oncoExTra™

EXACT SCIENCES

Report Date: MM/DD/YYYY

Patient:	Sample Patient	Ordering Client:	Medical Center
Sex at Birth:	Female	Specimen Type:	FFPE Block
DOB:	MM/DD/YYYY	Specimen Site:	Lung
Medical Record #:	MR 000000	Tumor Collection Date:	MM/DD/YYYY
Client Accession #:	CA 000000	Normal Collection Date:	MM/DD/YYYY
Ordering Physician:	Sample Physician	Received Date:	MM/DD/YYYY

Results Snapshot

Analytes sequenced: DNA+RNA+IHC
Actionable Targets: 5 IHC Tested: 1
TMB: Intermediate PD-L1: See Below
MSI: Stable
Clinical Trials: Yes

Diagnosis: **Lung Cancer**

KEY BIOMARKER FINDINGS

KEY BIOMARKERS	FDA-APPROVED DRUGS -for patient's cancer[1]	FDA-APPROVED DRUGS -for another cancer[1]	DRUGS PREDICTED NON-BENEFICIAL/ REDUCED BENEFIT	POTENTIAL CLINICAL TRIALS
TUMOR GENOMIC ALTERATIONS				
ARID1A (S2249*)				Yes
CD74/ROS1 (Fusion)	crizotinib, entrectinib	cabozantinib, ceritinib, lorlatinib		Yes
NF1 (Q369*)		binimetinib, everolimus, temsirolimus, trametinib		Yes
TP53 (I195T)				Yes
TUMOR MUTATION BURDEN (TMB)				
INTERMEDIATE (8 mut/Mb)				No
MICROSATELLITE STATUS (MSI)				
STABLE				No
IHC RESULTS				
PD-L1 (22C3): Low	atezolizumab, durvalumab, nivolumab, nivolumab + ipilimumab, pembrolizumab	dostarlimab-gxly		

HIGH INTEREST BIOMARKERS

As part of the OncoExTra test, key biomarkers relevant in the patient's tumor type have been assessed: ***NTRK1, NTRK2, NTRK3, RET, BRAF, ALK, EGFR, ERBB2, KRAS, MET, ROS1, PD-L1****. If clinically pertinent event(s) in these biomarkers have been identified, the biomarker(s) will appear within the 'Key Biomarker Findings' section of the report. If Biomarkers from this list do not appear, clinically pertinent event(s) have not been identified or fell outside of the OncoExTra reporting thresholds (please see Disclaimer Limitations information).*

[1]The prescribing information for the FDA-approved therapeutic option may not include the associated Key Biomarker.

ADDITIONAL SIGNIFICANT ALTERATIONS

MAP2K4 (R228I)	No

*NOTE: The CD74/ROS1 fusion was detected at both the RNA level and as a structural translocation at the DNA level in the sample. The CD74/ROS1 fusion event is reported in the Key Biomarker Findings section of the report, and the structural translocation at the DNA level of the same is listed in the VUS section to avoid repetition of contents related to therapy and clinical trials.

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Genomic Alterations Detail

Genomic Alteration		Therapeutic Implication	
Alteration:	ARID1A (S2249*)	**Drug**	**Status**
Alteration Type:	Stop Gain	See Clinical Trials Section	
Coordinate:	chr1:27107135		
Allele Frequency:	24%		
Origin:	DNA		
Read Depth:	465		
Location:	20/20		

Biomarker Summary

The AT Rich Interactive Domain 1A (ARID1A) gene encodes BAF250A/ARID1A protein, a member of SWI/SNF ATP-dependent chromatin remodeling complex (Reisman D et al., 2009; PMID: 19234488). ARID1A functions as a tumor suppressor and gate keeper gene; it regulates several biological processes, including cell cycle progression, DNA replication, methylation, and DNA repair, as well as plays a critical role in preventing genomic instability (Clapier CR and Bradley RC., 2009; PMID: 19355820). A subset of non-small cell lung carcinomas harbors mutations in ARID1A, which is implicated in modulating response to immunotherapy in diverse tumors. In one study, ARID1A mutations were present in 7.5% of lung cancers, of which 69% were loss-of-function mutations. Complete loss of ARID1A expression correlated with ARID1A premature-truncating mutations with evidence of biallelic inactivation. Both ARID1A mutations and aberrant expression correlated with a lack of EGFR mutations, frequent TP53 mutations, and increased mutational burden. Further, in patients with ARID1A-mutant tumors, aberrant ARID1A expression correlated with worse overall survival. Lung tumors with diffuse loss of ARID1A expression were predominantly adenocarcinomas, poorly differentiated, almost exclusively from smokers, and enriched for mismatch repair deficiency (Hung YP et al., 2020; PMID: 32572156). Pre-clinical studies have shown that ARID1A-deficient cells show an increased sensitivity to treatment with PARP inhibitors and inhibitors of the PI3K/AKT pathway (Shen J et al., 2015; PMID: 26069190, Samartzis EP et al., 2014; PMID: 24979463). EZH2 inhibition is another therapeutic option for ARID1A-mutated tumors, as it exploits the PRC2 (polycomb repressive complex 2) dependency in these malignancies, leading to synthetic lethality and tumor cell death (Bitler BG et al., 2015; PMID: 25686104, Alldredge JK and Eskander RN, 2017; PMID: 29093822, Kim KH et al., 2015; PMID: 26552009). Lastly, the multi-kinase inhibitor dasatinib has also been shown to cause synthetic lethality in ARID1A-deficient tumors in vitro and in vivo, via the p21/RB1 pathway, and is being evaluated in clinical trials (Miller RE et al., 2016; PMID: 27364904).

Molecular Function

The tumor sample harbors a stop-gain mutation which is predicted to result in premature truncation of the protein, leading to loss of function (Weigert O et al., 2011; PMID: 22585168). Loss of ARID1A function has been correlated with increased cell proliferation, tumor infiltration, higher tumor grade, and poor overall patient survival (Abe H et al., 2012; PMID: 22915242).

