Medical Policy

Blue Cross Blue Shield Blue Care Network of Michigan

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***Current Policy Effective Date: 9/1/24**
(See policy history boxes for previous effective dates)

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

Description/Background

Genetic testing of circulating tumor DNA (ctDNA) 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 (NSCLC), detection of "driver mutations" or resistance variants is important for selecting patients for targeted therapy. The tests discussed herein are intended for use in patients with advanced (stage III or IV) non-small-cell lung cancer.

PREDICTIVE BIOMARKERS IN NON-SMALL-CELL LUNG CANCER

Several predictive genetic biomarkers have been identified for non-small-cell lung cancer (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 Food and Drug Administration (FDA) for NSCLC. Guidelines generally suggest analysis of either the primary NSCLC tumor or of a metastasis for the presence of a set of driver mutations to select 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.

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 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*ex14skipping 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 97subjects (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 *HER2* (trastuzumab, afatinib), *MET* (crizotinib), and *RET* (cabozantinib, vandetanib). Human epidermal growth factor receptor 2 (*HER2*) is a member of the HER (EGFR) family of TK receptors and has no specific ligand. When activated, it forms dimers with other EGFR family members. *HER2* is expressed in approximately 25% of NSCLC. *RET* (rearranged during transfection) is a proto-oncogene that encodes a receptor tyrosine kinase growth factor.

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 a 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.

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 circulating tumor DNA (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 an 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 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 (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.

Regulatory Status

In June 2016, cobas® EGFR Mutation Test v2 (Roche Molecular Systems), a real-time PCR test, was approved by 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).(78) 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).(79) 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, 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.

Foundation Medicine’s FoundationACT™ uses hybrid capture based NGS to detect variants in over 60 genes for targeted therapy in metastatic cancer.

Guardant Health markets the Guardant360 CDx ® test. This test uses NGS to identify variants in 73 genes associated with several different cancers.

Circulogene Theranostics’ 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.

Biocept 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.

Biodesix’s GeneStrat® uses droplet digital PCR to analyze cell-free DNA and RNA to identify specific driver variants for which targeted therapy is available for NSCLC.

Resolution Bio offers ctDx-Lung™ uses NGS to detect single nucleotide variants, insertions and deletions, fusions, and copy number variants in approximately 20 genes targeted by a specific FDA-approved therapy or therapies in clinical trials.

Sysmex OncoBEAM™offers liquid biopsies using BEAMing technology to detect variants in *EGFR*, *KRAS*, and *BRAF* for NSCLC as well as liquid biopsies for breast, melanoma, and colorectal cancer.

Medical Policy Statement

The effectiveness and clinical utility of circulating tumor DNA of individual genes, listed multiple gene panels when more than 5 genes are tested, and FDA approved companion diagnostic tests for the management of non-small-cell lung cancer (liquid biopsy) have been established. They may be considered a useful therapeutic option when indicated.

Inclusionary and Exclusionary Guidelines

Inclusions:
Analyzing cell-free/circulating tumor DNA (ctDNA) alterations in the *ALK, EGFR, BRAF V600E, KRAS*, *ROS1, NTRK, MET exon14 skipping, PD-L1, ERBB2 (HER-2), and RET* gene using **ONE** of the following methods:

1. Individual genes
2. Targeted multi-gene panels
3. FDA approved companion diagnostic tests (e.g. Cobas® EGFR [Epidermal Growth Factor Receptor) Mutation Test v.2, FoundationOne® Liquid CDx, Guardant360® CDx)

when **ALL** of the following apply:

- Advanced stage III or IV non-small-cell lung cancer
- Clinical circumstances reflect **ONE** of the following:
    1. Patient is medically unfit for invasive tissue sampling, **OR**
    2. Following pathologic (biopsy) confirmation of a NSCLC diagnosis there is insufficient material for molecular analysis and follow-up tissue-based analysis is planned for all patients in which an oncogenic driver is not identified
- Used to detect ctDNA for targeted therapy benefit **or** to identify patients who will not benefit from further molecular testing

Exclusions:

- Use of circulating tumor DNA (ctDNA) for any indications not mentioned above.
- Cell-free testing when the patient already meets criteria for treatment based on known biomarker status. (e.g., patient has already had testing or testing is not required).

Policy Guidelines

The tests discussed herein are intended for use in patients with advanced (stage III or IV) non-small-cell lung cancer. 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, or osimertinib. The 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 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).

CPT/HCPCS Level II Codes *(Note: The inclusion of a code in this list is not a guarantee of coverage. Please refer to the medical policy statement to determine the status of a given procedure.)*

Established codes:

81191	81192	81193	81194	81210	81235
81275	81276	81401	81404	81405	81406
81445	81455	81479	88346	88350	0239U
0242U	0326U				

Other codes (investigational, not medically necessary, etc.):

0179U

PLA codes are considered investigational/experimental until the laboratory test the code represents is formally documented as Established in an Interim Medical Policy or Joint Uniform Medical Policy document.

Covered CPT codes may be used to represent and reimburse testing for incremental codes or multi-target codes.

Note: Individual policy criteria determine the coverage status of the CPT/HCPCS code(s) on this policy. Codes listed in this policy may have different coverage positions (such as established or experimental/investigational) in other medical policies.

Rationale

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.

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

Selecting Targeted Therapy

Clinical Context and Test Purpose

The purpose of identifying targetable oncogenic "driver mutations" such as 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 questions addressed in this evidence review are:

1. How accurately does liquid biopsy detect driver or 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 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 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 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. (24)

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.

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
For patients who can undergo tissue biopsy, the beneficial outcomes of a true-positive liquid biopsy result are avoidance of tissue biopsy and its associated complications. In the National Lung Screening Trial, which enrolled 53,454 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%.(3)

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

Potential Harmful Outcomes
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 for chemotherapy over EGFR TKIs for patients without *EGFR*-sensitizing variants was true in both the first- and second-line setting.(4)

In the 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).(5) 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 IM power 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).(6)

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

Study Selection Criteria
For the evaluation of 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.

