| 2.04.143 | Circulating Tumor DNA for Management of Non-Small-Cell Lung Cancer (Liquid Biopsy) | | |
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| **Original Policy Date:** | June 1, 2018 | **Effective Date:** | March 1, 2022 |
| **Section:** | 2.0 Medicine | **Page:** | Page 1 of 65 |

## Policy Statement

Circulating tumor DNA (ctDNA or liquid biopsy) analysis (genetic testing) may be **medically necessary** for some genes under limited circumstances. ctDNA testing is limited to 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 section) when an initial diagnostic biopsy sample (or there is progression of the cancer despite treatment) has insufficient tissue available to complete testing (or the testing is inconclusive) and the alternative is a second invasive biopsy.

**Alternative to Individual Testing**
**Any** of the following panel tests may be considered **medically necessary** as alternatives to the individual genes noted below (including those considered investigational as stand-alone tests) when the medically necessary criteria is met for ctDNA testing, either after diagnosis or after progression of the cancer despite treatment:

I. cobas® EGFR Mutation Test v2
II. FoundationOne® Liquid CDx
III. Guardant360® CDx or LDT
IV. OncoBEAM™ Lung1
V. OncoBEAM™ Lung2
VI. InVision First-Lung
VII. Resolution ctDx Lung (ResBio)

**Note**: 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, which is investigational by itself) and should use a miscellaneous CPT code of 81455 (sometimes incorrectly 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).

***Epidermal Growth Factor Receptor (EGFR)* Testing**
When included in one of the approved panel tests, analysis of somatic variants in exons 19 through 21 (e.g., exon 19 deletions, L858R, T790M) within the epidermal growth factor receptor (EGFR) gene, using plasma specimens to detect circulating tumor DNA (ctDNA), may be considered **medically necessary** as an alternative to tissue biopsy to predict treatment response to an *EGFR* tyrosine kinase inhibitor (TKI) therapy (e.g., erlotinib [Tarceva], gefitinib [Iressa], afatinib [Gilotrif], dacomitinib [Vizimpro], or osimertinib [Tagrisso]).

At progression, analysis of the *EGFR* T790M resistance variant for targeted therapy with osimertinib using ctDNA from plasma specimens may be considered **medically necessary** in patients when tissue biopsy to obtain new tissue is not feasible, e.g., in those who do not have

enough tissue for standard molecular testing using formalin-fixed paraffin-embedded tissue, do not have a biopsy-amenable lesion, or cannot undergo biopsy.

Unless included in one of the approved panel tests, analysis of other *EGFR* variants within exons 22 to 24, or other applications related to NSCLC, is considered **investigational**.

**Other Genes**
Plasma tests for oncogenic driver variants deemed medically necessary on tissue biopsy (see Blue Shield of California Medical Policy: Molecular Analysis for Targeted Therapy of Non-Small-Cell Lung Cancer) may be considered **medically necessary** to predict treatment response to targeted therapy for patients meeting **all** of the following criteria:

I. Patient does not have sufficient tissue for standard molecular testing using formalin-fixed paraffin-embedded tissue
II. Follow-up tissue-based analysis is planned should no driver variant be identified via plasma testing

***ALK* Testing**
Unless included in one of the approved panel tests, analysis of somatic rearrangement variants of the *ALK* gene using plasma specimens to detect ctDNA or RNA is considered **investigational** as an alternative to tissue biopsy to predict treatment response to ALK inhibitor therapy (e.g., crizotinib [Xalkori], ceritinib [Zykadia], alectinib [Alecensa], or brigatinib [Alunbrig]) in patients with NSCLC.

***BRAF* V600E Testing**
Unless included in one of the approved panel tests, analysis of the *BRAF* V600E variant using plasma specimens to detect ctDNA is considered **investigational** as an alternative to tissue biopsy to predict treatment response to BRAF or MEK inhibitor therapy (e.g., dabrafenib [Tafinlar], trametinib [Mekinist]) in patients with NSCLC.

***ROS1* Testing**
Unless included in one of the approved panel tests, analysis of somatic rearrangement variants of the *ROS1* gene using plasma specimens to detect ctDNA or RNA is considered **investigational** as an alternative to tissue biopsy to predict treatment response to ALK inhibitor therapy (crizotinib [Xalkori]) in patients with NSCLC.

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

***RET* Rearrangement Testing**
Analysis of genetic 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.

***NTRK* Gene Fusion Testing**
Analysis of *NTRK* gene fusions 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. Note that 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)*.

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

***KRAS* Testing**
Unless included in one of the approved panel tests, analysis of somatic variants of the *KRAS* gene using plasma specimens to detect ctDNA is considered **investigational** as a technique to predict treatment nonresponse to anti-EGFR therapy with tyrosine kinase inhibitors and for the use of the anti-EGFR monoclonal antibody cetuximab in NSCLC.

***HER2* Testing**
Unless included in one of the approved panel tests, analysis of alterations in the *HER2* gene using plasma specimens to detect ctDNA for targeted therapy in patients with NSCLC is considered **investigational**.

**Measurement of Residual Disease (MRD) or Initial Diagnosis**
The use of CtDNA for measuring residual disease or monitoring after treatment or for making an initial diagnosis (instead of using a tissue sample) is considered **investigational**.

***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 a panel.

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

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

## Policy Guidelines

The tests discussed herein are intended for use in patients with advanced (stage III or IV) non-small-cell lung cancer. These 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. Patients with sensitizing variants of the tyrosine kinase domain of the *EGFR* gene are considered good candidates for treatment with erlotinib, gefitinib, afatinib, dacomitinib, or osimertinib. The U.S. Food and Drug Administration approval for the cobas® EGFR Mutation Test v2 states that patients who are negative for *EGFR* exon 19 deletions or L858R variant based on the plasma test should be reflexed to routine biopsy and testing using formalin-fixed paraffin-embedded tissue. Plasma tests for other oncogenic driver variants deemed medically necessary on tissue biopsy (see Blue Shield of California Medical Policy: Molecular Analysis for Targeted Therapy of Non-Small-Cell Lung Cancer) may also be appropriate for patients who do not have enough tissue for standard molecular testing using formalin-fixed paraffin-embedded tissue do not have a biopsy-amenable lesion, cannot undergo biopsy, or have indeterminate histology (in whom an adeno-carcinoma component cannot be excluded); however this is only appropriate if follow-up tissue-based analysis is planned should no driver variant be identified.

FoundationACT™ was rebranded as FoundationOne® Liquid CDx in late 2018. FoundationOne® Liquid CDx is a “liquid biopsy” test that analyzes portions of DNA that make it into the blood-stream from solid tumors (circulating tumor DNA [ctDNA]). FoundationOne® CDx is used for solid tumor biopsies by taking DNA directly from tumor tissue specimens (using a formalin-fixed paraffin embedded [FFPE] technique).

**Genetics Nomenclature Update**
The Human Genome Variation Society nomenclature is used to report information on variants found in DNA and serves as an international standard in DNA diagnostics. It is being implemented for genetic testing medical evidence review updates starting in 2017 (see Table PG1). The Society's nomenclature is recommended by the Human Variome Project, the Human Genome Organization, and by the Human Genome Variation Society itself.

The American College of Medical Genetics and Genomics and the Association for Molecular Pathology standards and guidelines for interpretation of sequence variants represent expert opinion from both organizations, in addition to the College of American Pathologists. These recommendations primarily apply to genetic tests used in clinical laboratories, including genotyping, single genes, panels, exomes, and genomes. Table PG2 shows the recommended standard terminology-"pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign"-to describe variants identified that cause Mendelian disorders.

**Table PG1. Nomenclature to Report on Variants Found in DNA**

| Previous | Updated | Definition |
|---|---|---|
| Mutation | Disease-associated variant | Disease-associated change in the DNA sequence |
| | Variant | Change in the DNA sequence |
| | Familial variant | Disease-associated variant identified in a proband for use in subsequent targeted genetic testing in first-degree relatives |

**Table PG2. ACMG-AMP Standards and Guidelines for Variant Classification**

| Variant Classification | Definition |
|---|---|
| Pathogenic | Disease-causing change in the DNA sequence |
| Likely pathogenic | Likely disease-causing change in the DNA sequence |
| Variant of uncertain significance | Change in DNA sequence with uncertain effects on disease |
| Likely benign | Likely benign change in the DNA sequence |
| Benign | Benign change in the DNA sequence |

ACMG: American College of Medical Genetics and Genomics; AMP: Association for Molecular Pathology.

## Coding

There is a PLA code for the Resolution ctDx Lung assay:

- **0179U**: Oncology (non-small cell lung cancer), cell-free DNA, targeted sequence analysis of 23 genes (single nucleotide variations, insertions and deletions, fusions without prior knowledge of partner/breakpoint, copy number variations), with report of significant mutation(s)

There are no specific CPT codes for most ctDNA tests other than some specific PLA codes. These tests would likely be reported using any existing CPT molecular pathology code(s) that is applicable (81161-81355 and 81400-81408), or 81445, 81455 (for solid tumor testing), along with or as a single code, the unlisted molecular pathology procedure code (81479). Solid tumor test codes may be used as the closest available code to ctDNA tests in some cases.

In addition to the following specific code for the *EGFR* gene (solid tumor, not ctDNA)*:

- **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)

*Some of the following codes are solid tumor panels (not ctDNA) which also include the *EGFR* gene:

- **81445**: Targeted genomic sequence analysis panel, solid organ neoplasm, DNA analysis, and RNA analysis when performed, 5-50 genes (e.g., ALK, BRAF, CDKN2A, EGFR, ERBB2, KIT, KRAS, NRAS, MET, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, if performed
- **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

There is a Tier 1 CPT code to more accurately describe single-nucleotide polymorphism (SNP) array-derived copy number (CN) for neoplasia. This uses the patient's chromosomal microarray (CMA) results to look for abnormalities:

- **81277**: Cytogenomic neoplasia (genome-wide) microarray analysis, interrogation of genomic regions for copy number and loss-of-heterozygosity variants for chromosomal abnormalities

Detection and quantification of circulating tumor cells (such as for measurable residual disease) would be reported using the following codes:

- **86152**: Cell enumeration using immunologic selection and identification in fluid specimen (e.g., circulating tumor cells in blood)
- **86153**: Cell enumeration using immunologic selection and identification in fluid specimen (e.g., circulating tumor cells in blood); physician interpretation and report, when required)

There is a CPT code to be used as a companion diagnostic test, representing FoundationOne Liquid CDx®:

- **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

***Effective April 1, 2021***, there is a new CPT code that represents Guardant360 CDx by Guardant Health. Per the manufacturer, this is a gene sequencing panel approved for use in advanced solid tumor cancer patients to help determine therapeutic options.

- **0242U**: Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free circulating DNA analysis of 55-74 genes, interrogation for sequence variants, gene copy number amplifications, and gene rearrangements

## Description

Genetic testing of circulating tumor DNA and circulating tumor cells in peripheral blood (referred to as "liquid biopsy") potentially offers a noninvasive alternative to tissue biopsy for therapeutic decisions and prognosis in patients with cancer. For patients with non-small-cell lung cancer, the detection of "driver mutations" or resistance variants is important for selecting patients for targeted therapy.

## Related Policies

- Circulating Tumor DNA and Circulating Tumor Cells for Cancer Management (Liquid Biopsy)
- Molecular Analysis for Targeted Therapy or Immunotherapy of Non-Small-Cell Lung Cancer
- Proteomic Testing for Systemic Therapy in Non-Small-Cell Lung Cancer

## 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

In June 2016, cobas EGFR Mutation Test v2 (Roche Molecular Systems), a real-time PCR test, was approved by the FDA through the premarket approval process (P150047).[2] This plasma test is a real-time PCR test approved as a companion diagnostic aid for selecting NSCLC patients who have *EGFR* exon 19 deletions, and L858R substitution variants, for treatment with erlotinib. A premarket approval supplement expanded the indication to include the test as a companion diagnostic for treatment with gefitinib and osimertinib in 2018 (P120019/S019). Patients who test negative for the variants detected should be referred for (or "reflexed" to) routine biopsy with tissue testing for *EGFR* variants.

In August 2020, Guardant360® CDx (Guardant Health), a qualitative next generation sequencing-based diagnostic of circulating cell-free DNA in plasma, was approved by the FDA through the premarket approval process (P200010).[3] The plasma test is approved as a companion diagnostic for selecting NSCLC patients who have *EGFR* exon 19 deletions, L858R substitution variants, or T790M variants, for treatment with osimertinib. Patients who test negative for the variants detected should be referred for (or "reflexed" to) routine biopsy with tissue testing for *EGFR* variants. Testing for T790M using plasma specimens is most appropriate for consideration in patients for whom a tumor biopsy cannot be obtained, as the efficacy of osimertinib has not been established in T790M plasma-positive, tissue-negative or unknown patient populations.

In August 2020, FoundationOne® Liquid CDx (Foundation Medicine), a qualitative next generation sequencing-based diagnostic for circulating cell-free DNA in plasma, was approved by the FDA through the premarket approval process (P190032).[4] The plasma test is approved as a companion diagnostic for selecting NSCLC patients who have *EGFR* exon 19 deletions and *EGFR* exon 21 L858R substitution variants, for treatment with gefitinib, osimertinib, or erlotinib. Patients who test negative for the variants detected should be referred for (or "reflexed" to) routine biopsy with tissue testing for *EGFR* variants. Prior versions of FoundationOne Liquid CDx were previously marketed as FoundationACT and FoundationOne laboratory developed test (LDT).

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments. Several companies market tests that detect tumor markers from peripheral blood, including TKI-sensitizing variants for NSCLC. Laboratories that offer laboratory-developed tests must be licensed by the Clinical Laboratory Improvement Amendments for high-complexity testing. To date, the FDA has chosen not to require any regulatory review of this test. Clinical laboratories accredited through the College of American Pathologists enroll in proficiency testing programs to measure the accuracy of the test results. There are currently no College of American Pathologists proficiency testing programs available for ctDNA testing to ensure the accuracy of ctDNA laboratory-developed tests.

## Rationale

### Background

#### Predictive Biomarkers in Non-Small-Cell Lung Cancer

Several predictive genetic biomarkers have been identified for NSCLC. Somatic genome alterations known as "driver mutations" are usually transformative variants arising in cancer cells in genes encoding for proteins important in cell growth and survival. Randomized controlled trials have demonstrated improved efficacy, often in conjunction with decreased toxicity, of matching targeted therapies to patients with specific driver mutations. Several such targeted

therapies are approved by the U.S. Food and Drug Administration (FDA) for NSCLC. Guidelines generally suggest the analysis of either the primary NSCLC tumor or of metastasis for the presence of a set of driver mutations to select an appropriate treatment.

**Genetic Biomarkers With FDA Approved Targeted Therapies**
The list of targeted therapies approved for NSCLC is evolving. Currently, there are FDA approved targeted therapies for epidermal growth factor receptor (*EGFR*) variants, anaplastic lymphoma kinase (*ALK*) translocations, *ROS1* translocations, and *BRAF* variants for NSCLC. Companion diagnostics using tissue samples have also been FDA approved to identify the associated driver mutations for the targeted therapies. The evaluation of molecular analysis of tissue samples for targeted therapy of NSCLC is found in Blue Shield of California Medical Policy: Molecular Analysis for Targeted Therapy of Non-Small-Cell Lung Cancer.

***EGFR* Variants**
Specific *EGFR* variants confer sensitivity to treatment with tyrosine kinase inhibitors (TKIs), such as erlotinib, gefitinib, afatinib, dacomitinib, and osimertinib; the most common variants are deletions in exons 19 and an exon 21 substitution variant (L858R). These variants are referred to as TKI-sensitizing variants and are found in approximately 10% of white patients and up to 50% of Asian patients. The prevalence of *EGFR* variants is not well characterized in other ethnic or racial groups but is estimated to be 10% to 15% in studies including general U.S. populations. TKIs are indicated as first-line treatment for patients with sensitizing variants; progression-free survival is improved with the use of TKIs. Patients receiving TKIs have fewer treatment-related adverse events than patients receiving cytotoxic chemotherapy.

