PATIENT INFORMATION		REFERRAL INFORMATION	
NAME		CLINIC NAME **German Oncology Center**	
Gender		CLINIC ID	
DATE OF BIRTH		REFERRING CLINICIAN **Dr. Tsechelides**	
TEST INDICATIONS **Lung cancer**		CLINIC EMAIL	

SAMPLE INFORMATION

ORDER NUMBER	LAB NUMBER	DATE OF COLLECTION	DATE RECEIVED
			25/05/2020

CANCER TEST SELECTED

☒ LUNG Liquid Biopsy ☐ COLORECTAL Liquid Biopsy

TEST RESULTS

POSITIVE
At least 1 clinically significant variant identified

RESULT SUMMARY

BIOMARKER FINDINGS

MSI-H
☐ DETECTED ☒ NOT-DETECTED

GENOMIC FINDINGS

Gene	Variant Detected	Allele Fraction	FDA/EMA Approved Therapies (In patient's indication)*	FDA/EMA Approved Therapies (In other indications)**	Clinical Trials
KRAS	c.182T>A, (p.Gln61Leu)	1.9%			48
RET	c.2428G>T, (p.Gly810Cys)	3.3%	NCCN ✓ Selpercatinib NCCN ✓ Pralsetinib	NCCN Cabozantinib NCCN Vandetanib	30
DDR2	c.2395G>A, (p.Glu799Lys)	0.7%			2
TP53	c.745T>A, (p.Arg249Trp)	2.2%			6

✓ Approved in indication ✗ Contra-indication NCCN Included in NCCN guidelines

*List of FDA and/or EMA approved drugs in the patient's cancer type

**List of FDA and/or EMA approved drugs in other tumor types. Therapies that are included in the NCCN guidelines for the patient's cancer type are clearly indicated above.

Note: Clinical trials listed in this report are retrieved from clinicaltrials.gov[1] and only include not yet recruiting and recruiting trials for the indicated cancer type and gene. The list of therapies and clinical trials included in this report may not be complete and/or exhaustive. Therapies contained in this report are FDA/EMA approved, however information on drug approvals for different indications is updated regularly, based on new evidence, and may not reflect the current status at any time. This report should not be used as the sole basis when making treatment decisions, instead it should be regarded as a supplementary source of information for guiding therapy decisions. All treatment decisions remain the full and final responsibility of the treating clinician.

INTERPRETATIONS

Microsatellite Instability

MSI-H: Not detected
There is insufficient evidence of genomic instability at the microsatellite regions tested in the patient tumor sample tested.

KRAS c.182T>A, (p.Gln61Leu)

Variant details

The KRAS c.182T>A, (p.Gln61Leu) substitution is classified as Tier1 variant which has been recognized as a variant of strong clinical significance in Lung cancer. This is a missense mutation on exon 3, resulting to the substitution of Glutamine to Leucine at position 61 of the KRAS gene (NM_033360.4). This mutation has been reported in lung cancer patients in the ICGC database (MU91987), Cosmic database (COSM553) and ClinVar database (ID:45116).

Gene information and significance

Mutations in the RAS family of proteins are frequently observed across cancer types[7]. The KRAS gene provides instructions for making a protein called K-Ras, involved in the signaling pathway known as the RAS/MAPK pathway. The signaling pathway is regulating cell proliferation, maturation, and differentiation. The KRAS gene belongs to a class of genes known as oncogenes. When mutated they have the potential to cause abnormal cell growth and proliferation leading to cancer.

Approved therapies and Clinical trials available

Currently, there are no approved targeted therapies for Non-Small Cell Lung cancer (NSCLC) patients, with this specific KRAS mutation. However, there are at present 48 clinical trials investigating treatment options for *KRAS* mutant NSCLC. Notably, a phase I/II study of the CDK4/6 inhibitor Palbociclib (PD-0332991) in combination with MEK inhibitor Binimetinib (MEK162) for patients with advanced KRAS mutant NSCLC NCT03170206. For more information on the abovementioned and other clinical trials regarding *KRAS* mutated NSCLC please visit clinicaltrials.gov.

