Washington University Physicians
Washington University School of Medicine in St. Louis

Genomics and Pathology Services
CORTEX Building, Suite 302,4320 Forest Park Ave. St. Louis, MO 63108
Phone: (314) 737-7337 | (866) 450-7697 | Fax: (314) 737-7336
Email: gps@wustl.edu | Website: gps.wustl.edu
CLIA #26D0698285 | CAP #27556-03

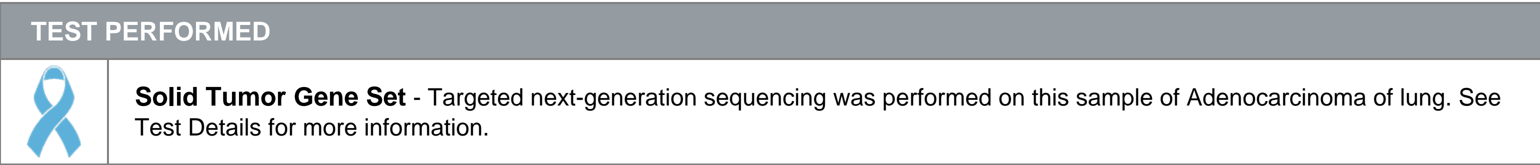
PATIENT INFORMATION

Name:		**Accession Number:**	
Date of Birth:		**MR#:**	
Gender:	Male	**Ordering Physician:**	
Disease:	Adenocarcinoma of lung		

Specimen Type:	Formalin-fixed paraffin-embedded tissue specimen	**Date Collected:**	
Indication:	Lung Cancer	**Date Ordered:**	
Specimen Quality:	Adequate	**Date Accessioned:**	

REVIEW STATUS: Final

TEST PERFORMED



Solid Tumor Gene Set - Targeted next-generation sequencing was performed on this sample of Adenocarcinoma of lung. See Test Details for more information.

CLINICALLY RELEVANT RESULTS SUMMARY

Variants that are deemed clinically significant are listed here.

Variants Detected	In patient tumor type: FDA approved therapies, prognostic information, or other course of action	In another tumor type: FDA approved therapies, prognostic information, or other course of action
EGFR p.L747_T751del	✓	
EGFR p.T790M	✓	

CLINICALLY RELEVANT INTERPRETATIONS

Interpretations of variants that are deemed clinically significant are listed here.

Variants Detected	Interpretation
EGFR p.L747_T751del	An activating deletion in kinase domain of *EGFR*, p.L747_P751del was identified. This deletion is well recognized and frequently reported in lung cancer (COSMIC). Mutations in *EGFR* predict the efficacy of treatment with EGFR tyrosine kinase inhibitors such as erlontinib, gefitinib and afatinib. Patients with *EGFR* exon 19 deletions treated with EGFR tyrosine kinase inhibitors show a marked radiographic response rate in prospective trials, including randomized phase III trials (Fukuoka M, et al.; J Clin Oncol 29; 2866-74; 2011 Jul 20); (Maemondo M, et al.; N Engl J Med 362; 2380-8; 2010 Jun 24); (Mitsudomi T, et al.; Lancet Oncol 11; 121-8; 2010 Feb); (Rosell R, et al.; Lancet Oncol 13; 239-46; 2012 Mar); (Yang JC, et al.; Lancet Oncol 13; 539-48; 2012 May); (Sequist LV, et al.; J Clin Oncol 31; 3327-34; 2013 Sep 20). These patients with *EGFR* mutant-harboring tumors have shown a longer progression-free survival on EGFR TKI therapy than those who receive non-TKI-inclusive chemotherapy. In general *EGFR* mutant tumors have a better prognosis when compared to those with *EGFR* wild type tumors (Marks JL, et al.; J Thorac Oncol 3; 111-6; 2008 Feb);(Kosaka T, et al.; J Thorac Oncol 4; 22-9; 2009 Jan). Thus, this variant is clinically actionable in lung cancer.
EGFR p.T790M	A non-synonymous *EGFR* p.T790M mutation was identified. This mutation is commonly identified in lung cancer (COSMIC) and is seen as a second mutation in about 50% of *EGFR*-mutant lung cancers that have developed acquired resistance to standard EGFR tyrosine kinase inhibitor (TKI) therapies. Consequently, this variant is widely recognized as a mechanism of EGFR TKI resistance (Pao W, et al.; PLoS Med 2; e73; 2005 Mar), (Kobayashi S, et al.; N Engl J Med 352; 786-92; 2005 Feb 24). Currently, several third-generation EGFR TKIs are in development to overcome the resistance conferred by this variant (Liao BC, Lin CC, Yang JC; Curr Opin Oncol; 2015 Mar;27(2):94-101) (Gray J, Haura E; 2014 Dec;3(6):360-2). Clinical correlation is advised.