***ALK* and *ROS1* Translocations**
*ALK* rearrangements confer resistance to TKIs. Approximately 4% of patients have *ALK* rearrangements. The TKI crizotinib, an inhibitor of ALK, ROS1, and mesenchymal-epithelial transition (MET) tyrosine kinases, is indicated in patients with *ALK*-positive tumors. In randomized trials comparing crizotinib with standard chemotherapy in *ALK*-positive patients, crizotinib has been associated with improved progression-free survival, response rates, lung cancer symptoms, and quality of life. *ROS1* rearrangements develop in 1% to 2% of patients. For such patients, crizotinib has been shown to be effective, with response rates of about 70%.

***BRAF* Variants**
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. *BRAF* or *MEK* inhibition with TKIs (e.g., vemurafenib/dabrafenib or trametinib) was originally approved by the FDA for treatment of unresectable or metastatic melanoma with *BRAF* V600 variants but the combination of dabrafenib and trametinib was expanded to include treatment of metastatic NSCLC in 2017.

***MET* Variants**
C-MET, the hepatocyte growth factor (HGF) receptor, is a tyrosine kinase receptor that is involved in cell survival and proliferation. *MET* (mesenchymal-epithelial transition) amplification is one of the critical events for acquired resistance in *EGFR*-mutated adenocarcinomas refractory to EGFR TKIs. *MET* amplification occurs in 2% to 4% of treatment-naive NSCLC and *MET* and *EGFR* commutations occur in 5% to 20% of NSCLC tumors with acquired resistance to EGFR TKIs. *MET* exon 14 (*MET*ex14) skipping mutations occur in approximately 3-4% of adenocarcinomas and 1-2% of patients with other NSCLC histologies. Higher frequencies are observed in older women who are nonsmokers. *METex14* genomic alterations do not typically overlap with *EGFR*, *ROS1*, *BRAF*, and *ALK* variants. Several types of *MET*ex14 skipping mutations can occur, including mutations, base substitutions, and deletions. *MET* inhibition with capmatinib was granted accelerated approval by the FDA in 2020 for treatment of metastatic NSCLC in patients positive for *MET*ex14 skipping mutations based on results from an open-label, non-randomized, phase 2 trial in 97 subjects (NCT02414139). Among 28 treatment-naive patients, the overall

response rate (ORR) was 68% with a response duration of 12.6 months. Among 69 previously treated patients, the ORR was 41% with a response duration of 9.7 months. Patients in this study were wild-type for *EGFR* variants and negative for *ALK* rearrangements,

#### *RET* Fusions

*RET* (rearranged during transfection) is a proto-oncogene that encodes a receptor tyrosine kinase growth factor. *RET* fusions occur in 0.6% to 2% of NSCLCs and 1.2% to 2% of adenocarcinomas. *RET* inhibition with pralsetinib was granted accelerated approval by the FDA in 2020 for treatment of metastatic *RET*-fusion-positive NSCLC. Approval was based on results from an open-label, non-randomized phase 1/2 trial in 114 patients (NCT03037385). Among 27 treatment-naive patients, the ORR was 70% with 58% of responses lasting 6 months or longer in duration. Among 87 patients previously treated with chemotherapy, the ORR was 57% with 80% of responses lasting 6 months or longer in duration. RET inhibition with selpercatinib was granted accelerated approval by the FDA in 2020 for the treatment of RET fusion-positive metastatic NSCLC and advanced or metastatic medullary thyroid cancer. Approval for NSCLC was based on results from an open-label, non-randomized phase 1/2 trial in 144 patients (NCT03157128). Among 39 treatment-naive patients, the ORR was 85% with 58% of responses lasting 6 months or longer in duration. Among 105 patients previously treated with platinum chemotherapy, the ORR was 64% with 81% of responses lasting 6 months or longer in duration.

### Genetic Biomarkers With Off-Label Targeted Therapies

Proposed targeted therapies may be used off-label for genetic alterations in human epidermal growth factor receptor 2 (trastuzumab, afatinib), *MET* (crizotinib), and *RET* (cabozantinib, vandetanib). Human epidermal growth factor receptor 2 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. Human epidermal growth factor receptor 2 is expressed in approximately 25% of NSCLC.

### Genetic Biomarkers Without Targeted Therapies

The most common predictive variant in North American populations is *KRAS*, occurring in 20% to 25% of NSCLC. Patients with *KRAS* variants have shorter survival than those without *KRAS* variants, and thus *KRAS* is a prognostic marker. It also predicts a lack of TKI efficacy. Because *KRAS* variants are generally not found with other tumor biomarkers, *KRAS* testing might identify patients who would not benefit from further molecular testing. Targeted therapies are under investigation for *KRAS*-variant NSCLC.

### Tyrosine Kinase Inhibitor-Resistance Variants

#### *EGFR* Variants

The *EGFR* variant T790M has been associated with acquired resistance to TKI therapy. When the T790M variant is detected in tissue biopsies from patients with suspected resistance to TKI therapy, osimertinib is recommended as second-line therapy. The use of osimertinib as first-line therapy for patients who have *EGFR*-sensitizing variants was approved by the FDA in 2018 on the basis of the randomized, double-blind phase 3 FLAURA trial (see Table 6).

## Treatment Selection

### Tissue Biopsy as a Reference Standard

The standard for treatment selection in NSCLC is biomarker analysis of tissue samples obtained by biopsy or surgery. However, a lung biopsy is invasive with a slow turnaround time for obtaining results. Tissue biopsy may also be an imperfect reference standard due to inadequate sampling, tumor heterogeneity, or other factors.

### Technologies for Detecting Circulating Tumor DNA

Cell-free DNA in blood is derived from nonmalignant and malignant cell DNA. The small DNA fragments released into the blood by tumor cells are referred to as ctDNA. Most ctDNA is derived from apoptotic and necrotic cells, either from the primary tumor, metastases or circulating tumor cells.[1.] Unlike apoptosis, necrosis is considered a pathologic process,

generating larger DNA fragments due to incomplete and random digestion of genomic DNA. The length or integrity of the circulating DNA can potentially distinguish between apoptotic and necrotic origins. The ctDNA can be used for genomic characterization of the tumor and identification of the biomarkers of interest.

Detection of ctDNA is challenging because cell-free DNA is diluted by nonmalignant circulating DNA and usually represents a small fraction (<1%) of total cell-free DNA. Therefore, methods up to 500 to 1000 times more sensitive than standard sequencing approaches (e.g., Sanger) are needed.

Sensitive and specific methods are available to detect ctDNA and identify single nucleotide variants, duplications, insertions, deletions, and structural variants. Examples of methods are as follows:

- Denaturing high-performance liquid chromatography involves polymerase chain reaction (PCR) followed by denaturing plus hybridization and then separation.
- Peptide nucleic acid-locked nucleic acid PCR suppresses wild-type *EGFR* followed by enrichment for mutated *EGFR*.
- Amplification refractory mutation system PCR generates different-sized PCR products based on the allele followed by separation of PCR fragments to determine the presence of variants.
- BEAMing combines emulsion PCR with magnetic beads and flow cytometry.
- Digital genomic technologies, such as droplet digital PCR, allow for the enumeration of rare variants in complex mixtures of DNA.

Genetic testing of ctDNA can be targeted at specific genes or at commonly found, acquired, somatic variants ("hotspots") that occur in specific cancers, which can impact therapy decisions (e.g., *EGFR* and *ALK* in NSCLC); such testing can also be untargeted and may include array comparative genomic hybridization, next-generation sequencing, and whole exome and genome sequencing. Panel testing for specific genetic variants that may impact therapy decisions in many different cancers can also be performed.

**Literature Review**

Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose. Evidence reviews assess the evidence on whether a test is clinically valid and clinically useful. Technical reliability is outside the scope of these reviews, and credible information on technical reliability is available from other sources.

**Selecting Targeted Therapy**

**Clinical Context and Test Purpose**

The purpose of identifying targetable oncogenic "driver mutations" such as epidermal growth factor receptor (*EGFR*) variants in patients who have non-small-cell lung cancer (NSCLC) is to inform a decision whether patients should receive a targeted therapy vs another systemic therapy. Patients have traditionally been tested for driver mutations using samples from tissue biopsies.

Figures 1 and 2 show how liquid biopsy could be used to select first-line and second-line treatments in patients with advanced NSCLC with reflex to tissue biopsy and how it would potentially affect outcomes. The testing strategy in Figure 1 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 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.[5,]

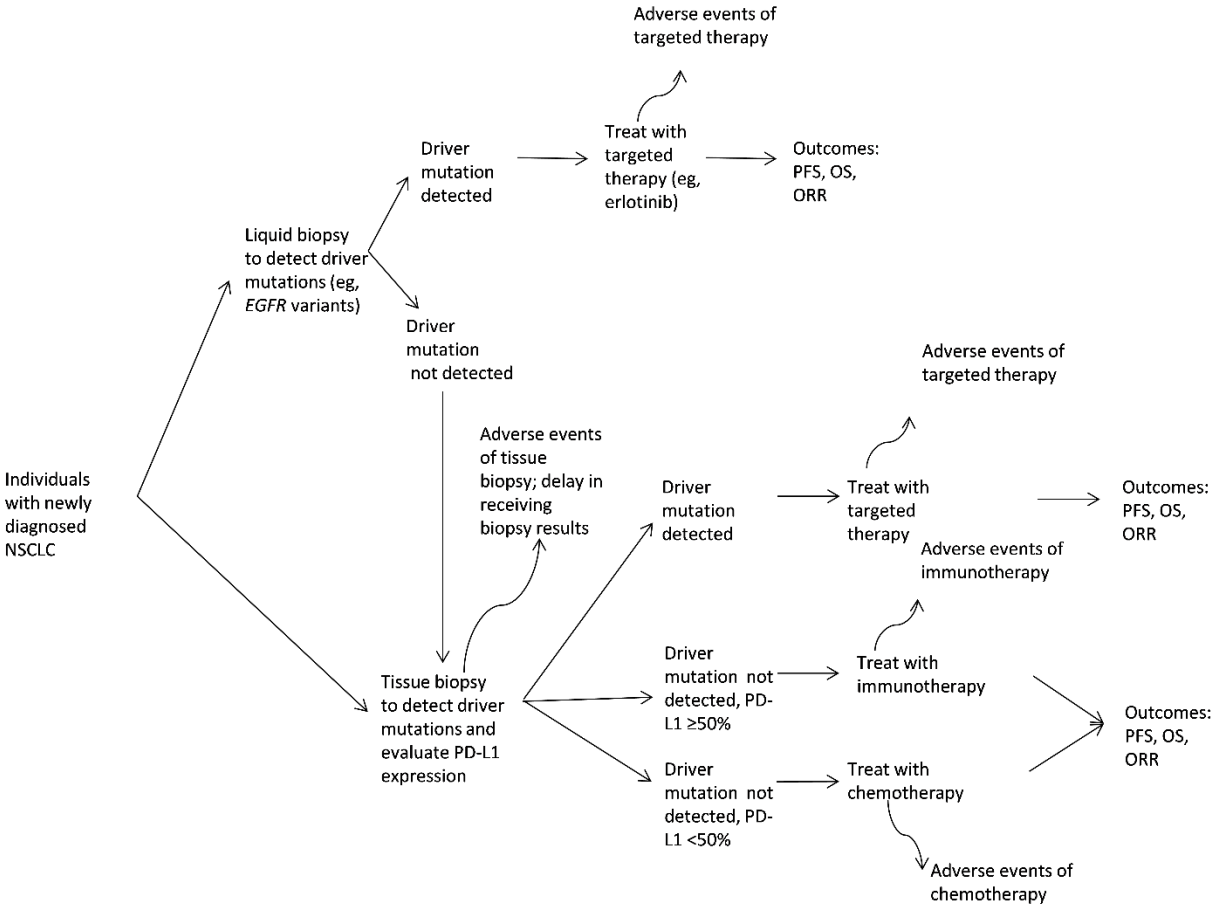
The evidence is considered separately for the different biomarkers. Studies have evaluated liquid biopsy for biomarkers that detect *EGFR* tyrosine kinase inhibitor (TKI) sensitization, concentrating on the *EGFR* exon 19 deletion and *EGFR* L858R variants. Studies have also evaluated separately biomarkers 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***

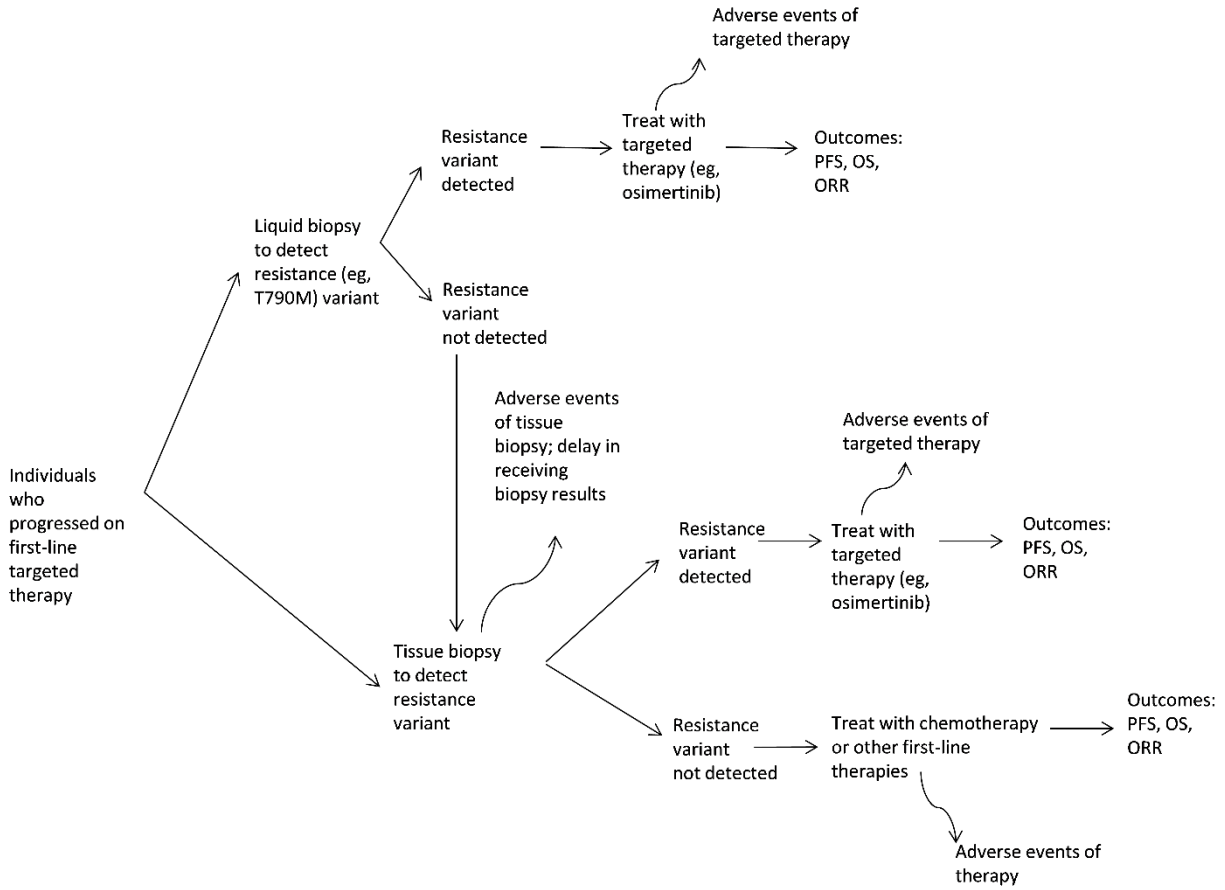
The relevant comparator of interest is testing for variants using tissue biopsy.
The testing strategy in Figure 1 is based on the reflex testing strategy suggested in the FDA approval for the cobas test. Some guidelines have suggested that testing with a liquid biopsy should be used when testing with tissue biopsy is not feasible.

