtecan is a HER3-targeted ADC that has demonstrated activity in tumors with both known and unknown EGFR TKI resistance mechanisms across a range of HER3 protein expression.¹² In December 2021, patritumab deruxtecan received FDA breakthrough therapy designation for treatment of patients with metastatic or locally advanced *EGFR*-mutated NSCLC with disease progression on or after treatment with a third-generation TKI and platinum-based therapies.¹³
- Hepatocyte growth factor receptor (MET)-targeted ADCs: Various MET oncogenic driver alterations in NSCLC have been discussed elsewhere in this atlas (see Chapter 13). Telisotuzumab vedotin monotherapy demonstrated promising antitumor activity in patients with MET-overexpressing tumors as assessed by immunohistochemistry (IHC)¹⁴ and is under active investigation in a number of settings, including

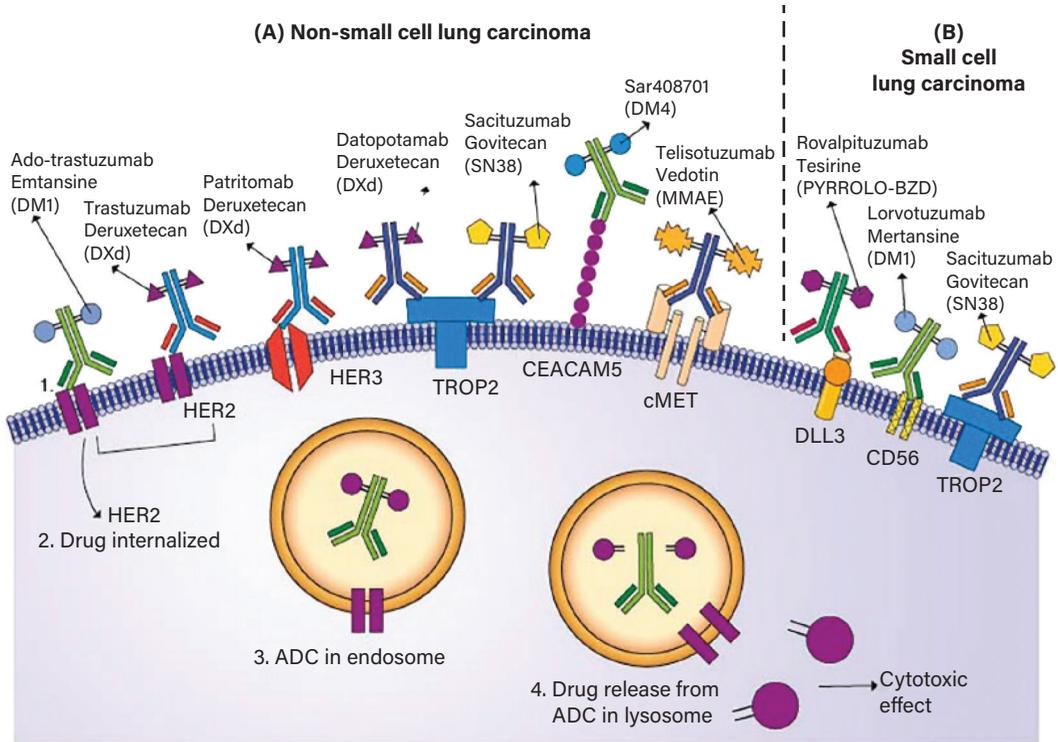


Figure 17-2. Mechanisms of various antibody-drug conjugates (ADCs) being studied in non-small cell and small cell lung carcinoma (Source: Desai et al.⁴ © 2021 Elsevier B.V. All rights reserved.)

EGFR-mutant NSCLC demonstrating MET overexpression.¹⁵ In January 2022, telisotuzumab vedotin received FDA breakthrough therapy designation for treatment of patients with advanced/metastatic *EGFR*-wild type, nonsquamous NSCLC with high levels of MET, whose disease progressed on or after platinum-based therapy.¹⁶

- Trophoblast cell surface antigen 2 (TROP2)-targeted ADCs: TROP2 is a transmembrane glycoprotein overexpressed in many tumors including lung cancer. Datopotamab deruxetecan is a TROP2-targeted ADC that is under investigation in NSCLCs, both with¹⁷ or without¹⁸ actionable genomic alterations. Sagituzumab govitecan is another TROP2-targeted ADC that is under investigation in a number of clinical trials for NSCLC⁴ and has also demonstrated activity in patients with SCLC in a basket trial.¹⁹
- Carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5, or CD66e)-targeted ADCs: CEACAM5 is a glycoprotein that demonstrates increased expression in some lung cancers. SAR408701 (tusamitamab ravtansine) showed promising antitumor activity and is being evaluated in a phase 3 trial for CEACAM5-positive nonsquamous NSCLC.²⁰
- NOTCH ligand delta-like protein 3 (DLL3)-targeted ADCs: DLL3 is highly expressed in most SCLCs. Rovalpituzumab tesirine showed modest activity against DLL3-expressing SCLCs in a third-line setting in a phase 2 trial,²¹ but exhibited inferior overall survival rates compared with topotecan in the second-line setting.²² Investigation of rovalpituzumab tesirine and other DLL3-targeted ADCs in various SCLC treatment settings is ongoing.⁴

Novel Agents Targeting Oncogenic Mutations

Multiple efforts are ongoing to effectively target oncogenic mutations commonly recognized in lung carcinomas such as *KRAS* non-G12C and *PIK3CA* variants (see Chapter 14). These therapeutic strategies show variable results and include directly targeting the mutant protein(s) using small molecule inhibitors or modulating downstream activation signals such as MEK, AKT, mTOR, SOS1, and SHP2.²³⁻²⁵ Additional studies are ongoing to target genes frequently co-mutated in lung carcinomas and associated with intracellular metabolic alterations and worse prognosis such as *STK11* and *KEAP1*.^{23,24,26} To date, these therapies are under investigation and have not received regulatory approval for clinical use.

Novel Immunostimulatory Therapies

Although myriad new immunotherapy strategies have been proposed to treat patients with solid tumors, monoclonal antibodies targeting 1 or more co-stimulatory T-cell/natural killer (NK)-cell receptors or immune suppressive enzymes are currently being evaluated in patients with NSCLC such as LAG3, TIM3, TIGIT, NKG2A, and CD73.²⁷⁻³² Currently, these therapies are being tested in different treatment settings and have not received regulatory approval for clinical use.

New Technologies for Molecular Analysis with Diagnostic Potential

The increasing incorporation of therapeutic modalities and targets has been paralleled by the rapid development of novel molecular analysis tools to interrogate the expression/location of such therapeutic targets or identify additional markers with biomarker potential. These developments include (1) the expansion of the multiplexing capacity of assays to simultaneously accommodate numerous markers, (2) increase in the sensitivity of the marker(s) identification to improve the limit of detection/quantification, (3) the implementation of high-dimensional molecular analysis tools with preservation of spatial context and/or single-cell resolution, and (4) interrogating tumor morphology using artificial intelligence to predict mutation or gene fusion events. Marked progress in computational methods to systematically extract information and integrate high-dimensional data are commonly required to interpret the results.

Quantitative and Spatial Molecular Analysis of Tumor Samples

Traditionally used platforms for in situ analysis of molecular markers in fixed histology sections with preservation of morphologic context include chromogenic IHC and in situ hybridization (ISH or fluorescence ISH [FISH]) of DNA segments or messenger RNA (mRNA) transcripts. The expanded use of digital pathology has increased the use of computational tools, such as artificial intelligence, to quantify the IHC/ISH/FISH staining using tissue segmentation steps, coupled to enumeration of individual cells and measurement of the signal intensity in user-defined tissue compartments. The advantages of these analyses include their quantitative output, objective nature, and enhanced reproducibility relative to semi-quantitative pathologist-based signal scoring. The automation of signal assessment from IHC stained slides has been explored for therapeutic targets such as HER2 and PD-L1.³³⁻³⁷ The major limitations for such platforms include challenges with the implementation, achieving

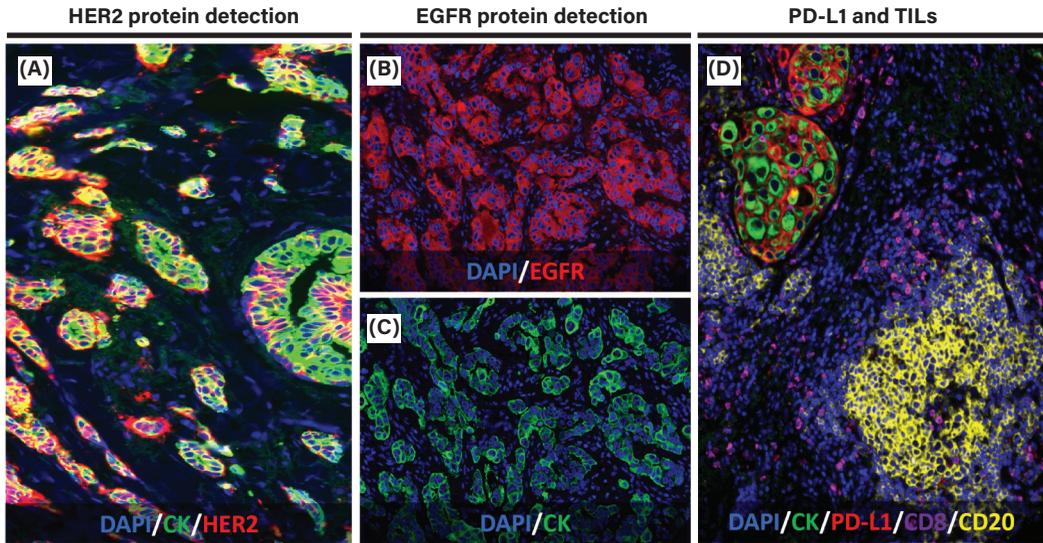


Figure 17-3. Localized analysis of therapeutic targets and tumor infiltrating lymphocytes (TILs) in human NSCLC using multiplex immunofluorescence. **(A)** Expression and co-localization of HER2 protein (*red*) in cytokeratin-positive tumor cells (CK, *green*). Nuclei are stained with DAPI. **(B)** Staining of EGFR protein (*red*) with membranous/perinuclear pattern in malignant lung adenocarcinoma cells. **(C)** Same tumor and field of view as in panel B stained with cytokeratin (*green*) and DAPI (*blue*). **(D)** Poorly differentiated lung carcinoma with simultaneous staining for DAPI (*blue*), cytokeratin (*green*), PD-L1 (*red*), CD8 (*magenta*), and CD20+ TILs (*yellow*).

consistent and rapid tissue/cell segmentation, and the presence of uncertainties about optimal reporting of continuous scores and regulatory framework.

Multiplex immunofluorescence (mIF) has been increasingly used in translational research and for biomarker identification. This method is conceptually similar to IHC and uses antibodies conjugated with fluorophores to detect proteins or nucleic acids in tissue slides. However, and different from IHC, mIF is an emissive signal generation method that increases its sensitivity and dynamic range.^{38,39} In addition, the mIF signal is generally evaluated using multispectral microscopes containing specific fluorescence filters, allowing for the objective separation of signal channels and independent measurement of multiple markers included in the same assay/panel.

