Molecular Analysis for Targeted Therapy of Non-Small Cell Lung Cancer

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DESCRIPTION

Note: This policy does not address circulating tumor DNA (ctDNA) for the management of Non-Small Cell Lung Cancer, see medical policy 02.04.79 Circulating Tumor DNA DNA for Management of Non-Small Cell Lung Cancer

Over half of patients with non-small cell lung cancer (NSCLC) present with advanced and therefore incurable disease. Treatment in this setting has been with platinum-based chemotherapy. The identification of specific, targetable oncogenic “driver mutations” in a subset of NSCLCs has results in a reclassification of lung tumors to include molecular subtypes that may direct targeted therapy depending on the presence of specific variants.
from DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tumor samples from routine biopsy or surgical resection specimens.

Lung cancer is the leading cause of cancer death in the United States. In 2020, an estimated 235,760 new cases (119,100 in men and 116,660 in women) of lung and bronchial cancer will be diagnosed, and 131,880 deaths (69,410 in men and 62,470 in women) are estimated to occur because of the disease. Only 19% of all patients with lung cancer are alive 5 years or more after diagnosis. However, much progress has been made recently for lung cancer such as screening, minimally invasive techniques for diagnosis and treatment, and advances in radiation therapy (RT) including stereotactic ablative radiotherapy (SABR), targeted therapies, and immunotherapies. Patients with metastatic lung cancer who are eligible for targeted therapies or immunotherapies are now surviving longer; 5-year survival rates range from 15% to 50%, depending on the biomarker. (NCCN Version 5.2021).

Treatment options for non-small-cell lung cancer (NSCLC) depend on disease stage and include various combinations of surgery, radiotherapy, systemic therapy, and best supportive care. Unfortunately, in up to 85% of cases, the cancer has spread locally beyond the lungs at diagnosis, precluding surgical eradication. Also, up to 40% of patients with NSCLC present with metastatic disease. When treated with standard platinum-based chemotherapy, patients with advanced NSCLC have a median survival of 8 to 11 months and a 1-year survival of 30% to 45%. The identification of specific, targetable oncogenic “driver mutations” in a subset of NSCLCs has resulted in a reclassification of lung tumors to include molecular subtypes, which are predominantly of adenocarcinoma histology. The NCCN NSCLC Panel recommends testing certain molecular and immune biomarkers in all appropriate patients with metastatic NSCLC to assess whether patients are eligible for targeted therapies or immunotherapies based on data showing improvement in overall survival (OS) for patients receiving targeted therapies or immunotherapies compared with traditional chemotherapy regimens. (NCCN Non-Small Cell Lung Cancer Version 5.2021).

Predictive and Prognostic Biomarkers in Non-Small Cell Lung Cancer
Several biomarkers have emerged as predictive and prognostic markers for non-small cell lung cancer (NSCLC). A predictive biomarker is indicative of therapeutic efficacy because there is an interaction between the biomarker and therapy on patient outcome. A prognostic biomarker is indicative of patient survival independent of the treatment received because the biomarker is an indicator of the innate tumor behavior.

Predictive biomarkers include ALK fusion oncogene ([fusion between ALK and other genes; [i.e., echinoderm microtubule-associated protein-like 4]), ROS1 gene rearrangements, sensitizing EGFR gene variants (presence of EGFR exon 19 deletions or exon 21 L858R mutations), BRAF V600E point mutations, NTRK gene fusions, METex14 skipping mutations, RET rearrangements, and PD-L1 expression. Emerging predictive biomarkers include ERBB2 mutations, high-level MET amplifications, and tumor mutational burden (TMB).
The KRAS oncogene is a prognostic biomarker. The presence of KRAS mutations is prognostic of poor survival for patients with NSCLC when compared to the absence of KRAS mutations, independent of therapy. KRAS mutations are also predictive of lack of benefit from EGFR TKI therapy. EGFR, KRAS, ROS1, BRAF, METex14 skipping mutations, RET rearrangements and ALK genetic variants do not usually overlap thus, testing for KRAS mutations may identify patients who will not benefit from molecular testing.

Genetic Biomarkers with FDA-Approved Targeted Therapies
Somatic genome alterations known as “driver mutations” are usually transformative variants arising in cancer cells in genes encoding for proteins important in cell growth and survival. Randomized controlled trials have demonstrated improved efficacy, often in conjunction with decreased toxicity, of matching targeted therapies to patients with specific driver mutations. Several such targeted therapies are approved by the Food and Drug Administration (FDA) for NSCLC. Guidelines generally suggest analysis of either the primary NSCLC tumor or of a metastasis for the presence of a set of driver mutations to select appropriate treatment.

The list of targeted therapies approved for NSCLC is evolving. Currently, there are FDA-approved targeted therapies for epidermal growth factor receptor (EGFR) variants, anaplastic lymphoma kinase (ALK) rearrangements, ROS1 rearrangements, BRAF V600E variants, METex14 skipping mutations, RET rearrangements, PD-L1 receptor expression and NTRK gene fusions for NSCLC. Companion diagnostics using tissue samples have also been FDA-approved to identify the associated driver mutations for these targeted therapies. The current NCCN guideline Non-Small Cell Lung Cancer (Version 8.2020) the NSCLC Panel currently recommends the minimum of the following biomarkers being tested, including EGFR mutations, BRAF mutations, ALK fusions, ROS1 fusions, METex14 skipping mutations, RET rearrangements, and PD-L1 expression levels. Testing for KRAS mutation may identify patients who will not benefit from further molecular testing. KRAS mutations are also predictive of lack of benefit from EGFR TKI therapy. Also, per the current NCCN guideline there are also emerging biomarkers ERBB2 (HER2), high-level MET amplifications and tumor mutations burden (TMB) that are also susceptible to targeted therapies for the treatment of non-small cell lung cancer, particularly those therapies currently under investigation in clinical trials in which the targeted therapy agent may be FDA approved for other indications. The NCCN NSCLC Panel recommends molecular testing but strongly advises broader molecular profiling to identify these other rare drive variants for which targeted therapies may be available to ensure that patients receive the most appropriate treatment; patients may be eligible for clinical trials for some of these targeted agents. The NCCN NSCLC Panel recommends that broad molecular profiling be done using validated test(s) and done at properly accredited laboratories (minimum of Clinical Laboratory Improvement Amendments [CLIA] accreditation).
**EGFR Variants**

In patients with NSCLC, the most commonly found EGFR variants are deletions in exon 19 (Exon19del [with conserved deletion of the LREA sequence] in 45% of patients with EGFR variants) and a point mutation in exon 21 (L858R) in 40% of patients with EGFR variants. Both variants result in activation of the tyrosine kinase (TKI) domain, and both are associated with sensitivity to the small molecule EGFR TKIs, such as afatinib, erlotinib, dacomitinib, gefitinib, and osimertinib. These variants are referred to as sensitive EGFR variants. Other less common variants (10%) that are also sensitive to EGFR TKIs include exon 19 insertions, p.L861Q, p.G719X and p.S768I. Data suggest that patients harboring tumors without sensitizing EGFR variants should not be treated with EGFR TKIs in any line of therapy. These sensitizing EGFR variants are found in approximately 10% of Caucasian patients with NSCLC and up to 50% of Asian patients.

Most patients with sensitizing EGFR variants are nonsmokers or former light smokers with adenocarcinoma histology. Data suggest that EGFR mutations can occur in patients with adenosquamous carcinoma, which is harder to discriminate from squamous cell carcinoma in small specimens. Patients with pure squamous cell carcinoma are unlikely to have sensitizing EGFR mutations; those with adenosquamous carcinoma may have mutations. However, smoking status, ethnicity, and histology should not be used in selecting patients for testing. EGFR mutation testing is not usually recommended in patients who appear to have squamous cell carcinoma unless they are a former light or never smoker, if only a small biopsy specimen (e.g., not a surgical resection) was used to assess histology, or if the histology is mixed. The ESMO Guidelines specify that only patients with nonsquamous cell (i.e., adenocarcinoma) should be assessed for EGFR mutations. ASCO recommends that patients be tested for EGFR mutations.

The predictive effects of the drug-sensitive EGFR mutations are well defined. Patients with these mutations have a significantly better response to erlotinib, gefitinib, afatinib, Osimertinib or dacomitinib. Data show that EGFR TKI therapy should be used as first-line monotherapy in patients advanced NSCLC and sensitizing EGFR mutations documented before first-line systemic therapy (i.e., carboplatin/paclitaxel) (see Targeted Therapies in this Discussion). Progression-free survival (PFS) is longer with use of EGFR TKI monotherapy in patients with sensitizing EGFR mutations when compared with cytotoxic systemic therapy, although overall survival is not statistically different.

Non-responsiveness to EGFR TKI therapy is associated with KRAS and BRAF mutations and ALK or ROS1 gene fusions. Patients with EGFR exon 20 insertion mutations are usually resistant to erlotinib, gefitinib, afatinib, or dacomitinib, although there are rare exceptions. Patients typically progress after first-line EGFR TKI monotherapy. EGFR p.Thr790Met (T790M) is a mutations associated with acquired resistance to EGFR TKI therapy and has been reported in about 60% of patients with disease progression after initial response to erlotinib, gefitinib or afatinib. Most patients with sensitizing EGFR mutations become resistant to erlotinib, gefitinib or afatinib; PFS is about 9.7 to 13 months. Studies suggest T790M may rarely occur in patients who have previously received erlotinib, gefitinib or afatinib. Genetic counseling is recommended.
for patients with pre-treatment p.T790M, because this suggest the possibility of germline mutations and is associated with predisposition to familial lung cancer. Acquired resistance to EGFR TKIs may also be associated with histologic transformation from NSCLC to SCLC and with epithelial to mesenchymal transition. For the 2020 updated (Version 1), the NCCN NSCLC Panel suggest that a biopsy can be considered at progression to rule out SCLC transformation, acquired resistance an also be mediated by other molecular events, such as acquisition of ALK rearrangement, MET or ERBB2 amplification.

DNA mutational analysis is the preferred method to assess for EGFR status; IHC (immunohistochemistry) is not recommended for detecting EGFR mutations. Real-time PCR (polymerase chain reaction), Sanger sequencing (paired with tumor enrichment), and NGS (next generation sequencing) are the most commonly used methods to assess EGFR variant status. Direct sequencing of DNA corresponding to exons 18 to 21 (or just testing exons 19 and 21) is a reasonable approach; however, more sensitive methods are available. Mutation screening assays using multiplex PCR (i.e., Sequenom’s MassARRAY system, SnAPSHOT Multiplex System) can simultaneously detect more than 50- point mutations.

Osimertinib is a preferred first-line EGFR TKI option for patients with EGFR positive metastatic NSCLC. Erlotinib, gefitinib, afatinib or dacomitinib are “other recommended” EGFR TKI options for first-line therapy. Osimertinib is recommended (category 1) as secondline and beyond (subsequent) therapy for patients with EGFR T790M-positive metastatic NSCLC who have progressed on erlotinib, gefitinib, afatinib, or dacomitinib. Sensitizing EGFR mutations and ALK or ROS1 fusions are generally mutually exclusive. Thus, crizotinib, ceritinib, alectinib, brigatinib or lorlatinib are not recommended as subsequent therapy for patients with sensitizing EGFR mutations who relapse on EGFR TKI therapy. The phrase subsequent therapy was recently substituted for the terms second-line or beyond systemic therapy, because the line of therapy may vary depending on previous treatment with targeted agents.

**ALK Rearrangements**

About 5% of patients with NSCLC have ALK gene rearrangements. Patients with ALK rearrangements are resistant to EGFR TKIs but have similar clinical characteristics to those with EGFR mutations (e.g., adenocarcinoma histology, light or never smokers). ALK rearrangements are not routinely found in patients with squamous cell carcinoma. Patients with ALK gene rearrangements can have mixed squamous cell histology. It can be challenging to accurately determine histology in small biopsy specimens; thus, patients may have mixed squamous cell histology (or squamous components) instead of pure squamous cell.

Testing for ALK fusion in patients with metastatic nonsquamous NSCLC based on data showing the efficacy of alectinib, brigatinib, ceritinib, and crizotinib for ALK fusions and on the FDA approvals. If patients appear to have squamous cell NSCLC, then testing can
be considered if small biopsy specimens were used to assess histology, mixed histology was reported, or patients are light or never smokers. Alectinib is recommended as a preferred first-line therapy for patients with ALK rearrangement-positive metastatic NSCLC. Brigatinib and ceritinib are “other recommended” options, whereas crizotinib is “useful” in certain circumstances.” Patients with ALK rearrangements do not respond to ICIs.

Patients typically progress after first-line therapy with alectinib, brigatinib, crizotinib, or ceritinib. ALK or ROS1 fusions, RET rearrangements, BRAF mutations, METex14 skipping mutations, and sensitizing EGFR mutations are generally exclusive. Specific targeted therapy for RET rearrangements, BRAF mutations, METex14 skipping mutations, and sensitizing EGFR mutations is not recommended as subsequent therapy in patients with ALK or ROS1 fusions who relapse on alectinib, brigatinib, crizotinib, ceritinib, or lorlatinib.

**ROS1 Rearrangements**

Although ROS1 proto-oncogene 1 (ROS1) is a distinct receptor tyrosine kinase, it is very similar to ALK and members of the insulin receptor family. It is estimated that ROS1 gene rearrangements occur in about 1% to 2% of patients with NSCLC; they occur more frequently in those who are negative for EGFR mutations, KRAS mutations, and ALK gene rearrangements.

ROS1 testing is recommended in patients with metastatic non-squamous NSCLC or NSCLC NOS based on data showing the efficacy of crizotinib, ceritinib and entrectinib for patients with ROS1 fusions. ROS1 testing can be considered in patients with metastatic squamous cell carcinoma NSCLC if small biopsy specimens were used to assess histology or mixed histology was reported.

Crizotinib is very effective for patients with ROS1 fusions with response rates of about 70% to 80% including complete responses. Crizotinib, ceritinib or entrectinib are the preferred first line therapy for patients with ROS1 positive metastatic NSCLC because they are better tolerated, have been assessed in more patients and are approved by the FDA. Although entrectinib has better CNS penetratin than crizotinib, it is more toxic. If ROS1 fusions are discovered during first line systemic therapy (e.g. carboplatin/paclitaxel), then the planned therapy may be either completed or interrupted followed by crizotinib, ceritinib or entrectinib. Lorlatinib as a subsequent therapy option for select patients with ROS1 positive metastatic NSCLC who have progressed after treatment with crizotinib, ceritinib or entrectinib. Initial systemic therapy options that are used for adenocarcinoma or squamous cell carcinoma are also an option in this setting (i.e., carboplatin/paclitaxel). Patients with ROS1 rearrangements have a slight response (17%) to ICIs. Alectinib, brigatinib, and ceritinib are not recommended in patients with ROS1 fusions whose disease becomes resistant to crizotinib.
BRAF V600E Mutations

BRAF (v-RAF murine sarcoma viral oncogene homolog B) is a serine/threonine kinase that is part of the MAP/ERK signaling pathway. BRAF V600E is the most common of the BRAF point variants when considered across all tumor types; it occurs in 1% to 2% of patients with lung adenocarcinoma. Although other BRAF variants occur in patients with NSCLC at a rate approximately equal to p.V600E (unlike many other tumor types) specific targeted therapy is not available for these other variants. Patients with BRAF V600E variants are typically current or former smokers in contrast to those with EGFR variants or ALK rearrangements who are typically non-smokers. Variants in BRAF typically do not overlap with EGFR mutations, ALK rearrangements, or ROS1 rearrangements. Testing for BRAF V600E is recommended in patients with metastatic non-squamous NSCLC and may be considered in patients with squamous cell carcinoma NSCLC if small biopsy specimens were used to assess histology or mixed histology was reported.

Real time PCR (polymerase chain reaction), Sanger sequencing, and NGS (next generation sequencing) are the most commonly used methods to assess for BRAF variants.

Testing for BRAF mutations is recommended in patients with metastatic non-squamous NSCLC based on data showing the efficacy of dabrafenib plus trametinib for patients with BRAF V600E mutations and on the FDA approval. Dabrafenib plus trametinib is recommended for patients with BRAF V600E mutations. If combination therapy with dabrafenib/trametinib is not tolerated, single-agent therapy with dabrafenib or vemurafenib are “other recommended” agents. Chemotherapy regimens are also used for initial systemic therapy (i.e., carboplatin/pemetrexed for non-squamous NSCLC) and are “useful in certain circumstances.” Patients with BRAF mutations response (24%) to immune checkpoint inhibitors (ICIs).

PD-L1 Expression Levels

Human immune-checkpoint-inhibitor antibodies inhibit the PD-1 receptor or PD-L1, which improves antitumor immunity; PD-1 receptors are expressed on activated cytotoxic T-cells. Nivolumab (opdiva) and pembrolizumab (Keytruda) inhibit PD-1 receptors. Atezolizumab (tecentriq) and durvalumab (imfinzi) inhibit PD-L1. IHC (immunohistochemistry) testing for PD-L1 expression is recommended ideally before first line treatment (if clinically feasible) in all patients with metastatic NSCLC to assess whether the ICI regimens are an option based on clinical data showing the efficacy of these regimens.

The FDA approve companion diagnostic test for PD-L1 expression is based on tumor proportion score (TPS) and used to determine usage of pembrolizumab in patients with metastatic NSCLC. TPS is the percentage of viable tumor cells showing partial or complete membrane staining at any intensity. Testing for PD-L1 is not required for prescribing first-line therapy with atezolizumab plus chemotherapy regimens or for subsequent therapy with single agent nivolumab or atezolizumab.
Although it is not an optimal biomarker, PD-L1 expression is currently the best available biomarker to assess whether patients are candidates for PD1 or PD-L1 inhibitors (ICIs; also known as immune-oncology [IO] agents, immunotherapy). PD-L1 expression is continuously variable and dynamic; thus, a cutoff value for a positive result is artificial. Patients with PD-L1 expression levels just below and just above 50% will probably have similar responses. Unique anti-PD-L1 IHC assays have been developed for each one of the different ICIs (PD-L1 IHC 22C3 pharmDx [Keytruda] and PD-L1 IHC 28-8 pharmDx [opdiva]). The definition of a positive PD-L1 test result varies depending on which biomarker assay is used. Extensive effort has been undertaken to examine the cross-comparability or different clones with regard to each other to facilitate adoption of testing.

Clinicians should obtain molecular testing results for actionable biomarkers before administering first-line ICI therapy, if clinically feasible. Patients with metastatic NSCLC and PD-L1 expression levels of 1% or more but who also have a targetable driver oncogene molecular variant (e.g. EGFR, ALK, ROS1) should receive first-line targeted therapy for that oncogene and not first-line ICIs because targeted therapies yield higher response rates (e.g. Osimertinib, 80%) than ICIs (poor response rates) in the first-line setting, targeted therapy is better tolerated, and these patients are unlikely to respond to ICIs. At a minimum, EGFR and ALK status should be known before starting systemic therapy with ICI regimens; however, it is ideal if ROS1 and BRAF status are also known. If it is not feasible to do molecular testing, then patients are treated as though they do not have driver oncogenes.

First line treatment includes pembrolizumab (Keytruda)/carboplatin (or cisplatin)/pemetrexed regimens for patients with metastatic non-squamous NSCLC. If patients have not previously received a PD-1/PD-L1 inhibitor, single agent nivolumab (opdiva) is preferred as a subsequent therapy for patients with metastatic non-squamous or squamous cell NSCLC who have progressed on or after first line chemotherapy based on data from 2 phase 3 randomized trials (Checkmate-057 and Checkmate-017 and FDA approvals). Atezolizumab or pembrolizumab (Keytruda) may also be used for subsequent therapy based on overall survival rates, longer duration of response, and fewer adverse events when compared with cytotoxic chemotherapy.

**NTRK1/2/3 Gene Fusions**

NTRK gene fusions encode tropomyosin receptor kinase (TRK) fusion proteins (i.e., TRKA, TRKB, TRKC) that act as oncogenic drivers for solid tumors that includes lung. A diverse range of solid tumors in children and adults may be caused by NTRK gene fusion (i.e., NTRK1, NTRK2, NTRK3). It is estimated that NTRK gene fusions occur in 0.2% of patients with NSCLC and do not typically overlap with other oncogenic drivers such as EGFR, ALK or ROS1. Lacrotrectinb and entrectinib are oral TKIs that inhibit TRK across a diverse range of solid tumors in younger and older patients with NTRK gene-fusion positive disease.
NTRK gene fusion testing is recommended in patients with metastatic NSCLC based on clinical data and the approval of larotrectinib and entrectinib for patients with NTRK gene fusion positive disease. Larotrectinib and entrectinib are recommended as either first line or subsequent therapy are options for patients with NTRK gene fusion positive metastatic NSCLC based on data and FDA approvals.

**METex14 Skipping Mutations**
C-MET, the hepatocyte growth factor (HGF) receptor, is a tyrosine kinase receptor that is involved in cell survival and proliferation; oncogenic drive genomic alterations in MET include METex14 skipping mutations, MET gene copy number (GCN) gain or amplification, and MET overexpression. MET genomic alterations do not typically overlap with EGFR, ROS1, BRAF and ALK genetic variants. However, METex14 skipping mutations and MET amplification may occur together. METex14 skipping mutations occur in 3% to 4% of patients with adenocarcinomas NSCLC and 1% to 2% of patients with other NSCLC histologies. METex14 skipping mutations are more frequent in older women who are nonsmokers. Several different types of METex14 skipping mutations may occur, such as mutations, based substitutions, and deletions, which makes it difficult to test for other mutations. NGS and RT-PCR assays can be used to detect METex14 skipping mutations with MET amplification. Patients with METex14 skipping mutations have a modest response (16%) to immunotherapy, even those with high PD-L1 levels.

METex14 skipping mutation testing is recommended in patients with metastatic NSCLC based on data showing the efficacy of several agents for patients with METex14 skipping mutations and on the FDA approval for capmatinib.

**RET Rearrangements**
RET is a tyrosine kinase receptor that affects cell proliferation and differentiation. Rearrangements (fusions) may occur in NSCLC between the RET gene and other domains, especially kinesin family 5B (KIF5B) and coiled coil domain containing-6 (CCDC6), which lead to overexpression of the RET protein. RET rearrangements occur in about 1% to 2% of patients with NSCLC and are more frequent in patients with adenocarcinoma histology. In European patients, RET rearrangements occur in both smokers and nonsmokers. RET rearrangements do not typically overlap with EGFR, ROS1, BRAF, METex14 skipping and ALK genetic variants. However, a few studies suggest that RET rearrangements may infrequently overlap with EGFR and KRAS mutations. Patients with RET rearrangements have minimal response (6%) to immunotherapy.

RET rearrangement testing is recommended in patients with metastatic NSCLC based on data showing the efficacy of several agents for patients with RET rearrangements and on the FDA approval for selpercatinib (LOXO-292).
Prognostic Biomarkers in Non-Small Cell Lung Cancer

A prognostic biomarker is indicative of patient survival independent of the treatment received because the biomarker is an indicator of the innate tumor behavior.

