2.04.45	Molecular Analysis (Including Liquid Biopsy) for Targeted Therapy or Immunotherapy of Non-Small-Cell Lung Cancer		
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Section:	2.0 Medicine	**Page:**	Page 1 of 97

Policy Statement

Note: Starting on July 1, 2022 (per CA law SB 535) for commercial plans regulated by the California Department of Managed Healthcare and California Department of Insurance (PPO and HMO), health care service plans and insurers shall not require prior authorization for biomarker testing, including biomarker testing for cancer progression and recurrence, if a member has stage 3 or 4 cancer. Health care service plans and insurers can still do a medical necessity review of a biomarker test and possibly deny coverage after biomarker testing has been completed and a claim is submitted (post service review).

The use of tissue samples for analysis is generally preferred over plasma testing (liquid biopsy or circulating tumor DNA, ctDNA) when available. Panel testing of tissue samples is an acceptable alternative to individual testing when the quantity of tissue is limited.

Molecular analysis (genetic testing) is reserved for advanced (stage III or IV) or metastatic Non-Small-Cell Lung Cancer (NSCLC) including adenocarcinoma, large cell, squamous cell and NSCLC not otherwise specified (see Policy Guidelines) or if a targeted therapy dependent on genetic testing is being considered. Small panel testing including the following medically necessary genes may be considered as an alternative to individual testing and may be preferred when there is limited tissue available for testing.

I. Plasma tests for oncogenic driver variants deemed medically necessary on tissue biopsy may be considered **medically necessary** to predict treatment response to targeted therapy for patients meeting the following criteria:
   A. Patient does not have sufficient tissue for standard molecular testing using formalin-fixed paraffin-embedded tissue; AND
   B. Follow-up tissue-based analysis is planned when possible should no driver variant be identified via plasma testing.

***EGFR* Testing**

II. Analysis of somatic variants (in exons 18 through 21 (e.g., G719X, L858R, T790M, S6781, L861Q) within the epidermal growth factor receptor *(EGFR)* gene, may be considered **medically necessary** to predict treatment response to an EGFR tyrosine kinase inhibitor (TKI) therapy (e.g., erlotinib [Tarceva®], gefitinib [Iressa®], afatinib [Gilotrif®], or osimertinib [Tagrisso™]) in patients with advanced or high risk earlier stage (IB-IIIA) lung adenocarcinoma, large cell carcinoma, advanced squamous-cell non-small-cell lung cancer (NSCLC), and NSCLC not otherwise specified.

III. At progression (or when included in an initial panel), analysis of the EGFR T790M resistance variant for targeted therapy with osimertinib using tissue or ctDNA may be considered **medically necessary** in patients with advanced or high risk earlier stage (IB-IIIA) lung adenocarcinoma, large cell carcinoma, advanced squamous-cell non-small-cell lung cancer, and non-small-cell lung cancer not otherwise specified.

IV. Analysis of other *EGFR* variants within exons 22 to 24, or other applications related to NSCLC, is considered **investigational**.

***ALK* Testing**

V. Analysis of somatic rearrangement variants of the anaplastic lymphoma kinase (*ALK*) gene may be considered **medically necessary** to predict treatment response to ALK inhibitor therapy (e.g., crizotinib [Xalkori®], ceritinib [Zykadia™], alectinib [Alecensa®], or brigatinib [Alunbrig™]) in patients with advanced lung adenocarcinoma or in whom an adenocarcinoma component cannot be excluded (see Policy Guidelines section) or when included in a panel approved for other indications.

VI. Analysis of somatic rearrangement variants of the *ALK* gene is considered **investigational** in all other situations.

***BRAF* V600E Testing**

VII. Analysis of the somatic *BRAF* V600E variant may be considered **medically necessary** to predict treatment response to BRAF or MEK inhibitor therapy (e.g., dabrafenib [Tafinlar®] and trametinib [Mekinist®]), in patients with advanced lung adenocarcinoma or in whom an adenocarcinoma component cannot be excluded (see Policy Guidelines section) or when included in a panel approved for other indications.

VIII. Analysis of the somatic *BRAF V600E* variant is considered **investigational** in all other situations.

***ROS1* Testing**

IX. Analysis of somatic rearrangement variants of the *ROS1* gene may be considered **medically necessary** to predict treatment response to ALK inhibitor therapy (crizotinib [Xalkori]) in patients with advanced lung adenocarcinoma or in whom an adenocarcinoma component cannot be excluded (see Policy Guidelines section) or when included in a panel approved for other indications.

X. Analysis of somatic rearrangement variants of the *ROS1* gene is considered **investigational** in all other situations.

***KRAS* Testing**

XI. Analysis of somatic variants of the *KRAS* gene may be considered **medically necessary** to predict treatment response to sotorasib (Lumakras) in patients with advanced lung adenocarcinoma or in whom an adenocarcinoma component cannot be excluded (see Policy Guidelines section) or when included in a panel approved for other indications.

XII. All other uses of analysis of somatic variants of the *KRAS* gene are considered **investigational**.

***HER2* Testing**

XIII. Analysis of somatic alterations in the *HER2* gene in tissue for targeted therapy in patients with NSCLC is considered **investigational** unless included in a panel approved for other indications.

***NTRK* Gene Fusion Testing**

XIV. Analysis of somatic *NTRK* gene fusions in tissue may be considered **medically necessary** to predict treatment response to entrectinib (Rozlytrek) or larotrectinib (Vitrakvi) in patients with advanced lung adenocarcinoma or in whom an adenocarcinoma component cannot be excluded (see Policy Guidelines section) or when included in a panel approved for other indications.

XV. Analysis of somatic *NTRK* gene fusions is considered **investigational** in all other situations.

***RET* Rearrangement Testing**

XVI. Analysis of somatic alteration in the *RET* gene may be considered **medically necessary** to predict treatment response to pralsetinib (Gavreto) or selpercatinib (Retevmo) in patients with metastatic NSCLC or when included in a panel approved for other indications.

XVII. Analysis of somatic alterations in the RET gene is considered **investigational** in all other situations.

***MET* Exon 14 Skipping Alteration**

XVIII. Analysis of somatic alteration in tissue that leads to *MET* exon 14 skipping may be considered **medically necessary** to predict treatment response to capmatinib (Tabrecta) in patients with metastatic NSCLC.

XIX. Analysis of genetic alterations of the *MET* gene is considered **investigational** in all other situations.

PD-L1 Testing

XX. Programmed Death-Ligand 1 (PD-L1) testing may be considered **medically necessary** to predict treatment response to atezolizumab (Tecentriq), nivolumab (Opdivo) in combination with ipilimumab (Yervoy), or pembrolizumab (Keytruda) in patients with metastatic NSCLC.

Note: PD-L1 is a ligand not a gene, and testing may be requested separately if not part of the panel.

XXI. PD-L1 testing is considered **investigational** in all other situations.

Tumor Mutational Burden Testing

XXII. Analysis of tumor mutational burden for targeted therapy in patients with NSCLC is considered **investigational.**

NOTE: Refer to Appendix A to see the policy statement changes (if any) from the previous version.

Policy Guidelines

Tumor Mutational Burden or TMB, is defined as the number of somatic mutations per megabase of a genomic sequence, and varies by type of cancer. Whole exome sequencing-derived TMB was initially common but **large** panel sequencing-based estimates of TMB are increasingly common. TMB has been proposed to predict the efficacy of immune checkpoint inhibitors like pembrolizumab (Keytruda®) for a variety of cancers. A result of greater than 10 is considered to be a high TMB and less than10 is low.

These gene tests are intended for use in patients with advanced (stage III or IV) non-small-cell lung cancer.

Patients with either small deletions in exon 19 or a point mutation in exon 21 (L858R) of the tyrosine kinase domain of the epidermal growth factor receptor (*EGFR*) gene are considered good candidates for treatment with erlotinib, gefitinib or afatinib. Patients with wild-type variants are unlikely to respond to erlotinib or afatinib; for these patients, so other treatment options should be considered.

ctDNA tests: The cobas® test is a companion diagnostic for erlotinib (Tarceva®; OSI Pharmaceuticals, Melville NY). Guardant 360 has 2 similar tests, each about 70+ genes. The CDx version is a new FDA approved companion diagnostic for the EGFR exon 19 deletions, L858R and T790M mutation associated with using osimertinib (TAGRISSO®), and it includes SNV testing for NTRK1 and NTRK3 as well as fusion testing for NTRK1 and uses the CPT PLA code 0242U. The Guardant LDT is a laboratory

developed test, which tests for all 3 NTRK genes (NTRK1, NTRK2 and NTRK3), also includes MSI (Microsatellite Instability) and Tumor Mutational Burden (TMB) and should use a miscellaneous CPT code of 81455 (sometimes billed as 81479). Either test is acceptable for use with NSCLC. The FoundationOne Liquid CDx is a 300+ gene panel companion diagnostic for multiple treatments including those related to EGFR and includes MSI and TMB. It is billed using CPT code 0239U and has a similar gene panel to their solid tumor test (FoundationOne CDx).

NTRK testing can also be done using IHC (ImmunoHistoChemical, usually Pan-TRK IHC) or FISH testing if not done as part of a gene panel. NTRK fusions represent up to 1/30 NSCLCs (Vaishnavi et al. Nature Medicine 2013).
NTRK (neurotrophic tyrosine receptor kinase) gene fusions happen when a piece of chromosome containing the NTRK gene breaks off and joins (fuses) with a gene on another chromosome, producing abnormal proteins that can cause cancer cells to grow. It has been associated with cancers of brain, head and neck, thyroid, soft tissue, lung, and colon.

Guidelines from the National Comprehensive Cancer Network on non-small-cell lung cancer provide recommendations for biomarker testing. Guidelines are updated frequently; refer to the source document for current recommendations. The most recent guidelines (v.6.2021) recommend that *EGFR* variants, *ALK* rearrangement, and PD-L1 testing (category 1) as well as *KRAS*, *ROS1*, *BRAF, NTRK1/2/3, MET* Exon 14 skipping alteration, and *RET* testing (category 2A) be performed in the workup of non-small-cell lung cancer in patients with metastatic disease with histologic subtypes adenocarcinoma, large cell carcinoma, and non-small-cell lung cancer not otherwise specified. The guidelines add that testing should be conducted as part of broad molecular profiling.

The 2018 guidelines issued jointly by the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology have recommended the following:

> "One set of genes must be offered by all laboratories that test lung cancers, as an absolute minimum: EGFR, ALK, and ROS1. A second group of genes should be included in any expanded panel that is offered for lung cancer patients: BRAF, MET, RET, ERBB2 (HER2), and KRAS, if adequate material is available. KRAS testing may also be offered as a single-gene test to exclude patients from expanded panel testing. All other genes are considered investigational at the time of publication."

Many tests include variants beyond exons 19 through 21 of the epidermal growth factor receptor (*EGFR)* gene, and some tests additionally include variants in numerous other genes. Liquid biopsy (ctDNA) tests that are negative for variants of interest should be reflexed to routine biopsy and testing using formalin-fixed paraffin-embedded tissue when possible. However, the reason to use ctDNA testing is often due to difficulty obtaining adequate tissue, including when the patient is a poor candidate for undergoing a biopsy procedure.

Recommended Testing Strategies

Patients who meet criteria for genetic testing as outlined in the policy statements above should be tested for the variants specified.

- When tumor tissue is available, use of tissue for testing of any/all variants and biomarkers outlined in this policy is recommended, but is not required in all situations. In certain situations, circulating tumor DNA testing (liquid biopsy) may be an option.

Coding

The following CPT code is specific for testing for common variants of *EGFR*:

- **81235**: *EGFR (epidermal growth factor receptor)* (e.g., non-small cell lung cancer) gene analysis, common variants (e.g., exon 19 LREA deletion, L858R, T790M, G719A, G719S, L861Q)

If testing is done by immunohistochemical assay, the following CPT code would likely be reported:

- **88342**: Immunohistochemistry or immunocytochemistry, per specimen; initial single antibody stain procedure

If testing is done by fluorescence in situ hybridization, the following CPT code would likely be reported:

- **88365**: In situ hybridization (e.g., FISH), per specimen; initial single probe stain procedure

The following CPT codes are specific for testing for *KRAS*:

- **81275**: KRAS (Kirsten rat sarcoma viral oncogene homolog) (e.g., carcinoma) gene analysis; variants in exon 2 (e.g., codons 12 and 13)

- **81276**: KRAS (Kirsten rat sarcoma viral oncogene homolog) (e.g., carcinoma) gene analysis; additional variant(s) (e.g., codon 61, codon 146)

The following Molecular Pathology codes are to support Neurotrophic receptor tyrosine kinase (NTRK) gene testing:

- **81191:** NTRK1 (neurotrophic receptor tyrosine kinase 1) (e.g., solid tumors) translocation analysis
- **81192:** NTRK2 (neurotrophic receptor tyrosine kinase 2) (e.g., solid tumors) translocation analysis
- **81193:** NTRK3 (neurotrophic receptor tyrosine kinase 3) (e.g., solid tumors) translocation analysis
- **81194:** NTRK (neurotrophic-tropomyosin receptor tyrosine kinase 1, 2, and 3) (e.g., solid tumors) translocation analysis

The following CPT code has a listing for *RET* testing:

- **81404**: Molecular Pathology Procedure Level 5
  - *RET (ret proto-oncogene)* (e.g., multiple endocrine neoplasia, type 2B and familial medullary thyroid carcinoma), common variants (e.g., M918T, 2647_2648delinsTT, A883F)

The following CPT code has listings for both *KRAS* and *RET* testing:

- **81405**: Molecular Pathology Procedure Level 6
  - *KRAS (Kirsten rat sarcoma viral oncogene homolog)* (e.g., Noonan syndrome), full gene sequence
  - *RET (ret proto-oncogene)* (e.g., multiple endocrine neoplasia, type 2A and familial medullary thyroid carcinoma), targeted sequence analysis (e.g., exons 10, 11, 13-16)

The following CPT code has a listing for *BRAF* testing:

- **81406**: Molecular Pathology Procedure Level 7
  - *BRAF* (B-*Raf proto-oncogene, serine/threonine kinase*) (e.g., Noonan syndrome), full gene sequence

Testing for variants in the other genes listed above would be reported with the following code:

- **81479**: Unlisted molecular pathology procedure

Description

Over half of patients with non-small-cell lung cancer (NSCLC) present with advanced and therefore incurable disease. Treatment in this setting has been with platinum-based chemotherapy. The identification of specific, targetable oncogenic "driver mutations" in a subset of NSCLCs has resulted

in a reclassification of lung tumors to include molecular subtypes that may direct targeted therapy depending on the presence of specific variants.

Related Policies

- Comprehensive Genomic Profiling for Selecting Targeted Cancer Therapies

Benefit Application

Benefit determinations should be based in all cases on the applicable contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

Some state or federal mandates (e.g., Federal Employee Program [FEP]) prohibits plans from denying Food and Drug Administration (FDA)-approved technologies as investigational. In these instances, plans may have to consider the coverage eligibility of FDA-approved technologies on the basis of medical necessity alone.

Regulatory Status

Table 1 summarizes the FDA-approved targeted treatments for patients with NSCLC along with the concurrently approved companion diagnostic tests. (Note this information is current as of September 29, 2021. The FDA maintains a list of cleared or approved companion diagnostics at https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools

Table 1. FDA-Approved Targeted Treatments for NSCLC and Companion Diagnostic Tests

Treatment	Indication	FDA-Approved Companion Diagnostic Tests
Afatinib (Gilotrif)	• 2013: First line for patients with metastatic NSCLC whose tumors have EGFR exon 19 deletions or exon 21 (L858R) substitutions • 2016: Second line for patients with metastatic squamous NSCLC • 2018: First line for patients with nonresistant EGFR variants other than exon 19 or exon 21 NSCLC	• 2013: therascreen® EGFR Rotor-Gene Q polymerase chain reaction (RGQ PCR) kit (Qiagen) • 2017: FoundationOne CDx™ (Foundation Medicine) • 2021: ONCO/Reveal Dx Lung & Colon Cancer Assay (O/RDx-LCCA)
Alectinib (Alecensa)	• 2015: Second line for patients with ALK-positive metastatic NSCLC who have progressed on or are intolerant of crizotinib • 2017: Patients with ALK-positive metastatic NSCLC as detected by an FDA-approved test	• 2017: FoundationOne CDx™ (Foundation Medicine) • 2017: Ventana ALK (D5F3) CDx Assay • 2020: FoundationOne Liquid CDx
Amivantamab-vmjw (Rybrenant)	• 2021: adult patients with locally advanced or metastatic NSCLC with EGFR exon 20 insertion mutations, as detected by an FDA-approved test, whose disease has progressed on or after platinum-based chemotherapy	• 2021: Guardant360 CDx
Atezolizumab (Tecentriq)	• 2020: First-line treatment of adult patients with metastatic NSCLC whose tumors have high PD-L1 expression (PD-L1 stained ≥ 50% of tumor cells [TC ≥ 50%] or PD-L1 stained tumor-infiltrating immune cells covering ≥ 10% of the tumor area [IC ≥ 10%]), as determined by an	• 2020: VENTANA PD-L1

Treatment	Indication	FDA-Approved Companion Diagnostic Tests
	FDA approved test, with no EGFR or ALK genomic tumor aberrations. o in combination with bevacizumab, paclitaxel, and carboplatin, for the first line treatment of adult patients with metastatic non-squamous NSCLC with no EGFR or ALK genomic tumor aberrations o in combination with paclitaxel protein-bound and carboplatin for the first line treatment of adult patients with metastatic non-squamous NSCLC with no EGFR or ALK genomic tumor aberrations o for the treatment of adult patients with metastatic NSCLC who have disease progression during or following platinum-containing chemotherapy.	
Brigatinib (Alunbrig)	• 2017: Second line for patients with metastatic ALK-positive NSCLC who have progressed on or are intolerant of crizotinib • 2020: Treatment of adult patients with ALK-positive metastatic NSCLC as detected by an FDA-approved test	• 2020: Vysis ALK Break Apart FISH Probe Kit • 2020: FoundationOneCDX
Capmatinib (Tabrecta)	• 2020: metastatic NSCLC whose tumors have a mutation that leads to *MET* exon 14 skipping as detected by an FDA-approved test.	• 2020: FoundationOne CDx (Foundation Medicine) • 2021: FoundationOne Liquid CDx
Ceritinib (Zykadia)	• 2014: Second line for patients with ALK-positive metastatic NSCLC who have progressed on or are intolerant of crizotinib • 2017: First line for patients with ALK-positive metastatic NSCLC	• 2015: Ventana ALK (D5F3) CDx Assay (Ventana Medical Systems) • 2017: FoundationOne CDx™ (Foundation Medicine) • 2017: VENTANA ALK (D5F3) CDx Assay
Crizotinib (Xalkori)	• 2011: First line for patients with ALK- or ROS1-positive metastatic NSCLC	• 2011: Vysis ALK Break Apart FISH Probe Kit (Abbott Laboratories) • 2015: Ventana ALK (D5F3) CDx Assay (Ventana Medical Systems) • 2017: FoundationOne CDx™ (Foundation Medicine) • Oncomine Dx • 2017: VENTANA ALK (D5F3) CDx Assay
Crizotinib (Xalkori)	• 2016: Patients with ROS1-positive metastatic NSCLC	• 2017: Oncomine™ Dx Target Test (Thermo Fisher Scientific)
Dacomitinib (Vizimpro)	• 2018: First line for patients with metastatic NSCLC with EGFR exon 19 deletion or exon 21 (L858R) substitutions	• 2018: therascreen EGFR RGQ PCR Kit • 2021: ONCO/Reveal Dx Lung & Colon Cancer Assay (O/RDx-LCCA)
Dabrafenib (Tafinlar) plus trametinib (Mekinist)	• 2017: Used in combination for treatment of patients with metastatic NSCLC with BRAF V600E variant	• 2017: Oncomine™ Dx Target Test • 2017: FoundationOne CDx™ (Foundation Medicine)
Entrectinib (Rozlytrek)	• 2019: o Adult patients with metastatic NSCLC whose tumors are ROS1-positive o Adult and pediatric patients 12 years of age and older with	• No companion diagnostic

Treatment	Indication	FDA-Approved Companion Diagnostic Tests
	▪ solid tumors that have a NTRK gene fusion without a known acquired resistance mutation, ▪ are metastatic or where surgical resection is likely to result in severe morbidity, and have progressed following treatment or have no satisfactory alternative therapy	
Erlotinib (Tarceva)	• 2013: First line for patients with metastatic NSCLC whose tumors have EGFR exon 19 deletions or exon 21 (L858R) substitutions • 2010: Maintenance for patients with locally advanced or metastatic NSCLC whose disease has not progressed after 4 cycles of platinum-based chemotherapy • 2004: Second line for patients with locally advanced or metastatic NSCLC	• 2013: cobas® EGFR Mutation Test (tissue test) (Roche Diagnostics) • 2016: cobas® EGFR Mutation Test v2 (tissue or blood test) (Roche Diagnostics) • 2017: FoundationOne CDx™ (Foundation Medicine) • 2020: FoundationOne® Liquid CDx • 2021: ONCO/Reveal Dx Lung & Colon Cancer Assay (O/RDx-LCCA)
Gefitinib (Iressa)	• 2015: First line for patients with metastatic NSCLC whose tumors have EGFR exon 19 deletions or exon 21 (L858R) substitutions • 2003: Second line for patients with locally advanced or metastatic NSCLC	• 2015: therascreen® EGFR Rotor-Gene Q polymerase chain reaction (RGQ PCR) kit • 2017: Oncomine™ Dx Target Test • 2017: FoundationOne CDx™ (Foundation Medicine) • 2017: cobas® EGFR Mutation Test (tissue test) (Roche Diagnostics) • 2017: Oncomine Dx Target Test • 2020: FoundationOne® Liquid CDx • 2021: ONCO/Reveal Dx Lung & Colon Cancer Assay (O/RDx-LCCA)
Ipilimumab (Yervoy)	• Treatment of adult patients with metastatic NSCLC expressing PD-L1 (≥1%) as determined by an FDA-approved test, with no EGFR or ALK genomic tumor aberrations, as first-line treatment in combination with nivolumab • Treatment of adult patients with metastatic or recurrent NSCLC with no EGFR or ALK genomic tumor aberrations as first-line treatment, in combination with nivolumab and 2 cycles of platinum doublet chemotherapy	• PD-L1 IHC 28-8 PharmDx
Larotrectinib (Vitrakvi)	• 2018: Adult and pediatric patients with solid tumors that o have a NTRK gene fusion without a known acquired resistance mutation, o are metastatic or where surgical resection is likely to result in severe morbidity, and o have no satisfactory alternative treatments or that have progressed following treatment	• FoundationOne CDx (solid tumors, NTRK1/2/3 fusions)
Lorlatinib (Lorbrena)	• 2018: Patients with ALK-positive metastatic NSCLC whose disease has progressed on: o crizotinib and at least 1 other ALK inhibitor for metastatic disease; or o alectinib as the first ALK inhibitor therapy for metastatic disease; or	• No companion diagnostic

Treatment	Indication	FDA-Approved Companion Diagnostic Tests
	o ceritinib as the first ALK inhibitor therapy for metastatic disease	
Nivolumab (Opdivo) in combination with Ipilimumab (Yervoy)	• 2020: o adult patients with metastatic NSCLC expressing PD-L1 (≥1%) as determined by an FDA-approved test, with no EGFR or ALK genomic tumor aberrations, as first-line treatment in combination with ipilimumab o adult patients with metastatic or recurrent NSCLC with no EGFR or ALK genomic tumor aberrations as first-line treatment, in combination with ipilimumab and 2 cycles of platinum-doublet chemotherapy o patients with metastatic NSCLC and progression on or after platinum-based chemotherapy. Patients with EGFR or ALK genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving OPDIVO.	• PD-L1 IHC 28-8 PharmDx
Osimertinib (Tagrisso)	• 2015: Second line for patients with metastatic NSCLC whose tumors have EGFR T790M variants as detected by an FDA-approved test, who have not responded to EGFR-blocking therapy • 2018: First line for patients with metastatic NSCLC whose tumors have EGFR exon 19 deletions or exon 21 L858R variants • 2019: EGFR exon 19 deletion and EGFR exon 21 L858R alterations • 2020: adjuvant therapy after tumor resection in adult patients with NSCLC whose tumors have EGFR exon 19 deletions or exon 21 L858R mutations, as detected by an FDA-approved test	• 2015: cobas® EGFR Mutation Test v2 (blood test) • 2017: FoundationOne CDx™ (Foundation Medicine) • 2020: Guardant360 CDx • 2020: FoundationOne® Liquid CDx
Pembrolizumab (Keytruda)	• 2018: Monotherapy for the treatment of patients with metastatic NSCLC whose tumors express PD-L1 (TPS ≥1%) as determined by an FDA-approved test, with disease progression on or after platinum-containing chemotherapy; patients with EGFR or ALK genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving KEYTRUDA • 2020: For the treatment of adult and pediatric patients with unresectable or metastatic tumor mutational burden-high (TMB-H) [≥10 mutations/megabase (mut/Mb)] solid tumors, as determined by an FDA-approved test, that have progressed following prior treatment and who have no satisfactory alternative treatment options	• 2018: PD-L1 IHC 22C3 pharmDx • 2020: FoundationOne CDx
Pralsetinib (Gavreto)	• Adult patients with metastatic RET fusion-positive NSCLC as detected by an FDA approved test	• 2020: Oncomine Dx Target Test

Treatment	Indication	FDA-Approved Companion Diagnostic Tests
Selpercatinib (Retevmo)	• Adult patients with metastatic RET fusion-positive NSCLC	• No companion diagnostic specified
Sotorasib (Lumakras)	• Adult patients with KRAS G12C-mutated locally advanced or metastatic NSCLC, as determined by an FDA-approved test, who have received at least 1 prior systemic therapy	• 2021: Therascreen KRAS RGQ PCR kit • 2021: Guardant360 CDx
Tepotinib (Tepmetko)	• Adult patients with metastatic NSCLC harboring MET exon 14 skipping alterations.	• No companion diagnostic

Sources: U.S. Food and Drug Administration (2020)[7]; U.S. Food and Drug Administration (n.d.)[8]
ALK: anaplastic lymphoma kinase; *EGFR*: epidermal growth factor receptor; FDA: U.S. Food and Drug Administration; FISH: fluorescence in situ hybridization; MET: mesenchymal-epithelial transition; NSCLC: non-small-cell lung cancer; NTRK neurotrophic receptor tyrosine kinase; PCR: polymerase chain reaction.

Rationale

Background

Non-Small-Cell Lung Cancer

Treatment options for non-small-cell lung cancer (NSCLC) depend on disease stage and include various combinations of surgery, radiotherapy, systemic therapy, and best supportive care. Unfortunately, in up to 85% of cases, cancer has spread locally beyond the lungs at diagnosis, precluding surgical eradication. Also, up to 40% of patients with NSCLC present with metastatic disease.[1] When treated with standard platinum-based chemotherapy, patients with advanced NSCLC have a median survival of 8 to 11 months and 1-year survival of 30% to 45%.[2,3] The identification of specific, targetable oncogenic "driver mutations" in a subset of NSCLCs has resulted in a reclassification of lung tumors to include molecular subtypes, which are predominantly of adenocarcinoma histology. Testing for epidermal growth factor receptor (*EGFR*) variants and anaplastic lymphoma kinase (*ALK*) rearrangements is routine in clinical decision making for the treatment of NSCLC. The use of testing for other variants to direct targeted therapy continues to evolve.

EGFR Gene

EGFR, a receptor tyrosine kinase (TK), is frequently overexpressed and activated in NSCLC. Drugs that inhibit EGFR signaling either prevent ligand binding to the extracellular domain (monoclonal antibodies) or inhibit intracellular TK activity (small-molecule tyrosine kinase inhibitors [TKIs]). These targeted therapies dampen signal transduction through pathways downstream to the EGFR, such as the RAS/RAF/MAPK cascade. RAS proteins are G proteins that cycle between active and inactive forms in response to stimulation from cell surface receptors, such as EGFR, acting as binary switches between cell surface EGFR and downstream signaling pathways. These pathways are important in cancer cell proliferation, invasion, metastasis, and stimulation of neovascularization.

Variants in 2 regions of the *EGFR* gene (exons 18-24)-small deletions in exon 19 and a point variant in exon 21 (L858R)-appear to predict tumor response to TKIs such as erlotinib. Likewise, tumors with an acquired exon 20 (T790M) substitution variant appear to respond to osimertinib following the failure of TKI therapy.

The prevalence of *EGFR* variants in NSCLC varies by population, with the highest prevalence in nonsmoking Asian women with adenocarcinoma, in whom *EGFR* variants have been reported to be up to 30% to 50%. The reported prevalence in the white population is approximately 10%.

ALK Gene

ALK is a TK that, in NSCLC, is aberrantly activated because of a chromosomal rearrangement that leads to a fusion gene and expression of a protein with constitutive TK activity that has been

demonstrated to play a role in controlling cell proliferation. The *EML4-ALK* fusion gene results from an inversion within the short arm of chromosome 2.

The *EML4-ALK* rearrangement ("*ALK*-positive") is detected in 3% to 6% of NSCLC patients, with the highest prevalence in never-smokers or light ex-smokers who have adenocarcinoma.

BRAF Gene

RAF proteins are serine/threonine kinases that are downstream of RAS in the RAS-RAF-ERK-MAPK pathway. In this pathway, the *BRAF* gene is the most frequently mutated in NSCLC, in 1% to 3% of adenocarcinomas. Unlike melanoma, about 50% of the variants in NSCLC are non-V600E variants.[4,] Most *BRAF* variants occur more frequently in smokers.

ROS1 Gene

ROS1 codes for a receptor TK of the insulin receptor family and chromosomal rearrangements result in fusion genes. The prevalence of *ROS1* fusions in NSCLC varies from 0.9% to 3.7%.[4,] Patients with *ROS1* fusions are typically never-smokers with adenocarcinoma.

KRAS Gene

The *KRAS* gene (which encodes RAS proteins) can harbor oncogenic variants that result in a constitutively activated protein, independent of signaling from the EGFR, possibly rendering a tumor resistant to therapies that target the EGFR. Variants in the *KRAS* gene, mainly codons 12 and 13, have been reported in 20% to 30% of NSCLC, and occur most often in adenocarcinomas in heavy smokers.

KRAS variants can be detected by direct sequencing, PCR technologies, or NGS.

EGFR, *ALK*, *ROS1*, and *KRAS* driver mutations are considered to be mutually exclusive.

HER2 Gene

Human epidermal growth factor receptor 2 (*HER2*) is a member of the HER (EGFR) family of TK receptors and has no specific ligand. When activated, it forms dimers with other EGFR family members. *HER2* is expressed in approximately 25% of NSCLC. *HER2* variants are detected mainly in exon 20 in 1% to 2% of NSCLC, predominantly in adenocarcinomas in nonsmoking women.[4,] There are currently no targeted therapies specifically approved for this indication.

RET Gene

RET (rearranged during transfection) is a proto-oncogene that encodes a receptor TK growth factor. Translocations that result in fusion genes with several partners have been reported.[4,] *RET* fusions occur in 0.6% to 2% of NSCLCs and 1.2% to 2% of adenocarcinomas.[4,]

MET Gene

MET alteration is one of the critical events for acquired resistance in *EGFR*-mutated adenocarcinomas refractory to EGFR TKIs.[4,]

NTRK Gene Fusions

NTRK gene fusions encode tropomyosin receptor kinase fusion proteins that act as oncogenic drivers for solid tumors including lung, salivary gland, thyroid, and sarcoma. It is estimated that NTRK gene fusions occur in 0.2% of patients with NSCLC and do not typically overlap with other oncogenic drivers.[5,]

PD-1/PD-L1

Programmed cell ligand-1 (PD-L1) is a transmembrane protein expressed on the surface of multiple tissue types, including many tumor cells. Blocking the PD-L1 protein may prevent cancer cells from inactivating T cells.

Tumor Mutational Burden

Tumor mutational burden, a measure of gene mutations within cancer cells, is an emerging biomarker of outcomes with immunotherapy in multiple tumor types, including lung cancer.[6,]

Targeted Treatment and Immunotherapy

Targeted treatments and immunotherapy for the variants described above are summarized in Table 1.

