Circulating Tumor DNA Management of Non-Small Cell Lung Cancer (Liquid Biopsy)

Policy # 00597
Original Effective Date: 03/21/2018
Current Effective Date: 03/21/2018

Applies to all products administered or underwritten by Blue Cross and Blue Shield of Louisiana and its subsidiary, HMO Louisiana, Inc.(collectively referred to as the "Company"), unless otherwise provided in the applicable contract. Medical technology is constantly evolving, and we reserve the right to review and update Medical Policy periodically.

Note: Molecular Analysis for Targeted Therapy of Non-Small Cell Lung Cancer is addressed separately in medical policy 00452

Note: Proteomic Testing for targeted Therapy in Non-Small Cell Lung Cancer is addressed separately in medical policy 00446.

Note: Circulating Tumor DNA and Circulating Tumor Cells for Cancer Management (Liquid Biopsy) is addressed separately in medical policy 00497.

Note: Miscellaneous Genetic and Molecular Diagnostic Tests is addressed separately in medical policy 00577.

EGFR TESTING

When Services Are Eligible for Coverage
Coverage for eligible medical treatments or procedures, drugs, devices or biological products may be provided only if:

- *Benefits are available in the member's contract/certificate, and*
- *Medical necessity criteria and guidelines are met.*

Based on review of available data, the Company may consider 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 variant in exon 21 (L858R)— using the cobas®‡ EGFR Mutation Test v2 with plasma specimens to detect circulating tumor deoxyribonucleic acid (ctDNA) as an alternative to tissue biopsy to predict treatment response to an EGFR tyrosine kinase inhibitor (TKI) therapy in patients with non-small-cell lung cancer (NSCLC). The cobas test is a companion diagnostic for erlotinib (Tarceva®‡; OSI Pharmaceuticals, Melville NY) to be **eligible for coverage.**

When Services Are Considered Investigational
Coverage is not available for investigational medical treatments or procedures, drugs, devices or biological products.

Based on review of available data, the Company considers analysis of other *EGFR* sensitizing variants within exons 18 to 24 using ctDNA for applications related to NSCLC to be **investigational.***

Based on review of available data, the Company considers analysis of *EGFR* T790M resistance variant for targeted therapy with osimertinib using ctDNA or for other applications related to NSCLC to be **investigational.***

Based on review of available data, the Company considers analysis of 2 types of somatic mutations variants within the *EGFR* gene—small deletions in exon 19 and a point mutation variant in exon 21 (L858R)—using ctDNA for patients with advanced NSCLC of squamous cell type to be **investigational.***

Policy Guidelines

These tests are intended for use in patients with advanced NSCLC. Patients with either small deletions in exon 19 or a point variant in exon 21 (L858R) of the tyrosine kinase domain of the *EGFR* gene are considered good candidates for treatment with erlotinib, gefitinib or afatinib. The Food and Drug Administration (FDA) 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 may also be appropriate for patients who do not have enough tissue for standard molecular testing using formalin-fixed paraffin-embedded tissue, do not have a biopsy-amenable lesion, cannot undergo biopsy or have indeterminate histology (in whom an adenocarcinoma component cannot be excluded).

GENETICS NOMENCLATURE UPDATE

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

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

Table PG1. Nomenclature to Report on Variants Found in DNA

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

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

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

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

Background/Overview

PREDICTIVE BIOMARKERS IN NON-SMALL-CELL LUNG CANCER

Several predictive genetic biomarkers have been identified for NSCLC. Predictive biomarkers are particularly important because they can define response to specific treatments. Depending on which biomarkers are identified, patients may be eligible to receive the most effective treatment or to receive a treatment that is equally effective but with fewer adverse effects.

Tyrosine Kinase Inhibitor–Sensitizing Variants

EGFR Variants

Specific *EGFR* variants confer sensitivity to treatment with TKIs, such as erlotinib, gefitinib, and afatinib; the most common variants are deletions in exons 19 and an exon 21 substitution variant. 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 around 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 (PFS) is improved with the use of TKIs. Patients receiving TKIs have fewer treatment-related adverse effects than patients receiving cytotoxic chemotherapy.

ALK, ROS1, and KRAS Variants

Anaplastic lymphoma kinase (*ALK*) rearrangements also confer resistance to TKIs. Between 2% and 7% 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 PFS, 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%. Finally, the most common predictive variant in North American populations is *KRAS*. Patients with *KRAS* variants have shorter survival than those without KRAS variants, and thus *KRAS* is a prognostic marker. It also predicts 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.

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.

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. Unlike apoptosis, necrosis is considered a pathologic process, generating larger DNA fragments due to an incomplete and random digestion of genomic DNA. The length or integrity of the circulating DNA can potentially distinguish between apoptotic and necrotic origins. 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 that are up to 500 to 1000 times more sensitive than standard sequencing approaches (e.g., Sanger) are needed.

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

- Denaturing high performance liquid chromatography involves polymerase chain reaction (PCR) followed by denaturing plus hybridization and then separation.
- Peptide nucleic acid–locked nucleic acid PCR suppresses wild-type *EGFR* followed by enrichment for mutated *EGFR*.
- Amplification refractory mutation system (ARMS) 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 enumeration of rare variants in complex mixtures of DNA.

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

FDA or Other Governmental Regulatory Approval

U.S. Food and Drug Administration (FDA)

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 U.S. FDA has chosen not to require any regulatory review of this test. Clinical laboratories accredited through 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.

Genomic Health (Redwood City, CA) markets the Oncotype SEQ™‡ Liquid select. The test uses NGS to identify actionable genomic alterations for late-stage lung, breast, colon, melanoma, ovarian, and gastrointestinal cancers.

Guardant Health (Redwood City, CA) markets the Guardant360®‡ test. This test uses NGS to identify variants in 73 genes associated with several different cancers.

Circulogene Theranostics' (Birmingham, AL) liquid biopsy test uses a finger stick blood sample and NGS to monitor known tumor variants (≈3000) in 50 cancer-associated genes for targeted therapy. The test uses a proprietary method to recover necrotic and apoptotic cell death–associated cell-free DNA.

CancerIntercept®‡ (Pathway Genomics, San Diego, CA) is a 96-gene panel designed to detect variants in 9 driver genes involved primarily in breast, ovarian, lung, and colorectal cancers, as well as melanoma. The test uses PCR amplification of both the wild-type and variant DNA followed by enrichment of the variant and removal of the wild-type DNA using a proprietary technology after which variant DNA is sequenced using NGS.

Biocept (San Diego, CA) offers blood-based assays that target variants found in lung and breast cancers. The test uses a proprietary real-time quantitative PCR and, using Sanger sequencing, sequences the amplified product to confirm variants.

Foundation Medicine's (Cambridge, MA) FoundationACT™‡ uses hybrid capture-based NGS to detect variants in over 60 genes for targeted therapy in metastatic cancer.

Biodesix's (Boulder, CO) GeneStrat®‡ uses droplet digital PCR to analyze cell-free DNA and ribonucleic acid (RNA) to identify specific driver variants for which targeted therapy is available for NSCLC.