RET c.2428G>T, (p.Gly810Cys)

Variant details

The RET c.2428G>T, (p.Gly810Cys) substitution is classified as Tier1 variant which has been recognized as a variant of strong clinical significance in Lung cancer. This is a missense mutation on exon 14 of the RET gene (NM_020975.6), resulting in substitution of Glycine to Cysteine at position 810 of the protein sequence.

Gene information and significance

The *RET* gene provides instructions for producing a protein that is involved in signaling within cells and it is essential for the normal development of several kinds of nerve cells. The RET protein spans the

cell membrane, so that one end of the protein remains inside the cell and the other end projects from the outer surface of the cell. This positioning of the protein allows it to interact with specific factors outside the cell and to receive signals that help the cell respond to its environment. When molecules that stimulate growth and development (growth factors) attach to the RET protein, a complex cascade of chemical reactions inside the cell is triggered. These reactions instruct the cell to undergo certain changes, such as dividing or maturing to take on specialized functions. Somatic RET mutations are reported in 2.79% of lung cancer patients according to the COSMIC database.

Approved therapies and Clinical trials available

Currently, there are two approved targeted therapies for Non-Small Cell Lung cancer (NSCLC) patients with RET muations. Selpercatinib (kinase inhibitor) is indicated for NSCLC patients with metastatic RET fusions or specific RET mutations. Pralsetinib (kinase inhibitor) is indicated for NSCLC patients with RET fusion cancers. Additionally, there are two other targeted therapies, Cabozantinib and Vandetanib, that are indicated for RET-positive cancers but are indicated for other cancer types.

Regarding clinical trials, there are currently 30 clinical trials recruiting patients with NSCLC who have RET mutations or fusions. Notably, a phase II study with Cabozantinib in patients with RET positive NSCLC is currently recruiting (NCT04131543). For more information on the abovementioned and other clinical trials regarding *RET* mutated NSCLC please visit clinicaltrials.gov.

DDR2 c.2395G>A, (p.Glu799Lys)

Variant details

The DDR2 c.2395G>A, (p.Glu799Lys) substitution is classified as Tier2 variant which has been recognized as a variant of potential clinical significance in Lung cancer. This is a missense mutation on exon 17 of the DDR2 gene (NM_006182.4), resulting in a Glutamic acid to Lysine substitution in position 799 of the protein sequence.

Gene information and significance

The DDR2 gene provides instruction for making a domain of the receptor tyrosine kinase protein family. These receptors play a key role in the communication of cells with their microenvironment. The DDR2 protein activates pathways involved in cell adhesion and proliferation. Somatic DDR2 mutations are reported in 2.36% of lung cancer patients according to the COSMIC database.

Approved therapies and Clinical trials available

Currently, there are no approved targeted therapies for Non-Small Cell Lung cancer (NSCLC) patients with DDR2 mutations. In addition, there are 2 clinical trials recruiting patients with NSCLC who have DDR2 mutations. For more information on the abovementioned and other clinical trials regarding *DDR2* mutated NSCLC please visit clinicaltrials.gov.

TP53 c.745T>A, (p.Arg249Trp)

Variant details

The TP53 c.745T>A, (p.Arg249Trp) substitution is classified as Tier1 variant which has been recognized as a variant of strong clinical significance in Lung cancer. This is a missense mutation on exon 7 of the TP53 gene (NM_000546.6), resulting in a Arginine to Tryptophan substitution in position 249 of the protein sequence. This mutation has been reported in lung cancer patients in the ICGC database (MU824195).

Gene information and significance

The TP53 gene provides instruction for making a receptor protein called tumor protein p53 and acts as a tumor suppressor. This gene regulates cell division by keeping cell from growing and dividing uncontrollably. The loss of a tumor suppressor is most often through large deleterious events, such as frameshift mutations, or premature stop codons. In TP53 however, many of the observed mutations in cancer are found to be single nucleotide missense variants. Somatic TP53 mutations are reported in 41.52% of lung cancer patients according to the COSMIC database.