Table 1. Targeted Treatments and Immunotherapy for Non-Small-Cell Lung Cancer

Target	FDA-Approved Therapies
EGFR	• Gefitinib (Iressa), • Erlotinib (Tarceva), • Afatinib (Gilotrif) • Osimertinib (Tagrisso) • Dacomitinib (Vizimpro) • Amivantamab-vmjw (Rybrenant)
ALK	• Crizotinib (Xalkori) • Ceritinib (Zykadia) • Alectinib (Alecensa) • Brigatinib (Alunbrig) • Lorlatinib (Lorbrena)
BRAF	• Dabrafenib and trametinib combination
ROS1	• Crizotinib (Xalkori) • Ceritinib (Zykadia) • Lorlatinib (Lorbrena) • Entrectinib (Rozlytrek)
KRAS	• Sotorasib (Lumakras)
HER2	• No FDA-approved targeted treatements
RET	• Selpercatinib (Retevmo) • Pralsetinib (Gavreto)
MET	• Capmatinib (Tabrecta) • Tepotinib (Tepmetko
NTRK	• Larotrectinib (Vitrakvi) • Entrectinib (Rozlytrek)
PD-L1	• Pembrolizumab (Keytruda) • Nivolumab (Opdivo) in combination with ipilimumab (Yervoy) • Atezolizumab (Tecentriq)

Literature Review

Evidence reviews assess the clinical evidence to determine whether the use of technology improves the net health outcome. Broadly defined, health outcomes are the length of life, quality of life (QOL), and ability to function-including benefits and harms. Every clinical condition has specific outcomes that are important to patients and managing the course of that condition. Validated outcome measures are necessary to ascertain whether a condition improves or worsens; and whether the magnitude of that change is clinically significant. The net health outcome is a balance of benefits and harms.

To assess whether the evidence is sufficient to draw conclusions about the net health outcome of technology, 2 domains are examined: the relevance, and quality and credibility. To be relevant, studies must represent 1 or more intended clinical use of the technology in the intended population and compare an effective and appropriate alternative at a comparable intensity. For some conditions, the alternative will be supportive care or surveillance. The quality and credibility of the

evidence depend on study design and conduct, minimizing bias and confounding that can generate incorrect findings. The randomized controlled trial (RCT) is preferred to assess efficacy; however, in some circumstances, nonrandomized studies may be adequate. Randomized controlled trials are rarely large enough or long enough to capture less common adverse events and long-term effects. Other types of studies can be used for these purposes and to assess generalizability to broader clinical populations and settings of clinical practice.

Biomarker Testing Using Tissue Biopsy to Select Targeted Therapy for Advanced-Stage NSCLC
Clinical Context and Test Purpose
The purpose of identifying targetable oncogenic "driver mutations" in patients who have non-small-cell lung cancer (NSCLC) is to inform a decision whether patients should receive a targeted therapy versus another systemic therapy. Patients who present with advanced disease or recurrence following initial definitive treatment typically receive systemic therapy. Traditionally, systemic therapy was cytotoxic chemotherapy. However, certain patients may be good candidates for treatment with targeted therapies or immunotherapy. The goal of targeted therapies is to preferentially kill malignant cells without significant damage to normal cells so that there is improved therapeutic efficacy along with decreased toxicity.

The question addressed in this evidence review is this: Does testing for epidermal growth factor receptor (*EGFR*), *BRAF*, *KRAS*, or *HER2* variants; *ALK*, *ROS*, or *RET* rearrangements; *MET* alterations, or NTRK gene fusions improve outcomes in individuals with advanced-stage NSCLC who are being considered for targeted therapy?

The following PICO was used to select literature to inform this review.

Populations
The relevant population of interest are individuals with advanced NSCLC who are being considered for targeted therapy.

Interventions
The intervention of interest is testing for somatic genome alterations known as "driver mutations," specifically *EGFR*, *BRAF*, *KRAS*, *HER2* variants; *ALK*, *ROS*, or *RET* rearrangements; *MET alterations*, or NTRK gene fusions.

Comparators
The following practice is currently being used to target therapy for advanced-stage NSCLC: standard management without testing for driver mutations. Standard management consists primarily of chemotherapy, although some patients are candidates for immunotherapy.

Outcomes
Beneficial outcomes resulting from a true-positive test result are prolonged survival, reduced toxicity, and improved QOL associated with receiving a more effective and less cytotoxic targeted therapy than chemotherapy in those with driver mutations. Beneficial outcomes from a true negative result are prolonged survival associated with receiving chemotherapy in those without driver mutations.

Harmful outcomes resulting from a false-negative test result include shorter survival from receiving less effective and more cytotoxic chemotherapy in those with driver mutations; possible harmful outcomes resulting from a false-positive test result are a shorter survival from receiving potentially ineffective targeted treatment and delay in initiation of chemotherapy in those without driver mutations.

Due to the poor prognosis of advanced NSCLC, the duration of follow-up for the outcomes of interest is 6 months and 1 year.

Study Selection Criteria

Methodologically credible studies were selected using the following principles:

- To assess efficacy outcomes, comparative controlled prospective trials were sought, with a preference for RCTs;
- In the absence of such trials, comparative observational studies were sought, with a preference for prospective studies.
- To assess long-term outcomes and adverse events, single-arm studies that capture longer periods of follow-up and/or larger populations were sought.
- Studies with duplicative or overlapping populations were excluded.

Review of Evidence

The evidence is presented below, by variant (*EGFR*, *ALK*, *BRAF*, *ROS1*, *KRAS*, *HER2*, *RET*, *MET, NTRK)* and by recommended therapy.

EGFR Gene Variants

Somatic variants in the tyrosine kinase domain of the *EGFR* gene, notably small deletions in exon 19 and a point mutation in exon 21 (L858R, indicating substitution of leucine by arginine at codon position 858) are the most commonly found *EGFR* variants associated with sensitivity to EGFR tyrosine kinase inhibitors (TKIs; afatinib, erlotinib, gefitinib). These variants are referred to as sensitizing variants. Almost all patients who initially respond to an EGFR TKI experience disease progression. The most common of these secondary variants, called resistance variants, involves the substitution of methionine for threonine at position 790 (T790M) on exon 20.

EGFR Variant Frequency

Fang et al (2013) reported *EGFR* variants (all L858R) in 3 (2%) of 146 consecutively treated Chinese patients with early-stage squamous cell carcinoma (SCC).[9,] In a separate cohort of 63 Chinese patients with SCC who received erlotinib or gefitinib as second- or third-line treatment (63% never-smokers, 21% women), *EGFR* variant prevalence (all exon 19 deletion or L858R) was 23.8%.

In a comprehensive analysis of 14 studies involving 2880 patients, Mitsudomi et al (2006) reported *EGFR* variants in 10% of men, 7% of non-Asian patients, 7% of current or former smokers, and 2% of patients with nonadenocarcinoma histologies.[10,] Eberhard et al (2005)[11,] observed EGFR variants in 6.4% of patients with SCC and Rosell et al (2009)[12,] observed *EGFR* variants in 11.5% of patients with large cell carcinomas. Both studies had small sample sizes.

In 2 other studies, the acquired *EGFR* T790M variant has been estimated to be present in 50% to 60% of TKI-resistant cases in approximately 200 patients.[13,14,]

U.S. Food and Drug Administration Approved Companion Diagnostic Tests for *EGFR* Variants

EGFR-sensitizing and -resistance variants can be detected by direct sequencing, polymerase chain reaction (PCR) technologies, or next-generation sequencing (NGS). Gene sequencing is considered an analytic criterion standard. A report by the Canadian Agency for Drugs and Technologies in Health, conducted by Mujoomdar et al (2010) analyzed *EGFR* variants.[15,] Based on 11 observational studies, the report authors concluded that PCR-based approaches identify EGFR variants with a sensitivity equivalent to that of direct sequencing.

Several tests have been approved as companion diagnostics to detect *EGFR*-resistance variants (exon 19 deletions or exon 21 L858R substitutions) for at least 1 of the EGFR TKIs (afatinib, erlotinib, gefitinib, or osimertinib): the therascreen EGFR Rotor-Gene Q polymerase chain reaction (RGQ PCR) kit, cobas EGFR Mutation Test v1 and v2, Oncomine Dx Target Test, and FoundationOne CDx (Table 1). The cobas v2 test also is approved as a companion diagnostic to detect the T790M resistance variant to select patients for treatment with osimertinib.

The clinical validity of the therascreen RGQ PCR kit was demonstrated in a retrospective analysis of patients screened for a phase 3, open-label RCT comparing afatinib with chemotherapy in treatment-naive patients with stage IIIB or IV NSCLC, in which the *EGFR* variants for enrollment were determined using a clinical trial assay (CTA) conducted at central laboratories.[16,] The positive percent agreement (PPA) of therascreen versus CTA for detection of *EGFR*-sensitizing variants was 98% (95% confidence interval [CI], 95% to 99%) and negative percent agreement (NPA) was 97% (95% CI, 94% to 99%). Overall, a statistically significant efficacy benefit for afatinib versus chemotherapy was reported in the *EGFR*-positive patients as measured by the therascreen EGFR RGQ PCR Kit (hazard ratio [HR], 0.49; 95% CI, 0.35 to 0.69) that was similar to the efficacy in the overall population, which was *EGFR*-positive by the CTA (HR, 0.58; 95% CI, 0.43 to 0.78).

The clinical validity of the cobas EGFR Mutation Test v1 was demonstrated in a retrospective analysis of patients screened for a phase 3, open-label RCT comparing erlotinib with chemotherapy in treatment-naive patients with advanced NSCLC. In this RCT, the *EGFR* variants for enrollment were determined with a CTA at a central laboratory using Sanger sequencing first for determination of *EGFR* variants status, followed by confirmatory testing for exon 19 deletions and exon 21 L858R variants=.[17,]The PPA of cobas versus CTA for detection of *EGFR*-sensitizing variants was 94% (95% CI, 89% to 97%) and NPA was 98% (95% CI, 95% to 99%). Overall, a statistically significant efficacy benefit for erlotinib versus chemotherapy was reported in the *EGFR*-positive patients as measured by the cobas EGFR Mutation Test v1 (HR, 0.34; 95% CI, 0.21 to 0.54) that was similar to the efficacy in the overall population, which was *EGFR*-positive by the CTA (HR, 0.34; 95% CI, 0.23 to 0.49). The cobas EGFR Mutation Test v2 expanded the indication for the use of the cobas EGFR Mutation Test to include the detection of the exon 20 (T790M) substitution variant in NSCLC patients for whom osimertinib (Tagrisso) treatment is indicated.[18,] The clinical validity of the cobas EGFR Mutation Test v2 was demonstrated in retrospective analyses of patients enrolled in a phase 2, single-arm study of osimertinib for *EGFR*-sensitizing variant-positive metastatic NSCLC who had progressed following prior therapy with an approved EGFR TKI. The osimertinib response rate in the patients identified as *EGFR* T790M-positive by the cobas v2 test was 62% (95% CI, 55% to 69%).

The clinical validity of the Oncomine Dx Target Test was demonstrated in a retrospective analysis of patients screened for a phase 3, open-label RCT, which included newly diagnosed patients with stage IIIB or IV or recurrent NSCLC, in which the *EGFR* variant for enrollment was determined using therascreen.[19,] The PPA of Oncomine versus therascreen for detection of *EGFR*-sensitizing variants was 99% (95% CI, 93% to 100%) and NPA was 99% (95% CI, 96% to 100%). No data on the effectiveness of gefitinib in patients identified as *EGFR*-positive by Oncomine were reported.

The clinical validity of FoundationOne CDx was demonstrated by assessing the concordance of the test with results from mass spectrometry, gel sizing, fluorescence in situ hybridization (FISH), and immunohistochemistry of clinical tumor tissue specimens.[20,] Test sensitivity ranged from 95% to 99% across alteration types, with a positive predictive value exceeding 99%. No data on the effectiveness of targeted therapy in patients identified as *EGFR*-positive by FoundationOne CDx were reported.

Tyrosine Kinase Inhibitors

Combined Analyses

A meta-analysis by Lee et al (2013), which evaluated 23 trials of erlotinib, gefitinib, and afatinib in patients with advanced NSCLC, reported improved progression-free survival (PFS) in *EGFR* variant-positive patients treated with EGFR TKIs in the first- and second-line settings and for maintenance therapy.[21,] Comparators were with chemotherapy, chemotherapy and placebo, and placebo in the first-line, second-line, and maintenance therapy settings, respectively. Among *EGFR* variant-negative patients, PFS was improved using EGFR TKIs compared with placebo maintenance but not in the first- and second-line settings. Overall survival (OS) did not differ between treatment groups in either variant-positive or variant-negative patients. Statistical heterogeneity was not reported for any outcome.

A Blue Cross Blue Shield Association Technology Evaluation Center (TEC) Assessment (2007) evaluated *EGFR* variants and TKI therapy in advanced NSCLC.[22,] It concluded that there was insufficient evidence to permit conclusions about the clinical validity or utility of *EGFR* variant testing to predict erlotinib sensitivity or to guide treatment in patients with NSCLC. An updated Assessment (2010),with revised conclusions, indicated that *EGFR* variant testing has clinical utility in selecting or deselecting patients for treatment with erlotinib.[22,]

Other meta-analyses have confirmed the PFS and OS results and conclusions for *EGFR*-positive patients have been published.[23,22,24,25,26,]

Erlotinib

Systematic Reviews

Petrelli et al (2012) reported a meta-analysis (13 randomized trials) of 1260 patients with *EGFR*-mutated NSCLC who received TKIs for first-line, second-line, or maintenance therapy.[27,] The comparator was standard therapy. Overall, reviewers noted that the use of EGFR TKIs increased the chance of obtaining an objective response almost 2 fold compared with chemotherapy. Response rates were 70% versus 33% in first-line trials and 47% versus 28.5% in second-line trials. Tyrosine kinase inhibitors reduced the hazard of progression by 70% in all trials and by 65% in first-line trials; however, they did not improve OS.

Randomized Controlled Trials

A summary of the characteristics and results of 3 key RCTs establishing the superiority of erlotinib over chemotherapy in the first-line setting is given in Tables 3 and 4. The 3 RCTs included 555 patients with stage IIIB or IV NSCLC. All reported clinically and statistically significant improvements in PFS (HR range, 0.16 to 0.37) but no improvements in OS with erlotinib versus chemotherapy. Grade 3 or greater adverse events and serious adverse events occurred in fewer patients in the erlotinib groups.

Table 3. Characteristics of RCTs of First-Line Erlotinib versus Chemotherapy in *EGFR*-Variant NSCLC

Study; Trial	Countries	Sites	Dates	Participants	Interventions	
					Erlotinib	Chemotherapy
Wu et al (2015)[28,]; ENSURE (NCT01342965)	China, Malaysia, Philippines	30	2011-2012	217 patients with stage IIIB/IV NSCLC	110 assigned to erlotinib (150 mg qd)	117 assigned to gemcitabine (1250 mg/m^2) and cisplatin (75 mg/m^2)
Rosell et a (2012)[29,]; EURTAC (NCT00446225)	France, Italy, Spain	42	2007-2011	173 patients with stage IIIB/IV NSCLC	86 assigned to erlotinib (150 mg qd)	87 assigned to cisplatin (75mg/m^2), docetaxel (75 mg/m^2), or gemcitabine (1250 mg/m^2)
Zhou et a (2011, 2015)[28,29,]; OPTIMAL (NCT00874419)	China	22	NR	165 patients with stage IIIB/IV NSCLC	83 assigned to erlotinib (150 mg qd)	82 assigned to carboplatin (AUC5) and gemcitabine (1000 mg/m^2)

AUC5: area under the concentration-time curve of 5.0 mg/mL/min; *EGFR*: epidermal growth factor receptor; NR: not reported; NSCLC: non-small-cell lung cancer; qd: every day; RCT: randomized controlled trial.

Table 4. Results of RCTs of First-Line Erlotinib versus Chemotherapy in *EGFR*-Variant NSCLC

Trial	Median PFS, mo	Median OS, mo	Adverse Events, %		
			Serious	*Grade 3 or 4*	*%*
ENSURE (2015)[28,]					
N	217	217	214	214	
Erlotinib	11.0	26.3	2.7	· Overall	
				· Neutropenia	· 35.5
				· Leukopenia	· 0.9
				· Anemia	· 0.9
				· Rash	· 0.9
					· 6.4
Chemotherapy	5.5	25.5	10.6	· Overall	· 57.7
				· Neutropenia	· 25.0

Trial	Median PFS, mo	Median OS, mo		Adverse Events, %	
				· Leukopenia	· 14.4
				· Anemia	· 12.5
				· Rash	· 1
HR (95% CI)	0.34 (0.22 to 0.51)	0.91 (0.63 to 1.31)			
EURTAC (2012)[29,]					
Erlotinib	9.7 (8.4 to 12.3)	19.3	6	· Rash	· 13
				· Neutropenia	· 0
				· Increased AT concentrations	· 2
Chemotherapy	5.2 (4.4 to 5.8)	19.5	20	· Rash	· 0
				· Neutropenia	· 22
				· Increased AT concentrations	· 0
HR (95% CI)	0.37 (0.25 to 0.54)	1.04 (0.65 to 1.68)			
OPTIMAL (2011, 2015)[28,29,]					
N	154	154	155	155	
Erlotinib	13.1 (10.6 to 16.5)	22.8	2	· Neutropenia	· 0
				· Thrombocytopenia	· 0
Chemotherapy	4.6 (4.2 to 5.4)	27.2	14	· Neutropenia	· 42
				· Thrombocytopenia	· 40
HR (95% CI)	0.16 (0.10 to 0.26)	1.19 (0.83 to 1.71)			

AT: aminotransferase; CI: confidence interval; *EGFR*: epidermal growth factor receptor; HR: hazard ratio; NSCLC: non-small-cell lung cancer; OS: overall survival; PFS: progression-free survival; RCT: randomized controlled trial.

Many additional publications have provided data on *EGFR* variants in tumor samples obtained from NSCLC patients treated with erlotinib. Nine of these [9,30,31,32,33,34,35,36,37,] were nonconcurrent prospective studies of treatment-naive and previously treated patients who received erlotinib and were then tested for the presence or absence of variants. Four others were prospective, single-arm enrichment studies of variant-positive or wild-type patients treated with erlotinib. In 3 studies of *EGFR* variant-positive patients, the objective radiologic response was 40% to 70%, the median PFS was 8 to 14 months, and the median OS was 16 to 29 months.[10,38,39,] In patients with wild-type tumors, the objective radiologic response was 3.3%, PFS was 2.1 months, and OS was 9.2 months.[40,]

Gefitinib

Systematic Reviews

A Cochrane review by Sim et al (2018) compared the use of gefitinib with no therapy or chemotherapy as first-line, second-line, or maintenance therapy for NSCLC.[41,] The literature search was conducted in February 2017 and identified 35 RCTs (N=12,089 patients) for inclusion. For the general population of patients with NSCLC, gefitinib did not improve OS when given as first- or second-line therapy but did improve PFS when administered as maintenance therapy. In the subset of patients with *EGFR* variants, gefitinib improved PFS compared with first- and second-line chemotherapy and improved both OS and PFS when administered as maintenance therapy.

Randomized Controlled Trials

Three RCTs described in Tables 5 and 6 have compared gefitinib with chemotherapy in the first-line setting.[42,43,44,] The RCTs included 668 patients with stage IIIB or IV NSCLC and *EGFR*-sensitizing variants. All reported clinically and statistically significant improvement in PFS (HR range, 0.30 to 0.49) but no improvement in OS with gefitinib compared with chemotherapy. Grade 3 or greater adverse events occurred in fewer patients in the gefitinib groups. The Iressa Pan-Asia Study (IPASS) trial enrolled patients with and without *EGFR*-sensitizing variants. The investigators reported a significant interaction between treatment and *EGFR* variant status for PFS (interaction p<.001); PFS was longer for gefitinib in patients with *EGFR*-sensitizing variants and shorter for gefitinib in patients without *EGFR*-sensitizing variants. Another 3-arm RCT in Tables 4 and 5 compared a combination of chemotherapy plus gefitinib with chemotherapy alone and gefitinib alone.[43,] Patients in the combined treatment arm experienced longer OS compared with chemotherapy and gefitinib alone.

Wu et al (2017) conducted a post hoc subgroup analysis focusing on Asian patients in the IPASS trial who were randomized to gefitinib (n=88) or carboplatin/paclitaxel (n=98).[45,] The analysis found that patients with the *EGFR* variant who received gefitinib experienced longer PFS than patients receiving chemotherapy (HR, 0.5; 95% CI, 0.4 to 0.8).

Table 5. Characteristics of RCTs of First-Line Gefitinib versus Chemotherapy in *EGFR*-Variant NSCLC

Study; Trial	Countries	Sites	Dates	Participants	Description of Interventions		
					Gefitinib Alone	*Chemo Alone*	*Gefitinib Plus Chemo*
Han et al (2017)[43,]	China	1	2011-2015	121 patients with advanced lung adenocarcinoma	41 assigned to gefitinib (250 mg/d)	40 assigned to pemetrexed (500 mg/m^2) and carboplatin (AUC5)	40 assigned to pemetrexed (500 mg/m^2) and carboplatin (AUC5) and gefitinib (250 mg/d)
					Gefitinib		*Chemo*
Mok (2009)[42,]; IPASS (NCT00322452)	9 East Asian countries	87	2006-2007	1217 patients with stage IIIB/IV NSCLC (261 *EGFR*-positive)	609 assigned to gefitinib (250 mg/d)		608 assigned to paclitaxel (200 mg/m^2) and carboplatin (AUC5 or AUC6)
Mitsudomi (2010)[44,]; WJTOG3405[a]	Japan	36	2006-2009	177 patients with stage IIIB/IV or recurrent NSCLC	88 assigned to gefitinib (250 mg/d)		89 assigned to cisplatin (80 mg/m^2) and docetaxel (60 mg/m^2)
Maemondo (2010), [46,] Inoue (2013)[47,]; NEJ002	Japan	43	2006-2009	230 patients with stage IIIB/IV NSCLC or postoperative relapse	115 assigned to gefitinib (250 mg/d)		115 assigned to paclitaxel (200 mg/m^2) and carboplatin (AUC6)

AUC5: area under the concentration-time curve of 5.0 mg/mL/min; AUC6: area under the concentration time curve of 6.0 mg/mL/min; chemo: chemotherapy; *EGFR*: epidermal growth factor receptor; NSCLC: non-small-cell lung cancer; RCT: randomized controlled trial.
[a] West Japan Oncology Group 172 trial.

Table 6. Results of RCTs of First-Line Gefitinib versus Chemotherapy in *EGFR*-Variant NSCLC

Study	Median PFS, mo	Median OS, mo	Adverse Events, %	
			Serious	*Grade 3 or 4* *%*
Han et al (2017)[43,]			NR	
Gefitinib	5.7 (5.2 to 6.3)	25.8 (21.3 to 30.2)	· Liver dysfunction · Skin rash	· 2.4 · 9.8
Chemotherapy	11.9 (9.1 to 14.6)	24.3 (17.7 to 30.1)	· Neutropenia · Fatigue · Skin rash	· 12.5 · 5.0 · 9.8
Gefitinib plus chemotherapy	17.5 (15.3 to 19.7)	32.6 (25.5 to 39.8)	· Liver dysfunction · Neutropenia · Fatigue · Skin rash	· 10.0 · 10.0 · 7.5 · 10.0
TE (95% CI)	Combination vs chemotherapy: · 0.2 (0.1 to 0.3)	Combination vs chemotherapy: · 0.5 (0.2 to 0.9)		

Study	Median PFS, mo	Median OS, mo		Adverse Events, %	
	Combination vs gefitinib: · 0.5 (0.3 to 0.8) Gefitinib vs chemotherapy: · 0.3 (0.2 to 0.6)	Combination vs gefitinib: · 0.4 (0.2 to 0.7) Gefitinib vs chemotherapy: · 1.0 (0.6 to 1.8)			
WJTOG3405 (2010)[44,]					
N	172	172	NR	172	
Gefitinib	9.2 (8.0 to 13.9)	34.8 (26.0 to 39.5)		· ALT/AST elevation · Rash · Fatigue	· 27.5 · 2.3 · 2.3
Chemotherapy	6.3 (5.8 to 7.8)	37.3 (31.2 to 45.5)		· ALT/AST elevation · Fatigue · Neutropenia · Leukocytopenia · Anemia	· 2.3 · 2.3 · 84 · 50 · 17
TE (95% CI)	HR, 0.49 (0.34 to 0.71)	HR, 1.25 (0.88 to 1.78)			
NEJ002 (2010, 2013)[42,]					
N	224		NR	227	
Gefitinib	10.8	27.7		· Rash · Arthralgia · Pneumonitis · Aminotransferase elevation · Neutropenia	· 5.3 · 0.9 · 2.6 · 26.3 · 0.9
Chemotherapy	5.4	26.6		· Rash · Neuropathy · Arthralgia · Aminotransferase elevation · Neutropenia · Anemia · Thrombocytopenia	· 2.7 · 6.2 · 7.1 · 0.9 · 65.5 · 5.3 · 3.5
HR (95% CI)	0.30 (0.22 to 0.41)	0.89 (0.63 to 1.24)			
IPASS (2009)[42,]					
N	259[a]	259[a]	1196[b]		
Gefitinib	»9.6[c]	NR	16.3%	· Rash · Diarrhea · Neurotoxic effects · Neutropenia · Anemia · Leukopenia	· 3.1 · 3.8 · 0.3 · 3.7 · 2.2 · 1.5
Chemotherapy	»5.8[c]	NR	15.6%	· Rash · Diarrhea · Neurotoxic effects · Neutropenia · Anemia · Leukopenia	· 0.8 · 1.4 · 4.9 · 67.1 · 10.6 · 35.0
HR (95% CI)	0.48 (0.36 to 0.64)	0.78 (0.50 to 1.20)			

ALT: alanine aminotransferase; AST: aspartate aminotransferase; CI: confidence interval; *EGFR*: epidermal growth factor receptor; HR: hazard ratio; NR: not reported; NSCLC: non-small-cell lung cancer; OS: overall survival; PFS: progression-free survival; RCT: randomized controlled trial; TE: treatment effect.

[a] Analysis includes *EGFR*-positive only.

[b] Analysis includes all patients with safety data.

[c] Estimated from the figure.

Afatinib

Unlike erlotinib and gefitinib, which selectively inhibit EGFR, afatinib inhibits not only EGFR but also human epidermal growth factor receptor 2 (HER2) and HER4, and may have activity in patients with acquired resistance to TKIs. Such patients often harbor a T790M variant (substitution of threonine by methionine at codon 790) in *EGFR* exon 20. The efficacy and safety of afatinib were evaluated in the LUX-Lung series of studies.

LUX-Lung 3 was an RCT including 345 patients with stage IIIB or IV, *EGFR* variant-positive, lung adenocarcinoma who were previously untreated for advanced disease.[48,] Seventy-two percent of patients were Asian, 26% were white, and 90% (308 patients) had common *EGFR* variants (exon 19 deletion or L858R substitution variant in exon 21). Patients received afatinib or chemotherapy (cisplatin plus pemetrexed). In a stratified analysis of patients with common *EGFR* variants, the median PFS was 13.6 months for the afatinib group and 6.9 months for the chemotherapy group (HR, 0.47; 95% CI, 0.34 to 0.65; p=.001). The median PFS for the 10% of patients who had other *EGFR* variants was not reported, but the median PFS for the entire patient sample was 11.1 months in the afatinib group and 6.9 months in the chemotherapy group (HR, 0.58; 95% CI, 0.43 to 0.78; p=.001). The incidence of objective response in the entire patient sample was 56% in the afatinib group and 23% in the chemotherapy group (p=.001). With a median follow-up of 16.4 months, the median OS was not reached in any group; preliminary analysis indicated no difference in OS between the 2 treatment groups in the entire patient sample (HR, 1.12; 95% CI, 0.73 to 1.73; p=.60). Patients in the afatinib group reported greater improvements in dyspnea, cough, and global health status/QOL than those in the chemotherapy group.[49,] Grade 3 or higher diarrhea, rash, and paronychia (nail infection) occurred in 14%, 16%, and 11% of afatinib-treated patients, respectively, and in no patients in the chemotherapy group. Grade 3 or higher mucositis (primarily stomatitis) occurred in 9% of the afatinib group and 1% of the chemotherapy group.[48,] Similar results were reported by Wu et al (2014) in a phase 3 trial conducted in 364 Asian patients (Lux-Lung 6), which compared afatinib with gemcitabine plus cisplatin.[50,] Progression-free survival was 11.0 in the afatinib group and 5.6 months in the chemotherapy group (HR, 0.28; 95% CI, 0.20 to 0.39) and the response rates were 67% and 23%, respectively.

Three other published LUX-Lung studies evaluated patients with stage IIIB or IV lung adenocarcinoma who were previously treated for advanced disease, but design features limit interpretation of results.

- LUX-Lung 2 was a single-arm study (2012) of afatinib in 129 patients (87% Asian, 12% white) with *EGFR* variant-positive disease.[51,] Patients had been treated with chemotherapy but not with *EGFR*-targeted therapy; approximately half of the patients (enrolled after a protocol amendment) were chemotherapy-naive. Objective responses (primarily partial responses) were observed in 66% of 106 patients with common *EGFR* variants (exon 19 deletion or L858R) and in 39% of 23 patients with other *EGFR* variants. The median PFS was 13.7 months in patients with common *EGFR* variants and 3.7 months in patients with other *EGFR* variants (p not reported). Results for variant-negative patients were not reported.
- LUX-Lung 1 and LUX-Lung 4 enrolled patients who had progressed on previous treatment with erlotinib, gefitinib, or both for advanced disease. Neither study prospectively genotyped patients. In the LUX-Lung 1 double-blind RCT, 96 (66% Asian, 33% white) of 585 enrolled patients were *EGFR* variant-positive (76 common *EGFR* variant-positive).[52,] In this group, the median PFS was 3.3 months in the afatinib group and 1.0 month in the placebo group (HR, 0.51; 95% CI, 0.31 to 0.85; p=.009). In 45 variant-negative patients, the median PFS was 2.8 months in the afatinib group and 1.8 months in the placebo group, a statistically nonsignificant difference (p=.22), possibly due to small group sizes. LUX-Lung 4 was a single-arm study (2013) of afatinib in 62 Japanese patients.[53,] Objective responses occurred in 2 (5%) of 36 patients with common *EGFR* variants and in none of 8 patients with other *EGFR* variants (p>.05).

Osimertinib

In 2015, the U.S. Food and Drug Administration (FDA) granted accelerated approval to osimertinib for treatment of metastatic *EGFR* T790M variant-positive NSCLC patients who have progressed on or after EGFR TKI therapy.[54,] The therapy was approved with an FDA-approved companion test, the cobas EGFR Mutation Test v2, which is a blood-based genetic test to detect *EGFR* variants including the T790M variant. Approval was based on 2 multicenter, single-arm studies.[55,]

The osimertinib label describes the 2 studies.[54,] Eligible patients had metastatic *EGFR* T790M variant-positive NSCLC and had progressed on prior systemic therapy, including an EGFR TKI. Patients received osimertinib 80 mg once daily. The first study enrolled 201 patients; the second enrolled 210 patients. The major efficacy outcome measure of both trials was the objective response rate (ORR) assessed by a blinded, independent review committee. The median duration of follow-up was 4.2 months in the first study and 4.0 months in the second. The ORR was similar in the 2 studies. The pooled ORR was 59% (95% CI, 54% to 64%); 0.5% achieved a complete response and 59% achieved a partial response. The most common adverse reactions were diarrhea (42%), rash (41%), dry skin (31%), and nail toxicity (25%). Serious adverse reactions reported in 2% or more patients were pneumonia and pulmonary embolus. Fatal adverse reactions included the following: 4 patients with interstitial lung disease/pneumonitis; 4 patients with pneumonia, and 2 patients with cerebral vascular accident/cerebral hemorrhage.

One RCT has compared osimertinib with chemotherapy and is described in Tables 7 and 8. Osimertinib was associated with clinically and statistically significantly prolonged PFS and higher response rates than chemotherapy and had lower rates of grade 3 and 4 adverse events. However, interstitial lung disease-like adverse events and QT prolongation were more common with osimertinib. Another RCT described in Tables 6 and 7 compared osimertinib with other EGFR TKIs (gefitinib or erlotinib) as first-line therapy.[56,] The results suggested a reduced risk for central nervous system progression with osimertinib compared with other TKIs.

Table 7. Osimertinib Randomized Controlled Trial Characteristics in *EGFR*-Variant NSCLC

Study; Trial	Countries	Sites	Dates	Participants	Interventions	
					Osimertinib	Standard TKI
Reungwetwattana et al (2018)[56,];FLAURA (NCT02296125)	31 countries in North America, Europe, Australia, Asia	168	2014-2017	128 (of 556) patients with untreated advanced *EGFR*-positive NSCLC with available brain scans at baseline	61 assigned to osimertinib (80 mg/d)	67 assigned to gefitinib (250 mg/d) or erlotinib (150 mg/d)
					Osimertinib	Chemotherapy
Mok et al (2017)[57,]; AURA3 (NCT02151981)	18 countries in North America, Europe, Australia, Asia	126	2014-2015	419 patients with T790M-positive advanced NSCLC who had disease progression after first-line EGFR-TKI therapy	279 assigned to osimertinib (80 mg/d)	140 assigned to platinum pemetrexed (500 mg/m^2 of BSA) plus carboplatin (target AUC5 or cisplatin [75 mg/m^2])

AUC5: area under the concentration-time curve of 5.0 mg/mL/min; BSA: body surface area; *EGFR*: epidermal growth factor receptor; NSCLC: non-small-cell lung cancer; TKI: tyrosine kinase inhibitor.

