The NeoTYPE Lung Tumor Profile is performed by the sequencing of select exons of the genes listed unless another method is noted. AKT1, BRAF, EGFR, ERBB2, ERBB4, FGFR1, FGFR2, FGFR3, KIT, KRAS, MET, MET Exon 14 Deletion Analysis, NOTCH1, NRAS, PDGFRα, PIK3CA, PTEN, SMAD4, SMO, SRC, TP53, ALK FISH, HER2 FISH, MET FISH, PTEN FISH, RET FISH, ROS1 FISH, and PD-L1 IHC. Individual genes from a validated list of solid tumor genes can be added-on. Test orders include summary interpretation of all results together. FISH components of NeoTYPE Profiles may be ordered as “Tech-Only” by pathology clients who wish to perform the professional component. Tumor mutation burden (TMB) is an option that can be added as well.

**AKT1**

The AKT1 proto-oncogene, on chromosome 14, encodes a serine/threonine protein kinase (PKB) and a downstream effector of PI3K that plays a role in cell proliferation, survival, apoptosis, cell growth, glucose metabolism, genome stability, transcription, and neovascularization. AKT1 promotes constitutive activation of the mTOR signaling pathway and the glycolytic phenotype in multiple cancers. The most frequent AKT1 alteration observed in cancer is E17K in the pleckstrin homology domain. Amplification and overexpression of AKT1 have also been observed in certain cancers. Point mutations in AKT1 occur in lung cancer (0.6%), but more frequently in sqNSCLC (2-5%). In lung cancer, 1.01% have copy number gain in AKT1. Testing for AKT1 mutations can be useful for determining sensitivity to various drugs, such as PI3K/AKT/mTOR inhibitors, including everolimus. Constitutive activation of AKT1 is associated with resistance to chemotherapy or radiation therapy in a variety of cancers, including EGFR-TKIs in lung cancer. While no direct AKT inhibitor has been yet approved for cancer, FDA approved drugs sensitive to AKT1 include everolimus and temsirolimus. Preclinical data report inhibition of certain AKT1 mutations, including E17K, by AKT inhibitors. Various allosteric and ATP-competitive AKT inhibitors are currently in clinical trials.1-11

**ALK FISH**

The ALK gene, on chromosome 2, encodes a receptor tyrosine kinase involved in cell growth, transformation, and differentiation. Alterations in ALK constitutively activate the kinase regulating the JAK–STAT3, PI3K–AKT and RAS–MAPK pathways and driving tumorigenesis in various tissues. The most common ALK alterations are gene rearrangements detectable by FISH. In addition to fusions, various cancers harbor gain of function mutations in ALK, such as F1174L, D1091N, I1250T, and R1275. ALK-rearranged NSCLC represents 3-7% of all NSCLC. Eight percent of ALK-rearranged NSCLC are also EGF+ or KRAS+ mutated. ALK rearrangements are associated with response to crizotinib in approximately 60-70% of ALK+ patients. A number of point mutations, such as the F1174L, are known to be associated with resistance to ALK inhibitor therapy. Additionally, ALK copy number gain as well as activating mutations in other driver genes such as EGFR may be acquired resistance mechanisms in patients undergoing ALK inhibitor therapy. FDA approved drugs sensitive to ALK against NSCLC include ceritinib, alectinib, and crizotinib. Evidence suggests differential primary response to crizotinib depending on the ALK fusion partner in NSCLC. Heat shock protein 90 (HSP90) inhibitors present a potential line of treatment due to dependence of ALK fusions, such as EML4-ALK, on HSP90 for stability. Next-generation agents such as alectinib may salvage CNS metastasis in ALK+ patients treated with both crizotinib and ceritinib.12-22

**BRAF**

The BRAF gene, on chromosome 7, encodes a serine/threonine kinase that plays a role in cell proliferation, differentiation, and growth. The majority of the BRAF gain of function mutations alter residues in the kinase domain, most notably V600E, detectable by molecular testing. BRAF mutations and EGFR mutations are believed to be mutually exclusive. BRAF rearrangements, detectable by FISH, such as BRAF-KIAA1549, are also reported in some cancers. Amplifications are observed in certain cancers. Constitutive activation of BRAF has been observed in multiple cancers, including lung, where it leads to activation of the RAF/MEK/ERK pathway. Point mutations (1-4%) and copy number gain (1.43%) in BRAF are found in NSCLC. Prognosis associated with BRAF fusions is neutral in NSCLC when treated with chemotherapy. BRAF and MEK1/2 inhibitors are approved or under clinical evaluation as single agents or in combination for the treatment of BRAF mutant cancers. Some patients with V600E mutations have increased sensitivity to the BRAF inhibitors vemurafenib and dabrafenib. BRAF inhibition may ultimately result in resistance to BRAF or MEK inhibitors. Additionally, BRAF V600E gain is neutral in NSCLC when treated with chemotherapy.

