Date: 09/03/2016
Order Date: 08/19/2016
Specimen Number: TEST

50**SEQ**

Patient Information	Specimen Information	Physician Information
Name: Test Patient	**Type:** Liver, Needle Core Biopsies	**Institution:** Test Account **Referring Physician:** Dr. Test
DOB: 01/02/1234 **Gender** Female	**Collected:** 08/16/2016	**Final Reviewer:**Michael Weindel, M.D. and Benton Middleman, M.D.
MRN: TEST123456	**Received:** 08/19/2016	**Genomic Analyst:**Kristen Champion, PhD, FACMG
Disease Type: Breast Cancer	**Block ID:** TEST-123	
Diagnosis: Metastatic Poorly Differentiated Carcinoma of Breast Origin		

Summary of Findings

This specimen is positive for a likely pathogenic mutation in the ESR1 gene (c.1609T>G, p.Y537D). ESR1 mutations involving the ligand binding domain have been observed in the setting of ER-positive metastatic breast cancers that have developed resistance to antiestrogen therapy (Robinson et al. 2013; Toy et al. 2013). No established recommendations for use of targeted therapeutics are available based on these results. Relevant clinical trials may be available. Please note that there are multiple regions of low sequencing coverage potentially due to extensive chromosomal level genomic instability within this specimen. Please see below for a listing of these gene regions. Alterations present within these gene regions may not be reliably detected.

*** Molecular - Electronically Signed Out by Michael Weindel, MD on 09/01/2016

*** Final Approval - Electronically Signed Out by Michael Weindel, M.D. and Benton Middleman, M.D. on 09/01/2016

Date: 09/03/2016
Order Date: 08/19/2016
Specimen Number: TEST
Report Version: 1

50**SEQ**: Final Report

Patient Information	Specimen Information	Physician Information
Name: Test Patient **DOB:** 01/02/1234 **Gender:** Female **MRN:** TEST123456 **Disease Type:** Breast Cancer **Diagnosis:** Metastatic Poorly Differentiated Carcinoma of Breast Origin	**Type:** Liver, Needle Core Biopsies **Collected:** 08/16/2016 **Received:** 08/19/2016 **Block ID:** TEST-123	**Institution:** Test Account **Referring Physician:** Dr. Test **Final Reviewer:** Michael Weindel, MD **Genomic Analyst:** Kristen Champion, PhD, FACMG

SUMMARY

Clinical Information:

Metastatic poorly differentiated carcinoma of breast origin

Results Interpretation:

This specimen is positive for a likely pathogenic mutation in the ESR1 gene (c.1609T>G, p.Y537D). ESR1 mutations involving the ligand binding domain have been observed in the setting of ER-positive metastatic breast cancers that have developed resistance to antiestrogen therapy (Robinson et al. 2013; Toy et al. 2013). No established recommendations for use of targeted therapeutics are available based on these results. Relevant clinical trials may be available. Please note that there are multiple regions of low sequencing coverage potentially due to extensive chromosomal level genomic instability within this specimen. Please see below for a listing of these gene regions. Alterations present within these gene regions may not be reliably detected.

Genes Tested With Alterations: ESR1, KDR, TP53

Genes Tested Without Alterations: AKT1, ALK, APC, ATM, BRAF, CDH1, CDKN2A, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KIT, KRAS, MAP2K1, MET, MLH1, NOTCH1, NRAS, PDGFRA, PIK3CA, PIK3R1, PTEN, PTPN11, RB1, RET, ROS1, SMAD4, SMARCB1, SMO, SRC, STK11, VHL

Gene Regions That Failed Testing: BRAF (V600D), BRAF (V600E), BRAF (V600G), BRAF (V600K), BRAF (V600M), BRAF (V600R), PIK3CA (C695F), ATM_1 (Exon 8), ERBB4_6 (Exon 9), ERBB4_7 (Exon 15), PIK3CA_4 (Exon 7), PIK3CA_8 (Exon 14), RB1_1 (Exon 4), RB1_2 (Exon 6), RB1_3 (Exon 10), RB1_4 (Exon 11), RB1_9 (Exon 21), ROS1 (Exon 41), SMAD4_2 (Exon 4)