OTHER RESULTS- SEE ALSO "All Identified Variants: Detailed Information"

Interpretations of variants that are not under clinically relevant results are listed here. Variants for which no interpretation is available are listed in the "All Identified Variants: Detailed Information" section at the end of report.

TP53 - p.H193Y

A non-synonymous variant, TP53 p.H193Y was detected. This variant is deleterious to the L2/L3 structural motif of the DNA binding domain, and ultimately renders the translated protein non-functional, resulting in loss of tumor suppression. According to the IARC *TP53* Database, this particular variant has been previously documented in a variety of tumors. Clinical studies of *TP53* mutations in non-small cell lung cancer have shown that patients with these mutations tend to have worse progression-free survival and/or overall survival compared those with wild-type *TP53* (Steels E, et al.; Eur Respir J 18; 705-19; 2001 Oct, Imielinski M, et al.; Cell 150; 1107-20; 2012 Sep 14), although whether this is independent of other clinical factors is unclear (Huncharek M, Kupelnick B, Geschwind JF, Caubet JF; Cancer Lett 153; 219-26; 2000 May 29). Other studies showed no effect of *TP53* mutation on overall survival and prognosis in lung non-small cell lung cancers (Kosaka T, et al.; J Thorac Oncol 4; 22-9; 2009 Jan); (Scoccianti C, et al.; Eur Respir J 40; 177-84; 2012 Jul); (Imielinski M, et al.; Cell 150; 1107-20; 2012 Sep 14). Given the limited and somewhat conflicted data in the literature regarding these mutations, the clinical significance of this variant, if any, in this patient's cancer, is uncertain.

RB1 - c.2211+2T>C

A novel, single nucleotide variant was detected in RB1 and occurs at an exon/intron junction. This variant may lead to aberrant splicing. The c.2211+2T>C variant has not been reported as a somatic mutation in cancer (COSMIC database). Similar truncated variants (due to aberrant splicing or premature stop codons) in *RB1* have been seen in lung adenocarcinoma (TCGA data, cBioportal database) and, as a group, are generally regarded as significant, recurrent alterations (Cancer Genome Atlas Research Network; Nature; 2014 Jul 31;511(7511):543-50). RB1 is a tumor suppressor, and loss of expression or loss of function variants are often reported in a number of cancers. This is a novel variant without functional data, so the specific impact of this variant in this patient's disease remains uncertain.

No rearrangements involving *ALK*, *ROS1*, *RET*, *NTRK1*, *FGFR2*, or *FGFR3* were detected by next generation sequencing.

OTHER TEST RESULTS

Results from tests other than next-generation sequencing are listed here.

None performed.

TEST DETAILS

Solid Tumor Gene Set: Targeted next-generation sequencing was performed on this sample of Adenocarcinoma of lung.

Pathologist review (performed by) of an H&E stained section of the tissue block ( ) was used to guide microdissection of areas of viable tumor at the CAP accredited (7233522) and CLIA certified (26D2013203) Washington University AMP Core Labs (425 S. Euclid Ave, Campus Box 8024, 4th Floor West Bldg, St Louis, MO 63110)

DNA was extracted from the areas of viable disease-involved tissue for sequence analysis. The technical component of the testing passed all established laboratory QC metrics, except for coverage of the targeted exons and introns specified below under Exon and Intron Coverage Metrics, where variants may not have been reliably detected.