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mutations are resistant to EGFR therapies, such as cetuximab or panitumumab, as well as imatinib and sunitinib. While specific mutations and fusions, such as BRAF D594A/V and K483M, are insensitive to RAF inhibitors, they are sensitive to MEK inhibitors. BRAF fusions, like BRAF-KIAA1549, are resistant to first generation BRAF inhibitors, such as vemurafenib, but second generation BRAF inhibitors are being investigated. FDA approved drugs sensitive to BRAF include dabrafenib, vemurafenib, cobimetinib, and trametinib. Targeting the RAF/MEK/ERK pathway may be efficacious in BRAF mutant cancers.23-34

**EGFR**

The EGFR/ERBB1 gene, on chromosome 7, encodes a receptor tyrosine kinase, which plays a role in cell proliferation, differentiation, motility, survival, and tissue development. EGFR alterations, including overexpression, amplification, and mutation, are involved in development of numerous solid tumors. The most frequent EGFR mutations in cancer are in the kinase domain, including indels between residues 739-757 and mutations of L858, leading to constitutive activation. Lung cancer point mutations in EGFR occur 28.94% of the time, while copy number gain is found in 5.06% of lung cancers. EGFR mutations in lung cancer are associated with adenocarcinoma in female nonsmokers of Asian ethnicity. Specific point mutations are frequently encountered in NSCLC: G719, T790M, C797S, and L861, and have distinct therapeutic relevance. Lung cancer patients with mutations in exons 18, 19, and 21 may be sensitive to EGFR inhibitors, such as erlotinib and gefitinib. Acquired mutations in exon 20, such as T790M, are known to be resistant to first generation EGFR TKIs. Later generation EGFR TKIs, such as afatinib and osimertinib, were developed to counter resistant variants, including T790M. Other mutations, such as C797S, L844V, and L718Q, may be responsible for resistance to third generation TKIs. EGFR alterations may also drive resistance to ALK-targeted therapy. FDA approved EGFR inhibitors include osimertinib, gefitinib, erlotinib, necitumumab, and afatinib. Osimertinib is approved for the treatment of T790M lung cancer. Gefitinib is approved for metastatic NSCLC with EGFR exon 19 deletions or exon 21 (L858R) substitution mutations as detected by an FDA-approved test. Other FDA approved drugs sensitive to EGFR include lapatinib, vandetanib, cetuximab, and panitumumab. Several clinical trials are currently underway for treatment resistant lung cancer patients with EGFR mutations.35-50

**ERBB2 + FISH (HER2)**

ERBB2, on chromosome 17, encodes a growth factor receptor (HER2) that regulates proliferation and survival. ERBB2 lacks an identified ligand, can undergo ligand independent activation, and has been identified as a driver of tumorigenesis in cancers. ERBB2 alterations in cancer are predominantly overexpression and amplification that can be evaluated by FISH. Alterations in ERBB2 found in cancer also include insertions in the kinase domain or deletions in the extracellular domain. Large deletions in the extracellular domain of ERBB2 result in mutant products p95HER2 and Δ16HER2. Other commonly mutated residues include G309, S310, S335, L755, 777, and 842. HER2 activation is associated with poor prognosis in a number of cancer types, including NSCLC with co-expression of EGFR. After EGFR T790M, HER2 and MET amplifications are the most common findings of acquired resistance (10-20%) under first-generation EGFR TKIs in NSCLCs. FDA approved drugs sensitive to ERBB2 include trastuzumab, afatinib, lapatinib, and pertuzumab. The ratio of T790M/activating-mutations may predict the patients who will remain sensitive to third-generation TKIs longer. HER2+ status is associated with resistance to endocrine and chemotherapy regimens. Alterations, including 95HER2, Δ16HER2, L726, L755, P780, and small insertions in exon 20, are resistant to trastuzumab or lapatinib. In order to overcome resistance to first-generation EGFR/HER inhibitors, the second-generation EGFR/HER-TKIs, including afatinib, dacomitinib, and neratinib, irreversibly block enzymatic activation of EGFR, HER2, and HER4. Current development of dacomitinib is focused to TK1 treatment naïve, EGFR and HER2 mutant lung cancers. Dacomitinib inhibits the activity of both WT and mutant HER2 kinase. HSP90 is required for stabilization of HER2 and is suggested as a therapeutic target.51-64

**ERBB4**

The ERBB4 gene, on chromosome 2, encodes a receptor tyrosine kinase (HER4) that regulates MAPK and PI3K pathways to induce mitogenesis and differentiation. HER4 is activated by binding to neuregulins, EGF family members, and other factors. HER4 expression is involved in embryonic development and regulates astrogenesis in the brain. Missense mutations in ERBB4 are observed across all domains, including the extracellular domain, transmembrane domain, and the tyrosine kinase domain. HER4 activation via mutation (3.37%), amplification, or overexpression are observed in lung cancer. Activating alterations in HER4 have been shown to result in increased ERBB2-ERBB4 heterodimerization and activation of downstream signaling in lung cancer. ERBB4 is part of a targetable pathway and testing for ERBB4 mutations can be useful in determining a patient’s sensitivity to tyrosine kinase inhibitors.65-70