DRUG RESPONSE

Drugs Associated With Sensitivity For Patient's Tumor Type, Based on Genomic Analysis

Drug(s)	Response to Drug Based on Biomarkers (Source)	Related Biomarker(s)	Indication of the Drug Response	Drug Class	Drug FDA-Approved for Patient's Tumor Type	Additional Information
None						

Drugs Associated With Resistance, Based on Genomic Analysis

Drug(s)	Response to Drug Based on Biomarkers (Source)	Related Biomarker(s)	Indication of the Drug Response	Drug Class	Drug FDA-Approved for Patient's Tumor Type	Additional Information
None						

med fusion clin-labs • clin-trials
Advancing Clarity in Medicine

PBM PATHOLOGISTS BIO-MEDICAL LABORATORIES, L.L.P.

Date: 09/03/2016
Order Date: 08/19/2016
Specimen Number: TEST
Report Version: 1

50**SEQ**: Final Report

Drugs Associated With Sensitivity For Other Tumor Types, Based on Genomic Analysis						
Drug(s)	**Response to Drug Based on Biomarkers (Source)**	**Related Biomarker(s)**	**Indication of the Drug Response**	**Drug Class**	**Drug FDA-Approved for Patient's Tumor Type**	**Additional Information**
None						

SOURCES:
FDA: US Food and Drug Administration (www.fda.gov), **NCCN**: National Comprehensive Cancer Network (www.nccn.org), **ASCO**: American Society of Clinical Oncology (www.asco.org), **MCG**: My Cancer Genome (www.mycancergenome.org)

GENETIC ALTERATIONS

Detected Alterations of Known or Potential Pathogenicity					
Gene	**Gene Alteration**	**Significance**	**Therapeutic Implications***	**Additional Information**	**Methodology**
ESR1	Y537D c.1609T>G	Likely Pathogenic	Potentially relevant clinical trials	COSMIC: N/A Allele Frequency: 0.0% dbSNP: N/A	Mutational Analysis

Detected Alterations of Uncertain Significance					
Gene	**Gene Alteration**	**Significance**	**Therapeutic Implications***	**Additional Information**	**Methodology**
None					

Detected Alterations Known to be Benign or Likely to be Benign					
Gene	**Gene Alteration**	**Significance**	**Therapeutic Implications***	**Additional Information**	**Methodology**
KDR	Q472H c.1416A>T	Benign	N/A	COSMIC: COSM149673 Allele Frequency: 21.2% dbSNP: rs1870377	Mutational Analysis
TP53	P72R c.215C>G	Benign	N/A	COSMIC: COSM250061 Allele Frequency: 54.3% dbSNP: rs1042522	Mutational Analysis

***Therapeutic Implications:** Associated with drug response = related to drug sensitivity or resistance as described in Drug Response section of this report; Potentially relevant clinical trials = gene is related to a trial in the Clinical Trials section of this report
COSMIC: Mutation ID in the Catalogue Of Somatic Mutations in Cancer (http://cancer.sanger.ac.uk/)
Allele Frequency: Allele frequency of the alteration in the 1000 Genomes Project (http://www.1000genomes.org/)
dbSNP: RS number of alteration in dbSNP database (http://www.ncbi.nlm.nih.gov/SNP)

CLINICAL TRIALS

Overview of Clinical Trials That May Be Relevant Based On Results Of The Tumor Profile	
Trial Phase	**Number of Open, Enrolling Trials**
Phase 3	1
Phase 2	1

50**SEQ**: Final Report

Phase 1/Phase 2	5
Phase 1	9
N/A	1

For a full list of clinical trials which may be relevant for your patient, please follow this link to the US Government Clinical Trials website: Full List of Clinical Trials on www.clinicaltrials.gov

Below are potentially relevant targeted clinical trials for your patient based on the results of the tumor profile only.

