BERNHARDT LABORATORIES
A Sonic Healthcare Anatomic Pathology Practice

MOLECULAR PATHOLOGY REPORT

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CLIA # 10D0645099 ILLUMINATING THE PATH TO BETTER HEALTH

Patient: DOE, JOHN
DOB: 01/01/1922 Age: 100
MRN: 123456 Chart#:
Gender: M LMP:
Ph:
Ref. Case#: C22-1234

Case No: BMD2022-999999
Collection Date: 08/16/2022
Received Date: 08/29/2022
Report Date: 09/07/2022
Copy To:

Client: **HOSPITAL**
Physicians: TBD, MD
123 Main Street, Suite 123
JACKSONVILLE FL 12345
Ph: **(555) 123-1234**

Diagnosis: Adenocarcinoma

Primary Tumor Type: Colorectal **Tumor Content (%):** 50 **Block #:** A1

AuraSeq Comprehensive Test	RESULTS
POSITIVE Genes for Detected Clinical Variants:	***KRAS, PIK3CA, BCOR, TP53, ROS1-Fusion, CDKN2A(loss)***
NEGATIVE Genes (Undetectable, disease-specific):	*NRAS, HRAS, BRAF, ERBB2, and NTRK1/2/3-Fusions*
GENOMIC Signatures:	**TMB-H MSI-H HRD-NEG**
OTHER Relevant Findings:	**PD-L1>1% by IHC**

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)
CDKN2A Loss (0.7x) -	None	None	None	2
KRAS c.35G>C p.G12A	Ulixertinib (BVD-523)	Binimetinib (MEKTOVI) Cobimetinib (COTELLIC) Trametinib (MEKINIST) Selumetinib (KOSELUGO)	Cetuximab (ERBITUX) Panitumumab (VECTIBIX)	3
PIK3CA c.1030G>A p.V344M	None	None	Cetuximab (ERBITUX) Panitumumab (VECTIBIX)	4
PIK3CA c.3140A>G p.H1047R	None	Alpelisib (PIQRAY) Idelalisib (ZYDELIG)	Cetuximab (ERBITUX) Panitumumab (VECTIBIX)	4
BCOR c.4376A>G p.N1459S	None	None	None	2

Summary of Clinically Significant Variants (continued)

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)
TP53 c.818G>A p.R273H	None	None	None	2
ROS1-Fusion CD74(6) - ROS1(34) -	None	Crizotinib (XALKORI) Entrectinib (ROZLYTREK) Repotrectinib (TPX-0005)	None	4
HRD Score=0 NEGATIVE	None	None	None	5
MSI - MSI-HIGH	Pembrolizumab (KEYTRUDA) Nivolumab (OPDIVO)	None	None	6
TMB 17 mut/MB TMB-HIGH	Pembrolizumab (KEYTRUDA)	None	None	9

Tier I and II Variant Details

Gene Symbol	Exon	Nucleotide Change	Amino Acid Change	VAF (%)	Effect on Protein	Category
KRAS	2	c.35G>C	p.G12A	22.3	Gain-of-function	Tier I - Level A

The KRAS (homologous to the oncogene from the Kirsten rat sarcoma virus) protein and other members of the RAS family are central mediators downstream of growth factor receptor signaling and therefore are critical for cell proliferation, survival, and differentiation. Somatic missense mutations in the KRAS gene lead to single amino acid substitutions and are generally independent of EGFR mutations. The most frequent alterations are detected in codons 12 (~82% of all reported KRAS mutations) and 13 (~17%) in exon 2 of the KRAS gene. Mutations in other positions, such as codons 61 and 146, have also been reported; however, these alterations account for a minor proportion (1–4%) of KRAS mutations. KRAS mutations in codons 12 and 13 appear to play a major role in the progression of colorectal cancer (CRC). KRAS mutations are found in approximately 30-40% of patients with CRC, and it has been shown that tumors harboring KRAS gene mutations in exons 2, 3, or 4 are associated with poor response to EGFR targeted therapies such as panitumumab or cetuximab (1,2). A subsequent study, however, has shown that the use of cetuximab was associated with longer overall survival (OS) in CRC patients with tumors harboring the KRAS NM_ 004985.3: c.38G>A (p.Gly13Asp) mutation, commonly known as p.G13D, than other KRAS-mutated tumors (3).
References: 1- J Clin Oncol. 2008;26(3):374-9; 2- N Engl J Med. 2013;369(11):1023-34; 3- JAMA. 2010;304(16):1812-20.