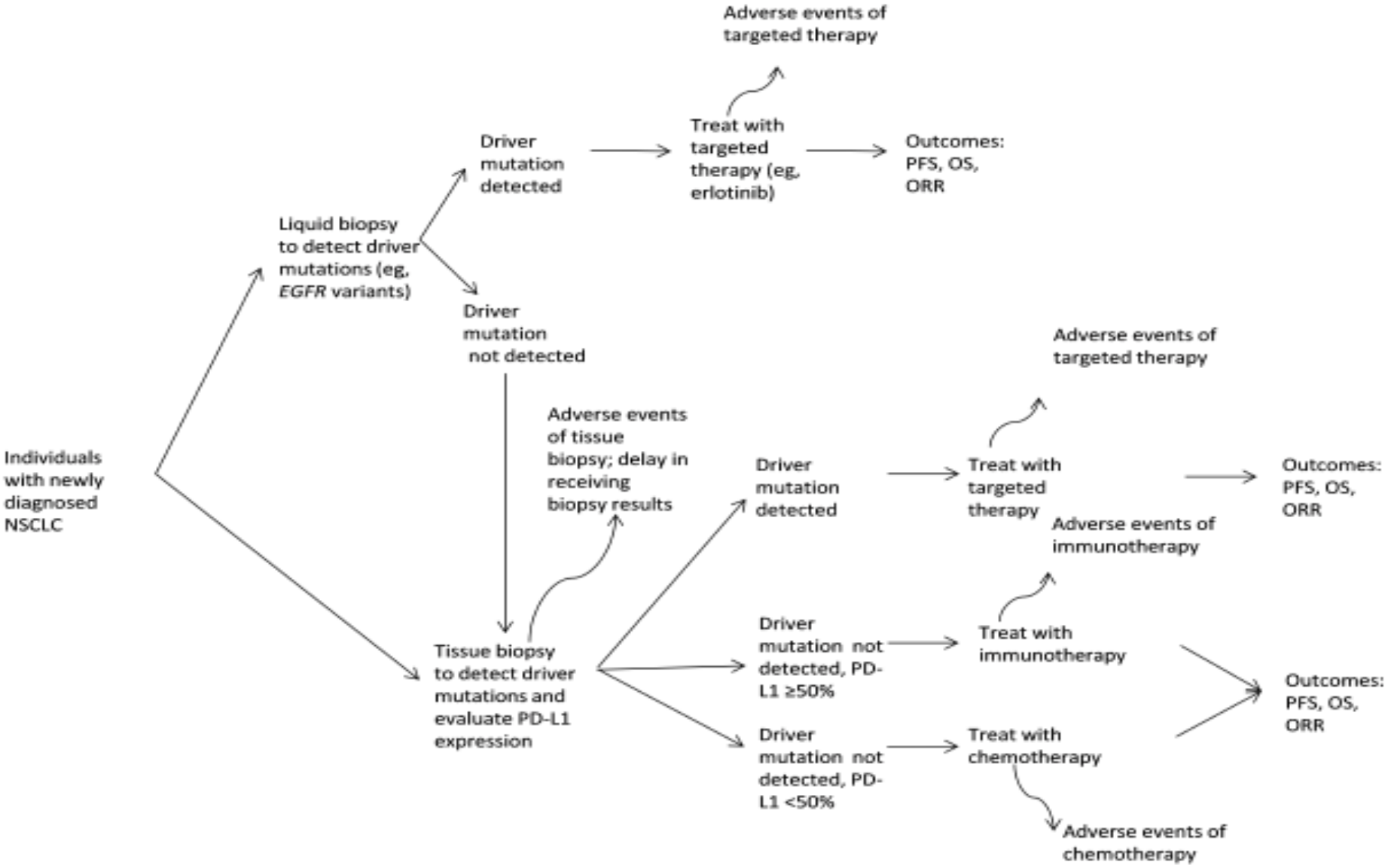
Technically Reliable
Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist. 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 United States. 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 United States. 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.(7) Therefore, studies in the United States or similar populations will be most informative regarding the clinical validity of tests marketed in the United States.

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, the clinical validity of each commercially

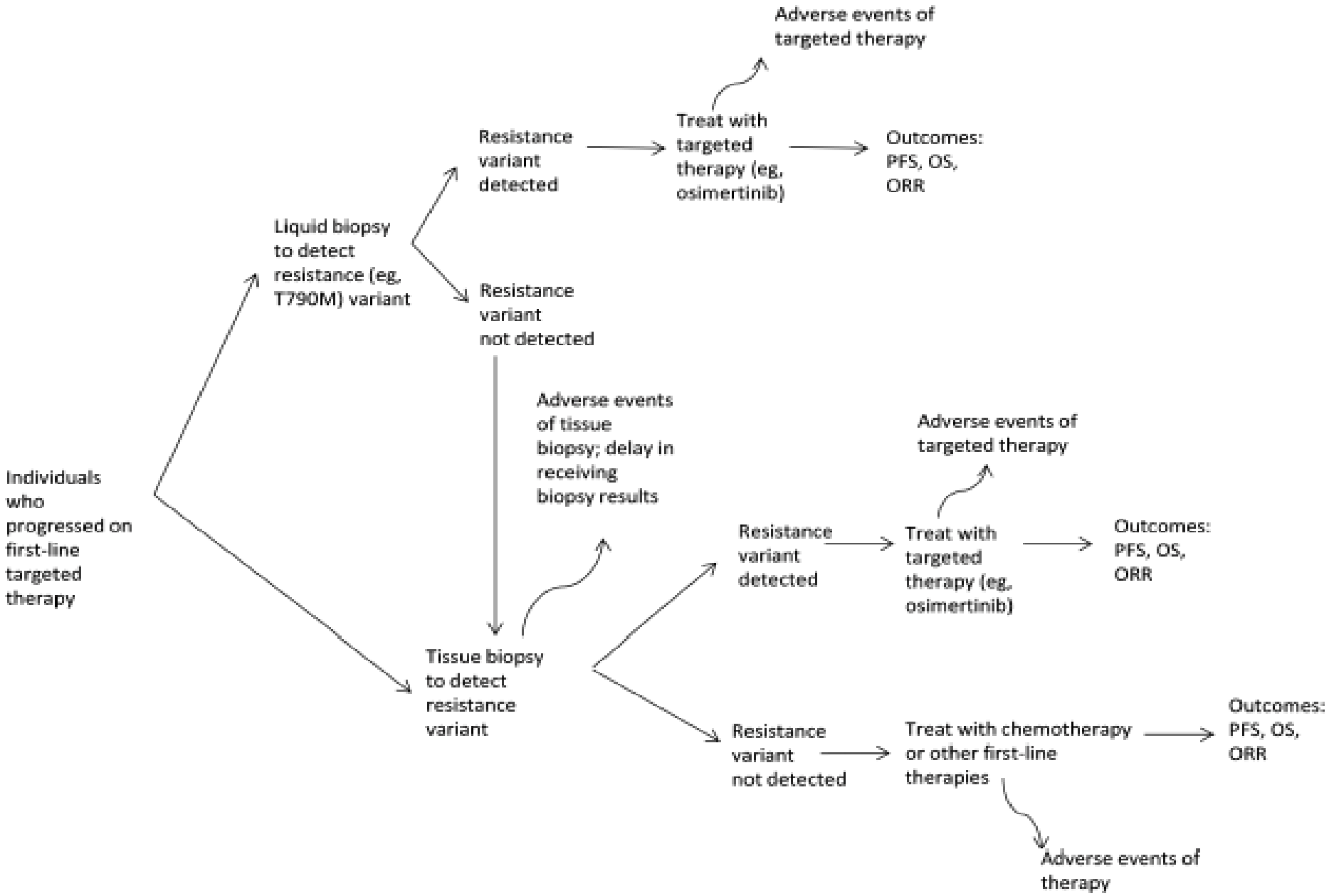
available test must be established independently. Table 1 summarizes some commercially available liquid biopsy tests, and this list may not be comprehensive.

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.