**FGFR1**

The FGFR1 gene, on chromosome 8, encodes a receptor tyrosine kinase, involved in cell division, cell growth and maturation, angiogenesis, wound healing, and embryonic development. The activation of FGFR1 (via amplification, missense mutation, or fusion) has been observed in multiple cancers and is associated with enhanced activation of the MAPK and PI3K/AKT pathways. The most frequently occurring FGFR1 alterations
in cancer are amplifications, such as in sqNSCLC (20%). The majority of gain of function mutations alter the tyrosine kinase domain. The most frequently mutated residues, 546 and 656, result in constitutive activation of the protein. FGFR1 translocations are also known in human cancers and belong to two distinct classes: type 1 fusions typically associated with hematomal malignancies and type 2 fusions found primarily in solid tumors. As per the COSMIC database, copy number gain in FGFR1 is observed in lung cancer (8.44%). Inhibition of FGFR1 is accomplished with FGFR inhibitors.71-74

**FGFR2**

The FGFR2 gene, on chromosome 10, encodes CD332 that is involved in wound healing, bone growth, embryonic development, proliferation, differentiation, survival, migration, and angiogenesis. FGFR2 has two alternatively spliced isoforms: FGFRlIIb and lIlc. The FG pathway consists of 22 FGFs and four transmembrane receptor tyrosine kinases (FGFR 1-4). FGFR family receptor tyrosine kinases promote activation of the RAS/MAPK and PI3K/AKT pathways. Mutations, rearrangements, deletions, and amplifications in FGFR2 are associated with certain cancers. While FGFR2 undergoes constitutive activation leading to an oncogenic function in most tissues, it has also been found to be downregulated or harbor loss of function mutations in some cases, hinting at a tumor suppressor role. Common oncogenic mutations are found in the linker region, such as S252 and P253, and the tyrosine kinase domain, such as N549 and N659. Mutations in FGFR2 are found in NSCLC (4%) and FGFR2 fusions are found in sqNSCLC. There are TKI sensitive FGFR2 mutations in sqNSCLC. Four agents (ponatinib, pazopanib, regorafenib, and lenvatinib) are FDA approved for use in cancer, although the approval was not based on their activity against FGFR. While there are no approved FGFR2 inhibitors, pan-FGFR TKIs, selective FGFR TKIs, and FGF-ligand traps are being evaluated in clinical trials for solid tumors harboring FGFR-activating alterations. FGFR2 signaling through FGFR1 causes resistance to EGFR inhibitors in lung cancer, and combination therapy with EGFR and FGFR inhibitors, such as AD4547, may overcome this resistance.75-88

**FGFR3**

The FGFR3 gene, on chromosome 4, encodes a receptor tyrosine kinase that promotes activation of the RAS/MAPK and PI3K/AKT pathways to regulate cell proliferation, survival, migration, and differentiation. The majority of the FGFR3 alterations observed in cancer are mutations that occur in the extracellular, transmembrane, and intracellular tyrosine kinase domains, particularly in residues 249, 373, 650, and result in constitutive activation of the receptor. FGFR3 copy number variations are rare. Alterations (half mutation and half amplification) of FGFR3 occur in 2% of lung adenocarcinoma and most often in sqNSCLC (4%). Gene fusions have also been observed in solid tumors, such as FGFR3-TACC3 in NSCLC (0.6%) and sqNSCLC (2.9%). FGFR inhibitors gefitinib and cetuximab increase expression of FGFR2 and FGFR3 in NSCLC cells contributing to the rapid development of EGFR treatment resistance. Evidence for effective therapeutic targeting of FGFR3 activation by kinase inhibitors is currently being explored. Treatment of NSCLC patients with combinations of EGFR and FGFR specific TKIs may enhance efficacy of EGFR inhibitors since EGFR inhibitors upregulate FGFR and lead to EGFR treatment resistance. FDA approved drugs sensitive to FGFR3 include pazopanib and ponatinib.76,89-98