KRAS

KRAS is a G-protein with GTPase activity that is part of the MAP/ERK pathway; point mutations in KRAS most commonly occur at codon 12. Data suggest that approximately 25% of patients with adenocarcinomas in a North American population have KRAS mutations; KRAS is the most common mutation in this population. KRAS mutation prevalence is associated with cigarette smoking. Patients with KRAS mutations appear to have a shorter survival than patients with wild-type KRAS (when the KRAS gene is found in its natural non-mutated [unchanged form]); therefore, KRAS mutations are prognostic biomarkers.

KRAS mutational status is also predictive of lack of therapeutic efficacy with EGFR TKIs; it does not appear to affect chemotherapeutic efficacy. KRAS mutations do not generally overlap with EGFR, ALK, ROS1 and BRAF genetic variants. Therefore, KRAS testing may identify patients who may not benefit from further molecular testing. Targeted therapy is not currently available for patients with KRAS mutations, although immune checkpoint inhibitors (immunotherapy) appear to be effective.

Emerging Biomarkers to identify Targeted Therapies for Patients with Metastatic Non-Small Cell Lung Cancer

Emerging predictive biomarkers include ERBB2 mutations. High-level MET amplifications, and tumor mutational burden (TMB)

High-Level MET Amplification

In NSCLC, amplification of MET typically occurs in about 2% to 5% of newly diagnosed adenocarcinomas.

The current NCCN Guideline for Non-Small Cell Lung Cancer Version 8.2020 states the following: “Other oncogenic driver variants are being identified such as high-level MET amplification, ERBB2 mutations and TMB. Targeted agents are available for patients with NSCLC who have these other genetic variants, although they are FDA approved for other indications. Thus, the NCCN NSCLC Panel recommends molecular testing but strongly advises broad molecular profiling to identify these other rare driver variants for which targeted therapies may be available to ensure that patients receive the most appropriate treatment; patients may be eligible for clinical trials for some of these targeted agents.”

ERBB2 (HER2)

ERBB2 (HER2) including both amplification and mutations have been classified as oncogenic drivers that contribute to 2% to 6% of lung adenocarcinomas. ERBB2 (HER2) amplification is also an important mechanism for acquired resistance to EGFR tyrosine kinase inhibitors (TKI).
The current NCCN Guideline for Non-Small Cell Lung Cancer Version 8.2020 states the following: “Other oncogenic driver variants are being identified such as high-level MET amplification, ERBB2 mutations and TMB. Targeted agents are available for patients with NSCLC who have these other genetic variants, although they are FDA approved for other indications. Thus, the NCCN NSCLC Panel recommends molecular testing but strongly advises broad molecular profiling to identify these other rare driver variants for which targeted therapies may be available to ensure that patients receive the most appropriate treatment; patients may be eligible for clinical trials for some of these targeted agents.”

**Tumor Mutation Burden (TMB)**
Tumor mutational burden (TMB) is an emerging biomarker that may be helpful for identifying patients with metastatic NSCLC who are eligible for first line therapy with nivolumab with or without ipilimumab. However, there is no consensus on how to measure TMB. TMB is the number of somatic mutations in a tumor’s exome.

The current NCCN Guideline for Non-Small Cell Lung Cancer Version 5.2021 states the following: “Other oncogenic driver variants are being identified such as high-level MET amplification, ERBB2 mutations and TMB. Targeted agents are available for patients with NSCLC who have these other genetic variants, although they are FDA approved for other indications. Thus, the NCCN NSCLC Panel recommends molecular testing but strongly advises broad molecular profiling to identify these other rare driver variants for which targeted therapies may be available to ensure that patients receive the most appropriate treatment; patients may be eligible for clinical trials for some of these targeted agents.”

**Treatment Selection**

**Tissue Biopsy as a Reference Standard**
The standard of care (SOC) for treatment selection in NSCLC is biomarker analysis of tissue samples obtained by tissue biopsy. Although tumor testing has been primarily focused on use of formalin-fixed paraffin-embedded (FFPE) tissues, increasingly, laboratories accept other specimen types, notably cytopathology preparations not processed by FFPE methods. Although testing on cell blocks is not included in the FDA approval for multiple companion diagnostic assays, testing on these specimen types is highly recommended when it is the only or best material.

While tissue biopsy is required to verify a cancer diagnosis and determine histology, there is often insufficient tissue for genotyping with expert centers reporting rates up to 25%, especially when a gene-by-gene sequential testing approach is utilized. Once tissue is exhausted options include a repeat biopsy or more often treating the patient empirically with standard chemotherapy when the patient may have benefited from targeted therapy.
Testing for Molecular Biomarkers in Non-Small Cell Lung Cancer

Molecular testing is used to test for genomic variants associated with oncogenic driver events for which targeted therapies are available; these genomic variants (also known as molecular biomarkers) include gene mutations and fusions. The various molecular testing methods that may be used to assess for the different biomarkers are described below. Per NCCN guideline Non-Small Cell Lung Cancer Version 8.2020, broad molecular profiling systems may be used to simultaneously test for multiple biomarkers:

- Next-generation sequencing (NGS) is used in clinical laboratories. Not all types of alterations are detected by individual NGS assays or combination(s) of assays.
- It is recommended at this time that when feasible, testing be performed via a broad, panel-based approach, most typically performed by next generation sequencing (NGS). For patients who, in broad panel testing don’t have identifiable driver oncogenes (especially in never smokers), consider RNA-based NGS if not already performed, to maximize detection of fusion events.
- Real-time polymerase chain reaction (PCR) can be used in a highly targeted fashion (specific mutations targeted). When this technology is deployed, only those specific alterations that are targeted by the assay are assessed.
- Sanger sequencing requires the greatest degree of tumor enrichment. Unmodified Sanger sequencing is not appropriate for detection of mutations in tumor samples with less than 25% to 30% tumor after enrichment is not appropriate for assays in which identification of subclonal events (i.e., resistance mutations) is important. If Sanger sequencing is utilized, tumor enrichment methodologies are nearly always recommended.
- Other methodologies may be utilized, including multiplex approaches not listed above (i.e. SNaPshot, MassARRAY).
- Fluorescence in situ hybridization (FISH) analysis is utilized for many assays examining copy number, amplifications, and structural alterations such as gene rearrangements.
- Immunohistochemistry (IHC) is specifically utilized for some specific analytes and can be useful surrogate or screening assays for others.

Commercially Available Tests

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<tr>
<th>Test</th>
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<tr>
<td>Cobas EGFR Mutation Test:</td>
<td>Roche Molecular Diagnostics</td>
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<tr>
<td>Is a real-time PCR test for the</td>
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<td>qualitative detection of exon 19</td>
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<td>deletions and exon 21 (L858R)</td>
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<td>(EGFR) gene in DNA derived from</td>
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<td>(FFPET) human non-small cell lung</td>
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<td>cancer (NSCLC) tumor tissue. The</td>
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<td>test is intended to be used as</td>
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<td>an aid in selecting patients with</td>
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<td>NSCLC for whom Tarceva® (erlotinib), an EGFR tyrosine kinase inhibitor (TKI), is indicated.</td>
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**FoundationOne CDx:** Is a next generation sequencing based in vitro diagnostic device for detection of substitutions, insertion and deletion alterations (indels), and copy number alterations (CNAs) in 324 genes and select gene rearrangements, as well as genomic signatures including microsatellite instability (MSI) and tumor mutational burden (TMB) using DNA isolated from formalin-fixed paraffin embedded (FFPE) tumor tissue specimens. The test is intended as a companion diagnostic to identify patients who may benefit from treatment with the targeted therapies listed in accordance with the approved therapeutic product labeling.

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<tr>
<th>Non-small cell lung cancer Biomarker</th>
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<tr>
<td>EGFR exon 19 deletions and L858R alterations</td>
<td>Gilotrif (afatinib)</td>
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<td>EGFR exon 21 alterations</td>
<td>Iressa (gefitinib)</td>
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<td>EGFR exon 20 T790 alterations</td>
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**Oncomine DX Target Test:** The Oncomine Dx Target Test is a qualitative in vitro diagnostic test that uses targeted high throughput, parallel-sequencing technology to detect single nucleotide variants (SNVs) and deletions in 23 genes from DNA and fusions in ROS1 from RNA isolated from formalin-fixed, paraffin-embedded (FFPE) tumor tissue samples from patients with non-small cell lung cancer (NSCLC) using the Ion PGM Dx System.

**Thermo Fisher Scientific**
The test is indicated to aid in selecting NSCLC patients for treatment with the targeted therapies in accordance with the approved therapeutic product labeling.

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<th>Biomarker</th>
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<td>BRAF V600E</td>
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<td>ROS1 Fusions</td>
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<td>EGFR L858R exon 19 deletions</td>
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<td>Iressa (gefitinib)</td>
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**PD-L1 IHC 22C3 pharmDx:** Is a qualitative immunohistochemical assay using Monoclonal Mouse Anti-PD-L1, Clone 22C3 intended for use in the detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) tissue using EnVision FLEX visualization system on Autostainer Link 48. PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying NSCLC patients for treatment with Keytruda (pembrolizumab).

**PD-L1 IHC 28.8 pharmDx:** Is a qualitative immunohistochemical assay using Monoclonal Rabbit Anti-PD-L1, clone 28-8 intended for use in the detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) non-squamous non-small cell lung cancer (NSCLC), PD-L1 protein expression is defined as the percentage of tumor cells exhibiting positive membrane staining at any intensity. In non-squamous NSCLC, PD-L1 expression as detected by PD-L1 IHC 28-8 pharmDx may be associated with enhanced survival from Opdivo.

**Therascreen EGFR:** Real-time PCR test for the qualitative detection of exon 19 deletions and exon 21 (L858R) substitution mutations of the epidermal growth factor receptor (EGFR) gene in DNA derived from formalin-fixed paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) tumor tissue. The test is intended to be used to select patients with NSCLC for whom Dako North America, Inc

Qiagen Manchester, LTD
Gilotrif (afatinib) or Iressa (gefitinib) EGFR tyrosine kinase inhibitors (TKIs) are indicated.

**Ventana ALK CDx Assay:** Is intended for the qualitative detection of the anaplastic lymphoma kinase (ALK) protein in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung carcinoma (NSCLC) tissue stained with a BenchMark XT automated staining instrument. It is indicated as an aid in identifying patients eligible for treatment with Xalkori (crizotinib)

| **Roche Diagnostics** |

**Vysis ALK Break Apart FISH Probe Kit:** The Vysis ALK Break Apart FISH Probe Kit is indicated as an aid in identifying patients eligible for treatment with XALKORI® (crizotinib) and ALUNBRIG® (brigatinib) in accordance with the approved therapeutic product labeling.

| **Abbott Molecular** |

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**Selecting Targeted Therapy for Metastatic Non-Small Cell Lung Cancer (NSCLC)**

**Clinical Context and Test Purpose**

The purpose of identifying targetable oncogenic “driver mutations” in patients who have non-small cell lung cancer (NSCLC) is to inform a decision whether patients should receive a targeted therapy versus another systemic therapy. Patients who present with advanced disease or recurrence following initial definitive treatment typically receive systemic therapy. Traditionally, systemic therapy was cytotoxic chemotherapy. However, certain patients may be good candidates for treatment with targeted therapies or immunotherapy. The goal of targeted therapies is to preferentially kill malignant cells without significant damage to normal cells so that there is improved therapeutic efficacy along with decreased toxicity.

Patients traditionally been tested for driver mutations using samples from tissue biopsies.

**Patients**

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

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

**Intervention**

The intervention of interest is testing for somatic genome alterations known as “driver mutations,” using standard of care tissue biopsy specifically for EGFR, BRAF, KRAS,
ERBB2 (HER2) variants; ALK, ROS, RET rearrangements; METex14 skipping mutations, MET amplification, NTRK gene fusions, tumor burden mutations (TMB) or PD-L1 receptor expression.

**Comparator**
The following practice is currently being used to target therapy for metastatic NSCLC: standard management without testing for driver mutations. Standard management consists primarily of chemotherapy, although some patients are candidates for targeted therapy.

Testing for variants using tissue biopsy.

**Outcomes**
Beneficial outcomes resulting from a true-positive test result are prolonged survival, reduced toxicity, and improved quality of life (QOL) associated with receiving a more effective and less cytotoxic targeted therapy than chemotherapy in those with driver mutations. 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.

Due to the poor prognosis of metastatic NSCLC, the duration of follow-up for the outcomes of interest is six months to one year.

The Current NCCN guideline Non-Small Cell Lung Cancer Version 8.2020 includes the following:

**Targeted Therapies**
Specific targeted therapies are available for the treatment of eligible patients with metastatic NSCLC. Afatinib, alectinib, brigatinib, ceritinib, crizotinib, erlotinib, gefitinib, lacrotrectinib, and lorraineib are oral TKIs. Bevacizumab and ramucirumab are recombinant monoclonal antibodies that target the vascular endothelial growth factor (VEGF) or VEGF receptor, respectively. Cetuximab is a monoclonal antibody that targets EGFR. Erlotinib, gefitinib, afatinib and dacombitinib inhibit EGFR sensitizing mutations; Osimertinib inhibits both EGFR sensitizing mutations and T790M. Crizotinib inhibits ALK fusions, ROS1 fusions and MET tyrosine kinase (i.e. high-level MET amplification, METex14 skipping mutation). Ceritinib inhibits ALK fusions and IGF-1 receptor. Alectinib inhibits ALK and RET fusions. Brigatinib inhibits various ALK fusions and other targets. Lorlatinib inhibits ALK and ROS fusions. Debrafenib inhibits BRAF V600E mutations; trametinib inhibits MEK; both agents inhibit different kinases in the RAS/RAF/MEK/ERK pathway. Entrectinib and lacrotrectinib inhibit TRK fusion.
proteins. Capmatinib inhibits several MET tyrosine kinases including METex14 skipping mutations. Selpercatinib, cabozantinib and candetanib inhibit RET rearrangements. Other targeted therapies are being developed. Flare phenomenon may occur in some patients who discontinue targeted therapies for EGFR, ALK, or ROS1 genetic variants. If disease flare occurs, then the targeted therapies should be restarted.

It is important to note that targeted therapies are recommended for patients with metastatic NSCLC and specific oncogenic drivers independent of PD-L1 levels. Patients with metastatic NSCLC and PD-L1 expression levels of 1% or more but who also have targetable driver oncogene molecular variant (e.g. EGFR, ALK, ROS1) should receive first-line targeted therapy for that oncogene and not first-line ICIs, because targeted therapies yield higher response rates (e.g. Osimertinib 80%) than ICIs (poor response rates) in the first-line setting, targeted therapy is better tolerated, and these patients are unlikely to response to ICIs. For the 2020 update (version 1), the NCCN NSCLC Panel emphasizes that clinicians should obtain molecular testing results for actionable biomarkers before administering first-line therapy, if clinically feasible. At a minimum, EGFR and ALK status should be known before starting first-line systemic therapy, if clinically feasible; however, it is ideal if ROS1 and BRAF status are also known. It is not feasible to do molecular testing, then patients are treated as though they do not have driver oncogenes.

**FDA Approved Companion Diagnostic Tests for EGFR Variants**

*EGFR*-sensitizing and -resistance variants can be detected by direct sequencing, polymerase chain reaction (PCR) technologies, or next-generation sequencing (NGS). Gene sequencing is considered an analytic criterion standard.

Several tests have been approved as companion diagnostics to detect *EGFR*-resistance variants (exon 19 deletions or exon 21 L858R substitutions) for at least one of the EGFR TKIs (afatinib, erlotinib, gefitinib, dacomitinib, or osimertinib) see Regulatory Status below.

The clinical validity of the therascreen RGQ PCR kit was demonstrated in a retrospective analysis of patients screened for a phase 3, open-label RCT comparing afatinib with chemotherapy in treatment-naïve patients with stage IIIB or IV NSCLC, in which the *EGFR* variants for enrollment were determined using a clinical trial assay (CTA) conducted at central laboratories. The positive percent agreement (PPA) of therascreen vs CTA for detection of *EGFR*-sensitizing variants was 98% (95% confidence interval [CI], 95% to 99%) and negative percent agreement (NPA) was 97% (95% CI, 94% to 99%). Overall, a statistically significant efficacy benefit for afatinib vs chemotherapy was reported in the *EGFR*-positive patients as measured by the therascreen EGFR RGQ PCR Kit (hazard ratio [HR], 0.49; 95% CI, 0.35 to 0.69) that was similar to the efficacy in the overall population, which was *EGFR*-positive by the CTA (HR=0.58; 95% CI, 0.43 to 0.78).

The clinical validity of the cobas EGFR Mutation Test was demonstrated in a retrospective analysis of patients screened for a phase 3, open-label RCT comparing
erlotinib with chemotherapy in treatment-naive patients with advanced NSCLC. In this RCT, the \textit{EGFR} variants for enrollment were determined with a CTA at a central laboratory using Sanger sequencing first for determination of \textit{EGFR} variants status, followed by confirmatory testing for exon 19 deletions and exon 21 L858R variants. The PPA of cobas vs CTA for detection of \textit{EGFR}-sensitizing variants was 94\% (95\% CI, 89\% to 97\%) and NPA was 98\% (95\% CI, 95\% to 99\%). Overall, a statistically significant efficacy benefit for erlotinib vs chemotherapy was reported in the \textit{EGFR}-positive patients as measured by the cobas EGFR Mutation Test v1 (HR=0.34; 95\% CI, 0.21 to 0.54) that was similar to the efficacy in the overall population, which was \textit{EGFR}-positive by the CTA (HR=0.34; 95\% CI, 0.23 to 0.49). The cobas EGFR Mutation Test v2 expanded the indication for the use of the cobas EGFR Mutation Test to include the detection of the exon 20 (T790M) substitution variant in NSCLC patients for whom osimertinib (Tagrisso) treatment is indicated. The clinical validity of the cobas EGFR Mutation Test v2 was demonstrated in retrospective analyses of patients enrolled in a phase 2, single-arm study of osimertinib for \textit{EGFR}-sensitizing variant-positive metastatic NSCLC who had progressed following prior therapy with an approved \textit{EGFR} TKI. The osimertinib response rate in the patients identified as \textit{EGFR} T790M-positive by the cobas v2 test was 62\% (95\% CI, 55\% to 69\%).

The clinical validity of the Oncomine Dx Target Test was demonstrated in a retrospective analysis of patients screened for a phase 3, open-label RCT, which included newly diagnosed patients with stage IIIIB or IV or recurrent NSCLC, in which the \textit{EGFR} variant for enrollment was determined using therascreen. The PPA of Oncomine vs therascreen for detection of \textit{EGFR}-sensitizing variants was 99\% (95\% CI, 93\% to 100\%) and NPA was 99\% (95\% CI, 96\% to 100\%). No data on the effectiveness of gefitinib in patients identified as \textit{EGFR}-positive by Oncomine were reported.

The clinical validity of FoundationOne CDx was demonstrated by assessing concordance of the test with results from mass spectrometry, gel sizing, fluorescence in situ hybridization (FISH), and immunohistochemistry of clinical tumor tissue specimens (comparison to cobas EGFR Mutation Test, VENTANA ALK CDx Assay, Vysis ALK Break Apart FISH Test and Therascreen EGFR). Test sensitivity ranged from 95\% to 99\% across alteration types, with a positive predictive value exceeding 99\%.

**ALK Gene Rearrangements**

\textit{ALK} gene rearrangements most often consist of an inversion in chromosome 2 which leads to fusion with the echinoderm microtubule-associated protein like 4 (\textit{EML4}) gene and a novel fusion oncogene \textit{EML4-ALK}. This inversion causes abnormal expression and activation of \textit{ALK} tyrosine kinase.

**ALK Rearrangement Frequency**

About 5\% of patients with NSCLC have \textit{ALK} gene rearrangements.

**FDA Approved Companion Diagnostic Tests for ALK Rearrangements**
Several methods are available to detect ALK gene rearrangements or the resulting fusion proteins in tumor specimens including FISH, immunohistochemistry, reverse transcription polymerase chain reaction and NGS. Two tests have been approved by FDA as companion diagnostics to detect ALK rearrangements for treatment with crizotinib: the Vysis ALK Break Apart FISH Probe Kit and Ventana ALK (D5F3) CDx Assay.

The Vysis kit is a FISH-based assay. The clinical validity of the Vysis ALK Break Apart FISH Probe Kit was demonstrated in a retrospective analysis of patients screened for a phase 2, open-label single-arm study of crizotinib in patients with stage IIIB or IV NSCLC. The response rate for crizotinib in 136 ALK-positive patients was 50% (95% CI, 42% to 59%) with a median duration of response of 42 weeks (range, 6-42 weeks). The response rate for 19 ALK-negative patients was 26% (95% CI, 9% to 51%).

The Ventana assay is an immunohistochemical-based assay. The clinical validity of the Ventana ALK (D5F3) CDx Assay was demonstrated in a retrospective analysis of patients screened for an open-label RCT of crizotinib vs platinum-doublet chemotherapy in patients with stage IIIB or IV NSCLC. The concordance between the Ventana and Vysis tests were calculated using patient samples analyzed at an independent, central laboratory. The PPA was 86.0% (95% CI, 80.2% to 90.4%) and the NPA was 96.3% (95% CI, 94.7% to 97.4%). Overall, in 343 patients who were ALK-positive by the Vysis assay, crizotinib was associated with longer PFS compared with chemotherapy (HR=0.45; 95% CI, 0.36 to 0.60). In the subset of 141 patients who were also ALK-positive by the Ventana assay, the results were similar (HR=0.40; 95% CI, 0.25 to 0.64). In the 25 patients who were ALK-positive by the Vysis assay and ALK-negative by the Ventana assay, the relative effect of crizotinib was not clear (HR=1.71; 95% CI, 0.43 to 6.79).

**ROS1 Gene Rearrangements**

Although ROS1 proto-oncogene 1 (ROS1) is a distinct receptor tyrosine kinase, it is very similar to ALK and members of the insulin receptor family.

**ROS1 Gene Rearrangement Frequency**

It is estimated that ROS1 gene rearrangements occur in about 1% to 2% of patients with NSCLC; they occur more frequently in those who are negative for EGFR mutations, KRAS mutations, and ALK gene rearrangements.

**FDA Approved Companion Diagnostic Test for ROS1 Rearrangements**

Several methods are available to detect ROS1 translocations including FISH, immunohistochemistry, quantitative real-time reverse transcription-PCR, and some NGS panels. FISH is considered the standard method. The Oncomine Dx Target Test was FDA-approved in 2017 as a companion diagnostic to detect fusions in ROS1 to aid in selecting NSCLC patients for treatment with crizotinib (Xalkori). The Oncomine test is an NGS oncology panel that detects, among other variants, fusions in ROS1 from RNA isolated from FFPE tumor tissue samples. The clinical validity of the detection of ROS1 rearrangements by the test was evaluated by retrospective analysis of FFPE
NSCLC specimens obtained from patients enrolled in a *ROS1* cohort from an ongoing single-arm, phase 1 safety study of crizotinib in patients with advanced cancer. *ROS1* fusion status was determined by a validated FISH comparator test for the study. Concordance between the Oncomine Dx Target Test and the FISH test as well as clinical outcomes were reported in the Summary of Safety and Effectiveness Data. A total of 157 specimens were included. The PPA for Oncomine vs FISH was 80% (95% CI, 59 to 93) and NPA was 100% (95% CI, 97% to 100%). For all *ROS1*-positive patients, as originally detected for enrollment into the *ROS1* cohort, the response rate was 72% (95% CI, 58% to 84%). For *ROS1*-positive patients as detected by Oncomine, the response rate was 83% (95% CI, 36% to 99.6%).