In June 2016, cobas EGFR Mutation Test v2 (Roche Molecular Systems, Pleasanton, CA), a real-time PCR test, was approved by the U.S. FDA through the premarket approval process (P150047). This plasma test was 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. Patients who test negative for the variants detected should be referred for (or "reflexed" to) routine biopsy with tissue testing for *EGFR* variants. The previously approved version 2 of this test, which used tissue biopsy specimens, was also approved for detection of T790M variants in tissue, which are used to select patients to receive osimertinib. Approval of version 2 of the plasma test did not include detection of T790M variants.

Centers for Medicare and Medicaid Services (CMS)
There is no national coverage determination. In the absence of a national coverage determination, coverage decisions are left to the discretion of local Medicare carriers.

Rationale/Source

Assessment of diagnostic technology typically focuses on 3 categories of evidence: (1) analytic validity (test-retest reliability or interrater reliability); (2) clinical validity (sensitivity, specificity, positive and negative predictive values) in relevant populations of patients; and (3) clinical utility (i.e., demonstration that the diagnostic information can be used to improve patient outcomes).

Randomized controlled trials (RCTs) comparing treatment selection based on tumor biomarkers with plasma biomarkers would be most convincing evidence of clinical utility. Evidence on the ability of liquid biopsy to predict treatment response similar to, or better than, 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 on for those variants on standard biopsies respond to *EGFR* TKIs (i.e., erlotinib, gefitinib, afatinib, osimertinib), it would suggest that the standard biopsy was correct and the liquid biopsy results were truly false-negatives. If patients with positive liquid biopsies and negative tissue biopsies for *EGFR* variants respond to *EGFR* TKIs, it would suggest that the positive liquid biopsies were correct rather than false-positives.

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, 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.

CIRCULATING TUMOR DNA AND CIRCULATING TUMOR CELLS

Clinical Context and Test Purpose

The purpose of identifying targetable oncogenic "driver mutations" such as *EGFR* variants in patients who have NSCLC is to inform a decision whether patients should receive a targeted therapy 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 treatment in patients with advanced NSCLC with reflex to tissue biopsy and how it would potentially affect outcomes.
The questions addressed in this evidence review are:

1. How accurately does liquid biopsy detect EGFR TKI-sensitizing and TKI-resistance variants of interest in the relevant patient population (clinical validity)?
2. Does a strategy including liquid biopsy in patients with NSCLC improve the net health outcome compared with standard biopsy?

The following PICOTS were used to select literature to inform this review.

Patients

The target population consists of patients with NSCLC where tumor biomarker testing is indicated to select treatment. Patients may be treatment-naive, or being considered for a treatment change due to progression, recurrence, or suspected treatment resistance. Because it is not standard practice to do routine surveillance or periodic monitoring of treatment response with standard biopsy, this potential use of liquid biopsy was not evaluated in this evidence review.

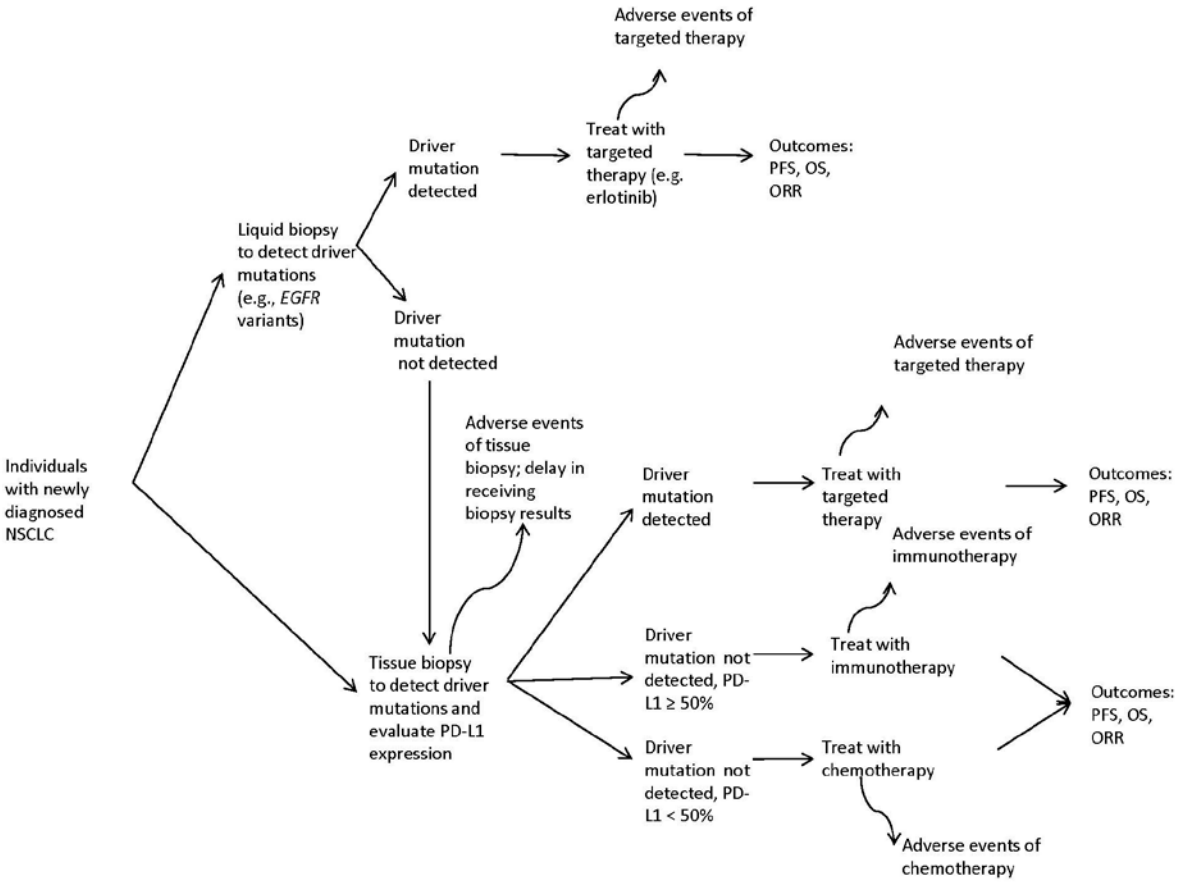
Interventions

The technology considered is analysis of tumor biomarkers in peripheral blood (liquid biopsy) to determine treatment selection. The comparator is analysis of tumor biomarkers for treatment selection using tumor tissue. Evidence was considered separately for the different biomarkers. Studies have evaluated liquid biopsy for biomarkers that detect *EGFR* TKI sensitization, concentrating on the *EGFR* exon 19 deletion and *EGFR* L858R variants. Studies have also evaluated separately biomarkers associated with TKI resistance, concentrating on the *EGFR* T790M variant.