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Genomic Alteration	
Alteration:	CD74/ROS1 (Fusion)
Alteration Type:	Fused Genes
Coordinate:	chr5:149784243; chr6:117645578
Transcript ID:	ENST00000009530.7; ENST00000368508.3
Origin:	RNA
Location:	E6; E34

Therapeutic Implication	
Drug	**Status**
cabozantinib (Cometriq)	PREDICTED BENEFICIAL
ceritinib (Zykadia)	PREDICTED BENEFICIAL
crizotinib (Xalkori)	PREDICTED BENEFICIAL
entrectinib (Rozlytrek)	PREDICTED BENEFICIAL
lorlatinib (Lorbrena)	PREDICTED BENEFICIAL

Biomarker Summary

The ROS proto-oncogene 1, receptor tyrosine kinase (ROS1) gene encodes an orphan receptor tyrosine kinase that may activates multiple pathways involved in cell survival and transformation (Davies KD et al., 2013; PMID: 23719267, Giustini NP and Bazhenova L., 2020; PMID: 32327173). ROS1 is activated by chromosomal rearrangement in a variety of human cancers, including NSCLC, cholangiocarcinoma, GBM, ovarian cancer, gastric adenocarcinoma, glioma, colorectal cancer, inflammatory myofibroblastic tumor, angiosarcoma, and epithelioid hemangioendothelioma (Davies K et al., 2013; PMID: 23719267, Dandan S et al., 2018; PMID: 30262706, Davare M et al., 2018; PMID: 30171048). The kinase domains of ALK and ROS1 share 77% amino acid identity within the ATP binding sites and ROS1 rearrangement can be therapeutically targeted by ALK inhibitors such as crizotinib, lorlatinib and ceritinib (Bergethon et al., 2012; PMID: 22215748, Yasuda et al., 2012; PMID: 22617245, Davies et al., 2012; PMID: 22919003, Jun et al., 2012; PMID: 22659450, Shaw et al., 2012; ASCO 2012, Abstract 7508, Chiari et al., 2014; PMID: 25087901). Xalkori (crizotinib) and Rozlytrek (entrectinib) are listed in the guidelines as the preferred first-line therapy for ROS1 rearranged non-small cell lung cancer. In a Phase I (TRIDENT-1) trial, repotrectinib (TPX-0005) treatment resulted in partial response in 21.6% (8/37) of patients with advanced solid tumors harboring ROS1 or NTRK fusions (Drilon A et al., 2018; PMID: 30093503).

Molecular Function

CD74/ROS1 fusion results from fusing exon 6 of CD74 to exon 34 of ROS1. CD74/ROS1 fusions account for 30% of all ROS1 fusions in non-small cell lung cancer (NSCLC) (Jun H et al., 2012; PMID: 22659450). Fusion genes involving the ROS1 gene have been frequently found to have breakpoints in exon 32, 34, or 35, thus retaining the kinase domain of ROS1. Previously, the same breakpoints involving the exon 6 of CD74 and exon 34 of ROS1 has been reported in NSCLC (Cai W et al., 2014; PMID: 24828671). CD74/ROS1 fusion protein was found to be tumorigenic as demonstrated by sustained growth and migratory potential in cells expressing the fusion protein. Further, Rat1 cells expressing CD74/ROS1 were found to be highly invasive and metastatic. Treatment of cells expressing this fusion protein by crizotinib lead to dose dependent reduction in growth (Jun H et al., 2012; PMID: 22659450). Clinical studies on lung cancer patients harboring CD74/ROS1 fusions have demonstrated clinical benefit in response to treatment with therapies that target ROS1, including crizotinib, ceritinib, cabozantinib, loratinib, and entrectinib (Shaw AT et al., 2014; PMID: 25264305, Drilon A et al., 2016; PMID: 26673800, Subbiah V et al., 2016; PMID: 26917690, Solomon BJ et al., 2018; PMID: 30413378, Sehgal K et al., 2020; PMID: 32776005).

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Genomic Alteration	
Alteration:	NF1 (Q369*)
Alteration Type:	Stop Gain
Coordinate:	chr17:29528097
Allele Frequency:	19%
Origin:	DNA
Read Depth:	415
Location:	10/58

Therapeutic Implication	
Drug	**Status**
binimetinib (Mektovi)	PREDICTED BENEFICIAL
everolimus (Afinitor)	PREDICTED BENEFICIAL
temsirolimus (Torisel)	PREDICTED BENEFICIAL
trametinib (Mekinist)	PREDICTED BENEFICIAL

Biomarker Summary

The neurofibromin 1 (NF1) gene encodes a tumor suppressor protein which contains a highly conserved functional GTPase activating domain, and functions to downregulate the activity of RAS, MAPK and mTOR pathways (Gerber PA et al., 2009; PMID: 19380279). Loss of NF1 leads to deregulation of several tumorigenic pathways, particularly the RAS (McGillicuddy LT et al., 2009; PMID: 19573811), and MAPK pathway (Ballester R et al., 1990; PMID: 2121371). Loss of function mutations, as well as copy number loss, of the NF1 gene is reported in several cancer types (Dodd RD et al., 2013; PMID: 23858101, Ding L et al., 2008; PMID: 18948947). In one study, targeted next generation sequencing of non-small cell lung cancer (NSCLC) samples revealed that approximately 10% of tumors harbored NF1 mutations, 15 NF1 mutations (25%) occurred with other oncogenic mutations, including BRAF (2/15), and NF1 tumor pathology was diverse, including both adenocarcinoma (36, 60%) and squamous cell carcinoma (10, 17%) (Redig AJ et al., 2016; PMID: 26861459). Neurofibromin contains a highly conserved functional GTPase activating domain and functions to downregulate the activity of RAS, MAPK and mTOR pathways. Loss of NF1 leads to deregulation of several tumorigenic pathways, particularly the RAS (McGillicuddy LT et al., 2009; PMID: 19573811) and MAPK pathway (Ballester R et al., 1990; PMID: 2121371). Based on results from clinical and pre-clinical studies, tumors with loss of NF1 may be sensitive to mTOR inhibitors, such as everolimus and temsirolimus, and MEK inhibitors (trametinib, cobimetinib and binimetinib) (Maertens O et al., 2013; PMID: 23171796, Dodd RD et al., 2013; PMID: 23858101), which are approved by the FDA. Clinical trials are ongoing for cancer patients with NF1 loss.

Molecular Function

The tumor sample harbors a stop-gain mutation in NF1, which is predicted to result in impaired or loss of functional NF1 protein. Loss of NF1 may result in the activation of several cellular pathways involved in regulation of proliferation and cell cycle control, such as Ras/Raf, PI3K/Akt, and mTOR (Brems H et al., 2009; PMID: 19410195, Morcos P et al., 1996; PMID: 8628317, Lodish MB and Stratakis CA, 2010; PMID: 20833335).

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Genomic Alteration	
Alteration:	TP53 (I195T)
Alteration Type:	Missense
Coordinate:	chr17:7578265
Allele Frequency:	28%
Origin:	DNA
Read Depth:	397
Location:	6/11