**Figure 1. Liquid and Tissue Biopsy in the Selection of First-Line Systemic Therapy for Advanced NSCLC**




*EGFR*: epidermal growth factor receptor; NSCLC: non-small-cell lung cancer; PD-L1: programmed death-1 ligand; PFS: progression-free survival; ORR: objective response rate; OS: overall survival.

**Figure 2. Liquid and Tissue Biopsy in the Selection of Second-Line Systemic Therapy for Advanced NSCLC**




NSCLC: non-small-cell lung cancer; PFS: progression-free survival; ORR: objective response rate; OS: overall survival.

### *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%.[6.]

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. The benefits of targeted therapy for patients with driver mutations in NSCLC are discussed in Blue Shield of California Medical Policy: Molecular Analysis for Targeted Therapy of Non-Small-Cell Lung Cancer.

**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.[7,]

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).[8,] 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).[9,]

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.

**Technically Reliable**
Assessment of technical reliability focuses on specific tests and operators and requires a review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

**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).

BCBSA staff performed 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.[10] 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).[11]

**Table 1. 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)[12] | 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)[13] | 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)[14] | 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)[15] | Patients with newly diagnosed or relapsed patients with advanced (stage IIIB, IV) NSCLC in U.S., Europe, and | Prospective | *EGFR*, *BRAF* | Plasma was collected within 60 d of tumor biopsy |

| Study | Study Population | Design | Variants Included[a] | Timing of Reference and Index Tests |
|---|---|---|---|---|
| | Australia between 2011 and 2013 | | | |
| Thress et al (2015)[16.] | 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 |
| Mok et al (2015)[17.] | Patients enrolled in a phase 3 RCT in Asian 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)[18.] | 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** | | | | |
| FDA SSED (2020)[14.] | 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 vs 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)[19.] | Patients with biopsy-proven, previously untreated, nonsquamous NSCLC (stage IIIB/IV) enrolled in the NILE study (Non-invasive versus Invasive Lung Evaluation at 1 of 28 North American centers between 2016 and 2018 | Prospective | *EGFR*, *ALK*, *ROS1*, *BRAF, MET, RET* | Unclear |
| Schwaederle et al (2017)[20.] | Patients with lung adenocarcinoma (86% with metastatic disease) from academic | Retrospective, consecutive | *EGFR*, *ALK*, *ROS1*, *BRAF* | Median time was 0.8 mo, range not given |

| Study | Study Population | Design | Variants Included[a] | Timing of Reference and Index Tests |
|---|---|---|---|---|
| | medical center in California between 2014 and 2015 | | | |
| Thompson et al (2016)[21] | 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)[22] | 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)[23] | 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)[15] | 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)[16] | 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)[24] | 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)[25] | 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** | | | | |
| Pritchet et al (2019)[26] | Patients with untreated, advanced NSCLC; primarily from cohorts enrolled in 2 | Prospective | *EGFR*, *ALK*, *ROS1*, *BRAF*, *MET* | Blood collected within 12 weeks of tissue biopsy and no therapy |

| Study | Study Population | Design | Variants Included[a] | Timing of Reference and Index Tests |
|---|---|---|---|---|
| | prospective US studies with 41 centers | | | between tissue and blood samples |
| Remon et al (2019)[27] | Patients with advanced NSCLC enrolled in single-center, prospective observational study in France. Patients were either treatment naive for advanced disease or who had 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)[28] | 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; 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 2 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 positive percent agreement and negative percent agreement, 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 2. 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)[12] | 562 | | No plasma sample; mainland China patients; withdrawn informed consent; invalid tests | | |

| Study | Initial N | Final N | Excluded Samples | Sensitivity (95% CI) | Specificity (95% CI) |
|---|---|---|---|---|---|
| *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)[13] | | | | | |
| *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)[14] | | | | | |
| *EGFR*-sensitizing variants | 601 | 431 | Insufficient plasma; invalid test result | 77 (71 to 82) | 98 (95 to 99) |
| Karlovich et al (2016)[15] | | | | | |
| *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)[16] | | | | | |
| *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)[17] | | | | | |
| *EGFR*-sensitizing variants | 397 | 238 | Insufficient plasma or tissue; invalid test result | 75 (65 to 83) | 96 (92 to 99) |
| Weber et al (2014)[18] | | | | | |
| *EGFR*-sensitizing and -resistance variants | 199[a] | 196 | Inadequate tumor tissue | 61 (41 to 78) | 96 (92 to 99) |
| **Guardant360 CDx** | | | | | |
| FDA SSED (2020)[29] | | | | | |
| *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)[12] | 562 | | No plasma sample; mainland China 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) |

| Study | Initial N | Final N | Excluded Samples | Sensitivity (95% CI) | Specificity (95% CI) |
|---|---|---|---|---|---|
| *EGFR* exon 20 (T790M, resistance) | | 207 | 51 (44 to 58) 66 (59 to 72) | 66 (59 to 72) | NA[d] |
| Leighl et al (2019)[19.] | 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)[20.] | | | | | |
| *EGFR* variants (various) | 88 | 34 | No tissue | 54 (25 to 81) | 90 (70 to 99) |
| Thompson et al (2016)[21.] | 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)[22.] | 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 |
| **OncoBEAM** | | | | | |
| Ramalingam et al (2018)[23.] | 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) |
| Karlovich et al (2016)[15.] | | | | | |
| *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)[16.] | | | | | |
| *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)[12.] | 562 | | No plasma sample; mainland China patients; withdrawn informed consent; invalid tests | | |

| Study | Initial N | Final N | Excluded Samples | Sensitivity (95% CI) | Specificity (95% CI) |
|---|---|---|---|---|---|
| *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)[24] | | | | | |
| *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)[25] | 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)[26] | 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)[27] | 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)[29] | 280 | | Samples in which there was insufficient plasma to process both replicates of the cobas reference test | | |
| *EGFR* exon 19 deletion (sensitizing)[e] | | 135 | | 95 (83 to 99)[c] (rep 1)<br>95 (83 to 99)[c] (rep 2) | 96 (89 to 99)[c] (rep 1)<br>96 (89 to 99)[c] (rep 2) |
| *EGFR* exon 21 substitution (L858R, sensitizing)[e] | | 133 | | 95 (83 to 99)[c] (rep 1)<br>100 (89 to 100)[c] (rep 2) | 96 (89 to 99)[c] (rep 1)<br>94 (86 to 97)[c] (rep 2) |
| *EGFR*-sensitizing (combined)[e] | | 177 | | 98 (91 to 100)[c] (rep 1)<br>98 (91 to 100)[c] (rep 2) | 96 (89 to 99)[c] (rep 1)<br>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 3 and 4) 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 3. 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)[12] | | | | | |
| **Cobas EGFR test** | | | | | |
| Jenkins et al (2017)[13] | | | | | |
| FDA SSED (2016)[14] | 4. Performed in Asia | | | | |
| Karlovich et al (2016)[15] | | | | | |
| Thress et al (2015)[16] | | | | | |
| Mok et al (2015)[17] | 4. Performed in Asia | | | | |
| Weber et al (2014)[18] | | | | | |
| **Guardant360 CDx** | | | | | |
| FDA SSED (2020)[29] | 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)[19] | | | | | |
| Schwaederle et al (2017)[20] | | | | | |
| Thompson et al (2016)[21] | | | | | |
| Villaflor et al (2016)[22] | | | | | |
| **OncoBEAM** | | | | | |
| Ramalingam et al (2018)[23] | 4. Performed in Asia | | | | |
| Karlovich et al (2016)[15] | | | | | |
| Thress et al (2015)[16] | | | | | |
| **Biodesix ddPCR** | | | | | |
| Mellert et al (2017)[24] | 3. Patient characteristics unclear | | | | |
| **ctDx-Lung** | | | | | |
| Paweletz et al (2016)[25] | 2. Unclear if same as current marketed version | | | | |
| **Invision** | | | | | |
| Pritchet et al (2019)[26] | 4: Calculation of performance characteristics only included subset of patients with at least 1 mutation | | | | |

| Study | Population[a] | Intervention[b] | Comparator[c] | Outcomes[d] | Duration of Follow-Up[e] |
|---|---|---|---|---|---|
| | detected by liquid biopsy | | | | |
| Remon et al (2019)[27.] | | | | | |
| **FoundationOne Liquid CDx** | | | | | |
| FDA SSED (2020)[28.] | 3. Eligibility criteria for retrospective-sourced plasma samples unclear<br>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 4. 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)[12.] | | | | | | |
| **Cobas EGFR test** | | | | | | |
| Jenkins et al (2017)[13.] | | | | | | |
| FDA SSED (2016)[14.] | | | | | | |
| Karlovich et al (2016)[15.] | | | | | | |
| Thress et al (2015)[16.] | | | 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)[17.] | | | 1. Time between blood and tissue sample | | | 1. Precision estimates not reported but calculated |

| Study | Selection[a] | Blinding[b] | Delivery of Test[c] | Selective Reporting[d] | Data Completeness[e] | Statistical[f] |
|---|---|---|---|---|---|---|
| | | | collection not described | | | based on data provided |
| Weber et al(2014)[18] | 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)[14] | | | 2. Time between tissue and plasma sample unclear; subset of samples collected after progression or treatment discontinuation | | | |
| Leighl et al (2019)[19] | | | 2.Time between tissue and plasma sample unclear | | | 1. Precision estimates not reported but calculated based on data provided |
| Schwaederle et al (2017)[20] | | | | | | 1. Precision estimates not reported but calculated based on data provided |
| Thompson et al (2016)[21] | | | 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)[22] | 1,2. Unclear how patients were selected | | 1.Time between tissue and blood collection was up 7 y, median 1.4 y | | | 1. Precision estimates not reported but calculated based on data provided |
| **OncoBEAM** | | | | | | |
| Ramalingam et al (2018)[23] | | | 1. Time between blood and tissue sample collection not described | | | |
| Karlovich et al (2016)[15] | | | | | | |
| Thress et al (2015)[16] | | | 1. Both samples collected after | | | 1. Precision estimates not |

| Study | Selection[a] | Blinding[b] | Delivery of Test[c] | Selective Reporting[d] | Data Completeness[e] | Statistical[f] |
|---|---|---|---|---|---|---|
| | | | progression and before next treatment but time between blood and tissue sample collection not described | | | reported but calculated based on data provided |
| **Biodesix ddPCR** | | | | | | |
| Mellert et al (2017)[24] | 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)[25] | 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)[26] | | | | | | 1. Precision estimates not reported but calculated based on data provided |
| Remon et al (2019)[27] | | | | | | |
| **FoundationOne Liquid CDx** | | | | | | |
| FDA SSED (2020)[28] | 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 5. 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 5. 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[13.]; FDA SSED[14.]; Karlovich[15.]; Thress[16.]; Mok[17.]; Weber[18.]) |
| Guardant360 CDx (NGS) | 63-100 | 96-100 | 5 | Long time between tissue and ctDNA tests (Leighl[19.];Thompson[21.]; Villaflor[22.]); unclear patient selection (Villaflor[22.]); variants not stratified by type in Schwaederle[20.]; very few limitations with Papadimitrakopoulou[12.]); outcomes from test versions combined (FDA SSED)[29.] |
| 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)[28.] |
| OncoBEAM | 63-82 | 67-100 | 3 | Few limitations identified (Karlovich[15.]; Thress[16.]; Rmalingam[23.]) Only a few negatives in Karlovich for estimating specificity. |
| Biodesix (ddPCR) | 70-100 | 100 (NR)[24.] | 2 | Patient characteristics and selection unclear; timing of blood and tissue samples unclear; precision estimates |

| Test (Method) | Comparison With Tissue Test | | | Study Quality |
|---|---|---|---|---|
| | | | | not provided (Mellert[24]; very few limitations with Papadimitrakopoulou[12]) |
| Resolution Bio ctDx-Lung | 89 (65 to 99)[b] | 100 (88 to 100)[b] | 1 | Several limitations identified (Paweletz[25]) |
| 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[26], Remon[27]) |

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.[30,]

**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, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary 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, BCBSA also considered 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 outcomes data for studies evaluating *EGFR* TKI-sensitizing and *EGFR* TKI-resistance variants are shown in Tables 6 and 7, respectively.

**Table 6. *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)[31]; newly diagnosed *EGFR*-positive and -negative patients treated with EGFR TKIs** | China | IV (85.6%) | ddPCR | PFS (95% CI), mo | | | |
| | | | | n | EGFR TKI | | p |
| | | | | 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)[29]; 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) | | <0.0001 |
| | | | | Liquid positive and tissue positive | | | |
| | | | | 304 | 0.41 (0.31 to 0.54) | | <0.0001 |
| **Zhang et al (2017)[32]; *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) | |

| Study/Patient Group | Country | Disease Stage | Technology Used to Detect ctDNA | Sample Sizes | Treatment Response | | |
|---|---|---|---|---|---|---|---|
| | | | | Tissue negative and liquid positive | | | |
| | | | | 3 | 133, 410, and 1153 | | |
| **FDA SSED (2016)[14]; 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)[33]; 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) | 0.14 |
| | | | | All patients with exon 19 deletion in tissue | | | |
| | | | | 56 | 30.4 (19.8 to 55.7) | 18.9 (10.4 to 36.2) | 0.22 |
| | | | | Patients with exon 19 deletion in both tissue and ctDNA | | | |
| | | | | 47 | 34.4 (22.9 to NR) | 19.9 (9.8 to 36.2) | 0.23 |
| | | | | Patients with exon 19 deletion in tissue but not ctDNA | | | |
| | | | | 9 | 13.0 (8.9 to 19.8) | 15.5 (0.3 to NR) | 0.87 |
| | | | | All patients with L858R variant in tissue | | | |
| | | | | 41 | 17.7 (6.3 to 26.8) | 17.5 (8.2 to 23.5) | 0.67 |
| | | | | 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) | 0.67 |
| | | | | Patients with L858R variant in tissue but not in ctDNA | | | |
| | | | | 12 | 29.4 (8.6 to 63.0) | 25.6 (16.1 to NR) | 0.64 |

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 6 (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 vs gefitinib or erlotinib in the first-line treatment of patients with locally advanced and metastatic NSCLC.[29] 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 vs LDT discordance; these imputed results did not significantly deviate from the original observations (HR=0.40-0.42). 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 biopsy.[31] 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, $P < 0.001$). 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 vs gemcitabine plus cisplatin as first-line treatment of NSCLC.[14] 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.[32] 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 vs 60 days for *EGFR*-negative subjects (p<0.001). 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 7. *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)[12]; AURA3 phase | Multinational[c] | Locally advanced | cobas (RT-PCR); | ORR (95% CI) (Osimertinib vs Chemotherapy) |

| Study/Patient Group | Country | Disease Stage | Technology Used to Detect ctDNA | Treatment Response | | |
|---|---|---|---|---|---|---|
| 3 trial of patients who progressed on EGFR TKI | | or metastatic | Guardant360 (NGS); Biodesix (ddPCR) | | | |
| | | | 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)[34]; AURA phase 1 trial of patients who progressed on EGFR TKI | Multinational[b] | Advanced | BEAMing | ORR (95% CI) (Osimertinib) | | |
| | | | | 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)[16]; 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% | |

| Study/Patient Group | Country | Disease Stage | Technology Used to Detect ctDNA | Treatment Response |
|---|---|---|---|---|
| Karlovich et al (2016)[15]; 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)[35]; 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 |
| | | | | 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 7).[16] 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.[34] Some patients may have overlapped with the Thress et al (2015) study.[16] 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.[15] 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 8). 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.[35] The ORR and PFS were provided for patients who were tissue positive and for patients who were liquid positive (see Table 9). 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 chemo-therapy after progression on *EGFR* TKI therapy.[12] 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 8). 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[36] and in treatment-naive patients[37]. 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.[38] 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.