Genes sequenced: *AKT1*, *AKT2*, *AKT3*, *ALK*, *ATM*, *BAP1*, *BRAF*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CDKN2A*, *CREBBP*, *CSF1R*, *CTNNB1*, *DDR2*, *EGFR*, *ERBB2*, *ERBB3*, *ERBB4*, *ESR1*, *FANCA*, *FGFR1*, *FGFR2*, *FGFR3*, *FGFR4*, *FLT1*, *FLT3*, *FLT4*, *GNAS*, *HRAS*, *IDH1*, *IDH2*, *JAK1*, *JAK2*, *KDR*, *KIT*, *KRAS*, *MAP2K1*, *MAP2K2*, *MET*, *MLH1*, *MTOR*, *MYC*, *NF1*, *NOTCH1*, *NOTCH2*, *NRAS*, *NTRK1*, *PALB2*, *PDGFRA*, *PDGFRB*, *PIK3CA*, *PTEN*, *RAD54B*, *RB1*, *RET*, *RIT1*, *ROS1*, *SMAD4*, *STK11*, *TP53*, *TSC1*, *TSC2* and *VHL*

Database Details: The versions/releases/builds/dates of the following databases were used to generate this report.

- Genomic Build: GRCh37.2
- Genomic Anotation Sources: NCBI RefSeq v37.2
- ClinVar: 20131230
- dbSNP: 138
- COSMIC: v68
- ExAC: v0.3
- dbNSFP: 3.0b2c
- NHLBI ESP: v.0.0.25. (February 7, 2014)

Exon and Intron Coverage Metrics: 100x coverage for > 95% of positions was not achieved for the targeted exons (coding + 2 bp) and introns (intron boundaries) listed below. Shown only for ordered genes.

Gene Transcript ID (Exon/Intron('))	*Gene* Transcript ID (Exon/Intron('))	*Gene* Transcript ID (Exon/Intron('))
AKT3 NM_181690 (13)	***NOTCH2*** NM_024408 (2)	***NTRK1*** NM_001007792 (2)
NTRK1 NM_002529 (7') NM_001007792 (8') NM_001012331 (7')		

METHODOLOGY

This test uses targeted next-generation sequencing to analyze all coding regions, and up to 10 base pairs of sequence flanking the coding regions, of the most inclusive annotated RefSeq transcript for each of the specified genes, as well as selected intronic regions. Target enrichment was performed using oligonucleotide-based targeted capture (xGen Lockdown Custom Target Capture Probes, Integrated DNA Technologies, and SeqCap EZ Hybridization and Wash Kit, Roche NimbleGen, Inc.) of whole genome shotgun sequencing libraries (KAPA Hyper Prep Kit and Kapa Library Amplification Kit, KAPA Biosystems, Inc.). Sequencing of enriched libraries was performed in multiplex on the Illumina HiSeq 2500 using the paired-end, 101 base-pair configuration.

Analysis and interpretation utilized the following software packages:

- Novoalign (version 3.02.00)
- samtools (version 0.1.19)
- picard (version 1.53)
- vcftools (0.1.6)
- GenomeAnalysis Toolkit (version 1.2)
- VarScan 2 (version 2.3.6)
- pindel (version 0.2.4d)
- BreakDancerMax (version 1.1r112)
- ClusterFast (version 1.0)
- Velvet (version 1.2.09)
- Blat (version 35)
- Variant Effect Predictor (VEP, part of Ensembl version 71), which uses Condel, SIFT (version 5.0.2), and polyPhen-2 (version 2.2.2)
- Integrative Genomics Viewer (version 2.1.24)
- IGV-tools (version 1.5.15)
- Internally developed Clinical Genomicist Workstation (version CGW_v3.0.3)
- All analysis was based on the human reference sequence UCSC build hg19 (NCBI build 37.2)

Variants are reported according to HGVS nomenclature (www.hgvs.org/mutnomen) and classified into five categories that are stratified by clinical actionability and previously reported data in the medical literature. Variations found in dbSNP (version 138 www.ncbi.nlm.nih.gov/projects/SNP/) which have > 1% minor allele frequency in at least one population except those that are also in OMIM, denoted as clinically relevant, used in a clinical diagnostic assay, or reported as a mutation in a publication are classified as known polymorphisms. Variants found in the NHLBI ESP (http://evs.gs.washington.edu/EVS/) database which have > 1% minor allele frequency in the total population are also classified as known polymorphisms.

