Medical Policy

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

Professional
Original Effective Date: December 21, 2018
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Populations	Interventions	Comparators	Outcomes
Individuals: • With advanced non-small-cell lung cancer	Interventions of interest are: • Testing for biomarkers of EGFR TKI sensitivity using circulating tumor DNA with the cobas EGFR Mutation Test v2	Comparators of interest are: • Testing for biomarkers of EGFR tyrosine kinase inhibitor sensitivity using tissue biopsy • No testing for biomarkers of EGFR tyrosine kinase inhibitor resistance	Relevant outcomes include: • Overall survival • Disease-specific survival • Test validity
Individuals: • With advanced non-small-cell lung cancer	Interventions of interest are: • Testing for biomarkers of EGFR TKI sensitivity using circulating tumor DNA with the Guardant360 or OncoBEAM tests	Comparators of interest are: • Testing for biomarkers of EGFR tyrosine kinase inhibitor sensitivity using tissue biopsy • No testing for biomarkers of EGFR tyrosine kinase inhibitor resistance	Relevant outcomes include: • Overall survival • Disease-specific survival • Test validity

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

DESCRIPTION

Genetic testing of circulating tumor DNA (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.

OBJECTIVE

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

BACKGROUND

Predictive Biomarkers in Non-Small-Cell Lung Cancer

Several predictive genetic biomarkers have been identified for 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, 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.

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). 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. *RET* fusions occur in 0.6% to 2% of NSCLCs and 1.2% to 2% of adenocarcinomas. *MET* 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* comutations occur in 5% to 20% of NSCLC tumors with acquired resistance to EGFR TKIs.

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. However, the use of osimertinib as a first-line therapy for patients who have *EGFR*-sensitizing variants is emerging and may prevent the development of T790M resistance.

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 in 2018 (P120019). Patients who test negative for the variants detected should be referred for (or "reflexed" to) routine biopsy with tissue testing for *EGFR* variants. A previously approved version 2 of this test, which used tissue biopsy specimens, was also approved for detection of T790M variants in tissue, which are used to select patients to receive osimertinib. Approval of version 2 of the plasma test did not include detection of T790M variants.

No other ctDNA tests have FDA approval. However, Foundation Medicine was granted FDA breakthrough device designation for FoundationACT™ in 2018.

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® 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.

POLICY

I. *EGFR* Testing

A. Except as noted below, analysis of 2 types of somatic sensitizing variants within the epidermal growth factor receptor (*EGFR*) gene—small deletions in exon 19 and a point mutation in exon 21 (L858R)—using the cobas EGFR Mutation Test v2, Guardant360 test, or OncoBEAM test with plasma specimens to detect circulating tumor DNA (ctDNA) may be considered **medically necessary** as an alternative to tissue biopsy to predict treatment response to an EGFR tyrosine kinase inhibitor (TKI) therapy in patients with advanced stage III or IV non-small-cell lung cancer (NSCLC). The cobas test is a companion diagnostic for erlotinib and gefitinib.

B. Analysis of other *EGFR*-sensitizing variants within exons 18 to 24 using ctDNA for applications related to NSCLC is considered **experimental / investigational**.

C. Analysis of the *EGFR* T790M resistance variant for targeted therapy with osimertinib using ctDNA or for other applications related to NSCLC is considered **experimental / investigational**.

D. Analysis of 2 types of somatic variants within the *EGFR* gene—small deletions in exon 19 and a point mutation in exon 21 (L858R)—using ctDNA is considered **experimental / investigational** for patients with advanced NSCLC of squamous cell type.

II. *ALK* Testing

A. Analysis of somatic rearrangement variants of the *ALK* gene using plasma specimens to detect ctDNA or RNA is considered **experimental / investigational** as an alternative to tissue biopsy to predict treatment response to ALK inhibitor therapy (eg, crizotinib [Xalkori], ceritinib [Zykadia], alectinib [Alecensa], or brigatinib [Alunbrig]) in patients with NSCLC.

III. *BRAF* V600E Testing

A. Analysis of the *BRAF* V600E variant using plasma specimens to detect ctDNA is considered **experimental / investigational** as an alternative to tissue biopsy to predict treatment response to BRAF or MEK inhibitor therapy (eg, dabrafenib [Tafinlar], trametinib [Mekinist]) in patients with NSCLC.

IV. *ROS1* Testing

A. Analysis of somatic rearrangement variants of the *ROS1* gene using plasma specimens to detect ctDNA or RNA is considered **experimental / investigational** as an alternative to tissue biopsy to predict treatment response to ALK inhibitor therapy (crizotinib [Xalkori]) in patients NSCLC.

V. *KRAS* Testing

A. Analysis of somatic variants of the *KRAS* gene using plasma specimens to detect ctDNA is considered **experimental / investigational** as a technique to predict treatment nonresponse to anti-EGFR therapy with tyrosine kinase inhibitors and for the use of the anti-EGFR monoclonal antibody cetuximab in NSCLC.

VI. Other Genes

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

Policy Guidelines

1. 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).

2. Genetics Nomenclature Update

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

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

Table PG1. Nomenclature to Report on Variants Found in DNA

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

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

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

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

RATIONALE

The most recent literature update was performed through August 8, 2018.

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

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

Selecting Targeted Therapy

Clinical Context and Test Purpose

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

Figures 1 and 2 show how liquid biopsy could be used to select first-line and second-line treatments in patients with advanced NSCLC with reflex to tissue biopsy and how it would potentially affect outcomes.

