Medical Policy

BlueCross BlueShield Kansas
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Title: Circulating Tumor DNA Management of Non-Small Cell Lung Cancer (Liquid Biopsy)

Professional
Original Effective Date: December 21, 2018
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Current Effective Date: March 29, 2021

Populations	Interventions	Comparators	Outcomes
Individuals: • With advanced non-small-cell lung cancer who are being considered for targeted therapy	Interventions of interest are: • Testing for biomarkers of EGFR TKI sensitivity using circulating tumor DNA with the cobas EGFR Mutation Test v2	Comparators of interest are: • Testing for biomarkers of EGFR tyrosine kinase inhibitor sensitivity using tissue biopsy • No testing for biomarkers of EGFR tyrosine kinase inhibitor sensitivity.	Relevant outcomes include: • Overall survival • Disease-specific survival • Test validity
Individuals: • With advanced non-small-cell lung cancer who are being considered for targeted therapy	Interventions of interest are: • Testing for biomarkers of EGFR TKI sensitivity using circulating tumor DNA with the Guardant360	Comparators of interest are: • Testing for biomarkers of EGFR tyrosine kinase inhibitor sensitivity using tissue biopsy	Relevant outcomes include: • Overall survival • Disease-specific survival • Test validity

Populations	Interventions	Comparators	Outcomes
	CDx, OncoBEAM, or InVision tests	• No testing for biomarkers of EGFR tyrosine kinase inhibitor sensitivity.	
Individuals: • With advanced non-small-cell lung cancer who are being considered for targeted therapy	Interventions of interest are: • Testing for biomarkers of EGFR TKI sensitivity using ctDNA with tests other than the cobas v2, Guardant360 CDx, OncoBEAM, and InVIsion	Comparators of interest are: • Testing for biomarkers of EGFR tyrosine kinase inhibitor sensitivity using tissue biopsy • No testing for biomarkers of EGFR tyrosine kinase inhibitor sensitivity.	Relevant outcomes include: • Overall survival • Disease-specific survival • Test validity
Individuals: • With advanced non-small-cell lung cancer who are being considered for targeted therapy	Interventions of interest are: • Testing for biomarkers other than EGFR using liquid biopsy to select targeted therapy	Comparators of interest are: • Testing for biomarkers other than EGFR using tissue biopsy to select targeted therapy • No testing for biomarkers of other than EGFR tyrosine kinase inhibitor sensitivity.	Relevant outcomes include: • Overall survival • Disease-specific survival • Test validity
Individuals: • With advanced non-small-cell lung cancer who progressed on EGFR tyrosine kinase inhibitors	Interventions of interest are: • Testing for biomarkers of EGFR tyrosine kinase inhibitor resistance using liquid biopsy	Comparators of interest are: • Testing for biomarkers of EGFR tyrosine kinase inhibitor resistance using tissue biopsy • No testing for biomarkers of EGFR tyrosine kinase inhibitor resistance	Relevant outcomes include: • Overall survival • Disease-specific survival • Test validity

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.

Objective

The objective of this evidence review is to determine whether testing for driver or resistance variants using circulating tumor DNA or other “liquid biopsies” in peripheral blood improves the net health outcome in individuals with non-small-cell lung cancer.

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 the BCBSKS medical policy *Molecular Analysis for Targeted Therapy or Immunotherapy 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 (eg, 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 (eg, 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 (eg, *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.

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.

POLICY

I. *EGFR* Testing

A. At diagnosis, 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 for example the cobas *EGFR* Mutation Test v2, Guardant360 CDx test, OncoBEAM test or InVisionFirst-Lung test with plasma specimens to detect circulating tumor DNA (ctDNA), may be considered **medically necessary** as an alternative to tissue biopsy (see Policy Guidelines) to predict treatment response to an EGFR tyrosine kinase inhibitor therapy (eg, erlotinib [Tarceva], gefitinib [Iressa], afatinib [Gilotrif], dacomitinib [Vizimpro], or osimertinib [Tagrisso]) in patients with advanced lung adenocarcinoma, large cell carcinoma, advanced squamous cell non-small-cell lung cancer, and non-small-cell lung cancer not otherwise specified.

