OncoStrands™ Essential Panel

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LifeStrands
Empowering lives with Genomics

PATIENT		PHYSICIAN	SPECIMEN	CASE
NAME M Z **DATE OF BIRTH** 06/07/19XX **DISEASE** Adenocarcinoma of lung **ADDRESS** xxxxxx xxxxx xxxxxx xxxxx	**SEX** Female **MRN#** -	**ORDERING PHYSICIAN** Dr. XXX XXXXXXX **FACILITY** xxxxxx Hospital **DATE ORDERED** 08/02/2023 **COPY TO** -	**EXT. SPECIMEN ID** C2300168 (xxxxxxx Pathology) **DATE RECEIVED** 14/02/2023 **SPECIMEN TYPE** Formalin-fixed paraffin-embedded tissue specimen **% TUMOR CELLULARITY** 30%	**ACCESSION#** A23Mxxxx **DATE REPORTED** 16/02/2023 **REVIEW STATUS** Final **REPORTED BY** Dr. Vivek Rathi

Report Summary

• A variant of strong clinical significance identified –

TIER 1A, KRAS p.G12C – Currently, Sotorasib is FDA approved for the treatment of advanced non-small cell lung carcinoma patients harbouring KRAS G12C variant, as described below.

• Pertinent negatives –

Mutations – no pathogenic variants detected in EGFR, BRAF, HER2, or MET (including MET exon 14 skipping variants).

Amplifications – no amplification of MET gene detected.

Fusions – no fusion transcripts detected in ALK, ROS1, RET, NTRK1, NTRK2 or NTRK3.

IA	IB	IIC	IID	Trials
1	0	0	0	2

Clinical Implications

TIER	VARIANT DETECTED (GENE/SYNTAX)	CLINICAL IMPACT		SELECT CLINICAL TRIALS
IA	KRAS p.G12C	**May benefit from:**	Pembrolizumab, Cemiplimab-rwlc, or Durvalumab + Tremelimumab-actl	2
		In Tumor Type:	Nonsquamous nonsmall cell neoplasm of lung, Squamous cell carcinoma of lung, Adenocarcinoma of lung, Non-small cell lung cancer, Large cell carcinoma of lung, or Non-small cell carcinoma	

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TIER	VARIANT DETECTED (GENE/SYNTAX)	CLINICAL IMPACT		SELECT CLINICAL TRIALS
		May benefit from:	Bevacizumab-adcd, Atezolizumab + Bevacizumab, Atezolizumab + Bevacizumab-maly, Atezolizumab + Bevacizumab-adcd, Bevacizumab-maly, Bevacizumab-bvzr, Atezolizumab + Bevacizumab-awwb, Bevacizumab-awwb, Atezolizumab + Bevacizumab-bvzr, Bevacizumab, or Atezolizumab	
		In Tumor Type:	Nonsquamous nonsmall cell neoplasm of lung, Adenocarcinoma of lung, or Large cell carcinoma of lung	
		May benefit from:	Sotorasib, Ipilimumab + Nivolumab, or Adagrasib	
		In Tumor Type:	Squamous cell carcinoma of lung, Adenocarcinoma of lung, Non-small cell lung cancer, Large cell carcinoma of lung, or Non-small cell carcinoma	
		May not benefit from:	Afatinib, Erlotinib, Osimertinib, or Gefitinib	
		In Tumor Type:	Non-small cell lung cancer or Non-small cell carcinoma	
		Unfavorable Prognosis in:	Malignant tumor of unknown origin or ill-defined site, Non-small cell lung cancer, Malignant tumor of unknown origin, or Non-small cell carcinoma	

Other Test Results

- Histopathology -
  EBUS TBNA Station 4R and 7 - adenocarcinoma.

- IHC for PD-L1 (Ventana, clone SP263)
  Positive membranous staining in less than 1% of the tumour cells.

Clinical Interpretations

KRAS	**p.G12C**	c.34G>T	Tier IA	NM_033360.2	VAF: 34.2%	Depth: 3357

GENE: KRAS, KRAS proto-oncogene, GTPase, is a member of the small GTPase superfamily and a key regulator of the MAPK, PI3K/AKT/mTOR pathways (PMID: 23622131) that plays a role in regulation of cell proliferation (PMID: 31988705). KRAS mutations are identified in a wide range of cancers (PMID: 28666118), including colorectal cancer (PMID: 31952666, PMID: 32241284), non-small cell lung cancer (PMID: 32062493, PMID: 32244355), and pancreatic cancer (PMID: 32005945).

