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

Date: 09/02/2016
Order Date: 07/15/2016
Specimen Number: TEST
Report Version: 1

LUNG**SEQ**Concurrent: Final Report

Patient Information	Specimen Information	Physician Information
Name: Test Patient	**Type:** Lung, right upper lobe	**Institution:** Test Account **Referring Physician:** Dr. Test
DOB: 01/02/1234 **Gender** Female	**Collected:** 07/14/2016	**Final Reviewer:** Benton Middleman, MD
MRN: Test123456	**Received:** 07/15/2016	**Genomic Analyst:** Kristen Champion, PhD, FACMG
Disease Type: Non-Small Cell Lung Cancer **Diagnosis:** Poorly differentiated adenocarcinoma	**Block ID:** TEST-1234	

Summary of Findings

This specimen is positive for an EGFR deletion mutation within exon 19. A positive result for this mutation has been shown to correlate with responsiveness to certain EGFR TKI cancer therapies in patients with non-small cell lung cancer including first-generation EGFR TKIs (erlotinib, gefitinib) and second-generation EGFR TKIs (afatinib, dacomitinib, neratinib). In phase I trials, third-generation EGFR TKIs, such as rociletinib and osimertinib, have demonstrated efficacy in patients with EGFR-mutated NSCLC who have progressed on prior TKI therapy (Soria et al. 2013; Ranson et al. 2013).

*** Molecular - Electronically Signed Out by Michael Weindel, MD on 07/26/2016

Fluorescence in situ hybridization (FISH) was negative for the ALK (2p23), ROS1 (6q22.1) and RET (10q11.21) gene rearrangements and for MET (7q31.2) oncogene amplification.

*** FISH - Electronically Signed Out by Michelle Shiller, D.O. on 07/25/2016

*** Final Approval - Electronically Signed Out by Benton Middleman, MD on 07/26/2016

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Molecular Cytogenetics Results*		
Nomenclature	**Total Cells**	**Abnormal Cells**
nuc ish(ALKx1~4)[99]	100	1 (1.0%)**
nuc ish(ROS1x1~3)[100]	100	0 (0%)
nuc ish(RETx1~4)[100]	100	0 (0%)
Nomenclature* **	**MET/CEP7 Ratio**	**MET Signals/cell**
nuc ish(D7Z1x1~5,METx1~6)[100]	1.10	1.98

No. of Cells Analyzed: 400

No. of Cells Imaged: 8

No. of Staining Procedures: 4

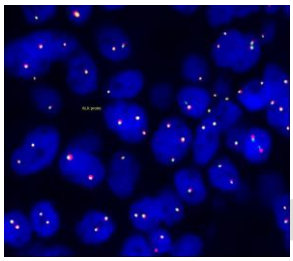
The cells semi-quantitatively analyzed by multiplex FISH were derived from paraffin embedded tissue (block 1D). FISH was performed using Vysis DNA probes (Abbott Molecular Inc., Des Plaines, IL 60018).

*Concurrent controls are run with most probes and the values are available upon request, as are our laboratory control reference ranges and published control reference ranges.

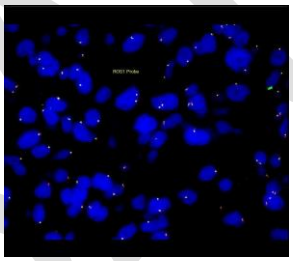
**This falls below the normal range of 15% established by published criteria for this probe.

***Criteria for MET amplification:
Not amplified: MET(7q31.2)/CEP7 <2.0 and MET signals/cell <5.0
Low level amplification: MET signals/cell >=5.0 and <6.0
High level amplification: MET(7q31.2)/CEP7 >=2.0, or MET signals/cell >=6.0,
or cells with MET cluster (>=15)/total cells examined >=10%

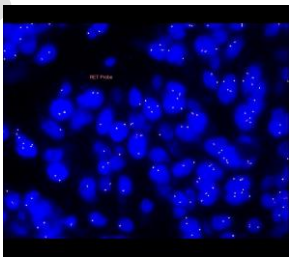
Normal ALK FISH



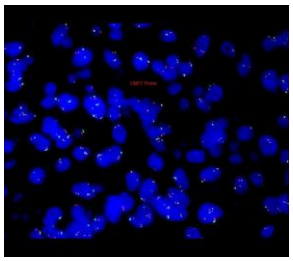
Normal ROS1 FISH



Normal RET FISH



Normal MET FISH



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Name: Test Patient **DOB:** 01/02/1234 **Gender:** Female **MRN:** Test123456 **Disease Type:** Non-Small Cell Lung Cancer **Diagnosis:** Poorly differentiated adenocarcinoma	**Type:** Lung, right upper lobe **Collected:** 07/14/2016 **Received:** 07/15/2016 **Block ID:** TEST-1234	**Institution:** Test Account **Referring Physician:** Dr. Test **Final Reviewer:** Michael Weindel, MD **Genomic Analyst:** Kristen Champion, PhD, FACMG

