2.04.151	Germline and Somatic Biomarker Testing (Including Liquid Biopsy) for Targeted Treatment and Immunotherapy in Breast Cancer		
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Policy Statement

***BRCA1 and BRCA2* Testing**

Genetic testing for *BRCA1* or *BRCA2* germline variants may be considered **medically necessary** to predict treatment response to PARP inhibitors (e.g., olaparib [Lynparza] and talazoparib [Talzenna]) for human epidermal receptor 2 (HER2)-negative metastatic and early stage, high-risk breast cancer (see Policy Guidelines).

Genetic testing of *BRCA1* or *BRCA2* germline or somatic variants in patients with breast cancer for guiding therapy is considered **investigational** in all other situations unless included in a panel approved under another policy.

***PIK3CA* Testing**

PIK3CA testing may be **medically necessary** to predict treatment response to alpelisib (Piqray) in patients with hormone receptor-positive, HER2-negative advanced or metastatic breast cancer (see Policy Guidelines).

PIK3CA testing of tissue is considered **investigational** in all other situations unless included in a panel approved under another policy.

***NTRK* Gene Fusion Testing**

Analysis of *NTRK* gene fusions may be considered **medically necessary** to predict treatment response to entrectinib (Rozlytrek) or larotrectinib (Vitrakvi) in patients with locally advanced or metastatic breast cancer that has progressed following standard treatment and who have no alternative treatment option (see Policy Guidelines).

Analysis of *NTRK* gene fusions is considered **investigational** in all other situations unless included in a panel approved under another policy.

PD-L1 Testing

PD-L1 testing may be considered **medically necessary** to predict treatment response to pembrolizumab (Keytruda) in patients with hormone receptor-negative/HER2-negative (triple negative) recurrent or metastatic breast cancer (see Policy Guidelines).

PD-L1 testing is considered **investigational** in all other situations, including to predict treatment response to atezolizumab (Tecentriq) unless included in a panel approved under another policy.

MSI-H/dMMR Testing

MSI-H/dMMR testing may be considered **medically necessary** to predict treatment response to pembrolizumab (Keytruda) in patients with unresectable or metastatic breast cancer that has progressed following standard treatment and who have no alternative treatment option (see Policy Guidelines).

MSI-H/dMMR testing is considered **investigational** in all other situations, including to predict treatment response to dostarlimab-gxly (Jemperli) unless included in a panel approved under another policy.

Ki-67 testing

Ki-67 testing to predict treatment response to abemaciclib (Verzenio) in patients with breast cancer is considered **investigational** unless included in a panel approved under another policy.

Tumor Mutational Burden Testing
Tumor mutational burden testing to predict response to immunotherapy in patients with breast cancer is considered **investigational.**

Circulating Tumor DNA Testing (Liquid Biopsy)
PIK3CA testing using FoundationOne Liquid CDx (FDA approved companion test) may be considered **medically necessary** to predict treatment response to alpelisib (Piqray) in patients with hormone receptor-positive, HER2 negative advanced or metastatic breast cancer (see Policy Guidelines) when there is insufficient tissue to be tested and an additional invasive procedure would be required otherwise.

Circulating tumor DNA testing is considered **investigational** in all other situations unless included in a panel approved under another policy, such as use in Non-Small Cell Lung Cancer (NSCLC).

Circulating Tumor Cell Testing
Analysis of circulating tumor cells to select treatment in patients with breast cancer is considered **investigational** (see Background section).

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

Policy Guidelines

See U.S. Food and Drug Administration labels, clinical trials, and NCCN guidelines for specific population descriptions. Descriptions varied slightly across sources.

Breast Cancer Risk Groups
In the OlympiA trial, patients with HER2-negative early-stage breast cancer (Clinical Stage I-III) and germline *BRCA1/2* mutations treated with (neo)adjuvant chemotherapy were considered at high risk of recurrent disease when the following eligibility criteria were met for treatment with olaparib (Tutt et al, 2021; PMID 34081848):

- Patients with triple-negative breast cancer who were treated with adjuvant chemotherapy were required to have axillary node-positive disease or an invasive primary tumor measuring at least 2 cm on pathological analysis. Patients treated with neoadjuvant chemotherapy were required to have not achieved pathological complete response.
- Patients treated with adjuvant chemotherapy for hormone receptor (HR)-positive, HER2-negative breast cancer were required to have at least 4 pathologically confirmed positive lymph nodes. Those treated with neoadjuvant chemotherapy were required to have not achieved a pathological complete response with a clinical stage, pathologic stage, estrogen receptor status, and tumor grade (CPS+EG) score of 3 or higher (Table PG1). This scoring system estimates relapse probability on the basis of clinical and pathological stage (CPS) and estrogen-receptor status and histologic grade (EG). Scores range from 0 to 6, with higher scores reflecting a worse prognosis.

Table PG1. CPS+EG Score[a,b]

Stage or Feature	Points
Clinical Stage (AJCC Staging)	
I	0
IIA	0
IIB	1
IIIA	1
IIIB	2
IIIC	2
Pathologic Stage (AJCC Staging)	
0	0
I	0

Stage or Feature	Points
IIA	1
IIB	1
IIIA	1
IIIB	1
IIIC	2
Receptor Status	
ER-negative	1
Nuclear Grade	
Nuclear grade 3	1

AJCC: American Joint Committee on Cancer; CPS+EG: clinical stage, pathologic stage, ER status, and tumor grade; ER: estrogen receptor.
[a] Adapted from Tung et al (2021; PMID 34343058).
[b] Add points for clinical stage, pathologic stage, ER status, and nuclear grade to yield a sum between 0 and 6.

Paired Genetic Testing

Testing for genetic changes in tumor tissue assesses somatic changes. However, most somatic testing involves a paired blood analysis in order to distinguish whether findings in tumor tissue are acquired somatic changes or inherited germline changes. As such, simultaneous sequencing of tumor and normal tissue can recognize potential secondary germline changes that may identify risk for other cancers as well as identify risk for relatives. Thus, some laboratories offer concurrent full germline and somatic testing or paired tumor sequencing and germline sequencing, through large panels of germline and somatic variants. For paired panel testing involving germline components, see Blue Shield of California Medical Policy: Genetic Cancer Susceptibility Panels Using Next-Generation Sequencing. For paired panel testing involving somatic components, see Blue Shield of California Medical Policy: Comprehensive Genomic Profiling for Selecting Targeted Cancer Therapies.

Genetics Nomenclature Update

The Human Genome Variation Society nomenclature is used to report information on variants found in DNA and serves as an international standard in DNA diagnostics. It is being implemented for genetic testing medical evidence review updates starting in 2017 (see Table PG2). The Society's nomenclature is recommended by the Human Variome Project, the HUman Genome Organization, and by the Human Genome Variation Society itself.

The American College of Medical Genetics and Genomics and the Association for Molecular Pathology standards and guidelines for interpretation of sequence variants represent expert opinion from both organizations, in addition to the College of American Pathologists. These recommendations primarily apply to genetic tests used in clinical laboratories, including genotyping, single genes, panels, exomes, and genomes. Table PG3 shows the recommended standard terminology- "pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign"- to describe variants identified that cause Mendelian disorders.

Table PG2. 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 PG3. 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-AMP: American College of Medical Genetics and Genomics and the Association for Molecular Pathology.

Genetic Counseling

Genetic counseling is primarily aimed at patients who are at risk for inherited disorders, and experts recommend formal genetic counseling in most cases when genetic testing for an inherited condition is considered. The interpretation of the results of genetic tests and the understanding of risk factors can be very difficult and complex. Therefore, genetic counseling will assist individuals in understanding the possible benefits and harms of genetic testing, including the possible impact of the information on the individual's family. Genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing. Genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

Note: The use of PARP inhibitors (e.g., Lynparza/olaparib or talazoparib) in HER2-negative metastatic breast cancer with a germline BRCA mutation, is sometimes based on germline rather than somatic mutations in BRCA. Both may be tested as well as HER2 somatic tumor testing. Myriad myChoice (CPT 0172U) may be used for somatic BRCA testing (esp. for ovarian cancer) and BRACAnalysis CDx (Myriad Genetic Laboratories) may be used for germline BRCA testing to help determine eligible patients.

Coding

The following CPT codes may be used for this genomic sequence analysis:

- **0037U**: Targeted genomic sequence analysis, solid organ neoplasm, DNA analysis of 324 genes, interrogation for sequence variants, gene copy number amplifications, gene rearrangements, microsatellite instability and tumor mutational burden (PLA for the FoundationOne CDx™ (F1CDx®) test)
- **0155U:** Oncology (breast cancer), DNA, PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha) (e.g., breast cancer) gene analysis (i.e., p.C420R, p.E542K, p.E545A, p.E545D [g.1635G>T only], p.E545G, p.E545K, p.Q546E, p.Q546R, p.H1047L, p.H1047R, p.H1047Y), utilizing formalin-fixed paraffin-embedded breast tumor tissue, reported as PIK3CA gene mutation status (PLA code for the therascreen® PIK3CA RGQ PCR Kit from QIAGEN)
- **0177U:** Oncology (breast cancer), DNA, PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha) gene analysis of 11 gene variants utilizing plasma, reported as PIK3CA gene mutation status (PLA code for the therascreen® PIK3CA RGQ PCR Kit test from QIAGEN)
- **81309:** PIK3CA (phosphatidylinositol-4, 5-biphosphate 3-kinase, catalytic subunit alpha) (e.g., colorectal and breast cancer) gene analysis, targeted sequence analysis (e.g., exons 7, 9, 20)

The following Molecular Pathology codes support Neurotrophic receptor tyrosine kinase (NTRK) gene testing:

- **81191:** NTRK1 (neurotrophic receptor tyrosine kinase 1) (e.g., solid tumors) translocation analysis
- **81192:** NTRK2 (neurotrophic receptor tyrosine kinase 2) (e.g., solid tumors) translocation analysis
- **81193:** NTRK3 (neurotrophic receptor tyrosine kinase 3) (e.g., solid tumors) translocation analysis
- **81194:** NTRK (neurotrophic-tropomyosin receptor tyrosine kinase 1, 2, and 3) (e.g., solid tumors) translocation analysis

Testing for variants in the other genes listed above would be reported with the following code:

- **81479**: Unlisted molecular pathology procedure

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

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

Effective October 1, 2021, there is a new CPT code that represents Oncosignal 7-Pathway version for Breast Cancer and Other Cancers by Protean BioDiagnostics. Per the manufacturer, this MAAA test is used after cancer diagnosis (various cancer types) to affect the course of treatment based on the activity of the signaling pathways tested by Oncosignal:

- **0262U:** Oncology (solid tumor), gene expression profiling by real-time RT-PCR of 7 gene pathways (ER, AR, PI3K, MAPK, HH, TGFB, Notch), formalin-fixed paraffin-embedded (FFPE), algorithm reported as gene pathway activity score

Description

Multiple biomarkers are being evaluated to predict response to targeted treatments and immunotherapy for patients with advanced or high-risk breast cancer. These include tissue-based testing as well as circulating tumor DNA and circulating tumor cell testing (known as liquid biopsy).

The objective of this evidence review is to examine whether biomarker testing for *BRCA* variants, *PIK3CA*, *NTRK gene fusions*, PD-L1, MSI-H/dMMR, Ki-67, TMB, circulating tumor DNA, or circulating tumor cells improves the net health outcome in patients with breast cancer who are considering targeted therapy or immunotherapy.

Related Policies

- Assays of Genetic Expression in Tumor Tissue as a Technique to Determine Prognosis in Patients with Breast Cancer
- Circulating Tumor DNA and Circulating Tumor Cells for Cancer Management (Liquid Biopsy)
- Comprehensive Genomic Profiling for Selecting Targeted Cancer Therapies
- Germline Genetic Testing for BRCA1 or BRCA2 for Hereditary Breast/Ovarian Cancer Syndrome and Other High-Risk Cancers

Benefit Application

Benefit determinations should be based in all cases on the applicable contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

Some state or federal mandates (e.g., Federal Employee Program [FEP]) prohibits plans from denying Food and Drug Administration (FDA)-approved technologies as investigational. In these instances, plans may have to consider the coverage eligibility of FDA-approved technologies on the basis of medical necessity alone.

Regulatory Status

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. Laboratories that offer laboratory-developed tests must be licensed by the Clinical Laboratory Improvement Amendments for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of these tests.

Table 1 summarizes available targeted treatments with FDA approval for breast cancer (including immunotherapy) and the FDA cleared or approved companion diagnostic tests associated with each. An up-to-date list of FDA cleared or approved companion diagnostics is available at https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools.