Potentially Relevant US Oncology Directed Clinical Trials

Phase	Trial Title	Genes	Location
None			

Sample of Other Potentially Relevant Clinical Trials

Phase	Trial Title	Genes	Location
Phase 1/Phase 2	A Study of GDC-0810 Single Agent or in Combination With Palbociclib and/or a Luteinizing Hormone-releasing Hormone (LHRH) Agonist in Women With Locally Advanced or Metastatic Estrogen Receptor Positive Breast Cancer (NCT01823835)	ESR1	Missouri
Phase 1	Safety Study of ALRN-6924 in Patients With Advanced Solid Tumors or Lymphomas (NCT02264613)	TP53	Texas
Phase 1	Study to Determine and Evaluate a Safe and Tolerated Dose of HDM201 in Patients With Selected Advanced Tumors That Are TP53wt (NCT02143635)	TP53	New York

ALTERATION DETAILS

ESR1 Description:

ESR1 (estrogen receptor 1, also known as ER) is a gene that encodes the estrogen receptor protein. ESR1 plays a role in pathogenesis of cancers such as breast, endometrial, and prostate cancer (Thomas and Gustafsson 2011).

Y537D
Frequency of ESR1 (ER) mutations in untreated breast cancer: Rare (TCGA 2012)
Frequency of ESR1 (ER) mutations in ER+ and/or PR+ invasive breast cancer: 12-55% (Jeselsohn et al. 2014; Merenbakh-Lamin et al. 2013; Robinson et al. 2013; Toy et al. 2013) in ER+ breast cancer with acquired resistance to antiestrogen therapy
Frequency of ESR1 (ER) expression in breast cancer: 70-80% (Allred et al. 2009)

References:
http://www.mycancergenome.org/content/disease/breast-cancer/esr1/314

Date: 09/03/2016
Order Date: 08/19/2016
Specimen Number: TEST
Report Version: 1

50**SEQ**: Final Report

TEST DETAILS

Targeted next generation sequencing (NGS) utilizes a Custom-Designed Ion AmpliSeq 50 gene panel.

Biomarkers Tested by Mutational Analysis:

AKT1 (NM_005163.2)	ALK (NM_004304.4)	APC (NM_001127510.2)	ATM (NM_000051.3)	BRAF (NM_004333.4)	CDH1 (NM_004360.3)	CDKN2A (NM_000077.4)
CSF1R (NM_005211.3)	CTNNB1 (NM_001904.3)	EGFR (NM_005228.3)	ERBB2 (HER2) (NM_004448.2)	ERBB4 (NM_005235.2)	ESR1 (NM_000125.3)	FBXW7 (NM_033632.3)
FGFR1 (NM_023110.2)	FGFR2 (NM_022970.3)	FGFR3 (NM_000142.4)	FLT3 (NM_004119.2)	GNA11 (NM_002067.4)	GNAQ (NM_002072.4)	GNAS (NM_001077488.2
HNF1A (NM_000545.5)	HRAS (NM_005343.2)	IDH1 (NM_005896.2)	IDH2 (NM_002168.2)	JAK2 (NM_004972.3)	JAK3 (NM_000215.3)	KDR (NM_002253.2)
KIT (NM_000222.2)	KRAS (NM_033360.3)	MAP2K1 (NM_002755.3)	MET (NM_001127500.1)	MLH1 (NM_000249.3)	NOTCH1 (NM_017617.3)	NRAS (NM_002524.3)
PDGFRA (NM_006206.4)	PIK3CA (NM_006218.2)	PIK3R1 (NM_181523.2)	PTEN (NM_000314.4)	PTPN11 (NM_002834.3)	RB1 (NM_000321.2)	RET (NM_020975.4)
ROS1 (NM_002944.2)	SMAD4 (NM_005359.5)	SMARCB1 (NM_003073.3)	SMO (NM_005631.4)	SRC (NM_005417.4)	STK11 (NM_000455.4)	TP53 (NM_000546)
VHL (NM_000551.3)						

Methodology

DNA is isolated from micro-dissected tumor tissue and amplified for mutational hotspot regions within 50 cancer-related genes. Mutation status is determined through semiconductor-based massively parallel sequencing (next generation sequencing) and sequence alignment to the hg19 reference genome (GRCh37). Synonymous substitution variants and variants occurring within intronic gene regions not affecting splicing junctions will be filtered out during data analysis. Clinically actionable variants will be reported and interpreted utilizing the GenomOncology software powered by the My Cancer Genome (TM) personalized cancer medicine knowledge-base. Non-actionable variants (including benign variants and variants of uncertain clinical significance) will be reported and classified using a classification system* adapted from the ACMG recommendations for standards for interpretation and reporting of sequence variations: Revisions 2007. Genet Med 2008 10(4), 294-300 and the ACMG/AMP/CAP Interpretation of Sequence Variants Workgroup draft document, August 2013.