**BRAF Point Mutations**

BRAF (v-RAF murine sarcoma viral oncogene homolog B) is a serine/threonine kinase that is part of the MAP/ERK signaling pathway.

**BRAF Point Mutations Frequency**

BRAF V600E is the most common of the BRAF point mutations when considered across all tumor types; it occurs in 1% to 2% of patients with lung adenocarcinoma. Although other BRAF mutations occur in patients with NSCLC at a rate approximately equal to pV600E (unlike many other tumor types), specific targeted therapy is not available for these other mutations. Patients with BRAF V600E mutations are typically current or former smokers in contrast to those with EGFR mutations or ALK fusion who are typically nonsmokers. Mutations in BRAF typically do not overlap with EGFR mutations, METex14 skipping mutations, RET rearrangements, ALK fusions, or ROS1 fusions.

**FDA Approved Companion Diagnostic Tests for BRAF Gene Mutations**

*BRAF* point mutations are detected by PCR sequencing or NGS methods.

The Oncomine Dx Target Test was FDA-approved in 2017 as a companion diagnostic to detect *BRAF* V600E variants to aid in selecting NSCLC patients for treatment with combination dabrafenib (Tafinlar) and trametinib (Mekinist) therapy. The Oncomine test is an NGS oncology panel that detects, among other variants, *BRAF* V600E variants from DNA isolated from formalin-fixed, paraffin-embedded (FFPE) tumor tissue samples. The detection of *BRAF* V600E variants by the test was evaluated by retrospective analyses of a phase 2, multicenter, nonrandomized study that included patients with a *BRAF* V600E variant who had progressed on prior treatment or were treatment-naive who were treated with dabrafenib in combination with trametinib in the study. Patients were screened for a *BRAF* V600E variant based on local lab tests used at each enrollment site. No FDA-approved test was available for detection of *BRAF* V600E variants in FFPE NSCLC specimens, so a validated PCR assay (*BRAF* V600 PCR Mutation Test) was used to estimate concordance. The concordance between the Oncomine test and the *BRAF* V600 PCR Mutation Test was 100% for PPA (95% CI, 95% to 100%) and 100% for NPA (95% CI, 97% to 100%). The response rate in the 57 previously treated patients in the study who were *BRAF*-positive by local lab test was 67% (95% CI, 53% to 79%)
compared with 73% (95% CI, 50% to 89%) for the 22 patients who were also BRAF-positive by Oncomine. The response rate in the 36 treatment-naive patients who were BRAF-positive by local lab test was 61% (95% CI, 44% to 77%) compared with 61% (95% CI, 39% to 80%) in the 23 patients who were also BRAF-positive by Oncomine. In June 2017, FDA approved an additional indication for use of dabrafenib and trametinib combination therapy in patients with NSCLC with BRAF V600E variant as detected by an FDA-approved test. The Oncomine Dx Target Test was approved as a companion diagnostic.

**FDA Approved Companion Diagnostic Tests for PD-L1 Expression**

The FDA approve companion diagnostic test for PD-L1 expression is based on tumor proportion score (TPS) and used to determine usage of pembrolizumab in patients with metastatic NSCLC. TPS is the percentage of viable tumor cells showing partial or complete membrane staining at any intensity. The NCCN NSCLC Panel recommends (category 1) IHC testing for PD-L1 expression ideally before first-line treatment (if clinically feasible) in all patients with metastatic NSCLC to assess whether the ICI regimens are an option based on clinical data showing the efficacy of these regimens. NCCN NSCLC Panel emphasizes that clinicians should obtain molecular testing results for actionable biomarkers before administering first-line ICI therapy, if clinically feasible.

Patients with metastatic NSCLC and PD-L1 expression levels of 1% or more but who also have a targetable driver oncogene molecular variant (e.g. EGFR, ALK, ROS1) should receive first-line targeted therapy for that oncogene and not first-line ICIs because targeted therapies yield higher response rates (e.g. Osimertinib, 80%) than ICIs (poor response rates) in the first-line setting, targeted therapy is better tolerated, and these patients are unlikely to respond to ICIs. The panel also added “ROS1 fusions” and “BRAF mutations” to the list of actionable biomarkers that need to be negative before administering PD-1 or PD-L1 inhibitors. At a minimum, EGFR and ALK status should be known before starting systemic therapy with ICI regimens; however, it is ideal if ROS1 and BRAF status are also known. If it is not feasible to do molecular testing, then patients are treated as though they do not have driver oncogenes.

(NCCN Non-Small Cell Lung Cancer Version 8.2020)

**Pembrolizumab (Keytruda)**

A phase 3 randomized trial (KEYNOTE-024) compared single agent pembrolizumab versus platinum-based chemotherapy as first-line therapy for patients with advanced non-squamous or squamous NSCLC and PD-L1 expression levels of 50% or more, but without EGFR mutations or ALK rearrangements. The FDA approved single agent pembrolizumab for first line therapy based on this trial. At 6 months, the rate of overall survival was 80.2% in the pembrolizumab group versus 72.4% in the chemotherapy group (HR for death; 0.60; 95% CI, 0.41-0.89; P = .005). Responses were higher for pembrolizumab than for chemotherapy (44.8% versus 27.8%). There were fewer severe treatment-related adverse events (grades 3-5) in patients receiving pembrolizumab compared with those receiving chemotherapy (26.6% versus 53.3%). Another phase 3
randomized trial (KEYNOTE-042) compared single agent pembrolizumab versus platinum-based chemotherapy as first line therapy for patients with advanced non-squamous or squamous NSCLC and PD-L1 expression levels of 1% or more, but without EGFR mutations or ALK rearrangements. Overall survival appeared to be similar in patients with PD-L1 levels of 1% to 49% who received single-agent pembrolizumab (13.4 months; 95% CI, 10.7-18.2) compared with chemotherapy (12.1 months; 95% CI, 11.0-14.0) in a subgroup analysis. However, overall survival was improved in patients with PD-L1 levels of 50% or more who received single-agent pembrolizumab (20.0 months; 95% CI, 15.4-24.9) compared with chemotherapy (12.2 months (10.4-14.2) (HR, 0.69; 95% CI, 0.56-0.85; P=.0003).

Single agent pembrolizumab (Keytruda) is recommended as a first line therapy option for patients with advanced non-squamous or squamous NSCLC, PD-L1 expression levels of 50% or more, no contraindications to immunotherapy and negative or unknown test results for EGFR mutations and ALK rearrangements based on phase 3 randomized controlled trials. Companion diagnostic test for pembrolizumab (Keytruda) is PD-L1 IHC 22C3 pharmDx and demonstrated clinical validity based on the above study.

**Nivolumab (Opdivo)**

Opdivo (nivolumab), in combination with ipilimumab, is indicated for the first-line treatment of adult patients with metastatic non-small cell lung cancer (NSCLC) whose tumors express PD-L1 (≥1%) as determined by an FDA-approved test PD-L1 IHC 28.8 pharmDx with no EGFR or ALK genomic tumor aberrations.

CHECKMATE-227 (NCT02477826) was a randomized, open-label, multi-part trial in patients with metastatic or recurrent NSCLC. The study included patients (18 years of age or older) with histologically confirmed Stage IV or recurrent NSCLC (per the 7th International Association for the Study of Lung Cancer [ASLC] classification), ECOG performance status 0 or 1, and no prior anticancer therapy. Patients were enrolled regardless of their tumor PD-L1 status. Patients with known EGFR mutations or ALK translocations sensitive to available targeted inhibitor therapy, untreated brain metastases, carcinomatous meningitis, active autoimmune disease, or medical conditions requiring systemic immunosuppression were excluded from the study. Patients with treated brain metastases were eligible if neurologically returned to baseline at least 2 weeks prior to enrolment, and either off corticosteroids, or on a stable or decreasing dose of <10 mg daily prednisone equivalents. IHC 28-8 pharmDx assay at a central laboratory. Randomization was stratified by tumor histology (non-squamous versus squamous). The evaluation of efficacy relied on the comparison between:

- **OPDIVO 3 mg/kg administered intravenously over 30 minutes every 2 weeks in combination with ipilimumab 1 mg/kg administered intravenously over 30 minutes every 6-weeks; or**
- **Platinum-doublet chemotherapy**
Chemotherapy regimens consisted of pemetrexed (500 mg/m²) and cisplatin (75 mg/m²) or pemetrexed (500 mg/m²) and carboplatin (AUC 5 or 6) for non-squamous NSCLC or gemcitabine (1000 or 1250 mg/m²) and cisplatin (75 mg/m²) or gemcitabine (1000 mg/m²) and carboplatin (AUC 5) (gemcitabine was administered on Days 1 and 8 of each cycle) for squamous NSCLC.

Study treatment continued until disease progression, unacceptable toxicity, or for up to 24 months. Treatment continued beyond disease progression if a patient was clinically stable and was considered to be deriving clinical benefit by the investigator. Patients who discontinued combination therapy because of an adverse event attributed to ipilimumab were permitted to continue OPDIVO as a single agent. Tumor assessments were performed every 6 weeks from the first dose of study treatment for the first 12 months, then every 12 weeks until disease progression or study treatment was discontinued. The primary efficacy outcome measure was OS. Additional efficacy outcome measures included PFS, ORR, and duration of response as assessed by BICR. In Part 1a, a total of 793 patients were randomized to receive either OPDIVO in combination with ipilimumab (n=396) or platinum-doublet chemotherapy (n=397). The median age was 64 years (range: 26 to 87) with 49% of patients 65 years and 10% of patients 75 years, 76% White, and 65% male. Baseline ECOG performance status was 0 (34%) or 1 (65%), 50% with PD-L1 ≥1%, 50%, 29% with squamous and 71% with non-squamous histology, 10% had brain metastases, and 85% were former/current smokers. The study demonstrated a statistically significant improvement in OS for PD-L1 ≥1% patients randomized to the OPDIVO and ipilimumab arm compared with the platinum-doublet chemotherapy arm. BICR-assessed PFS showed a HR of 0.82 (95% CI: 0.69, 0.97), with a median PFS of 5.1 months (95% CI: 4.1, 6.3) in the OPDIVO and ipilimumab arm and 5.6 months (95% CI: 4.6, 5.8) in the platinum-doublet chemotherapy arm. The BICR-assessed confirmed ORR was 36% (95% CI: 31, 41) in the OPDIVO and ipilimumab arm and 30% (95% CI: 26, 35) in the platinum-doublet chemotherapy arm. Median duration of response observed in the OPDIVO and ipilimumab arm was 23.2 months and 6.2 months in the platinum-doublet chemotherapy arm.

Companion diagnostic test for opdivo (nivolumab) is PD-L1 IHC 28.8 pharmDx and clinical validity was demonstrated based on the above study.

**NTRK 1/2/3 Gene Fusion**

NTRK gene fusions encode tropomyosin receptor kinase (TRK) fusion proteins (i.e., TRKA, TRKB, TRKC) that act as oncogenic drivers for solid tumors including lung, salivary gland, thyroid and sarcoma. A diverse range of solid tumors in children and adults may be caused by NTRK gene fusions (i.e., NTRK1, NTRK2, NTRK3). It is estimated that NTRK fusions occur in 0.2% of patients with NSCLC and do not typically overlap with other oncogenic drivers such as EGFR, ALK or ROS1.

The current NCCN guideline Non-Small Cell Lung Cancer Version 8.2020 recommends lacotrectinib (vitrakvi) and entrectinib (rozlytrek) (category 2A) as either first-line or
subsequent therapy options for patients with NTRK gene fusion-positive metastatic NSCLC based on data and the FDA approvals.

In three clinical trials, NTRK gene fusion assessment of efficacy was based on 55 patients with solid tumors including lung. Eighty-two percent of patients had metastatic disease and 18% had locally advanced, unresectable disease. Ninety-eight percent of patients had received prior treatment for their cancer, including surgery, radiotherapy, or systemic therapy. Of these, 82% (n = 45) received prior systemic therapy with a median of two prior systemic regimens and 35% (n = 19) received three or more prior systemic regimens. A total of 50 patients had NTRK fusions detected by NGS and 5 patients had NTRK gene fusions detected by FISH. Larotrectinib (vitrakvi) is an oral TKI that inhibit TRK fusion proteins across a diverse range of solid tumors in younger and older patients with NTRK gene fusion positive disease.

The efficacy of ROZLYTREK was evaluated in a pooled subgroup of adult patients with unresectable or metastatic solid tumors with a NTRK gene fusion enrolled in one of three multicenter, single-arm, open-label clinical trials: ALKA, STARTRK-1 (NCT02097810) and STARTRK-2 (NCT02568267). To be included in this pooled subgroup, patients were required to have progressed following systemic therapy for their disease, if available, or would have required surgery causing significant morbidity for locally advanced disease; measurable disease per RECIST v1.1; at least 6 months of follow-up after the first dose of ROZLYTREK; and no prior therapy with a TRK inhibitor. Patients received ROZLYTREK at various doses and schedules (94% received ROZLYTREK 600 mg orally once daily) until unacceptable toxicity or disease progression. Identification of positive NTRK gene fusion status was prospectively determined in local laboratories or a central laboratory using various nucleic acid-based tests. The major efficacy outcome measures were ORR and DOR, as determined by a BICR according to RECIST v1.1. Intracranial response according to RECIST v1.1 as evaluated by BICR. Tumor assessments with imaging were performed every 8 weeks. Efficacy was assessed in the first 54 adult patients with solid tumors with an NTRK gene fusion enrolled into these trials. The median age was 57 years (range: 21 to 83); female (59%); White (80%), Asian (13%) and Hispanic or Latino (7%); and ECOG performance status 0 (43%) or 1 (46%). Ninety-six percent of patients had metastatic disease, including 22% with CNS metastases, and 4% had locally advanced, unresectable disease. All patients had received prior treatment for their cancer including surgery (n = 43), radiotherapy (n = 36), or systemic therapy (n = 48). Thirty-four patients (63%) received prior systemic therapy for metastatic disease with a median of 1 prior systemic regimen and 17% (n = 9) received 3 or more prior systemic regimens. The most common cancers were sarcoma (24%), lung cancer (19%), salivary gland tumors (13%), breast cancer (11%), thyroid cancer (9%), and colorectal cancer (7%). A total of 52 (96%) patients had an NTRK gene fusion detected by NGS and 2 (4%) had an NTRK gene fusion detected by other nucleic acid-based tests. Eighty-three percent of patients had central laboratory confirmation of NTRK gene fusion using an analytically validated NGS test.
No companion diagnostic test is currently available related to Larotrectinib (vitrakvi) and entrectinib (rozlytrek). The current NCCN guideline Non-Small Cell Lung Cancer Version 8.2020 recommends larotrectinib (vitrakvi) and entrectinib (rozlytrek) (category 2A) as either first-line or subsequent therapy options for patients with NTRK gene fusion-positive metastatic NSCLC based on data and the FDA approvals. The recommendations also states, “to minimize tissue use and potential wastage, the NCCN NSCLC Panel recommends that broad molecular profiling be done as part of biomarker testing using a validated test(s) that assess a minimum of following potential genetic variants: EGFR mutations, BRAF mutations, METex14 skipping mutations, RET rearrangements, ALK fusions, and ROS1 fusions. Both FDA and laboratory developed test platforms are available that address the need to evaluate these and other analytes. Broad molecular profiling is also recommended to identify rare driver mutations for which effective therapy may be available, such NTRK gene fusion, high level MET amplification, ERBB2 mutations and TMB.”

**FDA Approved Companion Diagnostic Tests for METex14 Skipping Mutations**

METex14 skipping mutations occur in 3% to 4% of patients with adenocarcinomas NSCLC and 1% to 2% of patients with other NSCLC histologies. METex14 skipping mutations are more frequent in older women who are nonsmokers. NGS and RT-PCR assays can be used to detect METex14 skipping mutations with MET amplification.

The current NCCN guideline Non-Small Cell Lung Cancer Version 8.2020 states: “The NCCN NSCLC Panel recommends testing for METex14 skipping mutations (category 2A) in eligible patients with metastatic NSCLC based on data showing the efficacy of several agents for patients with METex14 skipping mutations and on the FDA approval for capmatinib (tabrecta).“

Capmatinib (tabrecta), is a kinase inhibitor indicated for the treatment of adult patients with metastatic non-small cell lung cancer (NSCLC) whose tumors have a mutation that leads to mesenchymal-epithelial transition (MET) exon 14 skipping as detected by an FDA-approved test FoundationOne CDx.

The efficacy of TABRECTA was evaluated in GEOMETRY mono-1, a multicenter, non-randomized, open-label, multi-cohort study (NCT02414139). Eligible patients were required to have NSCLC with a mutation that leads to MET exon 14 skipping, epidermal growth factor receptor (EGFR) wild-type and anaplastic lymphoma kinase (ALK) negative status, and at least one measurable lesion as defined by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Patients with symptomatic CNS metastases, clinically significant uncontrolled cardiac disease, or who received treatment with any MET or hepatocyte growth factor (HGF) inhibitor were not eligible for the study. Out of the 97 patients enrolled in GEOMETRY mono-1 following the central confirmation of MET exon 14 skipping by a RNA-based clinical trial assay, 78 patient samples were retested with the FDA-approved FoundationOne CDx (22 treatment-naïve and 56 previously treated patients) to detect mutations that lead to MET exon 14 skipping. Out of 78 samples retested with FoundationOne CDx, 73 samples were
evaluable (20 treatment-naïve and 53 previously treated patients), 72 (20 treatment-naïve and 52 previously treated patients) of which were confirmed to have a mutation that leads to MET exon 14 skipping, demonstrating an estimated positive percentage agreement of 99% (72/73) between the clinical trial assay and the FDA-approved assay. Patients received TABRECTA 400 mg orally twice daily until disease progression or unacceptable toxicity. The major efficacy outcome measure was overall response rate (ORR) as determined by a Blinded Independent Review Committee (BIRC) according to RECIST 1.1. An additional efficacy outcome measure was duration of response (DOR) by BIRC. The efficacy population included 28 treatment-naïve patients and 69 previously treated patients. The median age was 71 years (range: 49 to 90 years); 60% female; 75% White; 24% had Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) 0 and 75% had ECOG PS 1; 60% never smoked; 80% had adenocarcinoma; and 12% had CNS metastases. Amongst previously treated patients, 88% received prior platinum-based chemotherapy.

### Efficacy Results in GEOMETRY Mono-1

<table>
<thead>
<tr>
<th>Efficacy Parameters</th>
<th>Treatment – Naïve N=28</th>
<th>Previously Treated N=69</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overall Response Rate</strong></td>
<td>68% (48, 84)</td>
<td>41% (29, 53)</td>
</tr>
<tr>
<td><em>(95% CI)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete Response</td>
<td>4%</td>
<td>0</td>
</tr>
<tr>
<td>Partial Response</td>
<td>64%</td>
<td>41%</td>
</tr>
<tr>
<td><strong>Duration of Response</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>(DOR)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (months) (95% CI)</td>
<td>12.6 (5.5, 25.3)</td>
<td>9.7 (5.5, 13.0)</td>
</tr>
<tr>
<td>Patients with DOR ≥ 12 months</td>
<td>47%</td>
<td>32%</td>
</tr>
</tbody>
</table>

Abbreviations: CI = Confidence Interval.  
*Blinded Independent Review Committee (BIRC) review.*  
*Confirmed response.*  
*Clopper and Pearson exact binomial 95% CI.*  
*Based on Kaplan-Meier estimate.*  
*Companion diagnostic test FoundationOne CDx for Capmatinib (tabrecta) for METex 14 skipping mutation detection was clinically validated by the study above.*

### RET Rearrangements

RET rearrangements occur in about 1% to 2% of patients with NSCLC and are more frequent in patients with adenocarcinoma histology. In European patients, RET rearrangements occur in both smokers and nonsmokers. RET rearrangements do not typically overlap with EGFR, ROS1, BRAF, METex14 skipping and ALK genetic variants. However, a few studies suggest that RET rearrangements may infrequently
overlap with EGFR and KRAS mutations. FISH, RT-PCR, and NGS assays can be used to detect RET rearrangements.

Selpercatinib (Retevmo) is indicated for the treatment of adult patients with metastatic RET fusion-positive non-small cell lung cancer (NSCLC).

The efficacy of RETEVMO was evaluated in patients with advanced RET fusion-positive NSCLC enrolled in a multicenter, open-label, multi-cohort clinical trial (LIBRETTO-001, NCT03157128). The study enrolled patients with advanced or metastatic RET fusion-positive NSCLC who had progressed on platinum-based chemotherapy and patients with advanced or metastatic NSCLC without prior systemic therapy in separate cohorts. Identification of a RET gene alteration was prospectively determined in local laboratories using next generation sequencing (NGS), polymerase chain reaction (PCR), or fluorescence in situ hybridization (FISH). Adult patients received RETEVMO 160 mg orally twice daily until unacceptable toxicity or disease progression. The major efficacy outcome measures were confirmed overall response rate (ORR) and duration of response (DOR), as determined by a blinded independent review committee (BIRC) according to RECIST v1.1. Efficacy was evaluated in 105 patients with RET fusion-positive NSCLC previously treated with platinum chemotherapy enrolled into a cohort of LIBRETTO-001. The median age was 61 years (range: 23 to 81); 59% were female; 52% were White, 38% were Asian, 4.8% were Black, and 3.8% were Hispanic/Latino. ECOG performance status was 0-1 (98%) or 2 (2%) and 98% of patients had metastatic disease. Patients received a median of 3 prior systemic therapies (range 1–15); 55% had prior anti-PD-1/PD-L1 therapy. RET fusions were detected in 90% of patients using NGS (81.9% tumor samples 7.6% blood or plasma samples), 8.6% using FISH, and 1.9% using PCR.

Efficacy results for RET fusion-positive NSCLC are summarized in the table below

<table>
<thead>
<tr>
<th>Retevmo (n=105)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overall Response Rate</strong>¹ (95% CI)</td>
</tr>
<tr>
<td>Complete response</td>
</tr>
<tr>
<td>Partial response</td>
</tr>
</tbody>
</table>

**Duration of Response**

| Median in months (95% CI) | 17.5 (12, NE) |
| % with ≥6 months² | 81 |

¹ Confirmed overall response rate assessed by BIRC. ² Based on observed duration of response NE = not estimable

For the 58 patients who received an anti-PD-1 or anti-PD-L1 therapy, either sequentially or concurrently with platinum-based chemotherapy, an exploratory subgroup analysis of
ORR was 66% (95% CI: 52%, 78%) and the median DOR was 12.5 months (95% CI: 8.3, NE).

Among the 105 patients with RET fusion-positive NSCLC, 11 had measurable CNS metastases at baseline as assessed by BIRC. No patients received radiation therapy (RT) to the brain within 2 months prior to study entry. Responses in intracranial lesions were observed in 10 of these 11 patients; all responders had a DOR of ≥ 6 months.

Treatment-naïve RET Fusion-Positive NSCLC

Efficacy was evaluated in 39 patients with treatment-naïve RET fusion-positive NSCLC enrolled into a cohort of LIBRETTO-001.