Table 1. Examples of Commercial Liquid Biopsy Tests

Test	Regulatory Status	Technology	Classes of Variants Detected
cobas EGFR Mutation Test v2	FDA-approved PMA (P150047)	Real-time PCR	SNVs Insertions and deletions
Guardant360 CDx	LDT	NGS	SNVs Insertions and deletions Fusions CNVs
FoundationOne Liquid [c]	LDT	NGS	SNVs Insertions and deletions (1-40 bp) Rearrangements and fusions CNVs >20% <20%
Biocept	LDT	Real-time PCR	SNVs
Circulogene's (Ther anostics) liquid biopsy test	LDT	NGS	SNVs Insertions and deletions Fusions CNVs
Biodesix's GeneStrat	LDT	ddPCR	SNVs Fusions
Resolution Bio ctDx-Lung	LDT	NGS	SNVs Insertions and deletions CNVs Fusions
Sysmex OncoBEAM	LDT	BEAMing	SNVs Insertions and deletions

BEAM: beads, emulsions, amplification, and magnetics; 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.

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 2. Most have included testing for *EGFR* variants but a few included testing for less prevalent variants as well.

Table 2. 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)[80]	Patients harboring T790M mutation with locally advanced or metastatic NSCLC who had progressed on EGFR TKI therapy enrolled in AURA3 studies in U.S., Mexico, Canada,, Europe, Asia, and Australia	Retrospective	*EGFR*	Both tissue and blood samples collected at screening
Cobas EGFR test				
Jenkins et al (2017)[8,]	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)[10]	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)[11]	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)[12]	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)[13]	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)[81]	Patients with advanced and metastatic NSCLC with EGFR exon 19 deletions or exon 21 L858R mutations confirmed by the cobas EGFR Mutation Test enrolled in the FLAURA phase 3 study assessing the efficacy of osimertinib vs standard EGFR TKI therapy; patients enrolled in the NILE study were used to estimate the prevalence of CDx-positive, tissue-negative patients as no plasma from FLAURA tissue-negative patients was available	Retrospective	*EGFR*	Unclear
Leighl et al (2019)[83]	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)[14]	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)[15]	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)[18]	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)[17]	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)[10]	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)[11]	Patients with NSCLC enrolled in a multinational (including U.S.) phase 1 study who had progressed on an EGFR TKI therapy	Prospective	*EGFR*	Blood and tissue collected after progression and before next-line treatment; time between not specified
Biodesix ddPCR				

Mellert et al (2017)[18]	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)[19]	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 naïve 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)[82]	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 3 summarizes the results of clinical validity 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 detection of *EGFR*-resistance variants, fewer studies are available and estimates of specificity are more variable. For detection of less prevalent driver mutations, such as *ALK* and *ROS1* translocations and *BRAF*V600E, RET fusions, and MET exon 14 skipping, few publications are available and, in these publications, only a very few variants have been identified.

Table 3. 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)[80]	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)[8]					
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)[9]					
EGFR-sensitizing variants	601	431	Insufficient plasma; invalid test result	77 (71 to 82)	98 (95 to 99)
Karlovich et al (2016)[10,]					
EGFR-sensitizing variants	174	110	No matching tumor and plasma or inadequate tissue	73 (62 to 83)	100 (86 to 100)
EGFR exon 20 (T790M, resistance)	174	110		64 (45 to 80)	98 (91 to 100)
Thress et al (2015)[11,]					
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)[12,]					
EGFR-sensitizing variants	397	238	Insufficient plasma or tissue; invalid test result	75 (65 to 83)	96 (92 to 99)
Weber et al (2014)[13,]					

EGFR-sensitizing and -resistance variants	199[a]	196	Inadequate tumor tissue	61 (41 to 78)	96 (92 to 99)
Guardant360 CDx					
FDA SSED (2020)[82]					
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)[80]	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]
Leighl et al (2019)[83]	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,		223		90 (56 to 100)[c]	100 (98 to 100)[c]

sensitizing)					
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)[14]					
EGFR variants (various)	88	34	No tissue	54 (25 to 81)	90 (70 to 99)
Study	Initial N	Final N	Excluded Samples	Sensitivity (95% CI)	Specificity (95% CI)
Thompson et al (2016)[15],	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)[18]	68	31	No tissue		
EGFR-sensitizing				63 (24 to 91)[c]	96 (78 to 100)[c]
ROS1				None identified	None identified
BRAF V600E				None identified	None identified
OncoBEAM					
Ramalingam et al (2018)[17]	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)[10]					
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)[11]					
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)[80]	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)
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)[19]	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)[84]	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)
Study	Initial N	Final N	Excluded Samples	Sensitivity (95% CI)	Specificity (95% CI)
MET exon 14 skipping		48		33 (2 to 87)	100 (90 to 100)
FoundationOne Liquid CDx					
FDA SSED (2020)[87]	280		Samples in which there was insufficient plasma to process both replicates of the cobas reference test		

EGFR exon 19 deletion (sensitizing)[e]		135		95 (83 to 99)[c] (rep 1) 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: Food and Drug Administration; NR: not reported; SSED: Summary of Safety and Effectiveness Data.
[a] Unclear how many samples were eligible but not included
[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 gap tables (see Tables 4 and 5) are to display notable gaps 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 4. Relevance Gaps 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)[80]					
Cobas EGFR test					
Jenkins et al (2017)[8]					
FDA SSED (2016)[9]	4. Performed in Asia				
Karlovich et al (2016)[15]					
Thress et al (2015)[11]					
Mok et al (2015)[12]	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)[83]					
Schwaederle et al (2017)[14]					
Thompson et al (2016)[15]					
Villaflor et al (2016)[16]					
OncoBEAM					
Ramalingam et al (2018)[17]	4. Performed in Asia				
Karlovich et al (2016)[10]					

Thress et al (2015)[11]					
Biodesix ddPCR					
Mellert et al (2017)[18]	3. Patient characteristics unclear				
ctDx-Lung					
Paweletz et al (2016)[19]	2. Unclear if same as current marketed version				
Invision					
Pritchet et al (2019)[84]	4: Calculation of performance characteristics only included subset of patients with at least 1 mutation detected by liquid biopsy				
Remon et al (2019)[85]					
FoundationOne Liquid CDx					
FDA SSED (2020)[87]	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 evidence gaps stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
FDA: 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] Outcome 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 5. Study Design and Conduct Gaps 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)[80]						
Cobas EGFR test						
Jenkins et al (2017)[8]						
FDA SSED (2016)[9]						
Karlovich et al (2016)[10]						
Thress et al (2015)[11]			1. Both samples collected after progression and before next treatment but time between blood			1. Precision estimates not reported but calculated based on

			and tissue sample collection not described			data provided
Mok et al (2015)[12]			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)[13]	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)[81]			2. Time between tissue and plasma sample unclear; subset of samples collected after progression or treatment discontinuation			
Leighl et al (2019)[83]			2.Time between tissue and plasma sample unclear			1. Precision estimates not reported but calculated based on data provided
Schwaederle et al (2017)[14]						1. Precision estimates not reported but calculated based on data provided
Thompson et al (2016)[15]			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)[16]	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)[17]			1. Time between blood and tissue sample collection not described			

Karlovich et al (2016)[10]						
Thress et al (2015)[11]			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)[18]	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)[19]	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)[84]						1. Precision estimates not reported but calculated based on data provided
Remon et al (2019)[85]						
FoundationOne Liquid CDx						
Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
FDA SSED (2020)[82]	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 evidence gaps stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
FDA: 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 to 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 6. The cobas test has 6 studies, Guardant360 CDx has 5 studies and OncoBEAM have 3 studies and InVision has 2 studies, 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, and OncoBEAM tests have multiple studies of adequate quality to estimate the performance characteristics for *EGFR* TKI-sensitizing variants with sufficient precision.