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

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

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

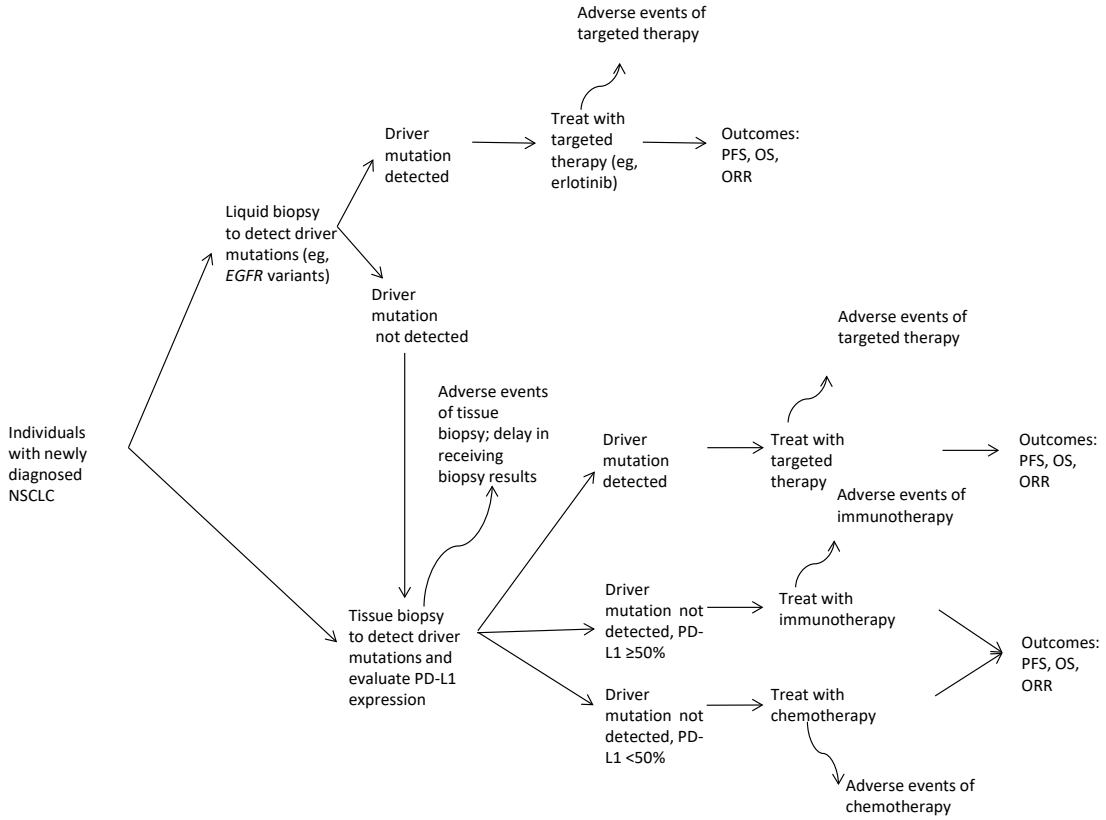
Interventions
The technology considered is an analysis of tumor biomarkers in peripheral blood (liquid biopsy) to determine treatment selection. The comparator is an analysis of tumor biomarkers for treatment selection using tumor tissue. The evidence is considered separately for the different biomarkers.

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.

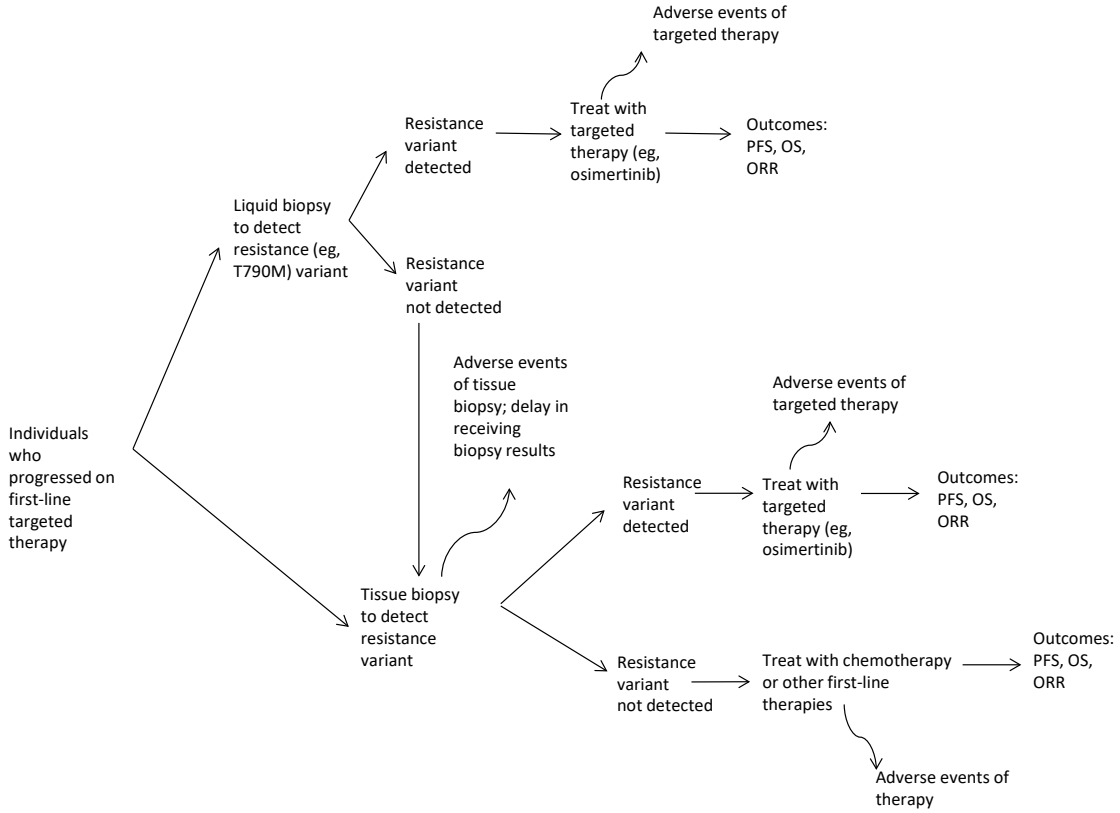
Comparators
The relevant comparator of interest is testing for variants using tissue biopsy.