The median age was 61 years (range 23 to 86); 56% were female; 72% were White, 18% were Asian, and 8% were Black. ECOG performance status was 0-1 in all patients (100%) and all patients (100%) had metastatic disease. RET fusions were detected in 92% of patients using NGS (69% tumor samples: 23% in blood) and 8% using FISH.

Efficacy results for treatment naïve RET fusion-positive NSCLC are summarized in the table below:

<table>
<thead>
<tr>
<th>Retevmo (n=105)</th>
<th>Overall Response Rate(^1) (95% CI)</th>
<th>64% (54%, 73%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Complete response</td>
<td>1.9%</td>
</tr>
<tr>
<td></td>
<td>Partial response</td>
<td>62%</td>
</tr>
<tr>
<td><strong>Duration of Response</strong></td>
<td>Median in months (95% CI)</td>
<td>17.5 (12, NE)</td>
</tr>
<tr>
<td></td>
<td>% with ≥ 6 months(^2)</td>
<td>81</td>
</tr>
</tbody>
</table>

\(^1\) Confirmed overall response rate assessed by BICR.  
\(^2\) Based on observed duration of response NE = not estimable

No companion diagnostic test is currently available related to selpercatinib (retevmo). The current NCCN guideline Non-Small Cell Lung Cancer Version 8.2020 states: “the NCCN NSCLC Panel recommends that broad molecular profiling be done as part of biomarker testing using a validated test(s) that assess a minimum of following potential genetic variants: EGFR mutations, BRAF mutations, METex14 skipping mutations, RET rearrangements, ALK fusions, and ROS1 fusions. Both FDA and laboratory developed test platforms are available that address the need to evaluate these and other analytes. Broad molecular profiling is also recommended to identify rare driver mutations for which effective therapy may be available, such NTRK gene fusion, high level MET amplification, ERBB2 mutations and TMB.”
KRAS Mutations
KRAS mutations can be detected by direct sequencing, PCR technologies, or NGS. Data suggest that approximately 25% of patients with adenocarcinomas in a North American population have KRAS mutations; KRAS is the most common mutation in this population. KRAS mutation prevalence is associated with cigarette smoking. KRAS mutational status is predictive of lack of therapeutic efficacy with EGFR TKIs; it does not appear to affect chemotherapeutic efficacy. KRAS mutations do not generally overlap with EGFR, ALK, ROS1 and BRAF genetic variants. Therefore, KRAS testing may identify patients who may not benefit from further molecular testing. Targeted therapy is not currently available for patients with KRAS mutations, although immune checkpoint inhibitors (ICIs) appear to be effective (pembrolizumab [Keytruda], nivolumab [opdivo], atezolizumab [tecentriq]), durvalumab [Imfinzi]).

Emerging Biomarkers to Identify Targeted Therapy for Patients with Metastatic NSCLC
Proposed targeted therapies may be used for genetic alterations in HER2 (ERBB2) (ado-trastuzumab emtansine), high level MET amplification n (crizotinib), and tumor mutational burden (TMB) (nivolumab [opdivo] + ipilimumab or nivolumab [opdivo]), there is no consensus on how to measure TMB. (NCCN Non-Small Cell Lung Cancer Version 5.2021)

The current NCCN guideline Non-Small Cell Lung Cancer Version 5.2021 states the following: “the NCCN NSCLC Panel recommends that broad molecular profiling be done as part of biomarker testing using a validated test(s) that assess a minimum of following potential genetic variants: EGFR mutations, BRAF mutations, METex14 skipping mutations, RET rearrangements, ALK fusions, and ROS1 fusions. Both FDA and laboratory developed test platforms are available that address the need to evaluate these and other analytes. Broad molecular profiling is also recommended to identify rare driver mutations for which effective therapy may be available, such NTRK gene fusion, high level MET amplification, ERBB2 mutations and TMB.”

Summary of Evidence
Non-small cell lung cancer (NSCLC) accounts for more than 85% of lung cancer and the majority of these patients present with advanced-stage disease and treated with systemic therapies. Great strides have been made in the development of therapies of such patients, including targeted therapies and immunotherapy. Targeted therapies require identification of specific molecular alterations in cancer and guidelines such as NCCN recommend broad molecular profiling using validated test(s) to assess for therapeutic targets.

The current NCCN guidelines for Non-Small Cell Lung Cancer Version 5.2021 includes the following:

  Molecular testing is used to test for genomic variants associated with oncogenic driver events for which targeted therapies are available; these genomic variants (also known as molecular biomarkers) include gene mutations and fusions.
To minimize tissue use and potential wastage the NCCN NSCLC Panel recommends that broad molecular profiling be done as part of biomarker testing using a validated test(s) that assess a minimum of following potential genetic variants: EGFR mutations, BRAF mutations, METex14 skipping mutations, RET rearrangements, ALK fusions, and ROS1 fusions. Both FDA and laboratory developed test platforms are available that address the need to evaluate these and other analytes. Broad molecular profiling is also recommended to identify rare driver mutations for which effective therapy may be available, such NTRK gene fusion, high level MET amplification, ERBB2 mutations and TMB. Although clinicopathologic features such as smoking status, ethnicity and histology are associated with specific genetic variants (i.e., EGFR mutations), these features should not be used to select patients for testing. Although the NCCN guidelines for NSCLC provide recommendations for individual markers that should be tested and recommend testing techniques, the guidelines do not endorse any specific commercially available biomarkers assays.

EGFR Mutations
- The NCCN NSCLCL Panel recommends testing for sensitizing EGFR mutations in patients with metastatic non-squamous NSCLC or NSCLC NOS based on data showing the efficacy of osimertinib, erlotinib, gefitinib, afatinib or dacomitinib and on FDA approval.
- Osimertinib is a preferred first-line EGFR TKI option for patients with EGFR positive metastatic NSCLC. Erlotinib, gefitinib, afatinib or dacomitinib are “other recommended” EGFR TKI options for first-line therapy. Osimertinib is recommended (category 1) as secondline and beyond (subsequent) therapy for patients with EGFR T790M-positive metastatic NSCLC who have progressed on erlotinib, gefitinib, afatinib, or dacomitinib. Sensitizing EGFR mutations and ALK or ROS1 fusions are generally mutually exclusive. Thus, crizotinib, ceritinib, alectinib,brigatinib or lorlatinib are not recommended as subsequent therapy for patients with sensitizing EGFR mutations who relapse on EGFR TKI therapy.

BRAF V600E Mutations
- Testing for BRAF mutations is recommended (category 2A) in patients with metastatic non-squamous NSCLC and may be considered in patients with squamous cell NSCLC (category 2A) if small biopsy specimens were used to assess histology or mixed histology was reported.
- The NCCN NSCLC Panel recommends testing for BRAF mutations in patients with metastatic non-squamous NSCLC based on data showing the efficacy of dabrafenib plus trametinib for patients with BRAF V600E mutations and on the FDA approval. For the 2020 update (Version 1), the NCCN Panel preference stratified first-line therapy for patients with BRAF V600E mutation-positive metastatic NSCLC. Dabrafenib plus trametinib is recommended (category 2A; preferred) for patients with BRAF V600E mutations. If combination therapy with dabrafenib/trametinib is not tolerated, single-agent therapy with dabrafenib or vemurafenib are “other
recommended” agents. Chemotherapy regimens are also used for initial systemic therapy (i.e., carboplatin/pemetrexed for non-squamous NSCLC) and are “useful in certain circumstances.”

**ALK Gene Rearrangements**
- The NCCN NSCLC Panel recommends testing for ALK fusion in patients with metastatic nonsquamous NSCLC based on data showing the efficacy of alectinib, brigatinib, ceritinib, and crizotinib for ALK fusions and on the FDA approvals. If patients appear to have squamous cell NSCLC, then testing can be considered if small biopsy specimens were used to assess histology, mixed histology was reported, or patients are light or never smokers.
- Alectinib is recommended as a preferred first-line therapy for patients with ALK rearrangement-positive metastatic NSCLC. The NCCN Panel preference stratified first-line therapy with brigatinib, ceritinib, or crizotinib for patients with ALK rearrangement-positive metastatic NSCLC. Brigatinib and ceritinib are “other recommended” options, whereas crizotinib is “useful” in certain circumstances.

**ROS1 Rearrangements**
- The NCCN NSCLC Panel recommends ROS1 testing (category 2A) in patients with metastatic nonsquamous NSCLC or NSCLC NOS based on data showing the efficacy of crizotinib, ceritinib, and entrectinib for patients with ROS1 fusions. ROS1 testing can be considered in patients with metastatic squamous cell NSCLC if small biopsy specimens were used to assess histology or mixed histology was reported.
- The NCCN NSCLC Panel recommends crizotinib, entrectinib or ceritinib (all are category 2A) as first-line therapy options for patients with ROS1-positive metastatic NSCLC based on the clinical trial data. The NCCN NSCLC Panel voted that crizotinib and entrectinib are preferred first-line therapy options for patients with ROS1-positive metastatic NSCLC because they are better tolerated, have been assessed in more patients, and are approved by the FDA. Although entrectinib has better CNS penetratin than crizotinib, it is more toxic. If ROS1 fusions are discovered during first-line systemic therapy (i.e., carboplatin/paclitaxel), then the planned therapy may be either completed or interrupted followed by crizotinib (preferred), entrectinib (preferred) or ceritinib.
- The NCCN NSCLC Panel recommends lorlatinib (category 2A) as a subsequent therapy option for select patients with ROS1-positive metastatic NSCLC who have progressed after treatment with crizotinib, entrectinib, or ceritinib. Initial systemic therapy options that are used for adenocarcinoma or squamous cell carcinoma are also an option in this setting (i.e., carboplatin/paclitaxel).

**NTRK 1/2/3 Gene Fusions**
- The NCCN NSCLC Panel recommends NTRK gene fusion testing in patients with metastatic NSCLC based on clinical trial data showing the efficacy of lacrotrectinib and entrectinib for patients with NTRK gene...
fusion-positive disease; however, clinical data are limited in NSCLC to support this recommendation. The NCCN NSCLC Panel recommends lacrotrectinib and entrectinib (both are category 2A) as either first-line or subsequent therapy options for patients with NTRK gene fusion-positive metastatic NSCLC based on data and the FDA approvals.

**METex 14 Skipping Mutations**
- The NCCN NSCLC Panel recommends testing for METex14 skipping mutations (category 2A) in eligible patients with metastatic NSCLC based on data showing the efficacy of several agents for patients with METex14 skipping mutations and on the FDA approval for capmatinib.

**RET Rearrangements**
- The NCCN NSCLC Panel recommends testing for RET rearrangements (category 2A) in eligible patients with metastatic NSCLC based on data showing the efficacy of several agents for patients with RET rearrangements and on the FDA approval for selpercatinib.

**KRAS Mutations**
- KRAS mutation prevalence is associated with cigarette smoking. Patients with KRAS mutations appear to have a shorter survival than patient with wild-type KRAS; therefore, KRAS mutations are prognostic biomarkers. KRAS mutational status is also predictive of lack of therapeutic efficacy with EGFR TKIs; it does not appear to affect chemotherapeutic efficacy. KRAS mutations do not generally overlap with EGFR, ROS1, BRAF and ALK genetic variants. Therefore, KRAS testing may identify patients who may not benefit from further molecular testing. KRAS mutations my infrequently overlap with EGFR mutations and RET rearrangements. Targeted therapy is not currently available for patients with KRAS mutations, although immune checkpoint inhibitors (ICIs) appear to be effective.

**PD-L1 Expression Levels**
- The NCCN NSCLC Panel recommends (category 1) IHC testing for PD-L1 expression ideally before first-line treatment (if clinically feasible) in all patients with metastatic NSCLC to assess whether the ICI regimens are an option based on clinical data showing the efficacy of these regimens.
- Although it is not an optimal biomarker, PD-L1 expression is currently the best available biomarker to assess whether patients are candidates for PD-1 or PD-L1 inhibitors (ICIs; also known as immune-oncology [IO] agents, immunotherapy).
- The NCCN NSCLC Panel emphasizes that clinicians should obtain molecular testing results for actionable biomarkers before administering first-line ICI therapy, if clinically feasible. Patients with metastatic NSCLC and PD-L1 expression levels of 1% or more but who also have a targetable driver oncogene molecular variant (e.g. EGFR, ALK, ROS1) should receive first-line targeted therapy for that oncogene and not first-line ICIs because targeted therapies yield higher response rates (e.g. osimertinib, 80%) than ICIs (poor response rates) in the first-line setting, targeted therapy is better
tolerated, and these patients are unlikely to respond to ICIs. The panel also added “ROS1 fusions” and “BRAF mutations” to the list of actionable biomarkers that need to be negative before administering PD-1 or PD-L1 inhibitors. At a minimum, EGFR and ALK status should be known before starting systemic therapy with ICI regimens; however, it is ideal if ROS1 and BRAF status are also known. If it is not feasible to do molecular testing, then patients are treated as though they do not have driver oncogenes.

**Emerging Biomarkers to Identify Novel Therapies for Patients with Metastatic NSCLC**

<table>
<thead>
<tr>
<th>Genetic Alterations (i.e. Driver Event)</th>
<th>Available Targeted Agents with Activity Against Drive Event in Lung Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-level Met amplification</td>
<td>Crizotinib</td>
</tr>
<tr>
<td></td>
<td>Capmatinib</td>
</tr>
<tr>
<td>ERBB2 (HER2) mutations</td>
<td>Ado-trastuzumab emtansine</td>
</tr>
<tr>
<td></td>
<td>Fam-trastuzumab deruxtecan-nxki</td>
</tr>
</tbody>
</table>

The current NCCN guideline Non-Small Cell Lung Cancer Version 5.2021 states the following: The NCCN NSCLC Panel recommends that broad molecular profiling be done as part of biomarker testing using a validated test(s), and currently recommends the following be included in the broad molecular profiling: ALK fusion oncogene, ROS1 gene fusions, EGRF gene mutations, BRAF V600E, NTRK fusion genes, METex14 skipping mutations, RET rearrangements and PD-L1 expression testing. The recommendation also includes that broad molecular profiling should include emerging biomarkers high-level MET amplification, ERBB2 (HER2) mutations and tumor mutation burden (TMB). This list of recommended biomarkers may be revised as new oncogenic driver variants are identified and new agents are approved.” Based on the literature review and FDA labeling for targeted therapies in the treatment of NSCLC companion diagnostic testing and its indications for biomarker/genetic/gene fusion testing are included as part of the FDA labeling, clinical validity was demonstrated in these tests see Regulatory Status information below. While there are currently no companion diagnostics tests for NTRK gene fusions or RET rearrangements it is recommended these biomarkers be included in the broad molecular profiling for targeted therapies in the treatment of NSCLC: NTRK gene fusions for lacosamide and entrectinib; and RET rearrangements for selpercatinib which was confirmed based on clinical studies for their FDA labeling. NCCN guideline also advises that for the emerging predictive markers ERBB2 (HER2) mutations, high-level MET amplifications, and tumor mutations burden) (TMB), broad molecular profiling should be completed to identify these other rare driver variants for which targeted therapies may be available to ensure that patients receive the most appropriate treatment; patients may be eligible for
clinical trials for some of these targeted agents. Therefore, the evidence is sufficient to determine biomarker/genetic/fusion gene testing using clinically validated test(s) (companion diagnostic testing) to predict treatment response to targeted therapy for the treatment of metastatic NSCLC results in meaningful improvement in net health outcomes (See Regulatory Status information below).

Practice Guideline and Position Statements

National Comprehensive Cancer Network (NCCN)
Non-Small Cell Lung Cancer Version 5.2021

Principles of Molecular and Biomarker Analysis

Molecular Diagnostic Studies in Non-Small Cell Lung Cancer
Numerous gene alterations have been identified that impact therapy selections. Testing of lung cancer specimens for these alterations is important for identification of potentially efficacious targeted therapies, as well as avoidance of therapies unlikely to provide clinical benefit.

Some selection approaches for targeted therapy include predictive immunohistochemical analysis, which are distinct from immunohistochemical studies utilized to identify tumor type and lineage.

Major elements of molecular testing that are critical for utilization and interpretation of molecular results include:
- Use of laboratory that is properly accredited, with a minimum of CLIA accreditation
- Understanding the methodologies that are utilize and the major limitations of those methodologies
- Understanding the spectrum of alterations tested (and those not tested) by a specific assay
- Knowledge of whether a tumor sample is subjected to pathologic review and tumor enrichment (i.e., microdissection, microdissection) prior to testing
- The types of samples accepted by the testing laboratory

Specimen Acquisition and Management:
- Although tumor testing has been primarily focused on use of formalin-fixed paraffin-embedded (FFPE) tissues, increasingly, laboratories accept other specimen types, notably cytopathology preparations not processed by FFPE methods. Although testing on cell blocks is not included in the FDA approval for multiple companion diagnostic assays, testing on these specimen types is highly recommended when it is the only or best material.
- A major limitation in obtaining molecular testing results for NSCLC occurs when minimally invasive techniques are used to obtain samples; the yield may be insufficient for molecular biomarker and histologic testing.
Therefore, bronchoscopists and interventional radiologists should procure sufficient tissue to enable all appropriate testing.

- When tissue is minimal, laboratories should deploy techniques to maximize tissue for molecular and ancillary testing, including dedicated histology protocols for small biopsies, including “up-front” slide sectioning for diagnostic and predictive testing.

Testing Methodologies

- Appropriate possible testing methodologies are indicated below for each analyst separately; however, several methodologies are generally considerations for use:
  - Next-generation sequencing (NGS) is used in clinical laboratories. Not all types of alterations are detected by individual NGS assays or combination(s) of assays.
  - It is recommended at this time that when feasible, testing be performed via a broad, panel-based approach, most typically performed by next generation sequencing (NGS). For patients who, in broad pane testing don’t have identifiable driver oncogenes (especially in never smokers), consider RNA-based NGS if not already performed, to maximize detection of fusion events.
  - Real-time polymerase chain reaction (PCR) can be used in a highly targeted fashion (specific mutations targeted). When this technology is deployed, only those specific alterations that are targeted by the assay are assessed.
  - Sanger sequencing requires the greatest degree of tumor enrichment. Unmodified Sanger sequencing is not appropriate for detection of mutations in tumor samples with less than 25% to 30% tumor after enrichment is not appropriate for assays in which identification of subclonal events (e.g., resistance mutations) is important. If Sanger sequencing is utilized, tumor enrichment methodologies are nearly always recommended.
  - Other methodologies may be utilized, including multiplex approaches not listed above (i.e. SNaPshot, MassARRAY).
  - Fluorescence in situ hybridization (FISH) analysis is utilized for many assays examining copy number, amplifications, and structural alterations such as gene rearrangements.
  - Immunohistochemistry (IHC) is specifically utilized for some specific analytes and can be useful surrogate or screening assays for others.

Molecular Targets for Analysis

- In general, the mutations/alterations described below are seen in a non-overlapping fashion, although between 1%-3% of NSCLC may harbor concurrent alterations.
- EGFR (Epidermal Growth Factor Receptor) Gene Mutations: EGFR is a receptor is a receptor tyrosine kinase normally found on the surface of
epithelial cells and is often overexpressed in a variety of human malignancies.

- The most commonly described mutations in EGFR (exon 19 deletions, p.L858R point mutation in exon 21) are associated with responsiveness to EGFR tyrosine kinase inhibitor (TKI) therapy; most recent data indicated that tumors that do not harbor a sensitizing EGFR mutation should not be treated with EGFR TKI in any line of therapy.

- Molecular testing for EGFR mutation to be performed on diagnostic biopsy or surgical resection sample to ensure the EGFR mutation results are available for adjuvant treatment decisions for patients with stage IIB-IIIA or high-risk stage IB-IIA NSCLC.

- Many of the less commonly observed alterations of EGFR, which cumulatively account for -10% of EGFR mutation positive NSCLC (e.g., exon 19 insertions, p>L861Q, p.G719X, p.S768I) are also associated with responsiveness to EGFR TKI therapy, although the number of studied patients is lower.

- EGFR exon 20 (EGFRex20) mutations are a heterogenous group, some of which are responsive to targeted therapy and that required detailed knowledge of the specific alteration
  - Most EGFRex20 alterations are a diverse group of in-frame duplication or insertion mutation.
    - These are generally associated with lack of response to EGFR TKI therapy, with select exceptions:
      - p.A763_Y764insFQEA, which is associated with sensitivity to TKI therapy.
      - p.A763_Y764insLQEA may be associated with sensitivity to TKI therapy
    - For this reason, the specific sequence of EGFRex20 insertion mutations is important and some assays will identify the presence of an EGFRex20 insertion without specifying the sequence. In this scenario, additional testing to further clarify the EGFRex20 insertion is indicated.

- EGFR p.T790M is most commonly observed as a mutation that arises in response to and as a mechanism of resistance to first- and second-generation EGFR TKI. In patients with progression on first or second generation TKI with p.T790M as the primary mechanism of resistant, third-generation TKIs are typically efficacious. If p.T790M is observed in the absence of prior EGFR TKI therapy, genetic counseling and possible germline genetic testing is warranted.
As use of NGS testing increases, additional EGFR variants are increasingly identified; however, the clinical implications of individual alterations are unlikely to be well established.

Some clinicopathologic features such as smoking status, ethnicity, and histology are associated with the presence of an EGFR mutation; however, these features should not be utilized in selecting patients for testing.

Testing methodologies: Real-Time PCR, Sanger sequencing (Ideally paired with tumor enrichment), and NGS are the most commonly deployed methodologies for examining EGFR mutations status.

ALK (Anaplastic Lymphoma Kinase) Gene Rearrangements: ALK is a receptor tyrosine kinase that can be rearranged in NSCLC, resulting in dysregulation and inappropriate signaling through the ALK kinase domain.

- The most common fusion partner seen with ALK is echinoderm microtubule-associated protein-like 4 (EML4), although a variety of other fusion partners have been identified.
- The presence of an ALK rearrangement is associated with responsiveness to ALK TKIs.
- Some clinicopathologic features such as smoking status and histology have been associated with the presence of an ALK rearrangement; however, these features should not be utilized in selecting patients for testing.
- Testing methodologies: FISH break-apart probe methodology was the first methodology deployed widely. IHC can be deployed as an effective screening strategy. FDA-approved ICH (ALK[D5F3] CDx Assay) can be utilized as a stand-alone test, not requiring confirmation by FISH. Numerous NGS methodologies can detect ALK fusions. Targeted real-time PCR assays are used in some settings, although it is unlikely to detect fusions with novel partners.

ROS1 (ROS proto-oncogene 1) Gene Rearrangements: ROS1 is a receptor tyrosine kinase that can be rearranged in NSCLC, resulting in dysregulation and inappropriate signaling through the ROS1 kinase domain.