SUMMARY

Clinical Information:

Poorly differentiated adenocarcinoma with mixed histologic patterns

Results Interpretation:

This specimen is positive for an EGFR deletion mutation within exon 19. A positive result for this mutation has been shown to correlate with responsiveness to certain EGFR TKI cancer therapies in patients with non-small cell lung cancer including first-generation EGFR TKIs (erlotinib, gefitinib) and second-generation EGFR TKIs (afatinib, dacomitinib, neratinib). In phase I trials, third-generation EGFR TKIs, such as rociletinib and osimertinib, have demonstrated efficacy in patients with EGFR-mutated NSCLC who have progressed on prior TKI therapy (Soria et al. 2013; Ranson et al. 2013).

Genes Tested With Alterations: EGFR

Genes Tested Without Alterations: AKT1, ALK, BRAF, ERBB2, KRAS, MAP2K1, MET, NRAS, PIK3CA, PTEN, RET, ROS1

Gene Regions That Failed Testing: None

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
Afatinib	Primary sensitivity (FDA, NCCN, ASCO, MCG)	EGFR Exon 19 Deletion	Non-Small Cell Lung Cancer	Kinase Inhibitors	Yes	
Erlotinib	Primary sensitivity (FDA, NCCN, ASCO, MCG)	EGFR Exon 19 Deletion	Non-Small Cell Lung Cancer	Kinase Inhibitors	Yes	
Gefitinib	Primary sensitivity (FDA, NCCN, ASCO, MCG)	EGFR Exon 19 Deletion	Non-Small Cell Lung Cancer	Kinase Inhibitors	Yes	

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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						

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						

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**
EGFR	EGFR Exon 19 Deletion	Pathogenic	Associated with drug response; Potentially relevant clinical trials	COSMIC: N/A Allele Frequency: 0.0% dbSNP: N/A	Mutational Analysis
ALK	Rearrangement Negative	N/A	None		Fluorescence in situ hybridization
RET	Rearrangement Negative	N/A	None		Fluorescence in situ hybridization
ROS1	Rearrangement Negative	N/A	None		Fluorescence in situ hybridization
MET	Negative (Not Amplified)	N/A	None		Fluorescence in situ hybridization

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**
None					

***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

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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 4	1
Phase 3	6
Phase 2/Phase 3	1
Phase 2	22
Phase 1/Phase 2	15
Phase 1	21
Phase 0	1
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 3	AZD9291 Versus Gefitinib or Erlotinib in Patients With Locally Advanced or Metastatic Non-small Cell Lung Cancer (NCT02296125)	EGFR	Texas
Phase 3	Erlotinib Hydrochloride in Treating Patients With Stage IB-IIIA Non-small Cell Lung Cancer That Has Been Completely Removed by Surgery (An ALCHEMIST Treatment Trial) (NCT02193282)	EGFR	Texas
Phase 2	Erlotinib Hydrochloride or Crizotinib and Chemoradiation Therapy in Treating Patients With Stage III Non-small Cell Lung Cancer (NCT01822496)	EGFR	Texas

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Phase 3	TIGER-3: Open Label, Multicenter Study of Rociletinib (CO-1686) Mono Therapy Versus Single-agent Cytotoxic Chemotherapy in Patients With Mutant EGFR NSCLC Who Have Failed at Least One Previous EGFR-Directed TKI and Platinum-doublet Chemotherapy (NCT02322281)	EGFR	Texas
Phase 1/Phase 2	A Study of BGB324 in Combination With Erlotinib in Patients With Non-Small Cell Lung Cancer (NCT02424617)	EGFR	Texas

ALTERATION DETAILS

EGFR Description:

EGFR (epidermal growth factor receptor, also known as ERBB1 and HER1) is a gene that encodes for the epidermal growth factor receptor protein. Missense mutations, deletions, and insertions are observed in cancers such as lung cancer and glioblastoma. Activating EGFR mutations increase the kinase activity of EGFR, leading to hyperactivation of downstream pro-survival signaling pathways (Sordella et al. 2004).