**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 American Society of Clinical Oncology with an expert consensus opinion that "Physicians may use plasma cfDNA 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 with advanced NSCLC who receive testing for biomarkers of *EGFR* TKIs 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 (DSS), and test validity. Current evidence does not permit determining whether cobas or tissue biopsy is more strongly associated with patient outcomes or treatment response. BCBSA identified 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 vs chemotherapy with high specificity. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals with advanced 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, DSS, and test validity. Current evidence does not permit determining whether liquid or tissue biopsy is more strongly associated with patient outcomes or treatment response. BCBSA identified 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 vs chemotherapy with high specificity. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals with advanced 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, DSS, 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. BCBSA found no RCTs providing evidence of the clinical utility of those methods of liquid biopsy. The evidence is insufficient to determine the effects of the technology on health outcomes.

For individuals with advanced 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, DSS, 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 the effects of the technology on health outcomes.

For individuals with advanced 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, DSS, 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 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. The evidence is insufficient to determine the effects of the technology on health outcomes. 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 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**
**Practice Guidelines and Position Statements**

**National Comprehensive Cancer Network**
National Comprehensive Cancer Network guidelines (v.8.2020) discuss the role of liquid biopsy in the management of non-small-cell lung cancer (NSCLC).[5] The guidelines state that cell-free/circulating tumor DNA testing should not be used in lieu of histologic tissue diagnosis. They also state that cfDNA testing can be used if the patient is not medically fit for tissue sampling or there is insufficient tissue for molecular analysis. If plasma-based analysis is used, follow-up with tissue-based analysis should be planned if plasma-based analysis is negative. The guidelines also state that at progression on erlotinib, afatinib, gefitinib or dacomitinib when testing for T790M, plasma-based testing should be considered and when plasma-based testing is negative, tissue-based testing is strongly recommended. Scheduling the biopsy concurrently with plasma testing referral may be considered.

The guidelines additionally state that if there is insufficient tissue to allow testing for *EGFR*, *ALK*, *ROS1*, *BRAF*, *MET*, and *RET*, repeat biopsy and/or plasma testing should be done. If not feasible, treatment should be guided by available results, and if mutation status is unknown, patients are treated as though they do not have driver oncogenes. Diagnosis of NSCLC should be guided by tissue. The guidelines do not endorse any specific commercially available test.

**International Association for the Study of Lung Cancer**
In 2018, the International Association for the Study of Lung Cancer published a statement paper on liquid biopsy for advanced non-small-cell lung cancer.[39] The work preparing the statement was supported by unrestricted grants from Guardant Health, Astra Zeneca, Biocept, and Roche. The statement made the following recommendations:

- "The criteria used to select treatment-naive patients for molecular testing of ctDNA [circulating tumor DNA] is the same used for molecular testing using DNA isolated from tissue."

- "Liquid biopsy can be considered at the time of initial diagnosis in all patients who need tumor molecular profiling, but it is particularly recommended when tumor tissue is scarce, unavailable, or a significant delay potentially greater than 2 weeks is expected in obtaining tumor tissue."

The following tests are acceptable to detect epidermal growth factor receptor (*EGFR*)-sensitizing variants and results are sufficient to start a first-line treatment with an EGFR tyrosine kinase inhibitor:

- Cobas EGFR Mutation Test v2.
- droplet digital polymerase chain reaction next-generation sequencing panels
- Multiplex panels using next-generation sequencing platforms could be considered to detect *EGFR*, *ALK*, *ROS1*, or *BRAF* variants and a positive result would be adequate to initiate first-line therapy.

A next-generation sequencing multiplex panel was preferred to detect T790M and other common resistance alterations. A positive result for *EGFR* T790M should be considered adequate to initiate osimertinib in the second-line setting.

**College of American Pathologists et al**

In 2018, the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology published a guideline on molecular testing for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors.[38,] The American Society of Clinical Oncology also endorsed the joint College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology guidelines with minor modifications.[40,]

The guidelines noted the following recommendation regarding liquid biopsy for activating *EGFR* mutations and a consensus opinion regarding liquid biopsy for the T790M resistance mutation.

- Recommendation: "In some clinical settings in which tissue is limited and/or insufficient for molecular testing, physicians may use a cfDNA assay to identify [activating] *EGFR* mutations."
- Expert Consensus Opinion: "Physicians may use plasma cfDNA 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."
- No recommendation: "There is currently insufficient evidence to support the use of circulating tumor cell molecular analysis for the diagnosis of primary lung adenocarcinoma, the identification of *EGFR* or other mutations, or the identification of *EGFR* T790M mutations at the time of *EGFR* TKI resistance."

**National Institute for Health and Care Excellence**

In 2018, the National Institute for Health and Care Excellence issued an innovation briefing on plasma *EGFR* mutation tests for adults with locally advanced or metastatic NSCLC.[41,] The briefing reviewed 7 ctDNA tests available in Europe and concluded:

- "The intended place in therapy would be as an alternative to tissue *EGFR* testing or before tumour testing to inform decisions about prescribing *EGFR*-TKIs. Plasma testing may be particularly useful for people whose disease has developed resistance to an *EGFR*-TKI and who could be offered second-line *EGFR*-TKIs, if appropriate, without having further tissue testing."
- "The main points from the evidence summarised in this briefing are from 7 non-UK-based prospective studies with 2,106 adults. They show that the diagnostic accuracy of plasma EGFR mutation testing has a similar specificity, but lower sensitivity, compared with tissue *EGFR* mutation testing in adults with locally advanced or metastatic NSCLC."

- "Key uncertainties around the evidence or technology are that tests for identifying *EGFR*-TKI mutations are rapidly evolving and there is no established gold-standard test against which to evaluate them."

### U.S. Preventive Services Task Force Recommendations
Not applicable.

### Medicare National Coverage
There is no national coverage determination. In the absence of a national coverage determination, coverage decisions are left to the discretion of local Medicare carriers.

### Ongoing and Unpublished Clinical Trials
Some currently ongoing and unpublished trials that might influence this review are listed in Table 8.

**Table 8. Summary of Key Trials**

| NCT No. | Trial Name | Planned Enrollment | Completion Date |
|---|---|---|---|
| *Ongoing* | | | |
| NCT01930474 | Analysis of Mechanism of Resistance to Chemotherapy by Sequencing of Plasma DNA | 200 | Dec 2018 (unknown) |
| NCT02894853[a] | Lung Cancer Early Molecular Assessment Trial | 1297 | Dec 2019 (unknown) |
| NCT02284633[a] | Use of a New Blood Test to Identify Response to Targeted Treatment in Patients With EGFR Mutated Lung Cancer | 250 | Jun 2020 (ongoing) |
| NCT02160366 | Profile Related Evidence to Determine Individualized Cancer Therapy (PREDICT) Program in Advanced Cancer Patients | 2000 | Sep 2020 (recruiting) |
| NCT03791034[a] | Prospective Feasibility Study of Cell Free Circulating Tumor DNA for the Diagnosis and Treatment Monitoring in Early-stage Non-small Cell Lung Cancer | 700 | Dec 2020 (recruiting) |
| NCT03465241 | Prospective, Open Clinical Study of Postoperative ctDNA Dynamic Monitoring and Its Role of Prognosis in Patients With Stage II to IIIA Non-small Cell Lung Cancer (NSCLC) Using Secondary Gene Sequencing (NGS) | 200 | Dec 2021 (recruiting) |
| NCT04238130 | Evaluation Perioperative Dynamic Changes in ctDNA From Patients of Non-Small-Cell Lung Cancer Following Resection for Relapse Prediction (EVOLUTION) | 200 | Jun 2023 (recruiting) |
| NCT03553550 | Role of Circulating Tumor DNA (ctDNA) From LIquid Biopsy in Early Stage NSCLC Resected Lung Tumor Investigation (LIBERTI) | 500 | Jun 2024 (recruiting) |
| NCT04178889 | Second Primary Lung Cancer Cohort Study (SPORT) | 850 | Dec 2024 (recruiting) |
| *Unpublished* | | | |
| NCT02418234 | Frequency and Abundance of T790M Mutation on Circulating Tumor DNA in Patients With Non-small Cell Lung Cancer After Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors Treatment Failure: a Perspective Observational Study | 314 | Nov 2017 (completed) |
| NCT03116633[a] | An Observational Multicenter Study to Evaluate the Performance and Utility of Inivata Liquid Biopsy Analysis Compared With Tissue Biopsy Analysis for Detection of Genomic Alterations in Patients With Lung Cancer | 34 | May 2018 (completed) |
| NCT02284633[a] | Blood sample monitoring of patients with EGFR mutated lung cancer | 250 | Dec 2018 |
| NCT02906852[a] | Prospective Observational Study to Evaluate the Performance of Inivata Liquid Biopsy Analysis Compared With Standard Tissue Biopsy Analysis for Detection of Genomic Alterations in Patients With Advanced Non-small Cell Lung Cancer | 264 | Dec 2018 (completed) |

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

# Appendix 1

**Assessment Systematic Review**

BCBSA staff performed a systematic review in 2017, as described in the Methods section (see below) and referred to herein as the "assessment systematic review." The search yielded 266 citations published between the existing published systematic reviews and February 2017. Nineteen studies published in that time frame met selection criteria and were included in the BCBSA assessment systematic review. The BCBSA review also included 35 of the 36 studies identified in 3 existing systematic reviews published in 2015. BCBSA staff did not select a 2007 study included in previous meta-analyses because it was published in Chinese.[42] In total, 55 studies with 6119 patients (range, 9-822 patients) were included.

Fifty-three studies reported on epidermal growth factor receptor (*EGFR*) tyrosine kinase inhibitor (TKI)-sensitivity variants or a combination of sensitivity and resistance variants. Two studies reported only on *EGFR* TKI-resistance variants (T790M). More than half (56%) included only advanced or recurrent non-small-cell lung cancer (NSCLC); 27% included all stages. The majority (75%) used plasma blood samples. Forty (73%) were performed solely in Asia. Various circulating tumor DNA (ctDNA)detection methods were used, with the amplification refractory mutation system being the most common. Study characteristics are shown in Appendix Table 1.

**Appendix Table 1. Characteristics of Studies Included in the Assessment's Systematic Review**

| Study | Year | Sample Size | Country | Plasma or Serum | Disease Stage | ctDNA Detection Method | *EGFR* Variants (Exons) |
|---|---|---|---|---|---|---|---|
| Zhang et al[32] | 2017 | 215 | China | Plasma | IIIB- IV | ddPCR | 19, 21 |
| Sacher et al[30] | 2016 | 174 | U.S. | Plasma | Recurrent, IIIB, IV | ddPCR | 19, 21, 20 |
| FDA SSED[14] | 2016 | 266 | China, Malaysia, Philippines | Plasma | IIIB- IV | cobas | 19, 21 |
| Ohira et al[43] | 2016 | 149 | Japan | Serum | I-IIIA | ddPCR | NR |
| Guo et al[44] | 2016 | 41 | China | Plasma | I-IV | NGS | NR |
| Sundaresan et al[45] | 2016 | 25 | U.S. | Plasma | IIIA-IV | cobas | 20 |
| Takahama et al[46] | 2016 | 41 | Japan | Plasma | Recurrence, IIIB, IV, inoperable | ddPCR | 19, 21, 18, 20 |
| Chen et al[47] | 2016 | 58 | China | Plasma | IA-IIA | NGS | 19, 21 |
| Que et al[48] | 2016 | 121 | China | Plasma | I-IV | DHPLC | 19, 21 |
| Vazquez et al[49] | 2016 | 174 | Spain | Serum | IIIB-IV | SARMS | 19, 21, 20, 18 |
| Han et al[50] | 2016 | 194 | Korea | Plasma | IIIB-IV | PNA clamping-assisted FMCA | 19, 21 |
| Thompson et al[21] | 2016 | 50 | U.S. | Plasma | II-IV | NGS | 19, 21, 20, 18 |
| Kimura et al[51] | 2016 | 24 | Japan | Plasma | NR | PointMan EGFR DNA enrichment kit, direct sequencing | 20 |
| Ma et al[52] | 2016 | 219 | China | Plasma | III-IV | ARMS | 19, 21, 20,18 |
| Oxnard et al[34] | 2016 | 216 | Multinational[a] | Plasma | Advanced | BEAMing | 19, 21, 20 |
| Xu et al[53] | 2016 | 41 | China | Plasma | III-IV | NGS | 19, 21, 20, 18 |
| Karachaliou et al[33] | 2015 | 147 | France, Italy, Spain | Serum | IIIB-IV | PNA-LNA | 19, 21 |
| Thress et al[16] | 2015 | 72 | U.S., Europe, Asia | Plasma | Advanced | cobas, BEAMing | 19, 21, 20 |
| Duan et al[54] | 2015 | 94 | China | Plasma | II-IV | SARMS | 19, 21, 20, 18 |
| Mok et al[17] | 2015 | 238 | China | Plasma | IIIB- IV | cobas | 19, 21, 20, 18 |
| Lam et al[55] | 2015 | 74 | Hong Kong | Plasma | III- IV | PNA-LNA | 19, 21 |

| Study | Year | Sample Size | Country | Plasma or Serum | Disease Stage | ctDNA Detection Method | *EGFR* Variants (Exons) |
|---|---|---|---|---|---|---|---|
| Jing et al[56.] | 2014 | 120 | China | Plasma | I-IV | HRM | 18-21 |
| Wang et al[57.] | 2014 | 134 | China | Plasma | Advanced | ARMS | 19, 21, 20 |
| Li et al[58.] | 2014 | 121 | China | Plasma, serum | I-IV | ARMS | 19, 21, 20 |
| Douillard et al[59.] | 2014 | 652 | Europe | Plasma | NR | ARMS | 19, 21, 20 |
| Weber et al[18.] | 2014 | 196 | Denmark | Plasma | I-IV | cobas | 19, 21, 20 |
| Kim HR et al[60.] | 2013 | 40 | Korea | Plasma | IIIA-IV | PNA-LNA | 19, 21 |
| Kim ST et al[61.] | 2013 | 57 | Korea | Serum | IIIB-IV | PNA-LNA | 19, 21, 20 |
| Lv et al[62.] | 2013 | 9 | China | Plasma | IIB-IIIA | DHPLC | 19, 21 |
| Akca et al[63.] | 2013 | 52 | Turkey | Serum | I-IV | Pyrosequencing, dideoxy sequencing | 19, 21 |
| Liu et al[64.] | 2013 | 86 | China | Plasma | Advanced | ARMS | 29 variants |
| Zhang et al[65.] | 2013 | 86 | China | Plasma | IIIB-IV | MEL | 19, 21, 20 |
| Zhao et al[66.] | 2013 | 111 | China | Plasma | I-IV | ME-PCR | 19, 21 |
| Goto et al[67.] | 2012 | 86 | Japan | Serum | Advanced | SARMS | 19, 21, 20 |
| Nakamura et al[68.] | 2012 | 70 | Japan | Plasma | I-IV | WIP-QP, MBP-QP | 19, 21 |
| Xu et al[69.] | 2012 | 34 | China | Serum | IIIB-IV | SARMS, DHPLC, ME-PCR | 19, 21 |
| Yam et al[70.] | 2012 | 37 | Hong Kong | Plasma | III-IV | PNA-LNA | 19, 21, 18 |
| Punnoose et al[71.] | 2012 | 28 | Australia, U.S. | Plasma | NR | SARMS | 19, 21, 20, 18 |
| Huang et al[72.] | 2012 | 822 | China | Plasma | I-IV | DHPLC | 19, 21 |
| Chen et al[73.] | 2012 | 30 | Taiwan | Plasma | NR | PNA-LNA | 19, 21 |
| Hu et al[74.] | 2012 | 24 | China | Serum | I-IV | HRM | 19, 21, 20,18 |
| Brevet et al[75.] | 2011 | 31 | U.S. | Plasma | III-IV | MSG, ME-PCR | 19, 21 |
| Jiang et al[76.] | 2011 | 58 | China | Serum | IIIB-IV | ME-PCR | 19, 21 |
| Sriram et al[77.] | 2011 | 64 | Australia | Serum | I-IV | ME-PCR | 19, 21 |
| Yasuda et al[78.] | 2011 | 23 | Japan | Serum | I-IV | PNA-LNA | 19, 21, 20, 18 |
| Taniguchi et al[79.] | 2011 | 44 | Japan | Plasma | Advanced | BEAMing | 19, 21, 20 |
| Song et al[80.] | 2010 | 50 | China | Serum | I-IIIA | Direct sequencing | 19, 21 |
| Bai et al[81.] | 2009 | 230 | China | Plasma | IIIB-IV | DHPLC | 19, 21 |
| Yung et al[82.] | 2009 | 35 | Hong Kong | Plasma | III-IV | ddPCR | 19, 21 |
| Mack et al[83.] | 2009 | 14 | U.S. | Plasma | IIIB-IV | SARMS | 19, 21, 20 |
| He et al[84.] | 2009 | 18 | China | Plasma | I-IV | ME-PCR | 19, 21 |
| Kuang et al[85.] | 2009 | 54 | U.S. | Plasma | Advanced | SARMS, direct sequencing | 19, 21 |
| Maheswaran et al[86.] | 2008 | 17 | U.K. | Plasma | NR | SARMS | 19, 21 |
| Kimura et al[87.] | 2007 | 42 | Japan | Serum | IIIB-IV | SARMS | 19, 21, 18 |
| Kimura et al[88.] | 2006 | 11 | Japan | Serum | IIIB-IV | SARMS | 19, 21 |