Multiple studies have analyzed the expression of therapeutic targets such as HER2, PD-L1, and EGFR within specific cell populations or tumor tissue compartments defined by simultaneous co-staining of additional markers⁴⁰⁻⁴² (Figure 17-3). Identification of specific cell populations with biomarker potential such as tumor infiltrating lymphocytes (TILs) is also possible.^{43,44} Current challenges for the systematic clinical use of mIF include the identification of assays with a clear biomarker value beyond clinical single-plex IHC tests, implementation of robust automated staining protocols in clinical laboratories, standardization in the signal scoring and reporting; as well as availability of a regulatory framework to support the clinical use.

Additional developments in spatial molecular imaging of tissue samples include the use of high-plex methods to simultaneously measure numerous proteins and/or mRNA transcripts using metal-labeling of antibodies or RNA probes followed by high-energy tissue ablation⁴⁵⁻⁴⁷ (Figure 17-4), cyclic staining protocols with or without microfluidics,⁴⁸⁻⁵⁰ and the use of molecular barcoding approaches to increase the assay multiplexing capacity and

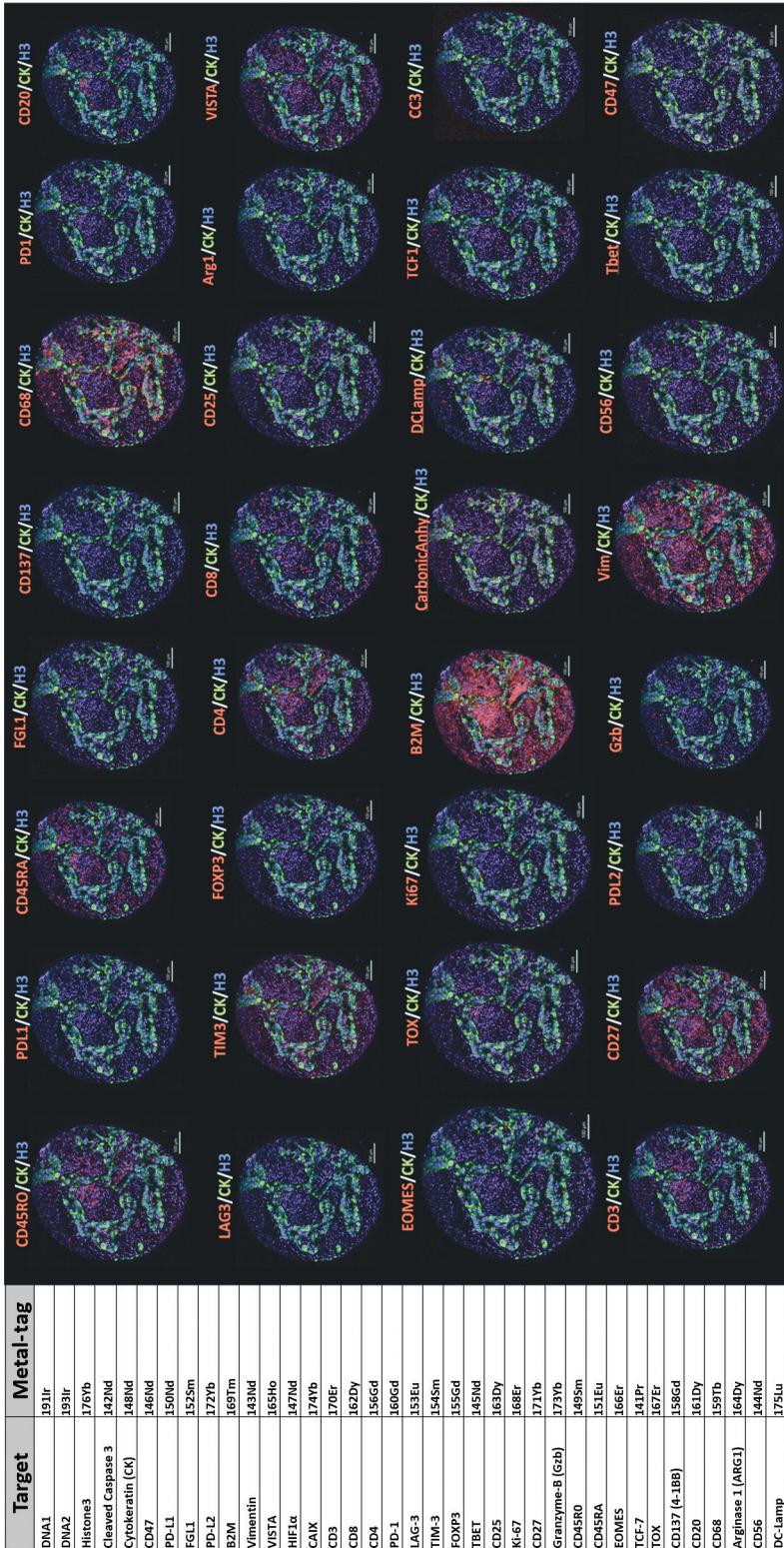


Figure 17-4. Simultaneous and localized detection of multiple protein markers using imaging mass cytometry. Lung adenocarcinoma sections were simultaneously stained with a panel containing 37 antibodies conjugated with metal isotopes to detect tumor and immune-cell markers as indicated in the inset table (left). Representative staining of the markers is shown in the microphotographs (right).

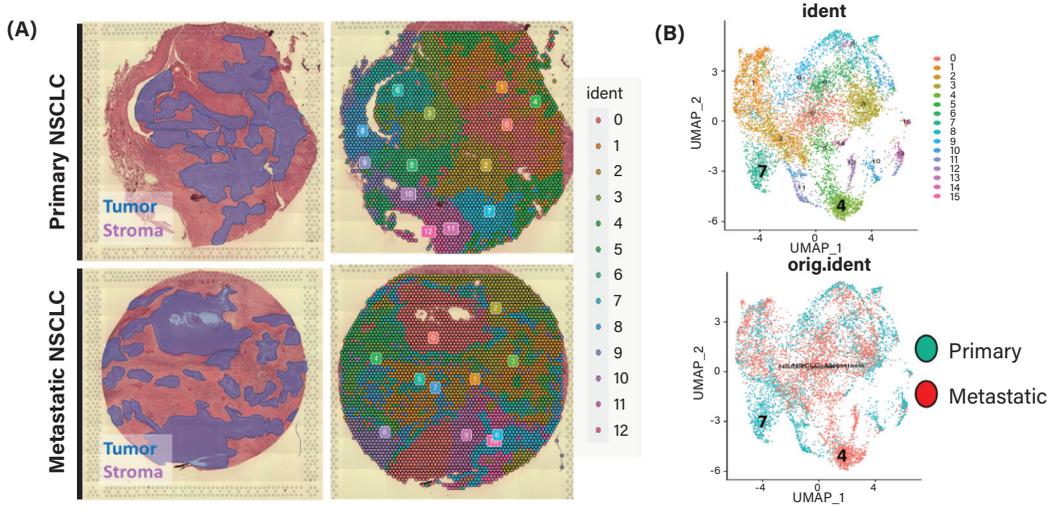


Figure 17-5. Spatial transcriptomics analysis of paired primary and metastatic NSCLC. **(A)** Fixed lung carcinoma sections were studied using hematoxylin and eosin stain (*left*) for morphologic context and using whole transcriptomic analysis with the Visium platform (*right*). **(B)** Unsupervised analysis identified individual multidimensional spatial transcriptomic clusters, some of which were differentially represented in the primary (*green*) and metastatic lesion (*red*). Specific mRNA transcripts and pathways were identified in each spatial cluster pointing to their cell type identity and biologic significance.

minimize molecular interference during staining.⁵¹⁻⁵⁷ These platforms allow for the simultaneous detection of numerous markers ranging from tens to hundreds of proteins and up to several thousand mRNA transcripts (Figure 17-5). Although most of these platforms are currently being used in the research setting, it is expected that they will progressively be incorporated into clinical-like environments. The capacity of these platforms to simultaneously detect proteins and nucleic acids with spatial resolution opens new possibilities to map therapeutic targets and actionable molecular alterations in tumor cells, as well as measurement of additional cell populations and tumor microenvironment features. The challenges for the implementation of these high-dimensional platforms include their elevated cost, limited throughput, difficult validation/standardization, and complex data analysis commonly requiring specialized personnel and resources.

Conclusion

New therapeutic targets and novel approaches for targeting them are constantly emerging. While existing technology, such as next-generation sequencing and IHC, may suffice for some, it remains possible that emerging, multiplex approaches will provide new data that might better inform selection of some drugs. Although increasingly complex technology allied to sophisticated tools for data analysis may provide biomarkers hitherto impossible to define, the practical implications of timely, cost-effective clinical delivery must always be borne in mind.

References

1. Pettinato MC. Introduction to antibody-drug conjugates. *Antibodies (Basel)*. 2021;10(4):42.
2. Tong JTW, Harris PWR, Brimble MA, Kavianinia I. An insight into FDA approved antibody-drug conjugates for cancer therapy. *Molecules*. 2021;26:5847.
3. Fu Z, Li S, Han S, Shi C, Zhang Y. Antibody drug conjugate: the “biological missile” for