Therapeutic Implication	
Drug	**Status**
See Clinical Trials Section	

Biomarker Summary

The tumor protein p53 (TP53) gene encodes a tumor suppressor protein with three main domains: transcriptional activation, DNA binding (DBD) and oligomerization, which keeps the cells under control by inducing cell cycle arrest, apoptosis, senescence, DNA repair or changes in metabolism when cells are stressed or damaged (Wang LH et al., 2018; PMID: 30562755, Yamamoto S and Iwakuma T., 2018; PMID: 30577483). In lung cancer, the squamous cell carcinomas (SCC), and large cell carcinomas (LCC) had a higher frequency of TP53 mutations compared to adenocarcinomas. The TP53 mutation profiles were different in the specific histologies and smoking categories, with a high number of frameshift variants in SCC. LCC with TP53 wild-type showed a significantly reduced survival, with an opposite tendency for AC and SCC. A recent study of non-small-cell lung cancer (NSCLC) patients demonstrated that TP53 mutations are highly recurrent in lung tumors wild type for EGFR or ALK somatic alterations, and mainly cluster in the DNA-binding domain (Zhao J et al., 2019; PMID: 30867754). According to this study, TP53 mutations correlated with clinical features such as adrenal and bone metastasis, infiltrative tumor growth. In addition, TP53 mutation status was a negative prognostic factor with shorter survival time for EGFR/ALK wild-type, advanced NSCLC patients (Zhao J et al., 2019; PMID: 30867754, Jiao XD et al., 2018; PMID: 30089598). Also, TP53 mutations in never-smokers differed from that of ever smokers, supporting the claim that lung cancer in never-smokers is a separate entity in lung cancer. The tobacco consumption was significantly higher among those carrying a TP53 mutation (Halvorsen AR, et al., 2016; PMID: 27242894). At present, there are no approved therapies targeting TP53 alterations, despite their high prevalence in cancer. Tumors with TP53 mutations may be sensitive to the Wee1 inhibitor MK-1775. WEE1 is a serine/threonine protein kinase that phosphorylates cyclin-dependent kinase 1 (Cdk1), and functions at the G2/M checkpoint of mitosis. Preclinical studies have demonstrated that cancer cell viability can be attenuated after Wee-1 inhibition. As such, cancer cells become sensitized to conventional therapy by Wee-1 inhibition, especially in cells with insufficient G1-arrest due to deficient p53 signaling (Wang Y et al., 2004; PMID: 14726685). Currently, clinical trials of WEE1 inhibitor are ongoing for patients with solid tumors (Hirai H et al., 2010; PMID: 20107315, Bridges KA et al., 2011; PMID: 21799033). A phase II study of WEE1 inhibitor AZD1775 plus carboplatin in patients with TP53-mutated refractory ovarian cancer showed median PFS and OS of 5.3 months and 12.6 months, with 2 patients demonstrating ongoing response for more than 31 and 42 months at data cutoff (Leijen S et al., 2016; PMID: 27998224). According to a study, TP53 mutations predict sensitivity to VEGF/VEGFR inhibitors in the clinic (Wheler JJ et al., 2016; PMID: 27466356).

Molecular Function

TP53 (I195T) is a hotspot missense mutation which lies within the L2 loop of DNA-binding domain (DBD) of the Tp53 protein (Bode AM and Dong Z, 2004; PMID: 15510160). It has been reported that I195 is critical for the proper folding and stabilization of DBD (Levine AJ and Vosburgh E, 2008; PMID: 18840714). In vitro studies have shown that I195T results in destabilization of the Tp53 protein, an increased rate of protein aggregation (Friedler A et al., 2003; PMID: 12700230), and reduced DNA binding ability relative to wild-type Tp53 (Friedler A et al., 2002; PMID: 11782540). High-throughput functional studies suggest that I195T leads to loss of Tp53 activity and function (Kato S et al., 2003; PMID: 12826609, Giacomelli AO et al., 2018; PMID: 30224644, Kotler E et al., 2018; PMID: 29979965).

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Additional Significant Alterations Detail

Additional Significant Alteration		Therapeutic Implication
Alteration:	MAP2K4 (R228I)	**Status**
Alteration Type:	Missense	See Clinical Trials Section
Coordinate:	chr17:12013741	
Allele Frequency:	31%	
Origin:	DNA	
Read Depth:	396	
Location:	6/11	

Biomarker Summary

The mitogen-activated protein kinase kinase 4 (MAP2K4) gene encodes a serine/threonine kinase that activates the ERK and JNK kinase pathways. This gene is a tumor suppressor, and loss-of-function mutations in this gene have been reported in multiple cancer types. Cancers that have lost MAP2K4 fail to activate JNK-JUN. Consequently, loss-of-function mutations in MAP2K4 confer sensitivity to MEK inhibition by disabling the JNK-JUN-mediated feedback loop upon MEK inhibition. In a panel of 168 Patient Derived Xenograft (PDX) tumors, MAP2K4 mutation status was found to be a strong predictor of response to MEK inhibition. PDX models having mutations in MAP2K4 were significantly more sensitive to binimetinib than their wild type counterparts, with only the mutant tumors showing a decrease in tumor volume over time (Xue Z et al., 2018; PMID: 29795445).

Molecular Function

MAP2K4 (R228I) is a missense mutation which lies within the protein kinase domain of the Map2k4 protein (Zang ZJ et al., 2011; PMID: 21097718). Although R228KI has not been functionally characterized, a different substitution at the same residue, R228K, results in decreased Map2k4 kinase activity compared to wild-type and demonstrates transforming activity in culture, suggesting it may result in a dominant-negative effect (Kan Z et al., 2010; PMID: 20668451). Loss of MAP2K4 function can result in impaired activity on downstream effector molecules such as c-Jun, p53, ELK1, ATF2 and several other transcription factors involved in apoptosis, cell survival, growth and differentiation (Whitmarsh AJ and Davis RJ., 2007; PMID: 17496914, Wagner EF and Nebreda AR., 2009; PMID: 19629069).

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Drug Evidence Detail

Literature Supporting Therapeutic Implication

Drug	Biomarker	Therapeutic Implication
cabozantinib (Cometriq)	CD74/ROS1 (Fusion)	PREDICTED BENEFICIAL

In a case report, a non-small cell lung cancer patient harboring CD74/ROS1 fusion was treated with cabozantinib, which significantly suppressed tumor growth and relieved respiration symptoms. The patient remained on cabozantinib for 1.5 years, then was treated with crizotinib after tumor progression.

https://pubmed.ncbi.nlm.nih.gov/32103985 *(Wang G et al., Onco Targets Ther. 2020 Feb 11;13:1171-1177)*

A 50 year-old female never smoker with metastatic lung adenocarcinoma involving the pleura was found to have CD74/ROS1 fusion on molecular profiling of the tumor. Patient was treated with crizotinib which resulted in a durable PR, but disease progressed at 18 months. After a total of 26 months on crizotinib, patient developed widespread disease progression. NGS analysis of a repeat biopsy from a growing retroperitoneal lymph node post-progression on crizotinib confirmed persistent expression of the CD74-ROS1 rearrangement. In addition, the deep-sequencing also revealed the another mutation D2033N in the ROS1 kinase domain, which was not detected in pre-crizotinib diagnostic sample from this patient. The D2033N mutation was present at an AF of 14%. Treatment with ROS1 inhibitor cabozantinib was initiated on a phase II clinical trial, which resulted in rapid PR by 4 weeks and confirmed at 8 weeks. At 12 weeks, near CR was achieved with a 92% reduction in disease burden, and patient remained on therapy through 8 months.

https://www.ncbi.nlm.nih.gov/pubmed/26673800 *(Drilon A et al., Clin Cancer Res. 2016 May 15;22(10):2351-8)*

Drug	Biomarker	Therapeutic Implication
ceritinib (Zykadia)	CD74/ROS1 (Fusion)	PREDICTED BENEFICIAL