Table 6. 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) Range, %		Available Studies	
	Sens	Spec		
Roche cobas EGFR Mutation Test v2	60-87	96-100	7	Very few gaps identified (Jenkins[8]; FDA SSED[9]; Karlovich[10]; Thress[11]; Mok[12]; Weber[13])
Guardant360 CDx (NGS)	63-100	90-100	5	Long time between tissue and ctDNA tests (Thompson[15]; Villaflor[16]); unclear patient selection (Villaflor[16]); variants not stratified by type in Schwaederle[14]. very few limitations with Papadimitrakopoulou[80],); outcomes from test versions combined (FDA SSED)[87],
FoundationOne Liquid [c] (NGS)	95-100	93-6	1	Non-inferiority trial with many limitations; no tissue-based comparator; non-inferiority margins not described (FDA SSED)[82],
OncoBEAM	63-82	67-100	3	Few gaps identified (Karlovich[10]; Thress[11]; Rmalingam[17]) Only a few negatives in Karlovich for estimating specificity.

Biodesix (ddPCR)	70-100	100 (NR)[18]	1	Patient characteristics and selection unclear; timing of blood and tissue samples unclear; precision estimates not provided (Mellert[18]); very few limitations with Papadimitrakopoulou[80])
Resolution Bio ctDx-Lung	89 (65 to 99)[b]	100 (88 to 100)[c]	1	Several gaps identified (Paweletz[19])
FoundationACT (NGS)	NA	NA	0	NA
Biocept (real-time 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[84], Remon[85])

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; 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 [AUROC], 0.96), a sensitivity of about 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 United States, 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 United States in the intended-use population for *EGFR* TKI-sensitizing variants. Estimates of specificity are consistently 90% or higher. Likewise, the OncoBEAM test has 3 studies using tissue biopsy in Asia, Europe, Australia, and the United States in the intended-use population, 2 of which provide precise estimates for specificity that are very high (>95%).

For tests other than the cobas test, Guardant360 CDx, and OncoBEAM 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* and *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 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.(20)

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 on 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.

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.

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, 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 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 7 and 8, respectively.

Table 7. *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)[86]; newly diagnosed *EGFR*-positive and -negative patients treated with EGFR TKIs	China	IV (85.6%)	ddPCR	PFS (95% CI), mo		
				n	EGFR TKI	p
				Tissue positive and liquid positive		
				26	15 (NR)	
				Tissue positive and liquid negative		
				12	11.5 (NR)	
				Tissue negative and liquid positive		
				5	NR	
				Tissue unknown and liquid positive		
				30	13 (NR)	
				Tissue negative and liquid negative		
				49	5.4 (NR)	
FDA SSED (2020)[87]; phase 3 FLAURA RCT in treatment-naive and *EGFR*-	Multinational[b]	IIIB, IV	Guardant360 CDx	PFS HR (95% CI) for Osimertinib vs Gefitinib or Erlotinib		

positive[a] patients							
				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)[21]; *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)[9]; 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
				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)[22]; 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			

Study/Patient Group	Country	Disease Stage	Technology Used to Detect ctDNA	Sample Sizes	Treatment Response		
				29	13.7 (2.6 to 21.9)	12.6 (7.1 to 23.5)	0.67
				Patients with L858R variant in tissue but not in ctDNA			
				12	29.4 (8.6 to 63.0)	25.6 (16.1 to NR)	0.64

CI: confidence interval; ctDNA: circulating tumor DNA; ddPCR: droplet digital polymerase chain reaction; *EGFR*: epidermal growth factor receptor; FDA: 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.

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

In Table 7 (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.(87) 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 andGuardant360 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.(87) 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.(9) 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 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.(21) 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 correct *EGFR* status.

Table 8. *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)[80]; AURA3 phase 3 trial of patients who progressed on EGFR TKI	Multinational[c]	Locally advanced or metastatic	cobas (RT-PCR); Guardant360 (NGS); Biodesix (ddPCR)	ORR (95% CI) (Osimertinib vs Chemotherapy)		
			Subgroup	n	Osimertinib	Chemotherapy
			T790M+, tissue	279, 140	71 (65 to 76)	31 (24 to 40)
			T790M+ liquid (cobas)	111, 48	76 (67 to 83)	45 (31 to 60)
			T790M+, liquid (Guardant360)	137, 53	68 (59 to 76)	40 (27 to 54)
			T790M-, liquid (cobas)	101, 47	71 (61 to 79)	28 (16 to 42)
			T790M-, liquid (Guardant360)	72, 29	78 (66 to 87)	17 (6 to 36)
				PFS HR (95% CI) (Osimertinib vs Chemotherapy)		
			T790M+, tissue	419	0.30 (0.23 to 0.41)	
			T790M+, liquid (cobas)	159	0.42 (0.29 to 0.63)	
			T790M+, liquid (Guardant360)	190	0.40 (0.28 to 058)	
			T790M-, liquid (cobas)	148	0.31 (0.20 to 0.48)	
			T790M-, liquid (Guardant360)	101	0.27 (0.15 to 0.49)	
				n	Outcomes	
Oxnard et al (2016)[23]; AURA phase 1 trial of	Multinational[b]	Advanced	BEAMing	ORR (95% CI) (Osimertinib)		

patients who progressed on EGFR TKI				
				Liquid positive, tissue positive
				108 64% (54% to 73%)
				Liquid positive, tissue negative
				18 28% (10% to 53%)
				Liquid negative, tissue positive
				45 69% (53% to 82%)
				Liquid negative, tissue negative
				40 25% (13% to 41%)
				PFS (95% CI), mo
				Liquid positive, tissue positive
				111 9.3 (8.3 to 10.9)
				Liquid positive, tissue negative
				18 4.2 (1.3 to 5.6)
				Liquid negative, tissue positive
				47 16.5 (10.9 to NC)
				Liquid negative, tissue negative
				40 2.8 (1.4 to 4.2)
Thress et al (2015)[11]; phase 1 AURA RCT in tissue *EGFR*-positive[a] with progression on EGFR TKI	Multinational[b]	Advanced	cobas; BEAMing ddPCR	ORR (Osimertinib)
				Tissue positive vs tissue negative
				65 61% vs 29%
				Liquid positive vs liquid negative
				72 59% vs 35%
				Liquid positive, tissue biopsy negative
				8 38%
Karlovich et al (2016)[10]; patients from observational study and a phase 1 dose- escalation part and a phase 2 study of rociletinib	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

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.