- targeted cancer therapy. *Signal Transduct Target Ther.* 2022;7:93.
4. Desai A, Abdayem P, Adjei AA, Planchard D. Antibody-drug conjugates: a promising novel therapeutic approach in lung cancer. *Lung Cancer.* 2022;163:96-106.
 5. Hotta K, Aoe K, Kozuki T, et al. A phase II study of trastuzumab emtansine in HER2-positive non-small cell lung cancer. *J Thorac Oncol.* 2018;13: 273-279.
 6. Peters S, Stahel R, Bubendorf L, et al. Trastuzumab emtansine (T-DM1) in patients with previously treated HER2-overexpressing metastatic non-small cell lung cancer: efficacy, safety, and biomarkers. *Clin Cancer Res.* 2019;25:64-72.
 7. Nakagawa K, Nagasaka M, Felip E, et al. Trastuzumab deruxtecan in HER2-overexpressing metastatic non-small cell lung cancer: interim results of DESTINY-Lung01. *J Thorac Oncol.* 2021;16(3S):S109-110.
 8. Li BT, Shen R, Buonocore D, et al. Ado-trastuzumab emtansine for patients with HER2-mutant lung cancers: results from a phase II basket trial. *J Clin Oncol.* 2018;36:2532-2537.
 9. Li BT, Smit EF, Goto Y, et al; DESTINY-Lung01 Trial Investigators. Trastuzumab deruxtecan in HER2-mutant non-small-cell lung cancer. *N Engl J Med.* 2022;386:241-251.
 10. FDA grants accelerated approval to fam-trastuzumab deruxtecan-nxki for HER2-mutant non-small cell lung cancer. US Food and Drug Administration; August 16, 2022. <https://www.fda.gov/drugs/resources-information-approved-drugs/fda-grants-accelerated-approval-fam-trastuzumab-deruxtecan-nxki-her2-mutant-non-small-cell-lung>.
 11. Mishra R, Patel H, Alanazi S, Yuan L, Garrett JT. HER3 signaling and targeted therapy in cancer. *Oncol Rev.* 2018;12:355.
 12. Jänne PA, Baik C, Su WC, et al. Efficacy and safety of patritumab deruxtecan (HER3-DXd) in EGFR inhibitor-resistant, EGFR-mutated non-small cell lung cancer. *Cancer Discov.* 2022;12:74-89.
 13. Tucker N. Patritumab deruxtecan granted FDA breakthrough therapy designation for metastatic or locally advanced EGFR+ NSCLC. Targeted Oncology; December 28, 2021. <https://www.targetedonc.com/view/patritumab-deruxtecan-granted-fda-breakthrough-therapy-designation-for-metastatic-or-locally-advanced-egfr-nsclc>.
 14. Strickler JH, Weekes CD, Nemunaitis J, et al. First-in-human phase I, dose-escalation and -expansion study of telisotuzumab vedotin, an antibody-drug conjugate targeting c-Met, in patients with advanced solid tumors. *J Clin Oncol.* 2018;36:3298-3306.
 15. Camidge DR, Barlesi F, Goldman JW, et al. Phase Ib study of telisotuzumab vedotin in combination with erlotinib in patients with c-Met protein-expressing non-small-cell lung cancer. *J Clin Oncol.* 2022;JCO2200739. doi:10.1200/JCO.22.00739.
 16. Tucker N. FDA grants breakthrough therapy designation to telisotuzumab vedotin for advanced EGFR wild type nsqNSCLC. Targeted Oncology; January 5, 2022. <https://www.targetedonc.com/view/fda-grants-breakthrough-therapy-designation-to-telisotuzumab-vedotin-for-advanced-egfr-wild-type-nsqnsclc>.
 17. Study of DS-1062a in advanced or metastatic non-small cell lung cancer with actionable genomic alterations (TROPION-Lung05). ClinicalTrials.gov identifier: NCT04484142. Updated December 1, 2022. <https://clinicaltrials.gov/ct2/show/NCT04484142>.
 18. Study of DS-1062a versus docetaxel in previously treated advanced or metastatic non-small cell lung cancer with or without actionable genomic alterations (TROPION-LUNG01). ClinicalTrials.gov identifier NCT04656652. Updated November 14, 2022. <https://clinicaltrials.gov/ct2/show/NCT04656652>.
 19. Bardia A, Messersmith WA, Kio EA, et al. Sacituzumab govitecan, a Trop-2-directed antibody-drug conjugate, for patients with epithelial cancer: final safety and efficacy results from the phase I/II IMMU-132-01 basket trial. *Ann Oncol.* 2021;32:746-756.
 20. SAR408701 versus docetaxel in previously treated, carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) positive metastatic non-squamous non-small cell lung cancer patients (CARMEN-LC03). ClinicalTrials.gov identifier: NCT04154956. Updated December 13, 2022. <https://clinicaltrials.gov/ct2/show/NCT04154956>.
 21. Morgensztern D, Besse B, Greillier L, et al. Efficacy and safety of rovalpituzumab tesirine in third-line and beyond patients with DLL3-expressing, relapsed/refractory small-cell lung cancer: results from the phase II TRINITY study. *Clin Cancer Res.* 2019;25:6958-6966.
 22. Blackhall F, Jao K, Greillier L, et al. Efficacy and safety of rovalpituzumab tesirine compared with topotecan as second-line

- therapy in DLL3-high SCLC: results from the phase 3 TAHOE study. *J Thorac Oncol.* 2021;16:1547-1558.
23. Jacobs F, Cani M, Malapelle U, et al. Targeting KRAS in NSCLC: old failures and new options for “non-G12c” patients. *Cancers (Basel).* 2021;13:6332.
 24. Cucurull M, Notario L, Sanchez-Cespedes M, et al. Targeting KRAS in lung cancer beyond KRAS G12C inhibitors: the immune regulatory role of KRAS and novel therapeutic strategies. *Front Oncol.* 2022;11:793121.
 25. Tan AC. Targeting the PI3K/Akt/mTOR pathway in non-small cell lung cancer (NSCLC). *Thorac Cancer.* 2020;11(3):511-518.
 26. Reck M, Carbone DP, Garassino M, Barlesi F. Targeting KRAS in non-small-cell lung cancer: recent progress and new approaches. *Ann Oncol.* 2021;32:1101-1110.
 27. Mamdani H, Matosevic S, Khalid AB, Durm G, Jalal SI. Immunotherapy in lung cancer: current landscape and future directions. *Front Immunol.* 2022;13:823618.
 28. Huo JL, Wang YT, Fu WJ, Lu N, Liu ZS. The promising immune checkpoint LAG-3 in cancer immunotherapy: from basic research to clinical application. *Front Immunol.* 2022;13:956090.
 29. Acharya N, Sabatos-Peyton C, Anderson AC. Tim-3 finds its place in the cancer immunotherapy landscape. *J Immunother Cancer.* 2020;8:e000911.
 30. Chauvin JM, Zarour HM. TIGIT in cancer immunotherapy. *J Immunother Cancer.* 2020;8:e000957.
 31. van Hall T, André P, Horowitz A, et al. Monalizumab: inhibiting the novel immune checkpoint NKG2A. *J Immunother Cancer.* 2019;7:263.
 32. Herbst RS, Majem M, Barlesi F, et al. COAST: an open-label, phase II, multidrug platform study of durvalumab alone or in combination with oleclumab or monalizumab in patients with unresectable, stage III non-small-cell lung cancer. *J Clin Oncol.* 2022;40:3383-3393.
 33. Lara H, Li Z, Abels E, et al. Quantitative image analysis for tissue biomarker use: a white paper from the Digital Pathology Association. *Appl Immunohistochem Mol Morphol.* 2021;29:479-493.
 34. Han Z, Lan J, Wang T, et al. A deep learning quantification algorithm for HER2 scoring of gastric cancer. *Front Neurosci.* 2022;16:877229.
 35. Bankhead P, Fernández JA, McArt DG, et al. Integrated tumor identification and automated scoring minimizes pathologist involvement and provides new insights to key biomarkers in breast cancer. *Lab Invest.* 2018;98:15-26.
 36. Cheng G, Zhang F, Xing Y, et al. Artificial intelligence-assisted score analysis for predicting the expression of the immunotherapy biomarker PD-L1 in lung cancer. *Front Immunol.* 2022;13:893198.
 37. Widmaier M, Wiestler T, Walker J, et al. Comparison of continuous measures across diagnostic PD-L1 assays in non-small cell lung cancer using automated image analysis. *Mod Pathol.* 2020;33:380-390.
 38. Rimm DL. What brown cannot do for you. *Nat Biotechnol.* 2006;24:914-916.
 39. Carvajal-Hausdorf DE, Schalper KA, Neumeister VM, Rimm DL. Quantitative measurement of cancer tissue biomarkers in the lab and in the clinic. *Lab Invest.* 2015;95:385-396.
 40. Velcheti V, Schalper KA, Carvajal DE, et al. Programmed death ligand-1 expression in non-small cell lung cancer. *Lab Invest.* 2014;94:107-116.
 41. McLaughlin J, Han G, Schalper KA, et al. Quantitative assessment of the heterogeneity of PD-L1 expression in non-small-cell lung cancer. *JAMA Oncol.* 2016;2:46-54.
 42. Dimou A, Agarwal S, Anagnostou V, et al. Standardization of epidermal growth factor receptor (EGFR) measurement by quantitative immunofluorescence and impact on antibody-based mutation detection in non-small cell lung cancer. *Am J Pathol.* 2011;179:580-589.
 43. Schalper KA, Brown J, Carvajal-Hausdorf D, et al. Objective measurement and clinical significance of TILs in non-small cell lung cancer. *J Natl Cancer Inst.* 2015;107:dju435.
 44. Lopez de Rodas M, Naginevi V, Ravi A, Datar IJ, et al. Role of tumor infiltrating lymphocytes and spatial immune heterogeneity in sensitivity to PD-1 axis blockers in non-small cell lung cancer. *J Immunother Cancer.* 2022;10:e004440.
 45. Giesen C, Wang HA, Schapiro D, et al. Highly multiplexed imaging of tumor tissues with subcellular resolution by mass cytometry. *Nat Methods.* 2014;11:417-422.
 46. Angelo M, Bendall SC, Finck R, et al. Multiplexed ion beam imaging of human breast tumors. *Nat Med.* 2014;20:436-442.
 47. Schulz D, Zanotelli VRT, Fischer JR, et al. Simultaneous multiplexed imaging of mRNA and proteins with subcellular resolution in

- breast cancer tissue samples by mass cytometry. *Cell Syst.* 2018;6:531.
48. Gerdes MJ, Sevinsky CJ, Sood A, et al. Highly multiplexed single-cell analysis of formalin-fixed, paraffin-embedded cancer tissue. *Proc Natl Acad Sci U S A.* 2013;110:11982-11987.
 49. Lin JR, Izar B, Wang S, et al. Highly multiplexed immunofluorescence imaging of human tissues and tumors using t-CyCIF and conventional optical microscopes. *Elife.* 2018;7:e31657.
 50. Migliozi D, Pelz B, Dupouy DG, et al. Microfluidics-assisted multiplexed biomarker detection for in situ mapping of immune cells in tumor sections. *Microsyst Nanoeng.* 2019;5:59.
 51. Vasaturo A, Galon J. Multiplexed immunohistochemistry for immune cell phenotyping, quantification and spatial distribution in situ. *Methods Enzymol.* 2020;635:51-66.
 52. Van TM, Blank CU. A user's perspective on GeoMx™ digital spatial profiling. *Immuno-oncol Technol.* 2019;1:11-18.
 53. Gupta S, Zugazagoitia J, Martinez-Morilla S, Fuhrman K, Rimm DL. Digital quantitative assessment of PD-L1 using digital spatial profiling. *Lab Invest.* 2020;100:1311-1317.
 54. Ståhl PL, Salmén F, Vickovic S, et al. Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. *Science.* 2016;353:78-82.
 55. Rodriques SG, Stickels RR, Goeva A, et al. Slide-seq: a scalable technology for measuring genome-wide expression at high spatial resolution. *Science.* 2019;363:1463-1467.
 56. Liu Y, Yang M, Deng Y, et al. High-spatial-resolution multi-omics sequencing via deterministic barcoding in tissue. *Cell.* 2020;183:1665-1681.e18.
 57. Taube JM, Akturk G, Angelo M, et al; Society for Immunotherapy of Cancer (SITC) Pathology Task Force. The Society for Immunotherapy of Cancer statement on best practices for multiplex immunohistochemistry (IHC) and immunofluorescence (IF) staining and validation. *J Immunother Cancer.* 2020;8:e000155.

Molecular Testing Results and the Role of Multidisciplinary Molecular Tumor Boards

18

By Paul Hofman, Chien-Feng Li, Andrew G. Nicholson, and Shani Shilo

Molecular testing in thoracic oncology is rapidly becoming increasingly complex and requires a well-structured approach to be translated into appropriate diagnostic or therapeutic recommendations.