ROS1 rearranged NSCLC patients (n=32) were enrolled in a phase II ceritinib trial. The ORR was 63% with 1 complete response and 19 partial responses. The median duration of response was 10.0 months. The median PFS was 19.3 months and the median OS was not reached at the time of the data cut off.

https://academic.oup.com/annonc/article/27/suppl_6/1205PD/2800071 *(Lim SM et al., Ann Oncol (2016) 27 (suppl_6): 1205PD)*

A 77 yr old male patient with NSCLC with ROS1 rearranged cancer was treated with ceritinib after progressing on crizotinib. Restaging scans showed a partial response (56% decrease) per RECIST1.1 and MRI showed that his brain metastases decreased as well.

https://www.ncbi.nlm.nih.gov/pubmed/26917690 *(Subbiah V et al., Proc Natl Acad Sci U S A. 2016 Mar 15;113(11):E1419-20)*

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Drug	Biomarker	Therapeutic Implication
crizotinib (Xalkori)	CD74/ROS1 (Fusion)	PREDICTED BENEFICIAL

In a phase 1 study of crizotinib in ROS1 rearranged (49/50) NSCLC patients reported that the objective response was 72%, with 3/50 (6%) complete responses and 33/50 (66%) PR and 9/50 (18%) had SD as best response. The median duration of response was 17.6 months and median PFS was 19.2 months, with 25 patients (50%) still in follow-up for progression. Among 30 tumors that were tested, 7 had ROS1 fusion of which 5 were known fusion partners and 2 were novel partner genes.

http://www.ncbi.nlm.nih.gov/pubmed/25264305 *(Shaw AT et al., N Engl J Med, 2014; 371(21):1963-71)*

In a case report of a 41-year-old Southeast-Asian, non-smoker, female diagnosed with NSCLC, genomic profiling of the tumor detected CD74-ROS1 fusion. Patient was switched to crizotinib from chemotherapy, which resulted in a significant radiographic and clinical response after 8 weeks, which continued till 4 months at the time the report was generated.

https://www.ncbi.nlm.nih.gov/pubmed/28115114 *(Wang V et al., J Thorac Oncol. 2017 Feb;12(2):e19-e20)*

Drug	Biomarker	Therapeutic Implication
entrectinib (Rozlytrek)	CD74/ROS1 (Fusion)	PREDICTED BENEFICIAL

A 22-year-old female patient with no prior medical history was diagnosed with metastatic lung adenocarcinoma. Subsequent evaluation with positron emission tomography/computed tomography scanning confirmed metastatic disease with multiple left lung nodules, left hilar lymphadenopathy, and bone lesions. Pathologic review of a core biopsy specimen from a lung lesion showed lung adenocarcinoma with predominant lepidic pattern. Molecular analysis identified a CD74-ROS1 fusion gene. The patient was treated with radiation therapy to the metastatic lesion in the right eye, followed by first-line crizotinib. Although there was initially a complete response, progression of disease in the brain was documented while she was receiving crizotinib therapy for 10 months. She was then treated with entrectinib for 34 months and after progression, she was treated with brigatinib but follow-up scans showed disease progression in the brain and several osseous disease sites (outside of the radiated field). At the time of brain and bone progression, a secondary ROS1 F2004V mutation was detected via circulating cell-free DNA testing. Besides the CD74-ROS1 fusion and the F2004V secondary mutation, no other mutations were detected in the ROS1 kinase domain or other genes. The patient was enrolled in a clinical trial (NCT03178071) of lorlatinib. Lorlatinib induced a dramatic response in the brain and the systemic disease sites, evident in the first disease evaluation with imaging studies. Disease remains in control 6 months later, while the patient continues receiving lorlatinib.

https://ascopubs.org/doi/full/10.1200/PO.19.00013 *(Dimou A. et al., JCO Precision Oncology 2019 :3,1-6)*

In Phase 1 dose escalation clinical study in patients with advanced solid tumors, 17 patients have been treated with entrectinib (rozlytrek; RXDX-101). A patient with neuroblastoma (ALK+) has a PR. Two patients have prolonged stabilization of their disease and remain on treatment; a patient with NSCLC (ALK+) in cycle 11, and a patient with pancreatic cancer (ROS1+) in cycle 8.

https://ascopubs.org/doi/10.1200/jco.2014.32.15_suppl.2502 *(De Braud FG et al., J Clin Oncol 32:5s, 2014 (suppl; abstr 2502))*

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Drug	Biomarker	Therapeutic Implication
lorlatinib (Lorbrena)	CD74/ROS1 (Fusion)	PREDICTED BENEFICIAL

A 22-year-old female patient with no prior medical history was diagnosed with metastatic lung adenocarcinoma. Subsequent evaluation with positron emission tomography/computed tomography scanning confirmed metastatic disease with multiple left lung nodules, left hilar lymphadenopathy, and bone lesions. Pathologic review of a core biopsy specimen from a lung lesion showed lung adenocarcinoma with predominant lepidic pattern. Molecular analysis identified a CD74-ROS1 fusion gene. The patient was treated with radiation therapy to the metastatic lesion in the right eye, followed by first-line crizotinib. Although there was initially a complete response, progression of disease in the brain was documented while she was receiving crizotinib therapy for 10 months. She was then treated with entrectinib for 34 months and after progression, she was treated with brigatinib but follow-up scans showed disease progression in the brain and several osseous disease sites (outside of the radiated field). At the time of brain and bone progression, a secondary ROS1 F2004V mutation was detected via circulating cell-free DNA testing. Besides the CD74-ROS1 fusion and the F2004V secondary mutation, no other mutations were detected in the ROS1 kinase domain or other genes. The patient was enrolled in a clinical trial (NCT03178071) of lorlatinib. Lorlatinib induced a dramatic response in the brain and the systemic disease sites, evident in the first disease evaluation with imaging studies. Disease remains in control 6 months later, while the patient continues receiving lorlatinib.

https://ascopubs.org/doi/full/10.1200/PO.19.00013 *(Dimou A. et al., JCO Precision Oncology 2019 :3,1-6)*

In a phase 2 global study of lorlatinib in patients (pts) with ALK+ or ROS1+, advanced, NSCLC, with or without CNS metastases, 276 pts were enrolled. Of these, 30 were ALK+ and treatment naive (EXP1); 59 were ALK+ and received previous crizotinib without (n=27; EXP2) or with (n=32; EXP3A) previous chemotherapy; 28 were ALK+ and received one previous non-crizotinib ALK TKI, with or without chemotherapy (EXP3B); 112 who were ALK+ with two (n=66; EXP4) or three (n=46; EXP5) previous ALK TKI with or without chemotherapy; and 47 were ROS1+ with any previous treatment (EXP6). In treatment-naive pts (EXP1), an objective response (OR) was achieved in 27/30 (90.0%) pts. Three pts had measurable baseline CNS lesions per independent central review, and objective intracranial responses were observed in two (66.7%). In ALK+ pts with at least one previous ALK inhibitor (EXP2-5), OR were achieved in 93/198 (47.0%) pts and objective intracranial response in those with measurable baseline CNS lesions in 51/81 (63.0%) pts. OR was achieved in 41/59 (69.5%) pts who had only received previous crizotinib (EXP2-3A), 9/28 (32.1%) pts with one previous non-crizotinib ALK TKI (EXP3B), and 43/111 (38.7%) pts with 2 or more previous ALK TKI (EXP4-5). Objective intracranial response was achieved in 20/23 (87.0%) pts with measurable baseline CNS lesions in EXP2-3A, 5/9 (55.6%) pts in EXP3B, and 26/49 (53.1%) pts in EXP4-5.