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



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

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



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

Outcomes

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

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

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

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

Potential Beneficial Outcomes: 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 IMpower 150 trial, the addition of the immunotherapy atezolizumab to the combination chemotherapy of bevacizumab, carboplatin, and paclitaxel improved PFS in a subset of 111 patients with *EGFR*-sensitizing variants or *ALK* translocations who had progressed on a prior targeted agent (median PFS, 9.7 months vs 6.1 months; HR=0.59; 95% CI 0.37 to 0.94).[6]

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

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

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).

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.

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	LDT	NGS	SNVs Insertions and deletions Fusions CNVs
FoundationACT	LDT	NGS	SNVs Insertions and deletions (1-40 bp) Rearrangements and fusions CNVs >20% <20%
Biocept	LDT	Real-time PCR	SNVs
Circulogene's (Theranostics) 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
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)[9]	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				
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* (70 total)	Time between tissue and blood collection ranged from 0 d to >2 y
Villaflor et al (2016)[16]	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

Study	Study Population	Design	Variants Included[a]	Timing of Reference and Index Tests
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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
GeneStrat				
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, 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

ctDNA: circulating tumor DNA; EGFR: epidermal growth factor receptor; FDA: Food and Drug Administration; NSCLC: non-small-cell lung cancer; SSED: Summary of Safety and Effectiveness Data; TKI: tyrosine kinase inhibitor.
[a] Noting *EGFR*, *ALK*, *ROS1*, and *BRAF* variants only.

Table 3 summarizes the results of clinical validity studies of liquid biopsy compared with tissue biopsy as a reference standard. 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 several clinical validity studies 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* variants, 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					
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]					

Study	Initial N	Final N	Excluded Samples	Sensitivity (95% CI)	Specificity (95% CI)
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)				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					
Schwaederle et al (2017)[14]					
EGFR variants	88	34	No tissue	54 (25 to 81)	90 (70 to 99)
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)[16]	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)
GeneStrat					
Mellert et al (2017)[18]					

Study	Initial N	Final N	Excluded Samples	Sensitivity (95% CI)	Specificity (95% CI)
EGFR exon 19 deletion (sensitizing)				95.9 (NR)	100 (NR)
EGFR exon 21 substitution (L858R, sensitizing)				100 (NR)	100 (NR)
EGFR exon 20 (T790M, resistance)				86.7 (NR)	100 (NR)
ALK fusion				~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]

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

The purpose of the gaps tables (see Tables 4 and 5) is 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]
Cobas EGFR test					
Jenkins et al (2017)[8]					
FDA SSED (2016)[9]	4. Performed in Asia				
Karlovich et al (2016)[10]					
Thress et al (2015)[11]					
Mok et al (2015)[12]	4. Performed in Asia				
Weber et al (2014)[13]					
Guardant360					
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]					
GeneStrat					
Mellert et al (2017)[18]	3. Patient characteristics unclear				

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
ctDx-Lung					
Paweletz et al (2016)[19]	2. Unclear if same as current marketed version				

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] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true positives, true negatives, false positives, false negatives cannot be determined).

Table 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]
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 and tissue sample collection not described			1. Precision estimates not reported but calculated based on 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						

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
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
GeneStrat						
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		1. Time between blood and tissue			1. Precision estimates not

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
	were selected		sample collection not described			reported but calculated based on data provided

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 and OncoBEAM each have 3 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. 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	75 (69 to 80)[b] 61-87	97 (95 to 98)[b] 96-100	6	Very few gaps identified (Jenkins[8]; FDA SSED[9]; Karlovich[10]; Thress[11]; Mok[12]; Weber[13])
Guardant360 (NGS)	79 (58 to 93) 54-79	100 (87 to 100) 90-100	3	Long time between tissue and ctDNA tests (Thompson[15]; Villaflor[16]); unclear patient selection (Villaflor[16]); Very few gaps with Schwaederle[14]
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 GeneStrat (ddPCR)	95.9 (NR)[18]	100 (NR)[18]	1	Patient characteristics and selection unclear; timing of blood and tissue samples unclear; precision estimates not provided (Mellert[18])
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

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.

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 75%, and a specificity above 95% 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 test has 3 studies using tissue biopsy as the reference standard performed in the United States in the intended-use population. 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, 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.

For tests of other, less prevalent, variants, such as *ALK* and *ROS1* translocations 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.

Fewer studies have examined the performance of liquid biopsy for detection of T790M variants associated with *EGFR* TKI resistance and several different tests were used in the studies. Detection of these variants is potentially important for liquid biopsy because this variant is of interest after 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, evidence was considered 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
Zhang et al (2017)[21]; *EGFR*-positive and -negative patients treated with EGFR TKIs	China	IIIB, IV	ddPCR		PFS (95% CI), d		
				n	Erlotinib	Chemotherapy	p
				Tissue positive vs tissue negative			
				215	342 (291 to 393)	60 (0 to 124)	215
				Tissue positive and liquid positive vs liquid negative			
				80	334 (298 to 371)	420 (100 to 740)	80
				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)			
				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			

Study/Patient Group	Country	Disease Stage	Technology Used to Detect ctDNA	Sample Sizes	Treatment Response		
				9	13.0 (8.9 to 19.8)	15.5 (0.3 to NR)	0.87
				All patients with L858R variant in tissue			
				41	17.7 (6.3 to 26.8)	17.5 (8.2 to 23.5)	0.67
				Patients with L858R variant in both tissue and in ctDNA			
				29	13.7 (2.6 to 21.9)	12.6 (7.1 to 23.5)	0.67
				Patients with L858R variant in tissue but not in ctDNA			
				12	29.4 (8.6 to 63.0)	25.6 (16.1 to NR)	0.64

CI: confidence interval; ctDNA: circulating tumor DNA; ddPCR: droplet digital polymerase chain reaction; *EGFR*: epidermal growth factor receptor; FDA: Food and Drug Administration; HR: hazard ratio; NR: not reported; OS: overall survival; PFS, progression-free survival; RCT: randomized controlled trial; rt-PCR: real-time polymerase chain reaction; SSED: Summary of Safety and Effectiveness; TKI: tyrosine kinase inhibitor.
[a] Exon 19 deletion or L858R variant.