Reporting of Molecular Testing Results

Molecular reports should list the names of the genes and specific loci, exons, or hotspots tested; intronic sequences analyzed for genes occurring in translocating generating fusion genes; and if relevant, the genome space covered to assess the tumor mutational burden and type of microsatellites tested to assess the microsatellite instability.¹ Interpretation of results must always be provided. Three scales for classifying molecular alterations based on potential clinical utility rank molecular targets into a level of clinical actionability:

1. Joint consensus recommendation of the Association for Molecular Pathology (AMP), American College of Medical Genetics and Genomics (ACMG), American Society of Clinical Oncology, and College of American Pathologists for reporting genetic variants in cancer
2. European Society for Medical Oncology scale for clinical actionability of molecular targets
3. OncoKB that distinguishes 4 levels of actionability and 3 levels of resistance¹⁻³

This scaling would facilitate eventual convergence toward common standards to annotate treatment decisions.⁴ All molecular findings need to be classified according to the most up-to-date evidence to distinguish benign or neutral alterations from pathogenic ones, even confirmed or likely. For this purpose, different online databases are available, including ClinVar, My Cancer Genome, Cosmic, or OncoKB.^{2,5-7} In particular, OncoKB is the first

somatic mutation archive approved by the US Food and Drug Administration.⁸ Of note, certain genomic alterations of potential medical significance may be identified which are unrelated to the primary purpose for testing (secondary findings), especially with larger panel testing. A list of secondary genetic findings (SF) has been recommended by the ACMG that should be reported in clinical genomic sequencing based on the phenotype, penetrance, and actionability.⁹⁻¹² Based on the updated ACMG SF v3.1 list, certain genes related to cancer phenotypes, cardiovascular phenotypes, inborn errors of metabolism phenotypes, and miscellaneous phenotypes (eg, hereditary TTR amyloidosis) should be reported.¹² Accordingly, the subjects with confirmed secondary findings should be referred for further evaluation and management for the increased disease risk.¹³

Multidisciplinary Molecular Tumor Boards and Interpretation of Tumor Biomarker Results

Multidisciplinary tumor boards, also known as *multidisciplinary teams* (MDTs), meet to evaluate patients in an evidence-based organizational approach to implement more effective practice in oncology. Different specialists take part in these different tumor boards. Usually, clinical oncologists, pathologists, thoracic surgeons, radiologists, radiation oncologists, pulmonologists, and nurses, among others, participate in MDTs in thoracic oncology. The same membership should also be involved in molecular tumor boards (MTBs), but alongside other specialists such as genetic pathologists, molecular scientists, biologists in information science, and pharmacists in oncology. Finally, as drugs can be accessed through clinical trials, a research/clinical trial coordinator may participate in the MTB. Thus, these boards are powerful tools to foster debate, discussion, and overall communication among different professionals, which is a major facilitator toward the development of precision thoracic oncology.

MTB meetings usually decide on the standard-of care (SOC) treatments for early or advanced lung cancer patients,¹⁴ thus MTBs are considered valuable tools in promoting the quality of care in thoracic oncology by reducing the vulnerability of a single person and promoting teams and strong systems. In addition, members of the MTB identify and discuss all potential therapeutic strategies based on genetic analyses for patients.¹⁵⁻¹⁸ Thus, an MTB may be consulted if patients with advanced lung cancer need to be included in clinical trials.^{16,19} MTBs provide recommendations that are always based on a multidisciplinary discussion, including specific molecular alterations in lung cancer as well as all features concerning the patient (eg, performance status and comorbidities).

Members of MTBs discuss the results from next-generation sequencing (NGS) along with any other relevant molecular tests, which may include reverse transcription polymerase chain reaction (RT-PCR) assays, array comparative genomic hybridization, or even whole-exome sequencing. MTBs usually deal with patients with difficult cases, and recommendations should be clearly documented and may include parameters such as:

- Driver mutations/copy number/structural variations, including fusion genes
- Druggable molecular alterations
- Microsatellite instability
- Tumor mutational burden
- Alterations indicating drug resistance

- Expression of proteins in tissue section, especially PD-L1
- MTB conclusions and recommendations
- Potential clinical trials

Treatment needs to be discussed according to the pathogenicity of the detected variant(s). The pathogenicity of a variant is determined by its disease-causing capacity.²⁰ For lung cancer, a variant is pathogenic when it is known to contribute to malignant transformation. In this context, pathogenicity is classified using an adapted version of the 2015 ACMG/AMP guidelines, which proposed a 5-tier scheme (pathogenic, likely pathogenic, variant of unknown or uncertain clinical significance [VUS], likely benign, benign).²¹ However, until now there has been no real (inter)national consensus on how the interpretation of pathogenicity should be made. Moreover, it is important to look for and to report the different pathogenic germline variants, as some of them can be associated with therapeutic relevance.²²

Some platforms can use innovative cloud-based virtual MTB technology, including a knowledge base and a collaborative environment for precision oncology-driven cancer care.²³ MTB could also be used as a key educational tool in teaching or university hospitals, where presentation of challenging cases initiate consultation.²⁴

Multidisciplinary Molecular Tumor Boards and the Quality of Care and Outcome of Patients with Lung Cancer

MTBs are critical, both at diagnosis and at tumor progression, for some patients, such as those with very rare or complex molecular alterations, patients with tumors carrying alterations without approval of targeted drugs in clinical practice, patients with oncogene-addicted neoplasms not responsive to available molecular drugs, or patients with rare thoracic neoplasms without recognized therapeutic approaches. Therefore, it is important to manage the turnaround time of both testing and the MTBs in generating output. An appropriate turnaround time is mandatory to guarantee initiation of targeted therapy in a beneficial timeframe. According to international guidelines, the testing for biomarkers mandatory for optimal initial treatment of advanced non-squamous non-small cell lung carcinoma must be completed within 10 working days.²⁵

At tumor progression, further discussion of the patient at the MTB may be appropriate. It may also be relevant to undertake additional molecular testing, possibly with larger gene panels; and thus, a balance is required between the urgency for treatment and the delay required in order to gather data and make an informed decision on the next treatment steps.

Moreover, adequate access to the MTB, facilitating best practices in thoracic oncology, should also provide benefit to the wider oncology community, and not only patients hospitalized in academic centers. The creation, therefore, of a virtual global MTB may be beneficial in thoracic oncology.²⁶ Patients receiving MTB-based therapy are better matched to the different genomic alteration than those having only a single physician choice.^{15,27} Consequently, those patients managed through an MTB (vs physician choice only) have significantly longer progression-free and overall survival rates.^{15,27}

Challenges of Multidisciplinary Tumor Boards in Thoracic Oncology

Despite the recommended organization of lung cancer patient care based on MTB discussion, some challenges still exist. Not every lung cancer service operates through an MTB.

This may be because of a lack of adequate resources, or even, in some situations, an unwillingness to open up management discussions. More pertinent to this discussion is whether it is possible to operate an MTB, and how any functioning MTB might interact with the thoracic MDT. Some services manage to integrate molecular data discussions into the main MDT, others organize separate meetings. In the latter scenario, the MTB may be a specialist thoracic tumor discussion or a general MTB considering a range of tumor cases, including lung cancer.

The issue of clinically important turnaround time for both molecular testing and MDT/MTB case assessment remains pertinent. Many patients are at risk of rapid clinical deterioration because of the progression of their underlying lung cancer, therefore even small delays may be highly detrimental. Yet the increasing number of biomarker-determined treatment options and the incompatibility of some of these with each other through increased toxicity, makes for a difficult wait while all required information is made available. The situation in cases with disease relapse can be even more difficult, with delays in obtaining repeat biopsies, repeating NGS analyses, and consideration of clinical trials (which usually requires mandatory repeat biopsy). Regarding clinical trials, complex inclusion criteria, lengthy screening procedures, and mandated central laboratory analysis may also add to delay, not least because many clinical trials, particularly industry-sponsored trials, require centralized laboratory testing for biomarker confirmation rather than accepting analyses performed in accredited local laboratories.

Despite there being molecular data for a patient that could support the use of either widely approved drugs or access to clinical trial or off-label treatment, some health systems offer relatively restricted access to treatments that may be available elsewhere; there is never universal access to trials that may leverage any appropriate molecular profile. Access to a drug may be a greater impediment to the delivery of biomarker-driven therapy than access to testing or appropriate discussion. MTBs may develop and use digital tools to help integrate clinical and genomic profiles and search for available clinical trials, and such developments could, in the future, use artificial intelligence to improve decision-making. Where resources allow, MTBs should include personnel facilitating access to drugs, as successfully carried out in the I-PREDICT trial.²⁸

There are, currently, no international guidelines for molecular test reporting and MTB operations reports, which leads to considerable heterogeneity from country to country and institution to institution.²⁹ Hence, considerable effort is required to better standardize the presentation and interpretation of molecular alterations in terms of actionability, therapeutic indications, and, later, the valuable recording of outcome data.

Finally, as the recommendations of the MTB may transcend guideline-based treatments, review, evaluation, and audit of the MTB operations and recommendations and their implementation is very important. Developing a registry of case reviews and outcomes can help this effort, should reinforce the educational value of MTBs, and assist in the evaluation of patterns of response in cohorts of lung cancer patients with rare actionable variants. In the end, MTB recommendations can benefit both the individual patient and, through research, future generations of lung cancer patients.