Table 8. Osimertinib Randomized Controlled Trial Results in *EGFR*-Variant NSCLC

Study	PFS, mo	OS, mo	ORR (95% CI)	Adverse Events, %		Prolongation of QT Interval, %
				Grade ≥3	ILD-Like	
AURA3(2017)[57,]						
N	419		419	415	415	415
Osimertinib	10.1	NR	71% (65 to 76)	23	4	4

Study	PFS, mo	OS, mo	ORR (95% CI)	Adverse Events, %		Prolongation of QT Interval, %
Platinum pemetrexed	4.4	NR	31% (24 to 40)	47	1	1
TE (95% CI)	HR, 0.30 (0.23 to 0.41)		OR, 5.4 (3.5 to 8.5)			
	PFS (N=128)					
	Median, mo	6-Mo (95% CI), %	12-Mo (95% CI), %	18-Mo (95% CI), %		ORR (95% CI), %
FLAURA (2018)[56,]						
Osimertinib	(16.5 to NC)	87 (74 to 94)	77 (62 to 86)	58 (40 to 72)		66 (52 to 77)
Other TKIs[a]	13.9 (8.3 to NC)	71 (57 to 81)	56 (42 to 68)	40 (25 to 55)		43 (31 to 56)
TE (95% CI)						2.5 (1.2 to 5.2)

CI: confidence interval; *EGFR*: epidermal growth factor receptor; HR: hazard ratio; ILD: interstitial lung disease; NC: not calculable; NR: not reported; NSCLC: non-small-cell lung cancer; OR: odds ratio; ORR: objective response rate; OS: overall survival; PFS: progression-free survival; TE: treatment effect.
[a] Erlotinib or gefitinib.

Comparative Effectiveness of *EGFR* TKIs

As the previous sections have shown, erlotinib, gefitinib, afatinib, and osimertinib all have improved efficacy compared with chemotherapy in patients who have NSCLC and *EGFR*-sensitizing variants and are well tolerated. Randomized controlled trials, as well as systematic reviews and meta-analyses of the RCTs, directly comparing the EGFR TKIs with each other and with chemotherapy, have been conducted. Several systematic reviews are summarized in Table 9.

Systematic Reviews

The systematic reviews and meta-analyses included overlapping trials. Randomized controlled trials included in the reviews and analyses differed in study design, treatments compared, and line of treatment (first-, second-, or third-line). In general, patients who are EGFR-positive and treated with TKIs experienced longer PFS than patients treated with chemotherapy. Meta-analyses comparing different TKIs reported inconsistent results, with some analyses finding various TKIs comparable and other analyses finding some TKIs more effective than others. Safety data were not consistently available among the RCTs, limiting adverse event comparisons among treatments.

Table 9. Summary of Systematic Reviews Comparing EGFR TKIs for the Treatment of NSCLC

Study	Study Dates	Design (No. of Studies)	No. of Patients	Line of Treatment	Treatments Compared	Conclusions
Lin et al (2018)[58,]	Nov 2017	RCT (11)	3145	First-line	Chemotherapy, afatinib, dacomitinib, erlotinib, gefitinib, osimertinib	· PFS: TKIs more effective than chemotherapy · Osimertinib, dacomitinib, and afatinib ranked highest probability of benefit among TKIs · Subgroup analyses comparing osimertinib with standard of care showed improvements in men, non-Asians, smokers, and those with del19 variants · Toxicity profiles similar for TKIs
Zhang et al (2018)[59,]	Oct 2017	RCT (40)	9376	First-, second-, and third-line	Erlotinib, gefitinib	· PFS: erlotinib and gefitinib comparable · Grade 3-5 adverse events more frequent with erlotinib
De Mello et al (2018)[60,]	Aug 2016	RCT (9)	3179	First-line	Chemotherapy, afatinib, erlotinib, gefitinib	· PFS: afatinib, erlotinib, and gefitinib more effective than chemotherapy · OS: afatinib, erlotinib, and

Study	Study Dates	Design (No. of Studies)	No. of Patients	Line of Treatment	Treatments Compared	Conclusions
						gefitinib comparable to chemotherapy
Crequit et al (2017)[61,]	Jun 2017	RCT (102)	36,058	Second-line	61 treatments (combinations of immunotherapy, chemotherapy, and afatinib, cabozantinib, erlotinib, gefitinib)	· OS: immunotherapy or pemetrexed plus erlotinib most effective · PFS: erlotinib plus cabozantinib most effective · Evidence for safety was insufficient
Wu et al (2017)[62,]	Jan 2017	RCT (12)	3341	Second- and third-line	Chemotherapy, PD-1/PD-L1 antibodies, erlotinib, gefitinib	· OS and PFS: PD-1/PD-L1 more effective than chemotherapy and erlotinib and gefitinib · OS and PFS: chemotherapy more effective than erlotinib and gefitinib
Yang et al (2017)[63,]	Dec 2016	Cohort (82) RCT (8)	17,621	First- and second-line	Afatinib, erlotinib, gefitinib	· PFS: gefitinib and erlotinib comparable regardless of line · Afatinib more effective than gefitinib and erlotinib as second- line treatment for advanced squamous NSCLC · Grade 3-4 adverse events comparable with afatinib and erlotinib; gefitinib adverse events lower
Zhang et al (2017)[64,]	Mar 2016	RCT (6)	1055	First-, second-, and third-line	Afatinib, dacomitinib, erlotinib, gefitinib, icotinib	· Therapeutic efficacy comparable among all 5 TKIs · Rank probabilities showed dacomitinib and afatinib had potentially better efficacy than erlotinib, gefitinib, and icotinib

EGFR: epidermal growth factor receptor; NSCLC: non-small-cell lung cancer; OS: overall survival; PD-1: programmed death-1; PD-L1: programmed death ligand-1; PFS: progression-free survival; RCT: randomized controlled trial; TKI: tyrosine kinase inhibitors.

Randomized Controlled Trials

Soria et al (2018) conducted a double-blind phase 3 trial comparing osimertinib with other TKIs (gefitinib or erlotinib) for the first-line treatment of patients with *EGFR*-positive advanced NSCLC.[65,] Median PFS was longer with osimertinib (18.9 months; 95% CI, 15.2 to 21.4 months) than with the other TKIs (10.2 months, 95% CI, 9.6 to 11.1 months; HR, 0.5, 95% CI, 0.4 to 0.6). Objective response rate did not differ significantly between osimertinib and the other TKIs. Follow-up was not long enough to adequately determine OS.

Two RCTs compared gefitinib with erlotinib in patients who had *EGFR*-sensitizing variants. Urata et al (2016) reported on a phase 3 RCT of 401 patients with *EGFR* variants randomized to gefitinib or erlotinib.[66,] The median PFS was 8.3 months (95% CI, 7.2 to 9.7 months) for patients receiving gefitinib and 10.0 months (95% CI, 8.5 to 11.2 months) for those receiving erlotinib. Rash was more common with erlotinib (18.1% vs. 2.2%) while both alanine aminotransferase elevation and aspartate amino-transferase elevation were more common with gefitinib (6.1% v.s 2.2% and 13.0% vs. 3.3%, respectively). Similarly, Yang et al (2017) reported a median PFS of 13.0 months for erlotinib and 10.4 months for gefitinib (HR, 0.81; 95% CI, 0.62 to 1.05) in 256 patients, with no differences in rates of grade 3 or 4 adverse events.[67,]

LUX-7 was a phase 2b, head-to-head trial of afatinib versus gefitinib for the treatment of first-line *EGFR* variant-positive (del19 and *L858R*) adenocarcinoma of the lung.[68,] LUX-7 randomized 319

patients in a 1:1 ratio to afatinib 40 mg/d or gefitinib 250 mg/d, stratified by variant type (del19 and L858R) and brain metastases (present vs. absent). In the overall population, PFS was significantly improved with afatinib than with gefitinib (HR, 0.73; 95% CI, 0.57 to 0.95; p=.02). Time-to-treatment failure also showed improvement in favor of afatinib (HR, 0.73; 95% CI, 0.58 to 0.92; p=.01). The ORR was significantly higher in the afatinib group (70% vs. 56%; p=.01). Several grade 3 or 4 adverse events were more common with afatinib than with gefitinib including diarrhea (13% vs. 1%) and rash (9% vs. 3%); liver enzyme elevations were more common with gefitinib (0% vs. 9%). Serious events occurred in 11% of patients in the afatinib group and 4% in the gefitinib group.

Section Summary: *EGFR* Gene Variants

Several RCTs, nonconcurrent prospective studies, single-arm enrichment studies, and meta-analyses of RCTs have demonstrated that patients with *EGFR*-sensitivity variants benefit from erlotinib, gefitinib, or afatinib therapy and patients with *EGFR*-resistance variant (T790M) benefit from osimertinib. Patient populations in these studies primarily had adenocarcinoma. Currently, there is little evidence to indicate that *EGFR* variant testing can guide treatment selection in patients with squamous cell histology. The FDA has approved several companion diagnostics for detecting *EGFR* variants to aid in selecting NSCLC patients for treatment with erlotinib, gefitinib, afatinib, and osimertinib.

Patients who are found to have wild-type tumors are unlikely to respond to erlotinib, gefitinib, or afatinib. These patients should be considered candidates for alternative therapies.

ALK Gene Rearrangements

ALK gene rearrangements most often consist of an inversion in chromosome 2, which leads to fusion with the echinoderm microtubule-associated protein like 4 (*EML4*) gene and a novel fusion oncogene *EML4-ALK*. This inversion causes abnormal expression and activation of ALK tyrosine kinase.[69,]

ALK Rearrangement Frequency

ALK rearrangements occur in 3% to 6% of NSCLC.

FDA-Approved Companion Diagnostic Tests for *ALK* Rearrangements

Several methods are available to detect *ALK* gene rearrangements or the resulting fusion proteins in tumor specimens including FISH, immunohistochemistry, reverse transcription-PCR of cDNA, and NGS. Two tests have been approved by the FDA as companion diagnostics to detect ALK rearrangements for treatment with crizotinib: the Vysis ALK Break Apart FISH Probe Kit and Ventana ALK (D5F3) CDx Assay.

The Vysis kit is a FISH-based assay. The clinical validity of the Vysis ALK Break Apart FISH Probe Kit was demonstrated in a retrospective analysis of patients screened for a phase 2, open-label single-arm study of crizotinib in patients with stage IIIB or IV NSCLC. The response rate for crizotinib in 136 *ALK*-positive patients was 50% (95% CI, 42% to 59%) with a median duration of response of 42 weeks (range, 6 to 42 weeks). The response rate for 19 *ALK*-negative patients was 26% (95% CI, 9% to 51%).

The Ventana assay is an immunohistochemical-based assay. The clinical validity of the Ventana ALK (D5F3) CDx Assay was demonstrated in a retrospective analysis of patients screened for an open-label RCT of crizotinib versus platinum-doublet chemotherapy in patients with stage IIIB or IV NSCLC. The concordance between the Ventana and Vysis tests were calculated using patient samples analyzed at an independent, central laboratory. The PPA was 86.0% (95% CI, 80.2% to 90.4%) and the NPA was 96.3% (9%% CI, 94.7% to 97.4%). Overall, in 343 patients who were *ALK*-positive by the Vysis assay, crizotinib was associated with longer PFS compared with chemotherapy (HR, 0.45; 95% CI, 0.36 to 0.60). In the subset of 141 patients who were also *ALK*-positive by the Ventana assay, the results were similar (HR, 0.40; 95% CI, 0.25 to 0.64). In the 25 patients who were *ALK*-positive by the

Vysis assay and *ALK*-negative by the Ventana assay, the relative effect of crizotinib was not clear (HR, 1.71; 95% CI, 0.43 to 6.79).

Companion diagnostic tests have been FDA approved to select patients with NSCLC for treatment with the ALK inhibitors ceritinib, alectinib, and brigatinib (Table 1).

Tyrosine Kinase Inhibitors

Crizotinib

The accelerated approval of crizotinib by the FDA was based on phase 1 and 2 trials in which crizotinib showed marked antitumor activity in patients with *ALK*-positive advanced NSCLC, with an ORR of 60% and PFS range from 7 to 10 months.[70,] These results were confirmed in 2 subsequent phase 3 trials.

A phase 3, open-label trial randomized 347 patients with previously treated, locally advanced, or metastatic *ALK*-positive lung cancer to oral crizotinib twice daily (n=173) or chemotherapy (n=174) every 3 weeks. All patients had received 1 platinum-based chemotherapy regimen before the trial. The extent of metastatic disease was 95% and 91% in patients in the crizotinib and chemotherapy groups, respectively, and tumor histology was adenocarcinoma in 95% and 94%, respectively. The primary endpoint was PFS. Patients in the chemotherapy group who experienced progressive disease were allowed to cross over to crizotinib as part of a separate study. The median PFS was 7.7 months in the crizotinib group and 3.0 months in the chemotherapy group (HR for progression or death with crizotinib, 0.49; 95% CI, 0.37 to 0.64; p<.001). Partial response rates with crizotinib were 65% (95% CI, 58% to 72%) and 20% (95% CI, 14% to 26%) with chemotherapy (p<.001). Interim analysis of OS showed no significant improvement with crizotinib compared with chemotherapy (HR for death in the crizotinib group, 1.02; 95% CI, 0.68 to 1.54; p=.54). The median follow-up for OS was 12.2 in the crizotinib group and 12.1 months in the chemotherapy group. Patients reported greater reductions in lung cancer symptoms and greater improvement in global QOL with crizotinib than with chemotherapy.

A phase 3, open-label trial compared crizotinib and chemotherapy in 343 previously untreated patients with *ALK*-positive advanced nonsquamous NSCLC.[71,] Patients were randomized to oral crizotinib twice daily or pemetrexed plus cisplatin or carboplatin every 3 weeks for up to 6 cycles. If there was disease progression for patients receiving chemotherapy, crossover to crizotinib was allowed. Progression-free survival was the primary endpoint; PFS was 10.9 months compared with 7.0 months for the groups that received crizotinib and chemotherapy, respectively (HR for progression or death with crizotinib, 0.45; 95% CI, 0.35 to 0.60; p<.001) and ORRs (complete and partial responses) were 74% and 45%, respectively (p<.001). The median OS was not reached in either group. The probability of 1-year survival with crizotinib was 84% and 79% with chemotherapy. Crizotinib was associated with greater patient-reported reductions in lung cancer symptoms and greater improvements in QOL.

Other ALK Inhibitors

Ceritinib has demonstrated superior efficacy concerning PFS when compared with chemotherapy in both the first-line and second-line (following crizotinib) settings in the ASCEND-4 and ASCEND-5 RCTs.[72,71,]

Alectinib was associated with response rates of approximately 50% in patients who had progressed on crizotinib in 2, phase 2 studies.[73,74,] Alectinib has also shown superior efficacy and lower toxicity when compared with crizotinib in the first-line setting in the ALEX and J-ALEX phase 3 RCTs.[75,76,]

Brigatinib has shown promise in early phase 1 and 2 studies with PFS of almost 13 months in patients with crizotinib-refractory disease.[77,78,] The FDA approval was granted to brigatinib in 2017 for the treatment of patients with *ALK*-positive NSCLC who have progressed on or are intolerant of

crizotinib. Approval was based on an open-label, multicenter clinical trial that reported a durable overall response rate.[79,]

Section Summary: *ALK* Gene Rearrangements

Crizotinib was granted accelerated approval by the FDA in 2011 for patients with locally advanced or metastatic NSCLC, based on ORRs observed in 2, single-arm trials. Two subsequent, phase 3 trials have shown superior PFS and tumor response rates and improved QOL in patients with crizotinib versus chemotherapy, in both previously untreated and untreated *ALK*-positive advanced NSCLC. The FDA has approved 2 companion diagnostics for detecting *ALK* gene rearrangements to aid in selecting NSCLC patients for treatment with crizotinib. Companion diagnostic tests have been FDA approved to select patients with NSCLC for treatment with ALK inhibitors.

BRAF Gene Variants

FDA-Approved Companion Diagnostic Tests for *BRAF* Variants

BRAF variants are detected by PCR sequencing or NGS methods. The Oncomine Dx Target Test was FDA-approved in 2017 as a companion diagnostic to detect *BRAF* V600E variants to aid in selecting NSCLC patients for treatment with combination dabrafenib (Tafinlar) and trametinib (Mekinist) therapy. The Oncomine test is an NGS oncology panel that detects, among other variants, *BRAF* V600E variants from DNA isolated from formalin-fixed, paraffin-embedded (FFPE) tumor tissue samples. The detection of *BRAF* V600E variants by the test was evaluated by retrospective analyses of a phase 2, multicenter, nonrandomized study that included patients with a *BRAF* V600E variant who had progressed on prior treatment or were treatment-naive who were treated with dabrafenib in combination with trametinib in the study. Patients were screened for a *BRAF* V600E variant based on local lab tests used at each enrollment site. No FDA-approved test was available for detection of *BRAF* V600E variants in FFPE NSCLC specimens so a validated PCR assay (BRAF V600 PCR Mutation Test) was used to estimate concordance. The concordance between the Oncomine test and the BRAF V600 PCR Mutation Test was 100% for PPA (95% CI, 95% to 100%) and 100% for NPA (95% CI, 97% to 100%). The response rate in the 57 previously treated patients in the study who were *BRAF*-positive by local lab test was 67% (95% CI, 53% to 79%) compared with 73% (95% CI, 50% to 89%) for the 22 patients who were also *BRAF*-positive by Oncomine. The response rate in the 36 treatment-naive patients who were *BRAF*-positive by local lab test was 61% (95% CI, 44% to 77%) compared with 61% (95% CI, 39% to 80%) in the 23 patients who were also *BRAF*-positive by Oncomine.

In June 2017, the FDA approved an additional indication for use of dabrafenib and trametinib combination therapy in patients with NSCLC with the *BRAF* V600E variant as detected by an FDA-approved test. The Oncomine Dx Target Test was approved as a companion diagnostic.

BRAF Inhibitors

Dabrafenib and Trametinib

The dabrafenib and trametinib product labels describe the results of an open-label, multicenter study of patients enrolled in 3 cohorts: cohorts A and B had received at least 1 previous platinum-based chemotherapy regimen with demonstrated disease progression but no more than 3 prior systemic regimens; cohort C could not have received prior systemic therapy for metastatic disease.[80,] Trial results for cohorts A,[81,] B,[82,] and C[83,] were reported by Planchard et al (2016, 2017) and are shown in Tables 10 and 11. Cohort A (n=78) received dabrafenib; cohorts B (n=57) and C (n=36) received dabrafenib and trametinib combination therapy.

The characteristics and results of key nonrandomized trials of BRAF or MEK inhibitors in NSCLC are described in Tables 9 and 10. In summary, the response rate for dabrafenib monotherapy in 78 patients who had progressed on chemotherapy was 33% at 11 months median follow-up while the response rate for 19 patients (17 of whom had progressed on chemotherapy) treated with vemurafenib monotherapy was 42% at 8 weeks. Response rates for dabrafenib and trametinib combination therapy were higher than 60% in patients who had progressed on prior treatment and

those who were treatment-naive. Toxicities were similar to those seen in melanoma patients taking BRAF or MEK inhibitors. Squamous cell carcinomas and other dermatological side effects were reported.

Table 10. Characteristics of Key Nonrandomized Trials of BRAF or MEK Inhibitors in *BRAF*-Variant NSCLC

Study; Trial	Study Type	Country	Dates	Participants	Treatment	Median FU, mo
Planchard et al (2017)[83,]NCT01336634	Single-arm, open-label phase 2 trial	9 countries in North America, Europe, Asia	2014-2015	Adults, stage IV, *BRAF* V600E variant, previously untreated	Dabrafenib (150 mg bid) plus trametinib (2 mg/d)	15.9
Planchardet al (2016)[81,] NCT01336634	Single-arm, open-label phase 2 trial	9 countries in North America, Europe, Asia	2011-2014	Adults, stage IV, *BRAF* V600E variant, progression after chemotherapy	Dabrafenib (150 mg bid)	10.7
Planchard et al (2016)[82,]; NCT01336634	Single-arm, open-label phase 2 trial	9 countries in North America, Europe, Asia	2013-2015	Adults, stage IV, *BRAF* V600E variant, progression after chemotherapy	Dabrafenib (150 mg bid) plus trametinib (2 mg/d)	11.6
Hyman et al (2015)[84,]; NCT01524978	Single-arm, open-label phase 2 trial	Germany, Spain, U.K., U.S., France	2012-2014	*BRAF* V600 variant-positive nonmelanoma cancers including NSCLC	Vemurafenib (960 mg bid)	6[a]

bid: twice a day; FU: follow-up; NSCLC: non-small-cell lung cancer.
[a] Estimated from a figure.

Table 11. Results of Key Nonrandomized Trials of BRAF or MEK Inhibitors in *BRAF*-Variant NSCLC

Study	Response (95% CI), %	PFS (95% CI), mo	Overall Survival (95% CI)	Adverse Events, %			
				Grade 3 or 4	%	Serious	%
Planchard et al (2017)[83,]							
	N=36	N=36	N=36				
	64 (46 to 79)	10.9 (7.0 to 16.6)[c]	At data cutoff: 24.6 mo At 2-y: 51% (33% to 67%)	· Overall · Pyrexia · Hypertension	· 7 · 11 · 11		
Planchard et al (2016)[81,]							
	N=78	N=78	N=78	N=78		N=78	
	33 (23 to 45)[a]	5.5 (3.4 to 7.3)	Median, 12.7 mo	· Overall · Cutaneous SCC · Asthenia · BCC	· 39 · 12 · 5 · 5	· Overall	· 42
Planchard et al (2016)[82,]							
	N=57	N=57	N=57	N=57		N=57	
	63 (49 to 76)	9.7 (6.9 to 19.6)	At 6 mo, 82%	· Overall · Neutropenia · Hyponatremia	· 49 · 9 · 7	· Overall · Pyrexia · Anemia · Cutaneous SCC	· 56 · 16 · 5 · 4
Hyman et al (2015)[84,]							
	N=19	N=20	N=20	N=95[b]			
	At 8 wk, 42 (20 to 67)	Median, 7.3 (3.5 to 10.8)	At 12 mo, 66% (36% to 85%)	· Overall · Rash · Fatigue · Arthralgia	· 73 · 16 · 12 · 4		

BCC: basal cell carcinoma; CI: confidence interval; NSCLC: non-small-cell lung cancer; PFS: progression-free survival; SCC: squamous cell carcinoma.
[a] The response rate in the U.S. Food and Drug Administration product label for this cohort was 27% (18% to 38%).

[b] Only reported for entire cohort including all cancer types.
[c] Investigator-assessed estimates. An independent committee assessment of PFS reported 14.6 months (95% CI, 7.0 to 22.1 months).

Case reports have also documented response to vemurafenib in patients with NSCLC and a *BRAF* variant.[85,84,]

Section Summary: *BRAF* Gene Variants

The FDA has approved a companion diagnostic for detecting *BRAF* variants to aid in selecting NSCLC patients for treatment with combination BRAF and MEK inhibitors, dabrafenib and trametinib. The clinical validity of the companion diagnostic was established in the Summary of Safety and Effectiveness Data document. The FDA expanded the indication for dabrafenib and trametinib to include the treatment of NSCLC patients whose tumors have a *BRAF* V600E variant based on a multicenter, single-arm study that included a cohort of 57 patients who had progressed on prior therapy and a cohort of 36 treatment-naive patients. Dabrafenib and trametinib combination therapy were effective in patients with a *BRAF* V600E variant, with a response rate of about 60% in both cohorts. Lower response rates were reported in other nonrandomized studies of BRAF inhibitor monotherapy in patients who had previously progressed on prior treatments.

ROS1 Gene Rearrangements

FDA-Approved Companion Diagnostic Tests for *ROS1* Rearrangements

Several methods are available to detect *ROS1* translocations including FISH, immunohistochemistry, quantitative real-time reverse transcription-PCR, and some NGS panels. FISH is considered the standard method. The Oncomine Dx Target Test was FDA-approved in 2017 as a companion diagnostic to detect fusions in *ROS1* to aid in selecting NSCLC patients for treatment with crizotinib (Xalkori). The Oncomine test is an NGS oncology panel that detects, among other variants, fusions in *ROS1* from RNA isolated from FFPE tumor tissue samples. The clinical validity of the detection of *ROS1* rearrangements by the test was evaluated by a retrospective analysis of FFPE NSCLC specimens obtained from patients enrolled in a *ROS1* cohort from an ongoing single-arm, phase 1 safety study of crizotinib in patients with advanced cancer. *ROS1* fusion status was determined by a validated FISH comparator test for the study. Concordance between the Oncomine Dx Target Test and the FISH test as well as clinical outcomes were reported in the Summary of Safety and Effectiveness Data. A total of 157 specimens were included. The PPA for Oncomine versus FISH was 80% (95% CI, 59 to 93) and NPA was 100% (95% CI, 97% to 100%). For all *ROS1*-positive patients, as originally detected for enrollment into the *ROS1* cohort, the response rate was 72% (95% CI, 58% to 84%). For *ROS1*-positive patients as detected by Oncomine, the response rate was 83% (95% CI, 36% to 99.6%).

Tyrosine Kinase Inhibitors

Crizotinib

In 2016, after an expedited review, the FDA expanded the indication for crizotinib to include the treatment of patients whose metastatic NSCLC tumors have a *ROS1* rearrangement. The approval was based on a 2014 multicenter, single-arm study that enrolled 50 patients with advanced NSCLC who tested positive for *ROS1* rearrangement.[86,] The study assessed an expansion cohort of the phase 1 PROFILE 1001 Trial. Patients were given oral crizotinib (250 mg twice daily) in continuous 28-day cycles; the median duration of treatment was 65 weeks. Characteristics and results of this and other nonrandomized studies are shown in Tables 12 and 13. A companion ROS1 biomarker diagnostic test was not approved at the time of the crizotinib indication expansion. However, the Oncomine Dx Target Test was FDA-approved in 2017 as a companion diagnostic to detect fusions in *ROS1* to aid in selecting NSCLC patients for treatment with crizotinib (Xalkori).

In summary, a nonrandomized trial and an observational study of crizotinib have shown response rates of greater than 70% in patients with *ROS*1 rearrangements, the majority of whom had progressed on prior therapy.

Ceritinib

One nonrandomized trial of ceritinib reported response rates of about 60%. Adverse events were similar to those seen in patients with *ALK* rearrangements using ALK TKIs. Common low-grade side effects included gastrointestinal side effects, visual impairment, and pain. Grade 3 or higher adverse events included liver function abnormalities and pneumonia.

Table 12. Characteristics of Key Nonrandomized Studies of Crizotinib or Ceritinib for *ROS1* Rearrangements in NSCLC

Study	Study Type	Country	Dates	Participants	Treatment	Follow-Up, mo
Lim et al (2017)[87,]	Open-label, single-arm, phase 2 study	Korea	2013-2016	Adults with *ROS1* rearrangement who had progressed on prior therapy, 94% crizotinib-naive	Ceritinib (750 mg/d)	14.0
Mazieres et al (2015)[88,]	Retrospective	6 European countries	NR	*ROS1* rearrangement, 97% had received previous chemotherapy	Crizotinib (250 mg bid)	NR
Shaw et al (2014)[86,]	Open-label, single-arm, expansion cohort of phase 1 study	Australia, Korea, U.S.	2010-2013	Adults with *ROS1* rearrangement, 86% had received prior therapy	Crizotinib (250 mg bid)	16.4

bid: twice a day; NR: not reported; NSCLC: non-small-cell lung cancer.

Table 13. Results of Key Nonrandomized Studies of Crizotinib or Ceritinib for *ROS1* Rearrangements in NSCLC

Study	Response (95% CI), %	Median PFS (95% CI), mo	OS (95% CI)	Adverse Events			
				Grade 3 or 4	%	All Grades	%
Lim et al (2017)[87,]							
	n=28	N=32	N=32	N=32		N=32	
	62 (45 to 77)	9.3 (0 to 22)	24 mo (5 to 43)	· Overall · Fatigue · Pneumonia · Hyperglycemia · Increased AST · Increased ALT	· 37 · 16 · 6 · 9 · 9 · 6	· Diarrhea · Nausea · Anorexia · Vomiting · Cough · Abdominal pain · Musculoskeletal pain	· 78 · 59 · 56 · 53 · 47 · 41 · 41
Mazieres et al (2015)[88,]							
	n=29	N=30		N=30			
	80 (NR)	9.1 (NR)	NR	· Grade 3 NR · Grade 4 or 5	· NR · 0	NR	
Shaw et al (2014)[86,]							
	N=50	N=50	N=50	N=50		N=50	
	72 (58 to 84)	19.2 (14.4 to not reached)	At 12 mo: 85% (72% to 93%)	·Hypophosphatemia · Neutropenia · Elevated ALT	· 10 · 10 · 4	· Visual impairment · Diarrhea · Nausea · Peripheral edema · Constipation · Vomiting	· 82 · 44 · 40 · 40 · 34 · 34

ALT: alanine aminotransferase; AST: aspartate aminotransferase; CI: confidence interval; NR: not reported; NSCLC: non-small-cell lung cancer: OS: overall survival; PFS: progression-free survival.

Kim et al (2013) reported on clinical outcomes in 208 never-smokers with NSCLC adenocarcinoma, according to *ROS1*-rearrangement status.[89] *ALK* rearrangements and *EGFR* variants were concurrently analyzed. The patients had clinical stages ranging from I to IV, but most were stage IV (41.3%). Of the 208 tumors, 3.4% (n=7) were *ROS1* rearranged. *ROS1* rearrangement was mutually exclusive from ALK rearrangement, but 1 of 7 ROS1-positive patients had a concurrent EGFR variant. Patients with *ROS1* rearrangement had a higher ORR and longer median PFS on pemetrexed than those without a rearrangement. In patients with *ROS1* rearrangement, PFS with EGFR TKIs was shorter than those patients without the rearrangement. None of the *ROS1*-positive patients received ALK inhibitors (e.g., crizotinib), which is the recommended targeted therapy for patients with NSCLC and this genetic alteration.

Entrectinib

Drilon et al (2020) conducted an analysis of 53 patients with *ROS-1* fusion-positive NSCLC enrolled in 3 ongoing clinical trials of entrectinib.[90] At median follow-up of 15.5 months (interquartile range 13.4 to 20.2), 41 of 53 patients had an objective response (77%; 95% CI 64% to 88%), with a median duration of response of 24.6 months (95% CI 11.4 to 34.8). In the safety-evaluable population 46 (34%) of 134 patients had grade 3 or 4 treatment-related adverse events. There were no treatment-related deaths. There is currently no FDA-approved companion diagnostic test for entrectinib.

Section Summary: *ROS1* Gene Rearrangements

The FDA has approved a companion diagnostic for detecting *ROS1* gene rearrangements to aid in selecting NSCLC patients for treatment with crizotinib. The clinical validity of the companion diagnostic was established in the Summary of Safety and Effectiveness Data document. The FDA expanded the indication for crizotinib to include the treatment of patients whose tumors have a *ROS1* rearrangement based on a multicenter, single-arm study including 50 patients, the majority of whom had progressed on prior therapy. Crizotinib was effective in patients with ROS1 rearrangements, with a response rate of about 70%. Similar response rates were reported in other nonrandomized studies of crizotinib and ceritinib. In an analysis of 53 patients with *ROS-1* fusion-positive NSCLC enrolled in 3 ongoing clinical trials of entrectinib, the ORR was 77%, with a median duration of response of 24.6 months. There is currently no FDA-approved companion diagnostic test for entrectinib.

KRAS Gene Variants

FDA-Approved Companion Diagnostic Test for *KRAS* Variants

KRAS variants can be detected by direct sequencing, PCR technologies, or NGS.

In 2021, FDA approved Therascreen KRAS RGQ PCR kit and Guardant360 CDx as companion diagnostic tests to select patients for treatment with the KRAS inhibitor, sotorasib.

There are no FDA approved companion diagnostic tests for detecting KRAS variants to select patients for treatment with EGFR TKI therapy.

RAS Inhibitor

Nonrandomized Trial

Skoulidis et al (2021) reported results of a phase 2, open-label trial of sotorasib in patients with KRAS variant NSCLC (Tables 14 and 15). Presence of the KRAS alteration in tissue was confirmed on central laboratory testing with the use of the therascreen KRAS RGQ PCR Kit. Among 124 patients evaluated for the primary outcome, 4 (3.2%) had a complete response and 42 (33.9%) had a partial response, with an acceptable safety profile. Median duration of response was 11.1 months (95% CI: 6.9 to not evaluable).