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 8).(11) 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.(23) Some patients may have overlapped with the Thress study (2015).(11) 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=.001), as was median PFS (16.5 months vs 2.8 months; p=.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=.004; PFS=4.2 months; p=.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.

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.(10) 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.

Papadimitrakopoulou et al (2020) compared outcomes in tissue-positive T790M patients enrolled in the AURA3 (A Phase III, Open Label, Randomized Study ofAZD9291 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.(80) 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. TheGuardant360 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.

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, or OncoBEAM tests), 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 (> 63%) with high specificities (>95%) for these 3 tests. 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 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 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 one study, eight 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, three 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 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 tests with established clinical validity such as the cobas EGFR Mutation Test v2, Guardant360 CDx, or OncoBEAM, can support its utility. The body of evidence has demonstrated moderate sensitivity (>63%), with high specificities (>96%). If 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.

Per National Comprehensive Cancer Network Guidelines (2022), testing for *EGFR, KRAS, ROS1*, *ALK,* and *BRAF* are predictive biomarkers that are helpful in determining the benefit/lack thereof of targeted therapy (category 2A). *EGFR, KRAS, ROS1* and *ALK* genetic alterations do not usually overlap, thus testing for *KRAS* mutations may identify patients who

will not benefit from further molecular testing. Targeted therapy is not currently available for patients with *KRAS* mutations. *KRAS* mutation is prognostic of poor survival for patients with NSCLC. *KRAS* mutations are also predictive of a lack of benefit from *EGFR* tyrosine Kinase Inhibitor (TKI) therapy.

The NCCN NSCLC Panel recommends capmatinib as either a first-line therapy or subsequent therapy option (category 2A; preferred) for patients with metastatic NSCLC who are positive for *METex14* skipping mutations based on preliminary data and the FDA approval. The NCCN NSCLC Panel also preference stratified regimens that are recommended for *METex14* skipping mutations and decided that capmatinib is a preferred first-line therapy or subsequent therapy option for *METex14* skipping mutation–positive metastatic NSCLC based on clinical trial data. Capmatinib may be used as subsequent therapy if not previously given as first-line therapy for *METex14* skipping mutation–positive metastatic NSCLC.

Selpercatinib was recommended as a first-line or subsequent therapy option (category 2A; preferred) for patients with metastatic NSCLC who are positive for *RET* rearrangements based on preliminary data and the FDA approval of selpercatinib for use in NSCLC. The NCCN NSCLC Panel also preference stratified the regimens that are recommended for *RET* rearrangements and decided that selpercatinib is a preferred first-line therapy or subsequent therapy option for *RET* rearrangement–positive metastatic NSCLC based on clinical trial data. The panel decided that cabozantinib (category 2A) is useful in certain circumstances as a first-line therapy option for *RET* rearrangements based on clinical trial data.

Single-agent pembrolizumab is recommended (category 1; preferred) as first-line therapy for eligible patients with metastatic NSCLC regardless of histology, PD-L1 expression levels of 50% or more, and with negative test results for EGFR, ALK, ROS1, METex14 skipping, and BRAF V600E (specific molecular) variants.

The NCCN NSCLC Panel recommended *NTRK* gene fusion testing in patients with metastatic NSCLC based on clinical trial data showing the efficacy of larotrectinib and entrectinib for patients with *NTRK* gene fusion--positive disease. Based on data from clinical trials and the FDA approvals, the NCCN NSCLC Panel recommended larotrectinib and entrectinib (category 2A) for use as a first-line or subsequent therapy option for patients with *NTRK* gene fusion--positive metastatic NSCLC. The NCCN Panel voted that larotrectinib and entrectinib are both preferred (category 2A) as first-line therapy for patients with *NTRK* gene fusion who have positive metastatic disease.(24)

According to the NCCN, *EGFR, ALK*, *ROS1* fusions, *RET* rearrangements, *METex14* skipping mutations, and *BRAF* mutations are on the list of actionable biomarkers that need to be negative before administering immunotherapy regimens to individuals with NSCLC.

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 overall survival, disease-specific survival, and test validity. Current evidence does not permit determining whether cobas or tissue biopsy is more strongly associated with patient outcomes or treatment response. 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 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 or OncoBEAM tests, the evidence includes several studies assessing the diagnostic characteristics of liquid biopsy compared with tissue. Relevant outcomes are overall survival, disease-specific survival, and test validity. Current evidence does not permit determining whether liquid or tissue biopsy is more strongly associated with patient outcomes or treatment response. BCBSA identified no RCTs providing evidence of the clinical utility of these tests. The Guardant360 CDx and OncoBEAM 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 or OncoBEAM 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, or OncoBEAM, the evidence includes studies assessing the diagnostic characteristics of liquid biopsy compared with tissue reference standard. Relevant outcomes are overall survival, disease-specific survival, and test validity. Given the breadth of molecular diagnostic methodologies available to assess ctDNA, the clinical validity of each commercially available test must be established independently. None of the commercially available tests other than the cobas, Guardant360 CDx, and OncoBEAM tests 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. 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 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. The relevant outcomes are overall survival, disease-specific survival, and test validity. Given

the breadth of molecular diagnostic methodologies available to assess ctDNA, the clinical validity of each commercially available test must be established independently.

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 a few studies assessing the diagnostic characteristics of liquid biopsy. Relevant outcomes are overall survival, disease-specific survival, and test validity. For variants that indicate *EGFR* TKI resistance and suitability for alternative treatments with osimertinib, liquid biopsy is moderately sensitive and moderately specific compared with a reference standard of tissue biopsy.

For individuals who may benefit from targeted therapy *EGFR, ROS1, ALK*, *BRAF* and *NTRK* are helpful in determining the best course of therapy. *KRAS* mutation is prognostic of poor survival as a targeted therapy is not currently available. The genetic alterations with *EGFR, ROS1, ALK,* and *KRAS* do not usually overlap, thus *KRAS* testing may identify patients who will not benefit from further molecular testing. KRAS mutations are also predictive of a lack of benefit from *EGFR* tyrosine Kinase Inhibitor (TKI) therapy.

Supplemental Information

PRACTICE GUIDELINES AND POSITION STATEMENTS

National Comprehensive Cancer Network
National Comprehensive Cancer Network guidelines discuss the role of liquid biopsy in the management of non-small-cell lung cancer (NSCLC).(24) 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
The International Association for the Study of Lung Cancer (2018) published a statement paper on liquid biopsy for advanced non-small-cell lung cancer.(25) The work preparing the statement was supported by unrestricted grants from Guardant Health, Astra Zeneca, Biocept, and Roche. The statement made the following recommendations:

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

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

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

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

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

U.S. Preventive Services Task Force Recommendations

Not applicable

Ongoing and Unpublished Clinical Trials

Some currently unpublished trials that might influence this review are listed in Table 9.

NCT No.	Trial Name	Planned Enrollment	Completion Date
Ongoing			
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.