References

- Li MM, Datto M, Duncavage EJ, et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *J Mol Diagn*. 2017;19(1):4-23. doi:10.1016/j.jmoldx.2016.10.002.
- Chakravarty D, Gao J, Phillips SM, et al. OncoKB: a precision oncology knowledge base. *JCO Precis Oncol*. 2017;2017:PO.17.00011. doi:10.1200/PO.17.00011.
- Mosele F, Remon J, Mateo J, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group. *Ann Oncol*. 2020;31(11):1491-1505. doi:10.1016/j.annonc.2020.07.014.
- Li X, Warner JL. A review of precision oncology knowledgebases for determining the clinical actionability of genetic variants. *Front Cell Dev Biol*. 2020;8:48. doi:10.3389/fcell.2020.00048.
- Holt ME, Mittendorf KF, LeNoue-Newton M, et al. My cancer genome: coevolution of precision oncology and a molecular oncology knowledge base. *JCO Clin Cancer Inform*. 2021;5:995-1004. doi:10.1200/CCI.21.00084.
- Landrum MJ, Chitipiralla S, Brown GR, et al. ClinVar: improvements to accessing data. *Nucleic Acids Res*. 2020;48(D1):D835-D844. doi:10.1093/nar/gkz972.
- Tate JG, Bamford S, Jubb HC, et al. COSMIC: the catalogue of somatic mutations in cancer. *Nucleic Acids Res*. 2019;47(D1):D941-D947. doi:10.1093/nar/gky1015.
- FDA recognizes Memorial Sloan-Kettering database of molecular tumor marker information. US Food and Drug Administration. November 10, 2021. www.fda.gov/drugs/resources-information-approved-drugs/fda-recognizes-memorial-sloan-kettering-database-molecular-tumor-marker-information.
- Green RC, Berg JS, Grody WW, et al; American College of Medical Genetics and Genomics. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet Med*. 2013;15(7):565-574. doi:10.1038/gim.2013.73.
- Kalia SS, Adelman K, Bale SJ, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. *Genet Med*. 2017;19(2):249-255. doi:10.1038/gim.2016.
- Miller DT, Lee K, Gordon AS, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2021 update: a policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2021;23(8):1391-1398. doi:10.1038/s41436-021-01171-4.
- Miller DT, Lee K, Abul-Husn NS, et al. ACMG SF v3.1 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2022;24(7):1407-1414. doi:10.1016/j.gim.2022.04.006.
- Katz AE, Nussbaum RL, Solomon BD, et al. Management of secondary genomic findings. *Am J Hum Genet*. 2020;107(1):3-14. doi:10.1016/j.ajhg.2020.05.002.
- Tan AC, Tan DSW. Targeted therapies for lung cancer patients with oncogenic driver molecular alterations. *J Clin Oncol*. 2022;40(6):611-625. doi:10.1200/JCO.21.01626.
- Larson KL, Huang B, Weiss HL, et al. Clinical outcomes of molecular tumor boards: a systematic review. *JCO Precis Oncol*. 2021;5:PO.20.00495. doi:10.1200/PO.20.00495.
- Rolfo C, Manca P, Salgado R, et al. Multidisciplinary molecular tumour board: a tool to improve clinical practice and selection accrual for clinical trials in patients with cancer. *ESMO Open*. 2018;3(5):e000398. doi:10.1136/esmoopen-2018-000398.
- Trédan O, Wang Q, Pissaloux D, et al; ProfILER investigators. Molecular screening program to select molecular-based recommended therapies for metastatic cancer patients: analysis from the ProfILER trial. *Ann Oncol*. 2019;30(5):757-765. doi:10.1093/annonc/mdz080.
- Tuxen IV, Rohrberg KS, Oestrup O, et al. Copenhagen Prospective Personalized Oncology (CoPPO)—clinical utility of using molecular profiling to select patients to phase I trials. *Clin Cancer Res*. 2019 Feb 15;25(4):1239-1247. doi:10.1158/1078-0432.CCR-18-1780.
- Basse C, Morel C, Alt M, et al. Relevance of a molecular tumour board (MTB) for patients' enrollment in clinical trials: experience of the Institut Curie. *ESMO Open*. 2018;3(3):e000339. doi:10.1136/esmoopen-2018-000339.

20. Andrivon D. Nomenclature for pathogenicity and virulence: the need for precision. *Phytopathology*. 1993;83:889-839.
21. Richards S, Aziz N, Bale S, et al; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424. doi:10.1038/gim.2015.30.
22. Cobain EF, Wu YM, Vats P, et al. Assessment of clinical benefit of integrative genomic profiling in advanced solid tumors. *JAMA Oncol*. 2021;7(4):525-533. doi:10.1001/jamaoncol.2020.7987.
23. Pishvaian MJ, Blais EM, Bender RJ, et al. A virtual molecular tumor board to improve efficiency and scalability of delivering precision oncology to physicians and their patients. *JAMIA Open*. 2019;2(4):505-515. doi:10.1093/jamiaopen/ooz045.
24. Harada S, Arend R, Dai Q, et al. Implementation and utilization of the molecular tumor board to guide precision medicine. *Oncotarget*. 2017;8(34):57845-57854. doi:10.18632/oncotarget.18471.
25. Lindeman NI, Cagle PT, Aisner DL, et al. 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. *Arch Pathol Lab Med*. 2018;142(3):321-346. doi:10.5858/arpa.2017-0388-CP.
26. Jain NM, Schmalz L, Cann C, et al. Framework for implementing and tracking a molecular tumor board at a National Cancer Institute-designated comprehensive cancer center. *Oncologist*. 2021;26(11):e1962-e1970. doi:10.1002/onco.13936.
27. Kato S, Kim KH, Lim HJ, et al. Real-world data from a molecular tumor board demonstrates improved outcomes with a precision N-of-One strategy. *Nat Commun*. 2020;11(1):4965. doi:10.1038/s41467-020-18613-3.
28. Sicklick JK, Kato S, Okamura R, et al. Molecular profiling of cancer patients enables personalized combination therapy: the I-PREDICT study. *Nat Med*. 2019;25(5):744-750. doi:10.1038/s41591-019-0407-5.
29. Koopman B, Groen HJM, Ligtenberg MJL, et al. Multicenter comparison of molecular tumor boards in The Netherlands: definition, composition, methods, and targeted therapy recommendations. *Oncologist*. 2021;26(8):e1347-e1358. doi:10.1002/onco.13580.

Molecular Biomarker Testing Algorithms

19

By Ming-Sound Tsao, Fernando Lopez-Rios, Erik Thunnissen, Jhanelle E. Gray, Yasushi Yatabe, Teh-Ying Chou, Justin F. Gainor, and Ivy Elkins

With an increasing number of new biomarker-guided therapies receiving approval for the treatment of patients with lung cancer, molecular testing algorithms in lung cancer continue to evolve. This chapter focuses mainly on testing algorithms that involve tumor tissue biopsies or resection specimens, as algorithms involving liquid biopsy are covered in Chapter 4.

Immunohistochemistry-based tests for predictive protein biomarkers are also included in this chapter as part of molecular testing. There are many potential algorithms that might achieve the required end. This chapter presents some possibilities, but also the principles on which other versions may be developed to suit local needs. Readers are referred to the relevant chapters for details of testing issues relating to specific genes.

Molecular Testing Algorithms in Lung Cancer Diagnosis

Predictive biomarker testing to guide therapies should only be conducted once a cancer diagnosis has been established; it should not be used to establish the diagnosis. In lung cancer, most molecular testing is conducted on biopsy specimens because 60% to 70% of patients are clinically diagnosed at an advanced stage (IIIB-IV). Throughout this process, whether limited or extensive testing is undertaken, there are 3 main principles in play:

1. Speed—turnaround time is paramount as patients with advanced lung cancer can deteriorate quickly.
2. Accuracy—important both in terms of cancer diagnosis and correct molecular profiling.
3. Appropriate testing—complete gene profiling to cover all of the targets for which drugs are available.

In this setting, it is critical that pathologists are aware of the Pathology Best Practice consideration, introduced in 2011 by the International Association for the Study of Lung Cancer (IASLC) multidisciplinary experts that recommended “tissue specimens should be managed not only for diagnosis but also to maximize the amount of tissue available for molecular studies.”¹ This principle has subsequently been adopted in the fourth and fifth editions of the World Health Organization (WHO) classification on lung and thoracic cancers.^{2,3} Consistent with this principle, algorithms (Figure 19-1) for the initial sectioning of formalin-fixed paraffin-embedded (FFPE) blocks of tumor biopsies have been introduced in previous IASLC atlases.^{4,5}

The principle also encourages that a minimum set of diagnostic markers for lung cancer pathologic classification be performed, but only when required, as the use of TTF1 and p40 can reduce the number of non-small cell lung carcinoma, not otherwise specified (NSCLC-NOS) cases to less than 5% to 10% (Figure 19-2).⁶ Along these lines, best practice recommendations for diagnostic immunohistochemistry in lung cancer have been released.⁷

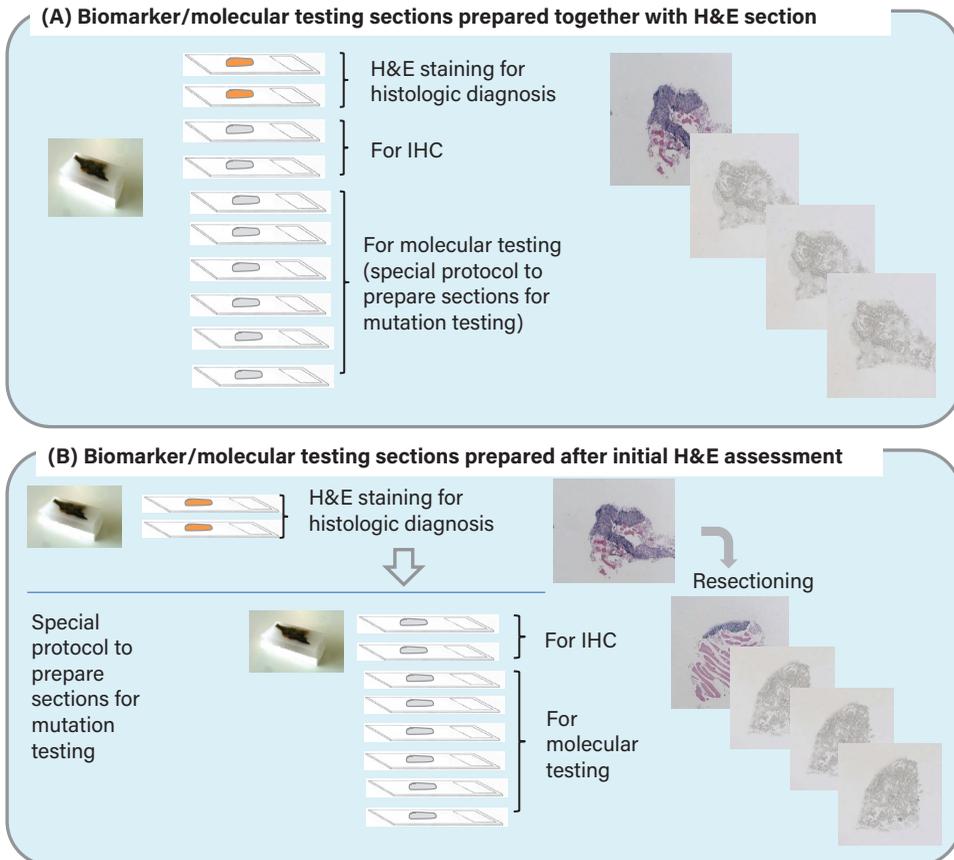


Figure 19-1. Strategies for maximizing tissue for diagnosis and molecular biomarker testing. The number of unstained sections to be prepared is determined by the institutional/departmental strategy for optimal tissue use to reduce tissue sample loss and turnaround time. Many laboratories cut sections for DNA/RNA extraction on a dedicated microtome to prevent cross-contamination. Abbreviations: H&E = hematoxylin and eosin; IHC = immunohistochemistry. (Source: Tsao et al.⁸)