B. At progression, analysis of the EGFR T790M resistance variant for targeted therapy with osimertinib using ctDNA using for example the cobas EGFR Mutation Testv2, Guardant360 CDx test, OncoBEAM test or InVisionFirst-Lung test with plasma specimens to detect circulating tumor DNA (ctDNA), may be considered **medically necessary** in patients with advanced lung adenocarcinoma, large cell carcinoma, advanced squamous cell non-small-cell lung cancer, and non-small-cell lung cancer not otherwise specified 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. (see Policy Guidelines).

C. Analysis of other EGFR variants within exons 22 to 24 or other applications related to NSCLC is considered **experimental / investigational**.

II. Other Genes

A. Plasma tests for oncogenic driver variants deemed medically necessary on tissue biopsy (the BCBSKS medical policy *Molecular Analysis for Targeted Therapy or Immunotherapy of Non-Small-Cell Lung Cancer.*) may be considered **medically necessary** to predict treatment response to targeted therapy for patients meeting the following criteria:

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

III. *ALK* Testing

A. Analysis of somatic rearrangement variants of the *ALK* gene using plasma specimens to detect ctDNA or RNA may be considered **medically necessary** to predict treatment response to ALK inhibitor therapy (eg, crizotinib [Xalkori], ceritinib [Zykadia], alectinib [Alecensa], or brigatinib [Alunbrig]) in patients with advanced lung adenocarcinoma, large cell carcinoma, advanced squamous cell non-small-cell lung cancer, and non-small-cell lung cancer not otherwise specified when tissue biopsy is medically contraindicated, or the quantity is not sufficient for testing.

B. Analysis of somatic rearrangement variants of the *ALK* gene using plasma specimens to detect ctDNA or RNA is considered **experimental / investigational** for all other applications related to NSCLC.

IV. *BRAF* V600E Testing

A. Analysis of the *BRAF* V600E variant using plasma specimens to detect ctDNA may be considered **medically necessary** to predict treatment response to BRAF or MEK inhibitor therapy (eg, dabrafenib [Tafinlar], trametinib [Mekinist]) in patients with advanced lung adenocarcinoma, large cell carcinoma, advanced squamous cell non-small-cell lung cancer, and non-small-cell lung cancer not otherwise specified when tissue biopsy is medically contraindicated, or the quantity is not sufficient for testing.

B. Analysis of the *BRAF* V600E variant using plasma specimens to detect ctDNA or RNA is considered **experimental / investigational** for all other applications related to NSCLC.

V. *ROS1* Testing

A. Analysis of somatic rearrangement variants of the *ROS1* gene using plasma specimens to detect ctDNA or RNA may be considered **medically necessary** to predict treatment response to ALK inhibitor therapy (crizotinib [Xalkori]) in patients with advanced lung adenocarcinoma, large cell carcinoma, advanced squamous cell non-small-cell lung cancer, and non-small-cell lung cancer not otherwise specified when tissue biopsy is medically contraindicated, or the quantity is not sufficient for testing.

B. Analysis of somatic rearrangement variants of the *ROS1* gene using plasma specimens to detect ctDNA or RNA is considered **experimental / investigational** for all other applications related to NSCLC.

VI. *MET* Testing

A. Analysis of the *MET* exon 14 skipping variant using plasma specimens to detect ctDNA is considered **experimental / investigational** as an alternative to tissue biopsy (see Policy Guidelines) to predict treatment response to MET inhibitor therapy (capmatinib [Tabrecta]) in patients with NSCLC.

VII. *RET* Testing

A. Analysis of somatic fusion variants of the *RET* gene using plasma specimens to detect ctDNA is considered **experimental / investigational** as an alternative to tissue biopsy (see Policy Guidelines) to predict treatment response to RET inhibitor therapy (eg, selpercatinib [Retevmo], pralsetinib [Gavreto]) in patients with NSCLC.

VIII. *KRAS* Testing

A. Analysis of somatic variants of the *KRAS* gene using plasma specimens to detect ctDNA is considered **experimental / 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.

IX. *HER2* Testing

A. Analysis of alterations in the *HER2* gene using plasma specimens to detect ctDNA for targeted therapy in patients with NSCLC is considered **experimental / investigational**.