Gene Symbol	Exon	Nucleotide Change	Amino Acid Change	VAF (%)	Effect on Protein	Category
PIK3CA	5	c.1030G>A	p.V344M	13	Gain-of-function	Tier I - Level A

Gene Symbol	Exon	Nucleotide Change	Amino Acid Change	VAF (%)	Effect on Protein	Category
PIK3CA	21	c.3140A>G	p.H1047R	19.4	Gain-of-function	Tier I - Level A

Phosphatidylinositol-4,5-bisphosphate 3-kinases (PI3K) are a family of lipid kinases involved in cell growth, proliferation, and survival, among other processes, by recruiting downstream signaling molecules such as AKT1. The PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha) gene encodes for the p110a catalytic subunit of the PI3K heterodimer. Mutant PIK3CA has been found in several tumors, including colorectal cancer (CRC), gliomas, gastric cancer, breast cancer, endometrial cancer, and lung cancer. Somatic mutations in PIK3CA have been found in 10-30% of CRC tumors (1). Multiple PI3K inhibitors, including buparlisib (2), taselisib (3), and copanlisib (4), are under investigation in patients with PIK3CA-mutated or PTEN-mutated solid tumors. Retrospective studies have suggested improved survival with postoperative aspirin use in patients whose CRC harbors a PIK3CA mutation (5).
References: 1- Science. 2004;304(5670):554.; 2- Gynecol Oncol. 2014;133(2):346-52; 3- Gynecol Oncol. 2014;135(2):312-7; 4- Ann Oncol. 2016;27(10):1928-40; 5- J Clin Oncol. 2013;31(34):4297-305.

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.

Gene Symbol	Exon	Nucleotide Change	Amino Acid Change	VAF (%)	Effect on Protein	Category
BCOR	10	c.4376A>G	p.N1459S	25.9	Loss-of-function	Tier II - Level C

The BCOR gene encodes the B-cell CLL/lymphoma 6 (BCL6) corepressor protein which potentiates transcriptional repression by BCL6 [PMID: 16943429]. BCOR also associates with class I and II histone deacetylases (HDACs) suggesting an alternate mechanism for BCOR mediated transcriptional repression independent of BCL6 [PMID: 10898795]. BCOR functions as a tumor suppressor gene [PMID: 30902969]. Higher mutational frequencies are reported in some solid tumors, including up to 15% of uterine cancer and 5-10% of colorectal cancer, stomach cancer, cholangiocarcinoma, and melanoma.

Gene Symbol	Exon	Nucleotide Change	Amino Acid Change	VAF (%)	Effect on Protein	Category
TP53	8	c.818G>A	p.R273H	7.3	Loss-of-function	Tier II - Level C

The TP53 tumor suppressor gene coding for a nuclear phosphoprotein involved in cellular stress responses is the most frequently mutated gene in human cancers (1). Somatic mutations in the TP53 gene are one of the most frequent alterations in human cancers. Most TP53 inactivating mutations are found within the highly conserved middle region (exons 5-8) that encodes for the DNA-binding domain, critical for the major function of TP53 protein as a transcriptional activator. The mutation spectrum of the TP53 gene varies from one tumor type to another with typical hot-spot codons for mutations. For instance, codons 157, 248, and 273 are frequently mutated in cigarette smoking-associated lung cancers. Several therapeutic approaches aim at the reactivation of p53 mutants as a rescue strategy, including small peptides or small molecules.
References: 1- Subcell Biochem. 2014;85:1-16.