- Numerous fusion partners are seen with ROS1, and common fusion partners include: CD74, SLC34A2, CCDC6, and FIG.
- The presence of a ROS1 rearrangement is associated with the responsiveness to oral ROS1 TKIs.
- Some clinicopathologic features such as smoking status and histology have been associated with the presence of ROS1 rearrangements; however, these features should not be utilized in selecting patients for testing.
- Testing methodologies: FISH break-apart probe methodology can be deployed; however, it may under-detect the FIG-ROS1 variant. IHC approaches can be deployed; however, IHC for ROS1 fusions has low specificity, and follow-up confirmatory testing is a necessary component of utilizing ROS1 IHC as a screening modality. Numerous NGS methodologies can detect ROS1 fusions, although DNA-based NGS may
under-detect ROS1 fusions. Targeted real-time PCR assays are utilized in some settings, although they are unlikely to detect fusions with novel partners.

BRAF (B-Raf proto-oncogene) point mutations: BRAF is a serine/threonine kinase that is part of the canonical MAP/ERK signaling pathway. Activating mutations in BRAF result in unregulated signaling through the MAP/ERK pathway.
- Mutations in BRAF can be seen in NSCLC. The presence of a specific mutation resulting in a change in amino acid position 600 (p.V600E) has been associated with responsiveness to combine therapy with oral inhibitors of BRAF and MEK.
- Note that other mutations in BRAF are observed in NSCLC, and the impact of those mutations on therapy selection is currently understated at this time.
- Testing methodologies: Real time PCR, Sanger sequencing (ideally paired with tumor enrichment), and NGS are commonly deployed methodologies for examining BRAF mutation status. While an anti-BRAF pV600E-specific monoclonal antibody is commercially available, and some studies have examined utilizing this approach, it should only be deployed after extensive validation.

KRAS (KRAS proto-oncogene) point mutations: KRAS is a G-protein with intrinsic GTPase activity, and activating mutations result in unregulated signaling through the MAP/ERK pathway.
- Mutations in KRAS are most commonly seen at codon 12, although other mutations can be seen in NSCLC.
- The presence of a KRAS mutation is prognostic of poor survival when compared to patients with tumors without KRAS mutation.
- Mutations in KRAS have been associated with reduced responsiveness to EGFR TKI therapy.
- Owing to the low probability of overlapping targetable alterations, the presence of a known activating mutations in KRAS identifies patients who are unlikely to benefit from further molecular testing.

- MET (mesenchymal–epithelial transition) exon 14 (METex14) skipping variants: MET is a receptor tyrosine kinase. A mutation that results in loss of exon 14 can occur in NSCLC. Loss of METex14 leads to dysregulation and inappropriate singling.
  - The presence of METex14 skipping mutation is associated with responsiveness to oral MET TKIs.
  - A broad range of molecular alterations lead to METex14 skipping.
  - Testing Methodologies: NGS – based testing is the primary method for detection or METex14 skipping events, with RNA – based NGS demonstrating improvements in detection. IHC is not a method for detection of METex14 skipping.

- RET (rearranged during transfection) Gene Rearrangements: RET is a receptor tyrosine kinase that can be rearranged in NSCLC, resulting in dysregulation and inappropriate signaling through the RET kinase domain.
Common fusion partners are KIF5B, NCOA4, and CCDC6; however, numerous other fusion partners have been identified.

The presence of a RET rearrangement is associated with responsiveness to oral RET TKIs regardless of fusion partner.

Testing Methodologies: FISH break-apart probe methodology can be deployed; however, it may under-detect some fusions. Targeted real-time reverse-transcriptase PCR assays are utilized in some settings, although they are unlikely to detect fusions with novel partners. NGS-based methodology has a high specificity, and RNA-based NGS is preferable to DNA-based NGS for fusion detection.

NTKR 1/2/3 (neurotropic tyrosine receptor kinase) gene fusions

NTKR 1/2/3 are tyrosine receptor kinases that are rarely rearranged in NSCLC as well as in other tumor types, resulting in dysregulation and inappropriate signaling.

Numerous fusion partners have been identified.

To date, no specific clinicopathologic features, other than the absence of other driver alterations, have been identified in association with these fusions.

Point mutations in NTRK 1/2/3 are generally non-activating and have not been studied in association with targeted therapy.

Testing Methodologies: Various methodologies can be used to detect NTRK gene fusions, including FISH, ICH, PCR and NGS; false negatives may occur. IHC methods are complicated by baseline expression in some tissues. FISH testing may require at least 3 probe sets for full analysis. NGS testing can detect a broad range of alterations. DNA-based NGS may under-detect NTRK-1 and NTRK-3 fusions.

In the event that a complete assessment for all biomarkers cannot be reasonably accomplished prior to initiation of therapy, consider repeat panel testing or selected biomarker testing at progression on first-line therapy is lesion can be accessed for sampling and testing.

Testing in the Setting of Progression on Targeted Therapy:

For many of the above listed analytes, there is growing recognition of the molecular mechanisms of resistance to therapy. Re-testing of a sample from a tumor that is actively progressing while exposed to targeted therapy can shed light on appropriate next therapeutic steps:

- For patients with an underlying EGFR sensitizing mutation who have been treated with EGFR TKI, minimum appropriate testing includes high-sensitivity evaluation for p.T790M; when there is no evidence of p.T790M, testing for alternate mechanisms of resistance (MET amplification, ERBB2 amplification) may be used to direct patients for additional therapies. The presence of p.T790M can direct patients to third-generation EGFR TKI therapy.
  - Assays for the detection of EGFR p.T790M should be designed to have an analytic sensitivity of a minimum of 5% allelic fraction. The original sensitizing mutation can be
utilized as an internal control in many assays to determine whether a p.T790M is within range of detection if present as sub-clonal event.

- For patients with underlying ALK rearrangement who have been treated with ALK TKI, it is unclear whether identification of specific tyrosine kinase domain mutation can identify appropriate next steps in therapy, although some preliminary data suggest that specific kinase domain mutations can impact next line of therapy.

PD-L1 (Programmed Death Ligand 1): PD-L1 is a co-regulatory molecule that can be expressed on tumor cells and inhibit T-cell-mediated cell death. T-cells express PD-1, a negative regulator, which binds to ligands including PD-L1 (CD274) or PD-L2 (CD273). In the presence of PD-L1, T-cell activity is suppressed.
  - Checkpoint inhibitor antibodies block the PD-1 and PD-L1 interaction, thereby improving the antitumor effects of endogenous T-cells.
  - IHC for PD-L1 can be utilized to identify disease most likely to respond to first-line anti PD-1/PD-L1.
    - Various antibody clones have been developed for IHC analysis of PD-L1 expression, and while several show relative equivalence, some do not.
    - Interpretation of PD-L1 IHC in NSCLC is typically focused on the proportion of tumor cells expressing membranous staining at any level and therefore is a linear variable, scoring systems may be different in other tumor types.
    - The FDA-approved companion diagnostics for PD-L1 guides utilization of pembrolizumab in patients with NSCLC and is based on the tumor proportion score (TPS). TPS is the percentage of viable tumor cells showing partial or complete membrane staining at any intensity.
    - The definition of positive and negative testing is dependent on the individual antibody and platform deployed, which may be unique to each checkpoint inhibitor therapy. The potential for multiple different assays for PD-L1 has raised concern among both pathologist and oncologists.
    - Although PD-L1 expression can be elevated in patients with oncogenic driver, targeted therapy for the oncogenic driver should take precedence over treatment with an immune checkpoint inhibitor.

Plasma Cell-Free/Circulating Tumor DNA Testing:
  - Cell-free/circulating tumor DNA testing should not be used in lieu of a histologic tissue diagnosis.
  - Some laboratories offer testing for molecular alternations examining nucleic acids in peripheral circulation, most commonly in processed plasma (sometimes referred to as “liquid biopsy”)
o Studies have demonstrated cell-free tumor DNA testing to generally have very high specificity, but significantly compromised sensitivity, with up to 30% false-negative rate.

o Standards for analytic performance characteristics of cell-free tumor DNA have not been established, and in contrast to tissue-based testing, no guidelines exist regarding the recommended performance characteristics of this type of testing.

o Cell-free tumor DNA testing can identify alterations that are unrelated to a lesion of interest, for example, clonal hematopoiesis of indeterminate potential (CHIP).

o The use of cell-free/circulating tumor DNA testing can be considered in specific clinical circumstances, most notably:
  ▪ If the patient is medically unfit for invasive tissue sampling
  ▪ In the initial diagnostic setting, if following pathologic confirmation of NSCLC diagnosis there is insufficient material for molecular analysis, cell-free/circulating tumor DNA should be used only if follow-up tissue-based analysis is planned for all patients in which an oncogenic driver is not identified.

### Emerging Biomarkers to Identify Novel Therapies for Patients with Metastatic NSCLC

<table>
<thead>
<tr>
<th>Genetic Alterations (i.e. Driver Event)</th>
<th>Available Targeted Agents with Activity Against Drive Event in Lung Cancer</th>
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<tbody>
<tr>
<td>High-level Met amplification</td>
<td>Crizotinib</td>
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<tr>
<td></td>
<td>Capmatinib</td>
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<tr>
<td>ERBB2 (HER2) mutations</td>
<td>Ado-trastuzumab emtansine</td>
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<td>Fam-trastuzumab deruxtecan-nxki</td>
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### Predictive and Prognostic Biomarkers

Several biomarkers have emerged as predictive and prognostic markers for NSCLC. A predictive biomarker is indicative of therapeutic efficacy, because there is an interaction between the biomarker and therapy on patient outcome. A prognostic biomarker is indicative of patient survival independent of the treatment received because the biomarkers is an indicated the innate tumor behavior (see KRAS Mutations at the end of this section. The NSCLC Panel recommends testing for certain molecular and immune
biomarkers in all appropriate patients with metastatic NSCLC to assess whether patients are eligible for targeted therapies or immunotherapies based on data showing improvement in overall survival for patients receiving targeted therapies or immunotherapies compared with traditional chemotherapy regimens.

Predictive biomarkers include the ALK fusion oncogene (fusion between ALK and other genes (i.e., echinoderm microtubule-associated protein-like 4), ROS1 gene fusions, sensitizing EGFR gene mutations, BRAF V600E point mutations, NTRK gene fusions, METex14 skipping mutations, RET rearrangements, and PD-L1 expression (see Principles of Molecular Biomarker Analysis in the NCCN guidelines for NSCLC). Emerging predictive markers include ERBB2 mutations, high-level amplifications, and tumor mutations burden) (TMB) (see Emerging Biomarkers to Identify Novel Therapies for Patients with Metastatic NSCLC). The presence of EGFR exon 19 deletions or exon 21 L858R mutations is predictive of treatment benefit from EGFR tyrosine kinase inhibitor (EGFR TKI) therapy (i.e., Osimertinib); therefore, these mutations are referred to as sensitizing EGFR mutations. The presence of EGFR exon 19 deletions (LREA0 or exon 21 L858R mutations does not appear to be prognostic of survival for patients with NSCLC, independent of therapy.

ALK fusion oncogenes (e.g., ALK gene fusions) and ROS1 fusions are predictive biomarkers that have been identified in a small subset of patients with NSCLC; both predict for benefit from targeted therapy such as crizotinib or ceritinib. Other gene fusions have recently been identified, such as ERBB2 (HER2) mutations that are susceptible to targeted therapies, particularly therapies currently under investigation in clinical trials.

Testing for ALK gene fusions and EGFR gene mutations is recommended (category 1 for both) in the NSCLC algorithm for patients with metastatic non-squamous NSCLC or NSCLC NOS so that patients with these genetic variants receive effective treatment with targeted agents. Testing for ROS1 fusions and BRAF mutations (both are category 2A) is also recommended in the NCCN Guidelines for non-squamous NSCLC or NSCLC NOS. Although rare, patients with ALK fusions or EGFR mutations can have mixed squamous cell histology. Therefore, testing for ALK fusions and EGFR mutations can be considered in select patients with metastatic squamous cell carcinoma if they are never smokers, small biopsy specimens were used for testing, or mixed histology was reported. Data suggest that EGFR mutations occur in patients with adenosquamous carcinoma at a rate similar to adenocarcinoma, which is harder to discriminate from squamous cell carcinoma in small specimens. Thus, testing for EGFR mutations and ALK fusions in recommended in mixed squamous cell lung specimens that contain an adenocarcinoma component, such as adenosquamous NSCLC or in samples in which an adenocarcinoma component cannot be excluded. The incidence of EGFR mutations is very low in patients with pure squamous cell history (.4%). Testing for ROS1 fusions or BRAF mutations is also recommended (category 2A) in patients with squamous cell carcinoma who have small biopsy specimens of mixed histology.
For patients with metastatic non-squamous NSCLC, the NCCN NSCLC Panel currently recommends the minimum of the following biomarkers should be tested, including EGFR mutations, BRAF mutations, ALK fusions, ROS1 fusions, METex14 skipping mutations, RET rearrangements, and PD-L1 expression levels. This list of recommended biomarkers may be revised as new oncogenic driver variants are identified and new agents are approved. The NCCN Guidelines for NSCLC provide recommendations for individual biomarkers that should be tested and recommend testing techniques but do not endorse any specific commercially available biomarker assay. Biomarker testing should be done at properly accredited laboratories (minimum of Clinical Laboratory Improvement Amendments [CLIA] accreditation). EGFR, KRAS, ROS1, BRAF, METex14 skipping mutations, RET rearrangements, and ALK genetic variants do not usually overlap thus testing for KRAS mutation may identify patients who will not benefit from further molecular testing. The KRAS oncogene is a prognostic biomarker. The presence of KRAS mutations is prognostic of poor survival for patients with NSCLC when compared to the absence of KRAS mutations, independent of therapy. KRAS mutations are also predictive of lack of benefit from EGFR TKI therapy.

Other oncogenic driver variants are being identified such as high-level MET amplification, ERBB2 mutations and TMB. TMB is emerging biomarker that may be helpful for identifying patients with metastatic NSCLC who are eligible for first line therapy with nivolumab with or without ipilimumab. However, there is no consensus on how to measure TMB. Targeted agents are available for patients with NSCLC who have these other genetic variants, although they are FDA approved for other indications. Thus, the NCCN NSCLC Panel recommends molecular testing but strongly advises broad molecular profiling to identify these other rare driver variants for which targeted therapies may be available to ensure that patients receive the most appropriate treatment; patients may be eligible for clinical trials for some of these targeted agents. Several online resources are available that describe NSCLC driver events such as My Cancer Genome.

Information about biomarker testing and plasma cell-free/circulating tumor DNA testing (so-called “liquid biopsy”) for genetic variants is included in the algorithm (See Principles of Molecular and Biomarker Analysis in the NCCN guidelines for NSCLC). Briefly, the pane feels that plasma cell-free/circulating tumor DNA testing should be sued to diagnose NSCLC; tissue should be used to diagnosis NSCLC. Standards and guideline for cell-free DNA (cfDNA)/circulating tumor DNS testing for genetic variants have not been established, there is up to 30% false-negative rate, and variants can be deterred that are not related to the tumor (i.e., clonal hematopoiesis of indeterminate potential [CHIP]). For example, an IDH1 mutation identified by cfDNA testing is likely unrelated to NSCLC, given exceptionally low incidence and is more likely to represent CHIP. Rare examples of CHIP with KRAS mutations have been described, suggesting caution in the interpretation of cfDNA findings. In addition, CHIP can be identified following prior chemotherapy or radiotherapy, further confounding interpretation of variants such as in TP53. Given the previous caveats, careful consideration is required to determine whether cfDNA findings reflect a true oncogenic driver or unrelated finding.
However, cfDNA testing can be used in specific circumstances if 1) the patient is not medically fit for invasive tissue sampling, or 2) there is insufficient tissue for molecular analysis and follow-up tissue-based analysis will be done if an oncogenic driver is not identified. Recent data suggest that plasma cell-free/circulating tumor DNA testing can be used to identify EGFR, ALK and other oncogenic biomarkers that would not otherwise be identified in patients with metastatic NSCLC.

**Testing for Molecular Biomarkers**

Molecular testing is used to test for genomic variants associated with oncogenic driver events for which targeted therapies are available; these genomic variants (also known as molecular biomarkers) include gene mutations and fusions. The various molecular testing methods that may be used to assess for the different biomarkers are described in the algorithm (see Principles of Molecular and Biomarker Analysis in the NCCN Guidelines for NSCLC). Broad molecular profiling systems may be used to simultaneously test for multiple biomarkers.

Next-generation sequencing (NGS) (also known as massively parallel sequencing) is a type of broad molecular profiling system that can detect panels of mutations and gene fusions if the NGS platforms have been designed and validated to detect these genetic variants. It is important to recognize that NGS requires quality control as much as any other diagnostic technique; because it is primer dependent, the pan of genes and abnormalities detected with NGS will vary depending on the design of the NGS platform. For example, some NGS platforms can detect both mutations and gene fusions, as well as copy number variation, but they are not uniformly present in all NGS assays being conducted either commercially or in institutional laboratories.

Other mutation screening assays are available for detecting multiple biomarkers simultaneously such as Sequenom’s MassARRAY system and SNaPshot Multiplex System which can detect more than 50- point mutations; NGS platforms can detect even more biomarkers, however, the multiplex polymerase chain reaction (PCR) system does not typically detect gene fusions. ROS1 and ALK gene fusions can be detected using fluorescence in situ hybridization (FISH), NGS and other methods (see ALK Gene Rearrangements and ROS1 Rearrangements in this Discussion and Principles of Molecular and Biomarker Analysis in the NCCN Guidelines for NSCLC).

To minimize tissue use and potential wastage the NCCN NSCLC Panel recommends that broad molecular profiling be done as part of biomarker testing using a validated test(s) that assess a minimum of following potential genetic variants: EGFR mutations, BRAF mutations, METex14 skipping mutations, RET rearrangements, ALK fusions, and ROS1 fusions. Both FDA and laboratory developed test platforms are available that address the need to evaluate these and other analytes. Broad molecular profiling is also recommended to identify rare driver mutations for which effective therapy may be available, such NTRK gene fusion, high level MET amplification, ERBB2 mutations and TMB. Although clinicopathologic features such as smoking status, ethnicity and histology are
associated with specific genetic variants (i.e., EGFR mutations), these features should not be used to select patients for testing. Although the NCCN guidelines for NSCLC provide recommendations for individual markers that should be tested and recommend testing techniques, the guidelines do not endorse any specific commercially available biomarkers assays.

**EGFR Mutations**

In patients with NSCLC, the most commonly found EGFR mutations are deletions in exon 19 (Exon19del [with conserved deletion of the LREA sequence] in 45% of patients with EGFR mutations) and a point-mutations in exon 21 (L858R in 40%). Both mutations result in activation of the tyrosine kinase domain, and both are associated with sensitivity to the small-molecule EGFR TKIs, such as erlotinib, gefitinib, afatinib, osimertinib, and dacomitinib (See Targeted Therapies in this Discussion). Thus, these drug-sensitive EGFR mutations are referred to as sensitizing EGFR mutations. Other less common mutations (10%) that are also sensitive to EGFR TKIs include exon 19 insertions, p.L861Q, p.G719X, and p.S768I (see Principles of Molecular and Biomarker Analysis in the NCCN Guidelines for NSCLC). Data suggest that patients harboring tumors without sensitizing EGFR mutations should not be treated with EGFR TKIs in any line of therapy. These sensitizing EGFR mutations are found in approximately 10% of Caucasian patients with NSCLC and up to 50% of Asian patients.

Most patients with sensitizing EGFR mutations are nonsmokers or former light smokers with adenocarcinoma histology. Data suggest that EGFR mutations can occur in patients with adenosquamous carcinoma, which is harder to discriminate from squamous cell carcinoma in small specimens. Patients with pure squamous cell carcinoma are unlikely to have sensitizing EGFR mutations; those with adenosquamous carcinoma may have mutations. However, smoking status, ethnicity, and histology should not be used in selecting patients for testing. EGFR mutation testing is not usually recommended in patients who appear to have squamous cell carcinoma unless they are a former light or never smoker, if only a small biopsy specimen (e.g., not a surgical resection) was used to assess histology, or if the histology is mixed. The ESMO Guidelines specify that only patients with nonsquamous cell (i.e., adenocarcinoma) should be assessed for EGFR mutations. ASCO recommends that patients be tested for EGFR mutations.

The predictive effects of the drug-sensitive EGFR mutations are well defined. Patients with these mutations have a significantly better response to erlotinib, gefitinib, afatinib, Osimertinib or dacomitinib. Data show that EGFR TKI therapy should be used as first-line monotherapy in patients advanced NSCLC and sensitizing EGFR mutations documented before first-line systemic therapy (i.e., carboplatin/paclitaxel) (see Targeted Therapies in this Discussion). Progression-free survival (PFS) is longer with use of EGFR TKI monotherapy in patients with sensitizing EGFR mutations when compared with cytotoxic systemic therapy, although overall survival is not statistically different.

Non-responsiveness to EGFR TKI therapy is associated with KRAS and BRAF mutations and ALK or ROS1 gene fusions. Patients with EGFR exon 20 insertion
mutations are usually resistant to erlotinib, gefitinib, afatinib, or dacomitinib, although there are rare exceptions (See Principles of Molecular and Biomarker Analysis in the NCCN Guidelines for NSCLC). Patients typically progress after first-line EGFR TKI monotherapy; subsequent therapy recommendations are described in the algorithm [see Second-Line and Beyond (Subsequent) Systemic Therapy in this Discussion and the NCCN Guidelines for NSCLC]. EGFR p.Thr790Met (T790M) is a mutations associated with acquired resistance to EGFR TKI therapy and has been reported in about 60% of patients with disease progression after initial response to erlotinib, gefitinib or afatinib. Most patients with sensitizing EGFR mutations become resistant to erlotinib, gefitinib or afatinib; PFS is about 9.7 to 13 months. Studies suggest T790M may rarely occur in patients who have previously received erlotinib, gefitinib or afatinib. Genetic counseling is recommended for patients with pretreatment p.T790M, because this suggest the possibility of germline mutations and is associated with predisposition to familial lung cancer. Acquired resistance to EGFR TKIs may also be associated with histologic transformation from NSCLC to SCLC and with epithelial to mesenchymal transition. For the 2020 updated (Version 1), the NCCN NSCLC Panel suggest that a biopsy can be considered at progression to rule out SCLC transformation acquired resistance an also be mediated by other molecular events, such as acquisition of ALK rearrangement, MET or ERBB2 amplification.

The NCCN NSCLC Panel recommends testing for sensitizing EGFR mutations in patients with metastastic nonsquamous NSCLC or NSCLC NOS based on data showing the efficacy of Osimertinib, erlotinib, gefitinib, afatinib or dacomitinib and on FDA approval. DNA mutational analysis is the preferred method to assess for EGFR status; IHC is not recommended for detecting EGFR mutations. Real-time PCR, Sanger sequencing (paired with tumor enrichment), and NGS are the most commonly used methods to assess EGFR mutation status (see Principles of Molecular and Biomarker Analysis in the NCCN Guidelines for NSCLC). Direct sequencing of DNA corresponding to exons 18 to 21 (or just testing for exons 19 and 21) is a reasonable approach; however, more sensitive methods are available. Mutation screening assays using multiplex PCR (e.g. Sequenom’s MassARRAY system, SNaPshot Multiplex system) can simultaneously detect more than 50-point mutations. NGS can also be used to detect EGFR mutations.

