Diagnosis: LUNG, LEFT UPPER LOBE, NEEDLE CORE BIOPSY OF MASS:
PRIMARY LUNG ADENOCARCINOMA.

Primary Tumor Site: Lung **Tumor Content (%):** 5 **Block #:** A1

AuraSeq Fusions Test **Results**

POSITIVE Genes for Detected Clinical Variants: ***ROS1-Fusion; PD-L1 by IHC (see below)***

NEGATIVE Genes (Undetectable, disease-specific): ***EGFR, BRAF, KRAS, METex14, ERBB2, and ALK, RET, FGFR, NTRK-Fusions***

Approved Therapies Associated with Response: ***2***

Summary of Clinically Significant Variants

Gene Variant	FDA Approved Therapies (for patient's tumor type)	FDA Approved Therapies (for other tumor types)	Therapies with Resistance	Potential Clinical Trials (by Gene)
ROS1-Fusion CD74(6) - ROS1(34) -	Crizotinib (XALKORI) Entrectinib (ROZLYTREK)	None	Afatinib (GILOTRIF) Erlotinib (TARCEVA) Gefitinib (IRESSA) Osimertinib (TAGRISSO)	NCT02465060 NCT02693535 NCT03994796 NCT04589845 NCT03391869

*****Full PD-L1 report attached to this NGS report*****

Tier I and II Variant Details

Gene Symbol	*Exon*	*Nucleotide Change*	*Amino Acid Change*	*VAF (%)*	*Effect on Protein*	*Category*
ROS1-Fusion	C6R34	CD74(6) - ROS1(34)	-	N/A	Gain-of-function	Tier I - Level A

The c-ROS proto-oncogene 1 (ROS1) gene is a human homolog of the avian sarcoma virus UR2 transforming gene v-ros and encodes a receptor tyrosine kinase (RTK) of the insulin receptor subfamily. C-ROS is an orphan RTK that is highly expressed in lung tissue. ROS1 has been best characterized as an oncogene in non-small cell lung cancer (NSCLC) and cholangiocarcinoma, where the cytoplasmic kinase domain of c-ROS is fused to other N-terminal partner genes (1). Phosphorylation of cytoskeleton proteins and activation of the PI3K pathway are mainly responsible for the transforming capacity of ROS1 gene fusions (1). Similar ROS1 fusions have been identified in NSCLC, cholangiocarcinoma, and ovarian cancer and are suggested to possess oncogenic potential (2). Crizotinib was originally designed as an inhibitor for c-MET and ALK kinases (3), and it also has inhibitory effect on ROS1 (4). Recent studies have also shown that lorlatinib has similar efficacy to other ROS1 inhibitors in ROS1-rearranged NSCLC patients (5) and that ceritinib demonstrated potent clinical activity in such patients (6).
References: 1- Expert Rev Anticancer Ther. 2012;12(4):447-56; 2- Proc Natl Acad Sci U S A. 2003;100(3):916-21; 3- Cancer Res. 2007;67 (9):4408-17; 4- Cancer Res. 2011;71(14):4920-31; 5- Nat Rev Clin Oncol. 2020;17(1):7; 6- J Clin Oncol. 2017;35(23):2613-2618.

Details on FDA approved Therapies (with Response or Resistance) and Clinical Trials

Crizotinib (XALKORI)

Crizotinib (XALKORI) is a kinase inhibitor indicated for the treatment of patients with metastatic non-small cell lung cancer (NSCLC) whose tumors are anaplastic lymphoma kinase (ALK) or ROS1-positive as detected by an FDA-approved test. For the complete drug label text, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/202570s023lbl.pdf

Entrectinib (ROZLYTREK)

Entrectinib (ROZLYTREK) is a kinase inhibitor indicated for the treatment of adult patients with metastatic non-small cell lung cancer (NSCLC) whose tumors are ROS1-positive. Also for adult and pediatric patients 12 years of age and older with solid tumors that have a neurotrophic tyrosine receptor kinase (NTRK) gene fusion without a known acquired resistance mutation, or aer metastatic or where surgical resection is likely to result in severe morbidity, and have progressed following treatment or have no satisfactory alternative therapy. For the complete drug label, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/212725s000lbl.pdf

Afatinib (GILOTRIF)

Afatinib (GILOTRIF), an inhibitor of the receptor tyrosine kinase epidermal growth factor receptor (EGFR). FDA approved for the treatment of patients with metastatic non-small cell lung cancer (NSCLC) who have tumors with EGFR exon 19 deletions or exon 21 (L858R) substitution mutations, as detected by an FDA-approved test. The safety and efficacy of the drug in patients who have other EGFR mutations have not been established. For the complete drug label, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/201292s000lbl.pdf