Gene Symbol	*Exon*	*Nucleotide Change*	*Amino Acid Change*	*VAF (%)*	*Effect on Protein*	*Category*
CDKN2A	N/A	Loss (0.7x)	-	N/A	Loss-of-function	Tier II - Level D

The majority of reported mutations in the CDKN2A gene affect the p16INK4a protein, which inhibits the CDK4/6-mediated phosphorylation of retinoblastoma protein (Rb). Alterations in the CDKN2A gene are associated with increased risk of a number of human malignancies including melanoma, pancreatic cancer, breast cancer, esophageal cancer, gallbladder carcinoma, endometrial carcinoma, head and neck squamous cell carcinoma, glioblastoma, and lung cancer, although less frequently. Mutations in this gene are inherited in an autosomal dominant manner and contribute to increased cancer risks, contributing to 10-39% of hereditary form of malignant melanoma (1). Somatic mutations of CDKN2A are present in up to 95% of pancreatic tumors (2). Currently there is no standard therapy option to treat tumors harboring germline or somatic CDKN2A alterations. However, loss of function mutation of CDKN2A leads to activation of CDK2/4/6, implicating CDK inhibitors as a logical therapeutic option for these patients.
References: 1- J Med Genet. 2007; 44:99-106; 2- Cancer Res 1997; 57: 3126–313.

Genomic Signature	*Exon*	*Nucleotide Change*	*Amino Acid Change*	*VAF (%)*	*Effect on Protein*	*Category*
HRD	-	Score=0	NEGATIVE	-	N/A	Tier II - Level D

Several cancer chemotherapy drugs mechanism of action is to produce excessive DNA damage causing cell death, which cancer cells overcome through repair of these lesions by several DNA repair pathways, such as homologous recombination (HR). Inhibitors of DNA repair pathways, such as a poly(ADP-ribose) polymerase inhibitor (PARPi) can be successfully used as targeted treatment of breast, ovarian, and other cancers that show homologous recombination deficiency (HRD). Genetic lesions causing HRD include germline and somatic BRCA1 and BRCA2 (BRCA1/2) mutations, as well as mutations in BRIP, CHEK2, RAD51, etc… The use of olaparib (LYNPARZA®), the first FDA-approved PARPi, has shown significantly longer progression free survival (PFS) in patients with BRCA1/2 mutations, and/or HRD-Positive, than non-mutant BRCA1/2 patients (1), and/or HRD-Negative. An HRD score based on gLOH, TAI, and LST is calculated to assess HRD status. Individual cutoffs of gLOH = 8%, TAI = 10%, and LST = 18% cannot accurately predict response to therapy. However, an HRD score > 42 and/or the presence of BRCA1/2 gene mutations, predicts the likelihood of response to neoadjuvant platinum-containing therapy in triple negative breast cancer (2). In addition, it has been shown that high-grade serous ovarian carcinoma cases carrying BRCA1/2 alterations exhibit HRD scores = 63 and show increased response to niraparib (ZEJULA®), another PARPi (3).
References: 1- Eur J Cancer. 2016;60:49-58; 2- Cancer. Clin Cancer Res. 2016;22(15):3764-73; 3- Rep. 2020;10(1):2757.

Genomic Signature	*Exon*	*Nucleotide Change*	*Amino Acid Change*	*VAF (%)*	*Effect on Protein*	*Category*
MSI	-	-	MSI-HIGH	-	N/A	Tier II - Level D

Microsatellite instability (MSI) has been shown to be due to DNA slippage in the process of replication, or mismatch of the basic group of slippage strand and complementary strand in the process of DNA replication and repair, resulting in one or more of the Short Tandem Repeats (STRs) missing or inserted. The normal tissue DNA repair system, called mismatch repair (MMR), can correct in the process of DNA replication errors. However, in cases with deficient MMR (dMMR), the possibility of gene mutation is increased, leading to the development of tumors (1). High microsatellite instability (MSI-H) has been typically observed in colorectal cancer (CRC) and, to a lesser extent, in other cancer types. MSI-H/dMMR tumors, which correlate with high mutation rates, respond well to immune checkpoint inhibitors (ICI). The FDA has approved PD-L1 (programed cell death ligand 1) blockade pembrolizumab (KEYTRUDA) to treat patients harboring MSI-H/dMMR tumors (2). Both MSI-H and high tumor mutational burden (TMB) represent the production of neoantigens, thus inducing effective anti-tumor immune responses.
References: 1- Li G-M. Cell Res. 2008;18(1):85-98. 2- Yu Y. Front Med. 2018;12(2):229-235.