Table 14. Nonrandomized Trial of Sotorasib for Patients with KRAS Variant NSCLC- Study Characteristics

Study	Study Type	Country	Dates	Participants	Treatment	Follow-Up
Skoulidis et al (2021)[91,]	Open-label, single-arm, phase 2 study	US, multiple European countries	2019 to 2020	Adults with previously treated, locally advanced or metastatic KRAS p.G12C–mutated NSCLC, confirmed with the use of the therascreen KRAS RGQ PCR Kit	Sotorasib 960 mg orally once daily	Median 15.3 months (1.1 to 18.4+)

NSCLC: non-smal-cell lung cancer; PCR: polymerase chain reaction.

Table 15. Nonrandomized Trial of Sotorasib for Patients with KRAS Variant NSCLC- Study Results

Study	Objective Response (Complete or Partial Response) (95% CI), %	Median PFS (95% CI), mo	OS (95% CI), mo	Adverse Events, n (%)
Skoulidis et al (2021)[91,]NCT03600883				
	n=124	n=124	n=126	n=126
	37.1 (28.6 to 46.2)	6.8 (95% CI 5.1 to 8.2)	12.5 (10.0 to not evaluable)	Grade 3: 53 (42.1) Grade 4: 4 (3.2) Grade 5: 20 (15.9) All grades: 125 (99.2)

CI: confidence interval; NSCLC: non-smal-cell lung cancer; OS: overall survival; PFS: progression-free survival.

Tyrosine Kinase Inhibitors

Data on the role of *KRAS* variants in NSCLC and response to erlotinib are available from post hoc analyses of phase 3 trials of TKIs in patients with wild-type (nonmutated) versus *KRAS*-mutated lung tumors; phase 2 trials; a large prospective study; retrospective single-arm studies; and meta-analyses.

Systematic Reviews

Pooled data on the relation between *KRAS* variants and response to EGFR TKI therapy are insufficient to determine an association between *KRAS* variant status and treatment effects on PFS or OS.

Pan et al (2016) published a meta-analysis of 41 studies (N = 13,103 patients) of prognostic and predictive values of a *KRAS* variant in NSCLC.[92,] Having a *KRAS* variant was significantly associated with poorer OS (HR, 1.6; 95% CI, 1.4 to 1.8) and disease-free survival (HR, 1.57; 95% CI, 1.2 to 2.1) in early-stage resected NSCLC, and with inferior outcomes of EGFR TKI treatment (relative risk, 0.21; 95% CI, 0.1 to 0.4) in advanced NSCLC. Having a *KRAS* variant was still significantly associated with poorer OS (HR, 1.4; 95 % CI, 1.2 to 1.6) and PFS (HR, 1.4; 95 % CI, 1.1 to 1.6) of EGFR TKIs when patients with *EGFR* variants were excluded.

Mao et al (2010) performed a meta-analysis of 22 studies in 1470 patients with NSCLC (1335 [91%] evaluable for response), 231 (17%) of whom had *KRAS* variants.[93,] Studies were heterogeneous in patient populations (smoking history, tumor histology, stage, ethnicity, treatment received) and response criteria. The primary endpoint was ORR, defined as the sum of complete and partial response. Objective response rates for patients with *KRAS* and wild-type *KRAS* variants were 3% and 26%, respectively. Incomplete reporting of survival data precluded meaningful assessment of the effect of *KRAS* status on survival in NSCLC patients treated with EGFR TKIs. Data for PFS and OS stratified by *KRAS* status were available in 8 studies. The median PFS in *KRAS*-mutated and wild-

type patients was 3.0 months and 3.9 months, respectively. The median OS in *KRAS*-mutated and wild-type patients was 4.7 and 10.7 months, respectively. However, only 2 studies presented HRs with 95% CIs for PFS and OS and, therefore, a pooled analysis to derive an overall HR was not performed.

Linardou et al (2008) performed a meta-analysis of 17 studies with 1008 patients, 165 (16.4%) of whom had a *KRAS* variant.[94,] Eligible studies reported response (complete or partial) stratified by *KRAS* variant status. Primary endpoints were sensitivity and specificity of *KRAS* testing, defined as *KRAS* variant carriers showing no response to erlotinib (stable disease or progressive disease) and *KRAS* wild-type patients showing a response, respectively. Sensitivity and specificity were assessed overall and in subgroups defined by TKI received (gefitinib and/or erlotinib), response criteria (Response Evaluation Criteria in Solid Tumors [RECIST] or World Health Organization), possible selection bias, and previous chemotherapy, if any. There was no significant difference in sensitivity or specificity across subgroups. The presence of a *KRAS* variant was associated with a lack of response to TKIs (sensitivity, 21%; 95% CI, 16% to 28%; specificity, 94%; 95% CI, 89% to 97%; positive likelihood ratio, 3.52; negative likelihood ratio, 0.84). (For the analysis, likelihood ratios were calculated using pooled estimates for sensitivity and specificity.) Reviewers concluded that *KRAS* variants conferred a high level of resistance to anti-EGFR therapies; however, this conclusion was tentative due to limitations of selected studies (e.g., lack of individual patient data, heterogeneity of response endpoints, treatment regimens, patient selection criteria, retrospective design of included studies). Furthermore, incomplete reporting of survival data precluded meaningful assessment of the effect of the *KRAS* variant on survival.

Retrospective Studies
Papadimitrakopoulou et al (2016) reported on the results of the A Biomarker-Integrated Targeted Therapy Study in Previously Treated Patients With Advanced Non-Small Cell Lung Cancer (BATTLE-2) phase 2 study.[95,] The BATTLE-2 program is an umbrella study evaluating the effects of targeted therapies focusing on *KRAS*-mutated cancers. Two hundred patients with advanced NSCLC tumors who did not have *EGFR* variants or *ALK* gene fusions whose cancer was refractory to more than 1 prior therapy were assigned to 1 of 4 arms using adaptive randomization: erlotinib (n=22), erlotinib plus MK-2206 (n=42), MK-2206 plus AZD6244 (n=75), or sorafenib (n=61), stratified by *KRAS* status. AZD6244 and MK2206 are targeted small-molecule drugs that inhibit MEK and AKT, respectively. Sorafenib is a multitargeted signal transduction inhibitor that inhibits raf-kinases, vascular endothelial growth factor receptor 2, platelet-derived growth factor receptor-B, and c-kit. Only 186 evaluable patients were included in analyses. The 8-week disease control rate was 20%, 25%, 62%, and 44% for the 4 treatment groups, respectively, in the *KRAS* variant-positive patients. For *KRAS* wild-type patients, disease control rate was 36%, 57%, 49%, and 47%, respectively. The median PFS did not differ by *KRAS* status.

Rulli et al (2015) reported on results from biomarker analyses in the Tarceva Italian Lung Optimization tRial (TAILOR) trial.[96,] TAILOR enrolled patients from 52 Italian hospitals and genotyped patients for *KRAS* and *EGFR* variant status. Wild-type *EGFR* patients (n=218) received first-line platinum-based chemotherapy and then were randomized at progression to erlotinib or docetaxel. *KRAS* variants were present in 23% of randomized patients. The presence of a *KRAS* variant was not associated with PFS (HR, 1.01; 95% CI, 0.71 to 1.41; p=.98) or OS (HR, 1.24; 95% CI, 0.87 to 1.77; p=.23). The treatment effect did not differ by *KRAS* status (test for interaction: OS p=.97; PFS p=.42).

In a phase 2 trial, Miller et al (2008) assessed response to erlotinib in 101 patients with lung bronchioloalveolar carcinoma (n=12) or adenocarcinoma, bronchioloalveolar subtype (n=89), according to *KRAS* variant status.[35,] Eighteen (18%) patients had *KRAS*-mutated tumors, and none responded to erlotinib (95% CI, 0% to 19%; p<.01). In patients without a *KRAS* variant, the response rate was 32%. The median OS in patients with *KRAS*-mutated tumor was 13 months and 21 months in patients with *KRAS* wild-type tumor (p=.30).

Zhu et al (2008) performed a post hoc subgroup analysis of *KRAS* variants in patients with advanced NSCLC who had failed standard chemotherapy and had been previously randomized to erlotinib or placebo.[39,] The original phase 3 trial (National Cancer Institute of Canada Clinical Trials Group Study BR.21) was the first to demonstrate a significant survival advantage with the use of an EGFR TKI in previously treated NSCLC patients.[97,] In post hoc analysis, 206 (28%) of the original 731 tumors were tested for *KRAS* variants, which were identified in 30 (15%) patients. Among the 206 tested patients, 118 (57%) were assessable for a response to erlotinib. Of 98 patients with wild-type *KRAS*, 10 (10.2%) responded to erlotinib; of 20 patients with a *KRAS* variant, 1 (5.0%) patient responded (HR [erlotinib vs. placebo] in patients with a *KRAS* variant, 1.67; 95% CI, 0.62 to 4.50; p=.31]; HR in wild-type patients, 0.69; 95% CI, 0.49 to 0.97; p=.03). In Cox regression, the interaction between *KRAS* variant status and treatment was not statistically significant (p=.09).

In a phase 2, multicenter, open-label study, Jackman et al (2007) evaluated treatment response to erlotinib in chemotherapy-naive patients 70 years of age or older who had advanced NSCLC.[38,] Of 80 patients eligible for treatment, 41 (51%) had *KRAS* variant analysis; 6 (15%) patients were variant-positive, none of whom responded to erlotinib. Five (14%) of 35 patients with wild-type *KRAS* had a partial response.

In a phase 2 trial, Giaccone et al (2006) studied the response to erlotinib in 53 chemotherapy-naive patients with advanced NSCLC.[37,] Histologic samples were available to assess *KRAS* variant status from 29 patients, 10 (34%) of whom had variants. All 10 were nonresponders to erlotinib (p=.125).

Pao et al (2005) were the first to suggest that patients with *KRAS*-mutated lung tumors were nonresponsive to treatment with EGFR TKIs.[98,] Thirty-six patients with bronchioloalveolar carcinoma underwent *KRAS* variant analysis; 9 (25%) were found to harbor *KRAS* variants. The response was by a single radiologist, blinded to patient outcome, using RECIST criteria. None of 9 patients with *KRAS*-mutated tumors responded to erlotinib (p=.553).

Eberhard et al (2005)[11,] performed a post hoc subgroup analysis of *KRAS* variants in previously untreated patients with advanced NSCLC who had been randomized in the phase 3 trial (TRIBUTE)[99,] to chemotherapy with or without erlotinib. Of the original 1079 patients, tumor DNA samples from 274 (25%) patients were sequenced for *KRAS* variants. Baseline demographics between patients with available tumor DNA and those without were balanced. *KRAS* variants were detected in 55 (21%) of 274 patients. The response rate for patients with wild-type *KRAS* was 26%, regardless of treatment. In patients with *KRAS*-mutated tumors, the response rate was 8% for those receiving chemotherapy with erlotinib and 23% for those receiving chemotherapy alone (p=.16; 95% CI for difference, -5% to 35%); the median OS was 4.4 months (95% CI, 3.4 to 12.9 months) in patients who received erlotinib and 13.5 months (95% CI, 11.1 to 15.9 months) in those who received chemotherapy alone (p=.019).

Observational Studies
Fiala et al (2013) retrospectively analyzed patients with NSCLC who underwent *EGFR*, *KRAS*, and *PIK3CA* (phosphatidylinositide-3-kinase catalytic subunit-alpha) variant testing.[100,] Of 215 patients tested, 16 (7.4%) had a *KRAS* variant. Of 174 tested patients treated with an EGFR TKI (erlotinib or gefitinib), median PFS in 14 *KRAS*-mutated patients was 1.3 months versus 2.0 months in *KRAS* wild-type patients (n=160 [92%]); the difference was not statistically significant (p=.120). Median OS in this treated group was 5.7 months in *KRAS*-mutated patients and 8.2 months in *KRAS* wild-type patients, a statistically significant difference (p=.039). The authors concluded that *KRAS* variant status might have a negative prognostic role but a predictive role was not confirmed.

Guan et al (2013) reported on 1935 consecutive patients with NSCLC who were treated at a single-institution in China.[101,] Patients with *KRAS* variants were randomized by the tumor, node, metastasis stage, time of the first visit within 1 year, and histology, to both *EGFR* variant-positive

and *KRAS/EGFR* wild-type patients. Seventy (4%) patients received EGFR TKI therapy. In this group, median PFS was 11.8 months and 2.0 months in patients with *EGFR* and *KRAS* variants, respectively, and 1.9 months in wild-type patients. Compared with wild-type patients, PFS was statistically longer in patients with *EGFR* variants (p<.001) but no different in patients with *KRAS* variants (p=.48). The authors observed that "the presence of an *EGFR* variant, but not a *KRAS* variant, was predictive of responsiveness to EGFR TKI treatment."

Boldrini et al (2009) reported on the association between *KRAS* and *EGFR* variant status and several clinical variables in 411 patients with lung adenocarcinoma and presented a subgroup analysis of tumor response in patients treated with erlotinib or gefitinib.[102,] *KRAS* variants were observed in 17.9% of all patients. The subset analysis comprised 21 women with stage IV disease who received a TKI as second- or third-line therapy and were assessed for radiographic tumor response using RECIST. The mean age of this subpopulation at the time of diagnosis was 60.8 years (range, 40 to 86 years). Nineteen (90%) of 21 women were *KRAS* wild-type, and of those, 8 (42%) showed a partial response, 4 (21%) had stable disease, and 7 (37%) had progressive disease. Two patients with *KRAS* variants had progressive disease.

Schneider et al (2008) reported on the relationship between clinical benefit and putative tumor markers in a subgroup of patients in a global open-label, single-arm study of erlotinib in advanced NSCLC, involving 7043 patients in 52 countries (the TRUST study).[36,] The subgroup was from German centers and comprised 311 patients with stage IIIB or IV disease who were treated using erlotinib because they had failed or were not medically suitable for standard first-line chemotherapy. Tumor response was assessed using RECIST. Seventeen (15%) patients had *KRAS* variants, and none responded to erlotinib; 2 patients had stable disease. The impact of *KRAS* variant status on OS (p=.06) and PFS (p not reported) was of borderline statistical significance. The authors concluded that their data did not support the selection of patients for treatment with erlotinib on the basis of tumor molecular characteristics.

Anti-*EGFR* Monoclonal Antibodies

Two, phase 3 trials (BMS099, FLEX) investigated platinum-based chemotherapy with and without cetuximab variant status and cetuximab treatment were performed for both trials.

In the multicenter, phase 3 BMS099 trial (2010), 676 chemotherapy-naive patients with stage IIIB or IV NSCLC were assigned to taxane and carboplatin with or without cetuximab.[103,] The primary endpoint was PFS; secondary endpoints were overall response rate, OS, QOL, and safety. The addition of cetuximab did not significantly improve PFS; however, there was a statistically significant improvement in overall response rate in the cetuximab group. The trend in OS favoring cetuximab was not statistically significant. A post hoc correlative analysis was conducted to identify molecular markers for the selection of patients most likely to benefit from cetuximab.[104,] Of the original 676 enrolled patients, 202 (29.9%) had tumor samples available for KRAS testing. KRAS variants were present in 35 (17%) patients. Among patients with wild-type *KRAS*, OS was similar for the cetuximab-containing arm (n=85) and the chemotherapy-alone arm (n=82) (HR, 0.93; 95% CI, 0.67 to 1.30; p=.68; median survival, 9.7 months and 9.9 months, respectively). Among patients with *KRAS* variants, OS was similar between the cetuximab-containing arm (n=13) and the chemotherapy-alone arm (n=22) (HR, 0.91; 95% CI, 0.45 to 2.07; p=.93; median survival, 16.8 months and 10.8 months, respectively). Overall, the study showed no significant treatment-specific interactions for the presence of *KRAS* variants and outcomes evaluated; treatment differences favoring the addition of cetuximab in the *KRAS*-mutated subgroup were consistent with those observed in the wild-type *KRAS* subgroup and in the overall study population. The authors concluded that the results did not support an association between *KRAS* variant status and lack of cetuximab benefit. However, the results should be interpreted with caution due to small subgroup sample sizes and the retrospective nature of the analysis.

In the open-label, randomized, phase 3 FLEX trial (2009), 1125 chemotherapy-naive patients with stage III or IV, NSCLC were randomized to chemotherapy plus cetuximab (n=557) or chemotherapy alone (n=568).[105,] The primary endpoint was OS. Patients who received chemotherapy plus cetuximab survived longer than those who received chemotherapy only (median OS, 11.3 months vs. 10.1 months, respectively; HR for death, 0.87; 95% CI, 0.76 to 1.00; p=.04). Subsequently, *KRAS* variant testing was performed on archived tumor tissue of 395 (35%) of 1125 patients.[106,] *KRAS* variants were detected in 75 (19%) tumors. Among patients with mutated *KRAS*, the median OS in the cetuximab-containing (n=38) and chemotherapy-alone arms (n=37) was similar (8.9 months vs. 11.1 months, respectively; HR, 1.00; 95% CI, 0.60 to 1.66; p=1.0). Among patients with wild-type *KRAS*, the median OS in the cetuximab-containing (n=161) and chemotherapy-alone arms (n=159) was similar (11.4 months vs. 10.3 months, respectively; HR, 0.96; 95% CI, 0.75 to 1.23; p=.74). Progression-free survival also was similar in the cetuximab-containing and chemotherapy-alone arms in patients with mutated (HR, 0.97; 95% CI, 0.76 to 1.24) and wild-type (HR, 0.84; 95% CI, 0.50 to 1.40) *KRAS*. Response rates in the cetuximab-containing arm in patients with *KRAS*-mutated and wild-type tumors were 36.8% and 37.3%, respectively (p=.96). Overall, there was no indication that *KRAS* variant status was predictive of cetuximab effect in NSCLC.

MEK Inhibitors

Two RCTs have compared a MEK inhibitor (with or without chemotherapy) with chemotherapy alone in patients with *KRAS*-positive advanced NSCLC after progression with first-line therapy.[106,107,] Trial characteristics and results are shown in Tables 16 and 17. MEK inhibitor therapy did not improve PFS compared with docetaxel alone; response rates were similar or marginally improved. Grade 3 or higher adverse events were more frequent with MEK inhibitor therapy compared with docetaxel.

Table 16. RCT Characteristics of MEK Inhibitors for *KRAS*-Variant NSCLC

Study; Trial	Countries	Sites	Dates	Participants	Interventions	
					MEK Inhibitor	Chemotherapy
Janne et al (2017)[107,]; SELECT1 (NCT01933932)	25 countries in North and South America, Australia, Europe	202	2013-2016	510 patients with advanced NSCLC and progression following first-line therapy	254 assigned to selumetinib (75 mg bid) plus docetaxel (75 mg/m^2)	256 assigned to docetaxel (75 mg/m^2)
Blumenschein et al (2015)[108,]; NCT01362296	U.S., Korea, 6 European countries	60	2011-2012	129 patients with stage IV NSCLC and progression following first-line platinum-containing chemotherapy	86 assigned to trametinib (2 mg/d)	43 assigned to docetaxel (75 mg/m^2)

bid: twice a day; NSCLC: non-small-cell lung cancer; RCT: randomized controlled trial.

Table 17. RCT Results for MEK Inhibitors for *KRAS*-Variant NSCLC

Study	PFS (95% CI%)	OS (95% CI%)	ORR (95% CI), %	Adverse Events, %		
				Grade ≥3	%	Serious
SELECT1 (2017)[107,]						
N	510	510	510	505		505
Selumetinib plus docetaxel	3.9 mo	8.7 mo	20.1	· Overall · Diarrhea · Asthenia · Dyspnea · Anemia · Neutropenia	· 67 · 7 · 9 · 8 · 5 · 7	49
Docetaxel	2.8 mo	7.9 mo	13.7	· Overall · Diarrhea · Asthenia · Dyspnea · Anemia · Neutropenia	· 45 · 3 · 3 · 2 · 4 · 4	32

Study	PFS (95% CI%)	OS (95% CI%)	ORR (95% CI), %	Adverse Events, %	
TE (95% CI)	HR, 0.93 (0.77 to 1.12)	HR, 1.05 (0.85 to 1.30)	OR, 1.61 (1.00 to 2.62)		
Blumenschein et al (2015)[108]					
N	129	129	129	130	130
Trametinib	12 wk	8 mo	12	· Overall · 41 · Rash · 6 · Diarrhea · 5 · Asthenia · 5 · Hypertension · 9 · Neutropenia · 0 · Decreased neutrophils · 0	37
Docetaxel	11 wk	Not reached	12	· Overall · 37 · Rash · 0 · Diarrhea · 2 · Asthenia · 0 · Hypertension · 0 · Neutropenia · 14 · Decreased neutrophils · 7	21
HR (95% CI)	1.14 (0.75 to 1.75)	0.97 (0.52 to 1.83)			

CI: confidence interval; HR: hazard ratio; NSCLC: non-small-cell lung cancer; OR: odds ratio; ORR: objective response rate; OS: overall survival; PFS: progression-free survival; RCT: randomized controlled trial; TE: treatment effect.

Section Summary: *KRAS* Gene Variants

In a phase 2 trial of sotorasib conducted in 126 patients with KRAS variant NSCLC confirmed with the use of the Therascreen KRAS RGQ PCR Kit, overall response was 37.1% (95% CI 28.6% to 46.2%) with an acceptable safety profile. In an analysis of secondary endpoints, PFS was 6.8 months (95% CI 5.1 to 8.2) and OS was 12.5 months (95% CI 10.0 to not evaluable).

Data on the role of *KRAS* variants in NSCLC and response to erlotinib are available from post hoc analysis of trials, observational studies, and meta-analyses. Although studies have shown that *KRAS* variants in patients with NSCLC confer a high level of resistance to TKIs, data are insufficient to assess any additional benefit to *KRAS* testing beyond *EGFR* testing.

A lack of response to EGFR monoclonal antibodies has been established in metastatic colorectal cancer, and the use of these drugs is largely restricted to patients with wild-type *KRAS*. The expectation that *KRAS* variant status also would be an important predictive marker for cetuximab response in NSCLC has not been shown. In 2 randomized trials with post hoc analyses of *KRAS* variant status and use of cetuximab with chemotherapy, *KRAS* variants did not identify patients who would benefit from anti-EGFR antibodies, because outcomes with cetuximab were similar regardless of *KRAS* variant status.

Two RCTs have compared a MEK inhibitor with docetaxel in patients with *KRAS*-positive advanced NSCLC who had progression following first-line therapy. The MEK inhibitor did not improve PFS compared with docetaxel; the response rate was marginally improved. Grade 3 or higher adverse events were more frequent with the MEK inhibitors.

HER2 Gene Variants

Mok et al (2016) reported on the biomarker subgroup analyses from the FASTACT-2 study.[109] FASTACT-2 is a multicenter, randomized, placebo-controlled, double-blind, phase 3 study of intercalated first-line erlotinib or placebo with gemcitabine and platinum, followed by maintenance therapy with erlotinib or placebo, for Asian patients with stage IIIB or IV NSCLC. In addition to

analyzing for *EGFR*, *HER2* and *HER3* biomarkers were analyzed by immunohistochemistry. Only *EGFR* variants (p<.001) were predictive of outcomes; *HER2 and HER3* biomarkers were not significant.

Shen et al (2015) retrospectively reviewed 111 patients from a Uygur population who received gefitinib 250 mg once daily and were evaluated for *HER2* expression.[110,] *HER2* overexpression was detected in 24 patients. The ORRs in patients with and without *HER2* overexpression were 29% and 14%, respectively (p=.12). The median PFS and OS in patients with and without *HER2* overexpression did not differ statistically significantly (PFS, 4.7 months vs. 3.9 months, p=.09; OS, 21 months vs. 19 months, p=.09).

Mazières et al (2013) reported on a retrospective review of a consecutive series of patients with NSCLC tested for a *HER2* variant and assessed clinicopathologic characteristics and patient outcomes by variant status.[111,] A *HER2* variant was identified in 65 (1.7%) of 3800 patients, and was mutually exclusive of other driver mutations (*EGFR*, *ALK*, *BRAF*), with the exception of a case in which both a *HER2* and a *KRAS* variant were identified. The patient population in which a *HER2* variant was found had a median age of 60 years (range, 31 to 86 years), 69% were women, and 52% were never-smokers. All tumors were adenocarcinomas, and 50% were stage IV (n=33). Patients with stage IV disease received conventional chemotherapy and, of these, 16 patients also received *HER2*-targeted therapy as additional lines of therapy (for a total of 22 evaluable individual anti-*HER2* treatments). Four patients had progressive disease, 7 had disease stabilization, and 11 with partial response. Progression-free survival for patients with *HER2* therapies was 5.1 months.

Section Summary: *HER2* Gene Variants

Studies of *HER2* variant testing have reported response rates and PFS in numbers of patients too small from which to draw conclusions.

RET Gene Testing

FDA-Approved Companion Diagnostic Tests for *RET* Gene Testing

Oncomine DxTarget is FDA approved as a companion diagnostic for pralsetinib for the treatment of metastatic RET fusion-positive NSCLC.[7,]

Kinase Inhibitors

In May 2020, FDA granted accelerated approval for selpercatinib for the treatment of adult patients with metastatic RET fusion-positive NSCLC. Approval was based on the overall response observed in a multicenter, open-label, multi-cohort clinical trial (LIBRETTO) in patients whose tumors had RET alterations (Tables 16 and 17).[90,] There is currently no FDA-approved companion diagnostic test for selpercatinib.

In September 2020, FDA approved pralsetinib for treatment of metastatic RET-fusion positive NSCLC along with the Oncomine Dx Target Test companion diagnostic. This indication was approved under the FDA's Accelerated Approval program, based on data from the phase I/II ARROW study (Tables 18 and 19). The ARROW study is an ongoing and not yet published in a peer review journal, but trial results are available in the FDA multi-discipline review of praseltinib.[112,] The FDA reviewers noted that for NSCLC, overall response rates may be considered an endpoint reasonably likely to predict clinical benefit when the treatment effect size is large and the responses are durable.

Table 18. Characteristics of Key Nonrandomized Trials of Kinase Inhibitors in *RET*-Fusion Positive NSCLC

Study; Citation	Study Type	Sites, Countries	Dates	Participants	Treatment	Median FU, mo
LIBRETTO NCT03157128	Single-arm, open-label phase 1-2 trial	65 centers in 12 countries	2017-2018	Patients with advanced *RET* fusion–positive NSCLC	Selpercatinib	12.1

Study; Citation	Study Type	Sites, Countries	Dates	Participants	Treatment	Median FU, mo
Drilon et al 2020[90],				• 105 who had previously received platinum-based chemotherapy • 39 previously untreated		
ARROW NCT03037385 FDA (2020)[112],	Multicohort, open-label phase 1-2 trial	53 centers in 11 countries	Data cutoff Nov 2019	Patients with metastatic *RET* fusion positive NSCLC • 87 previously treated with platinum-based chemotherapy • 27 previously untreated	Pralsetinib	10.5

NSCLC: non-small-cell lung cancer

Table 19. Results of Key Nonrandomized Trials of Kinase Inhibitors in *RET-Fusion Positive* NSCLC

Study	Response (95% CI), %	PFS (95% CI), mo	Adverse Events
LIBRETTO NCT03157128 Drilon et al 2020[90],	Previously treated: 64% (54% to 73%) Previously untreated: 85% (70% to 94%)	16.5 months (13.7 to NE)	Grade 3 or 4: • Hypertension (14%) • Increased ALT: (13%) • Hyponatrema (6%) • Lymphopenia (6%) Grade 5 (6 events in 4% of patients): • sepsis (n=2) • cardiac arrest, multiple organ dysfunction syndrome, pneumonia, and respiratory failure (1 patient each)
ARROW NCT03037385 FDA (2020)[112],	Previously treated: 57% (46% to 68%) Previously untreated: 70% (50% to 86%)	12.7 months (95% CI: 9.1 to NE)	Serious adverse reactions occurred in 45% of patients. Permanent discontinuation due to an adverse reaction occurred in 15% of patients. Grades 3 to 4 AEs: Fatigue (2.3%), constipation (1%), diarrhea (3.2%), hypertension (14%), cough (0.5%), pneumonia (8%)

AE: adverse event; ALT: alanine aminotransferase; CI: confidence interval; NE: not evaluable; NSCLC: non-small-cell lung cancer; PFS: progression-free survival.

Section Summary: *RET* Gene Testing

The FDA has approved a companion diagnostic (Oncomine Dx Target Test) for treating metastatic *RET*-fusion positive NSCLC with pralsetinib under accelerated approval based on studies of effect particularly among treatment naive patients (70% [95% CI, 50% to 86%]). The FDA has also approved selpercatinib for the treatment of adult patients with metastatic *RET* fusion-positive

NSCLC based on a multicenter, open-label, multicohort clinical trial in patients whose tumors had *RET* alterations, with high treatment naive effect (85% [95% CI, 70% to 94%]).

MET Gene Testing

FDA-Approved Companion Diagnostic Tests for *MET* Gene Testing

FoundationOne CDx and FoundationOne Liquid CDx are FDA approved as companion diagnostics for capmatinib for the treatment of NSCLC harboring MET with an exon 14 skipping alteration.[7,]

Capmatinib

In 2020, FDA approved the MET inhibitor capmatinib for treatment of adult patients with metastatic NSCLC whose tumors have an alteration that leads to MET exon 14 skipping. Approval was accelerated based on overall response rate and duration of response in the GEOMETRY mono-1 trial (NCT02414139)[113,] Tables 20 and 21 summarize characteristics and results of this trial.

Table 20. Characteristics of Key Nonrandomized Trials of Capmatinib in MET Alterations

Study; Trial	Study Type	Country	Dates	Participants	Treatment
GEOMETRY mono-1 NCT02414139 Wolf et al 2020[113,]	Multiple-cohort, phase 2 trial	NR	NR	364 patients with NSCLC • 97 patients had a *MET* exon 14 skipping alteration • 210 had MET amplification	Capmatinib

NR: not reported; NSCLC: non-small-cell lung cancer.

Table 21. Results of Key Nonrandomized Trials of Capmatinib in MET Alterations

Study	Overall Response Rate	Progression Free Survival (95% CI)	Median Duration of Response (95% CI)	Adverse Events
GEOMETRY mono-1 NCT02414139 Wolf et al 2020[113,]	Patients with *MET* exon 14 skipping alteration: • 41% (95% CI 29% to 53%) of 69 patients who had received 1 or 2 lines of therapy previously • 68% (95% CI 48% to 84%) of 28 patients who had not received treatment previously Patients with *MET* amplification: • Limited efficacy was observed in previously treated patients with *MET* amplification who had a gene copy number of less than 10 (overall response in 7% to 12% of patients) • Among patients with *MET* amplification and a gene copy number of 10 or higher, overall response was	Patients with *MET* exon 14 skipping alteration: Previously treated: 5.4 months (4.2 to 7.0) No previous treatment: 12.4 months (8.2 to not estimable) Patients with *MET* amplification: Previously treated: 4.1 months (2.9 to 4.8) No previous treatment: 4.2 months (1.4 to 6.9)	Patients with *MET* exon 14 skipping alteration: Previously treated: 9.7 months (5.6 to 13.0) No previous treatment: 12.6 months (5.6 to not estimable) Patients with *MET* amplification: Previously treated: 8.3 months (4.2 to 15.4) No previous treatment: 7.5 months (2.6 to 14.3)	Grade 3 or 4: 67% reported across all study cohorts (n=364). Most frequent (those occurring in ≥10% of patients) were peripheral edema, nausea, vomiting, and increased blood creatinine level. Treatment-related adverse events leading to discontinuation of treatment occurred in 39 patients (11%)

Study	Overall Response Rate	Progression Free Survival (95% CI)	Median Duration of Response (95% CI)	Adverse Events
	observed in 29% (95% CI, 19 to 41) of previously treated patients and in 40% (95% CI, 16 to 68) of those who had not received treatment previously			

CI: confidence interval.

Section Summary: *MET* Gene Testing

The GEOMETRY Mono-1 trial showed efficacy of capmatinib in patients with advanced NSCLC with a *MET* exon 14 skipping mutation, especially in treatment-naive patients (68% [95% CI, 48% to 84%]) and median duration of 12.6 months). Efficacy was also observed in pre-treated patients (overall response rate 41% [95% CI 29% to 53%] and median duration of 9.7 months).

NTRK Gene Fusions

FDA-Approved Companion Diagnostic Tests for *NTRK* Gene Fusions

There are currently no FDA-approved companion diagnostic tests for NTRK gene fusions.