ARMS: amplification refractory mutation system; BEAM: beads, emulsions, amplification, and magnetics; ctDNA: circulating tumor DNA; ddPCR: dropletdigital polymerase chain reaction; DHPLC: denaturing high performance liquid chromatography; *EGFR*: epidermal growth factor receptor; FDA: Food and Drug Administration; FMCA: fluorescence melting curve analysis; HRM: high-resolution melting; MBP-QP: mutation-biased polymerase chain reaction quenching probe; ME-PCR: mutant-enriched polymerase chain reaction; MEL: mutant-enriched liquid chip; MSG: multiplexed shotgun genotyping; NGS: next-generation sequencing; NR: not reported; PNA-LNA: peptide nucleic acid-locked nucleic acid; SARMS: Scorpion amplification refractory mutation system; SSED: Summary of Safety and Effectiveness Data; WIP-QP: wild inhibiting polymerase chain reaction and quenching probe.

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

BCBSA staff assessed the risk of bias for studies included in its assessment systematic review using QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies). QUADAS-2 ratings by study and summaries are shown in Appendix Table 3 and Appendix Figure 1. Because the method used to select patients was frequently not described in the selected studies and therefore staff could not determine whether included patients were selected randomly, consecutively, or as

convenience samples, the risk of bias for patient selection was rated as unclear in 33 (61%) studies. There were also concerns about the applicability of included studies because most were carried out in Asian countries with tests that may not be commercially available in the United States. Due to lack of information on whether results were interpreted without knowledge of the other test and how cutoffs were defined, the risks of bias for the index test and reference standard were unclear in 30% and 26% of the studies, respectively. The risk of bias for participant flow was high in 30% of studies and unclear in 14% of studies because of the length of time or lack of clarity about the length of time between collection of tissue and blood samples or because of the large number of exclusions from the analysis.

For *EGFR* TKI-sensitizing variants (or grouped *EGFR* variants when sensitizing variants were presented with resistance variant), the sensitivities ranged from 0% to 98% and specificities ranged from 71% to 100%. Sensitivities and specificities for each study are shown in Appendix Figures 2 and 3, respectively. The summary receiver operating characteristic (ROC) curve for *EGFR* TKI-sensitizing variants is shown in Appendix Figure 4 and indicates little trade-off between sensitivity and specificity. Overall, the area under the curve was 0.87, with a positive likelihood ratio of 11.1 (95% confidence interval [CI], 7.8 to 15.3), a negative likelihood ratio of 0.4 (95% CI, 0.3 to 0.5), and a diagnostic odds ratio (DOR) of 29 (95% CI, 19 to 43). The performance characteristics for subgroups related to disease stage, plasma vs serum, and ctDNA detection method are shown in Appendix Table 2. None of the covariates were statistically significant in the bivariate meta-regression model. Numerically, the cobas test had the highest area under the receiver operating characteristic curve (AUROC=0.96) and DOR (104.0; 95% CI, 57.5 to 173.0).

**Appendix Table 2. Overall and Subgroup Meta-Analytic Results for EGFR TKI-Sensitizing Variants**

| Subgroups | Studies | AUROC | Sensitivity (95% CI), % | Specificity (95% CI), % | PLR (95% CI)[a] | NLR (95% CI)[a] | DOR (95% CI)[a] |
|---|---|---|---|---|---|---|---|
| **Overall** | 53 | 0.87 | 64 (59 to 70) | 95 (93 to 96) | 11.10 (7.76 to 15.30) | 0.38(0.32 to 0.45) | 29.3 (18.7 to 43.4) |
| **Stage** | | | | | | | |
| **Only I-IIIA** | 4 | 0.70 | 14 (2 to 59) | 96 (78 to 99) | 5.10 (1.79 to 11.80) | 0.84 (0.56 to 0.98) | 6.2 (2.0 to 15.1) |
| **Mixed** | 15 | 0.86 | 61 (52 to 69) | 95 (91 to 97) | 9.93(6.03 to 15.50) | 0.42(0.32 to 0.53) | 24.4(12.4 to 43.7) |
| **Only III-IV, recurrence** | 30 | 0.89 | 68 (61 to 74) | 95 (92 to 97) | 11.20(7.03 to 16.80) | 0.35(0.28 to 0.43) | 32.7(18.7 to 53.4) |
| **Not reported** | 4 | 0.93 | 77 (59 to 89) | 89 (11 to 100) | 37.30(0.94 to 227.0) | 0.43(0.22 to 1.36) | 116.0(0.69 to 700) |
| **Blood product** | | | | | | | |
| **Plasma** | 39 | 0.87 | 66(61 to 71) | 94(92 to 96) | 10.10(6.84 to 14.60) | 0.36(0.30 to 0.43) | 28.3(17.4 to 43.7) |
| **Serum** | 14 | 0.86 | 54(36 to 71) | 97(93 to 98) | 15.10(6.08 to 31.30) | 0.49(0.30 to 0.69) | 33.8(9.6 to 85.7) |
| **Methods** | | | | | | | |
| **cobas** | 4 | 0.96 | 75(69 to 80) | 97(95 to 98) | 26.20(15.70 to 41.80) | 0.26(0.21 to 0.31) | 104.0(57.5 to 173.0) |
| **ddPCR** | 4 | 0.84 | 54(23 to 81) | 98(91 to 99) | 23.2 (4.79 to 72.90) | 0.49(0.13 to 0.86) | 59.6(7.7 to 230.0) |
| **BEAMing** | 3[b] | 0.76 | 80(74 to 85) | 97(92 to 99) | 17.30(3.78 to 53.80) | 0.23(0.15 to 0.34) | 85.1(11.7 to 310.0) |
| **ARMS** | 14 | 0.87 | 56(46 to 65) | 97(94 to 98) | 17.50(7.83 to 34.10) | 0.47 (0.37 to 0.57) | 38.7(14.7 to 83.6) |
| **DHPLC** | 5 | 0.86 | 66(49 to 80) | 88(84 to 92) | 5.59(3.58 to 8.15) | 0.35(0.23 to 0.49) | 17.4(7.4 to 34.9) |
| **ME-PCR** | 6 | 0.83 | 52(33 to 71) | 93(83 to 97) | 7.47(2.31 to 18.60) | 0.54(0.35 to 0.76) | 15.5(3.2 to 47.9) |
| **NGS** | 4 | 0.82 | 65(53 to 76) | 82(69 to 91) | 3.95(1.80 to 7.730) | 0.45(0.28 to 0.66) | 9.9(2.8 to 25.1) |
| **PNA-LNA** | 7 | 0.82 | 65(38 to 85) | 93(86 to 96) | 5.79(1.34 to 18.70) | 0.44(0.15 to 0.84) | 18.1(1.7 to 74.6) |

AUROC: area under the receiver operating characteristic curve; ARMS: amplification refractory mutation system; BEAM: beads, emulsions, amplification, and magnetics; CI: confidence interval; ddPCR: droplet digital polymerase chain reaction; DHPLC: denaturing high performance liquid chromatography; DOR: diagnostic odds ratio; *EGFR*: epidermal growth factor receptor; ME-PCR: mutant-enriched polymerase chain reaction; NGS: next-generation sequencing; NLR: negative likelihood ratio; PLR: positive likelihood ratio; PNA-LNA: peptide nucleic acid-locked nucleic acid; TKI: tyrosine kinase inhibitor.
[a] Markov chain Monte Carlo procedure used to generate PLR and NLR and DOR.
[b] Only 2 studies had data sufficient to calculate specificity, AUROC, PLR, NLR, and DOR.

Seven studies included performance characteristics for *EGFR* TKI-resistance variants. The sensitivities ranged from 50% to 92%, and the specificities ranged from 60% to 87% (see Appendix Figures 5-6). The overall area under the curve was 0.78, with positive likelihood, negative likelihood, and DOR of 2.5 (95% CI, 1.9 to 3.2), 0.4 (95% CI, 0.3 to 0.5), and 6 (95% CI, 4 to 9), respectively. The sensitivities and specificities for the individual studies reporting diagnostic performance of the T790M-resistance variant are shown in Appendix Figures 5 and 6, respectively. The summary ROC curve is shown in Appendix Figure 7.

## Methods

### Search Strategy

The PubMed database was searched (via PubMed) using the following search strategy: ((("lung neoplasms" OR "lung cancer") AND (EGFR OR erbB1 OR "epidermal growth factor receptor" OR "epidermal growth factor receptors") AND (serum OR plasma OR circulating) AND (mutation OR mutations)) OR ("circulating tumor dna" OR "circulating tumour DNA")) AND ("systematic review" OR meta-analysis OR random* OR prospective OR study OR trial).
The search was performed through February 8, 2017, limited to English-language articles on human subjects. The search was supplemented by a manual bibliography review of selected references, a review of data or literature reported on manufacturer websites, and ClinicalTrials.gov. Biodesix also provided a list of potential publications for consideration.

### Study Selection

We selected studies that permitted calculation of comparison (sensitivity, specificity) of liquid biopsy using tissue biopsy or other recognized reference standards. BCBSA attempted to find studies that estimated outcomes or treatment response of patients stratified by liquid biopsy results and tissue biopsy results, selected and analyzed in a manner to obtain comparable estimates of each test's discriminative capability.

### Data Abstraction and Bias/Quality Assessment

BCBSA abstracted relevant data describing patient populations and the diagnostic characteristics of liquid biopsy. We found no studies comparing diagnostic strategies using liquid biopsy with tissue biopsy.

The QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies) tool was used to assess the study risk of bias.[89] This tool assesses for risk of bias and concerns about applicability using 4 dimensions: Patient Selection, Index Test, Reference Standard, and Flow and Timing. Applicability concerns evaluate how well the studies address the question of interest in the systematic reviews. The QUADAS-2 team does not recommend assigning a summary "score" because of the "well-known problems associated with such scores."

### Meta-Analyses

Meta-analyses were conducted using the Reitsma et al (2005)[90] and Harbord et al (2007)[91] bivariate regression model for diagnostic test evaluations with R version 3.1.2.[92] An Markov chain Monte Carlo procedure was used to generate positive and negative likelihood ratio and diagnostic odds ratio for the bivariate model.[93] Meta-regression and subgroup analyses were used to examine sources of between-study heterogeneity. Covariates were chosen based on previous meta-analyses and included publication year, sample size (<30 or ≥30), stage (only not advanced stage, mixed stages, only advanced stage or not reported), blood product (plasma or serum), and index test method.

## Medical Advisory Panel Review

This Evidence Street Assessment was reviewed by the Blue Cross Blue Shield Association Medical Advisory Panel on September 28, 2017 (see Appendix 2). In the interest of maintaining the timeliness of the scientific information in this Assessment, literature search updates were performed subsequent to the Panel's review (see Search Strategy section above). If the search updates identified any additional studies that met the criteria for detailed review, the results of these studies were included in the tables and text where appropriate. There were no studies that would change the conclusions of this evidence review.

### Appendix Table 3. Study Quality Ratings Using QUADAS-2

| Study | Risk of Bias | | | | Applicability Concerns | | |
|---|---|---|---|---|---|---|---|
| | Patient Selection | Index Test | Reference Standard | Flow and Timing | Patient Selection | Index Test | Reference Standard |
| Kimura et al (2006) | ? | ☺ | ☺ | ☹ | ☹ | ☺ | ☺ |
| Kimura et al (2007) | ? | ☺ | ☺ | ☺ | ☹ | ☺ | ☺ |
| Maheswaran et al (2008) | ? | ? | ? | ☹ | ☹ | ☺ | ☺ |
| Bai et al (2009) | ☺ | ☺ | ☺ | ? | ☹ | ☺ | ☺ |
| Yung et al (2009) | ? | ☺ | ☺ | ? | ☹ | ☺ | ☺ |
| Mack et al (2009) | ? | ☺ | ☺ | ? | ☺ | ☺ | ☺ |
| He et al (2009) | ? | ☺ | ? | ☹ | ☹ | ☺ | ☺ |
| Kuang et al (2009) | ? | ☺ | ☺ | ☹ | ☹ | ☺ | ☺ |
| Song et al (2010) | ? | ☺ | ☺ | ☺ | ☹ | ☺ | ☺ |
| Brevet et al (2011) | ? | ☺ | ☺ | ☺ | ? | ☺ | ☺ |
| Jiang et al (2011) | ? | ? | ☺ | ☺ | ☹ | ☺ | ☺ |
| Sriram et al (2011) | ? | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Yasuda et al (2011) | ? | ? | ☺ | ☹ | ☹ | ☺ | ☺ |
| Taniguchi et al (2011) | ☹ | ☺ | ? | ? | ☹ | ☺ | ? |
| Goto et al (2012) | ☺ | ☺ | ☺ | ☺ | ☹ | ☺ | ☺ |
| Nakamura et al (2012) | ☹ | ☹ | ☺ | ? | ☹ | ☹ | ☺ |
| Xu et al (2012) | ? | ? | ☺ | ☹ | ☹ | ☺ | ☺ |
| Yam et al (2012) | ? | ? | ? | ? | ☹ | ☺ | ☺ |
| Punnoose et al (2012) | ☺ | ☺ | ? | ☺ | ☺ | ☺ | ☺ |
| Huang et al (2012) | ☺ | ☺ | ☺ | ☺ | ☹ | ☺ | ☺ |
| Chen et al (2012) | ? | ? | ☺ | ☹ | ☹ | ☺ | ☺ |
| Hu et al (2013) | ? | ? | ? | ? | ☹ | ☺ | ☺ |
| Kim HR et al (2013) | ? | ☺ | ☺ | ☺ | ☹ | ? | ☺ |
| Kim ST et al (2013) | ? | ☺ | ☺ | ☺ | ☹ | ☺ | ☺ |
| Lv et al (2013) | ? | ☺ | ☺ | ☹ | ☹ | ☺ | ☺ |
| Akca et al (2013) | ? | ☺ | ☺ | ☺ | ☹ | ? | ☺ |
| Liu et al (2013) | ? | ☺ | ☺ | ☹ | ☹ | ☺ | ☺ |
| Zhang et al (2013) | ? | ☺ | ☺ | ☺ | ☹ | ? | ? |
| Zhao et al (2013) | ? | ☺ | ☺ | ☺ | ☹ | ☺ | ☺ |
| Jing et al (2014) | ? | ? | ? | ? | ☹ | ☺ | ? |
| Wang et al (2014) | ☺ | ☺ | ☺ | ☺ | ☹ | ☺ | ☺ |
| Li et al (2014) | ? | ☺ | ? | ? | ☹ | ☺ | ☺ |
| Douillard et al (2014) | ☺ | ☺ | ☺ | ☺ | ☺ | ☺ | ☺ |
| Weber et al (2014) | ? | ☺ | ☺ | ☹ | ☺ | ☺ | ☺ |
| Karachaliou et al (2015) | ☺ | ? | ☺ | ☹ | ☺ | ☺ | ☺ |
| Thress et al (2015) | ☺ | ☺ | ☺ | ? | ☺ | ☺ | ☺ |
| Duan et al (2015) | ? | ☺ | ☺ | ? | ☹ | ☺ | ☺ |
| Mok et al (2015) | ☺ | ☺ | ☺ | ? | ☹ | ☺ | ☺ |
| Lam et al (2015) | ? | ? | ? | ☹ | ☹ | ☺ | ☺ |
| Sacher et al (2016) | ☺ | ☺ | ☺ | ☺ | ☺ | ☺ | ☺ |
| FDA SSED (2016) | ☺ | ☺ | ☺ | ☺ | ☹ | ☺ | ☺ |