Table 1. Targeted Treatments for Metastatic Breast Cancer and FDA Approved Companion Diagnostic Tests

Treatment	Class	Indications in Breast Cancer	Companion Diagnostic
Abemaciclib (Verzenio)	Cyclin-dependent kinase (CDK) 4/6 inhibitor	• In combination with endocrine therapy (tamoxifen or an aromatase inhibitor) for the adjuvant treatment of adult patients with HR-positive, HER2-negative, node-positive, early breast cancer at high risk of recurrence and a Ki-67 score ≥20% as determined by an FDA approved test. • In combination with an aromatase inhibitor as initial endocrine-based therapy for the treatment of postmenopausal women, and men, with HR-positive, HER2-negative advanced or metastatic breast cancer. • In combination with fulvestrant for the treatment of adult patients with HR-positive, HER2-negative advanced or metastatic breast cancer with disease progression following endocrine therapy. • As monotherapy for the treatment of adult patients with HR-positive, HER2-negative advanced or metastatic breast cancer with disease progression following endocrine therapy and prior chemotherapy in the metastatic setting.	Ki-67 IHC MIB-1 pharmDx (Dako Omnis)
Dostarlimab-gxly (Jemperli)	PD-1 blocking antibody	Adult patients with dMMR recurrent or advanced solid tumors, as determined by an FDA-approved test, that has progressed on or following prior treatment and who have no satisfactory alternative treatment options	VENTANA MMR RxDx Panel
Ado-trastuzumab emtansine (Kadcyla)	HER2-targeted antibody and microtubule inhibitor conjugate	As a single agent, for: • Treatment of patients with HER2-positive, metastatic breast cancer who previously received trastuzumab and a taxane, separately or in combination. Patients should have either: o received prior therapy for metastatic disease, or o developed disease recurrence during or within 6 months of completing adjuvant therapy.	FoundationOne CDx HER2 FISH pharmDx Kit HercepTest INFORM HER2 Dual ISH DNA Probe Cocktail PATHWAY anti-Her2/neu (4B5) Rabbit Monoclonal Primary Antibody

Treatment	Class	Indications in Breast Cancer	Companion Diagnostic
		• Adjuvant treatment of patients with HER2-positive early breast cancer who have residual invasive disease after neoadjuvant taxane and trastuzumab-based treatment.	
Alpelisib (Piqray)	Kinase inhibitor	In combination with fulvestrant for the treatment of postmenopausal women, and men, with HR positive, HER2 - negative, PIK3CA-mutated, advanced or metastatic breast cancer as detected by an FDA approved test following progression on or after an endocrine-based regimen	FoundationOne CDx FoundationOne Liquid CDx therascreen PIK3CA RGQ PCR Kit
Entrectinib (Rozlytrek)	Kinase inhibitor	Adult and pediatric patients 12 years of age and older with solid tumors that: • have a NTRK gene fusion without a known acquired resistance mutation, • are metastatic or where surgical resection is likely to result in severe morbidity, and • have progressed following treatment or have no satisfactory alternative therapy	No FDA approved companion diagnostic test
Larotrectinib (Vitrakvi)	Kinase inhibitor	Adult and pediatric patients 12 years of age and older with solid tumors that: • have a NTRK gene fusion without a known acquired resistance mutation, • are metastatic or where surgical resection is likely to result in severe morbidity, and • have progressed following treatment or have no satisfactory alternative therapy	FoundationOne CDx
Olaparib (Lynparza)	PARP inhibitor	Adult patients with deleterious or suspected deleterious germline BRCA mutated, HER2-negative metastatic breast cancer who have been treated with chemotherapy in the neoadjuvant, adjuvant or metastatic setting. Patients with HR -positive breast cancer should have been treated with a prior endocrine therapy or be considered inappropriate for endocrine therapy. Select patients for therapy based on an FDA approved companion diagnostic for Lynparza.	BRACAnalysis CDx
Pembrolizumab (Keytruda)	PD-L1-blocking antibody	• in combination with chemotherapy, for the treatment of patients with locally recurrent unresectable or metastatic TNBC whose tumors express PD-L1 as determined by an FDA approved test	PD-L1 IHC 22C3 pharmDx
		• Adult and pediatric patients with unresectable or metastatic, microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options	No FDA approved companion diagnostic test

Treatment	Class	Indications in Breast Cancer	Companion Diagnostic
		• Unresectable or metastatic tumor mutational burden-high (≥10 mutations/megabase) solid tumors, as determined by an FDA approved test, that have progressed following prior treatment and who have no satisfactory alternative treatment options.	FoundationOne CDx (Solid tumors TMB ≥ 10 mutations per megabase)
Pertuzumab (Perjeta)	HER2/neu receptor antagonist	• Use in combination with trastuzumab and docetaxel for treatment of patients with HER2-positive metastatic breast cancer who have not received prior anti-HER2 therapy or chemotherapy for metastatic disease. • Use in combination with trastuzumab and chemotherapy as o neoadjuvant treatment of patients with HER2-positive, locally advanced, inflammatory, or early stage breast cancer (either greater than 2 cm in diameter or node positive) as part of a complete treatment regimen for early breast cancer. o adjuvant treatment of patients with HER2-positive early breast cancer at high risk of recurrence	HER2 FISH pharmDx Kit HercepTest FoundationOne CDx
Talzenna (Talazoparib)	PARP inhibitor	Adult patients with deleterious or suspected deleterious germline BRCA-mutated HER2-negative locally advanced or metastatic breast cancer.	BRACAnalysis CDx
Trastuzumab (Herceptin)	HER2/neu receptor antagonist	The treatment of HER2-overexpressing breast cancer	Bond Oracle HER2 IHC System FoundationOne CDx HER2 CISH pharmDx Kit HER2 FISH pharmDx Kit HercepTest INFORM HER-2/neu INFORM HER2 Dual ISH DNA Probe Cocktail InSite Her-2/neu KIT PathVysion HER-2 DNA Probe Kit PATHWAY anti-Her2/neu (4B5) Rabbit Monoclonal Primary Antibody SPOT-LIGHT HER2 CISH Kit VENTANA HER2 Dual ISH DNA Probe Cocktail

dMMR: mismatch repair deficient; FDA: U.S. Food & Drug Administration; HER2: human epidermal growth factor receptor 2; HR: hormone receptor; MSI-H: microsatellite instability-high; NTRK: neurotrophic-tropomyosin receptor kinase; PD-1: programmed death receptor-1; D-L1: programmed death-ligand 1 ; PIK3CA: phosphatidylinositol 3-kinase catalytic alpha polypeptide; TNBC: triple-negative breast cancer
Sources: [17,18.]

In August 2021, Genentech voluntarily withdrew accelerated approval of atezolizumab (Tecentriq) for use in patients with PD-L1 positive, triple-negative breast cancer following FDA assessment of confirmatory trial results.

Rationale

Background

BRCA Variant Testing

The prevalence of *BRCA* variants is approximately 0.1% to 0.2% in the general population. The prevalence may be much higher for particular ethnic groups with characterized founder mutations (e.g., 2.5% [1/40] in the Ashkenazi Jewish population). Family history of breast and ovarian cancer is an important risk factor for the *BRCA* variant; additionally, age and ethnicity could be independent risk factors.

Several genetic syndromes with an autosomal dominant pattern of inheritance that features breast cancer have been identified. Of these, hereditary breast and ovarian cancer (HBOC) and some cases of hereditary site-specific breast cancer have in common causative variants in *BRCA* (breast cancer susceptibility) genes. Families suspected of having HBOC syndrome are characterized by an increased susceptibility to breast cancer occurring at a young age, bilateral breast cancer, male breast cancer, ovarian cancer at any age, as well as cancer of the fallopian tube and primary peritoneal cancer. Other cancers, such as prostate cancer, pancreatic cancer, gastrointestinal cancers, melanoma, and laryngeal cancer, occur more frequently in HBOC families. Hereditary site-specific breast cancer families are characterized by early-onset breast cancer with or without male cases, but without ovarian cancer. For this evidence review, BCBSA refers collectively to both as *hereditary breast and/or ovarian cancer*.

Germline variants in the *BRCA1* and *BRCA2* genes are responsible for the cancer susceptibility in most HBOC families, especially if ovarian cancer or male breast cancer are features. However, in site-specific cancer, BRCA variants are responsible only for a proportion of affected families. *BRCA* gene variants are inherited in an autosomal dominant fashion through maternal or paternal lineage. It is possible to test for abnormalities in *BRCA1* and *BRCA2* genes to identify the specific variant in cancer cases and to identify family members at increased cancer risk. Family members without existing cancer who are found to have *BRCA* variants can consider preventive interventions for reducing risk and mortality.

Young age of onset of breast cancer, even in the absence of family history, is a risk factor for *BRCA1* variants. Winchester (1996) estimated that hereditary breast cancers account for 36% to 85% of patients diagnosed before age 30.[1,] In several studies, BRCA variants were independently predicted by early age at onset, being present in 6% to 10% of breast cancer cases diagnosed at ages younger than various premenopausal age cutoffs (age range, 35-50 years).[1,2,3,4,] In cancer-prone families, the mean age of breast cancer diagnosis among women carrying *BRCA1* or *BRCA2* variants is in the 40s.[5,] In the Ashkenazi Jewish population, Frank et al (2002) reported that 13% of 248 cases with no known family history and diagnosed before 50 years of age had *BRCA* variants.[2,] In a similar study by Gershoni-Baruch et al (2000), 31% of Ashkenazi Jewish women, unselected for family history, diagnosed with breast cancer at younger than 42 years of age had *BRCA* variants.[6,] Other studies have indicated that early age of breast cancer diagnosis is a significant predictor of BRCA variants in the absence of family history in this population.[7,8,9,]

In patients with “triple-negative” breast cancer (i.e., negative for expression of estrogen, progesterone, and overexpression of human epidermal growth factor receptor 2 [HER2] receptors), there is an increased prevalence of *BRCA* variants. Pathophysiologic research has suggested that the physiologic pathway for the development of triple-negative breast cancer is similar to that for *BRCA*-associated breast cancer.[10,] Young et al (2009) studied 54 women with high-grade, triple-negative breast cancer with no family history of breast or ovarian cancer, representing a group that previously was not recommended for *BRCA* testing.[11,] Six BRCA variants (5 *BRCA1*, 1 *BRCA2*) were found, for a variant rate of 11%. Finally, Gonzalez-Angulo et al (2011) in a study of 77 patients with triple-negative breast cancer, reported that 15 patients (19.5%) had BRCA variants (12 in *BRCA1*, 3 in *BRCA2*).[12,]

PIK3CA Testing

Alterations in the protein coding gene *PIK3CA* (Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha) occur in approximately 40% of patients with hormone receptor (HR)-positive, HER2-negative breast cancer.

NTRK Gene Fusions

Neurotrophic-tropomyosin receptor kinase *(NTRK)* gene fusions encode tropomyosin receptor kinase fusion proteins that act as oncogenic drivers for solid tumors including lung, salivary gland, thyroid, and sarcoma. *NTRK* gene fusion findings might be more highly associated with rare breast cancer subtypes (eg secretory carcinoma).[13.].

Programmed Cell Death Ligand Protein-1

Programmed cell death ligand-1 (PD-L1) is a transmembrane protein expressed on the surface of multiple tissue types, including many tumor cells. Blocking the PD-L1 protein may prevent cancer cells from inactivating T cells.

Mismatch Repair Deficiency/Microsatellite Instability

Mismatch repair deficiency (dMMR) and high levels of microsatellite instability (MSI-H) describe cells that have alterations in certain genes involved in correcting errors made when DNA is replicated. dMMR tumors are characterized by a high tumor mutational load and potential responsiveness to anti-PD-L1-immunotherapy. MMR deficiency is most common in colorectal cancer, other types of gastrointestinal cancer, and endometrial cancer, but it may also be found in other cancers including breast cancer. Microsatellite instability testing is generally performed using polymerase chain reaction (PCR) for 5 biomarkers, although other biomarker panels and next generation sequencing are sometimes performed. High microsatellite instability is defined as 2 or more of the 5 biomarkers showing instability or more than 30% of the tested biomarkers showing instability depending on what panel is used. Microsatellite instability testing is generally paired with immunohistochemistry (IHC) assessing lack of protein expression from 4 DNA mismatch repair genes thereby reflecting dMMR.[14.]

Ki-67

Ki-67 is a nuclear protein used to detect and quantify the rate of tumor cell proliferation and has been investigated as a prognostic biomarker for breast cancer.[15.]

Tumor Mutational Burden

Tumor mutational burden (TMB), a measure of gene mutations within cancer cells, is an emerging biomarker of outcomes with immunotherapy in multiple tumor types. Initially, assessments of TMB involved whole exome sequencing (WES). More recently, targeted next generation sequencing (NGS) panels are being adapted to estimate TMB. Currently FoundationOne CDx is the only U.S. Food and Drug Administration (FDA) approved panel for estimating TMB, but others are in development.[16.]

Circulating Tumor DNA

Normal and tumor cells release small fragments of DNA into the blood, which is referred to as cell-free DNA. Cell-free DNA from nonmalignant cells is released by apoptosis. Most cell-free tumor DNA is derived from apoptotic and/or necrotic tumor cells, either from the primary tumor, metastases, or CTCs. Unlike apoptosis, necrosis is considered a pathologic process and generates larger DNA fragments due to incomplete and random digestion of genomic DNA. The length or integrity of the circulating DNA can potentially distinguish between apoptotic and necrotic origin. Circulating tumor DNA can be used for genomic characterization of the tumor.

Circulating Tumor Cells

Intact circulating tumor cells (CTCs) are released from a primary tumor and/or a metastatic site into the bloodstream. The half-life of a CTC in the bloodstream is short (1-2 hours), and CTCs are cleared through extravasation into secondary organs. Most assays detect CTCs through the use

of surface epithelial markers such as EpCAM and cytokeratins. The primary reason for in detecting CTCs is prognostic, through quantification of circulating levels.

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

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

Biomarker Testing Using Tissue Biopsy to Select Targeted Treatment
Clinical Context and Test Purpose
Breast cancer treatment selection is informed by tumor type, grade, stage, patient performance status and preference, prior treatments, and the molecular characteristics of the tumor such as the presence of driver mutations. One purpose of biomarker testing of patients who have advanced cancer is to inform a decision regarding treatment selection (e.g., whether to select a targeted treatment or standard treatment).

The question addressed in this evidence review is: Does biomarker testing of tumor tissue for *PIK3CA*, *NTRK* gene fusions, PD-L1, MSI-H/dMMR, Ki-67 or TMB or germline testing for *BRCA* variants improve the net health outcome in individuals with breast cancer?

The following PICO was used to select literature to inform this review.

Populations
The relevant population of interest is patients with advanced or metastatic breast cancer for whom the selection of treatment depends on the molecular characterization of the tumor.

Interventions
The technologies being considered are germline testing for *BRCA* variants, *PIK3CA*, *NTRK* gene fusions, PD-L1, MSI-H/dMMR, Ki-67 or TMB testing using tissue biopsy.

Comparators
Decisions about treatment in breast cancer are based on clinical characteristics.

Outcomes
The general outcomes of interest in oncology are overall survival, disease-specific survival, quality of life (QOL), treatment-related mortality and morbidity.

Beneficial outcomes resulting from a true-positive test result are prolonged survival, reduced toxicity, and improved QOL associated with receiving a more effective targeted therapy. Beneficial outcomes from a true negative result are prolonged survival associated with receiving chemotherapy in those without driver mutations.

Harmful outcomes resulting from a false-negative test result include shorter survival from receiving less effective and more cytotoxic chemotherapy in those with driver mutations; possible harmful outcomes resulting from a false-positive test result are a shorter survival from receiving potentially ineffective targeted treatment and delay in initiation of chemotherapy in those without driver mutations.

The overall response rate (ORR) may be used as a surrogate endpoint reasonably likely to predict clinical benefit in patients with refractory solid tumors. ORR can be measured by the proportion of patients with best overall confirmed response of complete response) or partial response by the Response Evaluation Criteria in Solid Tumors, version 1.1 (RECIST 1.1),[19.] or Response Assessment in Neuro-Oncology criteria,[20.] as appropriate by a blinded and independent adjudication committee.

There are clearly defined quantitative thresholds for the follow-up of patients in oncology trials. A general rule is a continuation of treatment until disease progression or unacceptable toxicity. Long-term follow-up outside of a study setting is conducted to determine survival status. The duration of follow-up for the outcomes of interest is 6 months and 1 year.