VARIANT: KRAS p.G12C is a missense alteration within the first "G box" domain of the K-Ras protein, one of several conserved regions responsible for GTP binding and hydrolysis; disruption of this region creates a protein that is defective for GTP hydrolysis and is therefore constitutively active (PMID: 6092920, PMID: 8439212, PMID: 15367757). KRAS p.G12C has been reported as the most common KRAS mutation in non-small cell lung carcinoma (NSCLC), and has been shown to have transforming ability and lead to activation of MEK and ERK signaling; in contrast to KRAS p.G12D, the p.G12C mutation has been reported not to result in activation of Akt (PMID: 21169473, PMID: 23313110, PMID: 22247021, PMID: 21306997).

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THERAPEUTICS: Currently, Sotorasib is FDA approved for treatment of advanced NSCLC patients harbouring this variant.

Sotorasib (Lumakras, Amgen, Inc.) was granted an accelerated FDA approval for advanced KRAS p.G12C-mutant NSCLC, based on the multicenter, single-arm, open-label CodeBreaK 100 clinical trial. A total of 129 patients (59 with NSCLC, 42 with colorectal cancer, and 28 with other tumors) were included in dose escalation and expansion cohorts. Patients had received a median of 3 (range, 0 to 11) previous lines of anticancer therapies for metastatic disease. No dose-limiting toxic effects or treatment-related deaths were observed. A total of 73 patients (56.6%) had treatment-related adverse events; 15 patients (11.6%) had grade 3 or 4 events. In the subgroup with NSCLC, 32.2% (19 patients) had a confirmed objective response (complete or partial response) and 88.1% (52 patients) had disease control (objective response or stable disease); the median progression-free survival was 6.3 months (range, 0.0+ to 14.9 [with + indicating that the value includes patient data that were censored at data cutoff]). Sotorasib showed encouraging anticancer activity in patients with heavily pretreated advanced solid tumors harboring the KRAS p.G12C mutation (CodeBreaK100; NCT03600883; PMID: 32955176).

The KRAS oncogene is a prognostic biomarker; the presence of KRAS mutations is prognostic of poor survival for patients with NSCLC when compared to the absence of KRAS mutations, independent of therapy. In a clinical trial of 215 patients with refractory NSCLC, patients with mutant KRAS p.G12V or KRAS p.G12C had worse progression-free survival compared with patients whose tumors had other mutant KRAS proteins or wild-type KRAS (PMID: 22247021). KRAS mutational status is also predictive of lack of therapeutic efficacy with EGFR-TKIs; however, it does not appear to affect chemotherapeutic efficacy. KRAS mutations do not generally overlap with EGFR mutations, ALK rearrangements, or ROS1 rearrangements (NCCN 'NSCLC' v.3.2022). It is important to note that not all mutant KRAS proteins affect patient survival or downstream signaling in a similar way (PMID: 22247021).

The significance of KRAS mutational analysis is becoming increasingly important with the further development of new therapies targeting downstream RAS pathways such as PI3K/AKT/mTOR and RAS/RAF/MEK. Immune checkpoint inhibitors appear to be effective for patients with KRAS mutations and MEK inhibitors are being investigated in clinical trials (NCCN 'NSCLC' v.3.2022). Results of clinical trials indicate that drugs targeting the MAPK pathway, such as sorafenib (FDA-approved for treatment of cancer), selumetinib, and trametinib, appear to be effective against KRAS-mutated non-small cell lung cancer patients (PMID: 26929424, PMID: 28492898). Studies including phase II trials have shown that RAF kinase inhibitor sorafenib is effective in treating chemotherapy pre-treated non-small cell lung cancer patients with activating KRAS mutations (PMID: 24166906, PMID: 23224737).

Clinical Trials

Clinical Trials associated with this patient's genomic profile and tumor type as displayed below.

TITLE	TRIAL IDENTIFIER	PHASE	VARIANT
Study of JDQ443 in Patients With Advanced Solid Tumors Harboring the KRAS G12C Mutation	NCT04699188	I/II	**KRAS** p.G12C c.34G>T
Study of Safety, Pharmacokinetics, and Antitumor Activity of BGB-3245 in Participants With Advanced or Refractory Tumors	NCT04249843	I	**KRAS** p.G12C c.34G>T

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Significant Negative Findings

Please refer to the Summary section of the report.