Intended Use

The 50SEQ cancer gene sequencing panel is intended to be used for the detection of somatic (non-inherited) hotspot mutations within clinically relevant genes in order to help predict response to targeted therapies and prioritize treatment for patients with solid tumor cancers. Results from this test may also be useful in some cases as a prognostic indicator. This test is not intended to be used for the detection of germline (inherited) mutations associated with inherited cancer syndromes such as Familial Adenomatous Polyposis syndrome, Juvenile Polyposis syndrome, Li-Fraumeni syndrome, PTEN hamartoma tumor syndromes, Von Hippel-Lindau syndrome, etc. This assay only examines tumor tissue and does not examine normal tissue from the patient, therefore detected mutations may be either somatic (non-inherited) or germline (inherited) and will not be distinguishable by this assay. If an inherited cancer syndrome is suspected in this patient, appropriate testing for that condition may be indicated. All results should be interpreted in conjunction with other clinical and pathological findings.

Limitations

The 50SEQ cancer panel is designed to detect somatic hotspot mutations within 50 cancer-related genes. Mutations occurring within regions of these genes that are not targeted by the assay specific design will not be detected. The limit of detection of this assay (the minimum percentage of mutant DNA that can be detected in a background of wild-type) is approximately 5 percent for single nucleotide substitution variants and 5-10% for insertion/deletion variants. Gene regions with low coverage (less than 250 reads) will be reported as "failed testing". Variants detected within regions of failed testing may be reported at the discretion of the laboratory director. Synonymous substitution variants and non-coding variants with the exception of variants occurring within splicing junctions will not be reported. Variants occurring within homopolymer regions, large insertion/deletion mutations, copy number variations, and complex mutations cannot be reliably detected.

*Variant Classification System

Pathogenic - Sequence variant is previously reported and recognized to be pathogenic (i.e. variant is reported in a curated mutational database with well-established *in vitro* or *in vivo* functional evidence that is supportive of a deleterious effect on the gene or gene product).

Likely Pathogenic - Sequence variant is previously unreported and is of the type which may be expected to be pathogenic (i.e. truncating variant, variant located within a mutational hotspot), multiple lines of computational evidence support a deleterious effect on the gene or gene product, and variant is absent (or at extremely low frequency) from control data sets such as 1000 genomes and Exome Sequencing Project. Or

Date: 09/03/2016
Order Date: 08/19/2016
Specimen Number: TEST
Report Version: 1

50**SEQ**: Final Report

TEST DETAILS

sequence variant is previously reported to be pathogenic in a reputable mutational database but without sufficient evidence to independently evaluate.

Uncertain Significance - There is insufficient evidence to further classify variant.

Likely Benign - Sequence variant is unreported and is of the type which is not expected to be pathogenic (i.e. intronic variant, synonymous change), multiple lines of computational evidence support a non-deleterious effect on the gene or gene product, or the sequence variant is reported in control data sets but MAF < 0.1%. Or sequence variant is previously reported to be benign in a reputable database but without sufficient evidence to independently evaluate.

Benign - Sequence variant is previously reported and recognized to be a neutral variant (i.e. variant appears in control data sets such as 1000 genomes and Exome Sequencing Project and has a MAF >= 0.1%).

Disclaimer

Laboratory test results should always be considered in the context of clinical observations and epidemiologic data. This test was developed and its performance characteristics determined by med fusion. It has not been cleared or approved by the U.S. Food and Drug Administration (FDA). 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 for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high complexity clinical laboratory testing.

Technical services performed at: med fusion, 2501 S State Hwy 121 Ste 1100, Lewisville, TX 75067 CLIA #45D2004217

Professional services performed at: Pathologists Bio-Medical Lab, 3600 Gaston Ave, Suite 261, Dallas, TX 75246