Comparators

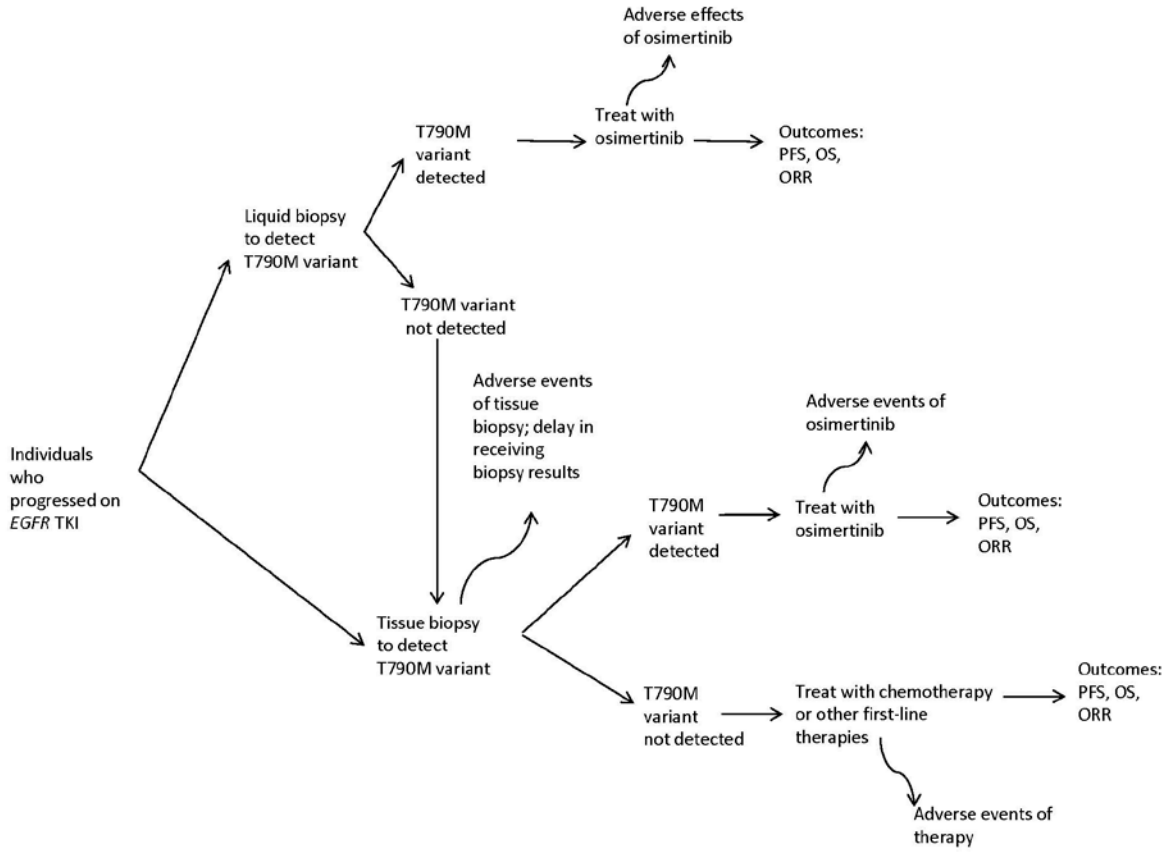
The relevant comparator of interest is testing for biomarkers of EGFR TKI sensitivity using tissue biopsy.

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



EGFR: epidermal growth factor receptor; NSCLC: non-small-cell lung cancer; PFS: progression-free survival; ORR: objective response rate; OS: overall survival; TKI: tyrosine kinase inhibitor.

Outcomes

The outcomes of interest are overall survival (OS) and cancer-related survival. Test performance measures, including sensitivity, specificity, and agreement with tissue biopsy, are also of interest as intermediate factors impacting treatment decisions.

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

Setting
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.

We evaluate evidence for the 1st, 2nd, and 5th PICO formulations (when tissue biopsy is possible) together in the following sections. The Section Summaries, Summary of Evidence, and review of TEC criteria will evaluate each PICO formulation separately. The conclusions regarding the 3rd and 4th PICO formulations (when tissue biopsy is not possible) derive from the evidence in the 1st and 2nd PICOs respectively.

Analytic Validity
For any test, it is important to establish its analytic validity—the ability of the test to measure accurately and reliably the characteristic of interest for which the test was designed to identify or measure. Assessment of analytic validity focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this review. We focus on the clinical validity and clinical utility.

Table 1 summarizes the commercially available ctDNA tests and laboratory-reported limits of detection for specific tests.

Table 1. Commercial Circulating Tumor DNA Tests

Test	Regulatory Status	Technology	Classes of Variants Detected		Limits of Detection
Roche cobas EGFR Mutation Test v2	FDA-approved PMA (P150047)	Real-time PCR	SNVs		75-100 copies/mL
			Insertions/deletions		75-100 copies/mL
Guardant360 (NGS)	LDT	NGS	SNVs		<0.1%
			Insertions/deletions		<0.1%
			Fusions		<0.1%
			CNVs		≥2.12 copies
FoundationACT (NGS)	LDT	NGS	SNVs		≥0.5%
			Insertions/deletions (1-40 bp)		≥1%
			Rearrangements/fusions		≥1%
			CNVs	>20%	≥8 copies
				<20%	Variable
Biocept (real-time PCR)	LDT	Real-time PCR	SNVs		NA
Genomic Health the Oncotype SEQ Liquid	LDT	NGS	SNVs		0.1%
			Insertions/deletions		0.1%

Test	Regulatory Status	Technology	Classes of Variants Detected	Limits of Detection
Select (NGS)			Fusions	0.1%
			CNVs	2.4 copies
Circulogene's (Theranostics) liquid biopsy test (NGS)	LDT	NGS	NA	NA
CancerIntercept (NGS)	LDT	NGS	NA	NA
Biodesix's GeneStrat (ddPCR)	LDT	ddPCR	SNVs	NA
			Fusions	0.2%

bp: base pairs; CNV: copy number variant; ddPCR: digital droplet polymerase chain reaction; EGFR: epidermal growth factor receptor; FDA: Food and Drug Administration; LDT: laboratory-developed test; NA: not applicable; NGS: next-generation sequencing; PCR: polymerase chain reaction; PMA: premarket approval; SNV: single-nucleotide variant.

Clinical Validity

Our clinical validity review focuses on evidence for *EGFR* variants in NSCLC and includes: (1) a systematic review and meta-analysis performed by BCBSA comparing the diagnostic performance of liquid biopsy with a tissue biopsy reference standard including studies published through early 2017, (2) 3 published meta-analyses, which were published in 2015, and (3) descriptions of selected studies published after the 2015 systematic reviews that compared the diagnostic performance of liquid biopsy with a tissue biopsy reference standard.

Assessment Systematic Review

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

Fifty-three studies have reported on *EGFR* TKI-sensitivity variants or a combination of sensitivity and resistance variants. Two studies have reported only on *EGFR* TKI-resistance variants (T790M). More than half (56%) included only advanced or recurrent NSCLC; 27% included all stages. The majority (75%) used plasma blood samples. Forty (73%) were performed solely in Asia. Various ctDNA detection methods were used, with ARMS being the most common. Study characteristics are shown in Table 2.