Genomic Signature	*Exon*	*Nucleotide Change*	*Amino Acid Change*	*VAF (%)*	*Effect on Protein*	*Category*
TMB	-	17 mut/MB	TMB-HIGH	-	N/A	Tier II - Level D

Tumor mutational burden (TMB) measures the number of somatic mutations per megabase of coding region of a tumor genome. It is postulated that highly mutated tumors are more likely to harbor neoantigens, which make them targets of activated immune cells. A TMB-HIGH status, corresponding to 10 or more mutations per megabase, has been shown, in several tumor types, to correlate with patient response to ICI (1), like pembrolizumab, and, in a clinical trial, TMB was more significantly associated with response rate than expression of PD-L1 by immunohistochemistry (2), demonstrating the clinical utility of this biomarker.
References: 1- Marabelle, et al. Lancet Oncol. 2020;21(10):1353-1365. 2- Rosenberg JE, et al. Lancet. 2016;387(10031):1909-20.

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

Pembrolizumab (KEYTRUDA)

Pembrolizumab (KEYTRUDA) is a monoclonal antibody that binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2, releasing PD-1 pathway-mediated inhibition of the immune response, including the anti-tumor immune response. Keytruda is specifically indicated for the treatment of patients with unresectable or metastatic melanoma, NSCLC with no EGFR or ALK alterations and PD-L1 expression = 1%, SCLC, HNSCC, and for the treatment of adult and pediatric patients with unresectable or metastatic, microsatellite instability-high (MSI-H) or mismatch repair deficient solid tumors, and for tumor mutational burden-high (TMB-H) cancers. For the complete drug label text, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/125514s084lbl.pdf

Nivolumab (OPDIVO)

Nivolumab (OPDIVO) is a human monoclonal antibody that blocks the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Binding of the PD-1 ligands, PD-L1 and PD-L2, to the PD-1 receptor found on T cells, inhibits T-cell proliferation and cytokine production. Opdivo is specifically indicated for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor. For the complete drug label, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/125554s022lbl.pdf.

Ulixertinib (BVD-523)

Ulixertinib (BVD-523) is a first-in-class and best-in-class small-molecule inhibitor of extracellular signal-regulated kinase (ERK) family kinases (ERK1 and ERK2) that is being developed as a novel anti-cancer drug. ERK kinases are downstream components of the mitogen-activated protein kinase (MAPK) signaling cascade (RAS-RAF-MEK-ERK). Ulixertinib has demonstrated promising early efficacy for patients with tumors harboring alterations in the MAPK pathway, including atypical (non-V600) BRAF alterations, for which there are currently no approved targeted agents. The FDA granted fast track (FT) designation for the treatment of patients with MAPK pathway aberrant cancer, including but not limited to KRAS, NRAS, HRAS, BRAF, MEK, and ERK mutations.

Binimetinib (MEKTOVI)

Binimetinib (MEKTOVI) is a kinase inhibitor indicated, in combination with encorafenib, for the treatment of patients with unresectable or metastatic melanoma with a BRAF V600E or V600K mutation. For the complete drug label, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/210498lbl.pdf

Cobimetinib (COTELLIC)

Cobimetinib (COTELLIC), is a MEK inhibitor, FDA-approved for the treatment of patients with unresectable or metastatic melanoma with a BRAF V600E or V600K mutation, in combination with vemurafenib. For the complete drug label, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/206192s000lbl.pdf

Trametinib (MEKINIST)

Trametinib (MEKINIST) is a MEK inhibitor, FDA-approved for the treatment of patients with unresectable or metastatic melanoma with BRAF V600E or V600K mutations, as detected by an FDA-approved test. For the complete drug label, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/204114s001lbl.pdf

Selumetinib (KOSELUGO)

Selumetinib (KOSELUGO) is a MEK1/2 inhibitor, approved by the FDA for neurofibromatosis type 1 with symptomatic, inoperable plexiform neurofibromas, and previously given investigational use only as adjuvant treatment for patients with stage III or IV differentiated thyroid cancer. For the complete drug label, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/213756s000lbl.pdf

Alpelisib (PIQRAY)

Alpelisib (PIQRAY) is an FDA approved kinase inhibitor indicated in combination with fulvestrant for the treatment of postmenopausal women, and men, with hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative, PIK3CA-mutated, advanced or metastatic breast cancer as detected by an FDA-approved test following progression on or after an endocrine-based regimen. For the complete drug label, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/212526s000lbl.pdf.