Larotrectinib

Drilon et al (2018) evaluated the effectiveness of larotrectinib in 55 patients with consecutively and prospectively identified tropomyosin receptor kinase (TRK) fusion-positive solid tumors, including 4 patients with lung tumors.[114,] The overall response rate was 80% (95% CI, 67 to 90). The median PFS had not been reached after a median follow-up duration of 9.9 months (range, 0.7 to 25.9). Responses were observed regardless of tumor type or age of the patient. The FDA approved larotrectinib for patients with TRK fusion-positive solid tumors based on these results.[115,] An updated analysis of 153 patients from this data set was consistent with the earlier analysis.[116,]

Entrectinib

Doebele et al (2020) published an analysis of 3 phase 1-2 trials of entrectinib in patients with advanced or metastatic NTRK fusion-positive solid tumors.[117,] Of 54 patients, 10 (19%) had NSCLC. AT a median follow-up of 12.9 months, 31 of 54 patients had an objective response (57%; 95% CI 43.2 to 70.8). Median duration of response was 10 months (95% CI 7.1 to not estimable). The most common grade 3 or 4 treatment-related adverse events in both safety populations were increased weight (7 [10%] of 68 patients in the NTRK fusion-positive safety population and in 18 [5%] of 355 patients in the overall safety-evaluable population) and anemia (8 [12%] and 16 [5%]). The most common serious treatment-related adverse events were nervous system disorders (3 [4%] of 68 patients and 10 [3%] of 355 patients). No treatment-related deaths occurred.

Section Summary: *NTRK* Gene Fusions

From studies of 55 patients with consecutively and prospectively identified *NTRK* fusion-positive solid tumors, including 4 patients with lung tumors, the overall response rate was 80% (95% CI, 67 to 90). The median PFS had not been reached after a median follow-up duration of 9.9 months (range, 0.7 to 25.9). Responses were observed regardless of tumor type or age of the patient. In an integrated analysis of 3 phase 1-2 trials in patients with NTRK solid tumors, 10 of whom had NSCLC, response was 57% (95% CI 43.2% to 70.8%) with an acceptable safety profile.

Immunotherapy for Advanced Non-Small-Cell Lung Cancer

Clinical Context and Test Purpose

The purpose of identifying PD-L1 expression and tumor mutational burden (TMB) in patients who have advanced NSCLC is to inform a decision whether patients should receive a immunotherapy

versus another systemic therapy. Patients who present with advanced disease or recurrence following initial definitive treatment typically receive systemic therapy. Traditionally, systemic therapy was cytotoxic chemotherapy. Targeted treatments are ineffective in patients whose tumors lack genetic alterations such as EGFR, ALK, BRAF, and ROS1 variants (driver mutations). However, a subset of these patients may be good candidates for treatment with immunotherapy. The goal of immunotherapy is to preferentially kill malignant cells without significant damage to normal cells so that there is improved therapeutic efficacy along with decreased toxicity.

The question addressed in this evidence review is this: Does testing for PD-L1 and TMB improve the net health outcome in individuals with advanced-stage NSCLC who are being considered for immunotherapy?

The following PICO was used to select literature to inform this review.

Populations
The relevant population of interest is individuals with advanced NSCLC who are being considered for immunotherapy.

Interventions
The interventions of interest are testing for PD-L1 and TMB.

Comparators
The following practice is currently being used to target therapy for advanced-stage NSCLC: standard management without testing for PD-L1 or TMB. Standard management consists primarily of chemotherapy.

Outcomes
Beneficial outcomes resulting from a true-positive test result are prolonged survival, reduced toxicity, and improved QOL associated with receiving a more effective and less cytotoxic targeted therapy than chemotherapy. Beneficial outcomes from a true negative result are prolonged survival associated with receiving chemotherapy in those whose tumors do not express PD-L1.

Harmful outcomes resulting from a false-negative test result include shorter survival from receiving less effective and more cytotoxic chemotherapy in those whose tumors express PD-L1; possible harmful outcomes resulting from a false-positive test result are a shorter survival from receiving potentially ineffective immunotherapy and delay in initiation of chemotherapy in those whose tumors do not express PD-L1.

Due to the poor prognosis of advanced NSCLC, the duration of follow-up for the outcomes of interest is 6 months and 1 year.

Study Selection Criteria
Methodologically credible studies were selected using the following principles:

- To assess efficacy outcomes, comparative controlled prospective trials were sought, with a preference for RCTs;
- In the absence of such trials, comparative observational studies were sought, with a preference for prospective studies.
- To assess long-term outcomes and adverse events, single-arm studies that capture longer periods of follow-up and/or larger populations were sought.
- Studies with duplicative or overlapping populations were excluded.

Review of Evidence
PD-L1 Testing

FDA Companion Diagnostic Tests for PD-L1
Companion diagnostic tests have been FDA-approved for PD-L1 testing for immunotherapy with atezolizumab, pembrolizumab, and the combination of nivolumab plus ipilimumab in patients with NSCLC.[7,]

Atezolizumab
Herbst et al (2020) published results of a phase 3, open label RCT of atezolizumab compared to platinum-based chemotherapy in 572 patients with NSCLC who had not previously received chemotherapy and who had PD-L1 expression on at least 1% of tumor cells or at least 1% of tumor-infiltrating immune cells (NCT02409342).[118,] In the subgroup of patients with tumors who had the highest expression of PD-L1 (205 patients), the median OS was longer by 7.1 months in the atezolizumab group than in the chemotherapy group (20.2 months vs. 13.1 months; HR for death, 0.59; p=.01). Atezolizumab treatment resulted in significantly longer OS than platinum-based chemotherapy among patients with NSCLC with high PD-L1 expression, regardless of histologic type. Grade 3 or 4 adverse events occurred in 30.1% and 52.5% of the patients in the atezolizumab group and the chemotherapy group, respectively.

Pembrolizumab
Reck et al (2016) published results of the KEYNOTE-024 Trial (NCT02142738), which compared pembrolizumab to platinum-based chemotherapy in 305 patients with NSCLC and PD-L1 expression on at least 50% of tumor cells.[119,] At a median follow-up of 11.2 months, PFS was longer with pembrolizumab compared with chemotherapy (median PFS, 10.3 vs. 6 months; HR, 0.50; 95% CI, 0.37 to 0.68). The median duration of response was not reached in the pembrolizumab group and was 6.3 months in the chemotherapy group.

Nivolumab in Combination with Ipilimumab
In the CHECKMATE 227 Trial (NCT02477826) reported by Hellmann et al (2019), among the patients with a PD-L1 expression level of 1% or more, the median duration of OS was 17.1 months (95% CI, 15.0 to 20.1) with nivolumab plus ipilimumab and 14.9 months (95% CI, 12.7 to 16.7) with chemotherapy (p=.007), with 2-year OS rates of 40.0% and 32.8%, respectively.[120,] The median duration of response was 23.2 months with nivolumab plus ipilimumab and 6.2 months with chemotherapy. First-line treatment with nivolumab plus ipilimumab resulted in a longer duration of OS than did chemotherapy in patients with NSCLC, independent of the PD-L1 expression level.

Section Summary: PD-L1 Testing
In RCTs, patients with high PD-L1 expression had longer PFS and fewer adverse events when treated with anti-PD-L1 monoclonal antibodies than with platinum chemotherapy. In the KEYNOTE trial, first-line treatment with nivolumab plus ipilimumab resulted in a longer duration of OS than did chemotherapy in patients with NSCLC, independent of the PD-L1 expression level.

Tumor Mutational Burden Testing to Select Patients for Immunotherapy
FDA-Approved Companion Diagnostic Test
There is no FDA approved companion diagnostic test for tumor mutational burden (TMB) to select patients for treatment with nivolumab plus ipilimumab. FoundationOne CDx is FDA approved as a companion diagnostic for use with pembrolizumab in patients with TMB-high (≥ 10 mutations per megabase) solid tumors.

Randomized Controlled Trial

Nivolumab plus Ipilimumab

In a subgroup analysis of the CHECKMATE 227 trial (NCT02477826), PFS was significantly longer with nivolumab plus ipilimumab than with chemotherapy among patients with NSCLC and a high TMB (≥10 mutations per megabase) (Tables 22 and 23).[6,]

In exploratory analyses, retrospective observational studies have reported an association between higher TMB and longer PFS[121,]and OS[122,] in patients receiving immunotherapy.

Table 22. Characteristics of RCT of Nivolumab Plus Ipilimumab in Patients with NSCLC and High Tumor Mutational Burden

Study; Trial	Dates	Participants	Interventions	
			Nivolumab plus ipilimumab	*Chemotherapy*
CHECKMATE 227 (NCT02477826) Hellmann et al (2018)[6,]NCT02477826	2015-2016	Adult patients with histologically confirmed squamous or nonsquamous stage IV or recurrent NSCLC who had received no previous systemic anticancer therapy as primary therapy for advanced or metastatic disease and high TMB (≥10 mutations per megabase)	N=139	N=160

NSCLC: non-small-cell lung cancer; RCT: randomized controlled trial; TMB: tumor mutational burden.

Table 23. Results of RCT of Nivolumab Plus Ipilimumab in Patients with NSCLC and High Tumor Mutational Burden

Study	1-year PFS	Median PFS (95% CI)	ORR (95% CI), %	Adverse Events, %	
Hellmann et al (2018)[6,] NCT02477826				Grade ≥3	%
N	299	299	299	294	
Nivolumab plus ipilimumab	42.6%	7.2 months (5.5 to 13.2)	45.3 (36.9 to 54.0)	• Any event • Any serious event • Any event leading to discontinuation	• 37 • 21 • 16
Chemotherapy	5.5%	5.5 months (4.4 to 5.8)	26.9 (20.2 to 34.4)	• Any event • Any serious event • Any event leading to discontinuation	• 36 • 11 • 6
Treatment Effect (95% CI)		HR, 0.58; 97.5% CI, 0.41 to 0.81; p<.001	Difference 18.4 (7.6 to 28.8)		

CI: confidence interval; HR: hazard ratio; NSCLC: non-small-cell lung cancer; ORR: objective response rate; PFS: progression-free survival; RCT: randomized controlled trial.

Pembrolizumab

Nonrandomized Trial

Marabelle et al (2020) reported the association of high TMB with response to pembrolizumab in patients with solid tumors enrolled in a prespecified exploratory analysis of the KEYNOTE-158 study (Table 24).[123,] High TMB was defined as >10 mutations per megabase according to the FoundationOne CDx panel. The proportion of patients with an objective response in the TMB-high group was 29%. At a median follow-up of approximately 3 years, the median duration of response

was not reached in the TMB-high group and was 33.1 months in the non-TMB-high group. Notably, TMB-high status was associated with improved response irrespective of PD-L1. Median PFS and OS did not differ between the high and non-high TMB groups. Objective responses were observed in 24 (35%; 95% CI 24 to 48) of 68 participants who had both TMB-high status and PD-L1-positive tumors (i.e., PD-L1 combined positive score of ≥1) and in 6 (21%; 8 to 40) of 29 participants who had TMB-high status and PD-L1-negative tumors.

Table 24. Association of TMB to Response to Pembrolizumab in Patients with Solid Tumors Enrolled in the KEYNOTE-158 Study

Study	Response	Median Duration of Response	Median PFS	Median OS (95% CI)	Adverse events
Marabelle et al (2020)[123],					
TMB >10 per megabase; N=102	Objective response: 29% (21 to 39%) Complete: 4%	Median not yet reached range 2·2+ to 34.8+ months	2.1 months (95% CI 2.1 to 4.1)	Median: 11.7 months (95% CI 9.1 to 19.1)	Deaths: 69/102 (68%)
TMB <10 per megabase; N=688	Objective response: 6% (5 to 8%) Complete: 2%	Median 33.1 months (4.0 to 35.7+)	2.1 months (2.1 to 2.2)	12.8 months (11.1 to 14.1)	534/688 (78%)

CI: confidence interval N: sample size; OS: overall survival; PFS: progression-free survival; TMB: tumor mutational burden.

Section Summary: Tumor Mutational Burden Testing

In a subgroup analysis of an RCT, PFS was significantly longer with nivolumab plus ipilimumab than with chemotherapy among patients with NSCLC and a high TMB (≥10 mutations per megabase). In exploratory analyses, retrospective observational studies have reported an association between higher TMB and longer PFS and OS in patients receiving immunotherapy. In a prespecified subgroup analysis of a nonrandomized trial of pembrolizumab in patients with various solid tumors, objective responses were observed in 24 (35%; 95% CI 24 to 48) of 68 participants who had both TMB-high status and PD-L1-positive tumors and in 6 (21%; 8 to 40) of 29 participants who had TMB-high status and PD-L1-negative tumors. In exploratory analyses, retrospective observational studies have reported an association between higher TMB and longer PFS and OS in patients receiving immunotherapy. These results need to be confirmed in additional, well-designed prospective studies.

Biomarker Testing Using Circulating Tumor DNA (Liquid Biopsy) to Select Targeted Therapy or Immunotherapy for Advanced-Stage Non-Small-Cell Lung Cancer

Selecting Targeted Therapy

Clinical Context and Test Purpose

The purpose of identifying targetable oncogenic "driver mutations" such as *EGFR* variants in patients who have NSCLC is to inform a decision whether patients should receive a targeted therapy versus another systemic therapy. Patients have traditionally been tested for driver mutations using samples from tissue biopsies.

One testing strategy is to use liquid biopsy to select first-line and second-line treatments in patients with advanced NSCLC, with reflex to tissue biopsy if the test is negative. This testing strategy is based on the reflex testing strategy suggested in the U.S. Food and Drug Administration (FDA) approval for the cobas test. Some guidelines have suggested a different testing strategy wherein testing with a liquid biopsy is considered only when testing with a tissue biopsy is not feasible.
The questions addressed in this evidence review are:

- How accurately does liquid biopsy detect driver or resistance variants of interest in the relevant patient population (clinical validity)?
- Does a strategy including liquid biopsy in patients with NSCLC improve the net health outcome compared with standard biopsy?

The following PICO was used to select literature to inform this review.

Populations
The target population consists of patients with NSCLC where tumor biomarker testing is indicated to select a treatment. Patients may be treatment-naive, or being considered for a treatment change due to progression, recurrence, or suspected treatment resistance.

Treatment recommendations for patients with advanced NSCLC are usually made in the tertiary care setting ideally in consultation with a multidisciplinary team of pathologists, thoracic surgeons, and oncologists.

Routine surveillance or periodic monitoring of treatment response as potential uses of the liquid biopsy were not evaluated in this evidence review.

Interventions
The technology considered is an analysis of tumor biomarkers in peripheral blood (liquid biopsy) to determine treatment selection. Several commercial tests are available and many more are in development. In contrast to tissue biopsy, guidelines do not exist establishing the recommended performance characteristics of liquid biopsy.

The evidence is considered separately for the different biomarkers. Studies have evaluated liquid biopsy for biomarkers that detect *EGFR* TKI sensitization, concentrating on the *EGFR* exon 19 deletion and *EGFR* L858R variants. Studies have also evaluated separately biomarkers associated with TKI resistance, concentrating on the *EGFR* T790M variant.

Studies have also assessed a liquid biopsy for detection of the *EML4-ALK* fusion oncogene and its variants, translocation between *ROS1* and other genes (most commonly *CD74*), *BRAF* variants occurring at the V600 position of exon 15, and other variants.

Comparators
The relevant comparator of interest is testing for variants using tissue biopsy.

Outcomes
The outcomes of interest are OS and cancer-related survival. In the absence of direct evidence, the health outcomes of interest are observed indirectly as a consequence of the interventions taken based on the test results.

In patients who can undergo tissue biopsy, given that negative liquid biopsy results are reflexed to tissue biopsy, a negative liquid biopsy test (true or false) does not change outcomes compared with tissue biopsy.

Similarly, in patients who cannot undergo tissue biopsy, a negative liquid biopsy test (true or false) should result in the patient receiving the same treatment as he/she would have with no liquid biopsy test so a negative liquid biopsy test does not change outcomes.

The implications of positive liquid biopsy test results are described below.

Potential Beneficial Outcomes with Positive Result
For patients who can undergo tissue biopsy, the beneficial outcomes of a true-positive liquid biopsy result are the avoidance of tissue biopsy and its associated complications. In the National Lung Screening Trial, which enrolled 53454 persons at high- risk for lung cancer at 33 U.S. medical centers, the percentage of patients having at least 1 complication following a diagnostic needle biopsy was approximately 11%.[124,]

For patients who cannot undergo tissue biopsy, the beneficial outcomes of a true-positive liquid biopsy result are receipt of a matched targeted therapy instead of chemotherapy and/or immunotherapy.

Potential Harmful Outcomes with Positive Result

The harmful outcome of a false-positive liquid biopsy result is incorrect treatment with a targeted therapy instead of immunotherapy and/or chemotherapy. In a meta-analysis of randomized controlled trials (RCTs) of EGFR TKIs vs chemotherapy in patients without *EGFR*-sensitizing variants, the overall median progression-free survival (PFS) was 6.4 months in patients assigned to chemotherapy vs 1.9 months in patients assigned to EGFR TKIs (hazard ratio [HR], 1.41; 95% confidence interval [CI], 1.10 to 1.81). The advantage of chemotherapy over EGFR TKIs for patients without *EGFR*-sensitizing variants was true in both the first- and second-line settings.[125,]
In the AZD9291 First Time In Patients Ascending Dose Study (AURA 1), single-arm, phase 1 trial of osimertinib, among 61 patients with *EGFR*-sensitizing variants who had progressed on an EGFR TKI but who did not have the *EGFR* T790M resistance variant, the response rate was 21% (95% CI, 12% to 34%) and median PFS was 2.8 months (95% CI, 2.1 to 4.3 months).[126,] There was no concurrent control group in AURA 1 for comparison of osimertinib with other second-line treatments among T790M-negative patients. However, in the IMpower 150 trial, the addition of the immunotherapy atezolizumab to the combination chemotherapy of bevacizumab, carboplatin, and paclitaxel improved PFS in a subset of 111 patients with *EGFR*-sensitizing variants or *ALK* translocations who had progressed on a prior targeted agent (median PFS, 9.7 months vs 6.1 months; HR=0.59; 95% CI 0.37 to 0.94).[127,]

Due to the poor prognosis of advanced NSCLC, the duration of follow-up for the outcomes of interest is 6 months and 1 year.

Study Selection Criteria

For the evaluation of the clinical validity of each test, studies that met the PICO criteria described above and the following eligibility criteria were considered:

- Reported on the performance characteristics (sensitivity and specificity) of the marketed version of the technology or included data sufficient to calculate sensitivity and specificity
- Included a suitable reference standard (tissue biopsy)
- Patient/sample clinical characteristics were described and patients were diagnosed with NSCLC
- Patient/sample selection criteria were described.
- At least 20 patients are included

Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Review of Evidence

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

A systematic review, including 55 studies reporting clinically validity of liquid biopsy compared with tissue biopsy for detection of *EGFR* TKI-sensitivity variants or resistance variants through February 2017. Details of that systematic review are found in Appendix 1. In brief, most studies were conducted in Asia, using tests not currently being marketed in the U.S.. There was high variability in performance characteristics, with sensitivities ranging from close to 0% to 98% and specificities ranging from 71% to 100%. Therefore, evidence will not be pooled across tests going forward and instead reviewed separately for tests marketed in the U.S.A systematic review by Wu et al (2015) noted sensitivity might

be lower in studies including non-Asian ethnicities (55%; 95% CI, 33% to 77%) compared with Asian ethnicities (68%; 95% CI, 57% to 79%), although the difference was not statistically significant.[128,] Therefore, studies in the U.S. or similar populations will be most informative regarding the clinical validity of tests marketed in the U.S.

As previously described, there are multiple commercially available liquid biopsy tests that detect *EGFR* and other variants using a variety of detection methods. Given the breadth of molecular diagnostic methodologies available and the lack of guidelines regarding the recommended performance characteristics of liquid biopsy,[5,] the clinical validity of each commercially available test must be established independently. The market is changing rapidly and all available tests may not be represented in the appraisal below.

Several clinical validity studies comparing liquid biopsy with tissue biopsy in patients who had advanced NSCLC for marketed tests have been published. Characteristics of the studies are shown in Table 1. Most have included testing for *EGFR* variants but a few included testing for less prevalent variants as well.

Evidence for the different variants is reviewed separately. Performance characteristics for detecting 1 type of variant (e.g., point mutations) may not represent performance to detect other types of variants (e.g., gene fusions).[129,]

Table 25. Characteristics of Clinical Validity Studies of Liquid Biopsy With Tissue Biopsy as the Reference Standard

Study	Study Population	Design	Variants Included[a]	Timing of Reference and Index Tests
Multiple tests				
Papadimitrakopoulou et al (2020) (AURA3)[130,]	Patients harboring T790M mutation with locally advanced or metastatic NSCLC who had progressed on EGFR TKI therapy enrolled in AURA3 studies in U.S., Mexico, Canada, Europe, Asia, and Australia	Retrospective	*EGFR*	Both tissue and blood samples collected at screening
Cobas EGFR test				
Jenkins et al (2017)[131,]	Patients with advanced NSCLC who had progressed on EGFR TKI therapy enrolled in AURA extension or AURA2 studies in U.S., Europe, Asia, and Australia	Retrospective	*EGFR* resistance	Both tissue and blood samples collected at screening/baseline
FDA SSED (2016)[132,]	Patients with stage IIIb/IV NSCLC enrolled in a phase 3 RCT in Asia between 2011 and 2012	Retrospective	*EGFR*	Both tissue and blood samples collected at screening
Karlovich et al (2016)[133,]	Patients with newly diagnosed or relapsed patients with advanced (stage IIIB, IV) NSCLC in U.S., Europe, and Australia between 2011 and 2013	Prospective	*EGFR*, *BRAF*	Plasma was collected within 60 d of tumor biopsy
Thress et al (2015)[134,]	Patients with NSCLC enrolled in a multinational (including U.S.) phase 1 study who had progressed on an EGFR TKI therapy	Prospective	*EGFR*	Blood and tissue collected after progression and before next-line treatment; time

Study	Study Population	Design	Variants Included[a]	Timing of Reference and Index Tests
				between not specified
Mok et al (2015)[135,]	Patients enrolled in a phase 3 RCT in Asia with stage IIIB/IV NSCLC	Prospective	*EGFR*	Tissue samples from diagnosis or resection or biopsy 14 d before first study dose. Blood collected within 7 d prior to first study dose
Weber et al (2014)[136,]	Patients in Denmark with NSCLC (84% stage IV) from 2008 to 2011	Retrospective	*EGFR*	Blood samples collected a median of 10.5 mo after diagnostic biopsy
Guardant360 CDx				
Palmero et al (2021)[137,]	Patients with treatment-naive NSCLC at 8 academic institutions in Spain	Prospective	*ALK, EGFR, ROS1, BRAF V600E, RET, MET exon 14 skipping variants, and ERBB2 (HER2), KRAS*	Pre-treatment blood collected at baseline. Tissue genotyping was performed as per the treating physician's choice and included FDA-approved companion diagnostics for EGFR and ALK, commercial and laboratory-developed tests for individual biomarkers, and more comprehensive NGS-based assays
FDA SSED (2020)[138,]	Patients with advanced and metastatic NSCLC with EGFR exon 19 deletions or exon 21 L858R mutations confirmed by the cobas EGFR Mutation Test enrolled in the FLAURA phase 3 study assessing the efficacy of osimertinib versus standard EGFR TKI therapy; patients enrolled in the NILE study were used to estimate the prevalence of CDx-positive, tissue-negative patients as no plasma from FLAURA tissue-negative patients was available	Retrospective	*EGFR*	Unclear
Leighl et al (2019)[139,]	Patients with biopsy-proven, previously untreated, nonsquamous NSCLC (stage IIIB/IV)	Prospective	*EGFR, ALK, ROS1, BRAF, MET, RET*	Unclear

Study	Study Population	Design	Variants Included[a]	Timing of Reference and Index Tests
	enrolled in the NILE study at 1 of 28 North American centers between 2016 and 2018			
Schwaederle et al (2017)[140,]	Patients with lung adenocarcinoma (86% with metastatic disease) from academic medical centers in California between 2014 and 2015	Retrospective, consecutive	*EGFR*, *ALK*, *ROS1*, *BRAF*	Median time was 0.8 mo, range not given
Thompson et al (2016)[141,]	Patients with NSCLC or suspected NSCLC (96% stage IV) from Pennsylvania between 2015 and 2016	Prospective, consecutive	*EGFR*, *ALK*, *ROS1*, *BRAF*	Time between tissue and blood collection ranged from 0 d to >2 y
Villaflor et al (2016)[142,]	Patients in Chicago with NSCLC (68% stage IV) who had undergone at least 1 ctDNA test at a single commercial ctDNA laboratory in 2014 and 2015	Retrospective, selection unclear	*EGFR*, *ROS1*, *BRAF*	Time between biopsy and blood draw ranged from 0 d to 7 y (median, 1.4 y)
OncoBEAM				
Ramalingam et al (2018)[143,]	Patients with locally advanced or metastatic NSCLC from the AURA study conducted in U.S., Europe, and Asia	Prospective	*EGFR*	Plasma was collected at baseline, time of tissue sample not specified
Karlovich et al (2016)[133,]	Patients with newly diagnosed or relapsed patients with advanced (stage IIIB, IV) NSCLC in U.S., Europe, and Australia between 2011 and 2013	Prospective	*EGFR*, *BRAF*	Plasma was collected within 60 d of tumor biopsy
Thress et al (2015)[134,]	Patients with NSCLC enrolled in a multinational (including U.S.) phase 1 study who had progressed on an EGFR TKI therapy	Prospective	*EGFR*	Blood and tissue collected after progression and before next-line treatment; time between not specified
Biodesix ddPCR				
Mellert et al (2017)[144,]	Patients in the test utilization data had lung cancer; unclear whether the samples in the clinical validity data were from patients with advanced NSCLC, patient characteristics are not described	Retrospective and prospective, selection unclear	*EGFR*, *ALK*	Timing not described
ctDx-Lung				
Paweletz et al (2016)[145,]	Patients in Boston with advanced NSCLC with a known tumor genotype, either untreated or progressive on therapy	Prospective	*EGFR*, *ALK*, *ROS1*, *BRAF*	Timing not described
InVision				

Study	Study Population	Design	Variants Included[a]	Timing of Reference and Index Tests
Pritchet et al (2019)[146,]	Patients with untreated, advanced NSCLC; primarily from cohorts enrolled in 2 prospective US studies with 41 centers	Prospective	*EGFR*, *ALK*, *ROS1*, *BRAF, MET*	Blood collected within 12 weeks of tissue biopsy and no therapy between tissue and blood samples
Remon et al (2019)[147,]	Patients with advanced NSCLC enrolled in single-center, prospective observational study in France. Patients were either treatment naive for advanced disease or who had a tissue-based molecular profile that failed or was not performed on the primary tissue sample (treated rescue cohort)	Prospective	*EGFR*, *BRAF, MET*	Time between tissue biopsy and blood collection less than 100 days; median time between tissue biopsy and liquid biopsy collection was 34 days.
FoundationOne Liquid CDx				
FDA SSED (2020)[148,]	Patients with NSCLC previously tested for EGFR mutations by the approved cobas EGFR Mutation Test v2 from unrelated clinical trials	Retrospective	*EGFR*	Timing not described; cobas plasma-based test results were used as the reference standard; no direct comparison to tissue

AURA3: A Phase III, Open Label, Randomized Study of AZD9291 Versus Platinum-Based Doublet Chemotherapy for Patients With Locally Advanced or Metastatic Non-Small Cell Lung Cancer Whose Disease Has Progressed With Previous Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor Therapy and Whose Tumours Harbour a T790M Mutation Within the Epidermal Growth Factor Receptor Gene; ctDNA: circulating tumor DNA; EGFR: epidermal growth factor receptor; FDA: U.S. Food and Drug Administration; NILE: Non-invasive versus Invasive Lung Evaluation; NSCLC: non-small-cell lung cancer; RCT: randomized controlled trial; SSED: Summary of Safety and Effectiveness Data; TKI: tyrosine kinase inhibitor.
[a] Noting *EGFR*, *ALK*, *ROS1*, *MET, RET,* and *BRAF* variants only.

Table 26 summarizes the results of clinical validation studies of liquid biopsy compared with tissue biopsy as a reference standard, with the exception of FoundationOne Liquid CDx, which was compared to cobas EGFR Mutation Test v2 in a non-inferiority study. Although tissue biopsy is not a perfect reference standard, the terms sensitivity and specificity will be used to describe the PPA and NPA, respectively. For detection of *EGFR*-sensitizing variants, the cobas test has multiple clinical validation studies of sufficient quality and the performance characteristics are well characterized with generally high specificity (>96%). For the detection of *EGFR*-resistance variants, fewer studies are available and estimates of specificity are more variable. For the detection of less prevalent driver mutations, such as *ALK* and *ROS1* translocations, *BRAF*V600E, RET fusions, and MET exon 14 skipping, few publications are available and, in these publications, very few variants have been identified.