Study Selection Criteria
Methodologically credible studies were selected using the following principles:

- To assess efficacy outcomes, comparative controlled prospective trials were sought, with a preference for randomized controlled trials (RCTs);
- In the absence of such trials, comparative observational studies were sought, with a preference for prospective studies.
- To assess long-term outcomes and adverse events, single-arm studies that capture longer periods of follow-up and/or larger populations were sought.
- Studies with duplicative or overlapping populations were excluded.

The evidence is presented below by biomarker (*BRCA*1/2, *PIK3CA*, *NTRK*, PD-L1, MIS-H/dMMR, Ki-67, TMB*)* and by recommended therapy.

Review of Evidence
***BRCA* Variants**

Food and Drug Administration Companion Diagnostic Tests
BRACAnalysis CDx is an FDA-approved companion diagnostic test for olaparib and talazoparib.

Randomized Controlled Trials
Numerous clinical trials have been conducted to evaluate the effectiveness of PARP inhibitors in individuals with hereditary breast and ovarian cancer (HBOC) Syndrome or other high-risk cancers confirmed to have a *BRCA1/2* mutation. Summarized below are the pivotal trials that supported the *BRCA* mutation-related FDA approved indications.

Olaparib
Tutt et al (2021) published results from the phase 3 multicenter, multinational, and double-blind OlympiA RCT, which evaluated the safety and efficacy of olaparib in patients with germline *BRCA1* or *BRCA2* pathogenic or likely pathogenic variants and high-risk, human epidermal growth factor receptor 2 (*HER2*)-negative primary early-stage breast cancer after definitive local treatment and neoadjuvant or adjuvant chemotherapy.[21.] Patients with triple-negative breast cancer who were treated with adjuvant chemotherapy were required to have axillary node-positive disease or an invasive primary tumor measuring at least 2 cm on pathological analysis. Patients treated with neoadjuvant chemotherapy were required to have not achieved pathological complete response. Patients treated with adjuvant chemotherapy for hormone receptor (HR)-positive, HER2-negative breast cancer were required to have at least 4 pathologically confirmed positive lymph nodes. Those treated with neoadjuvant chemotherapy were required to have not achieved a pathological complete response with a CPS+EG score of 3 or higher. This scoring system estimates relapse probability on the basis of clinical and pathological stage (CPS) and estrogen-receptor status and histologic grade (EG). Scores range from 0 to 6, with higher scores reflecting a worse prognosis. Approximately half of patients received adjuvant chemotherapy and half neoadjuvant chemotherapy, with the majority (93.7%) receiving a combination of an anthracycline and a taxane in their regimen. Patients with triple-negative disease comprised 82.2% of the trial population. Patients were randomized 1:1 to

treatment with twice daily 300 mg olaparib (n = 921) or placebo (n=915) for 52 weeks. At the prespecified interim analysis, 86% of the primary analysis target of 330 events of invasive disease or death in the intention-to-treat population were observed, with a median follow-up duration of 2.5 years (interquartile range [IQR], 1.5 to 3.5 y). The 3-year invasive disease-free survival was 85.9% in the olaparib group and 77.1% in the placebo group (difference, 8.8%; 95% CI, 4.5% to 13.0%). Invasive disease-free survival was significantly longer among patients receiving olaparib (hazard ratio [HR], 0.58; 99.5% CI, 0.41 to 0.82; p<.001). Distant disease-free survival at 3 years was 87.5% in the olaparib group and 80.4% in the placebo group (difference, 7.1%; 95% CI, 3.0% to 11.1%). This outcome was significantly longer among patients assigned to receive olaparib (HR, 0.57; 99.5% CI, 0.39 to 0.83; p<.001). While fewer deaths were reported in the olaparib group (59 versus 86) with a HR of 0.68 (99% CI, 0.44 to 1.05; p=.02), the between-group difference did not cross the prespecified multiple-testing procedure boundary for significance of p<.01. Subgroup analysis of invasive disease-free survival revealed treatment effects for olaparib over placebo that were consistent with those in the overall analysis population across all stratification groups and prespecified subgroups. Serious adverse events occurred in 8.7% and 8.4% of patients treated with olaparib and placebo, respectively. Adverse events leading to trial regimen discontinuation occurred in 9.9% and 4.2% of patients treated with olaparib and placebo, respectively.

OlympiAD is a phase 3 RCT in which patients with HER2-negative metastatic breast cancer and a germline *BRCA* variant were randomized to olaparib (n=205) or standard therapy (n=97).[22] *BRCA1/2* mutation was detected by BRACAnalysis testing. In its initial publication, Robson et al (2017) reported that after a median follow-up of 14.5 months, patients receiving olaparib experienced significantly longer progression-free survival (PFS) compared with patients receiving standard therapy (HR, 0.6; 95% CI, 0.4 to 0.8).[23] The rate of grade 3 or higher adverse events was lower in the group receiving olaparib (37%) compared with the group receiving standard therapy (51%). However, regarding overall survival, in their subsequent publication, Robson et al (2019) further reported that although improvement with olaparib was not significant overall (19.3 vs 17.1 months; HR, 0.90; 95% CI, 0.66 to 1.23) there may be a benefit in the subgroup of patients who had not received chemotherapy for metastatic disease (HR, 0.51; 95% CI, 0.29-0.90).[24]

Talazoparib
Litton et al (2018) published results from a phase 3, randomized, open-label trial of 431 patients with advanced breast cancer and a germline *BRCA1/2* mutation that compared talazoparib 1 mg once daily to standard single-agent therapy (EMBRACA).[25] *BRCA1/2* mutation was detected by BRACAnalysis testing. The primary endpoint was PFS. Median duration of follow-up for that endpoint was 11.2 months. Progression-free survival was significantly longer in the talazoparib group (8.6 months vs 5.6 months; HR 0.54, 95% CI, 0.41 to 0.71). The rate of overall grade 3 or higher adverse events was similar for talazoparib compared with the standard care (25.5% vs 25.4%), but hematologic grade 3-4 adverse events (primarily anemia) were more frequent for talazoparib (55% vs 38%) compared with nonhematologic grade 3-4 adverse events (32% vs 38%). Based on the European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire (QLQ-C30), compared to baseline, there was a significant improvement in the talazoparib group (+3.0; 95% CI, 1.2 to 4.8) and a significant decline in the standard therapy group (-5.4; 95% CI, -8.8 to -2.0). Although the trial was open-label, assessment of the primary outcome was based on blinded independent central review.

Section Summary: *BRCA* Variant Testing
No studies were identified that have directly compared health outcomes in patients with breast cancer who did and did not use *BRCA1* and *BRCA2* variant testing to guide systemic treatment decisions. Evidence for the use of testing for *BRCA1* and *BRCA2* variants in individuals with breast cancer consists of several placebo-controlled RCTs of PARP inhibitor drugs that have consistently demonstrated that, in individuals identified by genetic testing as having a *BRCA1* or *BRCA2* variant, treatment with PARP inhibitor drugs significantly improve PFS time. In individuals with a *BRCA1/2* mutation and either HER2-negative metastatic breast cancer or other advanced

breast cancer who were followed for 11-12 months, treatment with a PARP inhibitor drug resulted in a 40% to 46% lower risk of disease progression or death. In individuals with a *BRCA1/2* mutation and early-stage breast cancer at high-risk for recurrence, treatment with olaparib resulted in a 9% improvement in 3-year invasive disease-free survival.

PIK3CA

Food and Drug Administration Companion Diagnostic Tests

U.S. Food and Drug Administration (FDA) approved companion diagnostic tests for alpelisib in patients with *PIK3CA*-mutated breast cancer include both tissue-based and liquid biopsy assays (see Table 1). These tests are approved to measure 11 variants in the *PIK3CA* gene.

Randomized Controlled Trial

Andre et al (2019) reported results of SOLAR-1 (Clinical Studies of Alpelisib in Breast Cancer 1), a phase 3 trial to evaluate alpelisib plus fulvestrant in patients with HR-positive, HER2-negative advanced breast cancer who had received endocrine therapy previously.[26.] Patients were enrolled into 2 cohorts based on tumor-mutation status (*PIK3CA*-mutated vs not *PIK3CA*-mutated) and randomly assigned within cohorts to receive oral alpelisib plus fulvestrant or placebo plus fulvestrant. *PIK3CA* status was determined with the use of a tumor-tissue sample, and patients had to have adequate tumor tissue for central analysis of *PIK3CA* mutational status. The primary end point was progression-free survival in the cohort of patients with *PIK3CA*-mutated cancer.

Among patients with *PIK3CA*-positive tumors who received targeted therapy, PFS was 11.0 months (95% CI, 7.5 to 14.5), compared to 5.7 months (95% CI, 3.7 to 7.4) in *PIK3CA*-positive patients who received standard care (HR 0.65; 95% CI, 0.50 to 0.85). In contrast, the HR for PFS in the cohort without *PIK3CA*-mutated cancer was not significantly different for the active vs placebo groups.

Table 2. RCT of Alpelisib in Patients with *PIK3CA*-Mutated Breast Cancer- Characteristics

Study	Countries	Sites	Dates	Participants	Interventions		Endpoints	Median Duration of follow-up
					Active	***Comparator***		
Andre et al (2019)[26.] SOLAR-1 NCT02437318	Multiple, US, Asia, Europe (N=34)	198	2015-2018	Men and postmenopausal women with HR-positive, HER2-negative advanced breast cancer, eligible to receive further endocrine therapy after relapse or progression, and receiving or had received aromatase inhibitor treatment in the context of neoadjuvant or adjuvant therapy or for advanced disease.	Alpelisib plus fulvestrant n=169	Placebo plus fulvestrant n=172	Primary: PFS in the cohort of patients with *PIK3CA*-mutated cancer. Secondary: OS (not reported in the primary publication), overall response, clinical benefit (complete or partial response or stable disease for >6 months), safety	20.0 months (10.7 to 33.3)

HER2: human epidermal growth factor receptor 2; HR: hormone receptor; N: sample size; OS: overall survival; PIK3CA: phosphatidylinositol 3-kinase catalytic alpha polypeptide; PFS: progression-free survival; RCT: randomized controlled trial; SOLAR-1: Study Assessing the Efficacy and Safety of Alpelisib Plus

Fulvestrant in Men and Postmenopausal Women With Advanced Breast Cancer Which Progressed on or After Aromatase Inhibitor Treatment

Table 3. RCT of Alpelisib in Patients with *PIK3CA*-Mutated Breast Cancer- Results

Study	PFS (95% CI)	PFS at 12 months (95% CI)	Overall Response	Clinical Benefit	Adverse events- Grade 3 or 4
Andre et al (2019)[26] SOLAR-1NCT02437318					
N analyzed	341	341	341	182	571
Targeted therapy	11.0 months (95% CI, 7.5 to 14.5)	46.3%	45/169 26.6% (20.1 to 34.0)	104/169 61.5 (53.8 to 68.9)	Serious AEs: 34.9% Hyperglycemia:36.6% Rash: 9.9% Maculopapular rash: 8.8% Diarrhea: 6.7% Discontinuation due to AEs: 25.0% Death: 2.5%
Standard care	5.7 months (95% CI, 3.7 to 7.4)	32.9%	22/172 12.8 (8.2 to 18.7)	78/172 45.3 (37.8 to 53.1)	Serious AEs: 16.7% Hyperglycemia: 0.6 % Rash: 0.3% Maculopapular rash: 0.3% Diarrhea: 0.3% Discontinuation due to AEs:4.2% Death: 4.2%
HR (95% CI)	0.65; 95% CI, 0.50 to 0.85				
p	<.001				

CI: confidence interval; HR: hazard ratio; N: sample size; PFS: progression-free survival; PIK3CA: phosphatidylinositol 3-kinase catalytic alpha polypeptide; RCT: randomized controlled trial; SOLAR-1: Study Assessing the Efficacy and Safety of Alpelisib Plus Fulvestrant in Men and Postmenopausal Women With Advanced Breast Cancer Which Progressed on or After Aromatase Inhibitor Treatment

Section Summary: *PIK3CA* Testing

In a randomized, placebo-controlled trial of alpelisib compared to placebo in men and postmenopausal women with advanced breast cancer who had previously received endocrine therapy, PFS was longer among patients with *PIK3CA*-positive tumors who received targeted therapy, PFS was 11.0 months (95% CI, 7.5 to 14.5), compared to 5.7 months (95% CI, 3.7 to 7.4) in *PIK3CA*-positive patients who received standard care. In contrast, the hazard ratio for PFS in the cohort without *PIK3CA*-mutated cancer was not significantly different for the active vs placebo groups. The overall response rate was higher in patients with *PIK3CA*-positive tumors compared to the rate in the standard care group (26.6% [95% CI [20.1 to 34.0] vs 12.8% [8.2-18.7%]), with an acceptable side effect profile.

NTRK Gene Fusions

Food and Drug Administration Companion Diagnostic Tests

There is currently no FDA approved companion diagnostic test for entrectinib. FoundationOne CDX is an approved companion diagnostic test for larotrectinib.

Nonrandomized Trials of Targeted Treatment

Entrectinib

Doebele et al (2020) reported an analysis of data from 3 Phase 1-2 trials of entrectinib in patients with *NTRK*-fusion solid tumors (Table 4).[27] Of 54 patients included in the analysis, 6 had breast cancer (11%). Patients were assessed for eligibility for the 3 trials using either local molecular

profiling or central RNA-based next-generation sequencing to test for the presence of *NTRK* fusions. The primary endpoints were objective response and duration of response. PFS and overall survival were secondary endpoints.

Of the total cohort of 54 patients, 31 had an objective response (57%; 95% CI, 43.2–70.8) (Table 5). Four patients (7%) had a complete response and 27 a partial response (50%). Responses were recorded in all tumor types, including 5 (83%; 36–100) of 6 patients with breast cancer. Median PFS for the full cohort was 11 months (95% CI, 8.0–1) and median overall survival was 21 months (95% CI, 14.9 to not estimable). There were 7 serious treatment-related adverse events (10%), and 3 (4%) patients discontinued due to a treatment-related adverse event.

Table 4. Entrectinib in *NTRK*-Fusion-Positive Solid Tumors - Study Characteristics

Studies	Design	Countries	Sites	Dates	Participants	Intervention	Endpoints
Doebele et al (2020)[27]. STARTRK-1 (NCT02097810); STARTRK-2 (NCT02568267); ALKA-372–001 (EudraCT, 2012–000148–88)	Phase 1 (STARTRK-1 and ALKA) and Phase 2 (STARTRK-2)	STARTRK-1: US, Spain, Korea STARTRK-2: multiple (N=15) ALKA: Italy	STARTRK1:10 sites STARTRK-2: 150 sites ALKA: 2 sites	STARTRK-1: August 2014-May 2018 STARTRK-2: November 2015-ongoing ALKA: October 2012-March 2018	54 adults with metastatic or locally advanced *NTRK* fusion-positive solid tumors; included patients with 10 different tumor types and 19 different histologies (11%) had breast tumors	Entrectinib	Primary: objective response, duration of response Secondary: PFS, OS, clinical benefit rate. time to CNS progression, and safety.