Table 2. Characteristics of Studies Included in the Assessment's Systematic Review

Study	Year	Sample Size	Country	Plasma or Serum	Disease Stage	ctDNA Detection Method	*EGFR* Variants (Exons)
Kimura et al	2006	11	Japan	Serum	IIIB-IV	SARMS	19, 21
Kimura et al	2007	42	Japan	Serum	IIIB-IV	SARMS	19, 21, 18

Study	Year	Sample Size	Country	Plasma or Serum	Disease Stage	ctDNA Detection Method	*EGFR* Variants (Exons)
Maheswaran et al	2008	17	U.K.	Plasma	NR	SARMS	19, 21
Bai et al	2009	230	China	Plasma	IIIB-IV	DHPLC	19, 21
Yung et al	2009	35	Hong Kong	Plasma	III-IV	ddPCR	19, 21
Mack et al	2009	14	U.S.	Plasma	IIIB-IV	SARMS	19, 21, 20
He et al	2009	18	China	Plasma	I-IV	ME-PCR	19, 21
Kuang et al	2009	54	U.S.	Plasma	Advanced	SARMS, direct sequencing	19, 21
Song et al	2010	50	China	Serum	I-IIIA	direct sequencing	19, 21
Brevet et al	2011	31	U.S.	Plasma	III-IV	MSG, ME-PCR	19, 21
Jiang et al	2011	58	China	Serum	IIIB-IV	ME-PCR	19, 21
Sriram et al	2011	64	Australia	Serum	I-IV	ME-PCR	19, 21
Yasuda et al	2011	23	Japan	Serum	I-IV	PNA-LNA	19, 21, 20, 18
Taniguchi et al	2011	44	Japan	Plasma	Advanced	BEAMing	19, 21, 20
Goto et al	2012	86	Japan	Serum	Advanced	SARMS	19, 21, 20
Nakamura et al	2012	70	Japan	Plasma	I-IV	WIP-QP, MBP-QP	19, 21
Xu et al	2012	34	China	Serum	IIIB-IV	SARMS, DHPLC, ME-PCR	19, 21
Yam et al	2012	37	Hong Kong	Plasma	III-IV	PNA-LNA	19, 21, 18
Punnoose et al	2012	28	Australia, U.S.	Plasma	NR	SARMS	19, 21, 20, 18
Huang et al	2012	822	China	Plasma	I-IV	DHPLC	19, 21
Chen et al	2012	30	Taiwan	Plasma	NR	PNA-LNA	19, 21
Hu et al	2012	24	China	Serum	I-IV	HRM	19, 21, 20,18
Kim HR et al	2013	40	Korea	Plasma	IIIA-IV	PNA-LNA	19, 21
Kim ST et al	2013	57	Korea	Serum	IIIB-IV	PNA-LNA	19, 21, 20
Lv et al	2013	9	China	Plasma	IIB-IIIA	DHPLC	19, 21
Akca et al	2013	52	Turkey	Serum	I-IV	Pyrosequencing, dideoxy sequencing	19, 21
Liu et al[30]	2013	86	China	Plasma	Advanced	ARMS	29 variants
Zhang et al[31]	2013	86	China	Plasma	IIIB-IV	MEL	19, 21, 20
Zhao et al[32]	2013	111	China	Plasma	I-IV	ME-PCR	19, 21
Jing et al[33]	2014	120	China	Plasma	I-IV	HRM	18–21
Wang et al[34]	2014	134	China	Plasma	Advanced	ARMS	19, 21, 20
Li et al[35]	2014	121	China	Plasma, Serum	I-IV	ARMS	19, 21, 20
Douillard et al	2014	652	Europe	Plasma	NR	ARMS	19, 21, 20
Weber et al	2014	196	Denmark	Plasma	I-IV	cobas	19, 21, 20
Karachaliou et al	2015	147	France, Italy, Spain	Serum	IIIB-IV	PNA-LNA	19, 21
Thress et al	2015	72	U.S., Europe, Asia	Plasma	Advanced	cobas, BEAMing	19, 21, 20
Duan et al	2015	94	China	Plasma	II-IV	SARMS	19, 21, 20, 18
Mok et al	2015	238	China	Plasma	IIIB- IV	cobas	19, 21, 20,18
Lam et al	2015	74	Hong Kong	Plasma	III- IV	PNA-LNA	19, 21
Sacher et al	2016	174	U.S.	Plasma	Recurrent, IIIB, IV	ddPCR	19, 21, 20
FDA SSED	2016	266	China, Malaysia, Philippines	Plasma	IIIB- IV	cobas	19, 21
Ohira et al	2016	149	Japan	Serum	I-IIIA	ddPCR	NR

Study	Year	Sample Size	Country	Plasma or Serum	Disease Stage	ctDNA Detection Method	*EGFR* Variants (Exons)
Guo et al	2016	41	China	Plasma	I-IV	NGS	NR
Sundaresan et al	2016	25	U.S.	Plasma	IIIA-IV	cobas	20
Takahama et al	2016	41	Japan	Plasma	Recurrence, IIIB, IV, inoperable	ddPCR	19, 21, 18, 20
Chen et al	2016	58	China	Plasma	IA-IIA	NGS	19, 21
Que et al	2016	121	China	Plasma	I-IV	DHPLC	19, 21
Vazquez et al	2016	174	Spain	Serum	IIIB-IV	SARMS	19, 21, 20, 18
Han et al	2016	194	Korea	Plasma	IIIB-IV	PNA clamping-assisted FMCA	19, 21
Thompson et al	2016	50	U.S.	Plasma	II-IV	NGS	19, 21, 20, 18
Kimura et al	2016	24	Japan	Plasma	NR	PointMan EGFR DNA enrichment kit, direct sequencing	20
Ma et al	2016	219	China	Plasma	III-IV	ARMS	19, 21, 20,18
Oxnard et al	2016	216	Multinational[a]	Plasma	Advanced	BEAMing	19, 21, 20
Xu et al	2016	41	China	Plasma	III-IV	NGS	19, 21, 20, 18
Zhang et al	2017	215	China	Plasma	IIIB- IV	ddPCR	19, 21

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

BCBSA staff assessed the risk of bias for studies included in our assessment systematic review using QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies). Because the method used to select patients was frequently not described in the selected studies and therefore staff could not determine whether included patients were selected randomly, consecutively, or as convenience samples, the risk of bias for patient selection was rated as unclear in 33 (61%) studies. There were also concerns about the applicability of included studies because most were carried out in Asian countries with tests that may not be commercially available in the United States. Due to lack of information regarding whether results were interpreted without knowledge of the other test and how cutoffs were defined, the risks of bias for the index test and reference standard were unclear in 30% and 26% of the studies, respectively. The risk of bias for participant flow was high in 30% of studies and unclear in 14% of studies because of the length of time or lack of clarity about the length of time between collection of tissue and blood samples or because of the large number of exclusions from the analysis.

For *EGFR* TKI-sensitizing variants (or grouped *EGFR* variants when sensitizing variants were presented with resistance variant), the sensitivities ranged from 0% to 98% and specificities ranged from 71% to 100%. The summary receiver operating characteristic (ROC) curve for *EGFR* TKI-sensitizing variants indicates little trade-off between sensitivity and specificity. Overall, the area under the curve was 0.87, with

a positive likelihood ratio of 11.1 (95% confidence interval [CI], 7.8 to 15.3), a negative likelihood ratio of 0.4 (95% CI, 0.3 to 0.5), and a diagnostic odds ratio (DOR) of 29 (95% CI, 19 to 43). The performance characteristics for subgroups related to disease stage, plasma vs serum, and ctDNA detection method are shown in Table 3. None of the covariates were statistically significant in the bivariate meta-regression model. Numerically, the cobas test had the highest area under the receiver operating characteristic curve (AUROC; 0.96) and DOR (104.0; 95% CI, 57.5 to 173.0).