**KIT**

The v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (KIT) gene, on chromosome 4, encodes a receptor tyrosine kinase, which regulates RAS/RAF/MEK/ERK, PI3K/AKT, and JAK/STAT3 pathways, thereby playing roles in cell survival, proliferation, angiogenesis, and cell motility. Alterations, such as in frame deletions, insertions, amplifications, and missense mutations render the KIT receptor constitutively active. KIT mutations have been found in lung cancers as well as copy number gain (2.7%). Mutation detection can help identify which lung cancer patients may have a poorer prognosis. Patients with KIT mutations have been shown to be sensitive to tyrosine kinase inhibitors, such as imatinib and sunitinib. Exon 9, 13, 14, 17, and 18 mutations and point mutation at residue 816 present primary and acquired resistance to imatinib, sunitinib, sorafenib, and nilotinib. The multi-targeted TKI, sunitinib, has a promising clinical profile for SCLC. Crizotinib was originally developed as a c-MET inhibitor, was later found to inhibit ALK, and was established as first- and second-line treatment for ALK+ lung cancer. KIT alternations may cause secondary resistance to other multi-targeting TKIs such as crizotinib in lung cancer. FDA approved drugs sensitive to KIT include dasatinib, regorafenib, nilotinib, cabozantinib, sunitinib, axitinib, and imatinib. Newer approaches are being explored to address KIT mutations that are refractory to therapy with multi-targeting TKIs.99-108

**KRAS**

The KRAS gene, on chromosome 12, is one of three human RAS oncogenes that encodes the KRAS GTPase, which regulates proliferation, migration, and cell survival. KRAS alterations are gain of function mutations that result in constitutive activation of the RAS/RAF/MEK/ERK pathway. KRAS mutations in cancer are found in the phosphate binding loop/G1 motif (residues 10-17), the switch II domain (residues 57-67), and the G5 motif (residues 145-147), located at codons 12, 13 and 61. Point mutations in KRAS are found in 10-25% of
Point mutations in NOTCH1 are frequent in sqNSCLC. KRAS mutations are seldom seen together with EGFR or ALK alterations in lung cancer and are more frequently observed in former or current smokers compared to never smokers. As per the COSMIC database, copy number gain in KRAS is observed in lung cancer (5.23%). Amplification of KRAS is also observed in certain cancers. KRAS mutations are generally associated with poor prognosis in NSCLC. However, a recent large retrospective study found no difference in prognosis by KRAS exon 12 mutation in patients with early stage NSCLC, calling into question the role of KRAS mutations a prognostic biomarker. Testing for KRAS mutations can be useful in determining a patient’s sensitivity to tyrosine kinase inhibitors, such as MEK inhibitors. RAS mutations, such as in NSCLC, are associated with resistance to EGFR inhibitors, such as cetuximab, panitumumab, and erlotinib. FDA approved drugs sensitive to KRAS include sorafenib, regorafenib, palbociclib, cobimetinib, and trametinib. Several inhibitors of the RAF/MEK pathway, including selumetinib, are under clinical evaluation for the treatment of patients with KRAS mutated cancers, including lung cancer.\textsuperscript{129-145}

**MET + FISH & exon 14 deletion analysis**

The MET gene, on chromosome 7, encodes a transmembrane receptor tyrosine kinase (HGF) that regulates morphogenesis, regeneration, and survival. Occasional mutations in MET have been noted across the extracellular domain (residues 52-496), the juxtamembrane domain (residues 956-1093), and the tyrosine kinase domain (residues 1096-1355). The most frequently observed validated gain of function mutation in MET is Y1253D. Translocations of MET gene are rare, but have been noted in lung adenocarcinoma. Skipping of exon 14 and disruption of juxtamembrane domain activates MET in NSCLC and is sensitive to MET inhibitors. MET is amplified in 6% of sqNSCLC and copy number gain is seen in 2.7% of lung cancer. MET overexpression and amplification is generally associated with poor prognosis. Testing for MET mutations can be useful in determining a patient’s sensitivity to various tyrosine kinase inhibitors. MET gene amplification, as detected by FISH, is a known mechanism of acquired resistance to several receptor tyrosine kinase inhibitors such as HER2 and EGFR inhibitors. FDA approved drugs sensitive to MET include cabozantinib and crizotinib. Antibodies targeting the MET axis and small molecule MET inhibitors, such as volitinib and AMG337, are under clinical investigation.\textsuperscript{129-128}