This assay is designed to detect single nucleotide variants, small (less than 10bp) insertion/deletion (indel) events, selected larger insertion events, and limited structural rearrangement within selected genes (*KMT2A* (*MLL*), *ALK*, *ROS1*, *RET*, *NTRK1*, *FGFR2*, and *FGFR3*). Large insertions or deletions, complex indels, inversions, translocations, gene amplifications, other complex rearrangements, or copy number changes will not be detected by this assay. Variants located outside of targeted regions will not be detected. This assay does not determine variant causality, or whether a variant is inherited or somatically acquired.

Specificity, sensitivity, and positive predictive value (PPV) of this test to detect single nucleotide variants (SNV) in coding regions at an expected variant allele fraction (VAF) of 50% are estimated at 100.0% as determined by comparison of the genotypes at known single-nucleotide polymorphisms detected by this assay from HapMap DNA NA19129 to the genotypes at those positions reported by Complete Genomics (www.completegenomics.com/). Sensitivity for SNV in patient samples at VAFs of >= 7% is 98.9% (92/93). The limit of detection of this assay for SNV is 5%, with a 99.1% sensitivity for detection of SNV expected at a VAF of 5.0% at routinely achieved average coverage depths(~800-1200x),and an estimated 90% sensitivity for SNVs expected at a VAF of 5.0% at a genomic position with a coverage depth of 200-300x. Sensitivity for detecting insertions and deletions of 1-21 bp was 97.7% (43/44) relative to orthogonal clinical assays. sensitivity for detecting FLT3 ITD of > 70 bp was 100% (9/9) relative to orthogonal testing.

Inter- or intrachromosomal rearrangement events of the *KMT2A* (*MLL*), *ALK*, *ROS1*, *RET*, *NTRK1*, *FGFR2*, and *FGFR3* gene loci were assayed using ClusterFAST and Breakdancer. Sensitivity was 90.0% for detecting rearrangements across 20 patient samples and commercially available reference materials with *ALK*, *KMT2A (MLL)*, *ROS1*, or *RET* rearrangements as reported by FISH or other orthogonal testing. No rearrangement events were reported by ClusterFAST in genes previously reported as negative for rearrangement by FISH across 55 FISH assays, for a specificity of 100%. DNA level breakpoints are unique and may occur in areas of low sequence coverage resulting in reduced detection sensitivity. Should clinical concern for *ALK*, *KMT2A (MLL)*, *ROS1*, *RET*, *NTRK1*, *FGFR2*, or *FGFR3* rearrangements persist in the setting of negative sequencing results, correlation with FISH is recommended.

The six alignment or variant calling informatics tools used and their relevant parameters are detailed below.

1. Novoalign
Parameters: -o SAM -r none -t 254 -l 30 -e 100 -i 230 140 -a AGATCGGAAGAGCGGTTCAGCAGGAATGCCGAG AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTA -H -c 12 -f read1 read 2
2. Genome Analysis Tool Kit
CountCovariates: -l INFO -cov ReadGroupCovariate -cov QualityScoreCovariate -cov CycleCovariate -cov DinucCovariate
UnifiedGenotyper: -stand_call_conf 1.0 -stand_emit_conf 1.0 -dcov 5000 -G Standard -glm INDEL
VariantFiltration: default filters
3. VarScan 2
samtools mpileup -BA -d 9999 -Q 20 -q 30
mpileup2cns --variants 1 --output-vcf 1 --min-var-freq 0.01 --strand-filter 0
4. Pindel
sam2pindel: 270 sample 0
pindel: -c 1 -r true -t true -l false -k false -T true
5. Breakdancer_max
-r 2 -x5000
6. ClusterFast
-m1 2 -m2 1
sam2pindel: 270 H 0
pindel: -c ALL -l -k
velveth: [hashlength=]31 -fastq -shortPaired
velvetg: -cov_cutoff 3 -ins_length 300 -exp_cov 100 -ins_length_sd 60
blat/gfClient -minScore=2 -minIdentity=90

Note that it is possible that pathogenic variants may not be reported by one or more of the tools because of the parameters used. However, tool parameters were optimized to maximize specificity and sensitivity.