Osimertinib is a preferred first-line EGFR TKI option for patients with EGFR positive metastatic NSCLC. For the 2020 update (Version 1), the NCCN Panel preference stratified first-line therapy for patients with EGFR mutation positive metastatic NSCLC. Erlotinib, gefitinib, afatinib or dacomitinib are “other recommended” EGFR TKI options for first-line therapy. Osimertinib is recommended (category 1) as secondline and beyond (subsequent) therapy for patients with EGFR T790M-positive metastatic NSCLC who have progressed on erlotinib, gefitinib, afatinib, or dacomitinib. Sensitizing EGFR mutations and ALK or ROS1 fusions are generally mutually exclusive. Thus, crizotinib, ceritinib, alectinib, brigatinib or lorlatinib are not recommended as subsequent therapy for patients with sensitizing EGFR mutations who relapse on EGFR TKI therapy. The phrase subsequent therapy was recently substituted for the terms second-line or beyond
systemic therapy, because the line of therapy may vary depending on previous treatment with targeted agents.

**BRAF V600E Mutations**

BRAF (v-RAF murine sarcoma viral oncogene homolog B) is a serine-threonine kinase that is part of the MAP/ERK signaling pathway. BRAF V600E is the most common of the BRAF point mutations when considered across all tumor types; it occurs in 1% to 2% of patients with lung adenocarcinoma. Although other BRAF mutations occur in patients with NSCLC at a rate approximately equal to pV600E (unlike many other tumor types), specific targeted therapy is not available for these other mutations. Patients with BRAF V600E mutations are typically current or former smokers in contrast to those with EGFR mutations or ALK fusion who are typically nonsmokers. Mutations in BRAF typically do not overlap with EGFR mutations, METex14 skipping mutations, RET rearrangements, ALK fusions, or ROS1 fusions. Testing for BRAF mutations is recommended (category 2A) in patients with metastatic non-squamous NSCLC and may be considered in patients with squamous cell NSCLC (category 2A) if small biopsy specimens were used to assess histology or mixed histology was reported. Real time PCR, Sanger sequencing and NGS are the most commonly used methods to assess for BRAF mutations (see Principles of Molecular and Biomarker Analysis in the NCCN Guidelines for NSCLC).

The NCCN NSCLC Panel recommends testing for BRAF mutations in patients with metastatic non-squamous NSCLC based on data showing the efficacy of dabrafenib plus trametinib for patients with BRAF V600E mutations and on the FDA approval. For the 2020 update (Version 1), the NCCN Panel preference stratified first-line therapy for patients with BRAF V600E mutation-positive metastatic NSCLC. Dabrafenib plus trametinib is recommended (category 2A; preferred) for patients with BRAF V600E mutations. If combination therapy with dabrafenib/trametinib is not tolerated, single-agent therapy with dabrafenib or vemurafenib are “other recommended” agents. Chemotherapy regimens are also used for initial systemic therapy (i.e., carboplatin/pemetrexed for non-squamous NSCLC) and are “useful in certain circumstances.” Patients with BRAF mutations response (24%) to immune checkpoint inhibitors (ICIs).

**ALK Gene Rearrangements**

About 5% of patients with NSCLC have ALK gene rearrangements (also known as ALK fusions). Patients with ALK fusions are resistant to EGFR TKIs but have similar clinical characteristics to those with EGFR mutations, such as adenocarcinoma histology and being light or never smokers. ALK fusions are not routinely found in patients with squamous cell carcinoma. Patients with ALK gene fusions can have missed squamous cell histology. It can be challenging to accurately determine histology in small biopsy specimens; thus, patients may have mixed squamous cell histology (or squamous components) instead of pure squamous cell.

The NCCN NSCLC Panel recommends testing for ALK fusion in patients with metastatic nonsquamous NSCLC based on data showing the efficacy of alectinib, brigatinib,
certinib, and crizotinib for ALK fusions and on the FDA approvals. If patients appear to have squamous cell NSCLC, then testing can be considered if small biopsy specimens were used to assess histology, mixed histology was reported, or patients are light or never smokers. The different testing methods for ALK fusions are described in the algorithm (see Principles of Molecular and Biomarker analysis in the NCCN guidelines for NSCLC). A molecular diagnosis FISH test has been approved by the FDA for detecting ALK fusions. Rapid prescreening with IHC to assess for ALK fusions can be done. An IHC assay for ALK fusions has also been approved by the FDA. NGS can also be used to assess whether ALK fusions are present, if the platform has been appropriately designed and validated to detect ALK fusions.

Alectinib is recommended as a preferred first-line therapy for patients with ALK rearrangement-positive metastatic NSCLC. For the 2020 update (Version 1), the NCCN Panel preference stratified first-line therapy with brigatinib, ceritinib, or crizotinib for patients with ALK rearrangement-positive metastatic NSCLC. Brigatinib and ceritinib are “other recommended” options, whereas crizotinib is “useful” in certain circumstances.”. Patients with ALK rearrangements do not respond to ICIs.

Patients typically progress after first-line therapy with alectinib, brigatinib, crizotinib, or ceritinib. ALK or ROS1 fusions, RET rearrangements, BRAF mutations, METex14 skipping mutations, and sensitizing EGFR mutations are generally exclusive. Specific targeted therapy for RET rearrangements, BRAF mutations, METex14 skipping mutations, and sensitizing EGFR mutations is not recommended as subsequent therapy in patients with ALK or ROS1 fusions who replace on alectinib, brigatinib, crizotinib, ceritinib, or lorlatinib.

**ROS1 Rearrangements**

Although ROS proto-oncogene 1 (ROS1) is a distinct receptor tyrosine kinase, it is very similar to ALK and members of the insulin receptor family. It is estimated that ROS1 gene rearrangements (also known as ROS1 fusions) occur in about 1% to 2% of patient with NSCLC; they occur more frequently in those who are negative for EGFR mutations, KRAS mutations and ALK gene fusions. The NCCN NSCLC Panel recommends ROS1 testing (category 2A) in patients with metastatic nonsquamous NSCLC or NSCLC NOS based on data showing the efficacy of crizotinib, ceritinib, and entrectinib for patients with ROS1 fusions (see Principles of Molecular and Biomarker Analysis in the NCCN Guidelines for NSCLC). ROS1 testing can be considered in patients with metastatic squamous cell NSCLC if small biopsy specimens were used to assess histology or mixed histology was reported. Similar to testing for ALK fusions, testing for ROS1 fusions is done with FISH. NGS can also be used to assess whether ROS1 fusions are present, if the platform has been appropriately designed and validated to detect ROS1 fusions. Clinicians should use an appropriately validated test to detect ROS1 fusions.

Crizotinib is very effective for patients with ROS1 fusions with response rates of about 70% to 80% including complete responses. The NCCN NSCLC Panel recommends crizotinib, entrectinib or ceritinib (all are category 2A) as first-line therapy options for
patients with ROS1-positive metastatic NSCLC based on the clinical trial data. The NCCN NSCLC Panel voted that crizotinib and entrectinib are preferred first-line therapy options for patients with ROS1-positive metastatic NSCLC because they are better tolerated, have been assessed in more patients, and are approved by the FDA. Although entrectinib has better CNS penetratin than crizotinib, it is more toxic. If ROS1 fusions are discovered during first-line systemic therapy (i.e., carboplatin/paclitaxel), then the planned therapy may be either completed or interrupted followed by crizotinib (preferred), entrectinib (preferred) or certinib.

The NCCN NSCLC Panel recommends lorlatinib (category 2A) as a subsequent therapy option for select patients with ROS1-positive metastatic NSCLC who have progressed after treatment with crizotinib, entrectinib, or certinib. Initial systemic therapy options that are used for adenocarcinoma or squamous cell carcinoma are also an option in this setting (i.e., carboplatin/paclitaxel). Patients with ROS1 rearrangements have a slight response (17%) to ICIs. Alectinib, brigatinib, and ceritinib are not recommended in patients with ROS1 fusions whose disease becomes resistant to crizotinib. Studies are ongoing regarding new agents for patients with ROS1 fusions whose disease become resistant to crizotinib, ceritinib, or entrectinib. The phrase subsequent therapy was recently substituted for the terms second-line or beyond systemic therapy, because the line of therapy may vary depending on previous treatment with targeted agents.

**NTRK 1/2/3 Gene Fusions**

NTRK 1/2/3 gene fusions encode tropomyosin receptor kinase (TRK) fusion proteins (i.e., TRKA, TRKB, TRKC) that act as oncogenic drivers for solid tumors including lung, salivary gland, thyroid, and sarcoma. A diverse range of solid tumors in children and adults may be caused by NTRK gene fusions, (i.e., NTRK1, NTRK2, NTRK3). It is estimated that NTRK gene fusions occur in 0.2% of patients with NSCLC and do not typically overlap with other oncogenic drivers such as EGFR, ALK or ROS1. Various methods can be used to detect NTRK gene fusions, including FISH, IHC, NGS, and PCR assays (see Principles of Molecular and Biomarker Analysis in the NCCN Guidelines for NSCLC). DNA-based NGS may not detect some NTRK1 and NTRK3 fusions; RNA-based NGS may be considered to assess for fusions. In a clinical trial, NTRK gene fusions were detected with NGS (50 patients) and FISH (5 patients). Lacrotrectinib and entrectinib are oral TKIs that inhibit TRK across a diverse range of solid tumors in younger and older patients with NTRK gene-fusion positive disease.

The NCCN NSCLC Panel recommends NTRK gene fusion testing in patients with metastatic NSCLC based on clinical trial data showing the efficacy of lacrotrectinib and entrectinib for patients with NTRK gene fusion-positive disease; however, clinical data are limited in NSCLC to support this recommendation. The NCCN NSCLC Panel recommends lacrotrectinib and entrectinib (both are category 2A) as either first-line or subsequent therapy options for patients with NTRK gene fusion-positive metastatic NSCLC based on data and the FDA approvals. For the 2020 update (Version 1), the NCCN Panel voted that lacrotrectinib and entrectinib are both preferred (category 2A) as first-line therapy for patients with NTRK gene fusion-positive metastatic disease. A new
section was also added to the algorithm (see Principles of Molecular and Biomarker Analysis in the NCCN Guidelines for NSCLC). For example, if NRTK 1/2/3 testing was not included as part of a broad upfront panel, then NTRK 1/2/3 testing can be considered if the patients tumor is negative for the main oncogenic drivers (i.e. pan-negative for EGFR, ALK, ROS1 and BRAF drivers).

**METex14 Skipping Mutations**

C-MET, the hepatocyte growth factor (HGF) receptor, is a tyrosine kinase receptor that is involved in cell survival and proliferation; oncogenic drive genomic alterations in MET include METex14 skipping mutations, MET gene copy number (GCN) gain or amplification, and MET overexpression. MET genomic alterations do not typically overlap with EGFR, ROS1, BRAF and ALK genetic variants. However, METex14 skipping mutations and MET amplification may occur together. METex14 skipping mutations occur in 3% to 4% of patients with adenocarcinomas NSCLC and 1% to 2% of patients with other NSCLC histologies. METex14 skipping mutations are more frequent in older women who are nonsmokers.

Several different types of METex14 skipping mutations may occur, such as mutations, based substitutions, and deletions, which makes it difficult to test for other mutations. NGS and RT-PCR assays can be used to detect METex14 skipping mutations with MET amplification. Patients with METex14 skipping mutations have a modest response (16%) to immunotherapy, even those with high PD-L1 levels.

For the 2020 update (Version 4), the NCCN NSCLC Panel recommends testing for METex14 skipping mutations (category 2A) in eligible patients with metastatic NSCLC based on data showing the efficacy of several agents for patients with METex14 skipping mutations and on the FDA approval for capmatinib.

**RET Rearrangements**

RET is a tyrosine kinase receptor that affects cell proliferation and differentiation. Rearrangements (fusions) may occur in NSCLC between the RET gene and other domains, especially kinesin family 5B (KIF5B) and coiled coil domain containing-6 (CCDC6), which lead to overexpression of the RET protein. RET rearrangements occur in about 1% to 2% of patients with NSCLC and are more frequent in patients with adenocarcinoma histology. In European patients, RET rearrangements occur in both smokers and nonsmokers. RET rearrangements do not typically overlap with EGFR, ROS1, BRAF, METex14 skipping and ALK genetic variants. However, a few studies suggest that RET rearrangements may infrequently overlap with EGFR and KRAS mutations. FISH, RT-PCR, and NGS assays can be used to detect RET rearrangements. Patients with RET rearrangements have minimal response (6%) to immunotherapy.

For the 2020 update (Version 4), the NCCN NSCLC Panel recommends testing for RET rearrangements (category 2A) in eligible patients with metastatic NSCLC based on data showing the efficacy of several agents for patients with RET rearrangements and on the FDA approval for selpercatinib (LOXO-292).
KRAS Mutations
KRAS is a G-protein with GTPase activity that is part of the MAP/ERK; point mutations in KRAS most commonly occur at codon 12. Data suggest that approximately 25% of patients with adenocarcinomas in a North American population have KRAS mutations; KRAS is the most common mutation in this population. KRAS mutation prevalence is associated with cigarette smoking. Patients with KRAS mutations appear to have a shorter survival than patient with wild-type KRAS; therefore, KRAS mutations are prognostic biomarkers. KRAS mutational status is also predictive of lack of therapeutic efficacy with EGFR TKIs; it does not appear to affect chemotherapeutic efficacy. KRAS mutations do not generally overlap with EGFR, ROS1, BRAF and ALK genetic variants. Therefore, KRAS testing may identify patients who may not benefit from further molecular testing. KRAS mutations my infrequently overlap with EGFR mutations and RET rearrangements. Targeted therapy is not currently available for patients with KRAS mutations, although immune checkpoint inhibitors (ICIs) appear to be effective.

Testing for Immune Biomarkers: PD-L1 Expression Levels
Human ICI antibodies inhibit the PD-1 receptor or PD-L1, which improves antitumor immunity; PD-1 receptors are expressed on activated cytotoxic T-cells. Nivolumab and pembrolizumab inhibit PD-1 receptors. Atezolizumab and durvalumab inhibit PD-L1. The NCCN NSCLC Panel recommends (category 1) IHC testing for PD-L1 expression ideally before first-line treatment (if clinically feasible) in all patients with metastatic NSCLC to assess whether the ICI regimens are an option based on clinical data showing the efficacy of these regimens.

The FDA approve companion diagnostic test for PD-L1 expression is based on tumor proportion score (TPS) and used to determine usage of pembrolizumab in patients with metastatic NSCLC. TPS is the percentage of viable tumor cells showing partial or complete membrane staining at any intensity. Testing for PD-L1 is not required for prescribing first-line therapy with atezolizumab plus chemotherapy regimens or for subsequent therapy with single agent nivolumab or atezolizumab.

Although it is not an optimal biomarkers, PD-L1 expression is currently the best available biomarker to assess whether patients are candidates for PD-1 or PD-L1 inhibitors (ICIs; also known as immune-oncology [IO] agents, immunotherapy). PD-L1 expression is continuously variable and dynamic; thus, a cutoff value for a positive result is artificial. Patients with PD-L1 expression levels just below and just above 50% will probably have similar responses. Unique anti-PD-L1 IHC assays have been developed for each one of the different ICIs. The definition of a positive PD-L1 test result varies depending on which biomarker assay is used. Extensive effort has been undertaken to examine the cross-comparability or different clones with regard to each other to facilitate adoption of testing.

The NCCN NSCLC Panel emphasizes that clinicians should obtain molecular testing results for actionable biomarkers before administering first-line ICI therapy, if clinically
feasible. Therefore, the 2020 update (Version 1), the panel deleted “or unknown” regarding test results for certain actionable molecular biomarkers before administering PD-1 or PD-L1 inhibitors. Patients with metastatic NSCLC and PD-L1 expression levels of 1% or more but who also have a targetable driver oncogene molecular variant (e.g. EGFR, ALK, ROS1) should receive first-line targeted therapy for that oncogene and not first-line ICIs because targeted therapies yield higher response rates (e.g. Osimertinib, 80%) than ICIs (poor response rates) in the first-line setting, targeted therapy is better tolerated, and these patients are unlikely to respond to ICIs. For the 2020 update (version 1), the NCCN NSCLC Panel also deleted “or known” regarding test results for PD-L1 expression levels; the panel also added “ROS1 fusions” and “BRAF mutations” to the list of actionable biomarkers that need to be negative before administering PD-1 or PD-L1 inhibitors. At a minimum, EGFR and ALK status should be known before starting systemic therapy with ICI regimens; however, it is ideal if ROS1 and BRAF status are also known. If it is not feasible to do molecular testing, then patients are treated as though they do not have driver oncogenes.

**Targeted Therapies**

Specific targeted therapies are available for the treatment of eligible patients with metastatic NSCLC. Afatinib, alectinib, brigatinib, ceritinib, crizotinib, erlotinib, gefitinib, lacosertinib, and lorlatinib are oral TKIs. Bevacizumab and ramucirumab are recombinant monoclonal antibodies that target the vascular endothelial growth factor (VEGF) or VEGF receptor, respectively. Cetuximab is a monoclonal antibody that targets EGFR. Erlotinib, gefitinib, afatinib and dacomitinib inhibit EGFR sensitizing mutations; Osimertinib inhibits both EGFR sensitizing mutations and T790M. Crizotinib inhibits ALK fusions, ROS1 fusions and MET tyrosine kinase (e.g., high-level MET amplification, METex14 skipping mutation). Ceritinib inhibits ALK fusions and IGF-1 receptor. Alectinib inhibits ALK and RET fusions. Brigatinib inhibits various ALK fusions and other targets. Lorlatinib inhibits ALK and ROS fusions. Debrafenib inhibits BRAF V600E mutations; trametinib inhibits MEK; both agents inhibit different kinases in the RAS/RAF/MEK/ERK pathway. Entrectinib and lacosertinib inhibit TRK fusion proteins. Capmatinib inhibits several MET tyrosine kinases including METex14 skipping mutations. Selrecatinib, cabozantinib and candetanib inhibit RET rearrangements. Other targeted therapies are being developed (see Emerging Biomarkers to Identify in Novel Therapies for Patients with Metastatic NSCLC Guidelines for NSCLC). Flare phenomenon may occur in some patients who discontinue targeted therapies for EGFR, ALK, or ROS1 genetic variants. If disease flare occurs, then the targeted therapies should be restarted.

It is important to note that targeted therapies are recommended for patients with metastatic NSCLC and specific oncogenic drivers independent of PD-L1 levels. Patients with metastatic NSCLC and PD-L1 expression levels of 1% or more but who also have targetable driver oncogene molecular variant (e.g. EGFR, ALK, ROS1) should receive first-line targeted therapy for that oncogene and not first-line ICIs, because targeted therapies yield higher response rates (e.g. Osimertinib 80%) than ICIs (poor response rates) in the first-line setting, targeted therapy is better tolerated, and these patients are
unlikely to respond to ICIs. For the 2020 update (version 1), the NCCN NSCLC Panel emphasizes that clinicians should obtain molecular testing results for actionable biomarkers before administering first-line therapy, if clinically feasible. Therefore, the panel deleted “or unknown” regarding test results for actionable molecular biomarkers before administering PD-1 or PD-L1 inhibitors. At a minimum, EGFR and ALK status should be known before starting first-line systemic therapy, if clinically feasible; however, it is ideal if ROS1 and BRAF status are also known. It is not feasible to do molecular testing, then patients are treated as though they do not have driver oncogenes.

Targeted Therapy or Immunotherapy for Advanced Metastatic Disease

EGFR Mutation Positive (i.e., exon 19 deletion or L858R)

First line therapy
  o Afatinib
  o Erlotinib
  o Dacomitinib
  o Gefitinib
  o Osimertinib
  o Erlotinib + ramucirumab
  o Erlotinib + bevacizumab (nonsquamous)

Subsequent therapy
  o Osimertinib

EGFR exon 20 Insertion Mutation Positive

Subsequent therapy
  ▪ Amivantamab-vmjw

KRAS G12C Mutation Positive

Subsequent therapy
  ▪ Sotorasib

ALK Rearrangement Positive

First-line therapy
  o Alectinib
  o Brigatinib
  o Ceritinib
  o Crizotinib
  o Lorlatinib

Subsequent therapy
  o Alectinib
  o Brigatinib
  o Ceritinib
  o Lorlatinib

ROS1 Rearrangement Positive

First-line therapy
  o Certinib
o Crizotinib
o Entrectinib

• Subsequent therapy
  ▪ Lorlatinib
  ▪ Entrectinib

**BRAF V600E Mutation Positive**

First-line therapy
  o Dabrafenib/trametinib

Subsequent therapy
  o Dabrafenib/trametinib

**NTRK1/2/3 Gene Fusion Positive**

First-line/Subsequent therapy
  o Lacrotrectinib
  o Entrectinib

**MET Exon14 Skipping Mutation**

• First-line therapy/Subsequent therapy
  ▪ Capmatinib
  ▪ Crizotinib
  ▪ Tepotinib

**RET Rearrangement Positive**

First-line therapy/Subsequent therapy
  o Selpercatinib
  o Pralsetinib
  o Cabozantinib
  o Vandetanib

**PD-L1 ≥ 1%**

First-line therapy**
  o Pembrolizumab
  o Carboplatin or cisplatin/pemetrexed/pembrolizumab (nonsquamous)
  o Carboplatin/paclitaxel/bevacizumab/atezolizumab* (nonsquamous)
  o Carboplatin/(paclitaxel or albumin-bound paclitaxel)/pembrolizumab (squamous)
  o Carboplatin/albumin-bound paclitaxel/atezolizumab (nonsquamous)
  o Nivolumab/ipilimumab
  o Nivolumab + ipilimumab + pemetrexed + (carboplatin or cisplatin) (nonsquamous)
  o Nivolumab + ipilimumab + paclitaxel + carboplatin (squamous)

**PD-L1 ≥ 50% (in addition to above)**

• First-line therapy**
  ▪ Atezolizumab
  ▪ Cemiplimab-rwic
*An FDA approved biosimilar is an appropriate substitute for bevacizumab

**Continuation maintenance refers to the use of at least one of the agents given in first line, beyond 4-6 cycles, in the absence of disease progression.

**American Society of Clinical Oncology (ASCO)**

In 2017, the American Society of Clinical Oncology (ASCO) issued a guideline on systemic therapy for stage IV non-small cell lung cancer which included the following recommendations:

New or revised recommendations include the following. Regarding first-line treatment for patients with non–squamous cell carcinoma or squamous cell carcinoma (without positive markers, i.e., EGFR/ALK/ROS1), if the patient has high programmed death ligand 1 (PD-L1) expression, pembrolizumab should be used alone; if the patient has low PD-L1 expression, clinicians should offer standard chemotherapy. All other clinical scenarios follow 2015 recommendations. Regarding second-line treatment in patients who received first-line chemotherapy, without prior immune checkpoint therapy, if NSCLC tumor is positive for PD-L1 expression, clinicians should use single agent nivolumab, pembrolizumab, or atezolizumab; if tumor has negative or unknown PD-L1 expression, clinicians should use nivolumab or atezolizumab. All immune checkpoint therapy is recommended alone plus in the absence of contraindications. For patients who received a prior first line immune checkpoint inhibitor, clinicians should offer standard chemotherapy. For patients who cannot receive immune checkpoint inhibitor after chemotherapy, docetaxel is recommended; in patients with non-squamous NSCLC, pemetrexed is recommended. In patients with a sensitizing EGFR mutation, disease progression after first-line epidermal growth factor receptor tyrosine kinase inhibitor therapy, and T790M mutation, osimertinib is recommended; if NSCLC lacks the T790M mutation, then chemotherapy is recommended. Patients with ROS1 gene rearrangement without prior crizotinib may be offered crizotinib, or if they previously received crizotinib, they may be offered chemotherapy.