Table 3. Overall and Subgroup Meta-Analysis Results for *EGFR* TKI-Sensitizing Variants

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

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

[b] Only 2 studies had data sufficient to calculate specificity, AUROC, PLR, NLR, and DOR.

Seven studies included performance characteristics for *EGFR* TKI-resistance variants. The sensitivities ranged from 50% to 92%, and the specificities ranged from 60% to 87%. The overall area under the curve was 0.78, with positive likelihood, negative likelihood, and DOR of 2.5 (95% CI, 1.9 to 3.2), 0.4 (95% CI, 0.3 to 0.5), and 6 (95% CI, 4 to 9), respectively.

Published Systematic Reviews

As mentioned, 3 systematic reviews, comparing the diagnostic performance of ctDNA with that of a tissue reference for detection of *EGFR* variants, were reported in 2015 and are described below. Thirty-six unique studies were included in the 3 reviews, all of which were also included in the assessment systematic review. Reviews published in 2014 included studies contained in the 2015 reviews, so we do not discuss the earlier reviews further.

Mao et al (2015) conducted a systematic review and meta-analysis to assess whether blood sample could be used as a substitute for tumor tissue to detect *EGFR* variants for guiding treatment with TKIs in NSCLC. Reviewers selected 25 studies (total N=2605 patients). Nineteen were conducted in Asian countries and six in North America, Europe, or Australia. Stage of disease varied across studies, but most selected patients with stage III or IV NSCLC. There was heterogeneity across studies involving the blood tests (whether performed on plasma or serum), which could have affected sensitivity and specificity by source used. The pooled prevalence of all *EGFR* variants was 35.0% (95% CI, 26.0% to 44.0%). Pooled overall sensitivity, specificity, and concordance rates were 61%, 90%, and 79%, respectively (see Table 4). There were no major differences in the diagnostic performance in a subgroup analysis of variants in exons 19 and 21 compared with variants of all exons.

Qiu et al (2015) conducted a similar meta-analysis on the diagnostic performance of ctDNA in detecting *EGFR* variants in NSCLC patients. Reviewers identified 27 eligible studies, which included 3110 patients with NSCLC and who had *EGFR* variant status detected by ctDNA and tumor tissue. Most studies were conducted in Asia and most patients had advanced stage disease. Only six studies reported the exact collection time point of the tumor tissues and blood sample. Pooled sensitivity, specificity, and DOR were 62% (95% CI, 51% to 72%), 96% (95% CI, 93% to 98%), and 38.3 (95% CI, 21.1 to 69.4), respectively (see Table 4). The overall diagnostic performance, measured by summary ROC curve, was 0.91 (95% CI, 0.89 to 0.94).

Wu et al (2015) also conducted a meta-analysis comparing the diagnostic performance of ctDNA with that of a tissue reference in detecting *EGFR* variants in NSCLC patients. Twenty-six studies with 3256 patients were included in the review. Twenty studies were conducted in Asia, 17 included only advanced stage disease, and 19 were performed with plasma blood tests. Quality was assessed using the QUADAS-2 criteria and was described as a total methodologic quality score that ranged from 64% to 93% among selected studies. Pooled sensitivity, specificity, and DOR were 65% (95% CI, 54% to 74%), 97% (95% CI, 93% to 99%), and 69 (95% CI, 24 to 202), respectively (see Table 4). The overall area under the curve was 0.89 (95% CI, 0.86 to 0.91). In subgroup analysis, the sensitivity of ARMS method of detection (52%; 95%

CI, 35% to 69%) was significantly lower than that for non-ARMS methods (71%; 95% CI, 60% to 82%). The sensitivity was lower in studies including non-Asian ethnicities (55%; 95% CI, 33% to 77%) than Asian ethnicity (68%; 95% CI, 57% to 79%), although the difference was not statistically significant.

Table 4. Results of Meta-Analyses of Studies Evaluating Detection of *EGFR* Variants

Study and Patient Group	No. of Studies	Sensitivity (95% CI), %	Specificity (95% CI), %
Mao et al (2015)			
Pooled	24	61 (50 to 71)	90 (85 to 94)
Plasma sample	12	65 (51 to 77)	85 (76 to 91)
Serum sample	10	56 (35 to 75)	95 (89 to 98)
Tumor stage III-IV	10	80 (73 to 85)	91 (88 to 94)
Tumor stage I-IV	6	54 (44 to 63)	98 (95 to 99)
Qiu et al (2015)			
Pooled	27	62 (51 to 72)	96 (93 to 98)
Plasma sample	18	60 (93 to 98)	96 (93 to 98)
Serum sample	9	66 (43 to 81)	95 (86 to 99)
Tumor stage III-IV	14	52 (40 to 64)	96 (94 to 98)
Tumor stage I-IV	6	79 (42 to 95)	92 (75 to 98)
Wu et al (2015)			
Total	26	65 (54 to 74)	97 (93 to 99)
Plasma sample	19	62 (51 to 74)	96 (91 to 100)
Serum	7	73 (56 to 90)	100 (99 to 100)
Tumor stage III-IV	17	61 (48 to 74)	98 (96 to 100)
Tumor stage I-IV	9	72 (57 to 87)	95 (88 to 100)

CI: confidence interval; *EGFR*: epidermal growth factor receptor.

Individual Studies Published After 2015 Systematic Reviews

A subset of the individual studies published more recently and included in the Assessment Systematic Review is summarized in the following paragraphs. Sacher et al (2016) prospectively validated plasma droplet digital polymerase chain reaction (ddPCR) for the rapid detection of common *EGFR* and *KRAS* variants and the *EGFR* T790M-acquired resistance variant. The study included patients with advanced nonsquamous NSCLC who either had a new diagnosis for initial therapy or had developed acquired resistance to an EGFR kinase inhibitor and were scheduled for rebiopsy of the tumor. Patients underwent initial blood sampling with plasma ddPCR testing for *EGFR* exon 19 deletion as well as the *EGFR* L858R, *EGFR* T790M, and/or *KRAS* G12X variants between July 2014 and June 2015, at a National Cancer Institute–designated comprehensive cancer center. Tissue genotyping from a biopsy specimen was used as the reference standard for comparison with the plasma results. Rebiopsy was required for patients with acquired resistance to EGFR kinase inhibitors. There were 180 patients (62% female; median age, 62 years; range, 37-93 years), with 120 cases being newly diagnosed and 60 with acquired resistance. The sensitivity of plasma ddPCR was 82% (95% CI, 69% to 91%) for the *EGFR* 19 deletion, 74% (95% CI, 55% to 88%) for the *EGFR* L858R variant, 77% (95% CI, 60% to 90%) for the *EGFR* T790M variant, and lower at 64% (95% CI, 43% to 82%) for the *KRAS* variant. All specificity values (except T790M) were 100%, meaning that there were no false-positives for most tested variants. For the T790M variant, the specificity was 63% (95% CI, 38% to 84%).