Government Regulations
National:

Next Generation Sequencing (NGS). 100-3; Section **90.2**; version 2. Effective 1/27/20
Item/Service Description

A. General

Clinical laboratory diagnostic tests can include tests that, for example, predict the risk associated with one or more genetic variations. In addition, in vitro companion diagnostic laboratory tests provide a report of test results of genetic variations and are essential for the safe and effective use of a corresponding therapeutic product. **NEXT GENERATION SEQUENCING** (NGS) is one technique that can measure one or more genetic variations as a laboratory diagnostic test, such as when used as a companion in vitro diagnostic test.
This National Coverage Determination (NCD) is only applicable to diagnostic lab tests using NGS for somatic (acquired) and germline (inherited) cancer. Medicare Administrative Contractors (MACs) may determine coverage of diagnostic lab tests using NGS for RNA sequencing and protein analysis.

Indications and Limitations of Coverage

B. Nationally Covered Indications

1. Somatic (Acquired) Cancer

Effective for services performed on or after March 16, 2018, the Centers for Medicare & Medicaid Services (CMS) has determined that **NEXT GENERATION SEQUENCING** (NGS) as a diagnostic laboratory test is reasonable and necessary and covered nationally, when performed in a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory, when ordered by a treating physician, and when all of the following requirements are met:

a. Patient has:
   i. either recurrent, relapsed, refractory, metastatic, or advanced stage III or IV cancer; and
   ii. not been previously tested with the same test using NGS for the same cancer genetic content, and
   iii. decided to seek further cancer treatment (e.g., therapeutic chemotherapy).

b. The diagnostic laboratory test using NGS must have:
   i. Food & Drug Administration (FDA) approval or clearance as a companion in vitro diagnostic; and,
   ii. an FDA-approved or -cleared indication for use in that patient's cancer; and,
   iii. results provided to the treating physician for management of the patient using a report template to specify treatment options.

2. Germline (Inherited) Cancer

Effective for services performed on or after January 27, 2020, CMS has determined that NGS as a diagnostic laboratory test is reasonable and necessary and covered nationally for patients with germline (inherited) cancer, when performed in a CLIA-certified laboratory, when ordered by a treating physician and when all of the following requirements are met:

a. Patient has:
   i. ovarian or breast cancer; and,
   ii. a clinical indication for germline (inherited) testing for hereditary breast or ovarian cancer; and,
   iii. a risk factor for germline (inherited) breast or ovarian cancer; and

iv. not been previously tested with the same germline test using NGS for the same germline genetic content.

b. The diagnostic laboratory test using NGS must have all of the following:
   i. FDA-approval or clearance; and,
   ii. results provided to the treating physician for management of the patient using a report template to specify treatment options.

C. Nationally Non-Covered Indications

1. Somatic (Acquired) Cancer

Effective for services performed on or after March 16, 2018, NGS as a diagnostic laboratory test for patients with acquired (somatic) cancer are non-covered if the cancer patient does not meet the criteria noted in section B.1., above.

D. Other

1. Somatic (Acquired) Cancer

Effective for services performed on or after March 16, 2018, Medicare Administrative Contractors (MACs) may determine coverage of NGS as a diagnostic laboratory test for patients with advanced cancer only when the test is performed in a CLIA-certified laboratory, when ordered by a treating physician, and when the patient has:

a. either recurrent, relapsed, refractory, metastatic, or advanced stages III or IV cancer; and,
b. not been previously tested with the same test using NGS for the same cancer genetic content, and
c. decided to seek further cancer treatment (e.g., therapeutic chemotherapy).

2. Germline (Inherited) Cancer

Effective for services performed on or after January 27, 2020, MACs may determine coverage of NGS as a diagnostic laboratory test for patients with germline (inherited) cancer only when the test is performed in a CLIA-certified laboratory, when ordered by a treating physician, when results are provided to the treating physician for management of the patient and when the patient has:

a. any cancer diagnosis; and,
b. a clinical indication for germline (inherited) testing of hereditary cancers; and,
c. a risk factor for germline (inherited) cancer; and,
d. not been previously tested with the same germline test using NGS for the same germline genetic content.

(This NCD last reviewed January 2020)

Local:

LCD: MolDX: **Minimal Residual Disease Testing for Cancer** (L38835); Original date: 12/26/21; Revision date: 10/26/23

Coverage Indications, Limitations, and/or Medical Necessity

This Medicare contractor will provide limited coverage for minimally invasive molecular deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) tests that detect MINIMAL RESIDUAL DISEASE (MRD) in patients with a personal history of cancer.

This Contractor provides limited coverage for MRD testing in cancer when ALL of the following are true:

If Next-Generation Sequencing (NGS) methodology is used in testing, the conditions set by NCD 90.2 are fulfilled (summarized: the patient has advanced cancer; plans on being treated for said cancer, and has not been previously tested with the same test for the same genetic content) or are not applicable (the patient does not have cancer as defined below)

The patient has a personal history of cancer, the type and staging of which is within the intended use of the MRD test

The identification of recurrence or progression of disease within the intended use population of the test is identified in the National Comprehensive Cancer Network (NCCN) or other established guidelines as a condition that requires a definitive change in patient management.

The test is demonstrated to identify molecular recurrence or progression before there is clinical, biological, or radiographical evidence of recurrence or progression AND demonstrates sensitivity and specificity of subsequent recurrence or progression comparable with or superior to radiographical or other evidence (as per the standard of care for monitoring a given cancer type) of recurrence or progression.

To be reasonable and necessary, it must also be medically acceptable that the test being utilized precludes other surveillance or monitoring tests intended to provide the same or similar information unless they either (a) are required to follow-up or confirm the findings of this test or (b) are medically required for further assessment and management of the patient.

If the test is to be used for monitoring a specific therapeutic response, it must demonstrate the clinical validity of its results in published literature for the explicit management or therapy indication (allowing for the use of different drugs within the same therapeutic class, so long as they are considered ‘equivalent and interchangeable’ for the purpose of MRD testing, as determined by national or society guidelines).

Clinical validity (CV) of any analytes (or expression profiles) measured must be established through a study published in the peer-reviewed literature for the intended use of the test in the intended population.

The test is being used (a) in a patient who is part of the population in which the test was analytically validated and (b) according to the intended use of the test.

The MRD test [(unless it is a Food and Drug Administration (FDA)-approved and established standard-of-care single-gene polymerase chain reaction (PCR)] satisfactorily completes a technical assessment (TA) that will evaluate and confirm that the analytical validity, clinical validity, and clinical utility criteria set in this policy are met to establish the test as Reasonable and Necessary.

Tests utilizing a similar methodology or evaluating a similar molecular analyte to a test for which there is a generally accepted testing standard or for which existing coverage exists must demonstrate equivalent or superior test performance (i.e., sensitivity and/or specificity) when used for the same indication in the same intended-use population.

MRD testing often requires 2 types of assays to be performed as part of the service. First, a sample is taken from tumor diagnostic material to establish a baseline (solid and/or liquid) tumor signature as defined by the test methodology. This is followed by a series of assays run on a minimally invasive specimen (i.e., liquid biopsy or bone marrow aspirate) to detect the presence or recurrence of tumor based on the measured biomarkers, expression, or other analytes over various timepoints. Other approaches are also acceptable, based on the validity established for the individual test comprising the service. This series of assays comprises a single test when the patient is known to have cancer.