Erlotinib (TARCEVA)

Erlotinib (TARCEVA), is a kinase inhibitor of EGFR. FDA approved for first-line treatment of patients with metastatic non-small cell lung cancer (NSCLC) whose tumors have EGFR exon 19 deletions or exon 21 (L858R) substitution mutations as detected by an FDA-approved test. For the complete drug label, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2010/021743s14s16lbl.pdf

Gefitinib (IRESSA)

Gefitinib (IRESSA), a tyrosine kinase inhibitor of numerous kinases, including EGFR. FDA-approved for first-line treatment of patients with metastatic non-small cell lung cancer (NSCLC) whose tumors have epidermal growth factor receptor (EGFR) exon 19 deletions or exon 21 (L858R) substitution mutations as detected by an FDAapproved test. For the complete drug label, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/206995s000lbl.pdf

Osimertinib (TAGRISSO)

Osimertinib (TAGRISSO) is a selective kinase inhibitor of EGFR. FDA-approved for the treatment of patients with metastatic EGFR T790M mutation-positive non-small cell lung cancer (NSCLC), as detected by an FDA-approved test, who have progressed on or after EGFR TKI therapy. For the complete drug label, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/208065s000lbl.pdf

NCT02465060

Title: NCI-MATCH: Targeted Therapy Directed by Genetic Testing in Treating Patients With Advanced Refractory Solid Tumors, Lymphomas, or Multiple Myeloma. This phase II trial studies how well treatment that is directed by genetic testing works in patients with solid tumors or lymphomas that have progressed following at least one line of standard treatment or for which no agreed upon treatment approach exists. Genetic tests look at the unique genetic material (genes) of patients' tumor cells. Patients with genetic abnormalities (such as mutations, amplifications, or translocations) may benefit more from treatment which targets their tumor's particular genetic abnormality. Identifying these genetic abnormalities first may help doctors plan better treatment for patients with solid tumors, lymphomas, or multiple myeloma. For more information contact Comprehensive Cancer Centers of Nevada - Henderson, Henderson, NV, 89052. Principal Investigator: John A. Ellerton, phone: 805-474-9143.

NCT02693535

Title: TAPUR: Testing the Use of Food and Drug Administration (FDA) Approved Drugs That Target a Specific Abnormality in a Tumor Gene in People With Advanced Stage Cancer (TAPUR). The purpose of the study is to learn from the real world practice of prescribing targeted therapies to patients with advanced cancer whose tumor harbors a genomic variant known to be a drug target or to predict sensitivity to a drug. For more information contact: The Angeles Clinic and Research Institute, A Cedars-Sinai Affiliate, Los Angeles, CA, 90025; Contact: Saba Mukarram; phone: 310-231-2181; e-mail: smukarram@theangelesclinic.org; Principal Investigator: Omid Hamid, MD

NCT03994796

Title: Genetic Testing in Guiding Treatment for Patients With Brain Metastases.This phase II trial studies how well genetic testing works in guiding treatment for patients with solid tumors that have spread to the brain. Several genes have been found to be altered or mutated in brain metastases such as NTRK, ROS1, CDK or PI3K. Medications that target these genes such as abemaciclib, GDC-0084, and entrectinib may stop the growth of tumor cells by blocking some of the enzymes needed for cell growth.This study is sponsored by NCI and Genentech, Inc. For further informations contact Cancer Center at Saint Joseph's, Phoenix, AZ, 85004; phone: 602-406-8222; Principal Investigator: Richard L. Deming

NCT04589845

Title: Tumor-Agnostic Precision Immuno-Oncology and Somatic Targeting Rational for You (TAPISTRY) Platform Study.TAPISTRY is a Phase II, global, multicenter, open-label, multi-cohort study designed to evaluate the safety and efficacy of targeted therapies or immunotherapy as single agents or in rational, specified combinations in participants with unresectable, locally advanced or metastatic solid tumors determined to harbor specific oncogenic genomic alterations or who are tumor mutational burden (TMB)-high as identified by a validated next-generation sequencing (NGS) assay. Participants with solid tumors will be treated with a drug or drug regimen tailored to their NGS assay results at screening. Participants will be assigned to the appropriate cohort based on their genetic alteration(s). Treatment will be assigned on the basis of relevant oncogenotype, will have cohort-specific inclusion/exclusion criteria, and, unless otherwise specified, will continue until disease progression, loss of clinical benefit, unacceptable toxicity, participant or physician decision to discontinue, or death, whichever occurs first. This study is sponsored by Hoffmann-La Roche. For further informations contact: Comprehensive Cancer Centers of Nevada - Eastern Avenue, Las Vegas, NV, 89169; Reference Study ID Number: BO41932 www.roche.com/about_roche/roche_worldwide.htm 888-662-6728 (U.S. and Canada) Global-Roche-Genentech-Trials@gene.com.