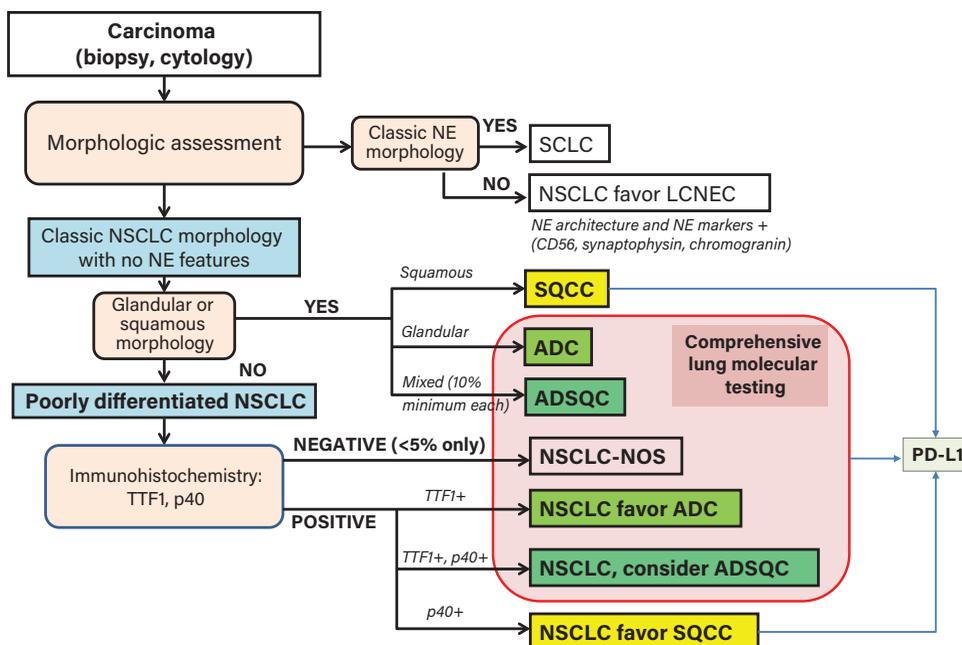


Figure 19-2. Diagnostic algorithm to guide predictive molecular testing in lung cancer. All nonsquamous NSCLC should undergo comprehensive molecular testing. In some settings, SQCC and LCNEC should also be considered for molecular testing (eg, SQCC in a nonsmoker or never-smoker). Abbreviations: ADC = adenocarcinoma; ADSQC = adenosquamous carcinoma; LCNEC = large cell neuroendocrine carcinoma; NE = neuroendocrine; NOS = not otherwise specified; NSCLC = non-small cell lung carcinoma; SCLC = small cell carcinoma; SQCC = squamous cell carcinoma. (Adapted from Travis et al and WHO Classification of Tumours Editorial Board.^{2,3})

Molecular Testing in Advanced-Stage Non-Small Cell Lung Carcinoma

The most commonly used testing algorithm is based on current National Comprehensive Cancer Network (NCCN), European Society for Medical Oncology (ESMO), and College of American Pathologists (CAP)/IASLC/Association for Molecular Pathology (AMP) guidelines, which are mainly for patients with advanced-stage NSCLC (see also Chapter 6). Figure 19-3 provides a graphical illustration of 2 options for testing workflows, with option A using a more comprehensive next-generation sequencing (NGS) panel assay as the primary technology for molecular profiling, and option B using a combination of single-analyte polymerase chain reaction (PCR)-based assays and immunohistochemistry/fluorescence in situ hybridization (IHC/FISH) (ALK, ROS1, RET, NTRK) assays. The choice of NGS panel in option A and individual marker assays in option B is largely determined by multiple local considerations, including the available funding mechanism, amount of tissue, technical/analytical platform and expertise for the various tests, local population prevalence of specific alterations (eg, *EGFR* mutations), number of approved marker-selected therapies, and the required turnaround time. In general, with a large number of molecular markers that need to be tested (eg, as currently recommended by the American Society of Clinical Oncology [ASCO], ESMO, NCCN), option A (NGS first) is more efficient and cost-effective than the combination of single analyte tests.⁹⁻¹¹

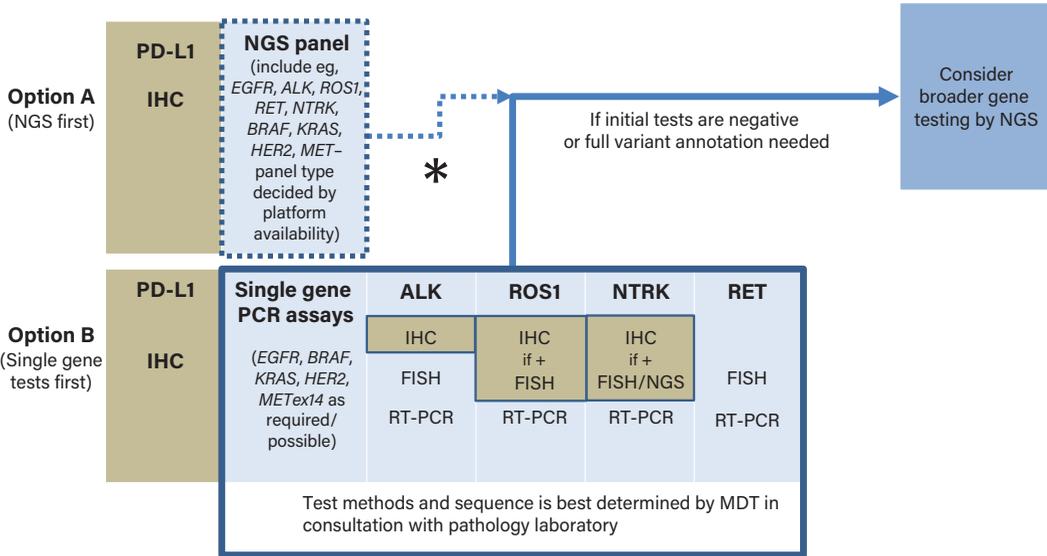


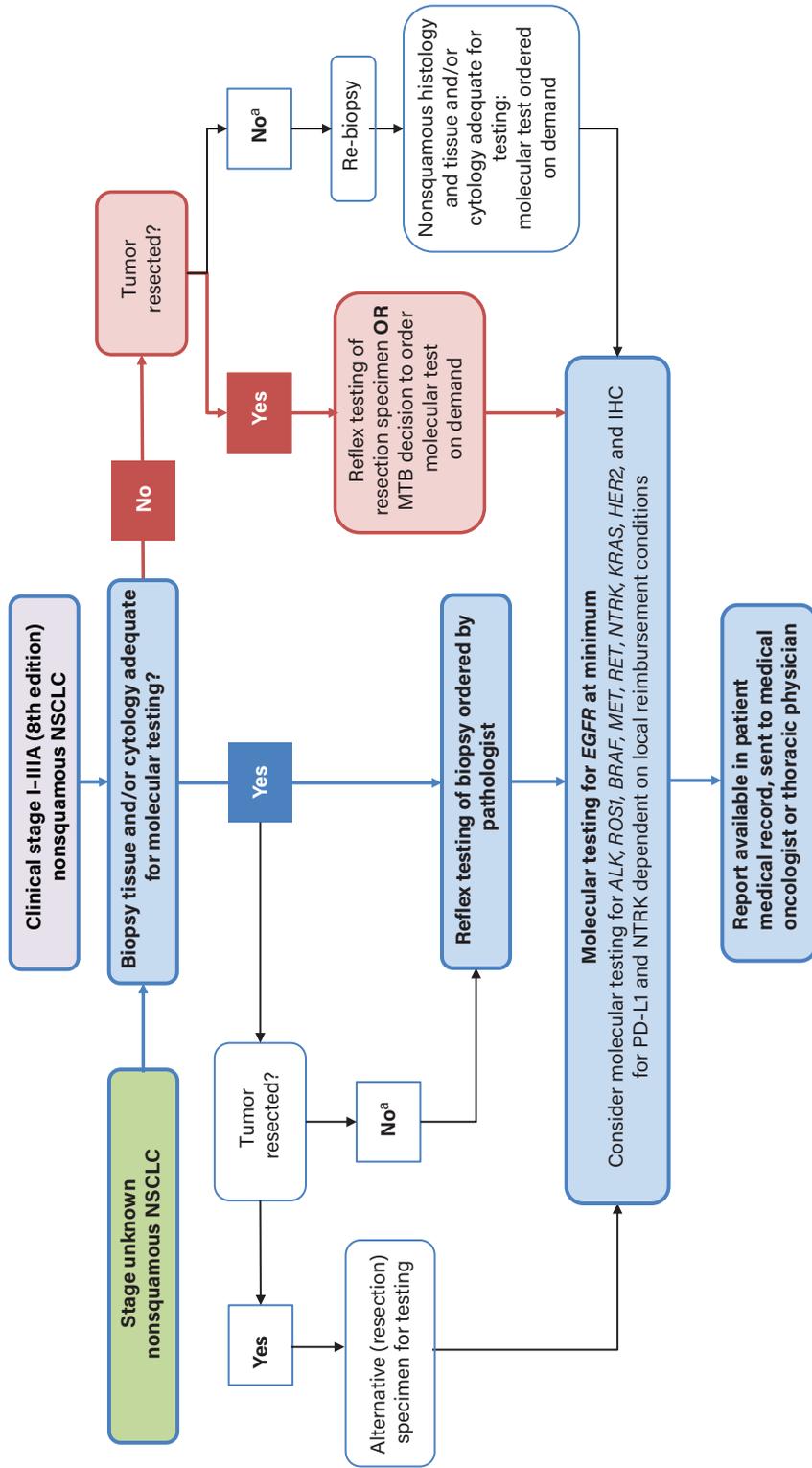
Figure 19-3. Testing workflows for patients with advanced NSCLC. (Option A) Up-front NGS and PD-L1 IHC is performed. Exact genes in the first NGS panels may vary and depend on available platforms/panels and DNA vs RNA approach. (Also see Chapter 5). (Option B) Single-analyte testing is initiated. Exact genes for single gene testing, assay choice, and order of testing depend on local resources, likelihood of identifying a mutation (local prevalence, eg, *EGFR* [see text]) and MDT preference. The tumor cell content in a specimen may also determine the optimal approach. Hybrid-capture NGS has sensitivity down to 20% tumor content. Sensitive targeted assays, such as droplet digital PCR, real-time PCR, and some amplicon-based NGS, have sensitivity down to 5%. (*asterisk*) A second larger NGS panel may also be considered, if possible, if the first NGS panel does not include additional genes/mutations with therapy available including in clinical trials. Abbreviations: FISH = fluorescence in situ hybridization; IHC = immunohistochemistry; MDT = multidisciplinary team; NGS = next-generation sequencing; PCR = polymerase chain reaction; RT-PCR = reverse transcription PCR.