In Table 7 (sensitizing variants), the SSED document supporting the approval of the cobas EGFR Mutation Test v2, reported clinical outcome data derived from a randomized phase 3 trial of erlotinib vs gemcitabine plus cisplatin as first-line treatment of NSCLC.[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
Oxnard et al (2016)[23]; AURA phase 1 trial of patients who progressed on EGFR TKI	Multinational[b]	Advanced	BEAMing		
				ORR (95% CI) (Osimertinib)	
				Liquid positive, tissue positive	
				108	64% (54% to 73%)
				Liquid positive, tissue negative	
				18	28% (10% to 53%)
				Liquid negative, tissue positive	
				45	69% (53% to 82%)
				Liquid negative, tissue negative	
				40	25% (13% to 41%)
				PFS (95% CI), mo	
				Liquid positive, tissue positive	
				111	9.3 (8.3 to 10.9)
				Liquid positive, tissue negative	
				18	4.2 (1.3 to 5.6)
				Liquid negative, tissue positive	
				47	16.5 (10.9 to NC)
				Liquid negative, tissue negative	
				40	2.8 (1.4 to 4.2)
Thress et al (2015)[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 roceiletinib	U.S., Australia, France, Poland	Advanced	BEAMing		
				ORR (95% CI) (Rociletinib)	
				Liquid positive, tissue positive	
				15	73 (51 to 96)
				Liquid positive, tissue negative	
				4	25 (0 to 67)
				Liquid negative, tissue positive	
				6	50 (10 to 90)
				Liquid negative, tissue negative	
				3	33 (0 to 87)

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=0.001), as was median PFS (16.5 months vs 2.8 months; p=0.001), which is consistent with false-negative ctDNA results. Among patients with T790M-positive ctDNA, ORR and median PFS were higher in 108 patients with T790M-positive tissue (ORR=64%; 95% CI, 54% to 73%; PFS=9.3 months) than in 18 patients with T790M-negative tissue (ORR=28%; 95% CI, 10% to 53%; p=0.004; PFS=4.2 months; p=0.0002) which is consistent with false-positive ctDNA results. The authors concluded that a T790-variant ctDNA assay could be used for osimertinib treatment decisions in patients with acquired *EGFR* TKI resistance and would permit avoiding tissue biopsy for patients with T790M-positive ctDNA results.

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.

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, 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 (range, 60%-80%) 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 1 study, 3 patients with negative tissue biopsies and positive liquid biopsies appeared to respond to *EGFR* TKI inhibitors.

The diagnostic characteristics of liquid biopsy for detection of T790M variants associated with *EGFR* TKI-inhibitor resistance, an indication for treatment with osimertinib, has shown that liquid biopsy is moderately sensitive and moderately specific and thus overall concordance is moderate. Using tissue testing of negative liquid biopsies would increase sensitivity, but because liquid biopsy is not highly specific, it would result in many false-positives. Because not enough data are available to determine whether these false-positives represent a faulty tissue reference standard or are correctly labeled as false-positives, outcomes for these patients are uncertain. In 1 study, 8 patients with negative tissue biopsies but positive liquid biopsies had low response rates consistent with those with negative tissue biopsies; and in the AURA study, 18 patients with liquid-positive, tissue-negative results had a low response rate, also consistent with negative tissue biopsy. In the TIGER-X study, 3 patients who were liquid-positive, tissue-negative had low response rates to rociletinib, similar to the other tissue-negative patients.

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

A chain of evidence based on the sensitivity and specificity of liquid biopsy for the detection of *EGFR* TKI-sensitizing variants for tests with established clinical validity such as the cobas EGFR Mutation Test v2, Guardant360, or OncoBEAM, can support its utility. The body of evidence has demonstrated sensitivity generally between 60% and 80%, with high specificities (>95%). 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 study with liquid-negative, tissue-positive results, these results are consistent with false-negative liquid biopsies. It is unclear whether false-positive liquid biopsies represent errors in the liquid biopsy or inadequacies of a tissue biopsy reference standard. In 3 studies, patients with negative tissue biopsies and positive liquid biopsies appeared not to have a high response to osimertinib or rociletinib, although sample sizes are very small.

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

SUMMARY OF EVIDENCE

For individuals with advanced NSCLC who receive testing for biomarkers of EGFR TKIs sensitivity using ctDNA with the cobas EGFR Mutation Test v2 (liquid biopsy), the evidence includes numerous studies assessing the diagnostic characteristics of liquid biopsy compared with tissue. Relevant outcomes are 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. No RCTs were identified 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 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. No RCTs were identified providing evidence of the clinical utility of these tests. The Guardant360 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 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, 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, 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. No RCTs were found to provide 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. None of the commercially available tests have multiple studies of adequate quality to estimate the performance characteristics with sufficient precision for variants other than *EGFR*. We found no RCTs providing evidence of the clinical utility of those of methods of liquid biopsy. The evidence is insufficient to determine the effects of the technology on health outcomes.

For individuals with advanced NSCLC who progressed on EGFR TKIs who receive testing for biomarkers of EGFR TKI resistance using liquid biopsy, the evidence includes 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. Given the moderate clinical sensitivity and specificity of liquid biopsy, using liquid biopsy alone or in combination with tissue biopsy might result in the selection of different patients testing positive for EGFR TKI resistance. It cannot be determined whether patient outcomes are improved. The evidence is insufficient to determine the effects of the technology on health outcomes.