LCD: MolDX: **Plasma-Based Genomic Profiling in Solid Tumors** (L38168); Original Effective Date: 3/15/20; Revision date: 10/26/23

This is a limited coverage policy for next generation sequencing (NGS) assays performed on solid tumor cell free DNA in plasma, from here on called “liquid biopsies.”

Criteria for Coverage
Guardant360 CDx ® is covered only when **all** of the following conditions are met:

- Patient has been diagnosed with a recurrent, relapsed, refractory, metastatic, or advanced solid tumor that did not originate from the central nervous system. Patients who would meet all of the indications on the FDA label for larotrectinib if they are found to have an NTRK mutation may be considered to have advanced cancer, **and**
- Patient has not previously been tested with the Guardant360® test for the same primary cancer. For a patient who has been tested previously using Guardant360® for a cancer, that patient may not be tested again unless he or she has a new primary cancer diagnosis. In a patient with previously tested primary cancer, who has evidence of new malignant growth, that growth may be considered to be a different primary cancer if it does not originate from the same cell line or it is physiologically different enough that it responds differently to treatment than the previously tested cancer, **and**
- Patient is untreated for the primary cancer being tested or the patient is not responding to treatment (e.g., progression or new lesions on treatment), **and**
- The patient has decided to seek further cancer treatment with the following conditions:
    - The patient is a candidate for further treatment with a drug that is either FDA-approved for that patient’s cancer, or has an NCCN 1 or NCCN 2A recommendation for that patient’s cancer, **and**
    - The FDA-approved indication or NCCN recommendation is based upon information about the presence or absence of a genetic biomarker tested for in the Guardant360® assay **and**
- Tissue-based CGP is infeasible (e.g., quantity not sufficient for tissue-based CGP or invasive biopsy is medically contraindicated) or specifically in NSLC Tissue-based CGP has shown no actionable mutations.

If no alteration is detected by Guardant360® or if ctDNA is insufficient/not detected, tissue-based genotyping should be considered.

Other liquid biopsies will be covered for the same indications if they display similar performance in their intended use applications to Guardant360®.

LCD: MolDX: **GUARDANT360 CDx ® Plasma-Based Comprehensive Genomic Profiling in Non-Small Cell Lung Cancer (NSCLC)** (L37671); Original effective date: 10/17/18; **RETIRED 3/14/2020**

Coverage Indications, Limitations, and/or Medical Necessity
This policy provides limited coverage for **GUARDANT**360 CDx ® (**GUARDANT** Health, Redwood City, CA), a plasma-based comprehensive somatic genomic profiling test (hereafter called CGP) for patients with Stage IIIB/IV non-small cell lung cancer (NSCLC):

- **At diagnosis-Untreated Patient**
  - when results for EGFR single nucleotide variants (SNVs) and (insertions and deletions (indels)); rearrangements in ALK and ROS1; and SNVs for BRAF are not available **AND** when tissue-based CGP is infeasible (i.e., quantity not sufficient for tissue-based CGP or invasive biopsy is medically contraindicated);

  OR
- **At progression-Treated Patient**
  - For patients progressing on or after chemotherapy or immunotherapy who have never been tested for EGFR SNVs and indels, and rearrangements in ALK and ROS1; and SNVs for BRAFs, and for whom tissue-based CGP is infeasible (i.e., quantity not sufficient for tissue-based CGP); **OR**
  - For patients progressing on any tyrosine kinase inhibitors (TKIs).

If no genetic alteration is detected by **GUARDANT**360, or if circulating tumor DNA (ctDNA) is insufficient/not detected, tissue-based genotyping should be considered.

LCD: **Inivata, InVisionFirst, Liquid Biopsy for Patients with Lung Cancer** (L37921)
Original effective date: 4/15/19; Revision date: 11/30/23

Coverage Indications, Limitations, and/or Medical Necessity
This test is a "**LIQUID BIOPSY**." It is intended to assist physicians caring for patients who suffer from a common form of lung cancer and who have advanced disease.

This policy provides limited coverage for InvisionFirst™ - Lung (Inivata, Research Triangle Park, NC) (hereafter InVision) a plasma-based, somatic comprehensive genomic profiling test (CGP) for patients with advanced (Stage IIIB/IV) non-small cell lung cancer (NSCLC):

- **At diagnosis** –
  - When results for EGFR single nucleotide variants (SNVs) and insertions and deletions (indels); rearrangements in ALK and ROS1; and SNVs for BRAF are not available AND when tissue-based CGP is infeasible [i.e., quantity not sufficient (QNS) for tissue-based CGP or invasive biopsy is medically contraindicated],

    or
- **At progression** –
  - For patients progressing on or after chemotherapy or immunotherapy who have not been tested for EGFR SNVs and indels; rearrangements in ALK and ROS1; and SNVs for BRAFs, and for whom tissue-based CGP is infeasible;

    or
  - For patients progressing on EGFR tyrosine kinase inhibitors (TKIs),

If no genetic alteration is detected by InVision or if circulating tumor DNA (ctDNA) is insufficient/not detected, tissue-based genotyping should be considered.

Local Coverage Article: **FDA approved EGFR Tests** (A55193); Original Effective Date: 2/16/17; Revision Date: 3/31/2022
Two tests have met the FDA criteria for EGFR genetic testing:

1. Effective 6/01/16
   cobas EGFR Mutation Test is a real-time PCR test for the qualitative detection of defined mutations of the epidermal growth factor receptor (EGFR) gene in non-small cell lung cancer (NSCLC) patients. Defined EGFR mutations are detected using DNA isolated from formalin-fixed paraffin embedded tumor tissue (FFPET) or circulating-free tumor DNA (cfDNA) from plasma derived from EDTA anticoagulated peripheral whole blood.

   The test is indicated as a companion diagnostic to aid in selecting NSCLC patients for treatment with the targeted therapies listed in the Table below in accordance with the approved therapeutic product labeling:

Drug	FFPET	Plasma
TARCEVA ® (erlotinib)	**EXON 19** deletions and L858R	**EXON 19** deletions and L858R
TAGRISSO ™ (osimertinib)	T790M	

   Patients with positive cobas® EGFR Mutation Test v2 test results using plasma specimens for the presence of EGFR **EXON 19** deletions or L858R mutations are eligible for treatment with TARCEVA® (erlotinib). Patients who are negative for these mutations by this test should be reflexed to routine biopsy and testing for EGFR mutations with the FFPET sample type.

2. Effective 7/12/13
   therascreen EGFR RGQ PCR kit for the detection of the epidermal growth factor receptor (EGFR) gene for non-small cell lung cancer (NSCLC) tumor tissue to help select patients with NSCLC for whom GILOTRIF™ (afatinib), an EGFR tyrosine kinase inhibitor (TKI), is indicated.