DISCLAIMER

Genetic testing using the methods applied at GPS@WUSTL is expected to be accurate. However, the chance of a false positive or false negative result due to laboratory errors incurred during any phase of testing cannot be completely excluded. GPS@WUSTL does not have control over the quantity, quality or provenance of specimens originating from outside institutions. Accordingly, GPS@WUSTL disclaims any and all responsibility and liability arising from a false positive or false negative result that may arise from an insufficient quantity of specimen, poor specimen quality, or contamination of specimen.

This Report was generated using the materials and methods described above, which required the use of various reagents, protocols, instruments, software, databases, and other items, some of which were provided or made accessible by third parties. A defect or malfunction in any such reagents, protocols, instruments, software, databases, and/or other items may compromise the quality or accuracy of the Report.

The Report has been created based on, or incorporates references to, various scientific manuscripts, references, and other sources of information, including without limitation manuscripts, references, and other sources of information that were prepared by third parties that describe correlations between certain genetic mutations and particular diseases (and/or certain therapeutics that may be useful in ameliorating the effects of such diseases). Such information and correlations are subject to change over time in response to future scientific and medical findings. WU makes no representation or warranty of any kind, expressed or implied, regarding the accuracy of the information provided by or contained in such manuscripts, references, and other sources of information. If any of the information provided by or contained in such manuscripts, references, and other sources is later determined to be inaccurate, the accuracy and quality of the Report may be adversely impacted. WU is not obligated to notify you of any impact that future scientific or medical research findings may have on the Report.

The Report must always be interpreted and considered within the clinical context, and a physician should always consider the Report along with all other pertinent information and data that a physician would prudently consider prior to providing a diagnosis to a patient or developing and implementing a plan of care for a patient. The Report should never be considered or relied upon alone in making any diagnosis or prognosis. The manifestation of many diseases are caused by more than one gene variant, a single gene variant may be relevant to more than one disease, and certain relevant gene variants may not have been considered in the Report. In addition, many diseases are caused or influenced by modifier genes, epigenetic factors, environmental factors, and other variables that are not addressed by the Report (or that are otherwise unknown). As such, the relevance of the Report should be interpreted in the context of a patient's clinical manifestations. The Report provided by WU is provided on an "AS IS" basis. WU makes no representation or warranty of any kind, expressed or implied, regarding the Report. In no event shall WU be liable for any actual damages, indirect damages, and/or special or consequential damages arising out of or in any way connected with the Report, your use of the Report, your reliance on the Report, or any defect or inaccurate information included within the Report.

This test was developed and its performance characteristics determined by Washington University School of Medicine. It has not been cleared or approved by the FDA. The laboratory is regulated under CLIA as qualified to perform high-complexity testing. This test is used for clinical purposes. It should not be regarded as investigational or for research.

All Identified Variants Detailed Information

Level 1- Predictive or prognostic in tumor type

Non-synonymous (Variants found : 2)

EGFR NM_005228:c.2369C>T	(chr7:g.55249071C>T) NP_005219:p.T790M
EGFR NM_005228:c.2238_2252del chr7(NM_201282):c.*106+d6140_*106+d6154del chr7(NM_201283):c.*108+d17824_*108+d17838del chr7(NM_201284):c.*501+d3730_*501+d3744del	(chr7:g.55242468_55242482del15) NP_005219:p.L747_T751del * * *

No established biological impact, non-coding region (Variants found : 0)

Synonymous (Variants found : 0)

Level 2- Predictive or prognostic in other tumor type(s)

Non-synonymous (Variants found : 0)

No established biological impact, non-coding region (Variants found : 0)

Synonymous (Variants found : 0)

Level 3- Reported in cancer or other disease

Non-synonymous (Variants found : 1)

TP53 NM_000546:c.577C>T NM_001126112:c.577C>T NM_001126113:c.577C>T NM_001126114:c.577C>T NM_001126115:c.181C>T NM_001126116:c.181C>T NM_001126117:c.181C>T	(chr17:g.7578272G>A) NP_000537:p.H193Y NP_001119584:p.H193Y NP_001119585:p.H193Y NP_001119586:p.H193Y NP_001119587:p.H61Y NP_001119588:p.H61Y NP_001119589:p.H61Y

No established biological impact, non-coding region (Variants found : 0)

Synonymous (Variants found : 0)

Level 4- Variant of uncertain significance

Non-synonymous (Variants found : 1)

RB1 chr13(NM_000321):c.2211+2T>C	(chr13:g.49037973T>C) NP_000312:p.?