PRACTICE GUIDELINES AND POSITION STATEMENTS

National Comprehensive Cancer Network

National Comprehensive Cancer Network guidelines (v.6.2018) on the management of non-small-cell lung cancer state that "if repeat biopsy is not feasible, plasma biopsy should be considered," but it is not stated to which biomarkers this statement applies.[24] In the text discussion of osimertinib, the guidelines state that "Data suggest that plasma genotyping (also known as liquid

biopsy or plasmas biopsy) may be considered instead of tissue biopsy to detect whether patients have T790M; however, if the plasma biopsy is negative, then tissue biopsy is recommended if feasible."

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.

Table 9. Summary of Key Trials

NCT No.	Trial Name	Planned Enrollment	Completion Date
Ongoing			
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	260	Oct 2018 (ongoing)
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	530	Nov 2018
NCT01930474	Analysis of plasma tumor DNA in lung cancer patients	200	Dec 2018
NCT02140463	Next generation personalized therapy with plasma DNA Trial 2 in refractory solid tumors (The NEXT-2 Trial)	260	Dec 2018
NCT02284633[a]	Blood sample monitoring of patients with EGFR mutated lung cancer	200	Sep 2019

NCT No.	Trial Name	Planned Enrollment	Completion Date
NCT02160366	Profile Related Evidence to Determine Individualized Cancer Therapy (PREDICT) Program in Advanced Cancer Patients	2000	Sep 2019
Unpublished			
NCT02620527[a]	Study of Concordance Between Circulating Tumor DNA Assay and Foundation One Tissue Analysis For Genomic Alterations	1400	Dec 2017 (completed)
NCT02418234	T790M Mutation on ctDNA in patients with NSCLC after EGFR-TKI failure	314	Nov 2017 (completed)
NCT01710605	Medico-economic interest of taking into account circulating tumor cells (CTCs) to determine the kind of first line treatment for metastatic, hormone-receptors positive breast cancer	1000	Mar 2016 (unknown)

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

CODING

The following codes for treatment and procedures applicable to this policy are included below for informational purposes. Inclusion or exclusion of a procedure, diagnosis or device code(s) does not constitute or imply member coverage or provider reimbursement. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

CPT/HCPCS

81235	EGFR (epidermal growth factor receptor) (eg, non-small cell lung cancer) gene analysis, common variants (eg, exon 19 LREA deletion, L858R, T790M, G719A, G719S, L861Q)
81479	Unlisted molecular pathology procedure
86152	Cell enumeration using immunologic selection and identification in fluid specimen (eg, circulating tumor cells in blood);
86153	Cell enumeration using immunologic selection and identification in fluid specimen (eg, circulating tumor cells in blood); physician interpretation and report, when required

ICD-10 Diagnoses

C34.01	Malignant neoplasm of right main bronchus
C34.02	Malignant neoplasm of left main bronchus
C34.11	Malignant neoplasm of upper lobe, right bronchus or lung
C34.12	Malignant neoplasm of upper lobe, left bronchus or lung
C34.2	Malignant neoplasm of middle lobe, bronchus or lung
C34.31	Malignant neoplasm of lower lobe, right bronchus or lung
C34.32	Malignant neoplasm of lower lobe, left bronchus or lung
C34.81	Malignant neoplasm of overlapping sites of right bronchus and lung
C34.82	Malignant neoplasm of overlapping sites of left bronchus and lung
C34.91	Malignant neoplasm of unspecified part of right bronchus or lung
C34.92	Malignant neoplasm of unspecified part of left bronchus or lung

REVISIONS

12-21-2018	Policy added to the bcbsks.com web site on 11-20-2018 with an effective date of 12-21-2018.