CNS: central nervous system; NTRK: neurotrophic-tropomyosin receptor kinase; OS: overall survival; PFS: progression-free survival; STARTRK-1: Study of Oral RXDX-101 in Adult Patients with Locally Advanced or Metastatic Cancer Targeting NTRK1, NTRK2, NTRK3, ROS1, or ALK Molecular Alterations; STARTRK-2: Basket Study of Entrectinib (RXDX-101) for the Treatment of Patients With Solid Tumors Harboring NTRK 1/2/3 (Trk A/B/C), ROS1, or ALK Gene Rearrangements (Fusions)

Table 5. Entrectinib in *NTRK*-Fusion-Positive Solid Tumors - Study Results

Studies	Objective Response	Duration of Response	Median PFS	Median OS	Adverse Events
Doebele et al (2020)[27]. STARTRK-1 (NCT02097810); STARTRK-2 (NCT02568267); ALKA-372–001 (EudraCT, 2012–000148–88)					
N analyzed	54	54	54	54	68
	31/54 (57%; 95% CI 43.2–70.8) • 4 (7%) complete response • 27 (50%) partial response. Responses were recorded in all tumor types, including 5 (83%; 36–100) of 6 patients with breast cancer,	10 months (95% CI 7.1 to not estimable)	11 months (95% CI 8.0–1)	21 months (95% CI 14.9 to not estimable)	7 serious treatment-related adverse events (10%) 3 (4%) patients discontinued due to a treatment-related adverse events deaths (9%)

CI: confidence interval; N: sample size; NTRK: neurotrophic-tropomyosin receptor kinase; OS: overall survival; PFS: progression-free survival; STARTRK-1: Study of Oral RXDX-101 in Adult Patients with Locally Advanced or Metastatic Cancer Targeting NTRK1, NTRK2, NTRK3, ROS1, or ALK Molecular Alterations; STARTRK-2: Basket Study of Entrectinib (RXDX-101) for the Treatment of Patients With Solid Tumors Harboring NTRK 1/2/3 (Trk A/B/C), ROS1, or ALK Gene Rearrangements (Fusions)

Larotrectinib

Hong et al (2020) reported an analysis of 3 phase 1-2 trials of larotrectinib in patients with *NTRK*-fusion positive solid tumors in adults and children.[28]

NTRK fusions were identified by next-generation sequencing, according to the procedures and analytic pipelines established by each laboratory, or by fluorescence in situ hybridization. The trials included adults and children with 17 different solid tumors. Five patients had breast cancer (3%).

These results were consistent with a preliminary analysis of data from these trials reported by Drilon et al in 2018. [29]

Table 6. Larotrectinib in *NTRK*-Fusion-Positive Solid Tumors - Study Characteristics

Study	Dates	Participants	Intervention	Endpoints
Hong et al (2020)[28] NCT02122913, NCT02637687, NCT02576431	May 2014-Feb 2019	159 adults and children with locally advanced or metastatic *NTRK*-fusion positive solid tumors. 5 (3%) with breast cancer	Larotrectinib	Primary: Objective response, as assessed by an independent radiology review committee Secondary: Overall response according to the investigator's assessment, duration of response, PFS, and safety.

NTRK: neurotrophic-tropomyosin receptor kinase; PFS: progression-free survival

Table 7. Larotrectinib in *NTRK*-Fusion-Positive Solid Tumors - Study Results

Study	Overall response	Duration of response	PFS	OS	Adverse events
Hong et al (2020)[28] NCT02122913, NCT02637687, NCT02576431					
N analyzed	153	108	159	159	260 (expanded safety population)
Targeted therapy	Overall: 79% (95% CI 72 to 85) Complete: 16% of the patients (24 patients) had a complete response, Partial: 63% (97) had a partial response, Stable disease: 12% (19) had stable disease, Progressive disease: 6% (9) had progressive disease. Not evaluated due to early withdrawal for clinical deterioration: 3% (4) Patients with breast cancer (n=4): 3 (75%; 19%-99%)	Median 35·2 months (95% CI 22.8–NE). At 12 months 80% (95% CI 71–89) NE for patients with breast cancer	Median: 28·3 months (95% CI 22·1–NE) At 12 months: 67% (95% CI 58–76)	Median: 44·4 months (95% CI 36·5–NE) Proportion surviving at 12 months: 88% (95% CI 83-94)	23 deaths (14%) at median follow-up of 13.9 months The most common grade 3 or worse treatment-emergent adverse events (regardless of attribution) were anemia (25 [10%] of 260 patients) and decreased neutrophil count (14 [5%]; table 4). The most common treatment-emergent serious adverse events were pneumonia (6 [2%] of 260 patients), pyrexia (6 [2%]), abdominal pain (5 [2%]), and diarrhea (5 [2%]);

CI: confidence interval; N: sample size; NE: not estimable; NTRK: neurotrophic-tropomyosin receptor kinase; OS: overall survival; PFS: progression-free survival; PFS: progression-free survival

Section Summary: *NTRK* Gene Fusion Testing
In an analysis of 159 patients with *NTRK*-fusion positive solid tumors who received larotrectinib, including 5 patients with breast tumors, the overall response rate was 79% (95% CI, 72-85). The median PFS was 28.3 months (95% CI, 22.1 to not estimable), and 67% of patients were progression-free at 12 months (95% CI, 58–76). In an integrated analysis of 3 phase 1-2 trials in 54 patients with *NTRK*-positive solid tumors who received entrectinib, 6 of whom had breast cancer, the overall response rate was 57% (95% CI, 43.2–70.8). At data cutoff, 16 (30%) of 54 patients had died, and the estimated median overall survival was 21 months (95% CI, 14.9 to not estimable). Responses were observed regardless of tumor type or age of the patient.

PD-L1 Testing
Food and Drug Administration Companion Diagnostic Tests
PD-L1 IHC 22C3 pharmDx is an approved companion diagnostic test to select patients with triple negative breast cancer for treatment with pembrolizumab.

Randomized and Nonrandomized Trials of Immunotherapy
Atezolizumab
Schmid et al (2018) reported results of a randomized, placebo-controlled trial of atezolizumab in combination with nab-paclitaxel for patients with metastatic or unresectable triple-negative breast cancer and PD-L1-positive tumors (defined as expression on ≥1% of tumor-infiltrating immune cells).[30.] PFS was longer in the group of PD-L1-positive patients who received targeted treatment, compared to those who received placebo (Table 9). However, the designated confirmatory trial, IMpassion131, did not demonstrate a statistically significant improvement in investigator-assessed PFS for atezolizumab plus paclitaxel compared with placebo plus paclitaxel in the PD-L1-positive population, with a median PFS of 5.95 and 5.72 months (HR, 0.82; 95% CI, 0.60 to 1.12), respectively.[31.] In August 2021, Genentech voluntarily withdrew accelerated approval of atezolizumab (Tecentriq) for use in patients with PD-L1 positive, triple-negative breast cancer following FDA assessment of these findings.

Table 8. RCT of Atezolizumab plus Nab-Paclitaxel in Patients with PD-L1-Positive Triple Negative Breast Cancer - Characteristics

Study	Countries	Sites	Dates	Participants	Interventions		Endpoints
					Active	Comparator	
Schmid et al 2018;[30.] IMpassion130 (NCT02425891)	41 (Europe US, Canada, Asia, Latin America, Australia)	246	Jun 2015-May 2017	Patients with metastatic or unresectable locally advanced triple-negative breast cancer with PD-L1–positive tumors (expression on tumor-infiltrating immune cells ≥1%)	n=185 Atezolizumab + Nab-Paclitaxel	n=184 Placebo + Nab-Paclitaxel	Primary: Investigator-assessed PFS and OS. Secondary: Rate and duration of objective response, safety

Impassion130: A Study of Atezolizumab in Combination with Nab-Paclitaxel Compared with Placebo with Nab-Paclitaxel for Participants with Previously Untreated Metastatic Triple-Negative Breast Cancer; n: sample size; OS: overall survival; PD-L1: programmed death-ligand 1 ; PFS: progression-free survival; RCT: randomized controlled trial

Table 9. RCT of Atezolizumab plus Nab-Paclitaxel in Patients with PD-L1-Positive Triple Negative Breast Cancer - Results

Study	Median PFS (95% CI)	PFS st 12 months	Median OS	2-Year Rate of OS	Objective response	Median Duration of Response	Adverse events
Schmid et al 2018;[30.] IMpassion130 (NCT02425891)							

Study	Median PFS (95% CI)	PFS st 12 months	Median OS	2-Year Rate of OS	Objective response	Median Duration of Response	Adverse events
Number analyzed	369	369	369	369	368		
Targeted therapy	7.5 months (6.7-9.2)	29.1%	25.0 months (22.6-NE)	53.5 (42.3-64.6)	58.9 (51.5–66.1)	8.5 months (7.3–9.7)	Deaths: 64/185 (34.6%)
Standard care	5.0 months (3.8-5.6)	16.4%	15.5 month (13.1-19.4)	36.6 (26.4-46.7)	42.6 (35.4–50.1)	5.5 months (3.7–7.1)	Deaths: 88/184 (47.8%)
HR (95% CI)	0.62; (95% CI, 0.49 to 0.78)		0.62 (95% CI, 0.45–0.86)		1.96 (1.29–2.98)	0.60 (0.43–0.86)	

CI: confidence interval; HR: hazard ratio; Impassion130: A Study of Atezolizumab in Combination with Nab-Paclitaxel Compared with Placebo with Nab-Paclitaxel for Participants with Previously Untreated Metastatic Triple-Negative Breast Cancer ; NE: not estimable; OS: overall survival; PD-L1: programmed death-ligand 1; PFS: progression-free survival; RCT: randomized controlled trial

Pembrolizumab

Randomized Controlled Trials

The efficacy of pembrolizumab plus chemotherapy compared to placebo plus chemotherapy for previously untreated, locally recurrent inoperable or metastatic triple-negative breast cancer (n=847) was evaluated in the KEYNOTE-355 study conducted by Cortes and coworkers (2020).[32.] Dual primary efficacy endpoints were PFS and overall survival in patients with PD-L1 combined positive score ≥1. This study formed the basis of pembrolizumab accelerated approval in patients with unresectable or metastatic triple-negative breast cancer and PD-L1 CPS ≥10.

Table 10. Pembrolizumab in Patients with PD-L1 Positive Triple Negative Breast Cancer - Randomized Study Characteristics

Study	Design	Participants	Intervention	Endpoints
Cortes et al (2020);[32.] KEYNOTE-355 NCT02819518	Randomized, placebo-controlled, double-blind, multicenter, phase 3	847 patients with previously untreated, locally recurrent inoperable or metastatic triple-negative breast cancer	• Pembrolizumab plus chemotherapy, 566 o PD-L1 CPS ≥1, 425 (75%) o PD-L1 CPS ≥10, 220 (39%) • Placebo plus chemotherapy, 281 o PD-L1 CPS ≥1, 210 (75%) o PD-L1 CPS ≥10, 220 (37%)	Primary: PFS, OS Secondary: Safety

CPS: combined positive score; OS: overall survival; PD-L1: programmed death ligand-1; PFS: progression-free survival.

Table 11. Pembrolizumab in Patients with PD-L1 Positive Triple Negative Breast Cancer - Randomized Study Results

Study	Median PFS, months					Grade ≥3 Adverse Events
Cortes et al (2020);[32.] KEYNOTE-355 NCT02819518	ITT	PD-L1 CPS <1	PD-L1 CPS ≥1	PD-L1 CPS <10	PD-L1 CPS ≥10	
N	847	211	636	524	323	

Study			Median PFS, months			Grade ≥3 Adverse Events
Pembrolizumab plus chemotherapy	7.5	6.3	7.6	5.8	9.7	Any adverse events, 438 (78%) Treatment-related adverse events, 383 (68%) Immune-mediated adverse events, 29 (5%)
Placebo plus chemotherapy	5.6	6.2	5.6	5.7	5.6	Any adverse events, 207 (74%) Treatment-related adverse events, 188 (67%) Immune-mediated adverse events, 0 (0%)
HR (95% CI)	0.82 (0.69 to 0.97)	1.08 (0.77 to 1.53)	0.74 (0.61 to 0.89)	0.94 (0.76 to 1.16)	0.65 (0.49 to 0.86)	

CPS: combined positive score; HR: hazard ratio; ITT: intention-to-treat; PD-L1: programmed death ligand-1; PFS: progression-free survival.

Nonrandomized Trials

Two nonrandomized, single-arm trials reported outcomes in a total of 111 patients with PD-L1 positive triple negative breast cancer treated with pembrolizumab (Tables 12 and 13).[33,34.]

Table 12. Pembrolizumab in Patients with PD-L1-Positive Triple Negative Breast Cancer - Study Characteristics

Study	Design	Participants	Intervention	Endpoints
Adams et al (2019)[33.] KEYNOTE-086 NCT02447003	Nonrandomized, multicohort, phase 2	84 patients with metastatic triple-negative breast cancer; 86.9% received prior (neo)adjuvant therapy; none had prior systemic therapy for metastatic disease	Pembrolizumab monotherapy	Primary: Safety Secondary: Objective response, disease control rate, duration of response, PFS, OS
Nanda et al 2016[34.] KEYNOTE-012 NCT01848834	Nonrandomized, multicohort, phase Ib	27 Patients with recurrent or metastatic PD-L1 positive triple-negative breast cancer. Most were heavily pretreated, having received therapy in both the early and advanced disease settings.	Pembrolizumab monotherapy	Primary: OR : defined as percentage of patients with a best overall response of complete response or partial response Secondary: PFS, duration of response, OS

OS: overall survival; PD-L1: programmed death-ligand 1; PFS: progression-free survival

Table 13. Pembrolizumab in Patients with PD-L1-Positive Triple Negative Breast Cancer - Study Results

Study	Response	Median PFS	Duration of Response	OS	Adverse Events
Adams et al (2019);[33.] NCT02447003					
N analyzed	84	84			84
Targeted therapy	Objective response rate: 21.4% (95% CI 13.9–31.4)	Median: 2.1 months (95% CI, 2.0–2.2) Rate at 6 months: 27.0%	Median: 10.4 months (range 4.2 to 19.2+)	Median 18.0 months (95% CI 12.9–23.0) 6-month rate 81.0% 12-month rate: 61.7%	53 (63.1%) patients experienced 1 or more treatment-related AE,8 (9.5%) with 1 or more grade 3 event. No grade 4 events, no AEs that led to death1 (1.2%) discontinued due

Study	Response	Median PFS	Duration of Response	OS	Adverse Events
					to AEs. Most common treatment-related AEs were fatigue (26.2%), nausea (13.1%), and diarrhea (11.9%)43 deaths (51.2%)
Nanda et al 2016;[34.] KEYNOTE-012 (NCT01848834)					
N Analyzed	27	22			
Targeted therapy	Overall response rate:18.5% (95% CI, 6.3 to 38.1) Complete response: 1 (3.7%) Partial response: 4 (14.8%)PD 13 (48.1%)	Median 1.9 months (95% CI, 1.7 to 5.5), 6 months PFS: 24.4%	Median not yet reached (range 15.0 to ≥47.3 weeks)	Median : 11.2 months (95% CI, 5.3 to [not reached]) 6 month rate: :66.7%12-month OS: 43.1%	56.3% of patients experienced at least one treatment-related toxicity, including 15.6% who experienced at least one grade 3 to 5 event. One patient died as a result of disseminated intravascular coagulation (DIC) accompanied by grade 4 decreased blood fibrinogen, both of which were considered by the investigator to be treatment related.