Policy Guidelines

1. The tests discussed herein, cobas EGFR Mutation Test v2, Guardant360 CDx test, Oncobeam test or InVisionFirst-Lung, are intended for use in patients with advanced lung adenocarcinoma, large cell carcinoma, advanced squamous cell non-small-cell lung cancer, and non-small-cell lung cancer not otherwise specified. 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 test for other oncogenic driver variants deemed medically necessary on tissue biopsy (the BCBSKS medical policy *Molecular Analysis for Targeted Therapy or Immunotherapy 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; however this is only appropriate if follow-up tissue-based analysis is planned should no driver variant be identified.

2. Genetics Nomenclature Update
   a. 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.
   b. 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.

RATIONALE

This evidence review has been updated with searches of the PubMed database. The most recent literature update was performed through July 20, 2020.

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.

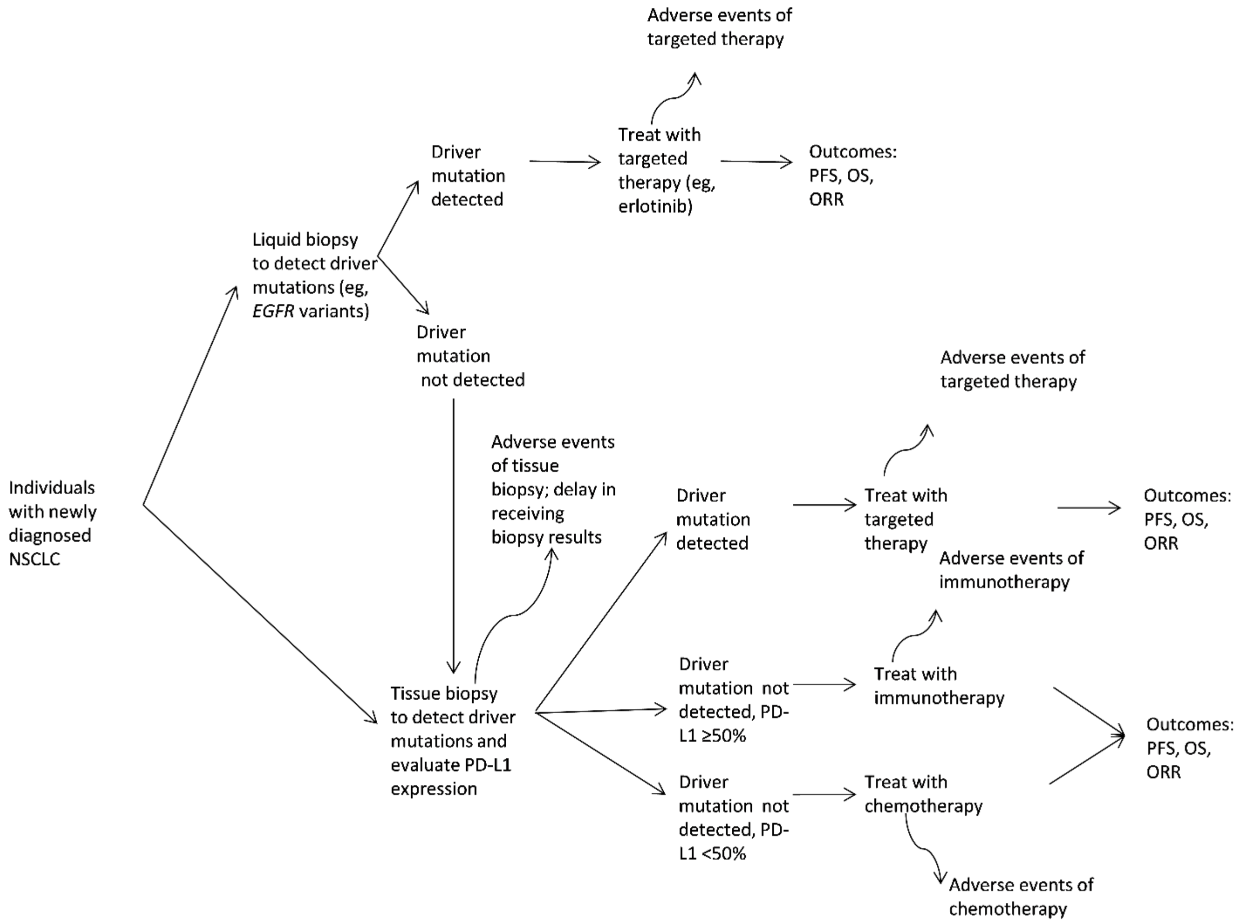
Studies have also assessed a liquid biopsy for detection of the EML4-ALK fusion oncogene and its variants, translocation between ROS1 and other genes (most commonly CD74), BRAF variants occurring at the V600 position of exon 15, and other variants.