LCD: **MolDX: Lab-Developed Tests for Inherited Cancer Syndromes in Patients with Cancer.** (L39040) Original effective date: 7/3/22. Revision date: 4/18/24
Coverage Indications, Limitations, and/or Medical Necessity

This policy describes and clarifies coverage for Lab-Developed Tests (LDTs), Federal Drug Administration (FDA)-cleared, and FDA-approved clinical laboratory tests in hereditary cancer tests including Next-Generation Sequencing (NGS) tests as allowable under the National Coverage Determination (NCD) 90.2, under section D describing Medicare Administrative Contractor (MAC) discretion for coverage. This policy's scope is specific for hereditary germline testing, and is exclusive of polygenic risk scores, solid tumor, hematologic malignancies, circulating tumor deoxyribonucleic acid (DNA) testing (ctDNA), and other acquired cancer-related tests.

Criteria for Coverage
All the following must be present for coverage eligibility:

- The patient must have:
  - Any cancer diagnosis
  - AND a clinical indication for germline (inherited) testing for hereditary cancer
  - AND a risk factor for germline (inherited) cancer
  - AND has not been previously tested for the same germline genetic content.
- The test has satisfactorily completed a Technical Assessment (TA) by Molecular Diagnostic Services Program (MolDX®) for the stated indications of the test.
- The test performed includes **at least** the minimum genetic content (genes or genetic variants) with definitive or well-established guidelines-based evidence required for clinical decision making for its intended use that can be reasonably detected by the test.
  - Because these genes and variants will change as the literature and drug indications evolve, they are listed separately in associated documents, such as the MolDX® TA forms.
  - A single gene or variant may be tested if it is the only gene or variant considered to be reasonable and necessary for a cancer type.
- If a previous test was performed with a similar/duplicative intended use, a subsequent test is only reasonable and necessary if the non-duplicative genetic content of the second test is reasonable and necessary.
- If the test is an NGS test, it must abide by all conditions listed in the NCD 90.2.

Situations in which a test should not be used, or coverage is denied:
The test in question will be non-covered if:

- It is an NGS test and does not fulfill all the criteria set forth in the NCD 90.2
- A previous test was performed for the same genetic content
- It is a panel or single gene test used to identify a known familial variant(s) that could be identified with a test targeted to that specific variant(s)
- It is a panel or single gene test used to confirm a variant(s) detected by somatic tumor testing that can be confirmed by a test targeted to that specific variant(s)
- A satisfactory TA is not completed
- For tests that are currently covered but a TA submission has not been made, providers must submit complete TA materials by the original effective date of the policy or coverage will be denied.

(The above Medicare information is current as of the review date for this policy. However, the coverage issues and policies maintained by the Centers for Medicare & Medicare Services [CMS, formerly HCFA] are updated and/or revised periodically. Therefore, the most current CMS information may not be contained in this document. For the most current information, the reader should contact an official Medicare source.)

Related Policies

- Circulating Tumor DNA and Circulating Tumor Cells for Selecting Targeted Therapy for Advanced Solid Cancers (Liquid Biopsy)
- Genetic Testing and Counseling
- Genetic Cancer Susceptibility Panels Using Next Generation Sequencing
- Genetic Testing-NGS Testing of Multiple Genes (Panel) for Malignant Conditions
- Genetic Testing: Microarray Testing for Cancers of Unknown Primary (CUP) Origin

- Genetic Testing - Somatic Biomarker Testing (Including Liquid Biopsy) for Targeted Treatment and Immunotherapy in Non-Small-Cell Lung Cancer (EGFR, ALK, BRAF, ROS1, RET, MET, KRAS, HER2, PD-L1, TMB)
- Somatic Biomarker Testing (Including Liquid Biopsy) for Targeted Treatment and Immunotherapy in Metastatic Colorectal Cancer (KRAS, NRAS, BRAF , MMR/MSI, HER2 , AND TMB)
- Proteomic Testing for Targeted Therapy in Non-Small-Cell Lung Cancer (NSCLC), e.g., VeriStrat®
- Miscellaneous Genetic and Molecular Diagnostic Tests

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The articles reviewed in this research include those obtained in an Internet based literature search for relevant medical references through May 6, 2024, the date the research was completed.

Joint BCBSM/BCN Medical Policy History

Policy Effective Date	BCBSM Signature Date	BCN Signature Date	Comments
9/1/20	8/18/20		Joint policy established
9/1/21	6/15/21		Routine maintenance. Added 81445 and 81455 as Established. Added 0179U and 0239U as E&I. No change in policy status. Added references 78-88 Added 0242U as E/I Added language under the coding section: • PLA codes are considered investigational/experimental until the laboratory test the code represents is formally documented as Established in an Interim Medical Policy or Joint Uniform Medical Policy document. • Covered CPT codes may be used to represent and reimburse testing for incremental codes or multi-target codes. Updated MPS – the highlighted portion is the update: • The effectiveness and clinical utility of circulating tumor DNA of individual genes and listed multiple gene panels when more than 5 genes are tested for the management of non-small-cell lung cancer (liquid biopsy) has been established. It may be considered a useful therapeutic option when indicated.
9/1/22	8/23/22		• BCBSA merged this policy with GT-Molecular analysis for targeted therapy or immunotherapy of NSCLS (Nov 2021) ○ We cover more than BCBSA (per NCCN guidelines), so policies are to remain separate (JUMP Dec 2021 discussion with review of GT-Mol anal for targeted therapy - NSCLC).

			• FDA approved companion test are covered • Follow NCCN recommendations ○ HER2 and PD-L1 covered
9/1/23	6/13/23		• Routine Maintenance • Added code 0326U (Guardant360) E/I EFD 7/1/22. • CODES 81455 and 81445 was REVISED on 1/1/23. • Vendor: N/A (ky)
9/1/24	6/11/24		• Routine maintenance • Per discussion on 5/20/24: moved code 0326U from E/I to EST. 6/2023 review 0326U was directed from CU to be EI; however, per discussion, the testing platform for Guardant is considered established for later stage solid cancer or liquid cancer. • Vendor: N/A (ky)

Next Review Date: 2nd Qtr, 2025

BLUE CARE NETWORK BENEFIT COVERAGE
POLICY: CIRCULATING TUMOR DNA FOR MANAGEMENT OF NON-SMALL-CELL LUNG CANCER (LIQUID BIOPSY)

I. Coverage Determination:

Commercial HMO (includes Self-Funded groups unless otherwise specified)	Covered; criteria apply
BCNA (Medicare Advantage)	Refer to the Medicare information under the Government Regulations section of this policy.
BCN65 (Medicare Complementary)	Coinsurance covered if primary Medicare covers the service.

II. Administrative Guidelines:

- The member's contract must be active at the time the service is rendered.
- Coverage is based on each member's certificate and is not guaranteed. Please consult the individual member's certificate for details. Additional information regarding coverage or benefits may also be obtained through customer or provider inquiry services at BCN.
- The service must be authorized by the member's PCP except for Self-Referral Option (SRO) members seeking Tier 2 coverage.
- Services must be performed by a BCN-contracted provider, if available, except for Self-Referral Option (SRO) members seeking Tier 2 coverage.
- Payment is based on BCN payment rules, individual certificate and certificate riders.
- Appropriate copayments will apply. Refer to certificate and applicable riders for detailed information.
- CPT - HCPCS codes are used for descriptive purposes only and are not a guarantee of coverage.
- *Duplicate (back-up) equipment is not a covered benefit.*