**Recommendations**

**First-Line Treatment for Patients**

- Patients with non–squamous cell carcinoma without a tumor EGFR-sensitizing mutation or ALK or ROS1 gene rearrangement and with a performance status (PS) of 0 or 1 (and appropriate PS of 2):
  - With high PD-L1 expression (tumor proportion score [TPS] 50%) and no contraindications, single-agent pembrolizumab is recommended (Evidence quality: high; Strength of recommendation: strong).
  - With low PD-L1 expression (TPS, 50%), a variety of combination cytotoxic chemotherapies (with or without bevacizumab if patients are receiving carboplatin and paclitaxel) are recommended (Platinum based [Evidence quality: high; Strength of recommendation: strong]; Non–platinum based [Evidence quality: intermediate; Strength of recommendation: weak]).
• There is insufficient evidence to recommend bevacizumab in combination with pemetrexed plus carboplatin.
• Other checkpoint inhibitors, combination checkpoint inhibitors, or immune checkpoint therapy with chemotherapy are not recommended.
• With PS of 2, combination or single-agent therapy or palliative care alone may be used (chemotherapy [Evidence quality: intermediate; Strength of recommendation: weak]; palliative care [Evidence quality: intermediate; Strength of recommendation: strong]).

Patients with squamous cell carcinoma without a tumor EGFR-sensitizing mutation or ALK or ROS1 gene rearrangement and with a PS of 0 or 1 (and appropriate PS of 2):
• With high PD-L1 expression (TPS 50%) and no contraindications, single-agent pembrolizumab is recommended (Evidence quality: high; Strength of recommendation: strong).
• With low PD-L1 expression (TPS, 50%), a variety of combination cytotoxic chemotherapies are recommended (Platinum based [Evidence quality: high; Strength of recommendation: strong]; Non-platinum based [Evidence quality: low; Strength of recommendation: weak]).
• Other checkpoint inhibitors, combination checkpoint inhibitors, or immune checkpoint therapy with chemotherapy are not recommended.

With PS of 2, combination or single-agent therapy or palliative care alone may be used (chemotherapy [Evidence quality: intermediate; Strength of recommendation: weak]; palliative care [Evidence quality: intermediate; Strength of recommendation: strong]).
• With squamous NSCLC treated with cisplatin and gemcitabine, the Panel neither recommends for nor recommends against the addition of necitumumab to chemotherapy.
• With sensitizing EGFR mutations, afatinib, erlotinib, or gefitinib is recommended (Evidence quality: high; Strength of recommendation: strong for each).
• With ALK gene rearrangements, crizotinib is recommended (Evidence quality: strong; Strength of recommendation: high).
• With ROS1 rearrangement, crizotinib is recommended (Type: informal consensus; Evidence quality: low; Strength of recommendation: weak).

Second-Line Treatment for Patients
Without a tumor EGFR-sensitizing mutation or ALK or ROS1 gene rearrangement and with PS of 0 or 1 (and appropriate PS of 2):
• In patients with high PD-L1 expression (TPS, 1%) and no contraindications who received first-line chemotherapy and have not received prior immune therapy, single-agent nivolumab, pembrolizumab, or atezolizumab is recommended (Evidence quality: high; Strength of recommendation: strong).
• In patients with negative or unknown tumor PD-L1 expression (TPS, 1%) and no contraindications who received first-line chemotherapy, nivolumab, or atezolizumab, a variety of combination cytotoxic chemotherapies are recommended (Evidence quality: high; Strength of recommendation: strong).
• Other checkpoint inhibitors, combination checkpoint inhibitors, and immune checkpoint therapy with chemotherapy are not recommended.
• In patients who received an immune checkpoint inhibitor as first-line therapy, a variety of combination cytotoxic chemotherapies are recommended (Platinum based [Evidence quality: high; Strength of recommendation: strong]. Non–platinum based [Informal consensus; Evidence quality: low; Strength of recommendation: strong]).

• In patients with contraindications to immune checkpoint inhibitor therapy after first-line chemotherapy, docetaxel is recommended (Evidence quality: intermediate; Strength of recommendation: moderate).

• In patients with non–squamous cell carcinoma who have not previously received pemetrexed, pemetrexed is recommended (Evidence quality: intermediate; Strength of recommendation: moderate).

**With sensitizing EGFR mutations:**

• In patients with disease progression after first-line therapy with an EGFR tyrosine kinase inhibitor (TKI) and the presence of the T790M resistance mutation, osimertinib is recommended (Evidence quality: high; Strength of recommendation: strong).

• If T790M mutation is not present, a platinum doublet is recommended (Type: informal consensus; Evidence quality: low; Strength of recommendation: strong).

• In patients who received an EGFR-TKI in the first-line setting, had an initial response, and subsequently experienced slow or minimal disease progression at isolated sites, EGFR-TKI with local therapy to the isolated sites is an option (Type: informal consensus; Evidence quality: insufficient; Strength of recommendation: weak).

**With ROS1 rearrangement:**

• In patients who have not received prior crizotinib, crizotinib is recommended (Type: informal consensus; Evidence quality: low; Strength of recommendation: moderate).

• In patients who have received prior crizotinib, platinum-based therapy in the second line with or without bevacizumab is recommended (Type: informal consensus; Evidence quality: insufficient; Strength of recommendation: moderate).

**With BRAF mutations:**

• In patients without prior immune checkpoint therapy and high PD-L1 expression (TPS, 1%), atezolizumab, nivolumab, or pembrolizumab is recommended (Type: informal consensus; Evidence quality: insufficient; Strength of recommendation: weak).

• In patients who have received prior immune checkpoint therapy, dabrafenib alone or in combination with trametinib in third line is an option (Type: informal consensus; Evidence quality: insufficient; Strength of recommendation: moderate).

**Third-Line Treatment for Patients**

• In patients without a tumor EGFR-sensitizing mutation or ALK or ROS1 gene rearrangement and with non–squamous cell carcinoma and PS of 0 or 1 (and appropriate PS of 2), who received chemotherapy with or without bevacizumab...
and immune checkpoint therapy, single-agent pemetrexed or docetaxel are options (Type: informal consensus; Evidence quality: low; Strength of recommendation: strong).

- In patients with tumor EGFR-sensitizing mutation(s) who have received at least one first-line EGFR-TKI and prior platinum-based chemotherapy, there are insufficient data to recommend immunotherapy in preference to chemotherapy (pemetrexed or docetaxel [Type: informal consensus; Evidence quality: insufficient; Strength of recommendation: weak]).

**Fourth-Line Treatment for Patients**

- Patients and clinicians should consider and discuss experimental treatment, clinical trials, and continued best supportive (palliative) care.

In 2018, the American Society of Clinical Oncology (ASCO) Expert Panel determined that the recommendations from the College of American Pathologists (CAP)/the International Association for the Study of Lung Cancer (IASLC)/the Association for Molecular Pathology (AMP) molecular testing guideline are clear, thorough, and based upon the most relevant scientific evidence. ASCO endorsed the guideline with minor modifications.

**Target Population**

Patients with advanced lung cancer (i.e., stage IV or other incurable lung cancer).

**Target Audience**

Medical or surgical oncologists, pathologists, thoracic surgeons, and specialists in pulmonary medicine or interventional radiology.

**Key Recommendations**

**2013 Recommendations that were reaffirmed or updated for 2018:**

1. Expert Consensus Opinion: Pathologists may use either cell blocks or smear preparations as suitable specimens for lung cancer biomarker molecular testing.

2. Expert Consensus Opinion: Laboratories should use, or have available at an external reference laboratory, clinical lung cancer biomarker molecular testing assays that are able to detect molecular alterations in specimens with as little as 20% cancer cells.

3. Strong Recommendation: Laboratories should not use epidermal growth factor receptor (EGFR) expression by immunohistochemistry (IHC) testing to select patients for EGFR-targeted TKI therapy.

4. Recommendation: Physicians should use molecular testing for the appropriate genetic targets on either primary or metastatic lung lesions to guide initial therapy selection.

5. Recommendation: Pathologists and laboratories should not use EGFR copy number analysis (i.e., fluorescent in situ hybridization or chemiluminescent in situ hybridization) to select patients for EGFR-targeted TKI therapy.
New 2018 Recommendations:

**Key Question 1: Which genes should be tested for patients with lung cancer?**

1. **Recommendation:** ROS1 testing should be performed on all patients with advanced lung adenocarcinoma, irrespective of clinical characteristics.
2. **Expert Consensus Opinion:** ROS1 IHC may be used as a screening test in patients with advanced lung adenocarcinoma; however, positive ROS1 IHC results should be confirmed by a molecular or cytogenetic method.
3. **Expert Consensus Opinion:** BRAF testing should be performed on all patients with advanced lung adenocarcinoma, irrespective of clinical characteristics.
4. **Expert Consensus Opinion:** RET molecular testing is not recommended as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include RET as part of larger testing panels performed either initially or when routine EGFR, ALK, BRAF, and ROS1 testing is negative.
5. **Expert Consensus Opinion:** ERBB2 (HER2) molecular testing is not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include ERBB2 (HER2) mutation analysis as part of a larger testing panel performed either initially or when routine EGFR, ALK, BRAF, and ROS1 testing is negative.
6. **Expert Consensus Opinion:** KRAS molecular testing is not indicated as a routine stand-alone assay as a sole determinant of targeted therapy. It is appropriate to include KRAS as part of larger testing panels performed either initially or when routine EGFR, ALK, BRAF, and ROS1 testing is negative.
7. **Expert Consensus Opinion:** MET molecular testing is not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include MET as part of larger testing panels performed either initially or when routine EGFR, ALK, BRAF, and ROS1 testing is negative.

**Key Question 2: What methods should be used to perform molecular testing?**

8. **Recommendation:** IHC is an equivalent alternative to FISH for ALK testing.

**CAP/IASLC/AMP Qualifying Statement:** *ALK IHC is an acceptable standard alternative to FISH, and treatment decisions can be made when IHC results are clearly positive, as manifested by strong granular cytoplasmic staining, with or without membrane accentuation, or negative; however, weak staining can be challenging to interpret, and the specificity of weak staining relative to FISH should be determined in each laboratory during validation.*

9. **Expert Consensus Opinion:** Multiplexed genetic sequencing panels are preferred where available over multiple single gene tests to identify other treatment options beyond EGFR, ALK, BRAF, and ROS1.
10. **Expert Consensus Opinion:** Laboratories should ensure that test results that are unexpected, discordant, equivocal, or otherwise of low confidence are confirmed or resolved by using an alternative method or sample.

**Key Question 3: Is molecular testing appropriate for lung cancers that do not have an adenocarcinoma component?**
11. Expert Consensus Opinion: Physicians may use molecular biomarker testing in tumors with:
   a. an adenocarcinoma component
   b. non-squamous, non–small-cell histology
   c. any non–small-cell histology when clinical features indicate a higher probability of an oncogenic driver (e.g., young age [, 50 years]; light or absent tobacco exposure).

**Key Question 4: What testing is indicated for patients with targetable mutations who have relapsed on targeted therapy?**

12. Strong Recommendation: In patients with lung adenocarcinoma who harbor sensitizing EGFR mutations and have progressed after treatment with an EGFR-targeted TKI, physicians must use EGFR T790M mutational testing when selecting patients for third-generation EGFR-targeted therapy.

13. Recommendation: Laboratories testing for EGFR T790M mutation in patients with secondary clinical resistance to EGFR-targeted kinase inhibitors should deploy assays capable of detecting EGFR T790M mutations in as little as 5% of viable cells.

14. No Recommendation: There is currently insufficient evidence to support a recommendation for or against routine testing for ALK mutational status for patients with lung adenocarcinoma with sensitizing ALK mutations who have progressed after treatment with an ALK-targeted TKI.

**Key Question 5: What is the role of testing for circulating cell-free DNA (cfDNA) for patients with lung cancer?**

15. No Recommendation: There is currently insufficient evidence to support the use of cfDNA molecular methods for the diagnosis of primary lung adenocarcinoma.

16. Recommendation: In some clinical settings in which tissue is limited and/or insufficient for molecular testing, physicians may use a cfDNA assay to identify EGFR mutations.

17. Expert Consensus Opinion: Physicians may use cfDNA methods to identify EGFR T790M mutations in patients with lung adenocarcinoma who have progression or secondary clinical resistance to EGFR-targeted TKIs; testing of the tumor sample is recommended if the plasma result is negative.

18. No Recommendation: There is currently insufficient evidence to support the use of circulating tumor cell molecular analysis for the diagnosis of primary lung adenocarcinoma, the identification of EGFR or other mutations, or the identification of EGFR T790M mutations at the time of EGFR TKI resistance.

**College of American Pathologists (CAP), International Association for the Study of Lung Cancer (IASLC) and Association for Molecular Pathology (AMP)**

In 2018, the College of American Pathologists (CAP), the International Association for the Study of Lung Cancer (IASLC) and the Association for Molecular Pathology (AMP) updated their molecular testing guidelines for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors.
Key Question 1: Which new genes should be tested for lung cancer patients?

<table>
<thead>
<tr>
<th>Guideline Statement</th>
<th>Strength of Recommendation</th>
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<tbody>
<tr>
<td>1. <em>ROSI</em> testing must be performed on all lung adenocarcinoma patients, irrespective of clinical characteristics.</td>
<td>Strong Recommendation</td>
</tr>
<tr>
<td>2. <em>ROS1</em> IHC may be used as a screening test in lung adenocarcinoma patients; however, positive <em>ROS1</em> IHC results should be confirmed by a molecular or cytogenetic method.</td>
<td>Expert Consensus Opinion</td>
</tr>
<tr>
<td>3. <em>BRAF</em> molecular testing is currently not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include <em>BRAF</em> as part of larger testing panels performed either initially or when routine <em>EGFR, ALK,</em> and <em>ROSI</em> testing are negative.</td>
<td>Expert Consensus Opinion</td>
</tr>
<tr>
<td>4. <em>RET</em> molecular testing is not recommended as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include <em>RET</em> as part of larger testing panels performed either initially or when routine <em>EGFR, ALK,</em> and <em>ROSI</em> testing are negative.</td>
<td>Expert Consensus Opinion</td>
</tr>
<tr>
<td>5. <em>ERBB2 (HER2)</em> molecular testing is not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include <em>ERBB2 (HER2)</em> mutation analysis as part of a larger testing panel performed either initially or when routine <em>EGFR, ALK,</em> and <em>ROSI</em> testing are negative.</td>
<td>Expert Consensus Opinion</td>
</tr>
<tr>
<td>6. <em>KRAS</em> molecular testing is not indicated as a routine stand-alone assay as a sole determinant of targeted therapy. It is appropriate to include <em>KRAS</em> as part of larger testing panels performed either initially or</td>
<td>Expert Consensus Opinion</td>
</tr>
</tbody>
</table>
when routine \( \text{EGFR}, \text{ALK}, \) and \( \text{ROS1} \) testing are negative.

7. \( \text{MET} \) molecular testing is not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include \( \text{MET} \) as part of larger testing panels performed either initially or when routine \( \text{EGFR}, \text{ALK}, \) and \( \text{ROS1} \) testing are negative.

**Key Question 2: What methods should be used to perform molecular testing?**

<table>
<thead>
<tr>
<th>Guideline Statement</th>
<th>Strength of Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>8. IHC is an equivalent alternative to FISH for ALK testing.</td>
<td>Recommendation</td>
</tr>
<tr>
<td>9. Multiplexed genetic sequencing panels are preferred over multiple single-gene tests to identify other treatment options beyond ( \text{EGFR}, \text{ALK}, ) and ( \text{ROS1} ).</td>
<td>Expert Consensus Opinion</td>
</tr>
<tr>
<td>10. Laboratories should ensure test results that are unexpected, discordant, equivocal, or otherwise of low confidence are confirmed or resolved using an alternative method or sample.</td>
<td>Expert Consensus Opinion</td>
</tr>
</tbody>
</table>

**Key Question 3: Is molecular testing appropriate for lung cancers that do not have an adenocarcinoma component?**

<table>
<thead>
<tr>
<th>Guideline Statement</th>
<th>Strength of Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>11. Physicians may use molecular biomarker testing in tumors with histologies other than adenocarcinoma when clinical features indicate a higher probability of an oncogenic driver.</td>
<td>Expert Consensus Opinion</td>
</tr>
</tbody>
</table>

**Key Question 4: What testing is indicated for patients with targetable mutations who have relapsed on targeted therapy?**

<table>
<thead>
<tr>
<th>Guideline Statement</th>
<th>Strength of Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 In lung adenocarcinoma patients who harbor sensitizing ( \text{EGFR} ) mutations and have progressed after treatment with an EGFR-</td>
<td>Strong Recommendation</td>
</tr>
</tbody>
</table>
targeted TKI, physicians must use \textit{EGFR} T790M mutational testing when selecting patients for third-generation EGFR-targeted therapy.

13. Laboratories testing for \textit{EGFR} T790M mutation in patients with secondary clinical resistance to EGFR-targeted kinase inhibitors should deploy assays capable of detecting \textit{EGFR} T790M mutations in as little as 5\% of viable cells.

14. There is currently insufficient evidence to support a recommendation for or against routine testing for \textit{ALK} mutational status for lung adenocarcinoma patients with sensitizing \textit{ALK} mutations who have progressed after treatment with an ALK-targeted TKI.

**Key Question 5: What is the role of testing for circulating cell-free DNA for lung cancer patients?**

15. There is currently insufficient evidence to support the use of circulating cfDNA molecular methods for the diagnosis of primary lung adenocarcinoma.

16. In some clinical settings in which tissue is limited and/or insufficient for molecular testing, physicians may use a cfDNA assay to identify \textit{EGFR} mutations.

17. Physicians may use cfDNA methods to identify \textit{EGFR} T790M mutations in lung adenocarcinoma patients with progression or secondary clinical resistance to EGFR-targeted TKI; testing of the tumor sample is recommended if the plasma result is negative.

18. There is currently insufficient evidence to support the use of circulating tumor cell molecular analysis for the diagnosis of primary lung adenocarcinoma, the identification of EGFR or other mutations, or the identification of \textit{EGFR} T790M mutations at the time of EGFR TKI resistance.

Abbreviations: ROS1, ROS Proto-Oncogene 1, Receptor Tyrosine Kinase; IHC, Immunohistochemistry; BRAF, B-Raf Proto-Oncogene, Serine/Threonine Kinase; EGFR,
Epidermal Growth Factor Receptor; ALK, RET, Ret Proto-Oncogene; ERBB2, Erb-B2 Receptor Tyrosine Kinase 2; HER2, human epidermal growth factor receptor 2; KRAS, MET, MET Proto-Oncogene, Receptor Tyrosine Kinase; FISH, fluorescence in situ hybridization; TKI, tyrosine kinase inhibitors; cfDNA, cell-free plasma DNA

**Regulatory Status**

**FDA Approved Targeted Treatment for NSCLC and Companion Diagnostic Tests**

A companion diagnostic device can be in vitro diagnostic device (IVD) or an imaging tool that provides information that is essential for the safe and effective use of a corresponding therapeutic product.

The use of an IVD companion diagnostic device with a specific therapeutic product is stipulated in the instructions for use in the labeling of both the diagnostic device and the corresponding therapeutic product, as well as in the labeling of any generic equivalents and biosimilar equivalents of the therapeutic product.

This table lists the clinically validated FDA approved companion diagnostic tests and its indication(s) for biomarker/genetic mutation/gene fusion testing related to targeted therapy in the treatment metastatic NSCLC.

This information is also available at the following: [https://www.fda.gov/media/119249/download](https://www.fda.gov/media/119249/download)

<table>
<thead>
<tr>
<th>Companion Diagnostic Test</th>
<th>Indications/Targeted Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>therascreen EGFR RGQ PCR Kit</td>
<td>Non-small cell lung cancer</td>
</tr>
<tr>
<td></td>
<td>• Iressa (gefitinib) - NDA 206995</td>
</tr>
<tr>
<td></td>
<td>• Gilotrif (afatinib) - NDA 201292</td>
</tr>
<tr>
<td></td>
<td>• Vizimpro (dacomitinib) - NDA 211288</td>
</tr>
<tr>
<td>cobas EGFR Mutation Test</td>
<td>Non-small cell lung cancer</td>
</tr>
<tr>
<td></td>
<td>• Tarceva (erlotinib) - NDA 021743</td>
</tr>
<tr>
<td></td>
<td>• Tagrisso (osimertinib) - NDA 208065</td>
</tr>
<tr>
<td></td>
<td>• Iressa (gefitinib) - NDA 206995</td>
</tr>
<tr>
<td>PD-L1 IHC 22C3 pharmDx</td>
<td>Non-small cell lung cancer (NSCLC)</td>
</tr>
<tr>
<td></td>
<td>• Keytruda (pembrolizumab) - BLA 125514</td>
</tr>
<tr>
<td>Test</td>
<td>Non-small cell lung cancer (NSCLC)</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>----------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>FoundationOne CDx</td>
<td>• Gilotrif (afatinib) - NDA 201292</td>
</tr>
<tr>
<td></td>
<td>• Iressa (gefitinib) - NDA 206995</td>
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<tr>
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</tr>
<tr>
<td></td>
<td>• Alecensa (alectinib) - NDA 208434</td>
</tr>
<tr>
<td></td>
<td>• Xalkori (crizotinib) - NDA 202570</td>
</tr>
<tr>
<td></td>
<td>• Zykadia (ceritinib) - NDA 205755</td>
</tr>
<tr>
<td></td>
<td>• Tafinlar (dabrafenib) - NDA 202806 in combination with Mekinist (trametinib) - NDA 204114</td>
</tr>
<tr>
<td></td>
<td>• Tabrecta (capmatinib) - NDA 213591</td>
</tr>
<tr>
<td>VENTANA ALK (D5F3) CDx Assay</td>
<td>Non-small cell lung cancer</td>
</tr>
<tr>
<td></td>
<td>• Zykadia (ceritinib) - NDA 205755</td>
</tr>
<tr>
<td></td>
<td>• Xalkori (crizotinib) - NDA 202570</td>
</tr>
<tr>
<td></td>
<td>• Alecensa (alectinib) - NDA 208434</td>
</tr>
<tr>
<td>Oncomine Dx Target Test</td>
<td>Non-small cell lung cancer</td>
</tr>
<tr>
<td>(ROS1 fusion, BRAFV600E and</td>
<td>• Tafinlar (dabrafenib) in combination with Mekinist (trametinib) – NDA 202806 and NDA 204114</td>
</tr>
<tr>
<td>EGFR mutations)</td>
<td>• Iressa (gefitinib) - NDA 206995</td>
</tr>
<tr>
<td></td>
<td>• Xalkori (crizotinib) - NDA 202570</td>
</tr>
<tr>
<td></td>
<td>• Gavreto (pralsetinib) - NDA 213721</td>
</tr>
<tr>
<td>Vysis ALK Break Apart FISH</td>
<td>Non-small cell lung cancer</td>
</tr>
<tr>
<td>Probe Kit</td>
<td>• Xalkori (crizotinib) – NDA 202570</td>
</tr>
<tr>
<td></td>
<td>• Alunbrig (brigatinib) - NDA 208772</td>
</tr>
</tbody>
</table>
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PD-L1 IHC 28-8 pharmDx Non-small cell lung cancer
- Opdivo (nivolumab) (BLA 125554) in combination with YERVOY (ipilimumab) (BLA 125377)

PRIOR APPROVAL

Not Applicable

POLICY

See Related Medical Policies
- 02.04.16 Circulating Tumor DNA and Circulating Tumor Cells for Cancer Management (Liquid Biopsies)
- 02.04.20 KRAS/NRAS and BRAF Mutation Analysis
- 02.04.55 Epidermal Growth Factor Receptor (EGFR) Testing
- 02.04.63 Expanded Genetic Panels to Identify Targeted Cancer Therapy
- 02.04.77 Proteomic Testing for Systematic Therapy in Non-Small Cell Lung Cancer
- 02.04.79 Circulating Tumor DNA for Management of Non-Small Cell Lung Cancer (Liquid Biopsy)

Single Gene Testing for Non-Small Cell Lung Cancer

EGFR Testing
Testing for EGFR (epidermal growth factor receptor) mutations using tumor tissue specimen (formalin-fixed paraffin-embedded tissue) may be considered medically necessary to predict treatment response to an EGFR tyrosine kinase inhibitor therapy (e.g., erlotinib [Tarceva], gefitinib [Iressa], afatinib [Gilotrif], dacomitinib [Vizimpro] or osimertinib [Tagrisso]) in patients with metastatic non-small cell lung cancer (NSCLC) who meet the following:
- Individual diagnosed with metastatic non-squamous NSCLC or NSCLC not otherwise specified (NOS); OR
- Individual diagnosed with metastatic squamous cell carcinoma who:
  - Have a mixed histology reported; or
  - If only a small biopsy specimen (e.g., not a surgical resection) was used to assess histology.