AE: adverse events; CI: confidence interval; OS: overall survival; PD-L1: programmed death-ligand 1; PFS: progression-free survival

Section Summary: PD-L1 Testing
In a placebo controlled trial of atezolizumab in combination with nab-paclitaxel for patients with PD-L1 positive triple negative breast cancer , median PFS (HR 0.62; 95% CI, 0.49 to 0.78) and overall survival 0.62 (95% CI, 0.45–0.86) were longer among patients who received the targeted immunotherapy. However, a designated confirmatory trial did not confirm these results and accelerated approval was withdrawn. In 2 nonrandomized trials of pembrolizumab for patients with PD-L1 positive triple negative breast cancer , the objective response rate was 21.4% (95% CI, 13.9 to 31.4) and 18.5% (95% CI, 6.3 to 38.1). In 1 randomized trial of pembrolizumab plus chemotherapy versus placebo plus chemotherapy for patients with triple negative breast cancer and PD-L1 combined positive score ≥10, the median PFS was 9.7 and 5.6 months, respectively (HR, 0.65; 95% CI, 0.49 to 0.86).

MSI-H/dMMR Testing
Food and Drug Administration Companion Diagnostic Tests
The Ventana MMR RxDx Panel is an FDA-approved test for the detection of dMMR to guide the use of dostarlimab-gxly (Jemperli) in solid tumors. There is no FDA approved test for the detection of MSI-H or dMMR for pembrolizumab (Keytruda). In clinical trials, the identification of MSI-H or dMMR tumor status for the majority of patients (135/149) was prospectively determined using local laboratory-developed, polymerase chain reaction (PCR) tests for MSI-H status or immunohistochemistry (IHC) tests for dMMR.

Nonrandomized Trials of Immunotherapy

Pembrolizumab

Marabelle et al (2020) reported results of a phase 2 trial of pembrolizumab in 233 previously treated patients with MSI-H solid tumors (Tables 14 and 15), 5 of whom had breast cancer.[35] The overall response rate, the primary outcome, was 34.3% (95% CI, 28.3% to 40.8%). Median PFS was 4.1 months (95% CI, 2.4 to 4.9 months) and median overall survival was 23.5 months (95% CI, 13.5 months to not reached). Treatment-related adverse events occurred in 151 patients (64.8%). Earlier, Le et al (2015) reported on a small (N = 41) phase 2 trial that compared response to pembrolizumab in patients with solid tumors that did or did not have mismatch repair.[36] Most of the patients had colorectal cancer, but a cohort of 9 patients with dMMR tumors that were not colorectal was included. In the full cohort, mismatch-repair status predicted clinical benefit of pembrolizumab, and patients with dMMR noncolorectal cancer had responses similar to those of patients with dMMR colorectal cancer.

Table 14. Pembrolizumab in Patients with MSI-H/dMMR-Positive Solid Tumors - Study Characteristics

Study	Countries	Sites	Dates	Design	Participants	Intervention	Endpoints
Marabelle et al (2020); [35] KEYNOTE-158 (NCT02628067)	Multiple (N=21)	81	Feb 2016-May 2018	Nonrandomized, open-label, multisite phase 2	233 patients 18 years or older with unresectable and/or metastatic incurable noncolorectal solid tumor with disease progression on or intolerance to prior standard therapy. 27 tumor types 5 patients had breast cancer (2.1%)	Pembrolizumab	Primary: Overall response rate Secondary: duration of response, PFS, OS, safety

dMMR: mismatch repair deficient; MSI-H: microsatellite instability-high; N: sample size; OS: overall survival; PFS: progression-free survival

Table 15. Pembrolizumab in Patients with MSI-H/dMMR-Positive Solid Tumors - Study Results

Study	Response	Duration of Response	PFS	OS	Adverse events
Marabelle et al (2020)[35] KEYNOTE-158 NCT02628067					
N analyzed	233				233
Targeted therapy	Overall response rate: 34.3% (95% CI, 28.3% to 40.8%) Complete: 23 (9.9%) Partial: 57 (24.5%)	Median: not reached, range, 2.9 to 31.3+ months Response 12 months or longer: 86.9% 24 months or longer: 77.6%	Median: 4.1 months (95% CI, 2.4 to 4.9 months) 12 months: 33.9% 24-months: 29.3%	Median: 23.5 months (95% CI, 13.5 months to not reached) 12 months: 60.7% 24-months: 48.9%	Overall, 151 patients (64.8%) had treatment-related adverse events and 34 (14.6%) had grade 3 to 5 treatment-related adverse events, one of which was grade 5 (pneumonia). Eighteen patients (7.7%) had serious treatment-

Study	Response	Duration of Response	PFS	OS	Adverse events
					related adverse events, and 22 (9.4%) discontinued treatment because of a treatment-related adverse event Deaths: 113 (48.5%)

CI: confidence interval; dMMR: mismatch repair deficient; MSI-H: microsatellite instability-high; N: sample size; OS: overall survival; PFS: progression-free survival

Dostarlimab-gxly

Patients with dMMR/MSI-H endometrial cancer (EC; n=103) or dMMR/MSI-H and/or polymerase epsilon (POLE)-mutant non-endometrial solid cancers (n=106) who had experienced disease progression for recurrent or advanced disease with no satisfactory alternative treatment options were evaluated in the multicenter, open-label GARNET trial, a phase 1 dose escalation and cohort expansion study of dostarlimab-gxly (Jemperli).[37,] Laboratory-developed tests using immunohistochemistry (IHC), polymerase chain reaction (PCR), or next generation sequencing (NGS) were used to prospectively determine patient variant status, and dMMR status was retrospectively confirmed with the marketed companion diagnostic test, the Ventana MMR RxDx Panel, a qualitative IHC test. Accelerated drug approval was based on an overall response rate of 41.6% (95% CI, 34.9%, 48.6%) for the full cohort, the primary efficacy outcome, as assessed at data cutoff with a median follow-up duration of 13.5 months. The median duration of response was 34.7 months, with 95.4% of patients achieving a duration of response ≥ 6 months. The confirmed overall response rate was 44.7% (95% CI, 34.9% to 54.8%) and 38.7% (29.4% to 48.6%) for EC and non-EC cohorts, respectively. One patient with breast cancer was enrolled in the study and achieved a complete response and ongoing duration of response of 16.8 months. Continued drug approval is subject to the results of confirmatory trials.

Section Summary: MSI-H/dMMR Testing

In a phase 2 trial of pembrolizumab in 233 previously treated patients with MSI-H solid tumors, the overall response rate was 34.3% (95% CI, 28.3% to 40.8%). Median PFS was 4.1 months (95% CI, 2.4 to 4.9 months) and median overall survival was 23.5 months (95% CI, 13.5 months to not reached). Treatment-related adverse events occurred in 151 patients (64.8%). A phase 1 dose escalation study of dostarlimab-gxly reported an overall response rate of 41.6% with a median duration of response of 34.7 months for a combined cohort of 209 patients with endometrial cancer and non-endometrial cancer solid cancers; however, enrollment of patients with breast cancer was limited to 1 individual.

Ki-67 Testing

FDA Companion Diagnostic Test

The Ki-67 IHC MIB-1 pharmDx (Dako Omnis) test is an FDA-approved companion diagnostic for abemaciclib (Verzenio).

Randomized Controlled Trial

Abemaciclib

Efficacy of abemaciclib was evaluated in the multicenter, randomized, open-label monarchE (NCT03155997) trial reported by Johnston and coworkers (2021).[38,] Adult men and women with HR-positive, HER2-negative, node-positive, early breast cancer with clinical and pathological features consistent with a high risk of recurrence were enrolled and randomized to receive either 2 years of abemaciclib plus physician's choice of standard endocrine therapy (n=2808) or endocrine therapy alone (n=2829). The primary efficacy outcome was invasive disease-free survival (IDFS). At the preplanned interim efficacy analysis, abemaciclib plus endocrine therapy demonstrated superior IDFS compared to endocrine therapy alone (HR, 0.75; 95% CI, 0.60 to

0.93; p=.01), with 2-year IDFS rates of 92.2% versus 88.75 respectively. Ki-67 index ≥ 20% was reported for 1262 (44.9%) and 1233 (43.6%) patients treated with abemaciclib plus endocrine therapy and endocrine therapy alone, respectively. In a secondary pre-planned efficacy analysis of patients with high risk of recurrence and retrospectively confirmed Ki-67 score ≥ 20% (n=2003), the study also demonstrated a statistically significant improvement in the primary efficacy outcome of IDFS (HR 0.626; 95% CI, 0.488-0.803; p=.0042). For patients receiving abemaciclib plus tamoxifen or an aromatase inhibitor, IDFS at 36 months was 86.1% (95% CI, 82.8% to 88.8%) compared to 79.0% at 36 months (95% CI, 75.3% to 82.3%) in patients receiving only tamoxifen or an aromatase inhibitor. At the time of IDFS, overall survival data was immature and not reported.

Efficacy of abemaciclib in the ITT population at median follow-up 19 months showed continued benefit in IDFS (HR = 0.71, 95% CI 0.58-0.87; nominal p<.001) with an absolute improvement of 3.0% in the 2-year IDFS rates (abemaciclib + ET: 92.3% versus ET alone: 89.3%), and benefit in DRFS (HR = 0.69, 95% CI 0.55 to 0.86; nominal p<.001) with absolute difference of 3.0% at 2 years (abemaciclib + ET: 93.8% versus ET alone: 90.8%). [39.] At 27 months, the benefit of abemaciclib held (IDFS HR = 0.70, 95% CI 0.59 to 0.82; nominal p<.0001 and DRFS HR = 0.69, 95% CI 0.57 to 0.83; nominal p<.0001). When assessing Ki-67-high and -low populations, abemaciclib + ET showed an IDFS benefit regardless of the Ki-67 index and for all follow-up time periods assessed. The 3-year IDFS rates in the control arm suggested that patients with Ki-67-high tumors had a higher risk of developing an IDFS event than those with Ki-67-low tumors (79.0% versus 87.2%, respectively), thus indicating the prognostic value of Ki-67. While Ki-67 was prognostic, the abemaciclib benefit was observed regardless of Ki-67 status. The data for IDFS among patients with 1-3 positive ALNs, tumor size <5cm, grade < 3, and high Ki-67 index (over 20%) remained immature.

Section Summary: Ki-67 Testing
Among patients with HR-positive, HER2-negative, node-positive, early breast cancer with clinical and pathological features consistent with a high risk of recurrence (n=5637), abemaciclib plus endocrine therapy demonstrated superior invasive disease-free survival compared to endocrine therapy alone (HR=0.75; p=.01). For the cohort of patients with Ki-67 score ≥ 20% (n=2003 [35.5%]), secondary analysis of invasive disease-free survival was also superior for the group receiving abemaciclib (HR=0.626; p=.0042). However, additional analyses showed the abemaciclib benefit was observed regardless of Ki-67 status. Further study is necessary to confirm whether an improved overall survival benefit is observed among patients with Ki-67 positive status.

Tumor Mutational Burden
Companion Diagnostic Test
FoundationOne is an FDA approved companion diagnostic test to measure TMB in patients with solid tumors being considered for pembrolizumab treatment.

Nonrandomized Trials
Pembrolizumab
Ott et al (2018) reported an exploratory analysis of the association between TMB and response to pembrolizumab. All patients in the study were PD-L1 positive.[40.]

Marabelle et al (2020) reported the association of high TMB to response to pembrolizumab in patients with solid tumors enrolled in a prespecified exploratory analysis of the KEYNOTE-158 study.[41.] High TMB was defined as >10 mutations per megabase according to the FoundationOne CDx panel. The proportion of patients with an objective response in the tTMB-high group was 29%. At a median follow-up of approximately 3 years, the median duration of response was not reached in the tTMB-high group and was 33·1 months in the non-tTMB-high group. Notably, TMB-high status was associated with improved response irrespective of PD-L1. Median PFS and overall survival did not differ between the high and non-high TMB groups. Objective responses were observed in 24 (35%; 95% CI, 24–48) of 68 participants who had both tTMB-high status and PD-L1-positive tumours (i.e., PD-L1 combined positive score of ≥1) and in 6

(21%; 8–40) of 29 participants who had tTMB-high status and PD-L1-negative tumors. The interpretation of these results is limited by the lack of enrollment of patients with breast cancer.