Comparators

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

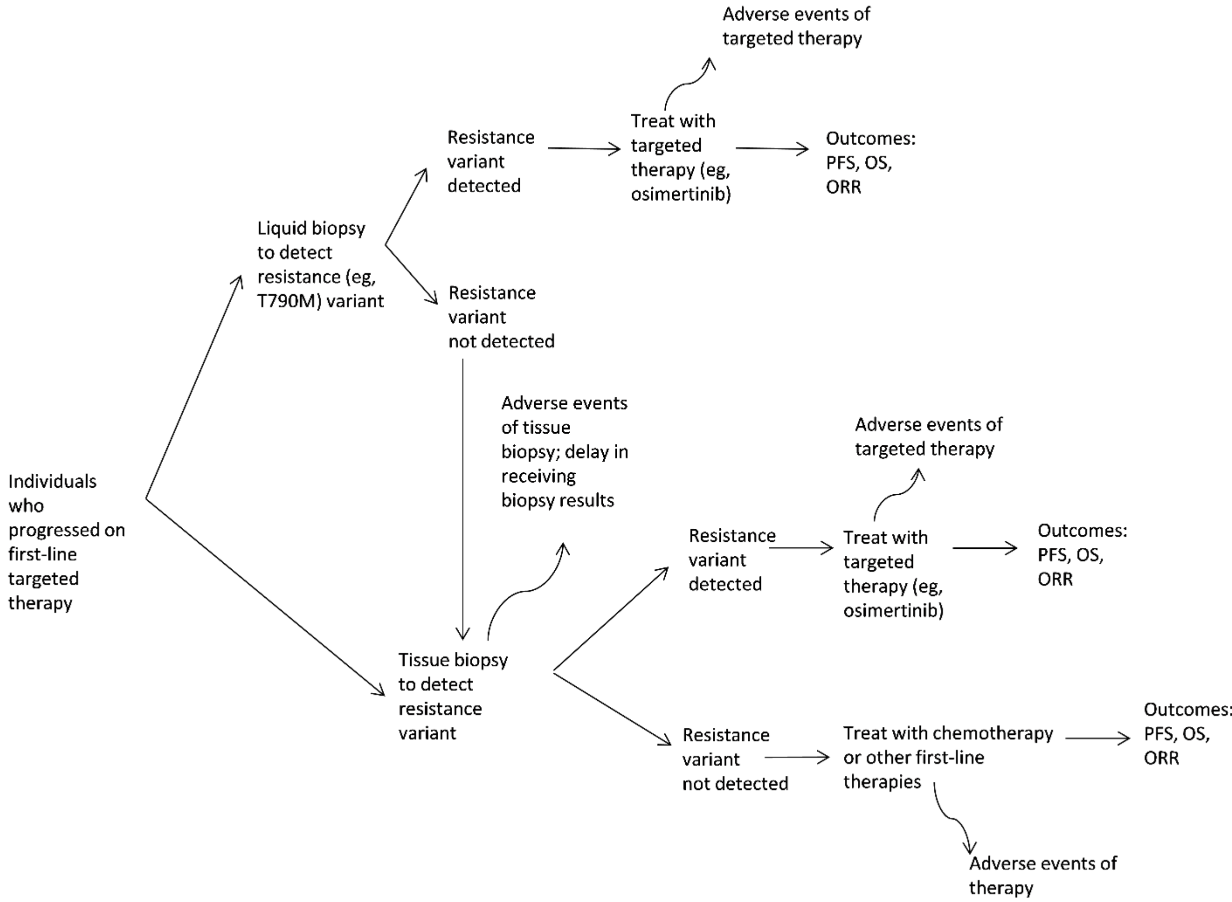
The testing strategy in Figure 1 is based on the reflex testing strategy suggested in the FDA approval for the cobas test. Some guidelines have suggested that testing with a liquid biopsy should be used when testing with tissue biopsy is not feasible.