Zhang et al (2017) evaluated the performance of ddPCR to detect *EGFR* variants in advanced NSCLC patients. Patients were from China, had stage III or IV NSCLC, and were treatment-naive at plasma collection. The overall prevalence of *EGFR* exon 19 deletion or L858R variants was 43%. The sensitivity for either variant was 61% (95% CI, not reported). Specificity was 97%. Sensitivities and specificities for these variants individually were similar to the pooled values. Patient characteristics significantly associated with the presence of plasma *EGFR* variants included nonsmoking (odds ratio, 3.24) and stage IV disease (odds ratio, 3.11). The sensitivity of ddPCR was calculated for separate groups of patients according to the quantity of cell-free DNA in the plasma samples, and the sensitivity was greater among those with greater quantities of cell-free DNA.

Mok et al (2015) evaluated the performance of real-time PCR to detect *EGFR* variants in patients with stage III or IV NSCLC enrolled in an RCT. Patients with and without *EGFR* variants were randomized to erlotinib plus chemotherapy or placebo plus chemotherapy. Plasma *EGFR* variants were assessed using the cobas EGFR Mutation Test (under development at the time of the trial). This test detected 41 *EGFR* variants, including the most common exon 19 deletion and L858R variants. The sensitivity of cobas for all *EGFR* variants was 75%, and specificity was 96% (95% CI, not reported).

In 2016, the U.S. Food and Drug Administration Summary of Safety and Effectiveness Data (FDA SSED) reported on the diagnostic characteristics of the cobas EGFR Mutation Test. The test is a real-time PCR test for the qualitative detection of defined variants of the *EGFR* gene. It detects 42 variants in exons 18, 19, 20, and 21, including the common exon 19 deletion and L858 variant used to select patients for erlotinib. Reported here are the diagnostic performance characteristics of the test. Selected patients were from the recruitment sample for the ENSURE study, an RCT conducted in Asia that compared erlotinib with cisplatin as first-line treatment for patients with stage IIIB or IV NSCLC. More than 94% of tumors were adenocarcinoma histology. Only patients with *EGFR* variants detected by tissue biopsy were enrolled. Of 647 patients screened for entry into the trial, 431 patients had paired liquid biopsy and tissue biopsy, the minimum 2 mL plasma volume required for liquid biopsy, and valid test results (10 patients were excluded due to invalid liquid biopsy results). For detection of the exon 19 deletion or the L858R variant, sensitivity was 77% (95% CI, 71% to 82%) and specificity was 98% (95% CI, 95% to 99%). For detection of exon 19 deletion alone, sensitivity was 81% (95% CI, 73% to 87%) and specificity was 99% (95% CI, 97% to 100%). For detection of L858R variant alone, sensitivity was 68% (95% CI, 58% to 77%) and specificity was 99% (95% CI, 97% to 100%).

Ohira et al (2016) evaluated the performance of ddPCR in detecting any of 15 variants, including *EGFR* variants, in patients with NSCLC at earlier stages of disease. A total of 150 matched tumor and serum samples from patients with stage I to IIIA disease were analyzed. Overall sensitivity and specificity for all variants were calculated in an unusual way in this study. The denominator for sensitivity was the number of variants identified in tissue biopsy rather than the number of patients with a positive variant. The sensitivity was very low because liquid biopsy only detected 5 of 155 variants detected on tissue biopsy. For *EGFR*, only 3 of 56 variants were detected. The authors suggested that their patient population (early-stage cancer) might have contributed to the low sensitivity of liquid biopsy.

Guo et al (2016) evaluated a panel of 50 biomarkers from 41 NSCLC patients pre- and postsurgery. Only results obtained before surgery are pertinent to this review. The method used to analyze plasma was minimally described as “a targeted sequencing approach.” Of the 41 patients, 65.9% had at least 1 of the 50 variants. The sensitivity of liquid biopsy was 69% (95% CI, 48% to 85%) and the specificity was 93% (95% CI, 66% to 100%). For the 30 patients with stage I or II disease, sensitivity was 75% (95% CI, 15% to 90%) and specificity was 90% (95% CI, 54% to 100%). For the 11 patients with stage III or IV disease, sensitivity was 50% (95% CI, 14% to 86%) and specificity was 100% (95% CI, 46% to 100%).

Sundaresan et al (2016) used the cobas EGFR Mutation Test to identify the presence of T790M variants in patients with clinical resistance to an *EGFR* TKI and undergoing a repeat biopsy. Forty-two patients enrolled who had advanced *EGFR* variant–positive NSCLC and clinical resistance to a TKI. The T790M variant was detected using the cobas EGFR Mutation Test v2. Twenty-five patients had paired biopsy and ctDNA specimens available. In these paired specimens, the prevalence of the T790M variant was 40% in the biopsy specimens. The study only reported concordance between the 2 results as 60% agreement (95% CI, 39% to 79%) (15/25 concordant results; κ=0.194). The data reported permits calculation of the sensitivity and specificity using tissue biopsy as the reference standard. We calculated the sensitivity of liquid biopsy to be 60% (95% CI, 26% to 88%) and specificity to be 60% (95% CI, 32% to 84%).

Takahama et al (2016) used ddPCR to identify T790M variants in patients suspected of having acquired resistance to TKIs. The study enrolled 260 patients with *EGFR* variant–positive NSCLC and acquired resistance to TKI therapy. Plasma was evaluated for both TKI-sensitizing variants and T790M variants using ddPCR. Forty-one patients had matching tissue or malignant blood specimens. The prevalence of TKI-sensitizing variants was 80% in tissue biopsies. The prevalence of the T790M variant was 76% in tissue biopsies. The sensitivity and specificity of liquid biopsy for TKI-sensitizing variants were 76% and 88% (95% CI, not reported), respectively. The sensitivity and specificity of liquid biopsy for T790M variant were 65% and 70%, respectively.

Thress et al (2015) compared several platforms used to evaluate liquid biopsy to detect *EGFR* variants. Matching tissue and liquid biopsies were obtained from patients enrolled in a phase 1 study assessing the experimental TKI agent AZD9291. All patients in these studies had initial TKI-sensitizing variants and evidence of disease progression while receiving TKI. We report results of the larger study sample, which was tested with the cobas EGFR Mutation Test and BEAMing ddPCR. For both liquid biopsy techniques, sensitivities and specificities were identical for detecting the exon 19 deletion (sensitivity, 82%; specificity, 97%; 95% CI, not reported) and L858R variant (sensitivity, 87%; specificity, 97%). For detection of the T790M variant, the cobas EGFR Mutation Test was 73% sensitive and 67% specific; BEAMing ddPCR was 81% sensitive and 58% specific.

Several additional studies with similar results have been published after the Assessment Systematic Review. Studies reporting on commercially available tests will be included in Table 5 in the following section and will be added to the QUADAS-2 ratings table in the appendix.

Commercially Available Tests

As previously described, there are multiple commercially available ctDNA tests that detect *EGFR* variants using a variety of detection methods. Given the breadth of molecular diagnostic methodologies available to assess ctDNA, the clinical validity of each commercially available test must be established independently. Published evidence of the clinical validity of the specific commercial tests is shown in Table 5. In summary, the cobas test has 4 studies of adequate quality to demonstrate the performance characteristics relative to a tissue test with tight precision estimates for specificity. Two other tests have promising preliminary results but none of the remaining available tests other than the cobas test have multiple studies of adequate quality to estimate the performance characteristics with sufficient precision.