https://www.ncbi.nlm.nih.gov/pubmed/30413378 *(Solomon BJ et al., Lancet Oncol. 2018 Dec;19(12):1654-1667)*

Drug	Biomarker	Therapeutic Implication
binimetinib (Mektovi)	NF1 (Q369*)	PREDICTED BENEFICIAL

An investigator-initiated, phase I, dose escalation study of the MEK inhibitor binimetinib combined with pexidartinib was conducted in patients with advanced or metastatic GIST who progressed on imatinib. The primary endpoint was phase II dose determination. A total of 2 patients were treated. Both patients had a best response of stable disease (SD) by RECIST. Progression-free survival (PFS) and overall survival (OS) were 6.1 and 14.6 months, respectively, in one patient with five prior lines of therapy. The second patient with NF1-mutant GIST had a 27% decrease in tumor burden by RECIST and remains on study after 19 months of treatment.

https://www.ncbi.nlm.nih.gov/pubmed/31213500 *(Rosenbaum E et al., Oncologist. 2019 Oct;24(10):1309-e983)*

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Drug	Biomarker	Therapeutic Implication
everolimus (Afinitor)	NF1 (Q369*)	PREDICTED BENEFICIAL

A Phase 1 trial on the combination of pazopanib and everolimus in advanced solid tumors, reported PR in 7.6% (4/52) of patients and SD ≥6 months in 19.2% (10/52) of patients. The clinical benefit rate in patients with activated PI3K/AKT/mTOR pathway was 27% (7/26) versus 26% (5/19) for patients without an alteration (p=0.764); However, 64% of those with clinical benefit rate and molecular testing done for alteration in the PI3K/AKT/mTOR pathway were positive. PR was reported in 33%(1/3) of patients with hepatocellular carcinoma carrying NF1 (R1241) mutation.*

https://pubmed.ncbi.nlm.nih.gov/23407561/ *(Rodrigues HV et al., Invest New Drugs, 2015; 33(3): 700-709)*

A total of 120 participants with a variety of advanced tumor types were included in a clinical study aimed to assess the effective delivery of precision oncology. Of these, 109 (90.8%) had successful molecular profiling (MP). Treatment on the basis of an actionable alteration (AA) was recommended by the MTB in 58% of patients (63 of 109) who had a successful MP result. One small-cell lung carcinoma patient with PTEN loss; NF1 E1206, who went through 1 prior line of treatment was reported to have a stable disease after treatment with everolimus.

https://ascopubs.org/doi/full/10.1200/PO.17.00220 *(Powell SF et al., JCO Precision Oncology - published online May 11, 2018)*

Drug	Biomarker	Therapeutic Implication
temsirolimus (Torisel)	NF1 (Q369*)	PREDICTED BENEFICIAL

A Phase II study of temsirolimus in 55 advanced NSCLC patients reported PR in 4 patients, SD ≥ 8 months in 14 patients, 24-week PFS of 25%, median PFS of 2.3 months and OS of 6.6 months.

https://pubmed.ncbi.nlm.nih.gov/22722792/ *(Reungwetwattana T et al., J Thorac Oncol, 2012; 7(5): 919-22)*

Phase I evaluating the combination of temsirolimus with thoracic radiation in patients with NSCLC reported PR in 37.5% (3/8) of patients and SD in 25% (2/8) of patients.

https://pubmed.ncbi.nlm.nih.gov/24373609/ *(Waqar SN et al., Clin Lung Cancer, 2014; 15(2): 119-23)*

Drug	Biomarker	Therapeutic Implication
trametinib (Mekinist)	NF1 (Q369*)	PREDICTED BENEFICIAL

Six children with sporadic pilocytic astrocytomas (PA) were treated with trametinib, a MEK inhibitor, following progression under conventional therapies. The median age at diagnosis was 2.3 years (y) old [range 11 months (m)- 8.5 y old]. KIAA1549-BRAF fusion was identified in five cases, and hotspot FGFR1/NF1/PTPN11 mutations in one. All patients received at least one previous line of chemotherapy (range 1-4). The median time on treatment was 11 m (range 4-20). Overall, we observed two partial responses and three minor responses as best response; three of these patients are still on therapy.

https://www.ncbi.nlm.nih.gov/pubmed/30097824 *(Kondyli M et al., J Neurooncol. 2018 Nov;140(2):435-444)*

A 56-y-old white male examined for a pigmented lesion of the vertex was diagnosed with melanoma. Patient was treated with pembrolizumab in a randomized trial but showed disease metastasis. Pt. was then treated with ipilimumab + nivolumab for 2 months followed by nivolumab for 3 months. The disease progressed with new metastases to different parts of the body. Chemotherapy was administered until further disease progression. Sequencing revealed 3 mutations in NF1 and two in PTPN11, both of which were considered as potential drivers. NF1 mutation included a frameshift (Q282fs), stop-gain (R440) and a splice site mutation (c.205-1G>C). PTPN11 mutation included a stop-gain mutation (W423*) and a missense mutation T468P. On the basis of the mutation, pt. was treated with trametinib, a MEKi and 6 weeks later, clinically evaluable cutaneous and subcutaneous lesions had visibly regressed and confirmed by PET/CT scan in which no new metastases were observed, and most lesions had regressed, indicating response to trametinib.*

https://ascopubs.org/doi/10.1200/PO.18.00028 *(Py C et al., JCO Precision Oncology 2018 :2, 1-11)*

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Drug	Biomarker	Therapeutic Implication
atezolizumab (Tecentriq)	PD-L1 (22C3) (Low)	PREDICTED BENEFICIAL

In an open-label, phase 2 randomised controlled trial, patients with NSCLC who progressed on post-platinum chemotherapy were stratified by PD-L1 tumor-infiltrating immune cells and randomly assigned (1:1) to receive intravenous atezolizumab 1200 mg (n=142) or docetaxel 75 mg/m(2) (n=135) once every 3 weeks. Overall survival in the intention-to-treat population was 12.6 months (95% CI 9.7-16.4) for atezolizumab versus 9.7 months (8.6-12.0) for docetaxel. Increasing improvement in overall survival was associated with increasing PD-L1 expression.

https://pubmed.ncbi.nlm.nih.gov/26970723 *(Fehrenbacher L et al., Lancet. 2016 Apr 30;387(10030):1837-46)*