For patients whose disease progresses either on or after TKI therapy, repeat EGFR testing using tumor tissue specimen (formalin-fixed paraffin-embedded tissue) to identify the
emergence of a T790M mutation may be considered **medically necessary** to determine whether further treatment with osimertinib [Tagrisso]) would be indicated.

**Note:** testing may be performed by multiple labs using cobas EGFR Mutation Test or Therascreen EGFR real time PCR testing.

Testing for other EGFR variants for all other indications in metastatic non-small cell lung cancer (NSCLC) not meeting the above criteria is considered **not medically necessary** based on current NCCN guidelines.

**ALK Rearrangements Testing**

Testing for ALK rearrangements (anaplastic lymphoma kinase (ALK) gene) using tumor tissue specimen (formalin-fixed paraffin-embedded tissue) may be considered **medically necessary** to predict treatment response to ALK inhibitor therapy (e.g., crizotinib [xalkori], ceritinib [zykadia], alectinib [alecensa], lorlatinib [lorbrena], or brigatinib [alunbrig]) in patients with metastatic non-small cell lung cancer (NSCLC) who meet the following:

- Individual diagnosed with metastatic non-squamous NSCLC or NSCLC not otherwise specified (NOS); **OR**
- Individual diagnosed with metastatic squamous cell carcinoma who:
  - Have mixed histology reported; **or**
  - If only a small biopsy specimen (e.g., not a surgical resection) was used to assess histology.

**Note:** Testing may be performed by multiple labs using VENTANA ALK CDx assay or Vysis ALK Break Apart FISH Probe Kit.

Testing for ALK rearrangements for all other indications in metastatic non-small cell lung cancer (NSCLC) not meeting the above criteria is considered **not medically necessary** based on current NCCN guidelines.

**ROS1 Rearrangements Testing**

Testing for ROS1 rearrangements (ROS1 gene) using tumor tissue specimen (formalin-fixed paraffin-embedded tissue) may be considered **medically necessary** to predict treatment response to ALK inhibitor therapy (crizotinib [xalkori]) in patients with metastatic non-small cell lung cancer (NSCLC) who meet the following:

- Individual diagnosed with metastatic non-squamous NSCLC or NSCLC not otherwise specified (NOS); **OR**
- Individual diagnosed with metastatic squamous cell carcinoma who:
  - Have mixed histology reported; or
  - If only a small biopsy specimen (e.g., not a surgical resection) was used to assess histology.
Testing for ROS1 rearrangements for all other indications in metastatic non-small cell lung cancer (NSCLC) not meeting the above criteria is considered not medically necessary based on current NCCN guidelines.

**BRAF V600E Testing**
Testing for BRAF V600E mutations using tumor tissue specimen (formalin-fixed paraffin-embedded tissue) may be considered medically necessary to predict treatment response to BRAF or MEK inhibitor therapy (e.g., dabrafenib [tafinlar] and trametinib [mekinist]), in patients with metastatic non-small cell lung cancer (NSCLC) who meet the following:

- Individual diagnosed with metastatic non-squamous NSCLC or NSCLC not otherwise specified (NOS); OR
- Individual diagnosed with metastatic squamous cell carcinoma who:
  - Have mixed histology reported; or
  - If only a small biopsy specimen (e.g., not a surgical resection) was used to assess histology.

Testing for BRAF V600E mutations for all other indications in metastatic non-small cell lung cancer (NSCLC) not meeting the above criteria is considered not medically necessary based on current NCCN guidelines.

**PD-L1 Expression Testing**
Testing for PD-L1 expression by immunohistochemistry using tumor tissue specimen (formalin-fixed paraffin-embedded tissue) in an individual diagnosed with metastatic non-small cell lung cancer (NSCLC) is considered medically necessary before therapy with pembrolizumab (Keytruda) and nivolumab (Opdivo).

Note: Testing may be performed by multiple labs using PD-L1 IHC 22C3 pharmDx or PD-L1/IHC 28.2 pharmDx.

Testing for PD-L1 expression by immunohistochemistry for all other indications in metastatic non-small cell lung cancer (NSCLC) is considered not medically necessary based on current NCCN guidelines.

**Tumor Mutations Burden (TMB)**
Testing for tumor mutations burden (TMB) using tumor tissue specimen (formalin-fixed paraffin-embedded tissue) may be considered medically necessary to predict treatment response to nivolumab (opdivo) in an individual diagnosed with metastatic non-small cell lung cancer (NSCLC).

Testing for tumor mutations burden (TMB) for all other indications in metastatic non-small cell lung cancer (NSCLC) not meeting the above criteria is considered not medically necessary based on current NCCN guidelines.

**METex14 Skipping Mutations**
Testing for METex 14 skipping mutations using tumor tissue specimen (formalin-fixed paraffin-embedded tissue) may be considered **medically necessary** to predict treatment response to capmatinib (tabrecta) in an individual diagnosed with metastatic non-small cell lung cancer (NSCLC).

Testing for METex 14 skipping mutations for all other indications in metastatic non-small cell lung cancer (NSCLC) not meeting the above criteria is considered **not medically necessary** based on current NCCN guidelines.

**High-Level MET Amplification**
Testing for high-level MET amplification using tumor tissue specimen (formalin-fixed paraffin-embedded tissue) may be considered **medically necessary** to predict treatment response to crizotinib (xalkori) in an individual diagnosed with metastatic non-small cell lung cancer (NSCLC).

Testing for high-level MET amplification for all other indications in metastatic non-small cell lung cancer (NSCLC) not meeting the above criteria is considered **not medically necessary** based on current NCCN guidelines.

**RET Rearrangements**
Testing for RET rearrangements using tumor tissue specimen (formalin-fixed paraffin-embedded tissue) may be considered **medically necessary** to predict treatment response to selpercatinib (retevmo) in an individual diagnosed with metastatic non-small cell lung cancer (NSCLC).

Testing for RET rearrangements for all other indications in metastatic non-small cell lung cancer (NSCLC) not meeting the above criteria is considered **not medically necessary** based on current NCCN guidelines.

**ERBB2 (HER2) Mutations**
Testing for ERBB2 (HER2) mutations using tumor tissue specimen (formalin-fixed paraffin-embedded tissue) may be considered **medically necessary** to predict treatment response to targeted therapy in an individual diagnosed with metastatic non-small cell lung cancer (NSCLC).

Testing for ERBB (HER2) mutations for all other indications in metastatic non-small cell lung cancer (NSCLC) not meeting the above criteria is considered **not medically necessary** based on current NCCN guidelines.

**NTKR 1/2/3 Gene Fusion Testing**
Testing for NTRK 1/2/3 gene fusions using tumor tissue specimen (formalin-fixed paraffin-embedded tissue) may be considered **medically necessary** to predict treatment response to lacotrectinib (vitrakvi) or entrectinib (rozlytrek) in an individual diagnosed with non-small cell lung cancer.
Testing for NTKR gene fusions for all other indications in metastatic non-small cell lung cancer (NSCLC) not meeting the above criteria is considered **not medically necessary** based on current NCCN guidelines.

**KRAS Testing**
Testing for KRAS (KRAS proto-oncogene) point mutations using tumor tissue specimen (formalin-fixed paraffin-embedded tissue) may be considered **medically necessary** to assess for reduced responsiveness to EGFR TKI therapy, identify patients who may not benefit from further molecular testing and for consideration of immune checkpoint inhibitors (immunotherapy), in an individual diagnosed with metastatic non-small cell lung cancer (NSCLC).

Testing for KRAS (KRAS proto-oncogene) point mutations for all other indications in metastatic non-small cell lung cancer (NSCLC) not meeting the above criteria is considered **not medically necessary** based on current NCCN guidelines.

**Other Single Genes Related to Non-Small Cell Lung Cancer**
Testing for the following genomic biomarkers using tumor tissue specimen (formalin-fixed paraffin-embedded tissue) to predict treatment response of targeted therapy in the treatment of metastatic non-small cell lung cancer as an individual biomarker marker, including but not limited to the following, is considered **investigational**, because the evidence is insufficient to determine the effects of the technology on net health outcomes and based on current NCCN guideline Non-Small Cell Lung Cancer Version 5.2021 these genetic biomarkers are currently not identified as gene alterations that impact targeted therapy selections for metastatic non-small cell lung cancer:

- AKT1
- APC Sequencing
- AR
- ARAF
- ARID1A
- ATM
- CCND1
- CCND2
- CCNE1
- CDH1
- CDK4
- CDK6
- CDKN2A
- CTNNB1
- DDR2
- ESR1
- E2H2
- FBXW7
- FGFR1
• FGFR2
• FGFR3
• GATA3
• GNA11
• GNAQ
• GNAS
• HNF1A
• HRAS
• IDH1
• IDH2
• JAK2
• JAK3
• KIT
• MAP2K1/MEK1
• MAP2K2/MEK2
• MAPK1/ERK2
• MAPK3/ERK1
• Microsatellite instability analysis
• MLH1
• MPL
• MSH2
• MSH6
• MTOR
• MYC
• NF1
• NFE2L2
• NOTCH1
• NPM1
• NRAS
• PDGFRA
• PIK3CA
• PMS2
• PTEN
• PTPN11
• RAF1
• RB1
• RHEB
• RHOA
• RIT1
• SMAD4
• SMO
• STK11
• TERT
Panel Testing for Non-Small Cell Lung Cancer

**FoundationOne CDx (0037U)**
FoundationOne CDx panel performed using tumor tissue specimen (formalin-fixed paraffin-embedded tissue) may be considered medically necessary for broad molecular profiling when ALL of the following criteria is met to predict treatment response to targeted therapy for the treatment of metastatic non-small cell lung cancer:

- The individual has been diagnosed with metastatic non-small cell lung cancer; AND
- The testing is being completed to determine the appropriate FDA labeled targeted therapy for the individual.

**Oncomine DX Target Test (0022U)**
Oncomine DX Target Test performed using tumor tissue specimen (formalin-fixed paraffin-embedded tissue) may be considered medically necessary to predict treatment response to targeted therapy in the treatment of metastatic non-small cell lung cancer (NSCLC) when ALL of the following criteria have been met:

- The individual has been diagnosed with metastatic non-small cell lung cancer; AND
- The testing is being completed to determine the appropriate FDA labeled targeted therapy for the individual.

**All Other Commercially Available Gene Panels (81445 and 81455)**
All other panel testing except as indicated above for FoundationOne CDx and Oncomine DX Target Test performed with standard of care tumor tissue specimen to predict treatment response to targeted therapy for individuals with metastatic non-small cell lung cancer (NSCLC) is considered investigational.

For individuals with metastatic non-small cell lung cancer (NSCLC) who receive testing for EGFR TKI-sensitizing variants and other genomic biomarkers for metastatic non-small cell lung cancer (NSCLC) using tumor tissue specimen (formalin-fixed paraffin-embedded tissue) to select a targeted therapy, given the breadth of molecular diagnostic methodologies available to assess tumor tissue specimen (formalin-fixed paraffin-embedded tissue) to select a targeted therapy, the clinical validity of each commercially available test must be established independently. At this time except for FoundationOne CDx and Oncomine DX Target Test none of the other commercially available tests have studies of adequate quality in demonstrating that this testing would produce outcomes to select targeted therapy. The current NCCN guideline Non-Small Cell Lung Cancer Version 5.2021 states: “The NCCN NSCLC Panel recommends that molecular profiling as part of biomarker testing use validated test(s).” The evidence is insufficient to determine the effects of the technology on net health outcomes.
Policy Guidelines

NCCN Guideline Version 5.2021 Non-Small Cell Lung Cancer

Targeted Therapy or Immunotherapy for Advanced Metastatic Disease

EGFR Mutation Positive (e.g. exon 19 deletion or L858R)
  • First line therapy
    o Afatinib
    o Erlotinib
    o Dacomitinib
    o Gefitinib
    o Osimertinib
    o Erlotinib + ramucirumab
    o Erlotinib + bevacizumab (nonsquamous)
  • Subsequent therapy
    o Osimertinib

EGFR exon 20 Insertion Mutation Positive
  • Subsequent therapy
    ▪ Amivantamab-vmjw

KRAS G12C Mutation Positive
  • Subsequent therapy
    ▪ Sotorasib

ALK Rearrangement Positive
  • First-line therapy
    o Alectinib
    o Brigatinib
    o Ceritinib
    o Crizotinib
    o Lorlatinib
  • Subsequent therapy
    o Alectinib
    o Brigatinib
    o Ceritinib
    o Lorlatinib

ROS1 Rearrangement Positive
  • First-line therapy
    o Certinib
    o Crizotinib
    o Entrectinib
• Subsequent therapy
  ▪ Lorlatinib
  ▪ Entrectinib

BRAF V600E Mutation Positive
• First-line therapy
  o Dabrafenib/trametinib
• Subsequent therapy
  o Dabrafenib/trametinib

NTRK 1/2/3 Gene Fusion Positive
• First-line/Subsequent therapy
  o Lacrotrectinib
  o Entrectinib

METExon14 Skipping Mutation
• First-line therapy/Subsequent therapy
  o Capmatinib
  o Crizotinib
  o Tepotinib

RET Rearrangement Positive
• First-line therapy/Subsequent therapy
  o Selpercatinib
  o Pralsetinib
  
  o Cabozantinib
  o Vandetanib

PD-L1 ≥ 1%
• First-line therapy**
  o Pembrolizumab
  o Carboplatin or cisplatin/pemetrexed/pembrolizumab (nonsquamous)
  o Carboplatin/paclitaxel/bevacizumab/atezolizumab* (nonsquamous)
  o Carboplatin/(paclitaxel or albumin-bound paclitaxel)/pembrolizumab (squamous)
  o Carboplatin/albumin-bound paclitaxel/atezolizumab (nonsquamous)
  o Nivolumab/ipilimumab
  o Nivolumab + ipilimumab + pemetrexed + (carboplatin or cisplatin) (nonsquamous)
  o Nivolumab + ipilimumab + paclitaxel + carboplatin (squamous)

PD-L1 ≥ 50% (in addition to above)
• First-line therapy**
  ▪ Atezolizumab
  ▪ Cemiplimab-rwic
Emerging Biomarkers to Identify Novel Therapies for Patients with Metastatic NSCLC

<table>
<thead>
<tr>
<th>Genetic Alterations (i.e. Driver Event)</th>
<th>Available Targeted Agents with Activity Against Drive Event in Lung Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-level Met amplification</td>
<td>Crizotinib</td>
</tr>
<tr>
<td></td>
<td>Capmatinib</td>
</tr>
<tr>
<td>ERBB2 (HER2) mutations</td>
<td>Ado-trastuzumab emtansine</td>
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<tr>
<td></td>
<td>Fam-trastuzumab deruxtecan-nxki</td>
</tr>
</tbody>
</table>

PROCEDURE CODES AND BILLING GUIDELINES

To report provider services, use appropriate CPT* codes, Alpha Numeric (HCPCS level 2) codes, Revenue codes, and/or ICD diagnosis codes.

- 81120 IDH1 (isocitrate dehydrogenase 1 [NADP+], soluble) (e.g., glioma), common variants (e.g., R132H, R132C)
- 81121 IDH2 (isocitrate dehydrogenase 2 [NADP+], mitochondrial) (e.g., glioma), common variants (e.g., R140W, R172M)
- 81173 AR (androgen receptor) (e.g., spinal and bulbar muscular atrophy, Kennedy disease, X chromosome inactivation) gene analysis; full gene sequence
- 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 receptor tyrosine kinase 1. 2. 3) (e.g. solid tumors) translocation analysis
- 81201 APC (adenomatous polyposis coli) (e.g., familial adenomatosis polyposis [FAP], attenuated FAP) gene analysis; full gene sequence
- 81210 BRAF (B-Raf proto-oncogene, serine/threonine kinase) (e.g., colon cancer, melanoma), gene analysis, V600 variant(s)
• 81235 EGFR (epidermal growth factor receptor) (e.g., non-small cell lung cancer) gene analysis, common variants (e.g., exon 19 LREA deletion, L858R, T790M, G719A, G719S, L861Q)
• 81270 JAK2 (Janus kinase 2) (e.g., myeloproliferative disorder) gene analysis, p.Val617Phe (V617F) variant
• 81272 KIT (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) (e.g., gastrointestinal stromal tumor [GIST], acute myeloid leukemia, melanoma), gene analysis, targeted sequence analysis (e.g., exons 8, 11, 13, 17, 18)
• 81275 KRAS (Kirsten rat sarcoma viral oncogene homolog) (e.g., carcinoma) gene analysis; variants in exon 2 (e.g., codons 12 and 13)
• 81276 KRAS (Kirsten rat sarcoma viral oncogene homolog) (e.g., carcinoma) gene analysis; additional variant(s) (e.g., codon 61, codon 146)
• 81277 Cytogenomic neoplasia (genome-wide) microarray analysis, interrogation of genomic regions for copy number and loss-of-heterozygosity variants for chromosomal abnormalities
• 81279 JAK2 (Janus Kinase 2) (e.g., myeloproliferative disorder) targeted sequence analysis (e.g., exons 12 and 13)
• 81293 MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) (e.g., hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; known familial variants
• 81295 MSH2 (mutS homolog 2, colon cancer, nonpolyposis type 1) (e.g., hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis
• 81298 MSH6 (mutS homolog 2, colon cancer, nonpolyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis
• 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
• 81310 NPM1 (nucleophosmin) (e.g., acute myeloid leukemia) gene analysis, exon 12 variants
• 81311 NRAS (neuroblastoma RAS viral [v-ras] oncogene homolog) (e.g., colorectal carcinoma), gene analysis, variants in exon 2 (e.g., codons 12 and 13) and exon 3 (e.g., codon 61)
• 81314 PDGFRA (platelet-derived growth factor receptor, alpha polypeptide) (e.g., gastrointestinal stromal tumor [GIST]), gene analysis, targeted sequence analysis (e.g., exons 12, 18)
• 81317 PMS2 (postmeiotic segregation increased 2 [S. cerevisiae]) (e.g., hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis
• 81321 PTEN (phosphatase and tensin homolog) (e.g., Cowden syndrome, PTEN hamartoma tumor syndrome) gene analysis; full sequence analysis
• 81400 Molecular pathology procedure, level 1
• 81401 Molecular pathology procedure level 2 (includes EML4/ALK)
• 81402 Molecular pathology procedure, level 3
• 81403 Molecular pathology procedure, level 4
• 81404 Molecular pathology procedure, level 5 (includes RET)
• 81405 Molecular pathology procedure, level 6 (e.g., analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis) (e.g., includes full sequence RET [ret proto-oncogene]) (e.g., multiple endocrine neoplasia, type 2A and familial medullary thyroid carcinoma)
• 81406 Molecular pathology procedure, Level 7
• 81455 Targeted genomic sequence analysis panel, solid organ or hematolymphoid neoplasm, DNA analysis, and RNA analysis when performed, 51 or greater genes (eg, ALK, BRAF, CDKN2A, CEBPA, DNMT3A, EGFR, ERBB2, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MLL, NPM1, NRAS, MET, NOTCH1, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, if performed
• 81479 Unlisted molecular pathology procedure
• 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 (eg, Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, per specimen, each single antibody stain procedure; using computer-assisted technology
• 0022U Targeted genomic sequence analysis panel, cholangiocarcinoma and non-small cell lung neoplasia, DNA and RNA analysis, 1-23 genes, interrogation for sequence variants and rearrangements, reported as presence/absence of variants and associated therapy(ies) to consider (Oncomine DX Target Test)
• 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 (FoundationOne CDx [F1CDx])

SELECTED REFERENCES
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- Linardou H, Dahabreh IJ, Kanaloupiti D, et al. Assessment of somatic k-RAS mutations as a mechanism associated with resistance to EGFR-targeted agents: a


- Janne PA, van den Heuvel MM, Barlesi F, et al. Selumetinib plus docetaxel compared with docetaxel alone and progression-free survival in patients with
KRAS-mutant advanced non-small cell lung cancer: the SELECT-1 Randomized Clinical Trial. JAMA. May 09 2017;317(18):1844-1853. PMID 28492898


- Cancer Therapeutics. Also available at https://www.aacrjournals.org
- Circulogene Liquid Biopsy Test. Also available at https://www.circulogene.com
- ClearID Biomarkers Expression Assays and ClearID Lung Cancer. Also available at https://cynvenio.com
- cobas EGFR Mutation Test. Also available at https://diagnostic.roche.com
- pharmDx PD-L1 IHC 28-8 and PD-L1 IHC 22C3. Also available at https://www.agilent.com
- Oncomine DX Target Test. ThermoFisher Scientific. Also available at https://www.oncomine.com
- Oncomine DX Target Test Physician Insert
- FDA approval for Vysis ALK Break Apart FISH Probe Kit
- VENTANA ALK (D5F3) CDx Assay
- FDA Approved Companion Diagnostic Testing Updated 9/10/2020
- FoundationOne CDx
- Therascreen EGFR PCR KIT

POLICY HISTORY

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<tr>
<td>November 2021</td>
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New information or technology that would be relevant for Wellmark to consider when this policy is next reviewed may be submitted to:
   Wellmark Blue Cross and Blue Shield
   Medical Policy Analyst
   PO Box 9232
   Des Moines, IA 50306-9232

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