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 the BCBSKS medical policy *Molecular Analysis for Targeted Therapy or Immunotherapy 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				

Study	Study Population	Design	Variants Included[a]	Timing of Reference and Index Tests
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 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
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	Retrospective	*EGFR*	Unclear

Study	Study Population	Design	Variants Included[a]	Timing of Reference and Index Tests
	prevalence of CDx-positive, tissue-negative patients as no plasma from FLAURA tissue-negative patients was available			
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 medical center in California between 2014 and 2015	Retrospective, consecutive	*EGFR*, *ALK*, *ROS1*, *BRAF*	Median time was 0.8 mo, range not given
Thompson et al (2016)[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				

Study	Study Population	Design	Variants Included[a]	Timing of Reference and Index Tests
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 US studies with 41 centers	Prospective	*EGFR*, *ALK*, *ROS1*, *BRAF*, *MET*	Blood collected within 12 weeks of tissue biopsy and no therapy between tissue and blood samples
Remon et al (2019)[27,]	Patients with advanced NSCLC enrolled in single-center, prospective observational study in France. Patients were either treatment naıve 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		
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)

Study	Initial N	Final N	Excluded Samples	Sensitivity (95% CI)	Specificity (95% CI)
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)
EGFR exon 20 (T790M, resistance)		207	51 (44 to 58) 66 (59 to 72)	66 (59 to 72)	NA[d]

Study	Initial N	Final N	Excluded Samples	Sensitivity (95% CI)	Specificity (95% CI)
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

Study	Initial N	Final N	Excluded Samples	Sensitivity (95% CI)	Specificity (95% CI)
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		
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)

Study	Initial N	Final N	Excluded Samples	Sensitivity (95% CI)	Specificity (95% CI)
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)
FounationOne Liquid CDx					
FDA SSED (2020)[29,]	280		Samples in which there was insufficient plasma		

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

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

The purpose of the limitations tables (see Tables 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,]					

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
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					

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Pritchet et al (2019)[26,]	4: Calculation of performance characteristics only included subset of patients with at least 1 mutation 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 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,]						

Study	Selection [a]	Blinding [b]	Delivery of Test[c]	Selective Reporting [d]	Data Completeness [e]	Statistical [f]
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 collection not described			1. Precision estimates not reported but calculated 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			

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

Study	Selection [a]	Blinding [b]	Delivery of Test[c]	Selective Reporting [d]	Data Completeness [e]	Statistical [f]
			collection not described			
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
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

Study	Selection [a]	Blinding [b]	Delivery of Test[c]	Selective Reporting [d]	Data Completeness [e]	Statistical [f]
						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 (ie, 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 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,])

Test (Method)	**Comparison With Tissue Test**			**Study Quality**
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 (ie, 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	

Study/Patient Group	Country	Disease Stage	Technology Used to Detect ctDNA	Sample Sizes	Treatment Response		
				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 (ie, 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)	
				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 (ie, tissue positive)			p

Study/Patient Group	Country	Disease Stage	Technology Used to Detect ctDNA	Sample Sizes	Treatment Response			
				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 (ie, 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				

Study/Patient Group	Country	Disease Stage	Technology Used to Detect ctDNA	Sample Sizes	Treatment Response		
				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 3 trial of patients who progressed on EGFR TKI	Multinational[c]	Locally advanced or metastatic	cobas (RT-PCR); Guardant360 (NGS); Biodesix (ddPCR)	ORR (95% CI) (Osimertinib vs Chemotherapy)		
			Subgroup	n	Osimertinib	Chemotherapy
			T790M+, tissue	279, 140	71 (65 to 76)	31 (24 to 40)
			T790M+ liquid (cobas)	111, 48	76 (67 to 83)	45 (31 to 60)
			T790M+, liquid (Guardant360)	137, 53	68 (59 to 76)	40 (27 to 54)
			T790M-, liquid (cobas)	101, 47	71 (61 to 79)	28 (16 to 42)
			T790M-, liquid (Guardant360)	72, 29	78 (66 to 87)	17 (6 to 36)
				PFS HR (95% CI) (Osimertinib vs Chemotherapy)		
			T790M+, tissue	419	0.30 (0.23 to 0.41)	