Drug	Biomarker	Therapeutic Implication
dostarlimab-gxly (Jemperli)	PD-L1 (22C3) (Low)	PREDICTED BENEFICIAL

In a phase I, muti-center, open-label, 2-part (dose escalation and cohort expansion) trial, 67 patients with recurrent/ advanced non-small cell lung cancer were treated with dostarlimab. Immune-related objective response rate (irORR) was 26.9%, including 2 complete and 16 partial responses. Responses were observed in 2 of 24 (16.7%) patients with PD-L1 TPS < 1%, 4 of 20 (20.0%) patients with PD-L1 TPS 1%-49% and 2 of 5 (40.0%) patients with PD-L1 TPS ≥ 50%. The median duration of response of 11.6 months (range: 2.8-19.4).

https://pubmed.ncbi.nlm.nih.gov/35729005 *(Moreno V et al., Clin Lung Cancer. 2022 May 23:S1525-7304(22)00115-2)*

Drug	Biomarker	Therapeutic Implication
durvalumab (Imfinzi)	PD-L1 (22C3) (Low)	PREDICTED BENEFICIAL

Patients in a randomized, double-blind multicenter phase III trial (PACIFIC) were randomized 2:1 to receive intravenous durvalumab (n = 473), or placebo (n = 236), every 2 weeks for up to 12 months. 63% of patients were assessable for PD-L1 expression on tumor cells (TC). Among patients with known PD-L1 status, 159 (35%) had TC ≥25%, 303 (67%) had TC ≥1%, and 144 (32%) had TC 1%–24%. Among patients with TC ≥25% a median PFS of 17.8 months with durvalumab versus 3.7 months with placebo was observed. Durvalumab improved OS versus placebo in the TC ≥1% subgroup [HR, 0.52 (95% CI, 0.38–0.70)] but not in the TC <1% subgroup [HR, 1.18 (95% CI, 0.73–1.89)].

https://pubmed.ncbi.nlm.nih.gov/32209338 *(Paz-Ares L et al., Ann Oncol. 2020 Jun;31(6):798-806)*

Drug	Biomarker	Therapeutic Implication
nivolumab (Opdivo)	PD-L1 (22C3) (Low)	PREDICTED BENEFICIAL

A randomized, open-label, international phase 3 study, assigned patients with nonsquamous non-small-cell lung cancer to nivolumab or docetaxel. Overall survival was longer with nivolumab than with docetaxel. The median overall survival was 12.2 months among 292 patients in the nivolumab group and 9.4 months among 290 patients in the docetaxel group. At 1 year, the overall survival rate was 51% with nivolumab versus 39% with docetaxel. With additional follow-up, the overall survival rate at 18 months was 39% with nivolumab versus 23% with docetaxel. The response rate was 19% with nivolumab versus 12% with docetaxel (P=0.02). Although progression-free survival did not favor nivolumab over docetaxel (median, 2.3 months and 4.2 months, respectively), the rate of progression-free survival at 1 year was higher with nivolumab than with docetaxel (19% and 8%, respectively). Nivolumab was associated with greater efficacy than docetaxel across all end points in subgroups defined according to prespecified levels of tumor-membrane expression (≥1%, ≥5%, and ≥10%) of the PD-1 ligand.

https://pubmed.ncbi.nlm.nih.gov/26412456 *(Borghaei H et al., N Engl J Med. 2015 Oct 22;373(17):1627-40)*

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Drug	Biomarker	Therapeutic Implication
nivolumab + ipilimumab (Opdivo + Yervoy)	PD-L1 (22C3) (Low)	PREDICTED BENEFICIAL

In an open-label, phase 1, multicohort study (CheckMate 012), patients with chemotherapy-naive advanced NSCLC were randomly assigned to treatment with 3 mg/kg nivolumab every two weeks and 1mg/kg ipilumumab either every 6 (n=31) or 12 weeks (n=30). In patients with 1% or greater tumor PD-L1 expression (21 [68%] of 31 patients in the every-6-weeks cohort and 23 [77%] of 30 in the every-12-weeks cohort), objective responses were achieved in 12 patients in the ipilimumab every-12-weeks cohort and 13 patients in the ipilimumab every-6-weeks cohort (57% in both cohorts). Median progression-free survival in these patients was 8.1 months (95% CI 5.6-not reached) in the ipilimumab every-12-weeks cohort (n=21) and 10.6 months (3.6-not reached) in the ipilimumab every-6-weeks cohort (n=23), and 24-week progression-free survival was 80% (55–92) and 65% (42–81), respectively. The magnitude of clinical benefit achieved with the combination treatment was enhanced with higher PD-L1 expression. Pooling the two cohorts, of the 13 patients with 50% or greater PD-L1 expression (who comprised 21% of the 61 PD-L1-evaluable patients), 12 had a confirmed objective response and one had an unconfirmed response.

https://pubmed.ncbi.nlm.nih.gov/27932067 *(Hellmann MD et al., Lancet Oncol. 2017 Jan;18(1):31-41)*

Drug	Biomarker	Therapeutic Implication
pembrolizumab (Keytruda)	PD-L1 (22C3) (Low)	PREDICTED BENEFICIAL

In a randomized, open-label, phase 2/3 study, patients with previously treated non-small cell lung cancer were allocated to receive pembrolizumab 2 mg/kg (n=345), pembrolizumab 10 mg/kg (n=346), or docetaxel (n=343). In the total population, median overall survival was 10.4 months with pembrolizumab 2 mg/kg, 12.7 months with pembrolizumab 10 mg/kg, and 8.5 months with docetaxel. Median progression-free survival was 3.9 months with pembrolizumab 2 mg/kg, 4.0 months with pembrolizumab 10 mg/kg, and 4.0 months with docetaxel. Among patients with at least 50% of tumor cells expressing PD-L1, overall survival was significantly longer with pembrolizumab 2 mg/kg than with docetaxel (median 14.9 months vs 8.2 months; HR 0.54, 95% CI 0.38–0.77; p=0.0002) and with pembrolizumab 10 mg/kg than with docetaxel (17.3 months vs 8.2 months; 0.50, 0.36–0.70; p<0.0001). Likewise, for this patient population, progression-free survival was significantly longer with pembrolizumab 2 mg/kg than with docetaxel (median 5.0 months vs 4.1 months; HR 0.59, 95% CI 0.44–0.78; p=0.0001) and with pembrolizumab 10 mg/kg than with docetaxel (5.2 months vs 4.1 months; 0.59, 0.45–0.78; p<0.0001).

https://pubmed.ncbi.nlm.nih.gov/26712084 *(Herbst RS et al., Lancet. 2016 Apr 9;387(10027):1540-1551)*

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IHC Score Detail

1 IHC Tested

IHC BIOMARKER	IHC Data	Status	Approved By
PD-L1 (22C3)	%TPS: 5-9	Low	Dr. Sample Pathologist (MM/DD/YYYY)