EGFR Exon 19 Deletion
Frequency of EGFR mutations in NSCLC: 10% in the USA and 35% in Asia (Lynch et al. 2004; Paez et al. 2004; Pao et al. 2004)
Frequency of EGFR exon 19 deletion mutations in EGFR-mutated NSCLC: 48% (Mitsudomi and Yatabe 2010)

References:
http://www.mycancergenome.org/content/disease/lung-cancer/egfr/21

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TEST DETAILS

This test utilizes a multiplexed genotyping panel to detect somatic hotspot mutations within 9 cancer-related genes and a FISH panel to detect gene rearrangements within the ALK, ROS1 and RET genes and amplification of the MET gene.

Biomarkers Tested by FISH:

ALK (NM_004304.4)	MET (NM_001127500.1)	RET (NM_020975.4)	ROS1 (NM_002944.2)

Biomarkers Tested by Mutational Analysis:

AKT1 (NM_005163.2)	BRAF (NM_004333.4)	EGFR (NM_005228.3)	ERBB2 (HER2) (NM_004448.2)	KRAS (NM_033360.3)	MAP2K1 (NM_002755.3)	NRAS (NM_002524.3)
PIK3CA (NM_006218.2)	PTEN (NM_000314.4)					

Methodology

The LungSEQ cancer panel consists of a genotyping panel to detect somatic hotspot mutations within 9 cancer-related genes and a FISH panel to detect gene rearrangements within the ALK, ROS1 and RET genes and amplification of the MET gene. Genotyping Panel - DNA is isolated from macro-dissected tumor tissue. The genotyping panel utilizes a combination of multiplexed genotyping by the SNaPshot(R) method (Life Technologies) and a triplex sizing assay by capillary electrophoresis to detect over 40 different recurrent mutations across 9 different genes implicated in non-small-cell lung cancer (NSCLC). The SNaPshot(R) technology consists of a multiplexed PCR step, followed by a single-base extension sequencing reaction in which allele-specific fluorescently-labeled probes interrogate each loci of interest. These different-sized probes are subsequently resolved by electrophoresis and analyzed by an automated DNA sequencer. This assay is capable of detecting the following pathogenic mutations: AKT1 (p.E17K); BRAF (p.G466V, p.G469A, p.L597V, p.V600E); EGFR (p.G719C/S/A, p.T790M, p.L858R, p.L861Q, exon 19 deletions, exon 20 insertions); HER2 (exon 20 insertions); KRAS (all mutations in codons 12, 13, and 61); MAP2K1 (p.Q56P, p.K57N, p.D67N); NRAS (all mutations in codon 61); PIK3CA (p.E542K, p.E545K/Q, p.H1047R); PTEN (p.R233X). The term "pathogenic" is used here to describe a sequence variant 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). FISH Analysis - Detection of ALK, ROS1, and RET gene rearrangements is based on examining fluorescent signal patterns for each probe in a minimum of 50 interphase nuclei of lung carcinoma cells. Detection of MET amplification is based on counting fluorescent signals for MET and CEP7 probes in a minimum of 50 interphase nuclei of lung carcinoma cells. Clinically actionable variants will be reported and interpreted utilizing the GenomOncology software powered by the My Cancer Genome(TM) personalized cancer medicine knowledge-base.

Intended Use

The LungSEQ Concurrent cancer panel is intended to be used for the detection of somatic hotspot mutations, gene rearrangements, and gene amplifications within clinically relevant genes in order to help predict response to targeted therapies and prioritize treatment for patients with non-small cell lung cancer (NSCLC). Approximately 50-60% of NSCLC patients are expected to be positive for one or more mutations on this assay according to the current literature (Sequist et al. Annals of Oncology 22: 2616-2624, 2011; Su et al. The Journal of Molecular Diagnostics, Vol. 13, No. 1, January 2011). All results should be interpreted in conjunction with other clinical and pathological findings.

Limitations

The LungSEQ Concurrent cancer panel is designed to detect somatic hotspot mutations within 9 cancer-related genes, gene rearrangements involving the ALK, ROS1 and RET genes, and gene amplification of the MET gene. Mutations occurring within regions of these genes that are not targeted by the assay specific design will not be detected. The absence of mutation does not rule out the possibility that other mutations that are not targeted by this assay may be present. The limit of detection of the genotyping assay (the minimum percentage of mutant DNA that can be detected in a background of wild-type) is approximately 5 percent.

Genotyping Panel: 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.

ROS1, RET, and MET by FISH: Laboratory test results should always be considered in the context of clinical observations. 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.

ALK by FISH: Laboratory test results should always be considered in the context of clinical observations. This FISH test is performed using a Vysis FDA modified ALK Probe Kit.

Technical services performed at: med fusion, 2501 South State Highway 121, Bld 12 Lewisville, TX 75067 CLIA#45D2004217
Professional services performed at: Pathologists Bio-Medical Lab, 3600 Gaston Ave, Suite 261, Dallas, TX 75246