Table 5. Published Evidence of Clinical Validity for Commercial Circulating Tumor DNA Tests, EGFR TKI–Sensitizing Variants

	Comparison With Tissue Test (95% CI)		
Test (Method)	**Studies Using Specific Commercial Test, %**	**No. of Available Studies**	**Study Quality**
Roche cobas EGFR Mutation Test v2	Sens, 75 (69 to 80)[a]	4	Most QUADAS criteria rated low risk of bias (Weber, Thress, Mok, FDA SSED)
	Spec, 97 (95 to 98)[a]		
Guardant360 (NGS)	Sens, 79 (58 to 93)	1	Most QUADAS criteria rated low risk of bias (Thompson)
	Spec, 100 (87 to 100)		
FoundationACT (NGS)	NA	0	NA
Biocept (real-time PCR)	NA	0	NA
Genomic Health the Oncotype SEQ Liquid Select (NGS)	NA	0	NA
Circulogene's (Theranostics) liquid biopsy test (NGS)	NA	0	NA
CancerIntercept (NGS)	NA	0	NA
Biodesix's GeneStrat (ddPCR)	Sens, 95.9 (NR)	1	Some QUADAS criteria rated high or unclear risk of bias (Mellert)
	Spec, 100 (NR)		

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; PCR: polymerase chain reaction; QUADAS: Quality Assessment of Diagnostic Accuracy Studies; Sens: sensitivity; Spec: specificity; SSED: Summary of Safety and Effectiveness Data; TKI: tyrosine kinase inhibitor.
[a] Pooled meta-analytically

Section Summary: Clinical Validity

The FDA SSED and our assessment systematic review have demonstrated that the cobas test has very high accuracy (AUROC=0.96), sensitivity of about 75%, and specificity above 95% for detection of *EGFR* TKI-sensitizing variants using tissue biopsy as the reference standard. The studies were performed in Asia, Europe, Australia, and the United States, primarily in patients with advanced disease of adenocarcinoma histology.

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

Fewer studies have examined the performance of liquid biopsy for 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 initiation of treatment, when biopsies may be more difficult to obtain. In our assessment systematic review, the 7 studies showed fair-to-good accuracy of liquid biopsy (AUROC=0.78), with moderate sensitivity (range, 60%-90%) and moderate specificity (range, 60%-90%). Unlike the high specificities demonstrated for other *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 tumor in the patient.

Clinical Utility
Depending on the analytic method, compared with 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 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, we 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 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
FDA SSED, phase 3 ENSURE RCT in tissue *EGFR*-positive[a]	China, Malaysia, Philippines	IIIB, IV	cobas					
					PFS HR (95% CI) for Chemotherapy vs Erlotinib			
				Overall (i.e., tissue positive)				
				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), EURTAC trial in tissue *EGFR*-positive[a]	France, Italy, Spain	IIIB, IV	Multiplex 5´ nuclease rt-PCR (TaqMan)					
					OS (95% CI) for Erlotinib vs Chemotherapy, mo			
				n	**Erlotinib**	**Chemotherapy**		**p**
				Overall (i.e., tissue positive)				
				97	25.8 (17.7 to 31.9)	18.1 (15.0 to 23.5)		0.14
				All patients with exon 19 deletion in tissue				
				56	30.4 (19.8 to 55.7)	18.9 (10.4 to 36.2)		0.22
				Patients with exon 19 deletion in both tissue and ctDNA				
				47	34.4 (22.9 to NR)	19.9 (9.8 to 36.2)		0.23
				Patients with exon 19 deletion in tissue but not ctDNA				
				9	13.0 (8.9 to 19.8)	15.5 (0.3 to NR)		0.87
				All patients with L858R variant in tissue				
				41	17.7 (6.3 to 26.8)	17.5 (8.2 to 23.5)		0.67
				Patients with L858R variant in both tissue and in ctDNA				
				29	13.7 (2.6 to 21.9)	12.6 (7.1 to 23.5)		0.67
				Patients with L858R variant in tissue but not in ctDNA				
				12	29.4 (8.6 to	25.6 (16.1 to NR)		0.64

Study/Patient Group	Country	Disease Stage	Technology Used to Detect ctDNA	Sample Sizes	Treatment Response		
					63.0)		
Zhang et al (2017), *EGFR*-positive and -negative patients treated with *EGFR* TKIs	China	IIIB, IV	ddPCR				
					PFS (95% CI), d		
				n			
				Tissue positive vs tissue negative			
				215	342 (291 to 393)	60 (0 to 124)	0.001
				Tissue positive and liquid positive vs liquid negative			
				80	334 (298 to 371)	420 (100 to 740)	0.15
				Tissue negative and liquid positive			
				3	133, 410, and 1153 days		

CI: confidence interval; ctDNA: circulating tumor DNA; ddPCR: droplet digital polymerase chain reaction; *EGFR*: epidermal growth factor receptor; FDA: Food and Drug Administration; HR: hazard ratio; 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.

In Table 6 (sensitizing variants), 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. However, only patients with *EGFR* variants detected from tissue biopsies were enrolled. In the overall study, erlotinib showed substantial improvement in PFS over chemotherapy (hazard ratio [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 the 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. 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. Although the numbers are small, the PFS values are consistent with response to TKIs and might represent tissue biopsies that did not reflect correct *EGFR* status.

Table 7. *EGFR* TKI-Resistance Variants: Osimertinib Treatment Response Stratified by Liquid and Tissue Biopsy

Study/Patient Group	Country	Disease Stage	Technology Used to Detect ctDNA	Treatment Response	
				n	Outcomes
Thress et al (2015), phase 1 AURA RCT in tissue *EGFR*-positive[a] with progression on EGFR-TKI	U.S., Australia, France, Germany, Italy, Japan, Korea, Spain, Taiwan, U.K.	Advanced	cobas; BEAMing ddPCR		
				Objective Response Rate	
				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%
Oxnard et al (2016), AURA phase 1 trial of patients who progressed on EGFR-TKI	U.S, Australia, France, Germany, Italy, Japan, Korea, Spain, Taiwan, U.K.	Advanced	BEAMing		
				Objective Response Rate (95% CI)	
				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%)
				Progression-Free Survival (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)

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; RCT: randomized controlled trial; TKI: tyrosine kinase inhibitor.
[a] Exon 19 deletion or L858R variant.

For *EGFR*-resistance variants, Thress et al (2015) examined response to the experimental therapeutic AZD9291 (osimertinib) by T790M status, determined using a tissue or liquid biopsy (see Table 7). 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. Some patients may have overlapped with Thress (2015). 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 T790-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.