Study/Patient Group	Country	Disease Stage	Technology Used to Detect ctDNA	Treatment Response	
			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)	

Study/Patient Group	**Country**	**Disease Stage**	**Technology Used to Detect ctDNA**	**Treatment Response**	
				Tissue positive vs tissue negative	
				65	61% vs 29%
				Liquid positive vs liquid negative	
				72	59% vs 35%
				Liquid positive, tissue biopsy negative	
				8	38%
Karlovich et al (2016)[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	

Study/Patient Group	Country	Disease Stage	Technology Used to Detect ctDNA	Treatment Response	
				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 chemotherapy 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 (eg, 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 (eg, 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" (ie, 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 MutationTest 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.

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)

NCT No.	Trial Name	Planned Enrollment	Completion Date
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.

CODING

The following codes for treatment and procedures applicable to this policy are included below for informational purposes. Inclusion or exclusion of a procedure, diagnosis or device code(s) does not constitute or imply member coverage or provider reimbursement. 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.

CPT/HCPCS

81210	BRAF (B-Raf proto-oncogene, serine/threonine kinase) (eg, colon cancer, melanoma), gene analysis, V600 variant(s)
81235	EGFR (epidermal growth factor receptor) (eg, non-small cell lung cancer) gene analysis, common variants (eg, exon 19 LREA deletion, L858R, T790M, G719A, G719S, L861Q)
81445	Targeted genomic sequence analysis panel, solid organ neoplasm, DNA analysis, and RNA analysis when performed, 5-50 genes (eg, 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 (eg, 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 (eg, circulating tumor cells in blood);
86153	Cell enumeration using immunologic selection and identification in fluid specimen (eg, circulating tumor cells in blood); physician interpretation and report, when required
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).
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

ICD-10 Diagnoses

Experimental / Investigational for all diagnoses related to this medical policy.

REVISIONS

12-21-2018	Policy added to the bcbsks.com website on 11-20-2018 with an effective date of 12-21-2018.
09-27-2019	Policy published to the bcbsks.com website on 08-28-2019 with an effective date of 09-27-2019.
	In Policy section:

	▪ In Item I A, added “when tissue biopsy is medically contraindicated or the quantity is not sufficient for testing” and removed “as an alternative to tissue biopsy” to read, “Except as noted below, analysis of 2 types of somatic sensitizing variants within the epidermal growth factor receptor (*EGFR*) gene—small deletions in exon 19 and a point mutation in exon 21 (L858R)—using the cobas EGFR Mutation Test v2, Guardant360 test, or OncoBEAM test with plasma specimens to detect circulating tumor DNA (ctDNA) may be considered medically necessary to predict treatment response to an EGFR tyrosine kinase inhibitor (TKI) therapy in patients with advanced stage III or IV non-small-cell lung cancer (NSCLC) when tissue biopsy is medically contraindicated or the quantity is not sufficient for testing. The cobas test is a companion diagnostic for erlotinib and gefitinib.” ▪ In Item II A, added “may be”, “medically necessary”, “advanced stage III or IV”, and “when tissue biopsy is medically contraindicated or the quantity is not sufficient for testing” and removed “experimental/investigational as an alternative to tissue biopsy” to read, “Analysis of somatic rearrangement variants of the *ALK* gene using plasma specimens to detect ctDNA or RNA may be considered medically necessary to predict treatment response to ALK inhibitor therapy (eg, crizotinib [Xalkori], ceritinib [Zykadia], alectinib [Alecensa], or brigatinib [Alunbrig]) in patients with advanced stage III or IV NSCLC when tissue biopsy is medically contraindicated or the quantity is not sufficient for testing.” ▪ Added new Item II B, “Analysis of somatic rearrangement variants of the *ALK* gene using plasma specimens to detect ctDNA or RNA is considered experimental / investigational for all other applications related to NSCLC.” ▪ In Item III A, added “may be”, “medically necessary”, “advanced stage III or IV”, “when tissue biopsy is medically contraindicated or the quantity is not sufficient for testing” and removed “experimental/investigational as an alternative to tissue biopsy” to read, “Analysis of the *BRAF* V600E variant using plasma specimens to detect ctDNA may be considered medically necessary to predict treatment response to BRAF or MEK inhibitor therapy (eg, dabrafenib [Tafinlar], trametinib [Mekinist]) in patients with advanced stage III or IV NSCLC when tissue biopsy is medically contraindicated or the quantity is not sufficient for testing.” ▪ Added new Item III B, “Analysis of the *BRAF* V600E variant using plasma specimens to detect ctDNA or RNA is considered experimental / investigational for all other applications related to NSCLC.” ▪ In Item IV A, added “may be”, “medically necessary”, “advanced stage III or IV”, “when tissue biopsy is medically contraindicated or the quantity is not sufficient for testing” and removed “experimental/investigational as an alternative to tissue biopsy” to read, “Analysis of somatic rearrangement variants of the *ROS1* gene using plasma specimens to detect ctDNA or RNA may be considered to predict treatment response to ALK inhibitor therapy (crizotinib [Xalkori]) in patients with advanced stage III or IV NSCLC when tissue biopsy is medically contraindicated or the quanity is not sufficient for testing.” C. Added new Item IV B, “Analysis of somatic rearrangement variants of the *ROS1* gene using plasma specimens to detect ctDNA or RNA is considered experimental / investigational for all other applications related to NSCLC.”
	Updated References section.
01-15-2021	• Updated Description Section
	• Updated Medical Policy Section • Revised Item I.A., with the following changes: (Content that is underlined was added, content with a strikethrough was removed.) “At diagnosis, ~~Except as noted below~~, analysis of ~~2 types of~~ somatic ~~sensitizing~~ variants ~~within the epidermal growth factor receptor (EGFR) gene□small deletions~~ in exons 18 through 21 (e.g., G719X, L858R, T790M, S6781, L861Q) within the epidermal growth factor receptor (EGFR), ~~19 and a point mutation in exon 21~~

	(L858R)-using for example the cobas EGFR Mutation Test v2, Guardant360 test, or OncoBEAM, or InVisionFirst-Lung test with plasma specimens to detect circulating tumor DNA (ctDNA) may be considered **medically necessary** as an alternative to tissue biopsy (see Policy Guidelines) to predict treatment response to an EGFR tyrosine kinase inhibitor (TKI) therapy (eg, erlotinib [Tarceva], gefitinib [Iressa], afatinib [Gilotrif], or osimertinib [Tagrisso]) in patients with advanced lung adenocarcinoma, large cell carcinoma, advanced squamous cell non-small-cell lung cancer, and non-small-cell lung cancer not otherwise specified. stage III or IV non-small-cell lung cancer (NSCLC) when tissue biopsy is medically contraindicated or the quantity is not sufficient for testing. The cobas test is a companion diagnostic for erlotinib and gefitinib." • Added Item I.B. • Item I.C., removed "sensitizing" "18" and "using ctDNA for" and added "22" and "or other" to read "Analysis of other EGFR variants within exons 22 to 24 or other applications related to NSCLC is considered experimental / investigational." • Removed Items I.D, "Analysis of the EGFR T790M resistance variant for targeted therapy with osimertinib using ctDNA or for other applications related to NSCLC is considered experimental / investigational." • Removed Item I.E., "Analysis of 2 types of somatic variants within the EGFR gene-small deletions in exon 19 and a point mutation in exon 21 (L858R)-using ctDNA is considered experimental / investigational for patients with advanced NSCLC of squamous cell type." • In Items II.A, III.A, and IV.A, replaced "stage III or IV NSCLC" with "advanced lung adenocarcinoma, large cell carcinoma, advanced squamous cell non-small-cell lung cancer, and non-small-cell lung cancer not otherwise specified." • In Policy guidelines, Replaced "advanced (stage III or IV) non-small-cell lung cancer" and "stage III or IV NSCLC" with "advanced lung adenocarcinoma, large cell carcinoma, advanced squamous cell non-small-cell lung cancer, and non-small-cell lung cancer not otherwise specified" to read "The tests discussed herein are intended for use in patients with advanced lung adenocarcinoma, large cell carcinoma, advanced squamous cell non-small-cell lung cancer, and non-small-cell lung cancer not otherwise specified." • Updated Code Section Added CPT 81210, 81445, 81455
	• Updated Rationale Section
	• Updated Reference Section
03-29-2021	• Updated Description Section
	• Updated Medical Policy Section • Item I.A.-The following chages were made. Strike through text was removed and underlined text was added: "At diagnosis, analysis of somatic variants in exons 18 19 through 21 (e.g., exon 19 deletions, G719X, L858R, T790M, S6781, L861Q) within the epidermal growth factor receptor (EGFR) gene, using for example the cobas EGFR Mutation Test v2, Guardant360 CDx test, OncoBEAM test or InVisionFirst-Lung test with plasma specimens to detect circulating tumor DNA (ctDNA), may be considered **medically necessary** as an alternative to tissue biopsy (see Policy Guidelines) to predict treatment response to an EGFR tyrosine kinase inhibitor (TKI) therapy (eg, erlotinib [Tarceva], gefitinib [Iressa], afatinib [Gilotrif], dacomitinib [Vizimpro], or osimertinib [Tagrisso]) in patients with advanced lung adenocarcinoma, large cell carcinoma, advanced squamous cell non-small-cell lung cancer, and non-small-cell lung cancer not otherwise specified." Item I.B.-The following chages were made. Strike through text was removed and underlined text was added: "At progression, analysis of the EGFR T790M resistance variant for targeted therapy with osimertinib using ctDNA using for example the cobas EGFR Mutation Testv2, Guardant360 CDx test, OncoBEAM test or InVisionFirst-Lung