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Clinical Trials Report

Potential trials based on genomic targets indicated in the OncoExTra™ Report

Genomic Alterations	Targeted Investigational Agents	Trial IDs
ARID1A (S2249*)	AKT inhibitors: (Afuresertib [GSK2110183], Capivasertib [AZD-5363], Ipatasertib [GDC-0068, RG-7440], Miransertib [ARQ092], ARQ751, MK-2206, Triciribine [TCN-P]), ATR inhibitors: (Berzosertib [M 6620, M6620, VX 970, VX970, VE-822], M4344 [VX-803], Ceralasertib [AZD6738], Elimusertib [BAY1895344], RP-3500), ATM kinase inhibitor: (M4076), BET/BRD4 inhibitors: (AZD5153, ZEN-3694 [BETi ZEN-3694], BMS-986158, CPI-0610, PLX51107), EZH2 or EED/PRC2 inhibitors: (Tazemetostat, Valemetostat [DS-3201b, DS-3201], PF-06821497, CPI-0209, EED/PRC2 inhibitor MAK683), mTOR inhibitors: (Everolimus, Temsirolimus, Sapanisertib [INK0128, MLN0128, TAK-228], Vistusertib [AZD2014]), PARP inhibitors: (Niraparib, Olaparib, Rucaparib, Talazoparib, Veliparib [ABT-888], Pamiparib [BGB-290], RBN-2397), Pan-PI3K inhibitors: (Apitolisib [GDC-0980, RG7422]), PI3K/mTOR dual kinase inhibitors: (BEZ235, Gedatolisib [PKI-587, PF-05212384], LY3023414), PIK3Cα/PIK3Cδ inhibitors: (Copanlisib), PIK3Cα inhibitors: (Serabelisib [INK1117, MLN1117, TAK-117]), PIK3Cα/PIK3Cδ/PIK3Cγ inhibitors: (Taselisib [GDC-0032, RG7604]), Src inhibitors: (Dasatinib)	NCT05023655 NCT02264678 NCT02484404 NCT05053971 NCT01582191 NCT03065062
CD74/ROS1 (Fusion)	Dual/Multikinase inhibitors: (Brigatinib, Crizotinib, Ceritinib], Ensartinib [X-396], Repotrectinib [TPX-0005], Lorlatinib, Entrectinib)	NCT01639508 NCT02693535 NCT02927340 NCT04589845 NCT03878524
MAP2K4 (R228I)	MEK inhibitors: (Binimetinib, Cobimetinib, Selumetinib, Trametinib, Mirdametinib [PD-0325901], E6201, Refametinib [BAY 869766, BAY86-9766, RDEA119], Pimasertib [AS703026, MSC1936369B], HL-085), ERK inhibitors: (LY3214996, KO-947, LTT462, Ulixertinib [BVD-523, VRT752271], ASN007, MK-8353),	Not recruiting for tumor type

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Genomic Alterations	Targeted Investigational Agents	Trial IDs
	Dual AKT/ERK inhibitors: (ONC-201), RAF-MEK dual inhibitors: (RO5126766)	
NF1 (Q369*)	EGFR/ERBB2 inhibitors: (Lapatinib), ERK inhibitors: (LY3214996, KO-947, LTT462, Ulixertinib [BVD-523, VRT752271], ASN007, MK-8353, ASTX029), FAK inhibitors: (Defactinib, GSK2256098), MEK inhibitors: (Binimetinib, Cobimetinib, Selumetinib, Trametinib, Mirdametinib [PD-0325901], E6201, Refametinib [BAY 869766, BAY86-9766, RDEA119], Pimasertib [AS703026, MSC1936369B], HL-085), mTOR inhibitors: (Everolimus, Temsirolimus, Sirolimus, Nab-Rapamycin, Ridaforolimus [AP23573, MK8669, Deforolimus], Vistusertib [AZD2014]), SRC inhibitors: (Dasatinib, Saracatinib [AZD0530]), TORC1/2 inhibitors: (Sapanisertib [INK0128, MLN0128, TAK-228])	NCT03520075 NCT05340621 NCT01582191 NCT03065062 NCT03878524 NCT04418167 NCT04683354
TP53 (I195T)	ATR inhibitors: (Berzosertib [M6620, VX-970, VE-822]), Small molecule inhibitor: (AMG 650), TP53 reactivator: (SGT-53), WEE1 inhibitors: (Adavosertib [AZD-1775, MK-1775])	NCT04216316 NCT04802174 NCT02595931

Disclaimer:

These clinical trial results were procured by keyword search on www.ClinicalTrials.gov, last updated on MM/DD/YYYY. The information contained in this site changes frequently and may be out of date. Search terms were based on alterations identified in the OncoExTra Report, drugs indicated in the OncoExTra Report, and the reported cancer type of the patient. The search strategy was not exhaustive and may not have retrieved every relevant trial for this patient. Healthcare professionals are encouraged to investigate other possibilities through additional searches at this site. The identified trials may have specific inclusion or exclusion criteria that would make a trial inappropriate for the patient. Consideration of any listed option should be made in the context of the patient's complete medical history.

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Variants of Unknown Significance

Alteration	Alteration Type	Allele Freq
ACAP2 (L768R)	Missense	7
ADCK5 (L554P)	Missense	6
ADNP2 (S910F)	Missense	38
AKAP13 (V2409A)	Missense	37
ALB (L55V)	Missense	30
ALDOC (R149H)	Missense	40
ANK3 (D2040N)	Missense	37
ATP5SL (N234S)	Missense	45
C18orf25 (c.1023-1G>A)	Splice Acceptor Variant	35
C9orf78 (G13D)	Missense	10
CAMSAP2 (H381Y)	Missense	30
CCZ1	Deletion	.
CD74_ROS1	Breakpoint: Translocation	.
CNPY3 (R121Q)	Missense	39
COL6A6 (D920N)	Missense	36
CPAMD8 (E787Q)	Missense	6
DAB1 (R124L)	Missense	7
FAT4 (A423S)	Missense	10
FGF2 (M218I)	Missense	33
FZD8 (D576N)	Missense	34
GNA13 (R200T)	Missense	36
HSP90AB1 (K64fs)	Frameshift	35
HSPB1 (W95*)	Stop Gain	78
KIF27 (Q843H)	Missense	34
MACC1 (S61F)	Missense	61
MROH8 (S456T)	Missense	36
MYLK (E969Q)	Missense	38
NFKBIA (c.834G>A)	Protein Protein Contact	41
NUP50	Breakpoint: Translocation	.
OR5T3 (S165N)	Missense	39
OR9G1 (R169C)	Missense	29
PAFAH1B2 (V122I)	Missense	35
POLR2J3	Deletion	.
PRAMEF1 (E416Q)	Missense	5
PRAMEF13 (E416Q)	Missense	34
PRRC2B (Q1997E)	Missense	5
PRSS12 (G826R)	Missense	17
PSD3	Breakpoint: Translocation	.
RECQL5 (S77C)	Missense	6
RP11-514P8.6	Deletion	.
RP11-514P8.7	Deletion	.
SENP8 (L6F)	Missense	35
SERPINB1 (*380Yext*?)	Stop Lost	34
SERPINB1 (D303N)	Missense	35
SLC2A4RG (R317H)	Missense	11
SPAG11B	Deletion	.
SVEP1 (T3461A)	Missense	32
TOMM40 (S320T)	Missense	43
TTC12 (S549C)	Missense	35
TULP4 (R1338Q)	Missense	25
USP36 (S1120G)	Missense	38
USP37 (L709V)	Missense	35
ZNF260 (E31Q)	Missense	40
ZNF503 (E105K)	Missense	36
ZNF589 (S242L)	Missense	40