REFERENCES

1. Alix-Panabieres C, Pantel K. Clinical applications of circulating tumor cells and circulating tumor DNA as liquid biopsy. *Cancer Discov.* May 2016;6(5):479-491. PMID 26969689
2. Food and Drug Administration (FDA). cobas® EGFR Mutation Test v2 (P150047). 2016; https://www.accessdata.fda.gov/cdrh_docs/pdf15/P150047a.pdf. Accessed September 24, 2018.
3. Aberle DR, Adams AM, Berg CD, et al. Reduced lung-cancer mortality with low-dose computed tomographic screening. *N Engl J Med.* Aug 4 2011;365(5):395-409. PMID 21714641
4. Lee JK, Hahn S, Kim DW, et al. Epidermal growth factor receptor tyrosine kinase inhibitors vs conventional chemotherapy in non-small cell lung cancer harboring wild-type epidermal growth factor receptor: a meta-analysis. *Jama.* Apr 9 2014;311(14):1430-1437. PMID 24715074
5. Janne PA, Yang JC, Kim DW, et al. AZD9291 in EGFR inhibitor-resistant non-small-cell lung cancer. *N Engl J Med.* Apr 30 2015;372(18):1689-1699. PMID 25923549
6. Socinski MA, Jotte RM, Cappuzzo F, et al. Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. *N Engl J Med.* Jun 14 2018;378(24):2288-2301. PMID 29863955
7. Wu Y, Liu H, Shi X, et al. Can EGFR mutations in plasma or serum be predictive markers of non-small-cell lung cancer? A meta-analysis. *Lung Cancer.* Jun 2015;88(3):246-253. PMID 25837799
8. Jenkins S, Yang JC, Ramalingam SS, et al. Plasma ctDNA analysis for detection of the EGFR T790M mutation in patients with advanced non-small cell lung cancer. *J Thorac Oncol.* Jul 2017;12(7):1061-1070. PMID 28428148
9. Food and Drug Administration. Summary of Safety and Effectiveness Data (SSED) cobas EGFR Mutation Test v2. 2016; https://www.accessdata.fda.gov/cdrh_docs/pdf15/P150047b.pdf. Accessed September 24, 2018.
10. Karlovich C, Goldman JW, Sun JM, et al. Assessment of EGFR mutation status in matched plasma and tumor tissue of NSCLC patients from a phase I study of rociletinib (CO-1686). *Clin Cancer Res.* May 15 2016;22(10):2386-2395. PMID 26747242
11. Thress KS, Brant R, Carr TH, et al. EGFR mutation detection in ctDNA from NSCLC patient plasma: A cross-platform comparison of leading technologies to support the clinical development of AZD9291. *Lung Cancer.* Dec 2015;90(3):509-515. PMID 26494259
12. Mok T, Wu YL, Lee JS, et al. Detection and dynamic changes of EGFR mutations from circulating tumor dna as a predictor of survival outcomes in NSCLC patients treated with first-line intercalated erlotinib and chemotherapy. *Clin Cancer Res.* Jul 15 2015;21(14):3196-3203. PMID 25829397
13. Weber B, Meldgaard P, Hager H, et al. Detection of EGFR mutations in plasma and biopsies from non-small cell lung cancer patients by allele-specific PCR assays. *BMC Cancer.* Apr 28 2014;14:294. PMID 24773774
14. Schwaederle MC, Patel SP, Husain H, et al. Utility of Genomic Assessment of Blood-Derived Circulating Tumor DNA (ctDNA) in Patients with Advanced Lung Adenocarcinoma. *Clin Cancer Res.* Sep 1 2017;23(17):5101-5111. PMID 28539465
15. Thompson JC, Yee SS, Troxel AB, et al. Detection of therapeutically targetable driver and resistance mutations in lung cancer patients by next-generation sequencing of cell-free circulating tumor DNA. *Clin Cancer Res.* Dec 01 2016;22(23):5772-5782. PMID 27601595
16. Villaflor V, Won B, Nagy R, et al. Biopsy-free circulating tumor DNA assay identifies actionable mutations in lung cancer. *Oncotarget.* Oct 11 2016;7(41):66880-66891. PMID 27602770
17. Ramalingam SS, Yang JC, Lee CK, et al. Osimertinib as first-line treatment of EGFR mutation-positive advanced non-small-cell lung cancer. *J Clin Oncol.* Mar 20 2018;36(9):841-849. PMID 28841389
18. Mellert H, Foreman T, Jackson L, et al. Development and clinical utility of a blood-based test service for the rapid identification of actionable mutations in non-small cell lung carcinoma. *J Mol Diagn.* May 2017;19(3):404-416. PMID 28433077

19. Paweletz CP, Sacher AG, Raymond CK, et al. Bias-corrected targeted next-generation sequencing for rapid, multiplexed detection of actionable alterations in cell-free DNA from advanced lung cancer patients. *Clin Cancer Res.* Feb 15 2016;22(4):915-922. PMID 26459174
20. Sacher AG, Paweletz C, Dahlberg SE, et al. Prospective validation of rapid plasma genotyping for the detection of EGFR and KRAS mutations in advanced lung cancer. *JAMA Oncol.* Aug 01 2016;2(8):1014-1022. PMID 27055085
21. Zhang Y, Xu Y, Zhong W, et al. Total DNA input is a crucial determinant of the sensitivity of plasma cell-free DNA EGFR mutation detection using droplet digital PCR. *Oncotarget.* Jan 24 2017;8(4):5861-5873. PMID 28052016
22. Karachaliou N, Mayo-de las Casas C, Queralt C, et al. Association of EGFR L858R mutation in circulating free DNA with survival in the EURTAC Trial. *JAMA Oncol.* May 2015;1(2):149-157. PMID 26181014
23. Oxnard GR, Thress KS, Alden RS, et al. Association between plasma genotyping and outcomes of treatment with osimertinib (AZD9291) in advanced non-small-cell lung cancer. *J Clin Oncol.* Oct 01 2016;34(28):3375-3382. PMID 27354477
24. National Comprehensive Cancer Network (NCCN). NCCN Clinical Practice Guidelines in Oncology: Non-small Cell Lung Cancer. Version 6.2018. https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf. Accessed September 9, 2018.
25. Rolfo C, Mack PC, Scagliotti GV, et al. Liquid biopsy for advanced non-small cell lung cancer (NSCLC): a statement paper from the IASLC. *J Thorac Oncol.* Sep 2018;13(9):1248-1268. PMID 29885479
26. Huang JS, Dong QG, Xu KL, et al. [Epidermal growth factor receptor mutation in serum circulating DNA and selective targeting therapy against lung cancer] [Chinese]. *Tumor.* 2007;27:968-972. PMID
27. Ohira T, Sakai K, Matsubayashi J, et al. Tumor volume determines the feasibility of cell-free DNA sequencing for mutation detection in non-small cell lung cancer. *Cancer Sci.* Nov 2016;107(11):1660-1666. PMID 27575703
28. Guo N, Lou F, Ma Y, et al. Circulating tumor DNA detection in lung cancer patients before and after surgery. *Sci Rep.* Sep 19 2016;6:33519. PMID 27641744
29. Sundaresan TK, Sequist LV, Heymach JV, et al. Detection of T790M, the acquired resistance EGFR mutation, by tumor biopsy versus noninvasive blood-based analyses. *Clin Cancer Res.* Mar 01 2016;22(5):1103-1110. PMID 26446944
30. Takahama T, Sakai K, Takeda M, et al. Detection of the T790M mutation of EGFR in plasma of advanced non-small cell lung cancer patients with acquired resistance to tyrosine kinase inhibitors (West Japan oncology group 8014LTR study). *Oncotarget.* Sep 06 2016;7(36):58492-58499. PMID 27542267
31. Chen KZ, Lou F, Yang F, et al. Circulating tumor DNA detection in early-stage non-small cell lung cancer patients by targeted sequencing. *Sci Rep.* Aug 24 2016;6:31985. PMID 27555497
32. Que D, Xiao H, Zhao B, et al. EGFR mutation status in plasma and tumor tissues in non-small cell lung cancer serves as a predictor of response to EGFR-TKI treatment. *Cancer Biol Ther.* Jan 2016;17(3):320-327. PMID 26785777
33. Vazquez S, Casal J, Afonso Afonso FJ, et al. EGFR testing and clinical management of advanced NSCLC: a Galician Lung Cancer Group study (GGCP 048-10). *Cancer Manag Res.* Feb 2016;8:11-20. PMID 26893581
34. Han JY, Choi JJ, Kim JY, et al. PNA clamping-assisted fluorescence melting curve analysis for detecting EGFR and KRAS mutations in the circulating tumor DNA of patients with advanced non-small cell lung cancer. *BMC Cancer.* Aug 12 2016;16:627. PMID 27519791
35. Kimura H, Nishikawa S, Koba H, et al. A rapid and sensitive method for detection of the T790M mutation of EGFR in plasma DNA. *Adv Exp Med Biol.* 2016;924:171-174. PMID 27753039
36. Ma M, Shi C, Qian J, et al. Comparison of plasma and tissue samples in epidermal growth factor receptor mutation by ARMS in advanced non-small cell lung cancer. *Gene.* Oct 10 2016;591(1):58-64. PMID 27370697
37. Xu S, Lou F, Wu Y, et al. Circulating tumor DNA identified by targeted sequencing in advanced-stage non-small cell lung cancer patients. *Cancer Lett.* Jan 28 2016;370(2):324-331. PMID 26582655
38. Duan H, Lu J, Lu T, et al. Comparison of EGFR mutation status between plasma and tumor tissue in non-small cell lung cancer using the Scorpion ARMS method and the possible prognostic significance