	test with plasma specimens to detect circulating tumor DNA (ctDNA), may be considered **medically necessary** in patients with advanced lung adenocarcinoma, large cell carcinoma, advanced squamous cell non-small-cell lung cancer, and non-small-cell lung cancer not otherwise specified when tissue biopsy ~~tumor tissue is not available and/or~~ 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. (see Policy Guidelines)." • Item V, "Other Genes" was reformatted to become Item III. HER2, MET, and RET were removed and now reads as follows "Plasma tests for oncogenic driver variants deemed medically necessary on tissue biopsy (see the BCBSKS medical policy Molecular Analysis for Targeted Therapy or Immunotherapy of Non-Small-Cell Lung Cancer.) may be considered **medically necessary** to predict treatment response to targeted therapy for patients meeting the following criteria: 1. Patient does not have sufficient tissue for standard molecular testing using formalin-fixed paraffin-embedded tissue; AND 2. Follow-up tissue-based analysis is planned should no driver variant be identified via plasma testing." • Items V, VI, and VIII were added. • Policy Guideline #1- The following chages were made. Strike through text was removed and underlined text was added: "The tests discussed herein, cobas EGFR Mutation Test v2, Guardant360 CDx test, Oncobeam test or InVisionFirst-Lung, are intended for use in patients with advanced lung adenocarcinoma, large cell carcinoma, advanced squamous cell non-small-cell lung cancer, and non-small-cell lung cancer not otherwise specified. 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 ~~epidermal growth factor receptor (~~*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. ~~However, the~~ Plasma test for other oncogenic driver variants deemed medically necessary on tissue biopsy (the BCBSKS medical policy Molecular Analysis for Targeted Therapy or Immunotherapy 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; however this is only appropriate if follow-up tissue-based analysis is planned should no driver variant be identified. ~~do not have a biopsy-amenable lesion, cannot undergo biopsy, or have indeterminate histology (in whom an adenocarcinoma component cannot be excluded).~~"
	• Updated Rationale Section
	• Updated Coding Section • Added CPT code 0179U • Removed the following ICD 10 diagnosis codes: C34.01, C34.02, C34.11, C34.12, C34.2, C34.31, C34.32, C34.81, C34.82, C34.91, C34.92
	• Updated Reference Section
04-01-2021	In Coding section: • Added CPT code 0242U

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