**NOTCH1**

The Notch homolog 1 (NOTCH1) gene, on chromosome 9, encodes a single-pass transmembrane receptor that regulates cell differentiation, growth, survival, apoptosis, migration, and invasion. NOTCH1 pathway components include NOTCH receptors (NOTCH1-4) and ligands (Jagged1, Jagged2, DLL1, DLL3, or DLL4). Upon the binding with NOTCH ligands, NOTCH1 is cleaved by proteases, including γ-secretases, to release its intracellular domain transcription cofactor. Point mutations in NOTCH1 frequent in sqNSCLC. NOTCH1 has predominantly been characterized as an oncogene, where gain of function mutations are seen in up to 10% of NSCLCs and where it plays a role in epithelial-mesenchymal transition (EMT) driven metastasis. For example, NOTCH1 is oncogenic in KRAS+ lung adenocarcinoma and under conditions of hypoxia where HIF1α activates NOTCH1 in lung adenocarcinoma cells. A tumor suppressor role for NOTCH1 has also been suggested in NSCLC and SCLC, dependent on context, disease subtype, co-mutation, and hypoxia. NOTCH1 is mutated in 8% of sqNSCLC, including loss of function truncating mutations occurring in 47% of NOTCH1 alterations in squamous cell carcinoma, including sqNSCLC. Specific mutations have been associated with gain of function in lung cancer: V2444fs, S2275fs, R2328W, and D1643H. The prognostic significance of NOTCH1 expression in sqNSCLC and adenocarcinoma is unclear, but NOTCH1 gain of function mutations in NSCLC are associated with poor outcome in the absence of TP53 mutations. NOTCH1 inhibitors may be useful for treating advanced stage NSCLC, whereas NOTCH1 inhibitors may promote invasion and metastasis of SCLC. Gamma-secretase inhibitors (GSIs) can interfere with the activation of NOTCH1, though first generation inhibitors have had limited success due to dose-limiting toxicity. Direct targeting of NOTCH1 using antibodies and small molecule inhibitors is under evaluation in lung cancer. Positive feedback loops between NOTCH, MYC, and AKT suggest that inhibitors of MYC or PI3K/AKT may help to prevent resistance to NOTCH1 inhibiting therapies.\textsuperscript{129-145}

**NRAS**

The NRAS gene, on chromosome 1, is one of the three human RAS oncogenes and encodes the NRAS GTPase that regulates proliferation, migration, and cell survival. Mutations in NRAS result in activation of the RAS/RAF/MEK/ERK pathway. The most frequent NRAS alterations observed in cancer are mutations at codons 12, 13, and 61 (90%), and within the phosphate binding loop/G1 motif (residues 10-17), the switch II region (residues 59-67), and the G5 motif (residues 145-147). Somatic mutations in NRAS is rarely (0.2-1%) reported in primary NSCLC, but their role in carcinogenesis has been proven. Smoking and environmental carcinogens are associated with the etiology of NRAS mutated lung cancer. NRAS mutations have been correlated with metastases of NSCLC (1.5%). Somatic mutations in NRAS are generally associated with poor response to standard therapies. MEK inhibitors, such as selumetinib, are effective in treating cancer patients with RAS mutations. NRAS mutations,
such as E63K0, are associated with resistance to anti-EGFR therapies, such as cetuximab and panitumumab, anti-BRAF therapies, such as vemurafenib and dabrafenib, ALK TKIs, and radiotherapy. Inhibitors of the RAS/RAF/MEK/ERK pathway are under clinical evaluation for the treatment of patients with NRAS mutated cancers. FDA approved drugs sensitive to NRAS include cobimetinib and trametinib.146-155

PDGFRA
Platelet-derived growth factor receptor alpha (PDGFRA) gene, on chromosome 4, is a member of the subfamily of type III receptor tyrosine kinases, which includes the KIT, PDGFRB, FLT3, and CSF-1 receptors. PDGFRA is activated upon ligand binding and impacts embryonal development, cell growth, proliferation, survival, migration, and apoptosis. Alterations of PDGFRA in cancer include point mutations, small indels, amplification, overexpression, and fusions. PDGFRA mutations cluster in exon 12 (juxtamembrane domain; JM domain) and exon 18 (TK domain). As per the COSMIC database, copy number gain in PDGFRA is found in lung cancer (2.03%). Approximately 3%-4% of lung carcinoma, particularly sqNSCLC, overexpresses PDGFRA, where it has been identified as a driver mutation. Testing for PDGFRA alterations by FISH may be useful in determining sensitivity to tyrosine kinase inhibitors, such as imatinib, sunitinib, and nilotinib. Specific PDGFRA mutants have been shown to be resistant to imatinib, sorafenib, and nilotinib, particularly D842V, in the TK domain. Concomitant activation of KRAS, NRAS or BRAF in PDGFRA altered tumors is also associated with imatinib resistance. FDA approved drugs sensitive to PDGFRA include sorafenib, regorafenib, olaratumab, sunitinib, pazopanib, dasatinib, axitinib, nilotinib, and imatinib. Although there are targeted treatments for lung adenocarcinoma, no personalized therapies currently exist for sqNSCLC. Several clinical trials are evaluating the effectiveness of nintedanib in NSCLC.145,156-162