Chain of Evidence
A chain of evidence, based on the sensitivity and specificity of liquid biopsy for the detection of *EGFR* TKI-sensitizing variants such as exon deletion 19 and L858R variants, for a test that has established clinical validity (e.g., the cobas test), can support its utility for the purpose of selecting treatment with *EGFR* TKIs (ie, erlotinib, gefitinib, afatinib). A robust body of evidence has demonstrated moderate sensitivity (range, 60%-80%) with high specificities (>95%). If 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 US populations with an assumed prevalence of *EGFR* TKI-sensitizing variants of 15% and a 75% sensitive and 97% specific liquid biopsy test (e.g., cobas), 86% of the patients would then require tissue testing to detect the remaining patients with variants; 3% would receive targeted therapy after liquid biopsy who would have received a different systemic therapy if tested with tissue biopsy; and 11% would appropriately receive targeted therapy following liquid biopsy without having to undergo tissue biopsy. In other populations such as Asians where the prevalence of *EGFR* TKI-sensitizing variants is 30% to 50%, the strategy would be more efficient, and a lower proportion of patients would be subject to repeat testing. There is extremely limited evidence on whether the “false-positives” (i.e., patients with positive liquid biopsy and negative tissue biopsy) might have been incorrectly identified as negative on tissue biopsy. In one study, three patients with negative tissue biopsies and positive liquid biopsies appeared to respond to *EGFR* TKI inhibitors.

The diagnostic characteristics of liquid biopsy for 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 many 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, 18 patients with negative tissue biopsies and positive liquid biopsies appeared not to have high response to osimertinib.

Section Summary: Clinical Utility

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 one study, three 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 a test with established clinical validity such as the cobas EGFR Mutation Test v2 can support its utility. A substantial body of evidence has demonstrated sensitivity of about 75%, with high specificities (>95%). If cobas 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 of patients with *EGFR* TKI-sensitizing variants.

For the other marketed tests that include detection of TKI-sensitizing variants, we lack sufficient evidence of clinical validity, 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 also little evidence on the comparative validity of tissue and liquid biopsies in discordant cases. Based on the apparent response to osimertinib from the AURA study with liquid negative, tissue-positive results, 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, 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.

SUMMARY OF EVIDENCE

For individuals with newly diagnosed NSCLC who are able to undergo tissue biopsy who receive testing for biomarkers of *EGFR* TKI sensitivity using ctDNA with the cobas EGFR Mutation Test v2 (liquid biopsy), the evidence includes numerous studies assessing the diagnostic characteristics of liquid biopsy compared with tissue. Relevant outcomes are OS, disease-specific survival, and test accuracy and validity. The cobas EGFR Mutation Test has adequate evidence of clinical validity for the *EGFR* TKI-sensitizing variants. The

FDA 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. Current evidence does not permit determining whether cobas or tissue biopsy is more strongly associated with patient outcomes or treatment response. We identified no RCTs providing evidence of the clinical utility of cobas. 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. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals with newly diagnosed NSCLC who are able to undergo tissue biopsy who receive testing for biomarkers of EGFR TKI sensitivity using ctDNA with tests other than the cobas EGFR Mutation Test v2, the evidence includes studies assessing the diagnostic characteristics of liquid biopsy compared with tissue reference standard. Relevant outcomes are OS, disease-specific survival, and test accuracy and 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 test have multiple studies of adequate quality to estimate the performance characteristics with sufficient precision. Current evidence does not permit determining whether liquid biopsy or tissue biopsy is more strongly associated with patient outcomes or treatment response. We found no RCTs providing evidence of the clinical utility of those of methods of liquid biopsy. The evidence is insufficient to determine the effects of the technology on health outcomes.

For individuals with newly diagnosed NSCLC who are not able to undergo tissue biopsy who receive testing for biomarkers of *EGFR* TKI sensitivity using ctDNA with the cobas EGFR Mutation Test v2 (liquid biopsy), the evidence includes numerous studies assessing the diagnostic characteristics of liquid biopsy compared with tissue. Relevant outcomes are OS, disease-specific survival, and test accuracy and validity. The cobas EGFR Mutation Test has adequate evidence of clinical validity for the *EGFR* TKI-sensitizing variants. Patients who cannot undergo tissue biopsy would likely otherwise receive chemotherapy. The cobas test can identify patients for whom there is a net benefit of targeted therapy versus chemotherapy with high specificity. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals with newly diagnosed NSCLC who are not able to undergo tissue biopsy who receive testing for biomarkers of EGFR TKI sensitivity using ctDNA with tests other than the cobas EGFR Mutation Test v2, the evidence includes studies assessing the diagnostic characteristics of liquid biopsy compared with tissue reference standard. Relevant outcomes are OS, disease-specific survival, and test accuracy and 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 test have multiple studies of adequate quality to estimate the performance characteristics with sufficient precision. The evidence is insufficient to determine the effects of the technology on health outcomes.

For individuals with NSCLC who progressed on EGFR TKI who receive testing for biomarkers of *EGFR* TKI resistance using ctDNA, the evidence includes a few studies assessing the diagnostic characteristics of liquid biopsy. Relevant outcomes are OS, disease-specific survival, and test accuracy and 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. The evidence is insufficient to determine the effects of the technology on health outcomes.

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Coding

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Circulating Tumor DNA Management of Non-Small-Cell Lung Cancer (Liquid Biopsy)

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Procedural Terminology which contains the complete and most current listing of CPT codes and descriptive terms. Applicable FARS/DFARS apply.

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Code Type	Code
CPT	81235, 81445, 81479, 86152, 86153
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*****Investigational – A medical treatment, procedure, drug, device, or biological product is Investigational if the effectiveness has not been clearly tested and it has not been incorporated into standard medical practice. Any determination we make that a medical treatment, procedure, drug, device, or biological product is Investigational will be based on a consideration of the following:

A. Whether the medical treatment, procedure, drug, device, or biological product can be lawfully marketed without approval of the U.S. Food and Drug Administration (FDA) and whether such approval has been granted at the time the medical treatment, procedure, drug, device, or biological product is sought to be furnished; or
B. Whether the medical treatment, procedure, drug, device, or biological product requires further studies or clinical trials to determine its maximum tolerated dose, toxicity, safety, effectiveness, or effectiveness as compared with the standard means of treatment or diagnosis, must improve health outcomes, according to the consensus of opinion among experts as shown by reliable evidence, including:
    1. Consultation with the Blue Cross and Blue Shield Association technology assessment program (TEC) or other nonaffiliated technology evaluation center(s);
    2. Credible scientific evidence published in peer-reviewed medical literature generally recognized by the relevant medical community; or
    3. Reference to federal regulations.

**Medically Necessary (or "Medical Necessity") - Health care services, treatment, procedures, equipment, drugs, devices, items or supplies that a Provider, exercising prudent clinical judgment, would provide to a patient for the purpose of preventing, evaluating, diagnosing or treating an illness, injury, disease or its symptoms, and that are:

A. In accordance with nationally accepted standards of medical practice;
B. Clinically appropriate, in terms of type, frequency, extent, level of care, site and duration, and considered effective for the patient's illness, injury or disease; and
C. Not primarily for the personal comfort or convenience of the patient, physician or other health care provider, and not more costly than an alternative service or sequence of services at least as likely to produce equivalent therapeutic or diagnostic results as to the diagnosis or treatment of that patient's illness, injury or disease.

For these purposes, "nationally accepted standards of medical practice" means standards that are based on credible scientific evidence published in peer-reviewed medical literature generally recognized by the relevant medical community, Physician Specialty Society recommendations and the views of Physicians practicing in relevant clinical areas and any other relevant factors.

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NOTICE: Medical Policies are scientific based opinions, provided solely for coverage and informational purposes. Medical Policies should not be construed to suggest that the Company recommends, advocates, requires, encourages, or discourages any particular treatment, procedure, or service, or any particular course of treatment, procedure, or service.