Table 16. Association of TMB to Response to Pembrolizumab in Patients with Solid Tumors Enrolled in the KEYNOTE-158 Study

Study	Response	Median Duration of Response	Median PFS	Median OS (95% CI)	Adverse events
Marabelle et al (2020);[41] NCT02628067					
TMB ≥10 per megabase; N=102	Objective Response: 29% (21-39%) Complete: 4%	Median not yet reached range 2·2+ to 34·8+ months	2.1 months (95% CI 2·1–4·1)	Median: 11·7 months (95% CI 9·1–19·1)	Deaths: 69/102 (68%)
TMB <10 per megabase; N=688	Objective Response: 6% (5-8%) Complete: 2%	Median 33·1 months (4·0 to 35·7+)	2·1 months (2·1–2·2)	12·8 months (11·1–14·1)	534/688 (78%)

CI: confidence interval N: sample size; OS: overall survival; PFS: progression-free survival; TMB: tumor mutational burden

Alva et al (2021) reported results from the Targeted Agent and Profiling Utilization Registry (TAPUR), a phase II pragmatic basket trial evaluating the efficacy of FDA-approved targeted therapies in patients with advanced tumors that harbor a genomic variant known to be a drug target or to predict treatment response.[42] In a cohort of 28 patients with metastatic breast cancer and TMB-H status treated with pembrolizumab, ten patients achieved disease control, defined as objective response or stable disease of at least 16 weeks duration, yielding a disease control rate of 37% (95% CI, 21% to 50%), a median PFS of 10.6 weeks (95% CI, 7.7 to 21.1 weeks), and a median overall survival of 30.6 weeks (95% CI, 18.3 to 103.3 weeks). The authors noted that TMB does not appear to be related to PFS or overall survival, but that the study was not powered to detect such an association. Additionally, TMB-H status was defined as ≥ 9 mut/Mb at the time of enrollment.

Section Summary: Tumor Mutational Burden
In a prespecified subgroup analysis of a nonrandomized trial of pembrolizumab in patients with various solid tumors, objective responses were observed in 24 (35%; 95% CI 24–48) of 68 participants who had both tTMB-high status and PD-L1-positive tumors and in 6 (21%; 8–40) of 29 participants who had tTMB-high status and PD-L1-negative tumors. In exploratory analyses, retrospective observational studies have reported an association between higher TMB and longer PFS and overall survival in patients receiving immunotherapy. These results need to be confirmed in additional, well-designed prospective studies.

Circulating Tumor DNA Testing to Select Targeted Treatment
Clinical Context and Test Purpose
The purpose of circulating tumor DNA testing in patients who have advanced or metastatic breast cancer is to inform a decision about selecting targeted treatment.

The question addressed in this evidence review is: Does biomarker testing using circulating tumor DNA improve the net health outcome in individuals with breast cancer?

The following PICO was used to select literature to inform this review.

Populations
The relevant population of interest is individuals with advanced or metastatic breast cancer.

Interventions
The test being considered is circulating tumor DNA testing.

Comparators

Tissue biopsy is used to make decisions about targeted treatment or immunotherapy for metastatic breast cancer.

Outcomes
Liquid biopsies are easier to obtain and less invasive than tissue biopsies. True-positive liquid biopsy test results lead to the initiation of appropriate treatment (e.g., targeted therapy) without a tissue biopsy. False-positive liquid biopsy test results lead to the initiation of inappropriate therapy, which could shorten progression-free survival.

In patients able to undergo a tissue biopsy, negative liquid biopsies reflex to tissue testing. In patients unable to undergo a tissue biopsy, a negative liquid biopsy result would not change empirical treatment. Therefore, health outcomes related to negative test results do not differ between liquid biopsy and tissue biopsy.

Study Selection Criteria
For the evaluation of clinical validity of the circulating tumor DNA test, studies that meet the following eligibility criteria were considered:

- Reported on the accuracy of the marketed version of the technology (including any algorithms used to calculate scores)
- Included a suitable reference standard (describe the reference standard)
- Patient/sample clinical characteristics were described
- Patient/sample selection criteria were described.

Companion Diagnostic Test for Targeted Treatment
FoundationOne Liquid is FDA approved as a companion diagnostic test for alpelisib (Piqray) for measuring 11 variants in the *PIK3CA* gene.

Clinical Validity
Woodhouse 2020 reported the clinical validity of FoundationOne liquid for detection of *PIK3CA* alterations through retrospective testing of plasma samples of patients enrolled in the SOLAR-1 trial.[43,]

All available plasma samples from patients collected at baseline prior to randomization into the SOLAR-1 clinical trial were tested with FoundationOne Liquid CDx, with results compared to tissue genotyping performing using the SOLAR-1 CTA. The positive predictive agreement and negative predictive agreement between FoundationOne Liquid CDx and the tissue-based CTA assay were 71.7% (95% CI 65.4%, 77.5%) and 100% (97.2%, 100%), respectively.

Table 17. Clinical Validity of FoundationOne Liquid CDx to detect *PIK3CA* Alterations- Results

Study	Study Population	Reference Standard	Initial N	Final N	Excluded Samples	Prevalence of Condition	Clinical Validity (95% Confidence Interval)	
							PPA	***NPA***
Woodhouse et al (2020)[43,]	Plasma samples from advanced or metastatic HR-positive, HER2-negative breast cancer patients enrolled in the SOLAR-1 trial	Tumor tissue PCR-based clinical trial assay	432	375	16	230 positive129 negative	71.7% (95% CI 65.4%, 77.5%)	100% (97.2%, 100%)

CI: confidence interval; HER2: human epidermal frowth factor receptor 2; HR: hormone receptor; N: sample size; NPA: negative predictive agreement; PCR: polymerase chain reaction; PIK3CA: phosphatidylinositol 3-kinase catalytic alpha polypeptide; PPA: positive predictive agreement

Clinical Utility

In the SOLAR-1 trial (discussed above in the section on *PIK3CA* testing), the clinical efficacy of alpelisib in combination with fulvestrant for the FoundationOne Liquid CDx-positive population was demonstrated with an estimated 54% risk reduction in disease progression or death in the alpelisib plus fulvestrant arm compared to the placebo plus fulvestrant arm (HR = 0.46, 95% CI, 0.30, 0.70).[26,]

Section Summary: Circulating Tumor DNA Testing
Clinical validity of the FoundationOne Liquid CDx test was demonstrated through retrospective testing of plasma samples of patients enrolled in the SOLAR-1 trial. The positive predictive agreement and negative predictive agreement between FoundationOne Liquid CDx and the tissue-based assay were 71.7% (95% CI 65.4%, 77.5%) and 100% (97.2%, 100%), respectively. Among the circulating tumor DNA-positive population, there was an estimated 54% risk reduction in disease progression or death in the alpelisib plus fulvestrant arm compared to the placebo plus fulvestrant arm (HR = 0.46, 95% CI, 0.30, 0.70).

Circulating Tumor Cell Testing to Select Targeted Treatment
Clinical Context and Test Purpose
The purpose of testing circulating tumor cells (CTC) in patients who have breast cancer is to inform a decision about selecting targeted treatment.

The question addressed in this evidence review is: Does CTC testing improve the net health outcome in individuals with breast cancer?

The following PICO was used to select literature to inform this review.

Populations
The relevant population of interest is individuals with recurrent or metastatic breast cancer.

Interventions
The test being considered is CTC testing.
The primary reason for CTCs would be to aid in decision-making about alternative treatment. CTC testing has been proposed as a method to guide the choice between chemotherapy and endocrine therapy as first-line treatment, or to change early to an alternative chemotherapy regimen in patients for whom chemotherapy has failed to reduce CTCs.

Comparators
Decisions about first-line treatment and alternative treatments in metastatic breast cancer are based on clinical evaluation and biopsy.

Outcomes
The general outcomes of interest in oncology are overall survival, disease-specific survival, quality of life, treatment-related mortality and morbidity.

Follow-up at 6-12 months is of interest to monitor outcomes.

Study Selection Criteria
For the evaluation of clinical validity of CTC test, studies that meet the following eligibility criteria were considered:

- Reported on the accuracy of the marketed version of the technology (including any algorithms used to calculate scores)
- Included a suitable reference standard (describe the reference standard)
- Patient/sample clinical characteristics were described
- Patient/sample selection criteria were described.

Clinical Validity

Systematic reviews and meta-analyses have described an association between CTCs and poor prognosis in metastatic breast cancer. [44,]

Clinical Utility

Randomized Controlled Trials

Two RCTs have evaluated the clinical utility of using CTC to guide treatment decisions in patients with metastatic breast cancer.

Smerage et al (2014) reported on the results of an RCT of patients with metastatic breast cancer and persistently increased CTC levels to test whether changing chemotherapy after 1 cycle of first-line therapy could improve overall survival.[45,]. Level of CTCs were enumerated using the CellSearch system. Five or more CTCs per 7.5 mL WB was considered an increased level, and it served as the cut point for separation of favorable versus unfavorable prognosis. Patients who did not have increased CTC levels at baseline remained on initial therapy until progression (arm A), patients with initially increased CTC levels that decreased after 21 days of therapy remained on initial therapy (arm B), and patients with persistently increased CTC levels after 21 days of therapy were randomized to continue initial therapy (arm C1) or change to an alternative chemotherapy (arm C2). There were 595 eligible and evaluable patients, 276 (46%) of whom did not have increased CTC levels (arm A). Of patients with initially increased CTC levels, 31 (10%) were not retested, 165 were assigned to arm B, and 123 were randomized to arms C1 or C2. There was no difference in median overall survival between arms C1 (10.7 months) and C2 12.5 months; p=0.98). CTC levels were strongly prognostic, with a median overall survival for arms A, B, and C (C1 and C2 combined) of 35 months, 23 months, and 13 months, respectively (p<.001). While the trial showed the prognostic significance of CTCs in patients with metastatic breast cancer, changing to an alternative chemotherapeutic regimen did not improve outcomes in patients whose CTCs were not reduced after 1 cycle of first-line chemotherapy.

More recently, Bidard et al (2021) reported on a noninferiority trial comparing CTC-driven vs clinician driven first-line therapy choice in patients with metastatic breast cancer.[46,] Median PFS was 15.5 months (95% CI, 12.7-17.3) in the CTC arm and 13.9 months (95% CI, 12.2-16.3) in the standard arm. The primary end point was met, with a hazard ratio of 0.94 (90% CI, 0.81-1.09).

Table 18. RCTs of CTC-Guided Treatment in Breast Cancer- Characteristics

Study	Countries	Sites	Dates	Participants	Interventions		Endpoints
					Active	Comparator	
Smerage et al (2014);[45,] NCT00382018			Oct 2006-Mar 2012	Women with histologically confirmed breast cancer and clinical and/or radiographic evidence of metastatic disease Persistent increased CTCs following 1 cycle of chemotherapy.	Changing chemotherapy after 1 cycle of first-line chemotherapy N=59	Continued initial therapy N=64	OS, PFS
Bidard et al (2021)[46,]	France	17	Feb 2012-Jul 2016	778 women with hormone-receptor positive, HER2-negative metastatic breast	CTC-driven treatment choice N=391	Clinician-driven treatment choice N=387	PFS, OS, rate of treatment changes, AEs

AEs: adverse events; CTC: circulating tumor cell; HER2: human epidermal growth factor receptor 2; N: sample size; OS: overall survival; PFS: progression-free survival; RCTs: randomized controlled trials

Table 19. RCTs of CTC-Guided Treatment in Breast Cancer- Results

Study	OS	PFS
Smerage et al (2014)[45.]		
N analyzed		
CTC-Directed Treatment	12.5 months	4.6 months
Standard care	10.7 months	3.5 months
HR (95% CI)	1.00 (95% CI, 0.69 to 1.47)	0.92 (95% CI, 0.64 to 1.32)
p	.98	.64
Bidard et al (2021)[46.]		
N analyzed		
CTC-directed treatment		15.5 months (12.7-17.3)
Standard care		13.9 months (12.2-16.3)
HR (95% CI)		0.94 (0.81 to 1.09)

CI: confidence interval; CTC: circulating tumor cell; HR: hazard ratio; N: sample size; OS: overall survival; PFS: progression-free survival; RCTs: randomized controlled trials

Section Summary: Circulating Tumor Cell Testing
Systematic reviews and meta-analyses have described an association between CTCs and poor prognosis in metastatic breast cancer, but evidence that CTC-driven treatment improves health outcomes is lacking. One RCT found no improvement in overall survival or PFS with CTC-driven treatment (early switching to a different chemotherapy regimen) compared to continuing initial therapy. A second RCT found that CTC-driven first-line therapy was noninferior to clinician-driven therapy in previously untreated patients with metastatic breast cancer (hazard ratio for PFS 0.94; 95% CI 0.81 to 1.09).

Summary of Evidence
For individuals with metastatic or high-risk, early stage HER2-negative breast cancer being considered for systemic therapy (i.e., poly(adenosine diphosphate–ribose) polymerase [PARP] inhibitors) who receive genetic testing for a *BRCA1* or *BRCA2* germline variant, the evidence includes randomized, placebo-controlled trials of olaparib and talazoparib. Relevant outcomes are overall survival, disease-specific survival, test validity, quality of life, and treatment-related morbidity. In individuals with a *BRCA1/2* mutation and either HER2-negative metastatic breast cancer or other advanced breast cancer who were followed for 11-12 months, treatment with a PARP inhibitor drug resulted in a 40% to 46% lower risk of disease progression or death. In individuals with a *BRCA1/2* mutation and early-stage breast cancer at high-risk for recurrence, treatment with olaparib resulted in a 9% improvement in 3-year invasive disease-free survival. Therefore, knowledge of *BRCA* variant status in individuals diagnosed with breast cancer may impact treatment decisions. The evidence is sufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with hormone receptor-positive, HER2 negative advanced or metastatic breast cancer who receive *PIK3CA* gene testing to select targeted treatment, the evidence includes a randomized, placebo-controlled trial of alpelisib compared to placebo in men and postmenopausal women with advanced breast cancer who had previously received endocrine therapy. Relevant outcomes include overall survival, disease-specific survival, test validity, quality of life, and treatment-related morbidity. Among patients with *PIK3C*A-positive tumors who received targeted therapy, PFS was 11.0 months (95% CI, 7.5 to 14.5), compared to 5.7 months (95% CI, 3.7 to 7.4) in *PIK3CA*-positive patients who received standard care. In contrast, the hazard ratio for PFS in the cohort without *PIK3CA*-mutated cancer was not significantly different for the active vs placebo groups. The overall response rate was higher in patients with *PIK3CA*-positive tumors compared to the rate in the standard care group (26.6% [95% CI 20.1- 34.0] vs 12.8% [8.2-18.7%]). The evidence is sufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with locally advanced or metastatic breast cancer being considered for immunotherapy who receive *NTRK* gene fusion testing, the evidence includes integrated