NCT03391869

Title: Randomized Phase III Trial of Local Consolidation Therapy (LCT) After Nivolumab and Ipilimumab for Immunotherapy-Naive Patients With Metastatic Non-Small Cell Lung Cancer (LONESTAR) -Strategic Alliance: BMS

Tier III: Variants of Unknown Significance (VUS)

None Detected

Methods and Limitations

Genomic DNA extracted from this patient's sample was used for multiplex PCR amplification using the Oncomine Focus Assay (OFA) DNA primers and Ion AmpliSeqTM reagents to detect somatic single nucleotide variants (SNVs) and small insertions and deletions (Indels) located in over 960 different hotspots (covered by 110 amplicons) on the 35 genes described in the Genes Tested for Hotspots section below. Also, somatic copy number variants (CNVs) for the 19 genes described in the Genes Tested for CNVs section below can be detected. In addition, total RNA was co-extracted from the same patient's sample and was reverse transcribed and amplified using the OFA RNA primers and Ion AmpliSeqTM reagents to detect fusions of 23 different gene drivers (listed in the Gene Fusion Drivers section below) with multiple partners, covering over 270 possible fusions. All these genes are involved in pathways that have been associated with cancer and response to certain targeted therapies. Amplicons were sequenced using next generation sequencing (NGS) technology on the Ion Torrent S5 XL sequencer and analyzed with Torrent Suite Software (version 5.8.0). The Feb. 2009 assembly of the human genome (hg19, GRCh37) is used as a reference. The DNA sequences for this panel of genes can be found at http://www.ncbi.nlm.nih.gov/refseq/rsg/ using the accession numbers listed in the Genes Tested sections below. Variant nomenclature is based on the convention recommended by the Human Genome Variation Society (http://varnomen.hgvs.org/). The genes included are not sequenced in their entirety. Somatic SNVs and small Indels outside the 110 amplicons included in this assay will not be detected. This assay performs with 100% (95%CI 76.8%-100.0%) sensitivity and 100% (95%CI 94.4%-100.0%) specificity for SNVs; 100% (95%CI 71.5%-100.0%) sensitivity and 100% (95%CI 93.3%-100.0%) specificity for small Indels; 100% (95%CI 82.4%-100.0%) sensitivity and 100% (95%CI 92.1%-100.0%) specificity for CNVs; and 100% (95%CI 47.8%-100.0%) sensitivity and 98.3% (95%CI 90.9%-100.0%) specificity for gene fusions. The specimen is judged undetectable for any of the tested genes if SNVs are present at a frequency below 5-6% of total DNA, at a mean coverage of 1000X or higher, and a minimum coverage of 500X. Small Indels are detected when present at a minimum frequency of 10% of total DNA, at a mean coverage of 2000X and a minimum coverage of 500X. CNVs can be detected at a minimum gain of 4x on samples with a minimum of 20% neoplastic nuclei. This is a qualitative assay so the variant allele frequency (VAF) values are estimated. This report includes variants, and their estimated VAF, classified based on a tiered evidence-based system according to AMP, ASCO, and CAP guidelines (PMID: 27993330), as follows:

- Tier I: Variants of Strong Clinical Significance (therapeutic, prognostic & diagnostic) with Level A (FDA approved therapies, and included in professional guidelines) or Level B (well-powered studies with consensus from experts in the field, including potential germline pathogenic variants associated with cancer predisposition) evidence.
- Tier II: Variants of Potential Clinical Significance (therapeutic, prognostic & diagnostic) with Level C (FDA approved therapies for different tumor types or investigational therapies, and/or included in multiple small published studies with some consensus) or Level D (preclinical trials or a few case reports without consensus) evidence.
- Tier III: Variants of Uncertain Significance (VUS).
- Benign and likely-benign variants (Tier IV), or UNDETECTABLE variants are NOT reported.

This test is performed on DNA isolated from the tumor sample only, therefore the somatic, or germline, nature of reported variants is inferred. FDA approved drugs and clinical trials listed in this report are referenced for information purposes only and are not guaranteed to offer clinical benefit to the patient. Treatment decision responsibilities reside entirely with the treating physician.