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IHC Thresholds

IHC Biomarker	IHC Clone	Negative	Equivocal/Not Significant	Positive
PD-L1 (22C3) TPS	22C3	0 and <1	Not Applicable	≥1 and ≤49 (Low) ≥50 (High)

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General Information

Methodology:
OncoExTra Test is a Next Generation Sequencing tumor/normal exome and tumor RNA Seq assay that provides for the detection of substitutions, insertions, deletions, copy number events, and fusions in tumor tissue. MET exon 14 skipping, AR-v7, and EGFRvIII variants are also detected in RNA. Genomic DNA is extracted from the patient's normal and tumor samples. The isolated DNA is then prepared using a custom xGen target capture (IDT). This library preparation includes shearing, purification, adaptor ligation and PCR amplification. Total RNA is extracted from the patient's tumor sample. The isolated RNA is then prepared using KAPA HyperPrep with Riboerase (Kapa Biosystems). Libraries are then clustered on a flow cell and sequenced using the Illumina NovaSeq 6000.
Sequence data are analyzed using various validated bioinformatics tools and custom Next Generation Sequencing pipeline NG2-LDT 1.1.2. The reference genome assembly used for alignment is NCBI GRCh37. Each tumor's cancer-specific mutations are then queried against a proprietary gene-drug database based on peer reviewed literature to identify potential therapeutic associations.
Copy number events (amplifications/deletions) reported are focal in nature (<25mb).
Allele frequency is dependent on tumor purity. Tumor purity is not taken into account when reporting allele frequencies.
Tumor Mutation Burden (TMB) is determined by measuring the number of somatic mutations occurring in sequenced genes, counting all mutations expected to change the amino acid sequence of the impacted protein. TMB results are rounded to the nearest integer and are classified as follows: TMB-High: ≥ 20 mutations per megabase (mut/Mb); TMB-Intermediate: 6-19 mut/Mb inclusive; TMB-Low: ≤ 5 mut/Mb. "Indeterminate" results may be due to poor sample quality or sequencing coverage. MSI is calculated by scanning certain indels indicative of microsatellite instability. If the number of these, exome wide, is ≥5, then the sample is declared to be "MSI-High". Otherwise, the sample is labelled "MSI-Stable".
Mean target coverage for tumor sample DNA averages 440x (unique reads). Tumor sample RNA averages 121 million reads.

Immunohistochemistry:
IHC testing is performed on formalin fixed paraffin-embedded tissue (FFPE) utilizing the detection method of avidin-biotin free polymer and is employed according to an optimized protocol. HER2 testing meets the 2018 ASCO-CAP HER2 testing guidelines in breast cancer and results are reported using the ASCO/CAP scoring criteria as defined as defined in the IHC Thresholds table appearing at the end of the report. For ER and PR, historical cut-offs for all non-breast tissues are followed.
The following are the antibody clones for each test: Anti HER2/neu (4B5); ER (SP1); PR (1E2).
These assays have not been validated on decalcified specimens.
External tissue controls are performed and reviewed on all stains for appropriate positive and negative immunoreactivity and found to be acceptable.
If HER2 by FISH is required, it is currently being performed by PhenoPath: 1737 Airport Way S, Ste 201 Seattle, WA 98134. HER2 FISH testing and scoring by PhenoPath is being completed according to the 2018 ASCO-CAP Guidelines, with its methodology listed in their final report. A copy of the final FISH report is stored and can be provided by Exact Sciences/GHI upon request.

Limitations:
Samples with a tumor content of less than 20% may have reduced sensitivity and lead to false negative results. It is also possible that the sample contains a mutation below our established limit of detection (1% allele frequency in hotspots, 5% in other regions), or in a region excluded by our assay.
Alterations present in repetitive or high GC content region or non-coding areas may not be detected. Indels larger than 40bp may not be detected. Copy number signal relative to background noise inherent in DNA from FFPE samples may affect sensitivity of reporting amplifications/deletions. Some gene rearrangements like internal tandem duplications (ITD) involving FLT3 and BCOR may not be reliably detected by the test.
The lack of a variant call does not necessarily indicate the absence of a variant since technical limitations to acquire data in some genetic regions may limit assay detection.
Given the nature of RNA isolated from FFPE, sequencing failures may be seen with highly degraded samples, as they may produce sequence reads too short to align informatically.
Previously unspecified fusions cannot be called by the informatics pipeline if the partner genes occur between two closely adjacent genes on the same strand of the same chromosome. In addition, some fusions that are important in hematolymphoid malignancies, including those involving IGH, are difficult to detect with short read sequencing and may be better detected by other modalities.

Disclaimer:
This report does not make any promise or guarantee that a particular drug or treatment regimen will be effective or helpful in the treatment of disease in any patient. This report also makes no promise or guarantee that a drug with a potential clinical benefit will in fact provide a clinical benefit or that a drug with potential lack of clinical benefit will in fact provide no clinical benefit. Exact Sciences expressly disclaims and makes no representation or warranties whatsoever relating, directly or indirectly, to this review of evidence or identified scientific literature, the conclusions drawn from it or any of the information set forth in this report that is derived from such review, including information and conclusions relating to therapeutic agents that are included or omitted from this report. This assay has not been validated on decalcified tissues. Results should be interpreted with caution given the possibility of false negative results on decalcified specimens.

The tests included in this report were developed, and their performance characteristics determined by Exact Sciences. They have not been cleared or approved by the US Food and Drug Administration. The test has been validated as a Laboratory Developed Test per institutional and applicable CLIA regulation (CLIA# 03D2048606) and College of American Pathology (CAP# 8869063) as qualified to perform high complexity clinical laboratory testing. Data interpretations are based on our current understanding of genes and variants as of the report date. Alterations are listed alphabetically and not in order of strength of evidence or appropriateness for the patient's disease. When the report does identify variants with therapeutic implications, this does not promise or guarantee that a particular drug or treatment regimen will be effective or helpful in the treatment of disease in any patient, and the selection of any drug for patient treatment is done at the discretion of the treating physician.

General genomic alterations should be considered in the context of the patient's history, risk factors and any previous genomic testing. Consideration of Variants of Unknown Significance (VUS) may associate with potential therapies in the future. Exact Sciences does not update reports or send notification regarding reclassification of these alterations.

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Electronically Signed By

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