analyses of nonrandomized trials of larotrectinib and entrectinib in patients with *NTRK*-fusion positive solid tumors. Relevant outcomes are overall survival, disease-specific survival, test validity, quality of life, and treatment-related morbidity. In an analysis of 159 patients with *NTRK*-fusion positive solid tumors who received larotrectinib, including 5 patients with breast tumors, the overall response rate was 79% (95% CI 72 to 85). The median PFS was 28·3 months (95% CI 22.·1 to not estimable), and 67% of patients were progression-free at 12 months (95% CI 58–76). In an integrated analysis of 3 phase 1-2 trials in 54 patients with *NTRK*-positive solid tumors who received entrectinib, 6 of whom had breast cancer, the overall response rate was 57% (95% CI, 43.2–70.8). At data cutoff, 16 (30%) of 54 patients had died, and the estimated median overall survival was 21 months (95% CI, 14.9 to not estimable. Responses were observed regardless of tumor type or age of the patient. The evidence is sufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with recurrent, metastatic, or unresectable hormone receptor-negative, HER2 negative (triple negative) breast cancer being considered for immunotherapy who receive PD-L1 testing, the evidence includes a RCT of atezolizumab and nonrandomized trials of pembrolizumab. Relevant outcomes include overall survival, disease-specific survival, test validity, quality of life, and treatment-related morbidity. In a placebo controlled trial of atezolizumab in combination with nab-paclitaxel for patients with PD-L1 positive TNBC, median PFS (HR 0.62; 95% CI, 0.49 to 0.78) and overall survival 0.62 (95% CI, 0.45–0.86) were longer among patients who received the targeted immunotherapy. However, these findings were not confirmed in a designated confirmatory trial and accelerated approval was withdrawn for atezolizumab. In 2 nonrandomized trials of pembrolizumab for patients with PD-L1 positive TNBC, the objective response rate was 21.4% (95% CI, 13.9 to 31.4) and 18.5% (95% CI, 6.3 to 38.1). In 1 randomized trial of pembrolizumab plus chemotherapy versus placebo plus chemotherapy for patients with TNBC and PD-L1 combined positive score ≥10, the median PFS was 9.7 and 5.6 months, respectively (HR, 0.65; 95% CI, 0.49 to 0.86). The evidence is sufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with unresectable or metastatic breast cancer who are being considered for pembrolizumab therapy who receive MSI-H/dMMR testing, the evidence includes nonrandomized trials in patients with solid tumors. Relevant outcomes include overall survival, disease-specific survival, test validity, quality of life, and treatment-related morbidity. In a phase 2 trial of pembrolizumab in 233 previously treated patients with MSI-H solid tumors, the overall response rate was 34.3% (95% CI, 28.3% to 40.8%). Median PFS was 4.1 months (95% CI, 2.4 to 4.9 months) and median overall survival was 23.5 months (95% CI, 13.5 months to not reached). Treatment-related adverse events occurred in 151 patients (64.8%). The evidence is sufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with recurrent or advanced breast cancer who are being considered for dostarlimab-gxly therapy who receive dMMR testing, the evidence includes nonrandomized trials in patients with solid tumors. Relevant outcomes include overall survival, disease-specific survival, test validity, quality of life, and treatment-related morbidity. A phase 1 dose escalation study of dostarlimab-gxly reported an overall response rate of 41.6% with a median duration of response of 34.7 months for a combined cohort of 209 patients with endometrial cancer and non-endometrial cancer solid cancers; however, enrollment of patients with breast cancer was limited to 1 individual. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with breast cancer who are being considered for abemaciclib therapy who receive Ki-67 testing, the evidence includes a randomized, controlled, open-label trial. Relevant outcomes include overall survival, disease-specific survival, test validity, quality of life, and treatment-related morbidity. Among patients with hormone receptor-positive, HER2-negative, node-positive, early breast cancer with clinical and pathological features consistent with a high risk of recurrence (n=5637), abemaciclib plus endocrine therapy demonstrated superior invasive disease-free survival compared to endocrine therapy alone (HR=0.75; p=.01). For the cohort of

patients with Ki-67 score ≥ 20% (n=2003 [35.5%]), secondary analysis of invasive disease-free survival was also superior for the group receiving abemaciclib (HR=0.626; p=.0042). However, additional analyses showed the abemaciclib benefit was observed regardless of Ki-67 status. Further study is necessary to confirm whether an improved overall survival benefit is observed among patients with Ki-67 'high' versus 'low' status. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with unresectable or metastatic breast cancer who are being considered for immunotherapy who receive tumor mutational burden (TMB) testing, the evidence includes prospective and retrospective subgroup analyses of nonrandomized trials. Relevant outcomes include overall survival, disease-specific survival, test validity, quality of life, and treatment-related morbidity. In a prespecified subgroup analysis of a nonrandomized trial of pembrolizumab in patients with various solid tumors, objective responses were observed in 24 (35%; 95% CI, 24–48) of 68 participants who had both tTMB-high status and PD-L1-positive tumors and in 6 (21%; 8–40) of 29 participants who had tTMB-high status and PD-L1-negative tumors. In exploratory analyses, retrospective observational studies have reported an association between higher TMB and longer PFS and overall survival in patients receiving immunotherapy. These results need to be confirmed in additional, well-designed prospective studies enrolling patients with breast cancer. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with hormone receptor-positive, HER2 negative advanced or metastatic breast cancer who receive circulating tumor DNA testing to select targeted treatment, the evidence includes a randomized, placebo-controlled trial of alpelisib compared to placebo in men and postmenopausal women with advanced breast cancer who had previously received endocrine therapy. Relevant outcomes include overall survival, disease-specific survival, test validity, quality of life, and treatment-related morbidity. Clinical validity of the FoundationOne Liquid CDx test was demonstrated through retrospective testing of plasma samples of patients enrolled in the SOLAR-1 trial. The positive predictive agreement and negative predictive agreement between FoundationOne Liquid CDx and the tissue-based assay were 71.7% (95% CI, 65.4%, 77.5%) and 100% (97.2%, 100%), respectively. Among the circulating tumor DNA-positive population, there was an estimated 54% risk reduction in disease progression or death in the alpelisib plus fulvestrant arm compared to the placebo plus fulvestrant arm (HR = 0.46, 95% CI, 0.30, 0.70). The evidence is sufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with metastatic breast cancer who receive CTC testing to guide treatment decisions, the evidence includes RCTs, observational studies, and systematic reviews. Relevant outcomes include overall survival, disease-specific survival, test validity, quality of life, and treatment-related morbidity. Systematic reviews and meta-analyses have described an association between CTCs and poor prognosis in metastatic breast cancer, but evidence that CTC-driven treatment improves health outcomes is lacking. One RCT found no improvement in overall survival or PFS with CTC-driven treatment (early switching to a different chemotherapy regimen) compared to continuing initial therapy. A second RCT found that CTC-driven first-line therapy was noninferior to clinician-driven therapy in previously untreated patients with metastatic breast cancer (hazard ratio for PFS 0.94; 95% CI 0.81 to 1.09).The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

Supplemental Information
The purpose of the following information is to provide reference material. Inclusion does not imply endorsement or alignment with the evidence review conclusions.

Practice Guidelines and Position Statements
Guidelines or position statements will be considered for inclusion in ‘Supplemental Information' if they were issued by, or jointly by, a US professional society, an international society with US

representation, or National Institute for Health and Care Excellence (NICE). Priority will be given to guidelines that are informed by a systematic review, include strength of evidence ratings, and include a description of management of conflict of interest.

National Comprehensive Cancer Network

Table 20 summarizes National Comprehensive Cancer Network guidelines (v.1.2022) on biomarker testing for the biomarkers included in this policy.[13.] The guidelines state that the use of circulating tumor cells or circulating tumor DNA in metastatic breast cancer is not yet included in algorithms for disease assessment and monitoring. For patients being considered for treatment with alpelisib, testing for *PIK3CA* with either tissue or liquid biopsy is recommended (category of evidence 2A).

Table 20. National Comprehensive Cancer Network Guidelines on Biomarker Testing for Targeted Treatment of Breast Cancer

Biomarker	Breast Cancer Subtype	FDA Approved Agents	Testing Recommendation	Targeted Therapy Category of Evidence	Targeted Therapy Category of Preference
BRCA1/2 mutations	Any	Olaparib Talazoparib	Patients with recurrent or metastatic breast cancer should be assessed for *BRCA1/2* mutations with germline sequencing to identify candidates for PARP inhibitor therapy. While olaparib and talazoparib are FDA-indicated in HER2-negative disease, NCCN supports use in any breast cancer subtype associated with a germline BRCA1 or BRCA2 mutation.	1	Preferred
PIK3CA	HR-positive/HER2-negative	Alpelisib + fulvestrant	For HR-positive/HER2-negative breast cancer, assess for *PIK3CA* mutations with tumor or liquid biopsy to identify candidates for alpelisib plus fulvestrant. PIK3CA mutation testing can be done on tumor tissue or ctDNA in peripheral blood (liquid biopsy). If liquid biopsy is negative, tumor tissue testing is recommended.	1	Preferred second-or subsequent-line therapy
PD-L1 expression (combined positive score ≥ 10)	Triple negative	Pembrolizumab + chemo therapy (albumin-bound paclitaxel, or gemcitabine and carboplatin)	For triple-negative breast cancer, assess PD-L1 expression using 22C3 antibody via immunohistochemistry. While available data are in the first-line setting, this regimen can be used for second and subsequent lines of therapy if PD-1/PD-L1 inhibitor therapy has not been previously used.	1	Preferred first-line therapy
NTRK fusion	Any	Larotrectinib Entrectinib	No specific testing recommendation. If a patient with recurrent/stage IV breast cancer presents with a tumor with an *NTRK* fusion, treatment	2A	Useful in certain circumstances

Biomarker	Breast Cancer Subtype	FDA Approved Agents	Testing Recommendation	Targeted Therapy Category of Evidence	Targeted Therapy Category of Preference
			with an *NTRK* inhibitor is an option if no satisfactory alternative treatments exist or that have progressed following treatment, treatment with an *NTRK* inhibitor is an option		
MSI-H/dMMR	Any	Pembrolizumab Dostarlimab-gxly	Biomarker detection via immunohistochemistry or PCR tissue block is recommended. If a patient with unresectable or metastatic MSI-H/dMMR breast cancer has progressed on or following prior treatment with no satisfactory alternative treatment options, pembrolizumab or dostarlimab-gxly are indicated.	2A	Useful in certain circumstances
TMB-H (≥10 mut/mb)	Any	Pembrolizumab	Biomarker detection via NGS is indicated in patients with unresectable or metastatic TMB-H tumors that have progressed following prior treatment and who have no satisfactory treatment options.	2A	Useful in certain circumstances

Source: Adapted from National Comprehensive Cancer Network guidelines on Breast Cancer (v.1.2022)[13,]

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

Medicare National Coverage
In January 2020, the Centers for Medicare and Medicaid Services (CMS) determined that next-generation sequencing (NGS) is covered for patients with breast or ovarian cancer when the diagnostic test is performed in a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory AND the test has approval or clearance by the U.S. Food and Drug Administration (CAG-00450R).[47,]

CMS states that local Medicare carriers may determine coverage of NGS for management of the patient for any cancer diagnosis with a clinical indication and risk factor for germline testing of hereditary cancers when performed in a CLIA-certified laboratory.

Ongoing and Unpublished Clinical Trials
Some currently unpublished trials that might influence this review are listed in Table 21.

Table 21. Summary of Key Trials

NCT No.	Trial Name	Planned Enrollment	Completion Date
Ongoing			
NCT04098640	Molecular Profiling Using FoundationOne CDx in Young (<50 Years of Age) Patients With Metastatic Breast Cancer (ML41263)	200	Jul 2021 (unknown)
NCT03197935[a]	A Phase III Randomized Study to Investigate the Efficacy and Safety of Atezolizumab (Anti-PD-L1 Antibody) in Combination With Neoadjuvant Anthracycline/Nab-	333	Oct 2022

NCT No.	Trial Name	Planned Enrollment	Completion Date
	Paclitaxel-Based Chemotherapy Compared With Placebo and Chemotherapy in Patients With Primary Invasive Triple-Negative Breast Cancer (IMpassion031)		
NCT03145961[a]	c-TRAK TN: A Randomised Trial Utilising ctDNA Mutation Tracking to Detect Minimal Residual Disease and Trigger Intervention in Patients With Moderate and High Risk Early Stage Triple Negative Breast Cancer	208	Dec 2022
NCT03213041[a]	I-CURE-1: A Phase II, Single Arm Study of Pembroluzimab Combined With Carboplatin in Patients With Circulating Tumor Cells (CTCs) Positive HER-2 Negative Metastatic Breast Cancer (MBC)	100	Jul 2023 (recruiting)
NCT02965755[a]	Individualized Molecular Analyses Guide Efforts in Breast Cancer - Personalized Molecular Profiling in Cancer Treatment at Johns Hopkins (IMAGE-II)	200	Jul 2023 (recruiting)
NCT02819518[a]	A Randomized, Double-Blind, Phase III Study of Pembrolizumab (MK-3475) Plus Chemotherapy vs Placebo Plus Chemotherapy for Previously Untreated Locally Recurrent Inoperable or Metastatic Triple Negative Breast Cancer (KEYNOTE-355)	882	Nov 2023
NCT02889978[a]	The Circulating Cell-free Genome Atlas Study (CCGA)	15000	Mar 2024
NCT02568267[a]	An Open-Label, Multicenter, Global Phase 2 Basket Study of Entrectinib for the Treatment of Patients With Locally Advanced or Metastatic Solid Tumors That Harbor NTRK1/2/3, ROS1, or ALK Gene Rearrangements (STARTRK-2)	700	Dec 2024 (recruiting)
NCT04591431	The Rome Trial - From Histology to Target: the Road to Personalize Target Therapy and Immunotherapy	384	Aug 2024 (recruiting)
NCT02693535[a]	Targeted Agent and Profiling Utilization Registry (TAPUR) Study	3581	Dec 2024 (recruiting)
NCT04720729	Chemotherapy Monitoring by Circulating Tumor DNA (ctDNA) in HER2 (Human Epidermal Growth Factor Receptor-2)- Metastatic Breast Cancer (MONDRIAN): a Phase 2 Study	214	Jun 2025 (recruiting)
NCT04526587	The Roswell Park Ciclib Study: A Prospective Study of Biomarkers and Clinical Features of Advanced/Metastatic Breast Cancer Treated With CDK4/6 Inhibitors	300	Jul 2025 (recruiting)
NCT04895358[a]	A Randomized, Double-blind, Placebo-controlled, Phase 3 Study of Pembrolizumab Plus Chemotherapy Versus Placebo Plus Chemotherapy for the Treatment of Chemotherapy-Candidate Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative (HR+/HER2-) Locally Recurrent Inoperable or Metastatic Breast Cancer (KEYNOTE-B49)	800	Oct 2027 (recruiting)
NCT02306096	SCAN-B: The Sweden Cancerome Analysis Network - Breast Initiative	20000	Aug 2031 (recruiting)

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

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Documentation for Clinical Review

Please provide the following documentation:

- History and physical and/or consultation notes including:
  - Clinical findings (i.e., pertinent symptoms and duration)
  - Current diagnoses and status (i.e., type of cancer, stage)
  - Family history, if applicable
  - Reason for test when applicable
  - Pertinent past procedural and surgical history (i.e., biopsies, resections, etc.)
  - Pertinent past genetic tests (i.e., somatic/tumor or germline test results including but not limited to HER2, PD-L1, MSI, BRCA, etc.)