Table 26. Results of Clinical Validity Studies of Liquid Biopsy With Tissue Biopsy as the Reference Standard

Study	Initial N	Final N	Excluded Samples	Sensitivity (95% CI)	Specificity (95% CI)
Cobas EGFR test					
Papadimitrakopoulou et al (2020) (AURA3)[130,]	562		No plasma sample; mainland China		

Study	Initial N	Final N	Excluded Samples	Sensitivity (95% CI)	Specificity (95% CI)
			patients; withdrawn informed consent; invalid tests		
EGFR exon 19 deletion (sensitizing)		216		84 (78 to 90)	99 (92 to 100)
EGFR exon 21 substitution (L858R, sensitizing)		216		60 (47 to 72)	100 (98 to 100)
EGFR exon 20 (T790M, resistance)		215		51 (44 to 58)	NA[d]
Jenkins et al (2017)[131,]					
EGFR exon 19 deletion (sensitizing)	710	551	No plasma sample	85 (81 to 89)	98 (95 to 100)
EGFR exon 21 substitution (L858R, sensitizing)				76 (69 to 82)	98 (96 to 99)
EGFR exon 20 (T790M, resistance)	710	551		61 (57 to 66)	79 (70 to 85)
FDA SSED (2016)[132,]					
EGFR-sensitizing variants	601	431	Insufficient plasma; invalid test result	77 (71 to 82)	98 (95 to 99)
Karlovich et al (2016)[133,]					
EGFR-sensitizing variants	174	110	No matching tumor and plasma or inadequate tissue	73 (62 to 83)	100 (86 to 100)
EGFR exon 20 (T790M, resistance)	174	110		64 (45 to 80)	98 (91 to 100)
Thress et al (2015)[134,]					
EGFR exon 19 deletion (sensitizing)	NR	72	Inadequate tumor tissue	82 (63 to 94)	97 (83 to 100)
EGFR exon 21 substitution (L858R, sensitizing)	NR	72		87 (66 to 97)	97 (85 to 100)
EGFR exon 20 (T790M, resistance)	NR	72		73 (57 to 86)	67 (45 to 84)
Mok et al (2015)[135,]					
EGFR-sensitizing variants	397	238	Insufficient plasma or tissue; invalid test result	75 (65 to 83)	96 (92 to 99)
Weber et al (2014)[136,]					
EGFR-sensitizing and -resistance variants	199[a]	196	Inadequate tumor tissue	61 (41 to 78)	96 (92 to 99)
Guardant360 CDx					
FDA SSED (2020)[149,]					
EGFR-sensitizing variants; FLAURA	556	380	No pretreatment plasma; invalid test result; informed consent withdrawn; China mainland patient	75 (70 to 79)	NR[d]
EGFR exon 19 deletion (sensitizing)		380		78 (72 to 83)	99 (96 to 100)
EGFR exon 21 substitution (L858R, sensitizing)		380		71 (62 to 78)	99 (97 to 100)
EGFR-sensitizing variants; NILE	92	88	No pretreatment plasma or tissue; informed consent withdrawn; invalid test result	100 (77 to 100)	99 (93 to 100)
Papadimitrakopoulou et al (2020) (AURA3)[130,]	562		No plasma sample; mainland China		

Study	Initial N	Final N	Excluded Samples	Sensitivity (95% CI)	Specificity (95% CI)
			patients; withdrawn informed consent; invalid tests		
EGFR exon 19 deletion (sensitizing)		208		79 (72 to 86)	99 (92 to 100)
EGFR exon 21 substitution (L858R, sensitizing)		208		63 (50 to 74)	100 (98 to 100)
EGFR exon 20 (T790M, resistance)		207		66 (59 to 72)	NA[d]
Leighl et al (2019)[139,]	307		No pretreatment ctDNA (4); no tissue genotyping (4); received prohibited treatment (8); metastatic disease not confirmed (4); squamous cell (5)		
EGFR exon 19 deletion (sensitizing)		223		81 (60 to 95)[c]	100 (98 to 100)[c]
EGFR exon 21 substitution (L858R, sensitizing)		223		90 (56 to 100)[c]	100 (98 to 100)[c]
ALK fusion		215		63 (24 to 91)[c]	100 (98 to 100)[c]
ROS1 fusion		153		0 (0 to 84)[c]	100 (98 to 100)[c]
BRAF V600E		92		100 (16 to 100)[c]	100 (96 to 100)[c]
MET exon 14 skipping		57		80 (30 to 99)[c]	98 (88 to 100)[c]
RET fusion		57		None identified	None identified
Schwaederle et al (2017)[140,]					
EGFR variants (various)	88	34	No tissue	54 (25 to 81)	90 (70 to 99)
Thompson et al (2016)[141,]	102	50	Insufficient tissue		
EGFR-sensitizing				79 (58 to 93)[c]	100 (87 to 100)[c]
EGFR-resistance				50 (7 to 93)[c]	87 (74 to 95)[c]
ALK fusion				None identified	None identified
ROS1 fusion				None identified	None identified
BRAF V600E				100 (2.5 to 100)[c]	100 (93 to 100)[c]
Villaflor et al (2016)[142,]	68	31	No tissue		
EGFR-sensitizing				63 (24 to 91)[c]	96 (78 to 100)[c]
ROS1				None identified	None identified
BRAF V600E				None identified	None identified
Palmero et al (2021)	199	186	No tissue or no blood sample collected pretreatment		
ALK				40.0 (5.3 to 85.0)	99.2 (95.7 to 99.9)
EGFR				66.7 (49.0 to 81.4)	100% (96.8% 100%)
Other variants				Not possible to calculate due to lack of matched tissue testing results or insufficient tissue sample	
OncoBEAM					
Ramalingam et al (2018)[143,]	60	51	Tissue or plasma not available		
EGFR exon 19 deletion (sensitizing)				82 (60 to 95)	100 (88 to 100)
EGFR exon 21 substitution (L858R, sensitizing)				63 (41 to 81)	96 (81 to 100)
EGFR exon 20 (T790M, resistance)				100 (40 to 100)	98 (89 to 100)

Study	Initial N	Final N	Excluded Samples	Sensitivity (95% CI)	Specificity (95% CI)
Karlovich et al (2016)[133,]					
EGFR-sensitizing variants	174	77	No matching tumor and plasma or inadequate tissue	82 (70 to 90)	67 (9 to 99)
EGFR exon 20 (T790M, resistance)	174	77		73 (58 to 85)	50 (26 to 74)
Thress et al (2015)[134,]					
EGFR exon 19 deletion (sensitizing)	NR	72	Inadequate tumor tissue	82 (63 to 94)	97 (83 to 100)
EGFR exon 21 substitution (L858R, sensitizing)				87 (66 to 97)	97 (85 to 100)
EGFR exon 20 (T790M, resistance)	NR	72		80 (65 to 91)	58 (36 to 78)
Biodesix ddPCR					
Papadimitrakopoulou et al (2020) (AURA3)[130,]	562		No plasma sample; mainland China patients; withdrawn informed consent; invalid tests		
EGFR exon 19 deletion (sensitizing)		190		73 (64 to 80)	100 (94 to 100)
EGFR exon 21 substitution (L858R, sensitizing)		189		70 (57 to 81)	98 (95 to 100)
EGFR exon 20 (T790M, resistance)		189		66 (59 to 72)	NA[d]
Mellert et al (2017)[144,]					
EGFR exon 19 deletion (sensitizing)		92		96 (NR)	100 (NR)
EGFR exon 21 substitution (L858R, sensitizing)		73		100 (NR)	100 (NR)
EGFR exon 20 (T790M, resistance)		55		87 (NR)	100 (NR)
ALK fusion		24		~85 (NR)	100 (NR)
ctDx-Lung					
Paweletz et al (2016)[145,]	NR	48	NR		
EGFR exon 19 deletion (sensitizing)				89 (65 to 99)[c]	100 (88 to 100)[c]
EGFR exon 21 substitution (L858R, sensitizing)				67 (9 to 99)[c]	100 (92 to 100)[c]
ALK fusion				67 (9 to 99)[c]	100 (92 to 100)[c]
ROS1 fusion				100 (16 to 100)[c]	100 (92 to 100)[c]
BRAF V600E				0 (0 to 98)[c]	100 (92 to 100)[c]
InVision					
Pritchet et al (2019)[146,]	264		Missing tissue or ctDNA testing		
EGFR exons 18-21		114		100 (75 to 100)[b,c]	100 (96 to 100)[b,c]
ALK/ROS1 fusions		234		40 (5 to 85)[b,c]	100 (98 to 100)[b,c]
BRAF V600E		109		100 (48 to 100)[b,c]	100 (97 to 100)[b,c]
MET exon 14 skipping		139		50 (14 to 86)[b,c]	100 (97 to 100)[b,c]
Remon et al (2019)[147,]	156		Missing tissue or ctDNA testing		
EGFR exons 18-21		78		88 (47 to 100)	98 (91 to 100)
BRAF V600E		75		50 (1 to 100)	100 (95 to 100)
MET exon 14 skipping		48		33 (2 to 87)	100 (90 to 100)
FoundationOne Liquid CDx					
FDA SSED (2020)[149,]	280		Samples in which there was insufficient plasma to process		

Study	Initial N	Final N	Excluded Samples	Sensitivity (95% CI)	Specificity (95% CI)
			both replicates of the cobas reference test		
EGFR exon 19 deletion (sensitizing)[e]		135		95 (83 to 99)[c] (rep 1) 95 (83 to 99)[c] (rep 2)	96 (89 to 99)[c] (rep 1) 96 (89 to 99)[c] (rep 2)
EGFR exon 21 substitution (L858R, sensitizing)[e]		133		95 (83 to 99)[c] (rep 1) 100 (89 to 100)[c] (rep 2)	96 (89 to 99)[c] (rep 1) 94 (86 to 97)[c] (rep 2)
EGFR-sensitizing (combined)[e]		177		98 (91 to 100)[c] (rep 1) 98 (91 to 100)[c] (rep 2)	96 (89 to 99)[c] (rep 1) 93 (85 to 97)[c] (rep 2)

CI: confidence interval; ctDNA: circulating tumor DNA; *EGFR*: epidermal growth factor receptor; FDA: U.S. Food and Drug Administration; NA: not applicable; NR: not reported; rep: replicate; SSED: Summary of Safety and Effectiveness Data.
[a] Unclear how many samples were eligible but not included
[b] Only included the subset of patients with at least 1 mutation detected by liquid biopsy
[c] Not reported; calculated based on data provided
[d] Not applicable; cannot calculate due to lack of mutation negative samples
[e] Compared to Roche cobas EGFr Mutation Test v2

The purpose of the limitations tables (see Tables 27 and 28) is to display notable limitations identified in each study. This information is synthesized as a summary of the body of evidence and provides the conclusions on the sufficiency of the evidence supporting the position statement.

Table 27. Study Relevance Limitations of Clinical Validity Studies of Liquid Biopsy With Tissue Biopsy as the Reference Standard

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Multiple tests					
Papadimitrakopoulou et al (2020) (AURA3)[130,]					
Cobas EGFR test					
Jenkins et al (2017)[131,]					
FDA SSED (2016)[132,]	4. Performed in Asia				
Karlovich et al (2016)[133,]					
Thress et al (2015)[134,]					
Mok et al (2015)[135,]	4. Performed in Asia				
Weber et al(2014)[136,]					
Guardant360 CDx					
FDA SSED (2020)[132,]	4. Plasma from FLAURA patients negative for *EGFR* mutations by tissue testing was not available to represent plasma-positive, tissue-negative portion of the intended use population	2. Two index test versions were combined		3. Performance characteristics not stratified according to respective Guardant360 test version	
Leighl et al (2019)[139,]					
Schwaederle et al (2017)[140,]					
Thompson et al (2016)[141,]					

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Villaflor et al (2016)[142,]					
OncoBEAM					
Ramalingam et al (2018)[143,]	4. Performed in Asia				
Karlovich et al (2016)[133,]					
Thress et al (2015)[134,]					
Biodesix ddPCR					
Mellert et al (2017)[144,]	3. Patient characteristics unclear				
ctDx-Lung					
Paweletz et al (2016)[145,]	2. Unclear if same as current marketed version				
InVision					
Pritchet et al (2019)[146,]	4: Calculation of performance characteristics only included subset of patients with at least 1 mutation detected by liquid biopsy				
Remon et al (2019)[147,]					
FoundationOne Liquid CDx					
FDA SSED (2020)[148,]	3. Eligibility criteria for retrospective-sourced plasma samples unclear 4. Differences in smoking status, race, and gender were observed between the study population and the FLAURA study patients		3. Test compared to approved plasma-based cobas test in non-inferiority study; no direct comparisons to tissue-based reference were conducted	1. Plasma from FLAURA study patients was not used and therefore survival outcomes were not reported	

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
FDA: U.S. Food and Drug Administration; SSED: Summary of Safety and Effectiveness Data.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity, and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true-positives, true-negatives, false-positives, false-negatives cannot be determined).

Table 28. Study Design and Conduct Limitations of Clinical Validity Studies of Liquid Biopsy With Tissue Biopsy as the Reference Standard

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Multiple tests						
Papadimitrakopoulou et al (2020) (AURA3)[130,]						
Cobas EGFR test						
Jenkins et al (2017)[131,]						
FDA SSED (2016)[132,]						
Karlovich et al (2016)[133,]						
Thress et al (2015)[134,]			1. Both samples collected after progression and before next treatment but time between blood and tissue sample collection not described			1. Precision estimates not reported but calculated based on data provided
Mok et al (2015)[135,]			1. Time between blood and tissue sample collection not described			1. Precision estimates not reported but calculated based on data provided
Weber et al(2014)[136,]	1,2. Unclear how patients were selected		2. Plasma not collected at time of tissue biopsy			1. Precision estimates not reported but calculated based on data provided
Guardant360 CDx						
FDA SSED (2020)[132,]			2. Time between tissue and plasma sample unclear; subset of samples collected after progression or treatment discontinuation			
Leighl et al (2019)[139,]			2.Time between tissue and plasma sample unclear			1. Precision estimates not reported but calculated based on data provided
Schwaederle et al (2017)[140,]						1. Precision estimates not reported but calculated

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
						based on data provided
Thompson et al (2016)[141],			1.Time between tissue and blood collection was up to >2 y, median not given			1. Precision estimates not reported but calculated based on data provided
Villaflor et al (2016)[142],	1,2. Unclear how patients were selected		1.Time between tissue and blood collection was up 7y, median 1.4 y			1. Precision estimates not reported but calculated based on data provided
OncoBEAM						
Ramalingam et al (2018)[143],			1. Time between blood and tissue sample collection not described			
Karlovich et al (2016)[133],						
Thress et al (2015)[134],			1. Both samples collected after progression and before next treatment but time between blood and tissue sample collection not described			1. Precision estimates not reported but calculated based on data provided
Biodesix ddPCR						
Mellert et al (2017)[144],	1,2. Unclear how patients were selected		1. Time between blood and tissue sample collection not described			1. Precision estimates not reported cannot be calculated based on data provided
ctDx-Lung						
Paweletz et al (2016)[145],	1,2. Unclear how patients were selected		1. Time between blood and tissue sample collection not described			1. Precision estimates not reported but calculated based on data provided
InVision						
Pritchet et al (2019)[146],						1. Precision estimates

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
						not reported but calculated based on data provided
Remon et al (2019)[147,]						
FoundationOne Liquid CDx						
FDA SSED (2020)[148,]	2. Selection unclear		1. Timing of index and reference tests not described		2. High number of samples excluded due to requirement for sufficient plasma for 2 replicates of reference test	1. Confidence intervals and/or p values not reported; confidence intervals for precision estimates not reported but calculated based on data provided; power calculations and non-inferiority margins not described

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
FDA: U.S. Food and Drug Administration; SSED: Summary of Safety and Effectiveness Data.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison with other tests not reported.

A summary of the previously described published evidence assessing the clinical validity of the specific commercial tests is shown in Table 29. The cobas test has at least 6 studies (n>1500), Guardant360 CDx has at least 5 studies (n> 800), OncoBEAM has at least 3 studies (n>200), and InVision has at least 2 studies (n>400), with the majority being of adequate quality to demonstrate the performance characteristics relative to a tissue test with tight precision estimates for specificity for EGFR TKI-sensitizing variants. The FoundationOne Liquid CDx test has 1 trial (n=177) reporting non-inferiority to the cobas test; however, direct comparisons to tissue-based testing were not conducted. Other tests have promising preliminary results but none of the remaining available tests other than the cobas, Guardant360 CDx, OncoBEAM, and InVision tests have multiple studies of adequate quality to estimate the performance characteristics with sufficient precision for *EGFR* TKI-sensitizing variants.

Table 29. Summary of Published Evidence[a] Assessing the Clinical Validity of Commercial Liquid Biopsy Tests for *EGFR* TKI-Sensitizing Variants

Test (Method)	Comparison With Tissue Test			Study Quality
	Studies Using Specific Commercial Test (95% CI) and/or Range, %		Available Studies	
	Sens	Spec		
Roche cobas EGFR Mutation Test v2 (RT-PCR)	60-87	96-100	7	Very few limitations identified (Jenkins[131]; FDA SSED[132]; Karlovich[133]; Thress[134]; Mok[135]; Weber[136])
Guardant360 CDx (NGS)	63-100	96-100	5	Long time between tissue and ctDNA tests (Leighl[139];Thompson[141]; Villaflor[142]); unclear patient selection (Villaflor[142]); variants not stratified by type in Schwaederle[140]; very few limitations with Papadimitrakopoulou[130]); outcomes from test versions combined (FDA SSED)[149]
FoundationOne Liquid[c] (NGS)	95-100	93-96	1	Non-inferiority trial with many limitations; no tissue-based comparator; non-inferiority margins not described (FDA SSED)[148]
OncoBEAM	63-82	67-100	3	Few limitations identified (Karlovich[133]; Thress[134]; Rmalingam[143]) Only a few negatives in Karlovich for estimating specificity.
Biodesix (ddPCR)	70-100	100 (NR)[144]	2	Patient characteristics and selection unclear; timing of blood and tissue samples unclear; precision estimates not provided (Mellert[144]; very few limitations with Papadimitrakopoulou[130])
Resolution Bio ctDx-Lung	89 (65 to 99)[b]	100 (88 to 100)[b]	1	Several limitations identified (Paweletz[145])
Biocept (RT-PCR)	NA	NA	0	NA
Circulogene (Theranostics) liquid biopsy test (NGS)	NA	NA	0	NA
InVIsion (Inivata) (NGS)	88-100	98 -100	2	Few limitations identified (Pritchett[146], Remon[147])

CI: confidence interval; ddPCR: digital droplet polymerase chain reaction; *EGFR*: epidermal growth factor receptor; FDA: Food and Drug Administration; NA: not applicable; NGS: next-generation sequencing; NR: not reported; RT-PCR: real-time polymerase chain reaction; Sens: sensitivity; Spec: specificity; SSED: Summary of Safety and Effectiveness Data; TKI: tyrosine kinase inhibitor.

[a] Meeting selection criteria

[b] For *EGFR* deletion 19.

[c] Compared to Roche cobas EGFr Mutation Test v2

Section Summary: Clinical Valid

The cobas test has very high accuracy (area under the receiver operating characteristic curve, 0.96), a sensitivity above 60%, and a specificity above 96% for detection of *EGFR* TKI-sensitizing variants using tissue biopsy as the reference standard. These estimates are consistent across several studies

performed using the test. The studies were performed in Asia, Europe, Australia, and the U.S., primarily in patients with advanced disease of adenocarcinoma histology. The Guardant360 CDx test has 5 studies using tissue biopsy as the reference standard performed in the U.S. in the intended-use population for *EGFR* TKI-sensitizing variants. Estimates of specificity are consistently 96% or higher. Likewise, the OncoBEAM test has 3 studies using tissue biopsy in Asia, Europe, Australia, and the U.S. in the intended-use population, 2 of which provide precise estimates for specificity that are very high (>96%). The InVision test has 2 studies using tissue biopsy as the reference standard in the U.S. and France in the intended-use population, both provide precise estimates for specificity (>96%).

For tests other than the cobas test, Guardant360 CDx, OncoBEAM, and InVision for detecting EGFR TKI-sensitizing variants, few studies were identified that evaluated the clinical validity of these commercially available tests for *EGFR* variants in NSCLC.

A single non-inferiority trial of FoundationOne Liquid CDx compared to the plasma-based cobas EGFR Mutation Test v2 was identified. However, this study does not meet selection criteria due to use of a non-tissue comparator and non-inferiority margins were not described in the FDA summary.

For tests of other, less prevalent, variants, such as ALK translocations, ROS1 translocations, RET fusions, MET exon 14 skipping, and BRAF V600E variants, few studies were identified that evaluated the clinical validity of any commercially available tests, and in these studies, very few variants were detected; therefore, performance characteristics are not well-characterized.

Few studies have examined the performance of liquid biopsy for the detection of T790M variants associated with *EGFR* TKI resistance and several different tests were used in the studies. Detection of these variants is potentially important for liquid biopsy because this variant is of interest after the initiation of treatment, when biopsies may be more difficult to obtain. Unlike the high specificities compared with tissue biopsy demonstrated for *EGFR* variants associated with TKI sensitivity, the moderate specificity means that liquid biopsy often detects T790M variants when they are not detected in tissue biopsy. Sacher et al (2016) suggested that these false-positives might represent tumor heterogeneity in the setting of treatment resistance, such that the T790M status of the biopsied site might not represent all tumors in the patient.[150,]

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, more effective therapy, or avoid unnecessary therapy or testing.

Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

No RCTs comparing management with and without liquid biopsy were identified.

Evidence on the ability of liquid biopsy to predict treatment response similar to, or better than, a tissue biopsy is also of interest. If the 2 tests are highly correlated, they are likely to stratify treatment response similarly overall. To understand the implications of "false-positive" and "false-negative" liquid biopsies for outcomes, patients who have discordant results on liquid biopsy and standard biopsy are of particular interest. If patients who are negative for *EGFR*-sensitizing or -resistance variants on liquid biopsies but positive for those variants on standard biopsies respond to EGFR TKIs (i.e., erlotinib, gefitinib, afatinib, osimertinib), it would suggest that the standard biopsy was correct and the liquid biopsy results were truly false-negatives. If patients with positive liquid biopsies and negative tissue biopsies for *EGFR* variants respond to EGFR TKIs, it would suggest that the positive liquid biopsies were correct rather than false-positives.

Chain of Evidence

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

The clinical utility might alternatively be established based on a chain of evidence. Assuming that tissue biomarkers are the standard by which treatment decisions are made, an agreement between liquid and tissue biopsies would infer that treatment selection based on liquid or tissue biopsies is likely to yield similar outcomes. Also, a liquid biopsy would reduce the number of patients undergoing tissue sampling and any accompanying morbidity.

Depending on the analytic method, compared with a tissue biopsy, liquid biopsy appears somewhat less sensitive with generally high specificity in detecting an *EGFR* TKI-sensitizing variant that can predict outcomes. This finding suggests that an *EGFR* TKI-sensitizing variant identified by liquid biopsy could be used to select a treatment with reflex to tissue biopsy. However, evidence directly demonstrating the predictive ability of liquid biopsy would be most convincing. Also, outcomes in patients who have discordant results on liquid and tissue biopsy are of particular interest.

Therefore, evidence on the ability of liquid biopsy to predict treatment response. Liquid biopsy could improve patient outcomes if it predicts treatment response similar to, or better than, tissue biopsy. Treatment response as measured by OS outcomes would be most informative. PFS can be difficult to interpret because of confounding influences in retrospective observational subgroup analyses. Response rate may be more informative than PFS.

Some studies were nested in nonrandomized designs or RCTs. This structure potentially permits comparing associations between liquid biopsy and tissue biopsy results with outcomes. Because it has already been demonstrated by the prior studies that liquid biopsy and tissue biopsy are moderately correlated, they should both be associated with either prognosis of disease or prediction of treatment response as has been demonstrated for tissue biopsy. However, if liquid biopsy results are more strongly associated with outcomes, it might be considered better than tissue biopsy (considered the reference standard). Although liquid biopsy had a high specificity for *EGFR*-sensitizing variants (>90%) in almost all studies, false-positives could be a concern in patient populations with a low prevalence of treatable variants. Known variability of tumor tissue sampling raises concern whether false-positive liquid biopsies represent cases in which the tissue biopsy is falsely negative.

Sufficient numbers of patients have not been studied in which all possible combinations of liquid biopsy and tissue biopsy results have been analyzed for associations with patient outcomes. Available patient outcome data for studies evaluating *EGFR* TKI-sensitizing and *EGFR* TKI-resistance variants are shown in Tables 30 and 31, respectively.

Table 30. *EGFR* TKI-Sensitizing Variants: Treatment Response Stratified by Liquid and Tissue Biopsy

Study/Patient Group	Country	Disease Stage	Technology Used to Detect ctDNA	Sample Sizes	Treatment Response	
				n	Outcomes	p
Guo et al (2019)[151]; newly diagnosed *EGFR*-positive and -negative patients treated with EGFR TKIs	China	IV (85.6%)	ddPCR	PFS (95% CI), mo		
				n	EGFR TKI	p

Study/Patient Group	Country	Disease Stage	Technology Used to Detect ctDNA	Sample Sizes	Treatment Response		
				Tissue positive and liquid positive			
				26	15 (NR)		
				Tissue positive and liquid negative			
				12	11.5 (NR)		
				Tissue negative and liquid positive			
				5	NR		
				Tissue unknown and liquid positive			
				30	13 (NR)		
				Tissue negative and liquid negative			
				49	5.4 (NR)		
FDA SSED (2020)[149,] ; phase 3 FLAURA RCT in treatment-naive and *EGFR*-positive[a] patients	Multinational[b]	IIIB, IV	Guardant360 CDx	PFS HR (95% CI) for Osimertinib vs. Gefitinib or Erlotinib			
				n	Osimertinib	Gefitinib or Erlotinib	p
				Overall (i.e., tissue positive)			
				556	0.46 (0.37 to 0.57)		<.0001
				Liquid positive and tissue positive			
				304	0.41 (0.31 to 0.54)		<.0001
Zhang et al (2017)[152,]; *EGFR*-positive and -negative patients treated with EGFR TKIs	China	IIIB, IV	ddPCR	PFS (95% CI), d (EGFR TKIs; 82% Gefitinib)			
				Tissue positive vs tissue negative			
				114	342 (291 to 393)	60 (0 to 124)	
				Tissue positive and liquid positive vs liquid negative			
				80	334 (298 to 371)	420 (100 to 740)	
				Tissue negative and liquid positive			
				3	133, 410, and 1153		
FDA SSED (2016)[153,]; phase 3 ENSURE RCT in tissue *EGFR*-positive[a]	China, Malaysia, Philippines	IIIB, IV	cobas	PFS HR (95% CI) for Chemotherapy vs. Erlotinib			
				Overall (i.e., tissue positive)			p
				179	0.33 (0.23 to 0.47)		
				Patients with positive tissue and liquid			
				137	0.29 (0.19 to 0.45)		
				Patients with positive tissue and negative liquid			
				42	0.37 (0.15 to 0.90)		
Karachaliou et al (2015)[154,]; EURTAC trial in tissue *EGFR*-positive[a]	France, Italy, Spain	IIIB, IV	Multiplex 5´ nuclease rt-PCR (TaqMan)	OS (95% CI) for Erlotinib vs. Chemotherapy, mo			
				n	Erlotinib	Chemotherapy	p
				Overall (i.e., tissue positive)			
				97	25.8 (17.7 to 31.9)	18.1 (15.0 to 23.5)	.14
				All patients with exon 19 deletion in tissue			
				56	30.4 (19.8 to 55.7)	18.9 (10.4 to 36.2)	.22

Study/Patient Group	Country	Disease Stage	Technology Used to Detect ctDNA	Sample Sizes	Treatment Response		
				Patients with exon 19 deletion in both tissue and ctDNA			
				47	34.4 (22.9 to NR)	19.9 (9.8 to 36.2)	.23
				Patients with exon 19 deletion in tissue but not ctDNA			
				9	13.0 (8.9 to 19.8)	15.5 (0.3 to NR)	.87
				All patients with L858R variant in tissue			
				41	17.7 (6.3 to 26.8)	17.5 (8.2 to 23.5)	.67
				Patients with L858R variant in both tissue and in ctDNA			
				29	13.7 (2.6 to 21.9)	12.6 (7.1 to 23.5)	.67
				Patients with L858R variant in tissue but not in ctDNA			
				12	29.4 (8.6 to 63.0)	25.6 (16.1 to NR)	.64

CI: confidence interval; ctDNA: circulating tumor DNA; ddPCR: droplet digital polymerase chain reaction; *EGFR*: epidermal growth factor receptor; FDA: U.S. Food and Drug Administration; HR: hazard ratio; NGS: next-generation sequencing; NR: not reported; OS: overall survival; PFS, progression-free survival; RCT: randomized controlled trial; rt-PCR: real-time polymerase chain reaction; SSED: Summary of Safety and Effectiveness; TKI: tyrosine kinase inhibitor.

[a] Exon 19 deletion or L858R variant.

[b] U.S., Australia, Canada, Europe, Brazil, Asia

In Table 31 (sensitizing variants), the SSED document supporting the approval of Guardant360 CDx reported clinical outcome data derived from the FLAURA study, a randomized phase 3 trial of osimertinib versus gefitinib or erlotinib in the first-line treatment of patients with locally advanced and metastatic NSCLC.[149,] Patients with *EGFR* variants detected from tissue biopsies were enrolled (N=556). A subset of pretreatment plasma samples were tested with an earlier test version, Guardant360 LDT, as part of an exploratory analysis of patients who had experienced disease progression or drug discontinuation (n=189). Pre-treatment plasma samples were only available for 252/556 patients (45%) who were not previously tested with Guardant360 LDT. To mitigate selection bias, results from both CDx and LDT tests were combined and reported as Guardant360 outcomes (n=441). An *EGFR*-sensitizing mutation was present in 304 and absent in 110 patients. Samples from 27 patients failed testing. The observed PFS for the Guardant360 population (HR, 0.41; 95% CI, 0.31 to 0.54) was similar to that observed in full FLAURA dataset (HR, 0.46; 95% CI, 0.37 to 0.57). Investigators utilized models to impute missing randomized data and consider the potential effect of Guardant360 CDx versus LDT discordance; these imputed results did not significantly deviate from the original observations (HR, 0.40 to 0.42). The SSED document also provided a concordance analysis between Guardant360 CDx and Guardant360 LDT test versions in NSCLC patients for *EGFR* exon 19 deletions, L858R, and T790M variants. Sensitivities were 96.7%, 98.1%, and 95.6%, respectively. Specificities were 98.1%, 97.2%, and 95.2%, respectively.

In Guo et al (2019), median PFS in the subset of newly diagnosed patients treated with EGFR TKIs (n=122) was compared for groups of patients with biomarker status determined by tissue biopsy and liquid biops.[151,] Patients with *EGFR* mutations in either tissue or liquid had a significantly improved PFS (13 months, n=68) compared to patients harboring wild-type *EGFR* in both tissue and liquid (5.4 months, n=49, p<.001). Two of 5 patients with tissue negative and liquid positive EGFR mutation status exhibited a PFS of 8 and 14 months, respectively. Overall PFS for this subset of patients was not reported.

The SSED document supporting the approval of the cobas EGFR Mutation Test v2 reported clinical outcome data derived from a randomized phase 3 trial of erlotinib versus gemcitabine plus cisplatin as first-line treatment of NSCLC.[132,] However, only patients with *EGFR* variants detected from tissue biopsies were enrolled. In the overall study, erlotinib showed substantial improvement in PFS over

chemotherapy (HR, 0.33; 95% CI, 0.23 to 0.47), consistent with the known efficacy of erlotinib in patients with a sensitizing *EGFR* variant. Among the subset of patients with positive liquid biopsy results (77% [137/179]), erlotinib showed a similar improvement in PFS (HR, 0.29; 95% CI, 0.19 to 0.45). However, the finding has limited meaning because all patients had positive tissue biopsies, thus showing a similar result. Those with negative liquid biopsies (n=42) also showed a similar magnitude of benefit of erlotinib (HR, 0.37; 95% CI, 0.15 to 0.90), which would be consistent with liquid biopsies being false-negatives.

In Zhang et al (2017), PFS in the subset of patients treated with *EGFR* TKIs (114/215) was compared for groups of patients with biomarker status determined by tissue biopsy and by liquid biopsy.[152,] The patients were primarily treated with gefitinib (n=94); 18 patients received erlotinib, 1 received icotinib, and 1 received afatinib. When patients were stratified by tissue biopsy EGFR status, PFS for *EGFR*-positive subjects was 342 days versus 60 days for *EGFR*-negative subjects (p<.001). Among the tissue biopsy-positive patients, there was no difference in PFS between those with positive (334 days) and negative liquid biopsies (420 days), consistent with the liquid biopsies being false-negatives. Three patients were tissue biopsy-negative, but liquid biopsy-positive; they had PFS with TKI treatment of 133, 410, and 1153 days, respectively. Although the numbers are small, the PFS values are consistent with a response to TKIs and might represent tissue biopsies that did not reflect the correct *EGFR* status.

Table 31. *EGFR* TKI-Resistance Variants: Treatment Response Stratified by Liquid and Tissue Biopsy

Study/Patient Group	Country	Disease Stage	Technology Used to Detect ctDNA	Treatment Response		
				n	Outcomes	
Papadimitrakopoulou et al (2020)[130,]; AURA3 phase 3 trial of patients who progressed on EGFR TKI	Multinational[c]	Locally advanced or metastatic	cobas (RT-PCR); Guardant360 (NGS); Biodesix (ddPCR)	ORR (95% CI) (Osimertinib vs. Chemotherapy)		
			Subgroup	n	Osimertinib	Chemotherapy
			T790M+, tissue	279, 140	71 (65 to 76)	31 (24 to 40)
			T790M+ liquid (cobas)	111, 48	76 (67 to 83)	45 (31 to 60)
			T790M+, liquid (Guardant360)	137, 53	68 (59 to 76)	40 (27 to 54)
			T790M-, liquid (cobas)	101, 47	71 (61 to 79)	28 (16 to 42)
			T790M-, liquid (Guardant360)	72, 29	78 (66 to 87)	17 (6 to 36)
				PFS HR (95% CI) (Osimertinib vs. Chemotherapy)		
			T790M+, tissue	419	0.30 (0.23 to 0.41)	
			T790M+, liquid (cobas)	159	0.42 (0.29 to 0.63)	
			T790M+, liquid (Guardant360)	190	0.40 (0.28 to 058)	
			T790M-, liquid (cobas)	148	0.31 (0.20 to 0.48)	
			T790M-, liquid (Guardant360)	101	0.27 (0.15 to 0.49)	
				n	Outcomes	
Oxnard et al (2016)[155,]; AURA phase 1 trial of	Multinational[b]	Advanced	BEAMing	ORR (95% CI) (Osimertinib)		

Study/Patient Group	Country	Disease Stage	Technology Used to Detect ctDNA	Treatment Response
patients who progressed on EGFR TKI				
				Liquid positive, tissue positive
				108 64% (54% to 73%)
				Liquid positive, tissue negative
				18 28% (10% to 53%)
				Liquid negative, tissue positive
				45 69% (53% to 82%)
				Liquid negative, tissue negative
				40 25% (13% to 41%)
				PFS (95% CI), mo
				Liquid positive, tissue positive
				111 9.3 (8.3 to 10.9)
				Liquid positive, tissue negative
				18 4.2 (1.3 to 5.6)
				Liquid negative, tissue positive
				47 16.5 (10.9 to NC)
				Liquid negative, tissue negative
				40 2.8 (1.4 to 4.2)
Thress et al (2015)[134]; phase 1 AURA RCT in tissue *EGFR*-positive[a] with progression on EGFR TKI	Multinational[b]	Advanced	cobas; BEAMing ddPCR	ORR (Osimertinib)
				Tissue positive vs. tissue negative
				65 61% vs 29%
				Liquid positive vs. liquid negative
				72 59% vs 35%
				Liquid positive, tissue biopsy negative
				8 38%
Karlovich et al (2016)[133]; patients from observational study and a phase 1 dose-escalation part and a phase 2 study of roceiletinib	U.S., Australia, France, Poland	Advanced	BEAMing	ORR (95% CI) (Rociletinib)
				Liquid positive, tissue positive
				15 73 (51 to 96)
				Liquid positive, tissue negative
				4 25 (0 to 67)
				Liquid negative, tissue positive
				6 50 (10 to 90)
				Liquid negative, tissue negative
				3 33 (0 to 87)
Helman et al (2018)[156]; patients who were tissue *EGFR* T790M-positive from the TIGER-X and TIGER-2 studies of roceiletinib	U.S.	Advanced or metastatic	Guardant360, NGS	ORR (95% CI) (Rociletinib)
				Tissue positive
				77 29.9% (20.0 to 41.4)
				Liquid positive

Study/Patient Group	Country	Disease Stage	Technology Used to Detect ctDNA	Treatment Response
				63 28.6% (17.9 to 41.3)
				PFS (95% CI), mo
				Tissue positive
				77 4.2 (3.9 to 5.7)
				Liquid positive
				63 4.1 (3.9 to5.6)

BEAM: beads, emulsions, amplification, and magnetics; CI: confidence interval; ctDNA: circulating tumor DNA; ddPCR: droplet digital polymerase chain reaction; *EGFR*: epidermal growth factor receptor; NC: not calculable; ORR: objective response rate; PFS: progression-free survival; RCT: randomized controlled trial; TKI: tyrosine kinase inhibitor.