Tier III - Variants of Uncertain Significance

No variants were reported for this classification tier.

Other Comments

Current FDA approved treatments for specific genetic alterations in metastatic non-small cell lung carcinoma -

ALK fusions - Crizotinib, ceritinib, alectinib, brigatinib, lorlatinib
BRAF V600E - Dabrafenib + trametinib
EGFR ex 19 del, L858Rm - Afatinib, dacomitinib, erlotinib, gefitinib, osimertinib
EGFR ex 20 insertions - Amivantamab
EGFR nonresistant mutations other than exon 19deletions and L858R - Afatinib
EGFR T790M - Osimertinib
EGFR G719 - Afatinib
KRAS G12C - Sotorasib
MET exon 14 skipping - Capmatinib, tepotinib
RET fusions - Pralsetinib, selpercatinib
ROS1 fusions - Crizotinib, entrectinib

FDA approved tumour agnostic indications -

NTRK1 or NTRK2 or NTRK3 fusions - Entrectinib, larotrectinib
MSI-H, TMB-H - Pembrolizumab

FDA-listed genetic alterations contraindicated for specific treatments with TRK inhibitors -

NTRK1 and NTRK3 known acquired resistance mutations (eg, NTRK1 G595R and G667C; NTRK3 F617L, G623R, and G696A)

FDA-approved combination treatments with non-targeted therapies for specific genetic alterations -

EGFR exon 19 deletions, L858R - Erlotinib + ramucirumab

Current NCCN recommended biomarkers for sequencing -

Mutations - BRAF, EGFR, HER2, KRAS, MET
Amplifications - MET
Fusions - ALK, NTRK1, NTRK2, NTRK3, RET, ROS1

Tier Definitions

Tier I-A: Approved therapy. Included in professional guidelines.
Tier I-B: Well-powered studies with consensus from experts in the field.
Tier II-C: Approved therapies for different tumour types or investigational therapies. Multiple small published studies with some

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consensus. Inclusion criteria for clinical trials.
Tier II-D: Limited clinical or preclinical studies.
Tier III (VUS): Variants of Unknown Clinical Significance.
Tier IV: Benign or likely benign variants (not included in the report)

Test Information

REPORTED GENES: AKT1, AKT2, AKT3, ALK, AR, ARAF, BRAF, CD274, CDK4, CDKN2A, CHEK2, CTNNB1, EGFR, ERBB2, ERBB3, ERBB4, ESR1, FGFR1, FGFR2, FGFR3, FGFR4, FLT3, GNA11, GNAQ, GNAS, HRAS, IDH1, IDH2, KIT, KRAS, MAP2K1, MAP2K2, MET, MTOR, NRAS, NRG1, NTRK1, NTRK2, NTRK3, NUTM1, PDGFRA, PIK3CA, PTEN, RAF1, RET, ROS1, RSPO2, RSPO3, SMO, TP53 **CGW VERSION:** CGW_v6.21.0.1 **DATABASE DETAILS:** The versions, releases, builds, dates of the following databases were used to generate this report: Genomic Build: GRCh37.p13 | Genomic Annotation Sources: NCBI RefSeq v105 | dbNSFP: 4.2c | NHLBI ESP: v.0.0.30 | gnomAD: r2.1 | COSMIC: v96 | ExAC: v1.0 | dbscSNV: v1.1 | ClinVar: 20220702 | dbSNP: 149 **ASSAY METHODOLOGY:**

ASSAY METHODS: This is a laboratory developed next generation sequencing (NGS)-based test utilising the Oncomine™ Precision Assay (OPA, Thermo Fisher Scientific) and running on the Genexus Integrated Sequencer (Thermo Fisher Scientific) to detect DNA and RNA based variants in formalin-fixed paraffin embedded (FFPE) samples. The OPA assay uses ThermoFisher's proprietary AmpliSeq™ enrichment chemistry enabling nucleic acid sequencing to be reliably performed with a small input (10 -20 ng) and is designed to detect multiple classes of variants including single nucleotide variants (SNVs), multi-nucleotide variants (MNVs), small insertions /deletions (Indels), copy number variations (CNVs), exonic variants and gene fusions in 50 selected cancer genes. The 50 gene panel includes detection of recurrent hotspot mutations in 45 genes, CNV detection in 14 genes, and the detection of gene fusions from RNA in 18 genes.