| Study | Risk of Bias | | | | Applicability Concerns | | |
|---|---|---|---|---|---|---|---|
| Ohira et al (2016) | ? | ☺ | ☺ | ☺ | ☹ | ☺ | ☺ |
| Guo et al (2016) | ? | ☺ | ? | ☺ | ☹ | ☺ | ☺ |
| Sundaresan et al (2016) | ? | ☺ | ? | ☺ | ☺ | ☺ | ☺ |
| Takahama et al (2016) | ☺ | ? | ☺ | ☹ | ☹ | ☺ | ☺ |
| Chen et al (2016) | ☺ | ☺ | ☺ | ☹ | ☹ | ☺ | ☺ |
| Que et al (2016) | ? | ? | ☺ | ? | ☹ | ☺ | ☺ |
| Vazquez et al (2016) | ☺ | ☺ | ☺ | ☺ | ☺ | ☺ | ☺ |
| Han et al (2016) | ☺ | ☺ | ? | ? | ☹ | ☺ | ☺ |
| Thompson et al (2016) | ☺ | ☺ | ☺ | ☹ | ☺ | ☺ | ☺ |
| Kimura et al (2016) | ? | ? | ? | ? | ☹ | ? | ☺ |
| Ma et al (2016) | ? | ? | ☺ | ☺ | ☹ | ☺ | ☺ |
| Oxnard et al (2016) | ☺ | ☺ | ? | ☺ | ☺ | ☺ | ☺ |
| Xu et al (2016) | ? | ☺ | ☺ | ? | ☹ | ☺ | ☺ |
| Zhang et al (2017) | ☹ | ? | ? | ☹ | ☹ | ☺ | ☺ |
| Mellert et al (2017) | ? | ☺ | ☺ | ☹ | ☹ | ☺ | ☺ |

☺: low risk; ☹: high risk;?: unclear risk.
FDA: Food and Drug Administration; SSED: Summary of Safety and Effectiveness Data.

**Figure 3: Appendix Figure 1. Summary of QUADAS-2 Quality Ratings by Domain**

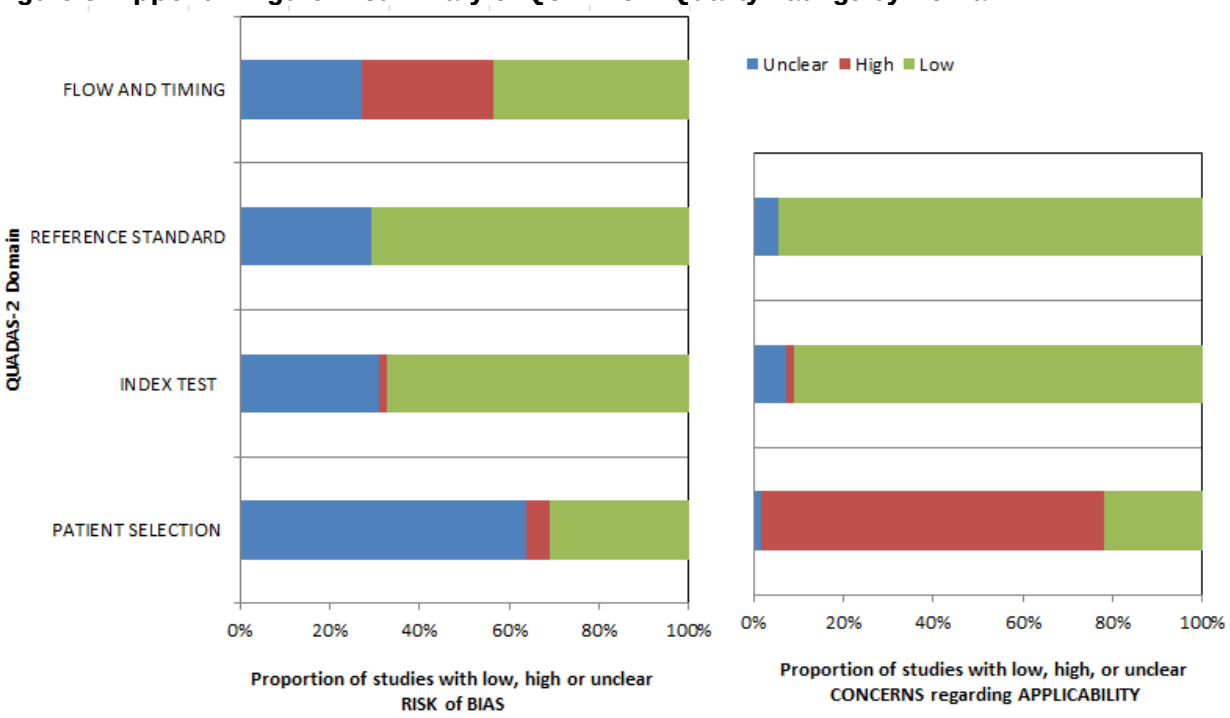
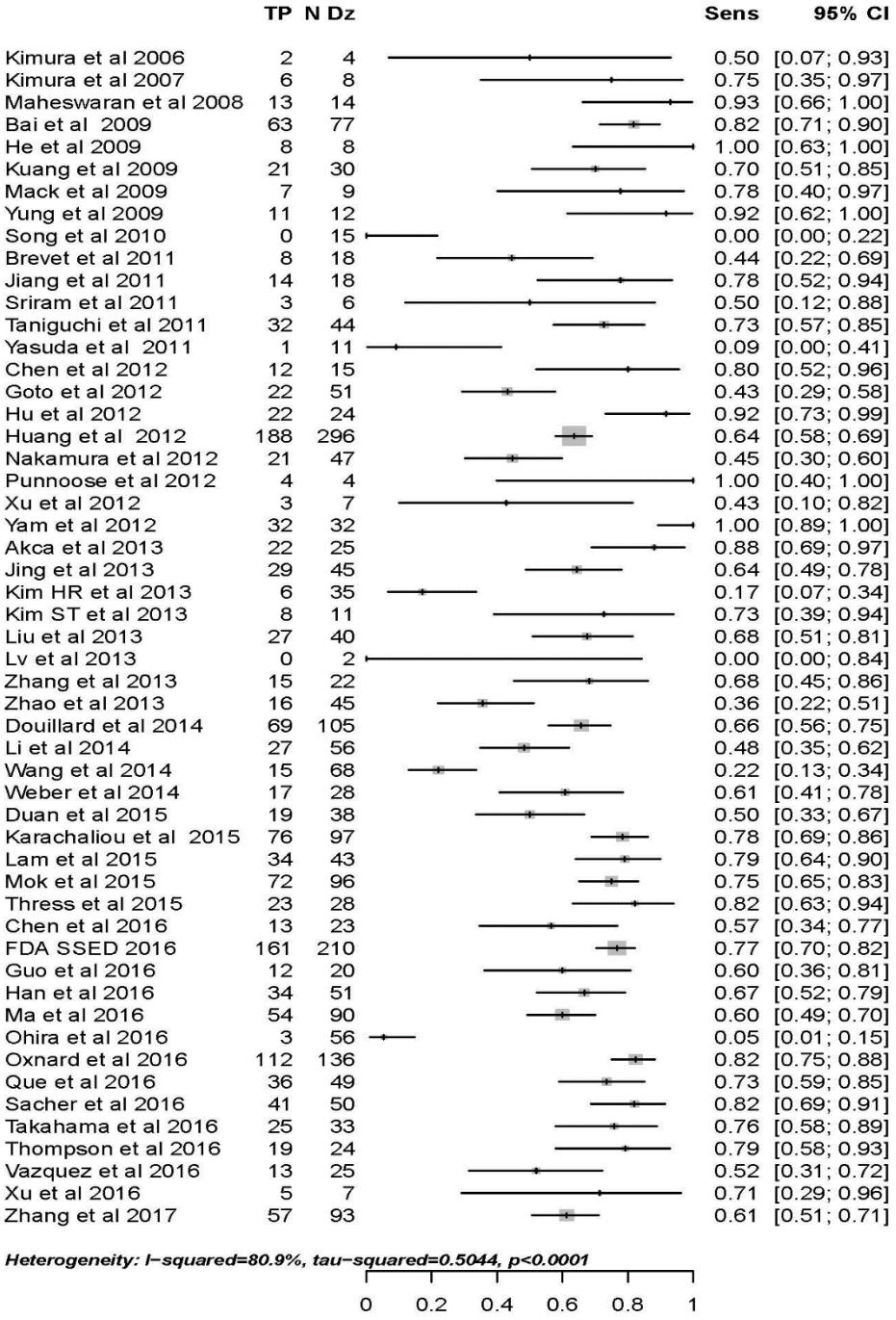
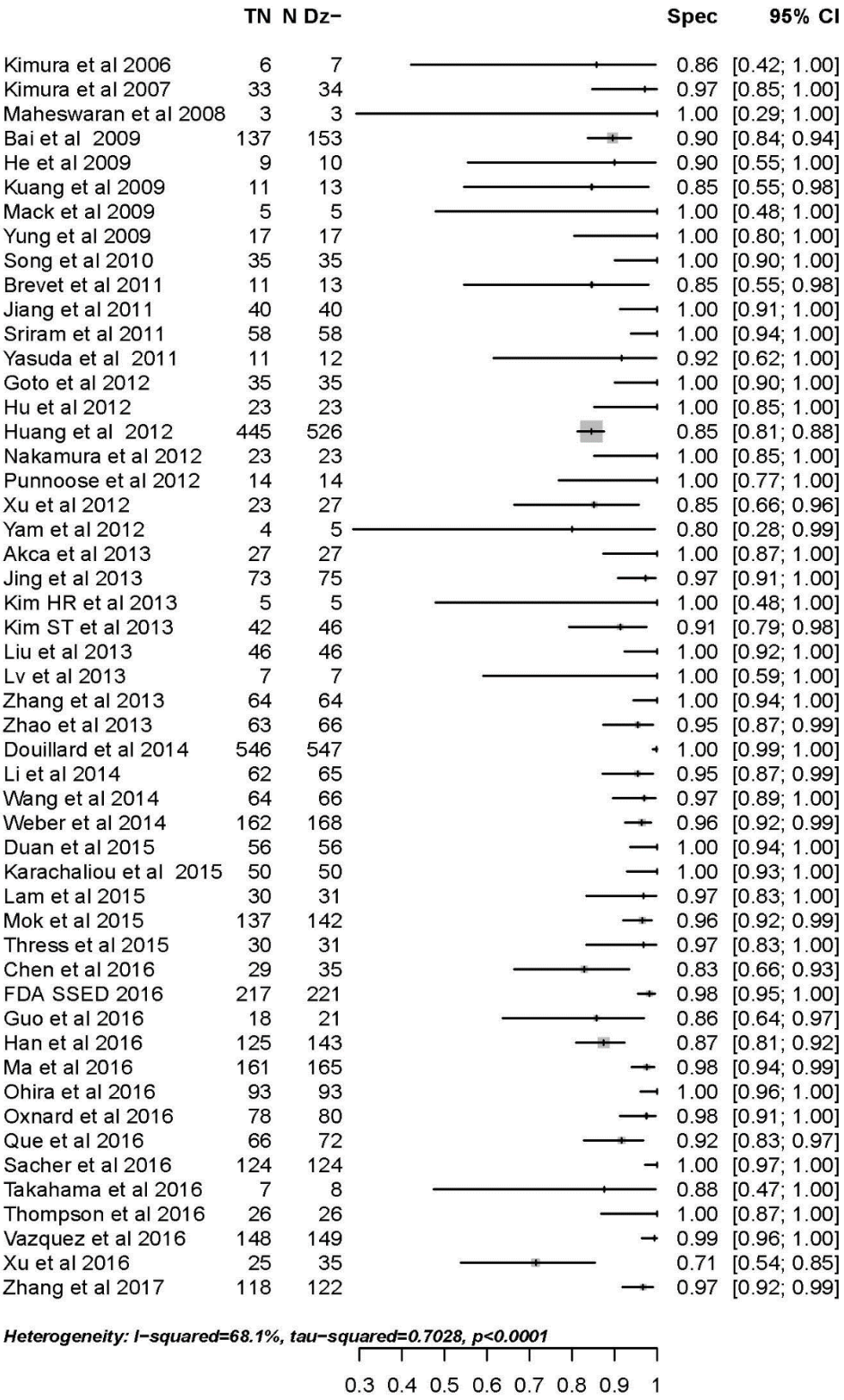