[a] Exon 19 deletion or L858R variant.

[b] U.S, Australia, France, Germany, Italy, Japan, Korea, Spain, Taiwan, U.K.

[c] U.S., Canada, Mexico, Europe, Asia, Australia

For *EGFR*-resistance variants, Thress et al (2015) examined the response to the experimental therapeutic AZD9291 (osimertinib) by T790M status, determined using a tissue or liquid biopsy (see Table 31).[134,] Patients were not selected for treatment based on T790M status, and there was only moderate concordance between tissue and liquid biopsies. Response rates by tissue biopsy variant identification (61% for positive variants vs 29% for negative variants) were qualitatively similar to the response rates by liquid biopsy variant identification (59% for positive variants vs 35% for negative variants). Formal statistical testing was not presented. However, the authors did report response rates for patients who had positive liquid biopsies but negative tissue biopsies. In these 8 patients, the pooled response rate was 38%. The number of patients is too small to make definitive conclusions but the response rate in these patients is closer to those for patients with negative variants than with positive variants. A source of additional uncertainty in these data is that the therapeutic responses to this experimental agent have not yet been well characterized.

Oxnard et al (2016) compared outcomes by T790M status for liquid biopsy and tissue biopsy in patients enrolled in the escalation and expansion cohorts of the phase 1 AURA study of osimertinib for advanced *EGFR*-variant NSCLC.[155,] Some patients may have overlapped with the Thress et al (2015) study.[134,] Among patients with T790M-negative ctDNA, objective response rate (ORR) was higher in 45 patients with T790M-positive tissue (69%; 95% CI, 53% to 82%) than in 40 patients with T790M-negative tissue (25%; 95% CI, 13% to 41%; p=0.001), as was median PFS (16.5 months vs 2.8 months; p=0.001), which is consistent with false-negative ctDNA results. Among patients with T790M-positive ctDNA, ORR and median PFS were higher in 108 patients with T790M-positive tissue (ORR=64%; 95% CI, 54% to 73%; PFS=9.3 months) than in 18 patients with T790M-negative tissue (ORR=28%; 95% CI, 10% to 53%; p=0.004; PFS=4.2 months; p=0.0002) which is consistent with false-positive ctDNA results. The authors concluded that a T790M-variant ctDNA assay could be used for osimertinib treatment decisions in patients with acquired *EGFR* TKI resistance and would permit avoiding tissue biopsy for patients with T790M-positive ctDNA results.

Karlovich et al (2016) compared outcomes by T790M status for liquid biopsy and tissue biopsy in patients enrolled in the TIGER-X phase 1/2 clinical trial of rociletinib and an observational study in patients with advanced NSCLC.[133,] Rociletinib was an EGFR inhibitor in development for the treatment of patients with *EGFR* T790M-mutated NSCLC but the application for regulatory approval was withdrawn in 2016. The ORR was provided by cross-categories of results of tissue and ctDNA testing (see Table 31). Although CIs overlapped substantially and sample sizes in the cross-categories were small, the ORR was quantitatively largest in patients positive for T790M in both tissue and ctDNA and smaller in patients who were T790M negative in tissue regardless of ctDNA positivity.

Helman et al (2018) compared outcomes in patients with positive T790M status for liquid biopsy and tissue biopsy in patients enrolled in the TIGER-X and TIGER-2 trials of rociletinib.[156,] The ORR and PFS

were provided for patients who were tissue positive and for patients who were liquid positive (see Table 31). Both ORR and PFS were similar for the 77 patients who were identified as positive for T790M by tissue biopsy and the 63 patients identified as positive by ctDNA. Thus, 63 of 77 patients (81.8%) who had been identified as positive by tissue biopsy were also identified as positive by liquid biopsy, and this did not affect outcomes for treatment with rociletinib. As noted above, the application for regulatory approval of rociletinib was withdrawn, limiting interpretation of the effect of rociletinib.

Papadimitrakopoulou et al (2020) compared outcomes in tissue-positive T790M patients enrolled in the AURA3 (A Phase III, Open Label, Randomized Study of AZD9291 Versus Platinum-Based Doublet Chemotherapy for Patients With Locally Advanced or Metastatic Non-Small Cell Lung Cancer Whose Disease Has Progressed With Previous Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor Therapy and Whose Tumours Harbour a T790M Mutation Within the Epidermal Growth Factor Receptor Gene) phase 3 trial of osimertinib vs platinum-pemetrexed chemotherapy after progression on *EGFR* TKI therapy.[130,] ORR and PFS HR was reported by mutation status as determined by both cobas and Guardant360 plasma tests compared to tissue as reference (see Table 31). PFS was prolonged in randomized patients (tissue T790M-positive) with a T790M-negative cobas plasma result in comparison with those with a T790M-positive plasma result in both osimertinib (median, 12.5 vs 8.3 months) and platinum-pemetrexed groups (median, 5.6 vs 4.2 months); similar outcomes were observed with Guardant360. The Guardant360 test demonstrated a significantly greater sensitivity for detection of the T790M variant compared to the cobas test ([66%, 95%CI, 59% to 72%] vs [51%, 95% CI, 44% to 58%]). Overall, patients with tissue-positive NSCLC and liquid-negative T790M status were associated with longer PFS, which may be attributable to a lower disease burden. Plasma T790M detection was associated with larger median baseline tumor size and the presence of extrathoracic disease. This observation is consistent with other studies that have observed improved plasma test sensitivity in patients with advanced stage disease[157,] and in treatment-naive patients[158,]. However, overall response rates (ORR) did not significantly differ between liquid-positive and liquid-negative groups in osimertinib-treated patients.

Merker et al (2018) reported a joint review on circulating tumor DNA for the American Society of Clinical Oncology and College of American Pathologists.[159,]The review was not specific to lung cancer but did make the following statements regarding the clinical utility of ctDNA testing for lung cancer:

- "At present, 1 PCR-based ctDNA assay for the detection of EGFR variants in patients with NSCLC has received regulatory approval in the United States and Europe, and PCR-based ctDNA assays for EGFR in NSCLC and KRAS in colorectal cancer are available for commercial use in Europe. These assays have demonstrated clinical validity, but the clinical utility in this setting is based on retrospective analyses."
- "Evidence demonstrated that, although positive EGFR testing results may effectively be used to guide therapy, undetected results should be confirmed with analysis of a tissue sample, if possible. Cases in which the variant is not detected in the ctDNA but is detected in the tissue sample are relatively common, so undetected ctDNA assay results should be confirmed in tumor tissue testing."
- "The challenges of demonstrating clinical utility are illustrated in NSCLC. A major potential issue is that the patient population selected for study inclusion may not be representative of those targeted for the intended clinical use of the ctDNA assay.."

A chain of evidence, based on the sensitivity and specificity of liquid biopsy for the detection of *EGFR* TKI-sensitizing variants such as exon deletion 19 and L858R variants, for a test that has established clinical validity (e.g., the cobas, Guardant360 CDx, OncoBEAM, or InVision tests), can support its utility for the purpose of selecting treatment with *EGFR* TKIs (e.g., erlotinib, gefitinib, afatinib, osimertinib). A robust body of evidence has demonstrated moderate sensitivity (>63%) with high specificities (>95%) for these 4 tests. If a liquid biopsy is used to detect *EGFR* TKI-sensitizing variants with referral (reflex) testing of tissue samples in those with negative liquid biopsies, then the sensitivity of the testing strategy will be equivalent to tissue biopsy, and the specificity will remain

between 95% and 100%. Tissue testing of biomarkers would be avoided in approximately two-thirds of patients with *EGFR* TKI-sensitizing variants. This strategy including tissue testing will be variably efficient depending on the prevalence of detected *EGFR* variants. For example, in U.S. populations with an assumed prevalence of *EGFR* TKI-sensitizing variants of 15% and a 75% sensitive and 97% specific liquid biopsy test (e.g., cobas), 86% of the patients would then require tissue testing to detect the remaining patients with variants; 3% would receive targeted therapy after liquid biopsy who would have received a different systemic therapy if tested with tissue biopsy; and 11% would appropriately receive targeted therapy following liquid biopsy without having to undergo tissue biopsy. In other populations such as Asians where the prevalence of *EGFR* TKI-sensitizing variants is 30% to 50%, the strategy would be more efficient, and a lower proportion of patients would be subject to repeat testing. There is extremely limited evidence on whether the "false-positives" (i.e., patients with positive liquid biopsy and negative tissue biopsy) might have been incorrectly identified as negative on tissue biopsy. In 1 study, 3 patients with negative tissue biopsies and positive liquid biopsies appeared to respond to *EGFR* TKI inhibitors.

The diagnostic characteristics of liquid biopsy for the detection of T790M variants associated with *EGFR* TKI-inhibitor resistance, an indication for treatment with osimertinib, has shown that liquid biopsy is moderately sensitive and moderately specific and thus overall concordance is moderate. Using tissue testing of negative liquid biopsies would increase sensitivity, but because liquid biopsy is not highly specific, it would result in false-positives. Because not enough data are available to determine whether these false-positives represent a faulty tissue reference standard or are correctly labeled as false-positives, outcomes for these patients are uncertain. In 1 study, 8 patients with negative tissue biopsies but positive liquid biopsies had low response rates consistent with those with negative tissue biopsies; and in the AURA study, 18 patients with liquid-positive, tissue-negative results had a low response rate, also consistent with negative tissue biopsy. In the TIGER-X study, 3 patients who were liquid-positive, tissue-negative had low response rates to rociletinib, similar to the other tissue-negative patients. However, although there is higher discordance in the liquid vs tissue results for the resistance variant, retrospective analyses have suggested that patients positive for T790M in liquid biopsy have outcomes with osimertinib that appear to be similar overall to patients positive by a tissue-based assay. In the AURA3 trial, T790M tissue-positive patients treated with osimertinib who were liquid-negative had longer median PFS compared to liquid-positive patients, a trend that may be associated with increased plasma test sensitivity in individuals with advanced disease.

Testing for ALK Rearrangements Using FoundationOne Liquid

FDA-Approved Companion Diagnostic Test

In 2021, FDA approved FoundationOne Liquid as a companion diagnostic to detect ALK rearrangements to select patients for treatment with alectinib. Tissue-based tests have previously been FDA approved for this indication and as companion diagnostics for other *ALK* inhibitors.

Clinical Validity

The evidence for the clinical validity of FoundationOne Liquid to detect ALK rearrangements in patients with NSCLC was assessed in an exploratory retrospective analysis of data from the ALEX trial, described in the FDA Summary of Safety and Effectiveness Data (SSED).[160,] The analysis compared results of tissue testing using the Ventana ALK IHC assay to results from Foundation ACT, a precursor of FoundationOne Liquid. Since all patients in the bridging study were ALK positive, only PPA could be calculated. The FDA summary notes that the poor agreement between the tissue and liquid tests (PPA 69.7%; 95% CI 58.1% to 79.8%) supports the reflex recommendations for plasma negative samples to an FDA-approved tissue test.

The SSED also provides concordance data for FoundationOne Liquid compared to the clinical trial assay (CTA) from the Blood First Assay Screening Trial (BFAST) (discussed below). However, since the CTA used in BFAST was a liquid test and there was no comparison to tissue biopsy, its relevance to the assessment of clinical validity of FoundationOne liquid is limited.

Table 32. Clinical Validity Studies of ALK Rearrangement Testing Using FoundationOne Liquid

Study	Design	Population	Results
ALEX[160,]	Exploratory retrospective analysis of data from a RCT comparing crizotinib to alectinib	76 patients with ALK-positive advanced NSCLC who were randomized to the alectinib arm of the RCT ALK-positive status was established using tissue biopsy (VENTANA ALK IHC test)	ALK positive on liquid biopsy: 53/76 ALK negative on liquid biopsy: 23/76 PPA (comparison of tissue biopsy to FoundationACT liquid, a precursor of FoundationOne Liquid): 69.7% (95% CI 58.1% to 79.8%)
B-FAST[160,]	Clinical bridging study to evaluate the concordance between ALK rearrangement status by the clinical trial assay (FoundationACT liquid) and FoundationOne liquid B-FAST is an ongoing, phase II/III open-label multi-cohort study designed to evaluate targeted therapies or immunotherapy.	Patients with advanced or metastatic NSCLC enrolled in the B-FAST trial.	Initial N: 287 Samples included: 249 Concordance between FoundationOne Liquid and Clinical Trial Assay (FoundationACT liquid) PPA: 84.0% (95% CI 73.7% to 91.4%) NPA: 100% (95% CI 97.9% to 100.0%)

NPA: negative percent agreement; NSCLC: non-small-cell lung cancer; PPA: positive percent agreement; RCT: randomized controlled trial

Table 33. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
ALEX[160,]	All patients had ALK-positive NSCLC on tissue biopsy	The test used was the precursor to the currently marketed test (Foundation ACT liquid)		Unable to calculate sensitivity and specificity from the study design	
B-FAST[160,]		The test used was the precursor to the currently marketed test (Foundation ACT liquid)	2. no comparison to tissue biopsy.		

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true positives, true negatives, false positives, false negatives cannot be determined).

Table 34. Study Design and Conduct Limitations

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
ALEX			Unclear when the liquid biopsy test performed			

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
B-FAST						

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison to other tests not reported.

Clinical Utility

The clinical utility of FoundationOne Liquid to select patients for targeted treatment with alectinib was assessed in BFAST, an ongoing, open-label Phase 2 trial of the association between blood-based NGS of genetic alterations and activity of targeted treatments in patients with advanced or metastatic NSCLC. Dziadziuszko et al (2021) reported data from the ALK-positive cohort from the study. Of 2119 patients screened, 119 (5.4%) had ALK-positive disease and 87 of these were enrolled and received alectinib (73.1%).

Study results are shown in Table 36. The overall response rate was 87.4% and the adverse event profile was consistent with previous phase 3 trials of alectinib.

Table 35. Nonrandomized Trial of ALK Rearrangement Testing Using FoundationOne Liquid to Select Patients for Targeted Treatment- Characteristics

Study	Study Type	Country	Dates	Participants	Treatment	Follow-Up
Dziadziuszko et al (2021)[161], BFAST NCT03178552	Non-randomized, open-label, multicohort	US, Multiple Asian and European countries	2017-2018	87 patients 18 years or older with stage IIIB or IV NSCLC and ALK rearrangements detected by FoundationACT blood-based NGS	Alectinib	12.6 months (range: 2.6 to 18.7)

NGS: next generation sequencing; NSCLC: non-small-cell lung cancer.

Table 36. Nonrandomized Trial of ALK Rearrangement Testing Using FoundationOne Liquid to Select Patients for Targeted Treatment- Results

Study	Overall Response Rate (95% CI)	PFS	OS	Adverse Events (% of Patients)
Dziadziuszko et al (2021)[161], BFAST NCT03178552	87.4% (78.5 to 93.5)	Median PFS not reached 6 month PFS: 90.7% (95% CI 84.5 to 96.8 12 month PFS: 78.4% (95% CI 69.1% to 87.7%)	9 deaths (14.9%) at data cutoff 6-month OS: 97.7% (94.6% to 100%) 12-month OS: 86.8% (79.6% to 94.1%)	Serious AEs: 24% Grade 3 or 4 AEs: 34%

AE: adverse event; CI: confidence interval; OS: overall survival; PFS: progression-free survival.
Limitations of this trial are summarized in Tables 37 and 38. Major limitations of this study include its nonrandomized, open-label design and small sample size.

Table 37. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-up[e]
Dziadziuszko et al (2021)[161,] BFAST NCT03178552			No comparison group	Primary outcome was response rate	

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Not clearly defined; 2. Version used unclear; 3. Delivery not similar intensity as comparator; 4.Not the intervention of interest.
[c] Comparator key: 1. Not clearly defined; 2. Not standard or optimal; 3. Delivery not similar intensity as intervention; 4. Not delivered effectively.
[d] Outcomes key: 1. Key health outcomes not addressed; 2. Physiologic measures, not validated surrogates; 3. No CONSORT reporting of harms; 4. Not establish and validated measurements; 5. Clinical significant difference not prespecified; 6. Clinical significant difference not supported.
[e] Follow-Up key: 1. Not sufficient duration for benefit; 2. Not sufficient duration for harms.

Table 38. Study Design and Conduct Limitations

Study	Allocation[a]	Blinding[b]	Selective Reporting[c]	Data Completeness[d]	Power[e]	Statistical[f]
Dziadziuszko et al (2021)[161,] BFAST NCT03178552	1. Not randomized	1. open-label				

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Allocation key: 1. Participants not randomly allocated; 2. Allocation not concealed; 3. Allocation concealment unclear; 4. Inadequate control for selection bias.
[b] Blinding key: 1. Not blinded to treatment assignment; 2. Not blinded outcome assessment; 3. Outcome assessed by treating physician.
[c] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[d] Data Completeness key: 1. High loss to follow-up or missing data; 2. Inadequate handling of missing data; 3. High number of crossovers; 4. Inadequate handling of crossovers; 5. Inappropriate exclusions; 6. Not intent to treat analysis (per protocol for noninferiority trials).
[e] Power key: 1. Power calculations not reported; 2. Power not calculated for primary outcome; 3. Power not based on clinically important difference.
[f] Statistical key: 1. Analysis is not appropriate for outcome type: (a) continuous; (b) binary; (c) time to event; 2. Analysis is not appropriate for multiple observations per patient; 3. Confidence intervals and/or p values not reported; 4. Comparative treatment effects not calculated.

Section Summary: Testing for ALK Rearrangements Using FoundationOne Liquid

The clinical validity of FoundationOne liquid was assessed in 1 exploratory retrospective analysis of data from an RCT comparing crizotinib to alectinib, and in 1 clinical bridging study that compared FoundationOne Liquid to another liquid biopsy test. There are no studies directly comparing FoundationOne liquid to tissue biopsy. Clinical validity has not been demonstrated in multiple well-designed and conducted studies; therefore, a chain of indirect evidence to show clinical utility cannot be established. One nonrandomized trial directly assessed the clinical utility of FoundationOne Liquid to select patients for treatment with alectinib, but this study was limited by its lack of a control group or comparison to tissue biopsy.

Testing for *MET* Exon 14 Skipping Alterations Using FoundationOne Liquid

FDA-Approved Companion Diagnostic Tests

In 2021, FDA approved FoundationOne Liquid as a companion diagnostic to detect Exon 14 skipping alterations to select patients for treatment with capmatinib. A tissue-based test (FoundationOne) was previously approved as a companion diagnostic for this indication.

Clinical Validity

The clinical validity of FoundationOne Liquid to detect MET Exon 14 skipping alterations in patients with NSCLC was assessed in a clinical bridging study using pre-treatment plasma samples and clinical outcome data from patients with NSCLC enrolled in the GEOMETRY mono-1 trial, an open-label, single arm, Phase 2 trial of targeted treatment with capmatinib (Table 39).[113,] The clinical bridging study is described in the SSED associated with FDA approval of FoundationOne Liquid as a companion diagnostic test for capmatinib.[160,] The SSED notes that based on the low PPA between F1LCDx and the tissue CTA (70.5%; 95% CI 59.1% to 80.3%), since the F1LCDx failed to detect a significant proportion of the patients, a reflex testing using tissue specimens to an FDA approved tissue test will be required, if feasible, if the plasma test is negative.

Table 39. Clinical Validity Study of MET Exon 14 Skipping Alterations Using FoundationOne Liquid

Study	Design	Population	Results
FDA 2021[160,]	Clinical bridging study	150 specimens from patients screened for enrollment into the GEOMETRY mono-1 study	PPA: 55/78 (70.5%; 95% CI, 59.1% to 80.3%) NPA: 100% (72/72; 95% CI, 95.0% to 100%) Overall predictive agreement: 127/150 (84.7%; 95% CI, 78.5% to 89.8%)

NPA: negative percent agreement; PPA: positive percent agreement.

Clinical Utility

There are no studies directly assessing the clinical utility of FoundationOne Liquid to detect MET Exon 14 skipping alterations to select patients for targeted treatment. Because the clinical validity of FoundationOne Liquid has not been established in multiple well-designed and well-conducted studies, a chain of indirect evidence to establish clinical utility cannot be completed.

Section Summary: Testing for MET Exon 14 Skipping Alterations Using FoundationOne Liquid

The clinical validity of FoundationOne liquid was assessed in 1 clinical bridging study that compared FoundationOne Liquid to tissue testing using data from a nonrandomized, open-label, phase 2 study of capmatinib therapy. There is no direct evidence of the clinical utility of FoundationOne Liquid to select patients for targeted therapy for capmatinib. Clinical validity has not been demonstrated in multiple well-designed and conducted studies and, therefore, a chain of indirect evidence to show clinical utility cannot be established.

Section Summary: Clinically Useful

There is little evidence on the comparative validity of tissue and liquid biopsies in discordant cases for *EGFR* TKI-sensitizing variants. Based on the apparent response to *EGFR* TKIs in patients with negative liquid biopsies and positive tissue biopsies in the FDA approval study, these results are consistent with false-negative liquid biopsies. It is unclear whether false-positive liquid biopsies represent errors in the liquid biopsy or inadequacies of a tissue biopsy reference standard. In 1 study, 3 patients with negative tissue biopsies but positive liquid biopsies for biomarkers indicating *EGFR* TKI sensitivity had apparent responses to *EGFR* TKIs, consistent with the tissue biopsies being incorrectly negative.

A chain of evidence based on the sensitivity and specificity of liquid biopsy for the detection of *EGFR* TKI-sensitizing variants for tests with established clinical validity such as the cobas EGFR Mutation Test v2, Guardant360 CDx, OncoBEAM, or InVision can support its utility. The body of evidence has demonstrated moderate sensitivity (>63%), with high specificities (>96%). If a liquid biopsy is used to detect *EGFR* TKI-sensitizing variants with reflex testing of tissue samples in those

with negative liquid biopsies, then the sensitivity of the testing strategy will be equivalent to tissue biopsy, and the specificity will be high. Therefore, outcomes should be similar, but tissue testing of biomarkers would be avoided in approximately two-thirds to three-quarters of patients with *EGFR* TKI-sensitizing variants.

For the other marketed tests that include detection of *EGFR* TKI-sensitizing variants and for liquid biopsy testing of other driver mutations, sufficient evidence of clinical validity is lacking, and thus a chain of evidence cannot be linked to support a conclusion that results for other ctDNA test methods will be similar to those for tissue biopsy.

For *EGFR* TKI-resistance variants, there is little evidence on the comparative validity of tissue and liquid biopsies in discordant cases. Based on the apparent response to osimertinib from the AURA and AURA3 studies with liquid-negative, tissue-positive results, these results are more consistent with false-negative liquid biopsies. In the AURA3 trial, patients with liquid-positive tests were associated with increased disease burden and increased plasma test sensitivity compared to liquid-negative patients. It is unclear whether false-positive liquid biopsies represent errors in the liquid biopsy or inadequacies of a tissue biopsy reference standard. In 3 studies, patients with negative tissue biopsies and positive liquid biopsies appeared not to have a high response to osimertinib or rociletinib. Sample sizes are very small for this scenario of discordance. Although the evidence is limited, the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology published joint guidelines endorsed by the American Society of Clinical Oncology with an expert consensus opinion that "Physicians may use plasma ctDNA methods to identify *EGFR* T790M mutations in lung adenocarcinoma patients with progression or secondary clinical resistance to *EGFR* targeted TKIs; testing of the tumor sample is recommended if the plasma result is negative." The National Comprehensive Cancer Network guidelines also state that at progression on erlotinib, afatinib, gefitinib or dacomitinib when testing for the T790M resistance variant, plasma-based testing should be considered and when plasma-based testing is negative, tissue-based testing is strongly recommended.

For tests of other, less prevalent, variants, such as ALK translocations, ROS1 translocations, RET fusions, MET exon 14 skipping, and BRAF V600E variants, few studies were identified that evaluated the clinical validity of any commercially available tests and in these studies, very few variants were detected; therefore, performance characteristics are not well characterized. Because sufficient evidence of clinical validity is lacking, a chain of evidence cannot be linked to support the conclusion that results for other variants using ctDNA test methods will be similar to those for tissue biopsy.

Summary of Evidence

For individuals who have advanced-stage NSCLC who are being considered for targeted therapy who receive testing for *EGFR* variants and *ALK* rearrangements, the evidence includes phase 3 studies comparing TKIs (e.g., afatinib, erlotinib, gefitinib, osimertinib, et al) with chemotherapy. Relevant outcomes are OS, disease-specific survival, test validity, QOL, and treatment-related morbidity. Studies have shown that TKIs are superior to chemotherapy regarding tumor response rate and PFS, with a reduction in toxicity and improvement in QOL. The evidence is sufficient to determine that the technology results in an improvement in the net health outcome.

For individuals who have advanced-stage NSCLC who are being considered for targeted therapy who receive testing for *BRAF* variants and *ROS1* rearrangements, the evidence includes nonrandomized trials and observational studies of BRAF and MEK inhibitors and crizotinib or ceritinib, respectively. Relevant outcomes are OS, disease-specific survival, test validity, QOL, and treatment-related morbidity. Studies have shown that combination therapy with dabrafenib and trametinib for *BRAF* V600E-variant NSCLC and crizotinib for NSCLC with *ROS1* rearrangements result in response rates of 60% and 70%, respectively, with acceptable toxicity profiles. In an analysis of 53 patients with *ROS-1* fusion -positive NSCLC enrolled in 3 ongoing clinical trials of entrectinib, the ORR was 77%, with a median duration of response of 24.6 months and acceptable toxicity. The

evidence is sufficient to determine that the technology results in an improvement in the net health outcome.

For individuals who have advanced-stage NSCLC who are being considered for targeted therapy who receive testing for *RET* or *MET* gene testing, the evidence includes nonrandomized trials of kinase inhibitors. Relevant outcomes are OS, disease-specific survival, test validity, QOL, and treatment-related morbidity. Studies have shown efficacy in PFS and duration of response for selpercatinib and pralsetinib in patients with RET-fusion positive NSCLC, and for capmatinib in patients with *MET* Exon 14 skipping alterations, with acceptable toxicity. The evidence is sufficient to determine that the technology results in an improvement in the net health outcome.

For individuals who have advanced-stage NSCLC who are being considered for targeted therapy who receive testing for *KRAS* as a technique to predict treatment nonresponse to anti-EGFR therapy with TKIs or testing for *HER2* variants to select the use of the anti-EGFR monoclonal antibody cetuximab (Erbitux), the evidence includes post hoc analysis of trials, observational studies, and meta-analyses. Relevant outcomes are OS, disease-specific survival, test validity, QOL, and treatment-related morbidity. Data on the role of *KRAS* variants in NSCLC and response to erlotinib are available from post hoc analysis of trials, observational studies, and meta-analyses. Although studies have shown that *KRAS* variants in patients with NSCLC confer a high level of resistance to TKIs, data are insufficient to assess any additional benefit to *KRAS* testing beyond *EGFR* testing. In 2 randomized trials with post hoc analyses of *KRAS* variant status and use of the anti-EGFR monoclonal antibody cetuximab with chemotherapy, *KRAS* variants did not identify patients who would benefit from anti-EGFR antibodies, because outcomes with cetuximab were similar regardless of *KRAS* variant status. Studies for *HER2* variant testing have reported response rates and PFS in numbers of patients too small from which to draw conclusions. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals who have advanced-stage NSCLC who receive testing for *KRAS* to select targeted treatment, the evidence includes a phase 2, open-label trial of sotorasib in patients with KRAS variant NSCLC. Relevant outcomes are OS, disease-specific survival, test validity, QOL, and treatment-related morbidity. Presence of the KRAS alteration in tissue was confirmed on central laboratory testing with the use of the therascreen KRAS RGQ PCR Kit. Among 124 patients evaluated for the primary outcome, 4 (3.2%) had a complete response and 42 (33.9%) had a partial response, with an acceptable safety profile. Median duration of response was 11.1 months (95% CI, 6.9 to not evaluable). The evidence is sufficient to determine that the technology results in an improvement in the net health outcome.

For individuals who have advanced-stage NSCLC who are being considered for targeted therapy who receive NTRK gene fusion testing, the evidence includes nonrandomized trials of larotrectinib and entrectinib in patients with solid tumors. Relevant outcomes are OS, disease-specific survival, test validity, QOL, and treatment-related morbidity. In 55 patients with consecutively and prospectively identified tropomyosin receptor kinase fusion-positive solid tumors who received larotrectinib, including 4 patients with lung tumors, the overall response rate was 80% (95% CI, 67 to 90). The median PFS had not been reached after a median follow-up duration of 9.9 months (range, 0.7 to 25.9). Responses were observed regardless of tumor type or age of the patient. In an integrated analysis of 3 phase 1-2 trials in patients with NTRK solid tumors who received entrectinib, 10 of whom had NSCLC, response was 57% (95% CI, 43.2% to 70.8%) with an acceptable safety profile. The evidence is sufficient to determine that the technology results in an improvement in the net health outcome.

For individuals who have advanced-stage NSCLC who are being considered for immunotherapy who receive PD-L1 testing, the evidence includes RCTs comparing immunotherapy to chemotherapy. Relevant outcomes are OS, disease-specific survival, test validity, QOL, and treatment-related morbidity. In RCTs, patients with high PD-L1 expression had longer PFS and fewer adverse events

when treated with anti-PD-L1 monoclonal antibodies than with platinum chemotherapy. In the KEYNOTE trial, first-line treatment with nivolumab plus ipilimumab resulted in a longer duration of OS than did chemotherapy in patients with NSCLC, independent of the PD-L1 expression level. The evidence is sufficient to determine that the technology results in an improvement in the net health outcome.

For individuals who have advanced-stage NSCLC who are being considered for immunotherapy who receive TMB testing, the evidence includes a RCT and retrospective observational studies. In a subgroup analysis of the KEYNOTE trial, PFS was significantly longer with nivolumab plus ipilimumab than with chemotherapy among patients with NSCLC and a high TMB (≥10 mutations per megabase). In exploratory analyses, retrospective observational studies have reported an association between higher TMB and longer PFS and OS in patients receiving immunotherapy. These results need to be confirmed in additional, well-designed prospective studies. Additionally, there is no consensus on how to measure TMB. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals who have advanced-stage NSCLC who receive testing for biomarkers of *EGFR* TKI sensitivity using ctDNA with the cobas EGFR Mutation Test v2 (liquid biopsy), the evidence includes numerous studies assessing the diagnostic characteristics of liquid biopsy compared with tissue. Relevant outcomes are OS, disease-specific survival, and test validity. Current evidence does not permit determining whether cobas or tissue biopsy is more strongly associated with patient outcomes or treatment response. No RCTs providing evidence of the clinical utility of cobas. The cobas EGFR Mutation Test has adequate evidence of clinical validity for the *EGFR* TKI-sensitizing variants. The U.S. Food and Drug Administration has suggested that a strategy of liquid biopsy followed by referral (reflex) tissue biopsy of negative liquid biopsies for the cobas test would result in an overall diagnostic performance equivalent to tissue biopsy. Several additional studies of the clinical validity of cobas have shown it to be moderately sensitive and highly specific compared with a reference standard of tissue biopsy. A chain of evidence demonstrates that the reflex testing strategy with the cobas test should produce outcomes similar to tissue testing while avoiding tissue testing in approximately two-thirds of patients with *EGFR* TKI-sensitizing variants. Patients who cannot undergo tissue biopsy would likely otherwise receive chemotherapy. The cobas test can identify patients for whom there is a net benefit of targeted therapy versus chemotherapy with high specificity. The evidence is sufficient to determine that the technology results in an improvement in the net health outcome.

For individuals who have advanced-stage NSCLC who receive testing for biomarkers of *EGFR* TKI sensitivity using ctDNA (liquid biopsy) with the Guardant360 CDx, OncoBEAM, or InVision tests, the evidence includes several studies assessing the diagnostic characteristics of liquid biopsy compared with tissue. Relevant outcomes are OS, disease-specific survival, and test validity. Current evidence does not permit determining whether liquid or tissue biopsy is more strongly associated with patient outcomes or treatment response. No RCTs providing evidence of the clinical utility of these tests. The Guardant360 CDx, OncoBEAM, and InVision tests have adequate evidence of clinical validity for the *EGFR* TKI-sensitizing variants. A strategy of liquid biopsy followed by referral (reflex) tissue biopsy of negative liquid biopsies for the tests would result in an overall diagnostic performance similar to tissue biopsy. A chain of evidence demonstrates that the reflex testing strategy with the Guardant360 CDx, OncoBEAM, or InVision tests should produce outcomes similar to tissue testing while avoiding tissue testing in approximately two-thirds of patients with *EGFR* TKI-sensitizing variants. Patients who cannot undergo tissue biopsy would likely otherwise receive chemotherapy. These tests can identify patients for whom there is a net benefit of targeted therapy versus chemotherapy with high specificity. The evidence is sufficient to determine that the technology results in an improvement in the net health outcome.