Molecular Testing in Stage-Unknown Reflex and/or Early Stage NSCLC

Reflex predictive biomarker testing is often routinely performed at the time of diagnosis (see also Chapter 6). In such a situation, the information on clinical staging may be incomplete or unknown to the pathologist who orders the biomarker test; thus the algorithm could be stage-agnostic, but it may also apply to patients with early stage (I-IIIa) NSCLC who potentially could undergo resection (Figure 19-4). Reflex testing has the advantage of making biomarker test results available sooner for therapeutic decision-making^{12,13} and, in the context of early disease, for identifying patients who may be candidates for neoadjuvant immunotherapy or adjuvant targeted therapy. Nonetheless, the whole multidisciplinary team should be aware of the challenges of predictive biomarker testing in the perioperative scenario.¹⁴

Molecular Testing in Special Circumstances and in Countries with High EGFR Mutation Prevalence

While guidelines (eg, NCCN, ESMO, CAP/IASLC/AMP, ASCO, etc) are often developed based on the latest clinical evidence available and are suited to countries with well-resourced health systems, there are many circumstances (Figure 19-5A) in which comprehensive molecular testing by NGS is not feasible or available. In such situations, alternate simplified or more practical testing algorithms may be adopted (Figure 19-5A). In places where *EGFR* sensitizing mutation is highly prevalent (eg, east Asian countries), testing algorithms that prioritize *EGFR*, and PD-L1, may also be more cost-effective^{15,16} (Figure 19-5B,C). However, the adoption of these alternate testing algorithms should only follow a multidisciplinary



^a Patient not suitable for surgery (unresectable tumor or patient medically inoperable) or declines surgery

Figure 19-4. Testing algorithm when tumor clinical stage is unknown at initial biopsy or when clinical staging indicates a potentially resectable early (stage I-IIIa) NSCLC. Abbreviations: IHC = immunohistochemistry; MTB = multidisciplinary tumor board; NSCLC = non-small cell lung carcinoma. (Adapted from Aggarwal et al.¹⁷)

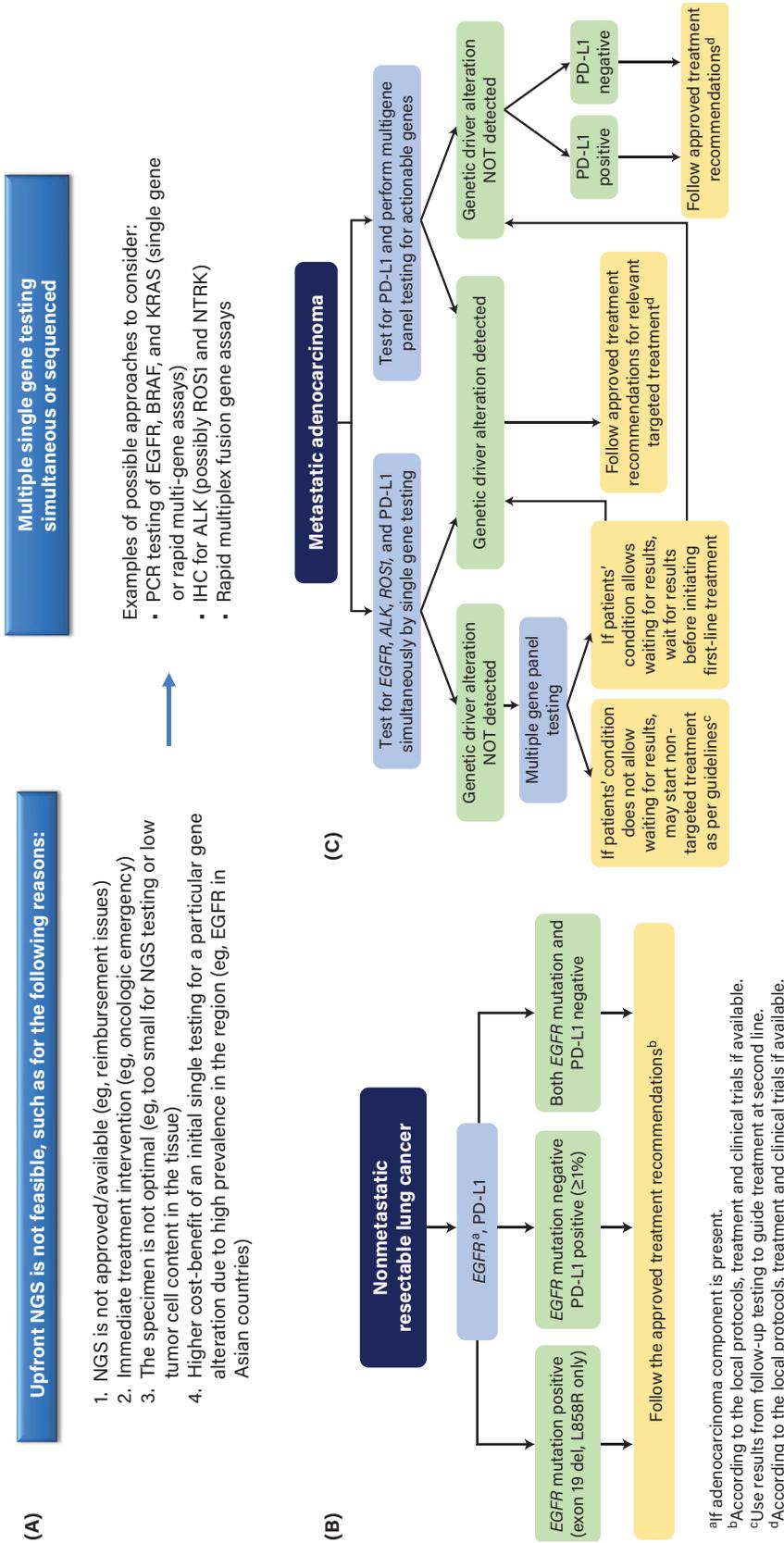


Figure 19-5. Alternate testing algorithms focus on maximizing the testing yield for the biomarker-driven therapies available to the affected patients. Abbreviations: IHC = immunohistochemistry; NGS = next-generation sequencing; PCR = polymerase chain reaction. (Adapted from Mitsudomi et al.¹⁵)

consensus decision. Another emerging, cost-saving practice, which is based on the mutual exclusivity of addictive oncogenic drivers, is to use a DNA-NGS panel first, and only when this fails to identify a driver mutation, would RNA-NGS for fusion genes (and possibly MET exon 14 skipping mutations) be undertaken. Any sequencing of testing does, however, mean delay in identifying those targets covered by later steps.

Conclusion

To achieve the most optimal benefit of precision oncology for patients with lung cancer, predictive biomarker testing is imperative. While comprehensive molecular testing that includes the use of NGS for *EGFR*, *ALK*, *ROS1*, *RET*, *NTRK*, *BRAF*, *KRAS*, *MET*, *HER2*, and PD-L1 by IHC is desirable, many circumstances may influence the practicality of this full testing algorithm. These include tissue availability, funding, available platforms, technical expertise, and not least the availability of the drugs for biomarker-driven treatment. It is generally recommended that the adoption of any testing algorithm be based on multidisciplinary input taking into account these factors.

References

1. Travis WD, Brambilla E, Noguchi M, et al. International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society International multidisciplinary classification of lung adenocarcinoma. *J Thorac Oncol*. 2011;6(2):244-285. doi:10.1097/JTO.0b013e318206a221.
2. Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG. *WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart*. 4th ed. International Agency for Research on Cancer; 2015.
3. WHO Classification of Tumours Editorial Board. *Thoracic Tumours*. WHO Classification of Tumours. Vol. 5. 5th ed. International Agency for Research on Cancer; 2021.
4. Nicholson AG, Kerr K, Wistuba I, Yatabe Y. Sample acquisition, processing, and general diagnostic procedures. In: Tsao MS, Hirsch FR, Yatabe Y, eds. *IASLC Atlas of ALK and ROS1 Testing in Lung Cancer*. 2nd ed. International Association for the Study of Lung Cancer; 2016.
5. Tsao MS, Kerr KM, Dacic S, Yatabe Y, Hirsch FR, eds. *IASLC Atlas of PD-L1 Immunohistochemistry Testing in Lung Cancer*. International Association for the Study of Lung Cancer; 2017.
6. Loo PS, Thomas SC, Nicolson MC, Fyfe MN, Kerr KM. Subtyping of undifferentiated non-small cell carcinomas in bronchial biopsy specimens. *J Thorac Oncol*. 2010;5(4):442-447. doi:10.1097/JTO.0b013e3181d40fac.
7. Yatabe Y, Dacic S, Borczuk AC, et al. Best practices recommendations for diagnostic immunohistochemistry in lung cancer. *J Thorac Oncol*. 2019;14(3):377-407. doi:10.1016/j.jtho.2018.12.005.
8. Tsao MS, Nicholson AG, Hirsch FR. Implementation of PD-L1 testing for personalized therapy for lung cancer. In: Tsao MS, Kerr KM, Dacic S, Yatabe Y, Hirsch FR, eds. *IASLC Atlas of PD-L1 Immunohistochemistry Testing in Lung Cancer*. International Association for the Study of Lung Cancer; 2017.
9. Pennell NA, Mutebi A, Zhou ZY, et al. Economic impact of next-generation sequencing versus single-gene testing to detect genomic alterations in metastatic non-small-cell lung cancer using a decision analytic model. *JCO Precis Oncol*. 2019;3:1-9. doi:10.1200/PO.18.00356.
10. Vanderpoel J, Stevens AL, Emond B, et al. Total cost of testing for genomic alterations associated with next-generation sequencing versus polymerase chain reaction testing strategies among patients with metastatic non-small cell lung cancer. *J Med Econ*. 2022;25(1):457-468. doi:10.1080/13696998.2022.2053403.
11. Tan AC, Lai GGY, Tan GS, et al. Utility of incorporating next-generation sequencing (NGS) in an Asian non-small cell lung cancer (NSCLC) population: incremental yield of actionable alterations and cost-effectiveness analysis. *Lung Cancer*. 2020;139:207-215. doi:10.1016/j.lungcan.2019.11.022.

12. Lim C, Tsao MS, Le LW, et al. Biomarker testing and time to treatment decision in patients with advanced nonsmall-cell lung cancer. *Ann Oncol.* 2015;26(7):1415-1421. doi:10.1093/annonc/mdv208.
13. Cheema PK, Menjak IB, Winterton-Perks Z, et al. Impact of reflex EGFR/ALK testing on time to treatment of patients with advanced nonsquamous non-small-cell lung cancer. *J Oncol Pract.* 2017;13(2):e130-e138. doi:10.1200/JOP.2016.014019.
14. Conde E, Hernandez S, Lopez-Rios F. Rethinking the role of biomarkers for operable non-small cell lung carcinoma: an effective collaboration with artificial intelligence algorithms. *Mod Pathol.* 2022;35(12):1754-1756. doi:10.1038/s41379-022-01167-8.
15. Mitsudomi T, Tan D, Yang JC, et al. Expert consensus recommendations on biomarker testing in metastatic and nonmetastatic NSCLC in Asia. *J Thorac Oncol.* 2022;S1556-0864(22)01905-0. doi:10.1016/j.jtho.2022.10.021.
16. Yang SC, Yeh YC, Chen YL, Chiu CH. Economic analysis of exclusionary EGFR test versus up-front NGS for lung adenocarcinoma in high EGFR mutation prevalence areas. *J Natl Compr Canc Netw.* 2022;20(7):774-782.e4. doi:10.6004/jnccn.2021.7120.
17. Aggarwal C, Bubendorf L, Cooper WA, et al. Molecular testing in stage I-III non-small cell lung cancer: approaches and challenges. *Lung Cancer.* 2021;162:42-53. doi:10.1016/j.lungcan.2021.09.003.

Summary and Future Perspectives

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In precision oncology, molecular testing is the engine that drives the implementation of biomarker-driven targeted therapies. In merely a decade, we have progressed from the necessity of testing a single marker (*EGFR*) in lung cancer to testing at least 10 markers (*EGFR*, *ALK*, *ROS1*, *BRAF*, *KRAS*, *RET*, *NTRK*, *MET*, *HER2*, PD-L1). Fortunately, remarkable advances in molecular testing technologies have developed in parallel. While previous International Association for the Study of Lung Cancer (IASLC) atlases on predictive biomarker testing in lung cancer have individually covered 1 or 2 markers (*ALK*, *ALK/ROS1*, *EGFR*, PD-L1), it is no longer practical or realistic to publish separate atlases on individual markers. This specific edition is an attempt to crystallize the state of the art and science in molecular testing in lung cancer for targeted therapies.