PD-L1 IHC
Programmed death-ligand 1 (PD-L1) is a transmembrane protein encoded by the CD274 gene, which plays a role in suppressing the immune system. PD-L1 binds to its receptor, PD-1, on activated T cells, B cells, NK cells, macrophages, epithelial cells, and myeloid cells, to modulate activation or inhibition. The binding of PD-L1 to PD-1 transmits an inhibitory signal which reduces the proliferation of CD8+ T cells and controls the accumulation of foreign antigen specific T cells through apoptosis. The result of PD-1 to PD-L1 binding is apoptosis and the exhaustion of activated immune cells. PD-L1 can be expressed on the surface of tumor cells and is upregulated by various solid tumor cells, shielding them from the endogenous antitumor response. Multiple solid tumor types, including NSCLC, co-opt this immune shield by expressing PD-L1 to generate an immunosuppressive tumor microenvironment and avoid T cell cytolysis. For the tumors classically associated with clinical responses to immune checkpoint inhibition with anti-PD-1 agents—including NSCLC—the range of PD-L1 IHC expression on tumor ranges from 14-100%. The PD-1/PD-L1 pathway is a critical therapeutic target for advanced NSCLC, particularly in earlier disease settings. While PD-L1 expression is being tested in clinical trials as a predictive biomarker for checkpoint inhibitor cancer immunotherapies, pathologists do not yet agree on the preferred test or definition of positive status. While PD-L1 expression is generally associated with negative prognosis in multiple tumor types, it is a positive prognostic factor when treatment is immunotherapy based. Multiple tumor types have demonstrated durable responses to immune checkpoint inhibition. Higher (than background) baseline tumor expression of PD-L1 is associated with better efficacy of pembrolizumab in a number of oncology indications, including NSCLC. Not all patients who respond to PD-1/PD-L1-based therapeutics express high levels of PD-1/PD-L1. Pembrolizumab, a PD1 inhibitor, is approved for first and second line PD-L1+ NSCLC. Nivolumab, a PD1 inhibitor, is approved to treat ALK/EGFR WT and mutant metastatic NSCLC. Atezolizumab, a PD-L1 inhibitor, is approved for the treatment of second line EGFR/ALK WT or mutant refractory NSCLC.163-176

PIK3CA
The PIK3CA gene, on chromosome 3, encodes the p110 alpha catalytic subunit of PI3K enzymes. PI3K is a lipid kinase that converts PIP2 to PIP3 to activate AKT. PI3KCA is a member of the PI3K/AKT/mTOR pathway, important in regulation of growth, proliferation, survival, differentiation, adhesion, and motility. Activating mutations or amplification in PIK3CA result in constitutively active PI3K. Most PIK3CA gain of function mutations occur within the kinase (particularly residues 1043, 1047, and H1049R), alpha-helical (particularly residues E542K, E545K, and 546), and C- (particularly residues 345 and 420) domains. Other key domains that are less frequently mutated are the adaptor and linker domains. The PIK3CA/mTOR pathway is dysregulated in 50-70% of NSCLC and PIK3CA mutations are detected in 1-5% of NSCLC. Copy number gain in PIK3CA is observed in lung cancer (16-20%), more frequently in sqNSCLC, and less frequently in SCLC (4.7%). PIK3CA is amplified in sqNSCLC (33-37%) and mutated (6.5-16%). PIK3CA activation is generally associated with poor prognosis. Tumors with constitutively active PI3K have been proposed to be sensitive to agents targeting the PI3K/AKT/mTOR pathway. Activation of the PI3K/Akt/mTOR pathway, such as mutant PIK3CA E545K, leads to resistance to ERBB-targeted therapies. Idelalisib is an FDA approved PI3K inhibitor for leukemia and lymphoma. Direct PI3K inhibitors under development include buparlisib, pilaralisib, pictilisib, alpelisib, tazalisib, CAL-101, and GDC-0941.177,186
PTEN + FISH

The tensin homolog gene (PTEN), on chromosome 10, is a member of the targetable PI3K/AKT/mTOR pathway, which encodes a tumor suppressor that plays a role in growth, migration, survival, cell cycle progression, chromosome stability, DNA repair, and apoptosis. PTEN plays dual lipid and protein phosphatase activities. The tumor suppressor role of PTEN is impaired by the disruption or loss of phosphatase domain (particularly residue R130 and R173) or the C2 domain (particularly R233). PTEN loss of function results in constitutive activation of the PI3K/AKT/mTOR pathway. PTEN loss of function and PIK3CA mutation are mutually exclusive in most cancers. Although PTEN mutations are rare in NSCLC, loss of PTEN expression, due to epigenetic silencing, occurs in 24-44% of NSCLC cases. Loss of PTEN expression is associated with poor prognosis, whereas high PTEN expression is associated with longer survival in NSCLC patients. Testing for PTEN alteration by FISH may be useful in identifying patients sensitive to PI3K/AKT/mTOR and FRAP/mTOR inhibitors, as well as agents that target double stranded break repair pathways. Activation of the PI3K/ATK/mTOR pathway as a result of PTEN loss may play a role in resistance to EGFR targeted therapies such as cetuximab and trastuzumab.8,187-194