For individuals who have advanced-stage NSCLC who receive testing for biomarkers of *EGFR* TKI sensitivity using ctDNA with tests other than the cobas EGFR Mutation Test v2, Guardant360 CDx,

OncoBEAM, or InVision tests, the evidence includes studies assessing the diagnostic characteristics of liquid biopsy compared with tissue reference standard. Relevant outcomes are OS, disease-specific survival, and test validity. Given the breadth of molecular diagnostic methodologies available to assess ctDNA, the clinical validity of each commercially available test must be established independently. None of the commercially available tests other than the cobas, Guardant360 CDx, OncoBEAM, and InVision tests have multiple studies of adequate quality to estimate the performance characteristics with sufficient precision. Current evidence does not permit determining whether a liquid biopsy or tissue biopsy is more strongly associated with patient outcomes or treatment response. No RCTs providing evidence of the clinical utility of those methods of liquid biopsy. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with advanced-stage NSCLC who are being considered for targeted therapy who undergo testing for ALK rearrangements using FoundationOne Liquid CDx, the evidence includes an exploratory retrospective analysis of data from a RCT comparing crizotinib to alectinib, and 1 clinical bridging study that compared FoundationOne Liquid to another liquid biopsy test. Relevant outcomes are OS, disease-specific survival, and test validity. There are no studies directly comparing FoundationOne liquid to tissue biopsy. Clinical validity has not been demonstrated in multiple well-designed and conducted studies, therefore, a chain of indirect evidence to show clinical utility cannot be established. One nonrandomized trial directly assessed the clinical utility of FoundationOne Liquid to select patients for treatment with alectinib, but this study was limited by its lack of a control group and no comparison to tissue biopsy. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with advanced-stage NSCLC who are being considered for targeted therapy who undergo testing for MET exon 14 skipping alterations using FoundationOne Liquid CDx, the evidence includes a clinical bridging study that compared FoundationOne Liquid to tissue testing using data from a nonrandomized, open-label phase 2 study of capmatinib therapy. There is no direct evidence of the clinical utility of FoundationOne Liquid to select patients for targeted therapy for capmatinib. Clinical validity has not been demonstrated in multiple well-designed and conducted studies, and therefore a chain of indirect evidence to show clinical utility cannot be established. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals who have advanced-stage NSCLC who receive testing for biomarkers other than *EGFR* using a liquid biopsy to select a targeted therapy, the evidence includes studies assessing the diagnostic characteristics of liquid biopsy compared with the tissue biopsy reference standard. Relevant outcomes are OS, disease-specific survival, and test validity. Given the breadth of molecular diagnostic methodologies available to assess ctDNA, the clinical validity of each commercially available test must be established independently. None of the commercially available tests have multiple studies of adequate quality to estimate the performance characteristics with sufficient precision for variants other than *EGFR*. We found no RCTs providing evidence of the clinical utility of those methods of liquid biopsy. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals who have advanced-stage NSCLC who progressed on *EGFR* TKIs who receive testing for biomarkers of *EGFR* TKI resistance using liquid biopsy, the evidence includes studies assessing the diagnostic characteristics of liquid biopsy. Relevant outcomes are OS, disease-specific survival, and test validity. For variants that indicate *EGFR* TKI resistance and suitability for alternative treatments with osimertinib, liquid biopsy is moderately sensitive and moderately specific compared with a reference standard of tissue biopsy. Given the moderate clinical sensitivity and specificity of liquid biopsy, using liquid biopsy alone or in combination with tissue biopsy might result in the selection of different patients testing positive for *EGFR* TKI resistance. It cannot be determined whether patient outcomes are improved. However, although there is higher discordance in the liquid versus tissue results for the resistance variant, retrospective analyses have suggested that patients positive for

T790M in liquid biopsy have outcomes with osimertinib that appear to be similar overall to patients positive by a tissue-based assay. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.. Although the evidence is limited, the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology published joint guidelines endorsed by the American Society of Clinical Oncology with an expert consensus opinion that physicians may use liquid biopsy (cell-free DNA) to identify *EGFR* T790M variants in patients with progression or resistance to *EGFR*-targeted TKIs and that testing of the tumor sample is recommended if the liquid biopsy result is negative. Similarly, the National Comprehensive Cancer Network guidelines also state that at progression on erlotinib, afatinib, gefitinib, or dacomitinib when testing for the T790M resistance variant, liquid biopsy should be considered and when a liquid biopsy is negative tissue-based testing is strongly recommended.

Supplemental Information

The purpose of the following information is to provide reference material. Inclusion does not imply endorsement or alignment with the evidence review conclusions.

Practice Guidelines and Position Statements

Guidelines or position statements will be considered for inclusion in 'Supplemental Information' if they were issued by, or jointly by, a US professional society, an international society with US representation, or National Institute for Health and Care Excellence (NICE). Priority will be given to guidelines that are informed by a systematic review, include strength of evidence ratings, and include a description of management of conflict of interest.

American College of Chest Physicians Guidelines

In 2013, the American College of Chest Physicians updated its evidence-based practice guidelines on the treatment of stage IV non-small-cell lung cancer (NSCLC).[162,] Based on a review of the literature, the College reported improved response rates, progression-free survival, and toxicity profiles with first-line erlotinib or gefitinib compared with first-line platinum-based therapy in patients with *EGFR* variants, especially exon 19 deletion and L858R. The College recommended, "testing patients with NSCLC for *EGFR* mutations at the time of diagnosis whenever feasible, and treating with first-line EGFR TKIs if mutation-positive."

American Society of Clinical Oncology

In 2021, the American Society of Clinical Oncology (ASCO) and Ontario Health published updated guidelines on therapy for stage IV NSCLC with driver alterations. [163,] The updated recommendations were based on a systematic review of randomized controlled trials from December 2015 to January 2020 and meeting abstracts from ASCO 2020. The recommendations include the following:

- All patients with nonsquamous NSCLC should have the results of testing for potentially targetable mutations (alterations) before implementing therapy for advanced lung cancer, regardless of smoking status, when possible.
- Targeted therapies against ROS-1 fusions, BRAF V600e mutations, RET fusions, MET exon 14 skipping mutations, and NTRK fusions should be offered to patients, either as initial or second-line therapy when not given in the first-line setting.
- Chemotherapy is still an option at most stages.

College of American Pathologists et al

In 2013, the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology published evidence-based guidelines for molecular testing to select patients with lung cancer for treatment with EGFR and ALK TKI therapy.[164,] Based on excellent quality evidence (category A), the guidelines recommended EGFR variant and *ALK* rearrangement testing in patients with lung adenocarcinoma regardless of clinical characteristics (e.g., smoking history).

In 2018, updated guidelines were published and added new EGFR and ALK recommendations.[165,] ROS1 testing is recommended for all patients with lung adenocarcinoma irrespective of clinical characteristics (strong recommendation). BRAF, RET, HER2, KRAS, and MET testing are not recommended as routine stand-alone tests, but may be considered as part of a larger testing panel or if EGFR, ALK, and ROS1 are negative (expert consensus opinion).

National Comprehensive Cancer Network Guidelines

Testing for Molecular Biomarkers

NCCN guidelines on NSCLC (v.6.2021) provide recommendations for individual biomarkers that should be tested, and recommend testing techniques. Guidelines are updated frequently; refer to the source document for current recommendations. The most recent guidelines (v.6.2021) include the following recommendations and statements related to testing for molecular biomarkers:

- Broad molecular profiling systems may be used to simultaneously test for multiple biomarkers.
- To minimize tissue use and potential wastage, the NCCN NSCLC Panel recommends that broad molecular profiling be done as part of biomarker testing using a validated test(s) that assesses potential genetic variants:
  - EGFR mutations
  - BRAF mutations
  - MET exon 14 skipping mutations
  - RET rearrangements
- Both FDA and laboratory-developed test platforms are available that address the need to evaluate these and other analytes
- Broad molecular profiling is also recommended to identify rare driver mutations for which effective therapy may be available, such as NTRK gene fusions, high-level MET amplification, ERBB2 mutations, and TMB.
- Clinicopathologic features should not be used to select patients for testing
- The guidelines do not endorse any specific commercially available biomarker assays.

Plasma Cell-Free/Circulating Tumor DNA Testing:

The NCCN guidelines on NSCLC (v.6.2021) include the following recommendations related to plasma cell-free/circulating tumor DNA testing.[166,]

- Plasma cell free/circulating tumor DNA testing should not be used to diagnose NSCLC; tissue should be used to diagnose NSCLC.
- Plasma cell free/circulating tumor DNA testing should not be used in lieu of a histologic tissue diagnosis, but cell-free/circulating tumor DNA testing can be considered in specific clinical circumstances, notably:
  - If the patient is medically unfit for invasive tissue sampling; or
  - In the initial diagnostic setting, if following pathologic confirmation of a NSCLC diagnosis there is insufficient material for molecular analysis, cell-free/circulating tumor DNA should be used only if follow-up tissue-based analysis is planned for all patients in which an oncogenic driver is not identified.

The guidelines also state:

- Standards for analytic performance characteristics of cell-free tumor DNA have not been established, and in contrast to tissue-based testing, no guidelines exist regarding the recommended performance characteristics of this type of testing

U.S. Preventive Services Task Force Recommendations

Not applicable.

Medicare National Coverage

The Centers for Medicare and Medicaid Services will cover diagnostic testing with next-generation sequencing for beneficiaries with recurrent, relapsed, refractory, metastatic cancer, or advanced stages III or IV cancer if the beneficiary has not been previously tested using the same next-generation sequencing test, unless a new primary cancer diagnosis is made by the treating physician, and if the patient has decided to seek further cancer treatment. The test must have a U.S. Food and Drug Administration approved or cleared indication as an in vitro diagnostic, with results and treatment options provided to the treating physician for patient management.[167,]

Ongoing and Unpublished Clinical Trials

Some currently ongoing trials that might influence this review are listed in Table 40.

Table 40. Summary of Key Trials

NCT No.	Trial Name	Planned Enrollment	Completion Date
Ongoing			
NCT01306045	Pilot Trial of Molecular Profiling and Targeted Therapy for Advanced Non-Small Cell Lung Cancer, Small Cell Lung Cancer, and Thymic Malignancies	469	Dec 2022
NCT03225664	BATTLE-2 Program: A Biomarker-Integrated Targeted Therapy Study in Previously Treated Patients With Advanced Non-Small Cell Lung Cancer	37 (actual)	Sep 2021
NCT02622581	Clinical Research Platform into Molecular Testing, Treatment and Outcome of Non-Small Cell Lung Carcinoma Patients (CRISP)	7500	Dec 2027
NCT02117167[a]	Intergroup Trial UNICANCER UC 0105-1305/ IFCT 1301: SAFIR02_Lung - Evaluation of the Efficacy of High Throughput Genome Analysis as a Therapeutic Decision Tool for Patients With Metastatic Non-small Cell Lung Cancer	999	Dec 2023
NCT02465060	Molecular Analysis for Therapy Choice (MATCH)	6452	Jun 2022
NCT02576431[a]	A Phase II Basket Study of the Oral TRK Inhibitor LOXO-101 in Subjects With NTRK Fusion-positive Tumors	200	Sep 2025
NCT02568267[a]	An Open-Label, Multicenter, Global Phase 2 Basket Study of Entrectinib for the Treatment of Patients With Locally Advanced or Metastatic Solid Tumors That Harbor NTRK1/2/3, ROS1, or ALK Gene Rearrangements	700	Dec 2024
NCT01639508	A Phase II Study of Cabozantinib in Patients With RET Fusion-Positive Advanced Non-Small Cell Lung Cancer and Those With Other Genotypes: ROS1 or NTRK Fusions or Increased MET or AXL Activity	86	Jul 2022
NCT03469960	A Randomized Phase 3 Trial Comparing Continuation Nivolumab-Ipilimumab Doublet Immunotherapy Until Progression Versus Observation in Treatment-naive Patients With PDL1-positive Stage IVNon-Small Cell Lung Cancer (NSCLC) After Nivolumab-Ipilimumab Induction Treatment	1360	May 2023
NCT03037385[a]	A Phase 1/2 Study of the Highly-selective RET Inhibitor, BLU-667, in Patients With Thyroid Cancer, Non-Small Cell Lung Cancer (NSCLC) and Other Advanced Solid Tumors	647	Feb 2024

NCT: national clinical trial.
[a] Denotes industry-sponsored or cosponsored trial.

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Documentation for Clinical Review

Please provide the following documentation:

- History and physical and/or consultation notes including:
  - Diagnosis and cancer stage
  - Previous treatment plan(s) and response(s)
  - Current treatment plan
  - Clinical justification for analysis testing
  - Previous biopsies and any tissue limitations or contraindications to repeat biopsy

Post Service (in addition to the above, please include the following):

- Analysis testing results

Coding

This Policy relates only to the services or supplies described herein. Benefits may vary according to product design; therefore, contract language should be reviewed before applying the terms of the Policy.

The following codes are included below for informational purposes. Inclusion or exclusion of a code(s) does not constitute or imply member coverage or provider reimbursement policy. Policy Statements are intended to provide member coverage information and may include the use of some codes for clarity. The Policy Guidelines section may also provide additional information for how to interpret the Policy Statements and to provide coding guidance in some cases.

Type	Code	Description
CPT®	0239U	Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free DNA, analysis of 311 or more genes, interrogation for sequence variants, including substitutions, insertions, deletions, select rearrangements, and copy number variations
	0326U	Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free circulating DNA analysis of 83 or more genes, interrogation for sequence variants, gene copy number amplifications, gene rearrangements, microsatellite instability and tumor mutational burden ***(Code effective 7/1/2022)***
	81191	NTRK1 (neurotrophic receptor tyrosine kinase 1) (e.g., solid tumors) translocation analysis
	81192	NTRK2 (neurotrophic receptor tyrosine kinase 2) (e.g., solid tumors) translocation analysis
	81193	NTRK3 (neurotrophic receptor tyrosine kinase 3) (e.g., solid tumors) translocation analysis
	81194	NTRK (neurotrophic-tropomyosin receptor tyrosine kinase 1, 2, and 3) (e.g., solid tumors) translocation analysis
	81235	EGFR (epidermal growth factor receptor) (e.g., non-small cell lung cancer) gene analysis, common variants (e.g., exon 19 LREA deletion, L858R, T790M, G719A, G719S, L861Q)
	81275	KRAS (Kirsten rat sarcoma viral oncogene homolog) (e.g., carcinoma) gene analysis; variants in exon 2 (e.g., codons 12 and 13)
	81276	KRAS (Kirsten rat sarcoma viral oncogene homolog) (e.g., carcinoma) gene analysis; additional variant(s) (e.g., codon 61, codon 146)
	81404	Molecular Pathology Procedure Level 5
	81405	Molecular Pathology Procedure Level 6
	81406	Molecular Pathology Procedure Level 7
	81455	Targeted genomic sequence analysis panel, solid organ or hematolymphoid neoplasm, DNA analysis, and RNA analysis when performed, 51 or greater genes (e.g., ALK, BRAF, CDKN2A, CEBPA, DNMT3A, EGFR, ERBB2, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MLL, NPM1, NRAS, MET, NOTCH1, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, if performed
	81479	Unlisted molecular pathology procedure
	88342	Immunohistochemistry or immunocytochemistry, per specimen; initial single antibody stain procedure
	88365	In situ hybridization (e.g., FISH), per specimen; initial single probe stain procedure

Type	Code	Description
HCPCS	None	

Policy History

This section provides a chronological history of the activities, updates and changes that have occurred with this Medical Policy.

Effective Date	Action
11/26/2014	BCBSA Medical Policy adoption
08/31/2015	Policy title change from Molecular Analysis for Targeted Therapy for Non-Small-Cell Lung Cancer Policy revision without position change
06/01/2016	Policy revision without position change
12/01/2016	Policy revision without position change
12/01/2017	Policy revision without position change
12/01/2018	Policy revision without position change
12/01/2019	Policy revision without position change
12/01/2020	Annual review. Policy statement updated
01/01/2021	Annual review. Policy statement, guidelines and literature updated. Policy title changed from Molecular Analysis for Targeted Therapy of Non-Small-Cell Lung Cancer to current one. Coding update.
03/01/2022	Annual review. No change to policy statement.
04/01/2022	Policy statement, guidelines and literature updated to combine with Circulating Tumor DNA Management of Non-Small-Cell Lung Cancer (Liquid Biopsy) 2.04.143. Policy title changed from Molecular Analysis for Targeted Therapy or Immunotherapy of Non-Small-Cell Lung Cancer to current one.
09/01/2022	Coding update

Definitions of Decision Determinations

Medically Necessary: Services that are Medically Necessary include only those which have been established as safe and effective, are furnished under generally accepted professional standards to treat illness, injury or medical condition, and which, as determined by Blue Shield, are: (a) consistent with Blue Shield medical policy; (b) consistent with the symptoms or diagnosis; (c) not furnished primarily for the convenience of the patient, the attending Physician or other provider; (d) furnished at the most appropriate level which can be provided safely and effectively to the patient; and (e) not more costly than an alternative service or sequence of services at least as likely to produce equivalent therapeutic or diagnostic results as to the diagnosis or treatment of the Member's illness, injury, or disease.

Investigational/Experimental: A treatment, procedure, or drug is investigational when it has not been recognized as safe and effective for use in treating the particular condition in accordance with generally accepted professional medical standards. This includes services where approval by the federal or state governmental is required prior to use, but has not yet been granted.

Split Evaluation: Blue Shield of California/Blue Shield of California Life & Health Insurance Company (Blue Shield) policy review can result in a split evaluation, where a treatment, procedure, or drug will be considered to be investigational for certain indications or conditions, but will be deemed safe and effective for other indications or conditions, and therefore potentially medically necessary in those instances.

Prior Authorization Requirements (as applicable to your plan)

Within five days before the actual date of service, the provider must confirm with Blue Shield that the member's health plan coverage is still in effect. Blue Shield reserves the right to revoke an authorization prior to services being rendered based on cancellation of the member's eligibility. Final determination of benefits will be made after review of the claim for limitations or exclusions.

Questions regarding the applicability of this policy should be directed to the Prior Authorization Department at (800) 541-6652, or the Transplant Case Management Department at (800) 637-2066 ext. 3507708 or visit the provider portal at www.blueshieldca.com/provider.

We are interested in receiving feedback relative to developing, adopting, and reviewing criteria for medical policy. Any licensed practitioner who is contracted with Blue Shield of California or Blue Shield of California Promise Health Plan is welcome to provide comments, suggestions, or concerns. Our internal policy committees will receive and take your comments into consideration.

For utilization and medical policy feedback, please send comments to: MedPolicy@blueshieldca.com

Disclaimer: This medical policy is a guide in evaluating the medical necessity of a particular service or treatment. Blue Shield of California may consider published peer-reviewed scientific literature, national guidelines, and local standards of practice in developing its medical policy. Federal and state law, as well as contract language, including definitions and specific contract provisions/exclusions, take precedence over medical policy and must be considered first in determining covered services. Member contracts may differ in their benefits. Blue Shield reserves the right to review and update policies as appropriate.

Appendix A

POLICY STATEMENT	
BEFORE Red font: Verbiage removed	**AFTER** Blue font: Verbiage Changes/Additions
Molecular Analysis for Targeted Therapy or Immunotherapy of Non-Small-Cell Lung Cancer 2.04.45 **Policy Statement:** **Note:** Starting on July 1, 2022 (per CA law SB 535) for commercial plans regulated by the California Department of Managed Healthcare and California Department of Insurance (PPO and HMO), health care service plans and insurers shall not require prior authorization for biomarker testing, including biomarker testing for cancer progression and recurrence, if a member has stage 3 or 4 cancer. Health care service plans and insurers can still do a medical necessity review of a biomarker test and possibly deny coverage after biomarker testing has been completed and a claim is submitted (post service review). The use of tissue samples for analysis is generally preferred over plasma testing (liquid biopsy or circulating tumor DNA, ctDNA) when available. Panel testing of tissue samples is an acceptable alternative to individual testing when the quantity of tissue is limited. Molecular analysis (genetic testing) is reserved for advanced (stage III or IV) or metastatic Non-Small-Cell Lung Cancer (NSCLC) including adenocarcinoma, large cell, squamous cell and NSCLC not otherwise specified (see Policy Guidelines) or if a targeted therapy dependent on genetic testing is being considered. Small panel testing including the following medically necessary genes may be considered as an alternative to individual testing and may be preferred when there is limited tissue available for testing. Plasma tests for oncogenic driver variants deemed medically necessary on tissue biopsy may be considered **medically necessary** to predict treatment response to targeted therapy for patients meeting the following criteria: I. Patient does not have sufficient tissue for standard molecular testing using formalin-fixed paraffin-embedded tissue; AND	**Molecular Analysis (Including Liquid Biopsy) for Targeted Therapy or Immunotherapy of Non-Small-Cell Lung Cancer 2.04.45** **Policy Statement:** **Note:** Starting on July 1, 2022 (per CA law SB 535) for commercial plans regulated by the California Department of Managed Healthcare and California Department of Insurance (PPO and HMO), health care service plans and insurers shall not require prior authorization for biomarker testing, including biomarker testing for cancer progression and recurrence, if a member has stage 3 or 4 cancer. Health care service plans and insurers can still do a medical necessity review of a biomarker test and possibly deny coverage after biomarker testing has been completed and a claim is submitted (post service review). The use of tissue samples for analysis is generally preferred over plasma testing (liquid biopsy or circulating tumor DNA, ctDNA) when available. Panel testing of tissue samples is an acceptable alternative to individual testing when the quantity of tissue is limited. Molecular analysis (genetic testing) is reserved for advanced (stage III or IV) or metastatic Non-Small-Cell Lung Cancer (NSCLC) including adenocarcinoma, large cell, squamous cell and NSCLC not otherwise specified (see Policy Guidelines) or if a targeted therapy dependent on genetic testing is being considered. Small panel testing including the following medically necessary genes may be considered as an alternative to individual testing and may be preferred when there is limited tissue available for testing. I. Plasma tests for oncogenic driver variants deemed medically necessary on tissue biopsy may be considered **medically necessary** to predict treatment response to targeted therapy for patients meeting the following criteria: A. Patient does not have sufficient tissue for standard molecular testing using formalin-fixed paraffin-embedded tissue; AND

POLICY STATEMENT	
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II. Follow-up tissue-based analysis is planned when possible should no driver variant be identified via plasma testing.	B. Follow-up tissue-based analysis is planned when possible should no driver variant be identified via plasma testing.
***EGFR* Testing** Analysis of somatic variants (in exons 18 through 21 (e.g., G719X, L858R, T790M, S6781, L861Q) within the epidermal growth factor receptor *(EGFR)* gene, may be considered **medically necessary** to predict treatment response to an EGFR tyrosine kinase inhibitor (TKI) therapy (e.g., erlotinib [Tarceva®], gefitinib [Iressa®], afatinib [Gilotrif®], or osimertinib [Tagrisso™]) in patients with advanced or high risk earlier stage (IB-IIIA) lung adenocarcinoma, large cell carcinoma, advanced squamous-cell non-small-cell lung cancer (NSCLC), and NSCLC not otherwise specified.	***EGFR* Testing** II. Analysis of somatic variants (in exons 18 through 21 (e.g., G719X, L858R, T790M, S6781, L861Q) within the epidermal growth factor receptor *(EGFR)* gene, may be considered **medically necessary** to predict treatment response to an EGFR tyrosine kinase inhibitor (TKI) therapy (e.g., erlotinib [Tarceva®], gefitinib [Iressa®], afatinib [Gilotrif®], or osimertinib [Tagrisso™]) in patients with advanced or high risk earlier stage (IB-IIIA) lung adenocarcinoma, large cell carcinoma, advanced squamous-cell non-small-cell lung cancer (NSCLC), and NSCLC not otherwise specified.
At progression (or when included in an initial panel), analysis of the EGFR T790M resistance variant for targeted therapy with osimertinib using tissue or ctDNA may be considered **medically necessary** in patients with advanced or high risk earlier stage (IB-IIIA) lung adenocarcinoma, large cell carcinoma, advanced squamous-cell non-small-cell lung cancer, and non-small-cell lung cancer not otherwise specified.	III. At progression (or when included in an initial panel), analysis of the EGFR T790M resistance variant for targeted therapy with osimertinib using tissue or ctDNA may be considered **medically necessary** in patients with advanced or high risk earlier stage (IB-IIIA) lung adenocarcinoma, large cell carcinoma, advanced squamous-cell non-small-cell lung cancer, and non-small-cell lung cancer not otherwise specified.
Analysis of other *EGFR* variants within exons 22 to 24, or other applications related to NSCLC, is considered **investigational**.	IV. Analysis of other *EGFR* variants within exons 22 to 24, or other applications related to NSCLC, is considered **investigational**.
***ALK* Testing** Analysis of somatic rearrangement variants of the anaplastic lymphoma kinase (*ALK*) gene may be considered **medically necessary** to predict treatment response to ALK inhibitor therapy (e.g., crizotinib [Xalkori®], ceritinib [Zykadia™], alectinib [Alecensa®], or brigatinib [Alunbrig™]) in patients with advanced lung adenocarcinoma or in whom an adenocarcinoma component cannot be excluded (see Policy Guidelines section) or when included in a panel approved for other indications.	***ALK* Testing** V. Analysis of somatic rearrangement variants of the anaplastic lymphoma kinase (*ALK*) gene may be considered **medically necessary** to predict treatment response to ALK inhibitor therapy (e.g., crizotinib [Xalkori®], ceritinib [Zykadia™], alectinib [Alecensa®], or brigatinib [Alunbrig™]) in patients with advanced lung adenocarcinoma or in whom an adenocarcinoma component cannot be excluded (see Policy Guidelines section) or when included in a panel approved for other indications.

POLICY STATEMENT	
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Analysis of somatic rearrangement variants of the *ALK* gene is considered **investigational** in all other situations.	VI. Analysis of somatic rearrangement variants of the *ALK* gene is considered **investigational** in all other situations.
***BRAF* V600E Testing** Analysis of the somatic *BRAF* V600E variant may be considered **medically necessary** to predict treatment response to BRAF or MEK inhibitor therapy (e.g., dabrafenib [Tafinlar®] and trametinib [Mekinist®]), in patients with advanced lung adenocarcinoma or in whom an adenocarcinoma component cannot be excluded (see Policy Guidelines section) or when included in a panel approved for other indications.	***BRAF* V600E Testing** VII. Analysis of the somatic *BRAF* V600E variant may be considered **medically necessary** to predict treatment response to BRAF or MEK inhibitor therapy (e.g., dabrafenib [Tafinlar®] and trametinib [Mekinist®]), in patients with advanced lung adenocarcinoma or in whom an adenocarcinoma component cannot be excluded (see Policy Guidelines section) or when included in a panel approved for other indications.
Analysis of the somatic *BRAF V600E* variant is considered **investigational** in all other situations.	VIII. Analysis of the somatic *BRAF V600E* variant is considered **investigational** in all other situations.
***ROS1* Testing** Analysis of somatic rearrangement variants of the *ROS1* gene may be considered **medically necessary** to predict treatment response to ALK inhibitor therapy (crizotinib [Xalkori]) in patients with advanced lung adenocarcinoma or in whom an adenocarcinoma component cannot be excluded (see Policy Guidelines section) or when included in a panel approved for other indications.	***ROS1* Testing** IX. Analysis of somatic rearrangement variants of the *ROS1* gene may be considered **medically necessary** to predict treatment response to ALK inhibitor therapy (crizotinib [Xalkori]) in patients with advanced lung adenocarcinoma or in whom an adenocarcinoma component cannot be excluded (see Policy Guidelines section) or when included in a panel approved for other indications.
Analysis of somatic rearrangement variants of the *ROS1* gene is considered **investigational** in all other situations.	X. Analysis of somatic rearrangement variants of the *ROS1* gene is considered **investigational** in all other situations.
***KRAS* Testing** Analysis of somatic variants of the *KRAS* gene may be considered **medically necessary** to predict treatment response to sotorasib (Lumakras) in patients with advanced lung adenocarcinoma or in whom an adenocarcinoma component cannot be excluded (see Policy Guidelines section) or when included in a panel approved for other indications.	***KRAS* Testing** XI. Analysis of somatic variants of the *KRAS* gene may be considered **medically necessary** to predict treatment response to sotorasib (Lumakras) in patients with advanced lung adenocarcinoma or in whom an adenocarcinoma component cannot be excluded (see Policy Guidelines section) or when included in a panel approved for other indications.
All other uses of analysis of somatic variants of the *KRAS* gene are considered **investigational**.	XII. All other uses of analysis of somatic variants of the *KRAS* gene are considered **investigational**.

POLICY STATEMENT	
BEFORE Red font: Verbiage removed	**AFTER** Blue font: Verbiage Changes/Additions
***HER2* Testing** Analysis of somatic alterations in the *HER2* gene in tissue for targeted therapy in patients with NSCLC is considered **investigational** unless included in a panel approved for other indications.	***HER2* Testing** XIII. Analysis of somatic alterations in the *HER2* gene in tissue for targeted therapy in patients with NSCLC is considered **investigational** unless included in a panel approved for other indications.
***NTRK* Gene Fusion Testing** Analysis of somatic *NTRK* gene fusions in tissue may be considered **medically necessary** to predict treatment response to entrectinib (Rozlytrek) or larotrectinib (Vitrakvi) in patients with advanced lung adenocarcinoma or in whom an adenocarcinoma component cannot be excluded (see Policy Guidelines section) or when included in a panel approved for other indications.	***NTRK* Gene Fusion Testing** XIV. Analysis of somatic *NTRK* gene fusions in tissue may be considered **medically necessary** to predict treatment response to entrectinib (Rozlytrek) or larotrectinib (Vitrakvi) in patients with advanced lung adenocarcinoma or in whom an adenocarcinoma component cannot be excluded (see Policy Guidelines section) or when included in a panel approved for other indications.
Analysis of somatic *NTRK* gene fusions is considered **investigational** in all other situations.	XV. Analysis of somatic *NTRK* gene fusions is considered **investigational** in all other situations.
***RET* Rearrangement Testing** Analysis of somatic alteration in the *RET* gene may be considered **medically necessary** to predict treatment response to pralsetinib (Gavreto) or selpercatinib (Retevmo) in patients with metastatic NSCLC or when included in a panel approved for other indications.	***RET* Rearrangement Testing** XVI. Analysis of somatic alteration in the *RET* gene may be considered **medically necessary** to predict treatment response to pralsetinib (Gavreto) or selpercatinib (Retevmo) in patients with metastatic NSCLC or when included in a panel approved for other indications.
Analysis of somatic alterations in the RET gene is considered **investigational** in all other situations.	XVII. Analysis of somatic alterations in the RET gene is considered **investigational** in all other situations.
***MET* Exon 14 Skipping Alteration** Analysis of somatic alteration in tissue that leads to *MET* exon 14 skipping may be considered **medically necessary** to predict treatment response to capmatinib (Tabrecta) in patients with metastatic NSCLC.	***MET* Exon 14 Skipping Alteration** XVIII. Analysis of somatic alteration in tissue that leads to *MET* exon 14 skipping may be considered **medically necessary** to predict treatment response to capmatinib (Tabrecta) in patients with metastatic NSCLC.
Analysis of genetic alterations of the *MET* gene is considered **investigational** in all other situations.	XIX. Analysis of genetic alterations of the *MET* gene is considered **investigational** in all other situations.

POLICY STATEMENT	
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PD-L1 Testing Programmed Death-Ligand 1 (PD-L1) testing may be considered **medically necessary** to predict treatment response to atezolizumab (Tecentriq), nivolumab (Opdivo) in combination with ipilimumab (Yervoy), or pembrolizumab (Keytruda) in patients with metastatic NSCLC. PD-L1 is a ligand not a gene, and testing may be requested separately if not part of the panel. PD-L1 testing is considered **investigational** in all other situations. Tumor Mutational Burden Testing Analysis of tumor mutational burden for targeted therapy in patients with NSCLC is considered **investigational.**	**PD-L1 Testing** XX. Programmed Death-Ligand 1 (PD-L1) testing may be considered **medically necessary** to predict treatment response to atezolizumab (Tecentriq), nivolumab (Opdivo) in combination with ipilimumab (Yervoy), or pembrolizumab (Keytruda) in patients with metastatic NSCLC. **Note:** PD-L1 is a ligand not a gene, and testing may be requested separately if not part of the panel. XXI. PD-L1 testing is considered **investigational** in all other situations. Tumor Mutational Burden Testing XXII. Analysis of tumor mutational burden for targeted therapy in patients with NSCLC is considered **investigational.**