Genomic DNA and total RNA were extracted using the commercial nucleic acid extraction kits from Qiagen or Zymo Research and were then quantified using the Qubit method. The automated Genexus sequencer performed library preparation, sequencing, and secondary analysis. Multiplex primer design and sample barcoding used in this NGS workflow enabled test results to be generated from multiple samples in a single NGS run.

SECONDARY ANALYSIS METHODS: DNA/RNA analysis was performed locally using the Genexus analysis platform. Variant files i.e., VCF/BAM files generated from the Genexus analysis pipeline were then uploaded to the Clinical Genomics Workspace (CGW) through PierianDx. Variant files were then parsed and combined into compatible formats and analysed using the CGW software platform using an in-house bioinformatics pipeline.

VARIANT CALLING: Variants are reported according to HGVS nomenclature (www.hgvs.org/mutnomen) and classified per the AMP classification system into tiers IA, IB, IIC, IID, III, and IV. These tiers are stratified by clinical utility ('actionability' for clinical decision-making as to diagnosis, prognosis, treatment options, and carrier status) and previously reported data in the medical literature. Variants found in gnomAD (https:// gnomad.broadinstitute.org/) that have ≥1% minor allele frequency (except those that are also in Clinvar denoted as clinically relevant, used in a clinical diagnostic assay, or reported as a mutation in a publication) are classified as known polymorphisms.

DNA/RNA VARIANTS (SNVS, INSERTIONS, DELETIONS, CNVS, AND GENE FUSIONS)
Based on the in-house validation using clinical samples and various types of reference standards, the assay can detect SNVs with 99.4% sensitivity and 100% specificity, short insertion/deletions with 100% sensitivity, and 100% specificity at ≥5% limit of detection. For the detection of copy number variation gain, the assays demonstrated 100% sensitivity and 100% specificity using the following CNV gain calling criteria; Not Detected (≤5 copies), Equivocal (>5, <10), and Detected (≥10). For the detection of gene fusions, the assay demonstrated 98.7% sensitivity and 100% specificity based on fusion calls having ≥20 copies.

Additional Notes:
- This assay is clinically validated for the detection of somatic variants in somatic tumour specimens.
- Variants located outside of the targeted regions or present below the limit of detection will not be detected.
- Synonymous and polymorphic variants are not reported.
- This assay cannot distinguish somatic variants from germline variants.
- This CNVs detected through this assay are just an estimate, thus orthogonal tests should be used to confirm the reported CNV results.
- This assay has not been fully validated for detection of CNV loss.
- 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.

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OncoStrands™Essential Panel Gene List

DNA Hotspot Genes

- *AKT1 AKT2 AKT3 ALK AR ARAF BRAF CDK4 CDKN2A CHEK2 CTNNB1 EGFR ERBB2 ERBB3 ERBB4 ESR1 FGFR1 FGFR2 FGFR3 FGFR4 FLT3 GNA11 GNAQ GNAS HRAS IDH1 IDH2 KIT KRAS MAP2K1 MAP2K2 MET MTOR NRAS NTRK1 NTRK2 NTRK3 PDGFRA PIK3CA PTEN RAF1 RET ROS1 SMO TP53*

Copy Number Variation (CNV) Genes

- *ALK AR CD274 CDKN2A EGFR ERBB2 ERBB3 FGFR1 FGFR2 FGFR3 KRAS MET PIK3CA PTEN*

Fusion (RNA) Genes

- *ALK AR BRAF EGFR ESR1 FGFR1 FGFR2 FGFR3 MET NRG1 NTRK1 NTRK2 NTRK3 NUTM1 RET ROS1 RSPO2 RSPO3*

DISCLAIMER:

This is a laboratory developed test, and its performance characteristics have been determined by LifeStrands Genomics. 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. LifeStrands Genomics 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. LifeStrands Genomics 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 is 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). This Report is based on a next generation sequencing assay which does not distinguish between somatic and germline variants. If a germline variant is in question, further testing may be recommended. As such, the relevance of the Report should be interpreted in the context of a patient's clinical manifestations. The Report provided by LifeStrands Genomics is provided on an AS IS basis. LifeStrands Genomics makes no representation or warranty of any kind, expressed or implied, regarding the Report. In no event shall LifeStrands Genomics 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. Medical knowledge annotation is constantly updated and reflects the current knowledge at the time.

Report electronically reviewed and signed out by: Dr. Vivek Rathi
Date Reported: 16/02/2023