**Figure 4: Appendix Figure 2. Sensitivities of Studies Including *EGFR* TKI-Sensitizing Variants**



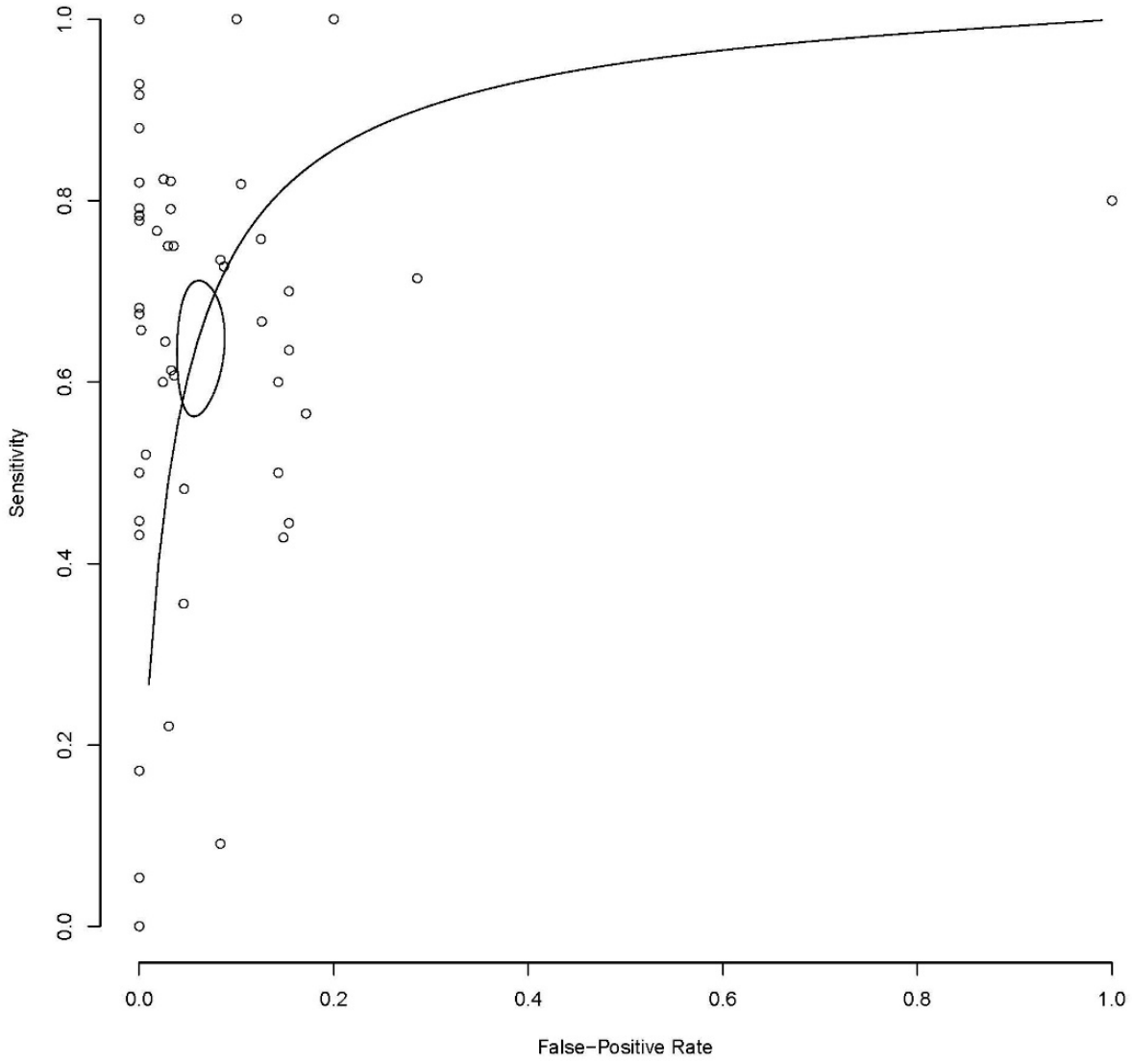

CI: confidence interval; *EGFR*: epidermal growth factor receptor; FDA: Food and Drug Administration; N Dz: number disease positive; Sens: sensitivity; SSED: Summary of Safety and Effectiveness Data; TKI: tyrosine kinase inhibitor; TP: true positive.

**Figure 5: Appendix Figure 3. Specificities of Studies Including *EGFR* TKI-Sensitizing Variants**



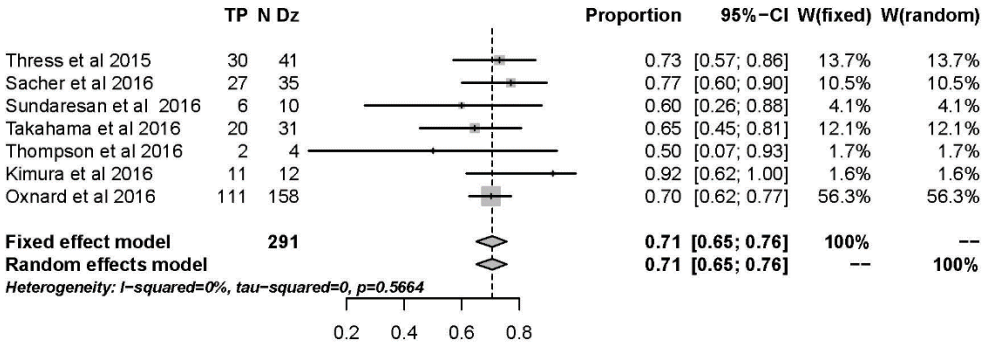
CI: confidence interval; *EGFR*: epidermal growth factor receptor; FDA: Food and Drug Administration; N Dz-: number disease negative; SSED: Summary of Safety and Effectiveness Data; Spec: specificity; TKI: tyrosine kinase inhibitor; TN: true negative.

**Figure 6: Appendix Figure 4. Summary ROC Curve for Studies Including *EGFR* TKI-Sensitizing Variants**



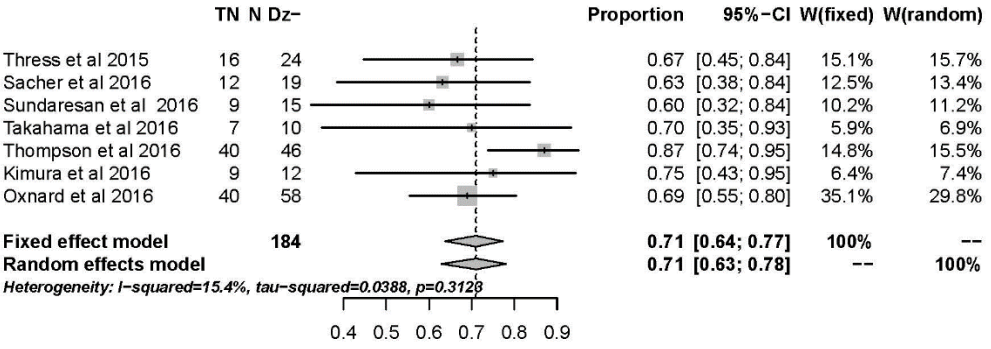

*EGFR*: epidermal growth factor receptor; ROC: receiver operating characteristic; TKI: tyrosine kinase inhibitor.

**Figure 7: Appendix Figure 5. Sensitivities of Studies Including *EGFR*-Resistance Variants**



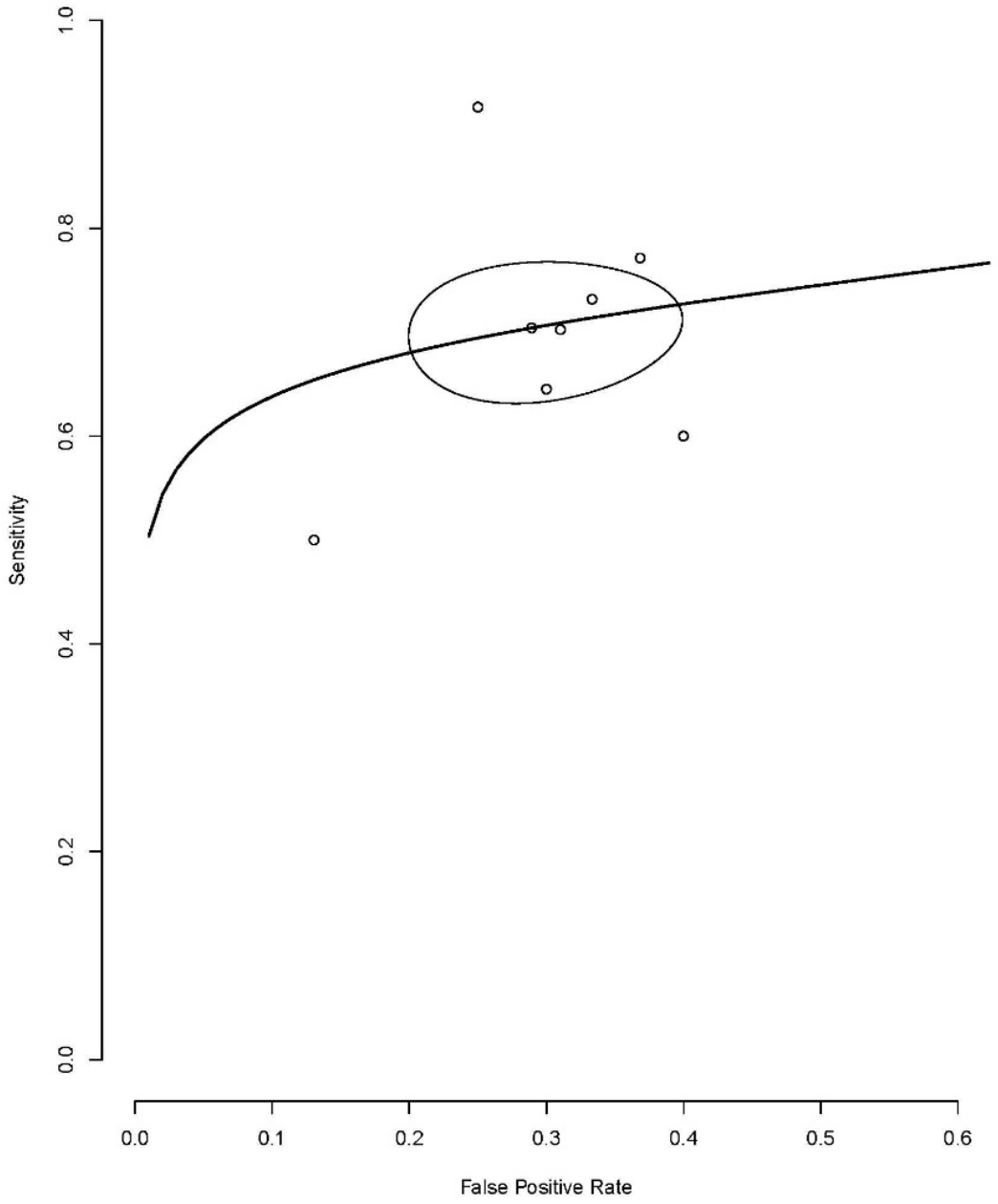

CI: confidence interval; *EGFR*: epidermal growth factor receptor; N Dz: number disease positive; TP: true positive.

**Figure 8: Appendix Figure 6. Specificities of Studies Including *EGFR*-Resistance Variants**




CI: confidence interval; *EGFR*: epidermal growth factor receptor; N Dz -: number disease negative; TN: true negative.

**Figure 9: Appendix Figure 7. Summary ROC Curve for Studies Including EGFR TKI-Resistance Variant (T790M)**




*EGFR*: epidermal growth factor receptor; ROC: receiver operating characteristic; TKI: tyrosine kinase inhibitor.

## Appendix 2

**Summary of Application of the Technology Evaluation Criteria**
Based on the available evidence, the Blue Cross Blue Shield Association Medical Advisory Panel made the following judgments in September 2017 about whether the assessment of biomarkers from circulating tumor DNA (ctDNA) meets the Blue Cross and Blue Shield Association TEC criteria.

**1. The technology must have final approval from the appropriate governmental regulatory bodies.**
Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments. Several companies market tests that detect tumor markers from peripheral blood, including tyrosine kinase inhibitor (TKI)-sensitizing variants for non-small-cell lung cancer. Laboratories that offer laboratory-developed tests must be licensed under the Clinical Laboratory Improvement Amendments for high-complexity testing. To date, the U.S. Food and Drug Administration (FDA) has chosen not to require any regulatory review of this test.

In June 2016, cobas EGFR Mutation Test v2 (Roche Molecular Systems), a real-time polymerase chain reaction test, was approved by FDA through the premarket approval process. This plasma test is approved as a companion diagnostic aid for selecting non-small-cell lung cancer patients who have epidermal growth factor receptor (*EGFR*) exon 19 deletions and L858R substitution variants for treatment with erlotinib. Patients who test negative for the *EGFR* variants detected should be referred for (or "reflexed" to) routine biopsy with tissue testing for *EGFR* variants. The previously approved version 2 of this test, which used tissue biopsy specimens, was also approved for detection of T790M variants in tissue, which are used to select patients to receive osimertinib. Approval of version 2 of the plasma test did not include the detection of T790M variants.

**2. The scientific evidence must permit conclusions concerning the effect of the technology on health outcomes.**
Numerous studies of patients in whom liquid biopsy and tissue biopsy results are available have demonstrated the diagnostic characteristics of liquid biopsy using a tissue biopsy as the reference standard. There is insufficient evidence on the association between liquid biopsy results and patient outcomes. Given this evidence and the pattern of diagnostic characteristics of liquid biopsy, separate conclusions on the effect of the technology on health outcomes were reached for different tests and biomarkers.

For detection of biomarkers of *EGFR* TKI sensitization, such as exon 19 deletion and L858R variants, the cobas EGFR Mutation Test v2 using real-time polymerase chain reaction technology is the only ctDNA test with demonstrated clinical validity compared with tissue biopsy. Given the breadth of molecular diagnostic methodologies available to assess ctDNA, the clinical validity of additional molecular methods must be established independently. Several meta-analyses and individual studies have demonstrated that the cobas liquid biopsy is moderately sensitive for *EGFR* variants associated with TKI sensitivity (range, 60%-80%), with high specificity (range, >90% to 100%) using tissue samples as the reference standard. The evidence is sufficient to reach a conclusion on the cobas EGFR Mutation Test v2.

The evidence demonstrating the clinical validity of other marketed tests that detect TKI-sensitizing variants using ctDNA is insufficient to reach a conclusion.

For detection of the T790M biomarker associated with *EGFR* TKI treatment resistance, the evidence demonstrating the clinical validity is insufficient to reach a conclusion.

**3. The technology must improve the net health outcome.**
For detection of *EGFR* TKI-sensitizing biomarkers, such as exon 19 deletion and L858R variants, a strategy of liquid biopsy using a test with proven clinical validity followed by reflex tissue testing will attain the same sensitivity as tissue testing and high specificity. This strategy will permit selection of patients appropriately for *EGFR* TKI treatment (i.e., erlotinib, gefitinib, afatinib) with a low false-positive rate. Depending on the prevalence of *EGFR* TKI-sensitizing biomarkers, a variable number of patients would avoid tissue testing. Use of the cobas EGFR Mutation Test v2 for detection of *EGFR* TKI-sensitizing biomarkers should improve the net health outcome.

It cannot be determined whether liquid biopsy using other ctDNA tests for detection of TKI-sensitizing biomarkers improves the net health outcome.

For detection of the T790M biomarker associated with *EGFR* TKI treatment resistance, liquid biopsy test characteristics of moderate sensitivity and specificity compared with tissue biopsy and uncertainty about outcomes for patients with discordant liquid and tissue biopsy do not translate into an osimertinib treatment selection strategy that will improve outcomes. It cannot be determined whether liquid biopsy for detection of TKI-resistance biomarkers improves the net health outcome.

**4. The technology must be as beneficial as any established alternatives.**
For detection of *EGFR* TKI-sensitizing biomarkers, a strategy of liquid biopsy using the cobas EGFR Mutation Test v2, which has proven clinical validity, followed by reflex tissue testing of negatives and appropriate selection of *EGFR* TKI therapy for patients testing positive, should attain patient outcomes as beneficial as a strategy of tissue biopsy testing alone.

For detection of TKI-sensitizing biomarkers using other marketed ctDNA tests for detection of TKI-sensitizing biomarkers, it is uncertain whether liquid biopsy alone or in combination with tissue biopsy testing will attain patient outcomes as beneficial as a strategy of tissue testing alone.

For detection of the T790M biomarker associated with *EGFR* TKI treatment resistance and for selection of treatment with osimertinib, it is uncertain whether liquid biopsy alone or in combination with tissue biopsy testing will attain patient outcomes as beneficial as a strategy of tissue testing alone.

**5. The improvement must be attainable outside the investigational settings.**
The cobas EGFR Mutation Test v2 is an FDA-approved companion diagnostic intended to be performed at a lab certified by CLIA and the College of American Pathologists. Therefore, conclusions concerning the clinical validity and clinical utility would be expected to apply outside the investigational setting for the detection of *EGFR* TKI-sensitizing variants.