It should be apparent that biomarker testing is complex and involves many stakeholders with expertise in sample acquisition and processing, testing technologies, assays development, implementation, and clinical utility. Critically, testing strategies must be relevant and accessible to patients who would benefit from the results. Therefore, the various chapters have purposefully been composed by a multidisciplinary team of coauthors. Some of the chapters included in this atlas have been covered in 1 or more previous IASLC atlases, as these represent fundamental requirements for best practice in molecular biomarker testing. These include specimen acquisition and pre-analytical issues of processing, reporting, and interpretation of test results and requirements for analytical quality assurance.

In this edition, we have expanded the chapter on testing technology into next-generation sequencing, the currently most advanced analytical platform for multiplex testing of large numbers of genomic aberrations in cancers. With the increasing complexity of clinical oncology whereby multiple drugs of similar class or different generations are available, we have included for each tractable gene, the description of state-of-the-art therapies to provide context for the biomarker testing described. The complexity of delivery of molecular biomarker

testing has also been covered in chapters on global perspectives in testing guidelines and algorithms, albeit rather simplistically to fit the nature of this publication.

There is little doubt that with rapid advances in our knowledge on the molecular pathobiology of lung cancer, nanotechnologies, computation, and informatics, molecular testing will continue to expand in scope, depth, and breadth. Such expansion has already started, and its progress will likely be rapid and exponential. Future challenges and potentials are hereby briefly discussed.

Future of Genomic Testing in Tumor Tissue

While liquid biopsy is presently the preferred first approach to identify potential resistance mechanisms in targeted therapies at therapeutic progression, there are efforts to elevate this approach to baseline testing at initial diagnosis.¹⁻³ The latter is still somewhat limited by assay sensitivity, especially for fusions and gene copy number,⁴ which might be overcome by additional technological advances, for example, use of plasma cell-free or exosome-derived RNA for detection of gene fusions.^{5,6}

While liquid biopsy seemingly might replace or render tumor tissue testing redundant, it is more likely that tissue and blood testing will evolve in parallel as complementary approaches.⁷ Tumor diagnosis, subtyping, and immunohistochemistry biomarkers are fundamental requirements. Currently, liquid biopsy has issues with sensitivity and specificity regarding some genomic alterations and cannot provide categorical tumor diagnosis and classification. The implementation of parallel tissue and liquid testing is feasible only if cost and funding are not an issue; yet this will likely be a major barrier for most patients worldwide, which requires deeper deliberation to develop future testing algorithms, including the challenge of assimilating the derived data and understanding the implications.

Minimal Residual Disease Detection and Monitoring Therapeutic Efficacy and Disease Progression

There is intense effort currently focused on the application of liquid biopsy for detecting minimal residual disease (MRD) and using MRD to monitor disease progression, prognosticate the likely outcome of patients, and potentially alter the course of therapy (see the “Clinical Application” section in Chapter 4). The noninvasive nature of this approach has accelerated the assessment for its clinical utility.⁸ Fundamental issues that need to be addressed to realize the promise of liquid biopsy for MRD testing include the sensitivity and specificity for such technology. While current works focus mainly on genomic DNA for testing, wider research using methylated DNA/RNA and circular RNA are ongoing.⁹⁻¹¹

Lung Cancer Early Detection

Current state-of-the-art technology for early lung cancer detection uses low-dose spiral computed tomography (CT), which remains considerably costly and low throughput, and has suboptimal specificity. Application of radiomics and artificial intelligence to enhance the performance of CT screening is an area of major research interest.¹² The addition of complementary tests with high sensitivity and specificity—such as for (epi)genomic markers in the blood or volatile organic compounds in the breath—could dramatically expand the ability to implement population-based early detection programs.^{13,14} Circulating tumor DNA is

being extensively investigated as a lung cancer early detection platform, yet the sensitivity and specificity is suboptimal for detection of early stage tumors.¹⁵ Similar to MRD detection, new markers involving methylated DNA and circular RNA are also being investigated.^{16,17}

Machine Learning and Artificial Intelligence

With increasingly comprehensive genomic testing requiring more sophisticated and time-consuming bioinformatics, it is likely that artificial intelligence and machine learning techniques will be increasingly used in clinical genomic diagnostics. This could potentially include various steps in genomic analyses such as variant calling and classification, genome annotation, and genotype-phenotype predictions.¹⁸

Testing in Other Lung Cancer Types and Thoracic Cancers

Aside from special circumstances, current practice in genomic profiling is largely focused on nonsquamous, non-small cell carcinomas of the lung, but the impact of predictive biomarker testing in squamous cell carcinomas, small cell carcinoma, and other thoracic malignancies, such as mesothelioma and thymic tumors, is yet to be fully realized. These will likely emerge when new therapeutic vulnerabilities and the corresponding therapies are identified, such as those involving tumor metabolism, oxidative stress response pathway, and cellular and noncellular components of the tumor microenvironment.¹⁹⁻²¹

Conclusion

In this *IASLC Atlas of Molecular Testing for Targeted Therapy in Lung Cancer*, we have provided an overview of the individual genes currently relevant to targeted therapy in lung cancer as well as the factors impacting their assessment such as specimen acquisition, assay techniques, approaches to testing algorithms, and global variations in molecular testing. We have provided an up-to-date outline of clinically relevant molecular testing that will be of value to a wide variety of lung cancer health care professionals and their patients that will hopefully lay the foundation for further advances in the clinical care of lung cancer.

References

1. Choudhury Y, Tan MH, Shi JL, et al. Complementing tissue testing with plasma mutation profiling improves therapeutic decision-making for patients with lung cancer. *Front Med (Lausanne)*. 2022;9:758464. doi:10.3389/fmed.2022.758464.
2. Park S, Olsen S, Ku BM, et al. High concordance of actionable genomic alterations identified between circulating tumor DNA-based and tissue-based next-generation sequencing testing in advanced non-small cell lung cancer: the Korean Lung Liquid Versus Invasive Biopsy Program. *Cancer*. 2021;127(16):3019-3028. doi:10.1002/cncr.33571.
3. Li BT, Janku F, Jung B, et al. Ultra-deep next-generation sequencing of plasma cell-free DNA in patients with advanced lung cancers: results from the Actionable Genome Consortium. *Ann Oncol*. 2019;30(4):597-603. doi:10.1093/annonc/mdz046.
4. Song P, Wu LR, Yan YH, et al. Limitations and opportunities of technologies for the analysis of cell-free DNA in cancer diagnostics. *Nat Biomed Eng*. 2022;6(3):232-245. doi:10.1038/s41551-021-00837-3.
5. Kok VC, Yu CC. Cancer-derived exosomes: their role in cancer biology and biomarker development. *Int J Nanomedicine*. 2020;15:8019-8036. doi:10.2147/IJN.S272378.
6. Larson MH, Pan W, Kim HJ, et al. Author correction: a comprehensive characterization of the cell-free transcriptome reveals tissue- and subtype-specific biomarkers for cancer detection. *Nat Commun*. 2022;13(1):2553. doi:10.1038/s41467-022-30329-0.

7. Rolfo C, Mack P, Scagliotti GV, et al. Liquid biopsy for advanced NSCLC: a consensus statement from the International Association for the Study of Lung Cancer. *J Thorac Oncol.* 2021;16(10):1647-1662. doi:10.1016/j.jtho.2021.06.017.
8. Chae YK, Oh MS. Detection of minimal residual disease using ctDNA in lung cancer: current evidence and future directions. *J Thorac Oncol.* 2019;14(1):16-24. doi:10.1016/j.jtho.2018.09.022.
9. Constâncio V, Nunes SP, Henrique R, Jerónimo C. DNA methylation-based testing in liquid biopsies as detection and prognostic biomarkers for the four major cancer types. *Cells.* 2020;9(3):624. doi:10.3390/cells9030624.
10. Yin H, Hong H, Yin P, et al. Increased levels of N6-methyladenosine in peripheral blood RNA: a perspective diagnostic biomarker and therapeutic target for non-small cell lung cancer. *Clin Chem Lab Med.* 2022;61(3):473-484. doi:10.1515/cclm-2022-1033.
11. Kristensen LS, Jakobsen T, Hager H, Kjems J. The emerging roles of circRNAs in cancer and oncology. *Nat Rev Clin Oncol.* 2022;19(3):188-206. doi:10.1038/s41571-021-00585-y.
12. Binczyk F, Prazuch W, Bozek P, Polanska J. Radiomics and artificial intelligence in lung cancer screening. *Transl Lung Cancer Res.* 2021;10(2):1186-1199. doi:10.21037/tlcr-20-708.
13. Seijo LM, Peled N, Ajona D, et al. Biomarkers in lung cancer screening: achievements, promises, and challenges. *J Thorac Oncol.* 2019;14(3):343-357. doi:10.1016/j.jtho.2018.11.023.
14. Scheepers MHMC, Al-Difaie Z, Brandts L, et al. Diagnostic performance of electronic noses in cancer diagnoses using exhaled breath: a systematic review and meta-analysis. *JAMA Netw Open.* 2022;5(6):e2219372. doi:10.1001/jamanetworkopen.2022.19372.
15. Abbosh C, Birkbak NJ, Swanton C. Early stage NSCLC—challenges to implementing ctDNA-based screening and MRD detection. *Nat Rev Clin Oncol.* 2018;15(9):577-586. doi:10.1038/s41571-018-0058-3.
16. Shen SY, Singhanian R, Fehring G, et al. Sensitive tumour detection and classification using plasma cell-free DNA methylomes. *Nature.* 2018;563(7732):579-583. doi:10.1038/s41586-018-0703-0.
17. Pedraz-Valdunciel C, Giannoukakos S, Giménez-Capitán A, et al. Multiplex analysis of CircRNAs from plasma extracellular vesicle-enriched samples for the detection of early-stage non-small cell lung cancer. *Pharmaceutics.* 2022;14(10):2034. doi:10.3390/pharmaceutics14102034.
18. Dias R, Torkamani A. Artificial intelligence in clinical and genomic diagnostics. *Genome Med.* 2019;11(1):70. doi:10.1186/s13073-019-0689-8.
19. Lau SCM, Pan Y, Velcheti V, Wong KK. Squamous cell lung cancer: current landscape and future therapeutic options. *Cancer Cell.* 2022;40(11):1279-1293. doi:10.1016/j.ccell.2022.09.018.
20. Hellyer JA, Padda SK, Diehn M, Wakelee HA. Clinical implications of KEAP1-NFE2L2 mutations in NSCLC. *J Thorac Oncol.* 2021;16(3):395-403. doi:10.1016/j.jtho.2020.11.015.
21. Suzuki J, Tsuboi M, Ishii G. Cancer-associated fibroblasts and the tumor microenvironment in non-small cell lung cancer. *Expert Rev Anticancer Ther.* 2022;22(2):169-182. doi:10.1080/14737140.2022.2019018.

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