Idelalisib (ZYDELIG)

Idelalisib (ZYDELIG), a PI3K inhibitor, is an FDA-approved drug for the treatment of patients with relapsed chronic lymphocytic leukemia (CLL), follicular lymphoma and small lymphocytic lymphoma (SLL). For the complete drug label, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/206545lbl.pdf

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 are 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

Repotrectinib (TPX-0005)

Repotrectinib (TPX-0005) is a small (low molecular weight), macrocyclic tyrosine kinase inhibitor of ROS1, TRK and ALK. Repotrectinib was designed to efficiently bind with the active kinase conformation and avoid steric interference from a variety of clinically resistant mutations. FDA has granted a breakthrough therapy (BT) designation to repotrectinib for the treatment of patients with ROS1-positive metastatic non–small cell lung cancer (NSCLC) who have been previously treated with one ROS1 TKI and have not received prior platinum-based chemotherapy. For further informations go to https://www.tptherapeutics.com/news-releases/news-release-details/turning-point-therapeutics-granted-breakthrough-therapy-0

Cetuximab (ERBITUX)

Cetuximab (ERBITUX), is a chimeric monoclonal antibody directed against EGFR. FDA approved alone or in combination to treat advanced squamous cell carcinoma of the head and neck and to treat K-Ras mutation negative, EGFR expressing metastatic colorectal cancer. For the complete drug label, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/125084s0228lbl.pdf

Panitumumab (VECTIBIX)

Panitumumab (VECTIBIX) is a recombinant, human monoclonal antibody that binds specifically to EGFR. FDA approved for the treatment of metastatic colorectal carcinoma (mCRC) patients with disease progression or following certain chemotherapy regimens. Vectibix is not recommended for the treatment of mCRC patients with KRAS mutations (codons 12 or 13), detected by an FDA-approved test. For the complete drug label, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/125147s080lbl.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 University of Florida Health Science Center, Gainesville, FL, 32610. Principal Investigator: Thomas J. George, phone: 888-823-5923; e-mail: ctsucontact@westat.com.

NCT02079740

Title: An Open Label, Two-Part, Phase Ib/II Study to Investigate the Safety, Pharmacokinetics, Pharmacodynamics, and Clinical Activity of the MEK Inhibitor Trametinib and the BCL2-Family Inhibitor Navitoclax (ABT-263) in Combination in Subjects With KRAS or NRAS Mutation-Positive Advanced Solid Tumors. Trametinib and Navitoclax in Treating Patients With Advanced or Metastatic Solid Tumors. Dana-Farber Cancer Institute, Boston, Massachusetts, United States, 02215. Contact: Ryan B. Corcoran Ph: 877-726-5130. Principal Investigator: Ryan B. Corcoran

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: Mayo Clinic, Jacksonville, FL, 32224; phone: 855-776-0015; Principal Investigator: Sani H. Kizilbash

NCT04632992

Title: MyTACTIC: An Open-Label Phase II Study Evaluating Targeted Therapies in Patients Who Have Advanced Solid Tumors With Genomic Alterations or Protein Expression Patterns Predictive of Response. Contact information: Reference Study ID Number: ML42439 (888-662-6728; global-roche-genentech-trials@gene.com)

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: University Cancer & Blood Center, LLC; Research, Athens, GA, 30607; 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.