No established biological impact, non-coding region (Variants found : 1)

MLH1 NM_000249:c.375A>G NM_001167617:c.81A>G NM_001167618:c.-349A>G NM_001167619:c.-257A>G	(chr3:g.37045960A>G) NP_000240:p.(=) NP_001161089:p.(=) * *

Synonymous (Variants found : 0)

Level 5- Known polymorphism

Non-synonymous (Variants found : 19)

PALB2 NM_024675:c.2590C>T	(chr16:g.23637715G>A) NP_078951:p.P864S
ERBB2 NM_001005862:c.1873A>G NM_004448:c.1963A>G	(chr17:g.37879588A>G) NP_001005862:p.I625V NP_004439:p.I655V
TSC1 NM_000368:c.965T>C NM_001162426:c.965T>C NM_001162427:c.812T>C	(chr9:g.135786904A>G) NP_000359:p.M322T NP_001155898:p.M322T NP_001155899:p.M271T
KDR NM_002253:c.1416A>T	(chr4:g.55972974T>A) NP_002244:p.Q472H
FANCA NM_000135:c.2426G>A	(chr16:g.89836323C>T) NP_000126:p.G809D
ALK NM_004304:c.4381A>G	(chr2:g.29416572T>C) NP_004295:p.I1461V
ATM NM_000051:c.5948A>G	(chr11:g.108183167A>G) NP_000042:p.N1983S
BRCA2 NM_000059:c.7397T>C	(chr13:g.32929387T>C) NP_000050:p.V2466A
BRIP1 NM_032043:c.2755T>C	(chr17:g.59763347A>G) NP_114432:p.S919P
ERBB2 NM_001005862:c.3418C>G NM_004448:c.3508C>G	(chr17:g.37884037C>G) NP_001005862:p.P1140A NP_004439:p.P1170A
FANCA NM_000135:c.1501G>A	(chr16:g.89849480C>T) NP_000126:p.G501S
FANCA NM_000135:c.796A>G NM_001018112:c.796A>G	(chr16:g.89866043T>C) NP_000126:p.T266A NP_001018122:p.T266A
FGFR4 NM_002011:c.407C>T NM_022963:c.407C>T NM_213647:c.407C>T	(chr5:g.176517797C>T) NP_002002:p.P136L NP_075252:p.P136L NP_998812:p.P136L
FGFR4 NM_002011:c.1162G>A chr5(NM_022963):c.1058-90G>A NM_213647:c.1162G>A	(chr5:g.176520243G>A) NP_002002:p.G388R * NP_998812:p.G388R
FLT3 NM_004119:c.680C>T	(chr13:g.28624294G>A) NP_004110:p.T227M
KDR NM_002253:c.1384T>G	(chr4:g.55973932A>C) NP_002244:p.L462V
MLH1 NM_000249:c.655A>G NM_001167617:c.361A>G NM_001167618:c.-69A>G NM_001167619:c.-69A>G	(chr3:g.37053568A>G) NP_000240:p.I219V NP_001161089:p.I121V * *
NTRK1 NM_001007792:c.-5G>A	(chr1:g.156785617G>A) NP_001007793:p.?
ROS1 NM_002944:c.500G>A	(chr6:g.117724379C>T) NP_002935:p.R167Q
No established biological impact, non-coding region (Variants found : 23)	

KRAS chr12(NM_004985):c.451-5617G>A NM_033360:c.483G>A	(chr12:g.25368462C>T) * NP_203524:p.(=)
Synonymous (Variants found : 59)	

Report Electronically reviewed and signed out by

Date Reported:

Amendments for Solid Tumor Gene Set

No Amendments