Because there is insufficient evidence supporting the clinical validity of other methods of assessing ctDNA TKI-sensitizing variants, conclusions concerning improved health outcomes outside the investigational setting cannot be made.

Because there is insufficient evidence supporting the clinical validity of methods of assessing ctDNA TKI-resistance variants, conclusions concerning improved health outcomes outside the investigational setting cannot be made.

Based on the above, liquid biopsy for detection of *EGFR* TKI-sensitive biomarkers using the cobas EGFR Mutation Test v2 meets the Blue Cross Blue Shield Association TEC criteria.

Based on the above, liquid biopsy for detection of TKI-sensitive biomarkers using other methods does not meet the Blue Cross Blue Shield Association TEC criteria.

Based on the above, liquid biopsy for detection of biomarkers associated with TKI resistance with any method does not meet the Blue Cross Blue Shield Association TEC criteria.

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## Documentation for Clinical Review

**Please provide the following documentation:**

- History and physical and/or consultation notes including:
  - Clinical findings (i.e., pertinent symptoms and duration)
  - Comorbidities
  - Activity and functional limitations
  - Family history, if applicable
  - Reason for procedure/test/device, when applicable
  - Pertinent past procedural and surgical history
  - Past and present diagnostic testing and results
  - Prior conservative treatments, duration, and response
  - Treatment plan (i.e., surgical intervention)
- Consultation and medical clearance report(s), when applicable
- Genetic counseling/professional results (if applicable)
- Radiology report(s) and interpretation (i.e., MRI, CT, discogram)
- Laboratory results
- Other pertinent multidisciplinary notes/reports: (i.e., psychological or psychiatric evaluation, physical therapy, multidisciplinary pain management), when applicable

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

- Results/reports of tests performed
- Procedure report(s)

## 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®** | 0179U | Oncology (non-small cell lung cancer), cell-free DNA, targeted sequence analysis of 23 genes (single nucleotide variations, insertions and deletions, fusions without prior knowledge of partner/breakpoint, copy number variations), with report of significant mutation(s) |

| Type | Code | Description |
|---|---|---|
| | 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 |
| | 0242U | Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free circulating DNA analysis of 55-74 genes, interrogation for sequence variants, gene copy number amplifications, and gene rearrangements ***(Code effective 4/1/2021)*** |
| | 81210 | BRAF (B-Raf proto-oncogene, serine/threonine kinase) (e.g., colon cancer, melanoma), gene analysis, V600 variant(s) |
| | 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) |
| | 81277 | Cytogenomic neoplasia (genome-wide) microarray analysis, interrogation of genomic regions for copy number and loss-of-heterozygosity variants for chromosomal abnormalities |
| | 81445 | Targeted genomic sequence analysis panel, solid organ neoplasm, DNA analysis, and RNA analysis when performed, 5-50 genes (e.g., ALK, BRAF, CDKN2A, EGFR, ERBB2, KIT, KRAS, NRAS, MET, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, if performed |
| | 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 |
| | 86152 | Cell enumeration using immunologic selection and identification in fluid specimen (e.g., circulating tumor cells in blood); |
| | 86153 | Cell enumeration using immunologic selection and identification in fluid specimen (e.g., circulating tumor cells in blood); physician interpretation and report, when required |
| **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 |
|---|---|
| 06/01/2018 | BCBSA medical policy adoption |
| 12/01/2018 | Policy revision without position change |
| 08/01/2019 | Policy revision without position change |
| 02/01/2020 | Annual review. Policy statement, guidelines, literature and coding updated. |
| 03/01/2020 | Coding update |
| 08/01/2020 | Policy statement updated. Coding Update. |
| 11/01/2020 | Administrative update. Policy statement and guidelines updated. |
| 12/01/2020 | Policy statement and guidelines updated. |
| 01/01/2021 | Annual review. Policy statement, guidelines, literature and coding updated. |
| 07/01/2021 | Policy statement and guidelines updated. Coding update |
| 03/01/2022 | Annual review. No change to policy statement. |

## 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.

*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 (No changes) | |
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| **BEFORE** | **AFTER** |
| **Circulating Tumor DNA for Management of Non-Small-Cell Lung Cancer (Liquid Biopsy) 2.04.143**<br><br>**Policy Statement:**<br>Circulating tumor DNA (ctDNA or liquid biopsy) analysis (genetic testing) may be **medically necessary** for some genes under limited circumstances. ctDNA testing is limited to 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 section) when an initial diagnostic biopsy sample (or there is progression of the cancer despite treatment) has insufficient tissue available to complete testing (or the testing is inconclusive) and the alternative is a second invasive biopsy.<br><br>**Alternative to Individual Testing**<br>**Any** of the following panel tests may be considered **medically necessary** as alternatives to the individual genes noted below (including those considered investigational as stand-alone tests) when the medically necessary criteria is met for ctDNA testing, either after diagnosis or after progression of the cancer despite treatment:<br>I. cobas® EGFR Mutation Test v2<br>II. FoundationOne® Liquid CDx<br>III. Guardant360® CDx<br>IV. OncoBEAM™ Lung1<br>V. OncoBEAM™ Lung2<br>VI. InVision First-Lung<br>VII. Resolution ctDx Lung (ResBio)<br><br>**Note**: The cobas® test is a companion diagnostic for erlotinib (Tarceva®; OSI Pharmaceuticals, Melville NY). | **Circulating Tumor DNA for Management of Non-Small-Cell Lung Cancer (Liquid Biopsy) 2.04.143**<br><br>**Policy Statement:**<br>Circulating tumor DNA (ctDNA or liquid biopsy) analysis (genetic testing) may be **medically necessary** for some genes under limited circumstances. ctDNA testing is limited to 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 section) when an initial diagnostic biopsy sample (or there is progression of the cancer despite treatment) has insufficient tissue available to complete testing (or the testing is inconclusive) and the alternative is a second invasive biopsy.<br><br>**Alternative to Individual Testing**<br>**Any** of the following panel tests may be considered **medically necessary** as alternatives to the individual genes noted below (including those considered investigational as stand-alone tests) when the medically necessary criteria is met for ctDNA testing, either after diagnosis or after progression of the cancer despite treatment:<br>I. cobas® EGFR Mutation Test v2<br>II. FoundationOne® Liquid CDx<br>III. Guardant360® CDx or LDT<br>IV. OncoBEAM™ Lung1<br>V. OncoBEAM™ Lung2<br>VI. InVision First-Lung<br>VII. Resolution ctDx Lung (ResBio)<br><br>**Note**: The cobas® test is a companion diagnostic for erlotinib (Tarceva®; OSI Pharmaceuticals, Melville NY).<br>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 |

| POLICY STATEMENT (No changes) | |
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| **BEFORE** | **AFTER** |
| | 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, which is investigational by itself) and should use a miscellaneous CPT code of 81455 (sometimes incorrectly 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). |
| ***Epidermal Growth Factor Receptor* (*EGFR*) Testing**<br>When included in one of the approved panel tests, analysis of somatic variants in exons 19 through 21 (e.g., exon 19 deletions, L858R, T790M) within the epidermal growth factor receptor (EGFR) gene, using plasma specimens to detect circulating tumor DNA (ctDNA), may be considered **medically necessary** as an alternative to tissue biopsy to predict treatment response to an *EGFR* tyrosine kinase inhibitor (TKI) therapy (e.g., erlotinib [Tarceva], gefitinib [Iressa], afatinib [Gilotrif], dacomitinib [Vizimpro], or osimertinib [Tagrisso]).<br><br>At progression, analysis of the *EGFR* T790M resistance variant for targeted therapy with osimertinib using ctDNA from plasma specimens may be considered **medically necessary** in patients when tissue biopsy to obtain new tissue is not feasible, e.g., in those who do not have enough tissue for standard molecular testing using formalin-fixed paraffin-embedded tissue, do not have a biopsy-amenable lesion, or cannot undergo biopsy.<br><br>Unless included in one of the approved panel tests, analysis of other *EGFR* variants within exons 22 to 24, or other applications related to NSCLC, is considered **investigational**. | ***Epidermal Growth Factor Receptor* (*EGFR*) Testing**<br>When included in one of the approved panel tests, analysis of somatic variants in exons 19 through 21 (e.g., exon 19 deletions, L858R, T790M) within the epidermal growth factor receptor (EGFR) gene, using plasma specimens to detect circulating tumor DNA (ctDNA), may be considered **medically necessary** as an alternative to tissue biopsy to predict treatment response to an *EGFR* tyrosine kinase inhibitor (TKI) therapy (e.g., erlotinib [Tarceva], gefitinib [Iressa], afatinib [Gilotrif], dacomitinib [Vizimpro], or osimertinib [Tagrisso]).<br><br>At progression, analysis of the *EGFR* T790M resistance variant for targeted therapy with osimertinib using ctDNA from plasma specimens may be considered **medically necessary** in patients when tissue biopsy to obtain new tissue is not feasible, e.g., in those who do not have enough tissue for standard molecular testing using formalin-fixed paraffin-embedded tissue, do not have a biopsy-amenable lesion, or cannot undergo biopsy.<br><br>Unless included in one of the approved panel tests, analysis of other *EGFR* variants within exons 22 to 24, or other applications related to NSCLC, is considered **investigational**. |
| **Other Genes**<br>Plasma tests for oncogenic driver variants deemed medically necessary on tissue biopsy (see Blue Shield of California Medical Policy: Molecular Analysis for Targeted Therapy of Non-Small-Cell Lung Cancer) may be | **Other Genes**<br>Plasma tests for oncogenic driver variants deemed medically necessary on tissue biopsy (see Blue Shield of California Medical Policy: Molecular Analysis for Targeted Therapy of Non-Small-Cell Lung Cancer) may be |

| POLICY STATEMENT (No changes) | |
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| **BEFORE** | **AFTER** |
| considered **medically necessary** to predict treatment response to targeted therapy for patients meeting **all** of the following criteria:<br>I. Patient does not have sufficient tissue for standard molecular testing using formalin-fixed paraffin-embedded tissue<br>II. Follow-up tissue-based analysis is planned should no driver variant be identified via plasma testing<br><br>***ALK* Testing**<br>Unless included in one of the approved panel tests, analysis of somatic rearrangement variants of the *ALK* gene using plasma specimens to detect ctDNA or RNA is considered **investigational** as an alternative to tissue biopsy to predict treatment response to ALK inhibitor therapy (e.g., crizotinib [Xalkori], ceritinib [Zykadia], alectinib [Alecensa], or brigatinib [Alunbrig]) in patients with NSCLC.<br><br>***BRAF* V600E Testing**<br>Unless included in one of the approved panel tests, analysis of the *BRAF* V600E variant using plasma specimens to detect ctDNA is considered **investigational** as an alternative to tissue biopsy to predict treatment response to BRAF or MEK inhibitor therapy (e.g., dabrafenib [Tafinlar], trametinib [Mekinist]) in patients with NSCLC.<br><br>***ROS1* Testing**<br>Unless included in one of the approved panel tests, analysis of somatic rearrangement variants of the *ROS1* gene using plasma specimens to detect ctDNA or RNA is considered **investigational** as an alternative to tissue biopsy to predict treatment response to ALK inhibitor therapy (crizotinib [Xalkori]) in patients with NSCLC.<br><br>***MET* Exon 14 Skipping Alteration**<br>Analysis of genetic alteration that leads to MET exon 14 skipping may be considered **medically necessary** to predict treatment response to capmatinib (Tabrecta) in patients with metastatic NSCLC.<br><br>***RET* Rearrangement Testing** | considered **medically necessary** to predict treatment response to targeted therapy for patients meeting **all** of the following criteria:<br>I. Patient does not have sufficient tissue for standard molecular testing using formalin-fixed paraffin-embedded tissue<br>II. Follow-up tissue-based analysis is planned should no driver variant be identified via plasma testing<br><br>***ALK* Testing**<br>Unless included in one of the approved panel tests, analysis of somatic rearrangement variants of the *ALK* gene using plasma specimens to detect ctDNA or RNA is considered **investigational** as an alternative to tissue biopsy to predict treatment response to ALK inhibitor therapy (e.g., crizotinib [Xalkori], ceritinib [Zykadia], alectinib [Alecensa], or brigatinib [Alunbrig]) in patients with NSCLC.<br><br>***BRAF* V600E Testing**<br>Unless included in one of the approved panel tests, analysis of the *BRAF* V600E variant using plasma specimens to detect ctDNA is considered **investigational** as an alternative to tissue biopsy to predict treatment response to BRAF or MEK inhibitor therapy (e.g., dabrafenib [Tafinlar], trametinib [Mekinist]) in patients with NSCLC.<br><br>***ROS1* Testing**<br>Unless included in one of the approved panel tests, analysis of somatic rearrangement variants of the *ROS1* gene using plasma specimens to detect ctDNA or RNA is considered **investigational** as an alternative to tissue biopsy to predict treatment response to ALK inhibitor therapy (crizotinib [Xalkori]) in patients with NSCLC.<br><br>***MET* Exon 14 Skipping Alteration**<br>Analysis of genetic alteration that leads to MET exon 14 skipping may be considered **medically necessary** to predict treatment response to capmatinib (Tabrecta) in patients with metastatic NSCLC.<br><br>***RET* Rearrangement Testing** |

| POLICY STATEMENT (No changes) | |
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| **BEFORE** | **AFTER** |
| Analysis of genetic 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. | Analysis of genetic 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. |
| ***NTRK* Gene Fusion Testing**<br>Analysis of *NTRK* gene fusions 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. | ***NTRK* Gene Fusion Testing**<br>Analysis of *NTRK* gene fusions 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. Note that 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)*. |
| Analysis of *NTRK* gene fusions is considered **investigational** in all other situations. | Analysis of *NTRK* gene fusions is considered **investigational** in all other situations. |
| ***KRAS* Testing**<br>Unless included in one of the approved panel tests, analysis of somatic variants of the *KRAS* gene using plasma specimens to detect ctDNA is considered **investigational** as a technique to predict treatment nonresponse to anti-EGFR therapy with tyrosine kinase inhibitors and for the use of the anti-EGFR monoclonal antibody cetuximab in NSCLC. | ***KRAS* Testing**<br>Unless included in one of the approved panel tests, analysis of somatic variants of the *KRAS* gene using plasma specimens to detect ctDNA is considered **investigational** as a technique to predict treatment nonresponse to anti-EGFR therapy with tyrosine kinase inhibitors and for the use of the anti-EGFR monoclonal antibody cetuximab in NSCLC. |
| ***HER2* Testing**<br>Unless included in one of the approved panel tests, analysis of alterations in the *HER2* gene using plasma specimens to detect ctDNA for targeted therapy in patients with NSCLC is considered **investigational**. | ***HER2* Testing**<br>Unless included in one of the approved panel tests, analysis of alterations in the *HER2* gene using plasma specimens to detect ctDNA for targeted therapy in patients with NSCLC is considered **investigational**. |
| **Measurement of Residual Disease (MRD) or Initial Diagnosis**<br>The use of CtDNA for measuring residual disease or monitoring after treatment or for making an initial diagnosis (instead of using a tissue sample) is considered **investigational**. | **Measurement of Residual Disease (MRD) or Initial Diagnosis**<br>The use of CtDNA for measuring residual disease or monitoring after treatment or for making an initial diagnosis (instead of using a tissue sample) is considered **investigational**. |
| ***PD-L1* Testing**<br>Programmed Death-Ligand 1 (PD-L1) testing may be considered **medically necessary** to predict treatment response to atezolizumab | ***PD-L1* Testing**<br>Programmed Death-Ligand 1 (PD-L1) testing may be considered **medically necessary** to predict treatment response to atezolizumab |

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| **BEFORE** | **AFTER** |
| (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 a panel.<br><br>*PD-L1* gene testing is considered **investigational** in all other situations. | (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 a panel.<br><br>*PD-L1* gene testing is considered **investigational** in all other situations. |