NCT03600701

Title: Atezolizumab and Cobimetinib in Treating Patients With Metastatic, Recurrent, or Refractory Non-small Cell Lung Cancer. This phase II trial studies how well atezolizumab and cobimetinib work in treating patients with non-small cell lung cancer that has spread to other places in the body (metastatic), has come back (recurrent), or does not respond to treatment (refractory). Immunotherapy with monoclonal antibodies, such as atezolizumab, may help the immune system attack the cancer, and may interfere with the ability of tumor cells to grow and spread. Cobimetinib may stop the growth of tumor cells by blocking some of the enzymes needed for cell growth. Giving atezolizumab and cobimetinib may work better in treating patients with non-small cell lung cancer. For more information contact: Andreas N. Saltos ; ph: 800-679-0775 email: ClinicalTrials@moffitt.org

NCT03178552

Title: A Phase II/III Multicenter Study Evaluating the Efficacy and Safety of Multiple Targeted Therapies as Treatments for Patients With Advanced or Metastatic Non-Small Cell Lung Cancer (NSCLC) Harboring Actionable Somatic Mutations Detected in Blood (B-FAST: Blood-First Assay Screening Trial)

Tier III: Variants of Unknown Significance (VUS)

AKT1	c.73C>T	p.R25C

Methods and Limitations

Genomic DNA and RNA extracted from this patient's sample was used for multiplex PCR amplification using the Oncomine Comprehensive Assay Plus (OCA+) primers and Ion AmpliSeqTM reagents to detect the following alterations: single nucleotide variants (SNVs) and small insertions and deletions (Indels) in 165 genes (including hotspots); copy number variations (CNVs) for 333 genes and chromosomal level loss of heterozygozity (LOH); full coding DNA sequence of 227 genes (including BRCA1 and BRCA2 plus 44 other homologous recombination repair (HRR) genes); fusions of 51 different gene drivers with multiple partners covering over 1,300 possible intergenic and intragenic rearrangements, including NTRK1/2/3; microsatellite instability (MSI); tumor mutational burden (TMB); homologous recombination deficiency (HRD) status, based on genomic LOH (gLOH), telomeric allelic imbalance (TAI), and large-scale transitions (LST). Amplicons were sequenced using next generation sequencing (NGS) technology on the Ion Torrent S5 XL sequencer and analyzed with Torrent Suite Software (version 5.18). 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 this report. 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. The performance characteristics of this test are the following: SNVs [Sensitivity 100.0% (95%CI 95.8%-100.0%), Specificity 100.0% (95%CI 92.5%-100.0%)]; Small Indels [Sensitivity 100.0% (95%CI 85.8%-100.0%), Specificity 100.0% (95%CI 94.0%-100.0%)]; CNVs [Sensitivity 100.0% (95%CI 89.7%-100.0%), Specificity 100.0% (95%CI 93.6%-100.0%)]; Fusions: [Sensitivity 100.0% (95%CI 79.4%-100.0%), Specificity 100.0% (95% CI 94.2%-100.0%)]; MSI (for all tumor types except endometrial cancer): [Sensitivity 100.0% (95%CI 63.1%-100.0%), Specificity 100.0% (95%CI 75.3%-100.0%)]; MSI (for endometrial cancer) [Sensitivity 66.7% (95%CI 22.3% - 95.7%), Specificity 100.0% (95%CI 54.1%-100.0%)]; TMB [Sensitivity 100.0% (95%CI 54.1%-100.0%), Specificity 100.0% (95%CI 63.1%-100.0%)]; HRD [Sensitivity 100.0% (95%CI 54.1%-100.0%), Specificity 100.0% (95%CI 69.2%-100.0%)]. The specimen is judged undetectable for any of the tested genes if SNVs are present at a frequency below 4% of total DNA, at a mean coverage of 1,000X 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 1,000X 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) and copy number (CN) 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 studies 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.