of plasma EGFR mutation status. *Int J Clin Exp Pathol.* Jan 2015;8(10):13136-13145. PMID 26722512
39. Lam DC, Tam TC, Lau KM, et al. Plasma EGFR mutation detection associated with survival outcomes in advanced-stage lung cancer. *Clin Lung Cancer.* Nov 2015;16(6):507-513. PMID 26239567
40. Jing CW, Wang Z, Cao HX, et al. High resolution melting analysis for epidermal growth factor receptor mutations in formalin-fixed paraffin-embedded tissue and plasma free DNA from non-small cell lung cancer patients. *Asian Pac J Cancer Prev.* Jan 2014;14(11):6619-6623. PMID 24377577
41. Wang S, Han X, Hu X, et al. Clinical significance of pretreatment plasma biomarkers in advanced non-small cell lung cancer patients. *Clin Chim Acta.* Mar 20 2014;430:63-70. PMID 24378285
42. Li X, Ren R, Ren S, et al. Peripheral blood for epidermal growth factor receptor mutation detection in non-small cell lung cancer patients. *Transl Oncol.* Jun 2014;7(3):341-348. PMID 25180058
43. Douillard JY, Ostoros G, Cobo M, et al. Gefitinib treatment in EGFR mutated caucasian NSCLC: circulating-free tumor DNA as a surrogate for determination of EGFR status. *J Thorac Oncol.* Sep 2014;9(9):1345-1353. PMID 25122430
44. Kim HR, Lee SY, Hyun DS, et al. Detection of EGFR mutations in circulating free DNA by PNA-mediated PCR clamping. *J Exp Clin Cancer Res.* Aug 09 2013;32(1):50. PMID 23927790
45. Kim ST, Sung JS, Jo UH, et al. Can mutations of EGFR and KRAS in serum be predictive and prognostic markers in patients with advanced non-small cell lung cancer (NSCLC)? *Med Oncol.* Mar 2013;30(1):328. PMID 23307237
46. Lv C, Ma Y, Feng Q, et al. A pilot study: sequential gemcitabine/cisplatin and icotinib as induction therapy for stage IIB to IIIA non-small-cell lung adenocarcinoma. *World J Surg Oncol.* Apr 26 2013;11:96. PMID 23621919
47. Akca H, Demiray A, Yaren A, et al. Utility of serum DNA and pyrosequencing for the detection of EGFR mutations in non-small cell lung cancer. *Cancer Genet.* Mar 2013;206(3):73-80. PMID 23491080
48. Liu X, Lu Y, Zhu G, et al. The diagnostic accuracy of pleural effusion and plasma samples versus tumour tissue for detection of EGFR mutation in patients with advanced non-small cell lung cancer: comparison of methodologies. *J Clin Pathol.* Dec 2013;66(12):1065-1069. PMID 23888061
49. Zhang H, Liu D, Li S, et al. Comparison of EGFR signaling pathway somatic DNA mutations derived from peripheral blood and corresponding tumor tissue of patients with advanced non-small-cell lung cancer using liquidchip technology. *J Mol Diagn.* Nov 2013;15(6):819-826. PMID 23988622
50. Zhao X, Han RB, Zhao J, et al. Comparison of epidermal growth factor receptor mutation statuses in tissue and plasma in stage I-IV non-small cell lung cancer patients. *Respiration.* Jul 2013;85(2):119-125. PMID 22797485
51. Goto K, Ichinose Y, Ohe Y, et al. Epidermal growth factor receptor mutation status in circulating free DNA in serum: from IPASS, a phase III study of gefitinib or carboplatin/paclitaxel in non-small cell lung cancer. *J Thorac Oncol.* Jan 2012;7(1):115-121. PMID 21900837
52. Nakamura T, Sueoka-Aragane N, Iwanaga K, et al. Application of a highly sensitive detection system for epidermal growth factor receptor mutations in plasma DNA. *J Thorac Oncol.* Sep 2012;7(9):1369-1381. PMID 22858585
53. Xu F, Wu J, Xue C, et al. Comparison of different methods for detecting epidermal growth factor receptor mutations in peripheral blood and tumor tissue of non-small cell lung cancer as a predictor of response to gefitinib. *Onco Targets Ther.* Dec 2012;5:439-447. PMID 23251095
54. Yam I, Lam DC, Chan K, et al. EGFR array: uses in the detection of plasma EGFR mutations in non-small cell lung cancer patients. *J Thorac Oncol.* Jul 2012;7(7):1131-1140. PMID 22610259
55. Punnoose EA, Atwal S, Liu W, et al. Evaluation of circulating tumor cells and circulating tumor DNA in non-small cell lung cancer: association with clinical endpoints in a phase II clinical trial of pertuzumab and erlotinib. *Clin Cancer Res.* Apr 15 2012;18(8):2391-2401. PMID 22492982
56. Huang Z, Wang Z, Bai H, et al. The detection of EGFR mutation status in plasma is reproducible and can dynamically predict the efficacy of EGFR-TKI. *Thorac Cancer.* Nov 2012;3(4):334-340. PMID 28920271
57. Chen YM, Fan WC, Tseng PC, et al. Plasma epidermal growth factor receptor mutation analysis and possible clinical applications in pulmonary adenocarcinoma patients treated with erlotinib. *Oncol Lett.* Mar 2012;3(3):713-717. PMID 22740981