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

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

Coding

This Policy relates only to the services or supplies described herein. Benefits may vary according to product design; therefore, contract language should be reviewed before applying the terms of the Policy.

The following codes are included below for informational purposes. Inclusion or exclusion of a code(s) does not constitute or imply member coverage or provider reimbursement policy. Policy Statements are intended to provide member coverage information and may include the use of some codes for clarity. The Policy Guidelines section may also provide

additional information for how to interpret the Policy Statements and to provide coding guidance in some cases.

Type	Code	Description
CPT®	0037U	Targeted genomic sequence analysis, solid organ neoplasm, DNA analysis of 324 genes, interrogation for sequence variants, gene copy number amplifications, gene rearrangements, microsatellite instability and tumor mutational burden (PLA for the Foundation One CDx™ (F1CDx®) test)
	0048U	Oncology (solid organ neoplasia), DNA, targeted sequencing of protein-coding exons of 468 cancer-associated genes, including interrogation for somatic mutations and microsatellite instability, matched with normal specimens, utilizing formalin-fixed paraffin-embedded tumor tissue, report of clinically significant mutation(s) (PLA code for the MSK–IMPACT™ (Integrated Mutation Profiling of Actionable Cancer Targets), Memorial Sloan Kettering Cancer Center)
	0155U	Oncology (breast cancer), DNA, PIK3CA (phosphatidylinositol-4,5-bisphosphate 3- kinase, catalytic subunit alpha) (e.g., breast cancer) gene analysis (i.e., p.C420R, p.E542K, p.E545A, p.E545D [g.1635G>T only], p.E545G, p.E545K, p.Q546E, p.Q546R, p.H1047L, p.H1047R, p.H1047Y), utilizing formalin-fixed paraffin-embedded breast tumor tissue, reported as PIK3CA gene mutation status (PLA code for the therascreen® PIK3CA RGQ PCR Kit from QIAGEN)
	0177U	Oncology (breast cancer), DNA, PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha) gene analysis of 11 gene variants utilizing plasma, reported as PIK3CA gene mutation status (PLA code for the therascreen® PIK3CA RGQ PCR Kit test from QIAGEN)
	0211U	Oncology (pan-tumor), DNA and RNA by next-generation sequencing, utilizing formalin-fixed paraffin-embedded tissue, interpretative report for single nucleotide variants, copy number alterations, tumor mutational burden, and microsatellite instability, with therapy association (PLA code for the MI Cancer Seek™ – NGS Analysis from Caris MPI d/b/a Caris Life Sciences.)
	0239U	Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free DNA, analysis of 311 or more genes, interrogation for sequence variants, including substitutions, insertions, deletions, select rearrangements, and copy number variations
	0242U	Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free circulating DNA analysis of 55-74 genes, interrogation for sequence variants, gene copy number amplifications, and gene rearrangements ***(Code effective 4/1/2021)***
	0262U	Oncology (solid tumor), gene expression profiling by real-time RT-PCR of 7 gene pathways (ER, AR, PI3K, MAPK, HH, TGFB, Notch), formalin-fixed paraffin-embedded (FFPE), algorithm reported as gene pathway activity score ***(Code effective 10/1/2021)***
	81191	NTRK1 (neurotrophic receptor tyrosine kinase 1) (e.g., solid tumors) translocation analysis
	81192	NTRK2 (neurotrophic receptor tyrosine kinase 2) (e.g., solid tumors) translocation analysis
	81193	NTRK3 (neurotrophic receptor tyrosine kinase 3) (e.g., solid tumors) translocation analysis
	81194	NTRK (neurotrophic-tropomyosin receptor tyrosine kinase 1, 2, and 3) (e.g., solid tumors) translocation analysis

Type	Code	Description
	81301	Microsatellite instability analysis (e.g., hereditary non-polyposis colorectal cancer, Lynch syndrome) of markers for mismatch repair deficiency (e.g., BAT25, BAT26), includes comparison of neoplastic and normal tissue, if performed
	81309	PIK3CA (phosphatidylinositol-4, 5-biphosphate 3-kinase, catalytic subunit alpha) (e.g., colorectal and breast cancer) gene analysis, targeted sequence analysis (e.g., exons 7, 9, 20)
	81445	Targeted genomic sequence analysis panel, solid organ neoplasm, DNA analysis, and RNA analysis when performed, 5-50 genes (e.g., ALK, BRAF, CDKN2A, EGFR, ERBB2, KIT, KRAS, NRAS, MET, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, if performed
	81455	Targeted genomic sequence analysis panel, solid organ or hematolymphoid neoplasm, DNA analysis, and RNA analysis when performed, 51 or greater genes (e.g., ALK, BRAF, CDKN2A, CEBPA, DNMT3A, EGFR, ERBB2, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MLL, NPM1, NRAS, MET, NOTCH1, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, if performed
	86152	Cell enumeration using immunologic selection and identification in fluid specimen (e.g., circulating tumor cells in blood)
	86153	Cell enumeration using immunologic selection and identification in fluid specimen (e.g., circulating tumor cells in blood); physician interpretation and report, when required
	88360	Morphometric analysis, tumor immunohistochemistry (e.g., Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, per specimen, each single antibody stain procedure; manual
	88361	Morphometric analysis, tumor immunohistochemistry (e.g., Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, per specimen, each single antibody stain procedure; using computer-assisted technology
	81479	Unlisted molecular pathology procedure
HCPCS	None	

Policy History

This section provides a chronological history of the activities, updates and changes that have occurred with this Medical Policy.

Effective Date	Action
02/01/2021	New policy
06/01/2021	Coding update
11/01/2021	Coding update
03/01/2022	Annual review. Policy statement, guidelines and literature updated. Policy title changed from Biomarker Testing (Including Liquid Biopsy) for Targeted Treatment and Immunotherapy in Breast Cancer to current one.

Definitions of Decision Determinations

Medically Necessary: Services that are Medically Necessary include only those which have been established as safe and effective, are furnished under generally accepted professional standards to treat illness, injury or medical condition, and which, as determined by Blue Shield,

are: (a) consistent with Blue Shield medical policy; (b) consistent with the symptoms or diagnosis; (c) not furnished primarily for the convenience of the patient, the attending Physician or other provider; (d) furnished at the most appropriate level which can be provided safely and effectively to the patient; and (e) not more costly than an alternative service or sequence of services at least as likely to produce equivalent therapeutic or diagnostic results as to the diagnosis or treatment of the Member's illness, injury, or disease.

Investigational/Experimental: A treatment, procedure, or drug is investigational when it has not been recognized as safe and effective for use in treating the particular condition in accordance with generally accepted professional medical standards. This includes services where approval by the federal or state governmental is required prior to use, but has not yet been granted.

Split Evaluation: Blue Shield of California/Blue Shield of California Life & Health Insurance Company (Blue Shield) policy review can result in a split evaluation, where a treatment, procedure, or drug will be considered to be investigational for certain indications or conditions, but will be deemed safe and effective for other indications or conditions, and therefore potentially medically necessary in those instances.

Prior Authorization Requirements (as applicable to your plan)

Within five days before the actual date of service, the provider must confirm with Blue Shield that the member's health plan coverage is still in effect. Blue Shield reserves the right to revoke an authorization prior to services being rendered based on cancellation of the member's eligibility. Final determination of benefits will be made after review of the claim for limitations or exclusions.

Questions regarding the applicability of this policy should be directed to the Prior Authorization Department at (800) 541-6652, or the Transplant Case Management Department at (800) 637-2066 ext. 3507708 or visit the provider portal at www.blueshieldca.com/provider.

Disclaimer: This medical policy is a guide in evaluating the medical necessity of a particular service or treatment. Blue Shield of California may consider published peer-reviewed scientific literature, national guidelines, and local standards of practice in developing its medical policy. Federal and state law, as well as contract language, including definitions and specific contract provisions/exclusions, take precedence over medical policy and must be considered first in determining covered services. Member contracts may differ in their benefits. Blue Shield reserves the right to review and update policies as appropriate.

Appendix A

POLICY STATEMENT	
BEFORE **Red font: Verbiage removed**	**AFTER** **Blue font: Verbiage Changes/Additions**
Biomarker Testing (Including Liquid Biopsy) for Targeted Treatment and Immunotherapy in Breast Cancer 2.04.151	**Germline and Somatic Biomarker Testing (Including Liquid Biopsy) for Targeted Treatment and Immunotherapy in Breast Cancer 2.04.151**
Policy Statement:	**Policy Statement:** ***BRCA1 and BRCA2* Testing** Genetic testing for *BRCA1* or *BRCA2* germline variants may be considered **medically necessary** to predict treatment response to PARP inhibitors (e.g., olaparib [Lynparza] and talazoparib [Talzenna]) for human epidermal receptor 2 (HER2)-negative metastatic and early stage, high-risk breast cancer (see Policy Guidelines). Genetic testing of *BRCA1* or *BRCA2* germline or somatic variants in patients with breast cancer for guiding therapy is considered **investigational** in all other situations unless included in a panel approved under another policy.
***PIK3CA* Testing** *PIK3CA* testing may be **medically necessary** to predict treatment response to alpelisib (Piqray) in patients with hormone receptor-positive, HER2 negative advanced or metastatic breast cancer (see Policy Guidelines). *PIK3CA* testing of tissue is considered **investigational** in all other situations unless included in a panel approved under another policy.	***PIK3CA* Testing** *PIK3CA* testing may be **medically necessary** to predict treatment response to alpelisib (Piqray) in patients with hormone receptor-positive, HER2-negative advanced or metastatic breast cancer (see Policy Guidelines). *PIK3CA* testing of tissue is considered **investigational** in all other situations unless included in a panel approved under another policy.
***NTRK* Gene Fusion Testing** Analysis of *NTRK* gene fusions may be considered **medically necessary** to predict treatment response to entrectinib (Rozlytrek) or larotrectinib (Vitrakvi) in patients with locally advanced or metastatic breast cancer that has progressed following standard treatment and who have no alternative treatment option (see Policy Guidelines). Analysis of *NTRK* gene fusions is considered **investigational** in all other situations unless included in a panel approved under another policy.	***NTRK* Gene Fusion Testing** Analysis of *NTRK* gene fusions may be considered **medically necessary** to predict treatment response to entrectinib (Rozlytrek) or larotrectinib (Vitrakvi) in patients with locally advanced or metastatic breast cancer that has progressed following standard treatment and who have no alternative treatment option (see Policy Guidelines). Analysis of *NTRK* gene fusions is considered **investigational** in all other situations unless included in a panel approved under another policy.

POLICY STATEMENT	
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PD-L1 Testing PD-L1 testing may be considered **medically necessary** to predict treatment response to atezolizumab (Tecentriq) in patients with hormone receptor-negative/HER2-negative (triple negative) metastatic or unresectable breast cancer (see Policy Guidelines). PD-L1 testing may be considered **medically necessary** to predict treatment response to pembrolizumab (Keytruda) in patients with hormone receptor-negative/HER2-negative (triple negative) recurrent or metastatic breast cancer (see Policy Guidelines). PD-L1 testing is considered **investigational** in all other situations unless included in a panel or separately approved under another policy.	**PD-L1 Testing** PD-L1 testing may be considered **medically necessary** to predict treatment response to pembrolizumab (Keytruda) in patients with hormone receptor-negative/HER2-negative (triple negative) recurrent or metastatic breast cancer (see Policy Guidelines). PD-L1 testing is considered **investigational** in all other situations, including to predict treatment response to atezolizumab (Tecentriq) unless included in a panel approved under another policy.
MSI-H/dMMR Testing MSI-H/dMMR testing may be considered **medically necessary** to predict treatment response to pembrolizumab (Keytruda) in patients with unresectable or metastatic breast cancer that has progressed following standard treatment and who have no alternative treatment option (see Policy Guidelines). MSI-H/dMMR testing is considered **investigational** in all other situations unless included in a panel or separately approved under another policy.	**MSI-H/dMMR Testing** MSI-H/dMMR testing may be considered **medically necessary** to predict treatment response to pembrolizumab (Keytruda) in patients with unresectable or metastatic breast cancer that has progressed following standard treatment and who have no alternative treatment option (see Policy Guidelines). MSI-H/dMMR testing is considered **investigational** in all other situations, including to predict treatment response to dostarlimab-gxly (Jemperli) unless included in a panel approved under another policy. **Ki-67 testing** Ki-67 testing to predict treatment response to abemaciclib (Verzenio) in patients with breast cancer is considered **investigational** unless included in a panel approved under another policy.
Tumor Mutational Burden Testing Tumor mutational burden testing to predict response to immunotherapy in patients with breast cancer is considered **investigational.** **Circulating Tumor DNA** *PIK3CA* testing using FoundationOne Liquid CDx (FDA approved companion test) may be considered **medically necessary** to predict	**Tumor Mutational Burden Testing** Tumor mutational burden testing to predict response to immunotherapy in patients with breast cancer is considered **investigational.** **Circulating Tumor DNA Testing (Liquid Biopsy)**

POLICY STATEMENT	
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treatment response to alpelisib (Piqray) in patients with hormone receptor-positive, HER2 negative advanced or metastatic breast cancer (see Policy Guidelines) when there is insufficient tissue to be tested and an additional invasive procedure would be required otherwise. Circulating tumor DNA testing is considered **investigational** in all other situations unless included in a panel approved under another policy, such as use in Non-Small Cell Lung Cancer (NSCLC). **Circulating Tumor Cells** Analysis of circulating tumor cells to select treatment in patients with breast cancer is considered **investigational** (see Background section).	*PIK3CA* testing using FoundationOne Liquid CDx (FDA approved companion test) may be considered **medically necessary** to predict treatment response to alpelisib (Piqray) in patients with hormone receptor-positive, HER2 negative advanced or metastatic breast cancer (see Policy Guidelines) when there is insufficient tissue to be tested and an additional invasive procedure would be required otherwise. Circulating tumor DNA testing is considered **investigational** in all other situations unless included in a panel approved under another policy, such as use in Non-Small Cell Lung Cancer (NSCLC). **Circulating Tumor Cell Testing** Analysis of circulating tumor cells to select treatment in patients with breast cancer is considered **investigational** (see Background section).