RET FISH

Activation of the RET receptor is stimulated upon binding of glial cell line-derived neurotrophic factor (GDNF) family of ligands and promotes developmental processes such as kidney morphogenesis, maturation nerve cells, and spermatogenesis. Activation of RET results in promotion of PI3K/AKT, RAS/ERK and JAK/STAT pathways. Certain point mutations destabilize RET dimerization and result in constitutive activation of RET. RET gene fusions are found in 1-2% of adenocarcinoma type NSCLC and are generally mutually exclusive of mutations in EGFR, KRAS, ALK, and ROS1. Patients with RET rearrangements in NSCLC tend to be younger (≤60) and lack smoking history. Specific RET mutations, such as V804, may be responsible for insensitivity to TKIs such as vandetanib, motesanib, and cabozantinib, while retaining sensitivity to others, such as sunitinib and ponatinib. FDA approved drugs sensitive to RET include regorafenib, lenvatinib, ponatinib, cabozantinib, sorafenib, sunitinib, and vandetanib. Small molecule inhibitors targeting RET or downstream effectors RAF or MEK are under development for their efficacy in RET altered carcinoma.195-198

ROS1 FISH

The proto-oncogene receptor tyrosine kinase (ROS1) gene, on chromosome 6, encodes a member of the insulin receptor superfamily. Although very little is known about the function of wild-type ROS1, fusions have been found in a variety of cancers. ROS1 shares 77% amino acid sequence homology with ALK in the ATP binding sites of the tyrosine kinase domain, and ROS1 rearrangement is mutually exclusive to ALK rearrangement in NSCLC. A number of ROS1 fusions, detectable by FISH, have been identified in 1-2% of NSCLC: FIG-ROS1, SLC34A2-ROS1, CD74-ROS1, EZR-ROS1, TPM3-ROS1, SDC4-ROS1, LRRG3-ROS1, KDEL2-ROS1, and CCDC66-ROS1. ROS1 rearrangements share clinical and histological characteristics: never-smoking history, female, younger age, and adenocarcinoma with signet ring cell histology. ALK and ROS1 fusion tumors have a significantly shorter disease free survival, which does not translate into a short overall survival, since patients respond to targeted therapy, such as crizotinib. Two thirds of ROS1+ patients respond to crizotinib, approved in the first-line for NSCLC. Crizotinib resistant ROS1 G2032R mutants are sensitive to foretinib and cabozantinib. Patients ultimately develop secondary resistance to crizotinib and later generation therapies. Increased EGFR phosphorylation is detected in 44% of ALK- and ROS1-rearranged crizotinib resistant tumors, indicating that this mechanism may mediate resistance. Ceritinib, ASP3026, and brigatinib exhibit activity against ROS1 kinase, but fail to inhibit crizotinib resistant ROS1. There are a number of multi TKIs in evaluation for resistance to crizotinib, ceritinib, and alectinib: including merestinib, entrectinib, TAE684, and lorlatinib.18,199-212

SMAD4

Mothers against decapentaplegic homolog 4 (MAH4), on chromosome 18, encodes a putative tumor suppressor, SMAD4, which is involved in TGF-beta signaling, resulting in the regulation of cell growth, extracellular matrix (ECM), differentiation, and proliferation. SMAD4 is produced in skin, pancreatic, colon, uterine, epithelial cells, and fibroblasts. SMAD4 is composed of MH1, MH2, and linking domains. SMAD4 is regulated by miR-1285 and miR-27a, and GSK3. Mutations in SMAD4 occur predominantly in the MH2 domain (particularly D351 and R361) disrupting heteromerization and in the MH1 domain inhibiting DNA binding. SMAD4 alterations are mutually exclusive DNA repair machinery dysfunction. Loss of SMAD4 function has been shown to promote tumorigenesis in NSCLC. SMAD4 loss in tumors results in abrogated TGF-beta signaling, driving tumor progression, metastasis, and poor overall survival. Loss of SMAD4 function is associated with a better response to DNA topoisomerase inhibitors in NSCLC. Loss of SMAD4 is also associated with increased angiogenesis, suggesting a possible therapeutic role for anti-angiogenic agents.213-221
The SMO gene, on chromosome 7, encodes the G-protein-coupled smoothened receptor an oncogene that functions as a transducer of the hedgehog (Hh) pathway and promotes proliferation, embryonic development, cellular localization, and regeneration and maintenance of adult tissues. Normally, PTCH1 inhibits SMO. In the presence of Shh, PTCH1 inhibition results in activated SMO, resulting in the nuclear localization of GLI, which stimulates transcription of Hh target genes. Most activating mutations in SMO occur in the frizzled domain, particularly residues 412 and 535. Alterations in SMO has been observed lung cancer, where copy number is seen in 1.01% of cases. SMO inhibitors such as sonidegib, erismodegib, and vismodegib are approved for the treatment of basal cell carcinoma, which usually harbors a dysregulated hedgehog signaling axis. Vismodegib is a second generation cycloamine derivative that binds directly to SMO to prevent GLI activation and is under evaluation in SCLC. Erismodegib is a SMO antagonist shown to induce cell cycle arrest and apoptosis, decreasing the epithelial-mesenchymal transition and invasive potential of glioblastoma, prostate, and RCC, and is currently being studied in solid tumors. The GLI inhibitor arsenic trioxide is approved for the treatment of acute promyelocytic leukemia and has demonstrated anti-tumor activity in hedgehog pathway dysregulated solid tumors. Spontaneous mutations can develop as a response to some SMO inhibitors, such as vismodegib. Next generation inhibitors are being developed, such as itraconazole, and other agents that target SMO and GLI.²²²-²³⁰