Genes Tested

Genes Tested for Hotspots
AKT1 (NM_005163.2), ALK (NM_004304.4), AR (NM_000044.4), BRAF (NM_004333.4), CDK4 (NM_000075.3), CTNNB1 (NM_001904.3), DDR2 (NM_006182.2), EGFR (NM_005228.3), ERBB2 (NM_004448.2), ERBB3 (NM_001982.3), ERBB4 (NM_005235.2), ESR1 (NM_001122740.1), FGFR2 (NM_000141.4), FGFR3 (NM_001163213.1), GNA11 (NM_002067.2), GNAQ (NM_002072.3), HRAS (NM_005343.2), IDH1 (NM_005896.2), IDH2 (NM_002168.3), JAK1 (NM_002227.2), JAK2 (NM_004972.3), JAK3 (NM_000215.3), KIT (NM_000222.2), KRAS (NM_033360.3), MAP2K1 (NM_002755.3), MAP2K2 (NM_030662.3), MET (NM_001127500.1), MTOR (NM_004958.3), NRAS (NM_002524.4), PDGFRA (NM_006206.4), PIK3CA (NM_006218.2), RAF1 (NM_002880.3), RET (NM_020975.4), ROS1 (NM_002944.2), SMO (NM_005631.4).

Genes Tested for CNVs
ALK (NM_004304.4), AR (NM_000044.4), BRAF (NM_004333.4), CCND1 (NM_053056.2), CDK4 (NM_000075.3), CDK6 (NM_001145306.1), EGFR (NM_005228.3), ERBB2 (NM_004448.2), FGFR1 (NM_001174067.1), FGFR2 (NM_000141.4), FGFR3 (NM_001163213.1), FGFR4 (NM_213647.1), KIT (NM_000222.2), KRAS (NM_033360.3), MET (NM_001127500.1), MYC (NM_002467.4), MYCN (NM_005378.4), PDGFRA (NM_006206.4), PIK3CA (NM_006218.2).

Gene Fusion Drivers
ABL1 (NM_005157.4), ALK (NM_004304.4), AKT3 (NM_005465.5), AXL (NM_021913.5), BRAF (NM_004333.4), EGFR (NM_005228.3), ERBB2 (NM_004448.2), ERG (NM_182918.4), ETV1 (NM_004956.5), ETV4 (NM_001986.2), ETV5 (NM_004454.3), FGFR1 (NM_001174067.1), FGFR2 (NM_000141.4), FGFR3 (NM_001163213.1), MET (NM_001127500.1), NTRK1 (NM_001012331.1), NTRK2 (NM_006180.4), NTRK3 (NM_001012338.2), PDGFRA (NM_006206.4), PPARG (NM_138712.3), RAF1 (NM_001354689.1), RET (NM_020975.6), ROS1 (NM_002944.2).

Electronically Signed By:

Dinesh Pradhan M.D.

Signed Date: 05-21-2021

Electronically Signed By:

Catherine I. Dumur, Ph.D., HCLD(ABB)

Signed Date: 05-21-2021

Disclaimer

This test is a Laboratory Developed Test that was developed and its performance characteristics were determined by the Bernhardt Laboratories at Aurora Diagnostics. This test has not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA does not require this test to go through pre-market review. This test is used for clinical purposes and should not be regarded as investigational or for research. The Molecular Diagnostics Laboratory is certified under Clinical Laboratory Improvement Amendment of 1988 as qualified to perform high complexity clinical laboratory testing.

Testing Performed : Aurora Diagnostics , 5008 Mustang Road, Jacksonville, FL 32216. CLIA # 10D0645099.

PDL1 RESULTS:

Specimen: Lung LEFT UPPER LOBE MASS

PDL1 Clone 22C3

Diagnosis

PD-L1 IMMUNOHISTOCHEMISTRY (clone 22C3; KEYTRUDA®) (performed on block A1)

Results:

PD-L1 Tumor Proportion Score (TPS): 3 %
Interpretation: Positive for PD-L1 expression (> or = 1%)

Assay Comment

Staining for programmed death ligand-1 (PD-L1, Clone 22C3) was performed at LMC Pathology Services.Stain was scored by a pathologist using manual microscopy. The percentage of tumor cells with staining is reported following the FDA-approved protocol.

Intended Use

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), gastric or gastroesophageal junction (GEJ) adenocarcinoma, cervical cancer and urothelial carcinoma tissues using EnVision FLEX visualization system on Autostainer Link 48.

PD-L1 protein expression in NSCLC is determined by using Tumor Proportion Score (TPS), which is the percentage of viable tumor cells showing partial or complete membrane staining at any intensity. The specimen should be considered to have PD-L1 expression if TPS > or = 1% and high PD-L1 expression if TPS > or = 50%.

PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying NSCLC patients for treatment with KEYTRUDA (pembrolizumabñ). See the KEYTRUDA product label for expression cutoff values guiding therapy in specific clinical circumstances.

Some of the these immunohistochemical stains may not have been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88) as qualified to perform high complexity clinical laboratory testing.

Electronically Signed By:

Trevor Caldwell M.D.

Signed Date: 05-11-2021