Genes Tested*

Genes Tested for Hotspots

ABL1, ABL2, ACVR1, AKT1, AKT2, AKT3, ALK, AR, ARAF, ATP1A1, AURKA, AURKC, AXL, BCL2,, BCL2L12, BCL6, BCR, BMP5, BRAF, BTK, CACNA1D, CARD11, CBL, CCND1, CCND2, CCND3, CCNE1,, CD79B, CDK4, CDK6, CHD4, CSF1R, CTNNB1, CUL1, CYSLTR2, DDR2, DGCR8, DROSHA, E2F1, EGFR,, EIF1AX, EPAS1, ERBB2, ERBB3, ERBB4, ESR1, EZH2, FAM135B, FGF7, FGFR1, FGFR2, FGFR3, FGFR4,, FLT3, FLT4, FOXA1, FOXL2, FOXO1, GATA2, GLI1, GNA11, GNAQ, GNAS, H3F3A, H3F3B, HIF1A,, HIST1H2BD, HIST1H3B, HRAS, IDH1, IDH2, IKBKB, IL6ST, IL7R, IRF4, IRS4, KDR, KIT, KLF4, KLF5,, KNSTRN, KRAS, MAGOH, MAP2K1, MAP2K2, MAPK1, MAX, MDM4, MECOM, MED12, MEF2B,, MET, MITF, MPL, MTOR, MYC, MYCN, MYD88, MYOD1, NFE2L2, NRAS, NSD2, NT5C2, NTRK1,, NTRK2, NTRK3, NUP93, PAX5, PCBP1, PDGFRA, PDGFRB, PIK3C2B, PIK3CA, PIK3CB, PIK3CD,, PIK3CG, PIK3R2, PIM1, PLCG1, PPP2R1A, PPP6C, PRKACA, PTPN11, PTPRD, PXDNL, RAC1, RAF1,, RARA, RET, RGS7, RHEB, RHOA, RICTOR, RIT1, ROS1, RPL10, SETBP1, SF3B1, SIX1, SIX2, SLCO1B3,, SMC1A, SMO, SNCAIP, SOS1, SOX2, SPOP, SRC, SRSF2, STAT3, STAT5B, STAT6, TAF1, TERT,, TGFBR1, TOP1, TPMT, TRRAP, TSHR, U2AF1, USP8, WAS, XPO1, ZNF217, and ZNF429

HRR Genes

ABRAXAS1, ATM, ATR, BAP1, BARD1, BLM, BRCA1, BRCA2, BRIP1, CDK12, CHEK1, CHEK2, FANCA, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, MRE11, NBN, PALB2, PARP1, PARP2, PARP3, POLD1, POLE, PPP2R2A, PTEN, RAD50, RAD51, RAD51B, RAD51C, RAD51D, RAD52, RAD54L, RNASEH2A, RNASEH2B, RNASEH2C, RPA1, SLX4, TP53, XRCC2, and XRCC3.

Gene Fusion Drivers

AKT2, ALK, AR, AXL, BRAF, BRCA1, BRCA2, CDKN2A, EGFR, ERBB2, ERBB4, ERG, ESR1, ETV1, ETV4, ETV5, FGFR1, FGFR2, FGFR3, FGR, FLT3, JAK2, KRAS, MDM4, MET, MYB, MYBL1, NF1, NOTCH1, NOTCH4, NRG1, NTRK1, NTRK2, NTRK3, NUTM1, PDGFRA, PDGFRB, PIK3CA, PPARG, PRKACA, PRKACB, PTEN, RAD51B, RAF1, RB1, RELA, RET, ROS1, RSPO2, RSPO3, and TERT.

**For a complete list of genes covered by this test visit: https://www.sonichealthcareusa.com/ap/testing-solutions/auraseq/*

Electronically Signed By:

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

Signed Date: 09-08-2022

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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.

The testing was performed at Bernhardt Laboratories 3728 Philips Highway, Suite 64, Jacksonville, FL 32207.

PDL1 RESULTS:

Specimen: Colon S22-123456

PDL1 Clone 22C3

Diagnosis

PD-L1 IMMUNOHISTOCHEMISTRY (clone 22C3; KEYTRUDA) (Performed on Block A2)
Results: PDL1 Combined Positive Score (CPS): 10%
Interpretation: Positive for PD-L1 expression (> or = to 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 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 gastric or GEJ adenocarcinoma is determined by using Combined Positive Score (CPS), which is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100. The specimen should be considered to have PD-L1 expression if CPS > or = 1.

PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying gastric or GEJ adenocarcinoma patients for treatment with ßKEYTRUDA (pembrolizumabñ). See the KEYTRUDA product label for specific clinical circumstances guiding PD-L1 testing.

Decal Disclaimer

This assay has not been validated on decalcified tissues. Results should be interpreted with caution given the likelihood of false negativity on decalcified specimens.

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.

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Signed Date: 09-08-2022