The SRC gene, a proto-oncogene on chromosome 20, encodes a tyrosine kinase that plays a role embryonic development, cell growth, immune response, cell adhesion, cell cycle progression, apoptosis, migration, and transformation. Alterations in SRC lead to increased proliferation by upregulating the RAS/MAPK, PI3K/AKT, and STAT pathways. SRC is critical for proper ubiquitination and degradation of EGFR. While overexpression and amplification of SRC are more frequently observed, activating mutations are infrequent. Truncations within the C-terminal region of SRC that result constitutive activation are also observed. EGFR and SRC are often co-overexpressed in human cancers, including in NSCLC. SRC upregulation has been suggested as a mechanism for EGFR and ALK mutant treatment resistance in NSCLC. Mutant EGFR cells, such as EGFR Y845, are sensitive to SRC inhibitors such as dasatinib, PP1, or SKI-606. Inhibiting SRC could inhibit proliferation in ceritinib resistant ALK+ NSCLC cells, as ceritinib upregulates SRC in NSCLC. FDA approved drugs sensitive to SRC include dasatinib and bosutinib. Combinatorial therapeutic approaches may aid in obtaining greater benefit from SRC inhibition.²³¹-²⁴⁰

Checkpoint proteins, such as PD-1, PD-L1, CTLA-4, and LAG-3, can be coopted by cancer to suppress autoimmunity and evade apoptosis. Immunotherapies, designed to restore the immune surveillance system to fight their cancer have received regulatory approval across a number of cancer types. While these checkpoint based therapies can be remarkably effective in some patients, only a limited subset of patients will benefit (e.g. 20-40% of NSCLC). Tumors with a large number of somatic mutations may be particularly responsive to these immunotherapies, whether or not they also overexpress checkpoint proteins. Tumor mutational burden (TMB) is defined as the total number of mutations per coding area of a tumor genome and measuring this biomarker is clinically actionable. A subset of patients exhibit high TMB across almost all types of cancer, while each individual type of cancer has a characteristic TMB. Melanoma and NSCLC represent some of the highest TMB indications, which also show the best response to immunotherapies. Co-occurrence of specific mutations with TMB, particularly in DNA repair genes, are correlated with higher TMB, and presumably better response to immunotherapies. High TMB predicts cancer patients who will benefit from a durable clinical benefit from immunotherapy as well as chemotherapy. For example, high TMB is associated with better clinical benefit from pembrolizumab, a PD-1 inhibitor, in NSCLC. Monoclonal antibodies that target immune checkpoints are FDA approved for multiple disease types, including NSCLC, and include blinatumomab, nivolumab, pembrolizumab, atezolizumab, avelumab, ipilimumab, and durvalumab.¹⁶⁹,²⁴¹-²⁵²

The TP53 gene, on chromosome 17, encodes the tumor suppressor P53 that has been termed the “guardian of the genome” due to its central role in maintaining genome integrity. The most frequently reported alterations in TP53 are missense and frame shift mutations, though deletions are also reported. Mutations in TP53 are usually clustered in the DNA binding domain, particularly R175, G245, R248, R249, R273, and R282. Mutations in the tetramerization domain have been reported infrequently, including R317, K320, and R337. In addition to the loss of wild-type protein function, mutations of certain residues also confer oncogenic properties, or gain of function mutations, that promote survival, proliferation, and migration. TP53 mutations are detected in at least 50% of all adult tumors. Gain of function TP53 mutations are associated with poor prognosis in multiple cancers. Some drugs sensitize TP53 deficient cells to DNA damaging agents. However, the predictive significance of TP53 alterations for response to drugs is not conclusively established, due to the inconsistent
categorization of patients with loss or gain of function mutations in TP53 mutated tumors. The approved HDAC inhibitor, vorinostat, has been shown to mediate cell death in TP53 mutant tumors. While TP53 mutations have largely remained non-actionable, development of TP53 based therapeutics is an area of active research. Decreasing expression of mutant P53 in tumor cells by targeting HSP90 and histone deacetylases (HDACs), has been explored as a potential therapeutic avenue, including against TP53 mutant NSCLC. Several other approaches are under study across multiple tumor types as well, including WEE1 or ATR/CHK1 inhibitors, oncolytic viruses targeting TP53 mutated tumor cells, adenoviral delivery of wild-type TP53 into tumor cells, small chaperone peptides to stabilize wild-type P53, and targeting the MDM2-P53 interaction.

Please see our website neogenomics.com for a complete test description and printable specimen requirements.

References