58. Hu C, Liu X, Chen Y, et al. Direct serum and tissue assay for EGFR mutation in non-small cell lung cancer by high-resolution melting analysis. *Oncol Rep.* Nov 2012;28(5):1815-1821. PMID 22923193
59. Brevet M, Johnson ML, Azzoli CG, et al. Detection of EGFR mutations in plasma DNA from lung cancer patients by mass spectrometry genotyping is predictive of tumor EGFR status and response to EGFR inhibitors. *Lung Cancer.* Jul 2011;73(1):96-102. PMID 21130517
60. Jiang B, Liu F, Yang L, et al. Serum detection of epidermal growth factor receptor gene mutations using mutant-enriched sequencing in Chinese patients with advanced non-small cell lung cancer. *J Int Med Res.* 2011;39(4):1392-1401. PMID 21986139
61. Sriram KB, Tan ME, Savarimuthu SM, et al. Screening for activating EGFR mutations in surgically resected nonsmall cell lung cancer. *Eur Respir J.* Oct 2011;38(4):903-910. PMID 21349912
62. Yasuda H, Soejima K, Nakayama S, et al. Bronchoscopic microsampling is a useful complementary diagnostic tool for detecting lung cancer. *Lung Cancer.* Apr 2011;72(1):32-38. PMID 20813423
63. Taniguchi K, Uchida J, Nishino K, et al. Quantitative detection of EGFR mutations in circulating tumor DNA derived from lung adenocarcinomas. *Clin Cancer Res.* Dec 15 2011;17(24):7808-7815. PMID 21976538
64. Song G, Ren J, Zhang L, et al. Low correspondence of EGFR mutations in tumor tissue and paired serum of non-small-cell lung cancer patients. *Chin J Cancer Res.* 2010;22:27–31. PMID
65. Bai H, Mao L, Wang HS, et al. Epidermal growth factor receptor mutations in plasma DNA samples predict tumor response in Chinese patients with stages IIIB to IV non-small-cell lung cancer. *J Clin Oncol.* Jun 01 2009;27(16):2653-2659. PMID 19414683
66. Yung TK, Chan KC, Mok TS, et al. Single-molecule detection of epidermal growth factor receptor mutations in plasma by microfluidics digital PCR in non-small cell lung cancer patients. *Clin Cancer Res.* Mar 15 2009;15(6):2076-2084. PMID 19276259
67. Mack PC, Holland WS, Burich RA, et al. EGFR mutations detected in plasma are associated with patient outcomes in erlotinib plus docetaxel-treated non-small cell lung cancer. *J Thorac Oncol.* Dec 2009;4(12):1466-1472. PMID 19884861
68. He C, Liu M, Zhou C, et al. Detection of epidermal growth factor receptor mutations in plasma by mutant-enriched PCR assay for prediction of the response to gefitinib in patients with non-small-cell lung cancer. *Int J Cancer.* Nov 15 2009;125(10):2393-2399. PMID 19530244
69. Kuang Y, Rogers A, Yeap BY, et al. Noninvasive detection of EGFR T790M in gefitinib or erlotinib resistant non-small cell lung cancer. *Clin Cancer Res.* Apr 15 2009;15(8):2630-2636. PMID 19351754
70. Maheswaran S, Sequist LV, Nagrath S, et al. Detection of mutations in EGFR in circulating lung-cancer cells. *N Engl J Med.* Jul 24 2008;359(4):366-377. PMID 18596266
71. Kimura H, Suminoe M, Kasahara K, et al. Evaluation of epidermal growth factor receptor mutation status in serum DNA as a predictor of response to gefitinib (IRESSA). *Br J Cancer.* Sep 17 2007;97(6):778-784. PMID 17848912
72. Kimura H, Kasahara K, Shibata K, et al. EGFR mutation of tumor and serum in gefitinib-treated patients with chemotherapy-naive non-small cell lung cancer. *J Thorac Oncol.* Mar 2006;1(3):260-267. PMID 17409866
73. QUADAS-2. n.d.; http://www.bristol.ac.uk/social-community-medicine/projects/quadas/quadas-2/. Accessed September 24, 2018.
74. Reitsma JB, Glas AS, Rutjes AW, et al. Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. *J Clin Epidemiol.* Oct 2005;58(10):982-990. PMID 16168343
75. Harbord RM, Deeks JJ, Egger M, et al. A unification of models for meta-analysis of diagnostic accuracy studies. *Biostatistics.* Apr 2007;8(2):239-251. PMID 16698768
76. R Foundation. The R Project for Statistical Computing (version 3.1.2). 2014; https://www.r-project.org/. Accessed September 24, 2018.
77. Zwinderman AH, Bossuyt PM. We should not pool diagnostic likelihood ratios in systematic reviews. *Stat Med.* Feb 28 2008;27(5):687-697. PMID 17611957