Approved therapies and Clinical trials available

Currently, there are no approved targeted therapies for Non-Small Cell Lung cancer (NSCLC) patients with TP53 mutations. In addition, there are 6 clinical trials recruiting patients with NSCLC who have TP53 mutations. For more information on the abovementioned and other clinical trials regarding *TP53* mutated NSCLC please visit clinicaltrials.gov.

VARIANTS OF UNKNOWN SIGNIFICANCE

Variants of unknown significance have not been detected in this patient's sample.

Genes covered by Lung Liquid Biopsy Panel

Single Nucleotide Variants/ Insertions and Deletions	Copy Number Alterations	Rearrangements
AKT1, ALK, APC, ARAF, ATM, BRAF, BRCA2, CTNNB1, DDR2, EGFR, ERBB2, ERBB3, ERBB4, FBXW7, JAK2, KEAP1, KRAS, MAP2K1, MET, NRAS, PDGFRA, PIK3CA, POLE, PTEN, RAF1, SMAD4, STK11 TP53	EGFR, ERBB2, FGFR1, FGFR2, FGFR3, MET, PIK3CA	ALK, FGFR3, NTRK1, NTRK2, NTRK3, RET, ROS1

METHODOLOGY

Liquid Biopsy Lung Panel (RUO) is a Laboratory Developed Test (LTD) from NIPD Genetics Public Company Ltd for tumor molecular profiling by analyzing circulating tumor DNA (ctDNA). ctDNA is extracted from blood samples using a standardized methodology, followed by DNA library preparation. DNA enrichment for the genomic regions of interest is carried out using a solution-based hybridization method followed by next generation sequencing (NGS). Sequence data is aligned to a reference genome and variants are identified using proprietary bioinformatics pipelines. Liquid Biopsy Lung Panel (RUO) can be used for the identification of selected single nucleotide variants (SNVs), small insertions and deletions (Indels, ≤30bp), rearrangements and copy number variations (CNAs) depending on the test ordered. Tumor-related actionable and clinically relevant alterations are reported. Analysis and Interpretation is performed using but not limited to Varsome Clinical CE-IVD platform (ISO 13485) according to published knowledge at the time of testing. Genetic counselling for the clinical interpretation and significance of the results is recommended. The Liquid Biopsy Lung Panel (RUO) test development and performance evaluation was carried out by NIPD Genetics Public Company Limited, which is regulated under the Clinical Laboratory Improvement Act of 1998 (CLIA) as qualified to perform high-complexity testing. Liquid Biopsy Lung Panel (RUO) is intended only for research purposes. The test has not been cleared or approved by the U.S.Food and Drug Administration (FDA), which does not require this test to go through premarket FDA review.

TECHNICAL SPECIFICATIONS AND LIMITATIONS

NIPD Genetics Liquid Biopsy test is designed to detect selected targeted variants, including SNVs (Single Nucleotide Variants), Indels (Insertions and/or deletions), CNAs (Copy Number Changes), SVs (Structural Rearrangements) and MSI (MicroSatellite Instability) status, associated with cancer development. NIPD Genetics Liquid Biopsy test targets exonic and other hotspot regions in selected genes (listed above). Variants on the flanks or outside of the targeted regions are not intended to be detected by this assay. The analytical sensitivity for detecting sequence specific alterations such as SNVs and Indels depends on the true variant allele frequency (VAF) of the mutation and it is estimated at (i) 92% (81-97% at 0.05 sign. level) when the true VAF lies between 0.1% and 0.5%, and (ii) 100% (92-100% at 0.05 sign. level) when the true VAF is greater than 0.5%. The test cannot detect variants with VAF<0.1%. The estimated analytical specificity is >99.99%. SVs are reported when VAF exceeds 0.5% with an estimated sensitivity and specificity of 100% (66-100% at 0.05 sign. level) and 100% (93-100% at 0.05 sign. level), respectively. The test cannot detect SVs with VAF<0.5%. The limit of detection for detection of selected gene level CNAs is 2.8 copies (average copy number state of the tumor/normal admixture in cfDNA sample) with an estimated sensitivity and specificity of 100% (48-100% at 0.05 sign. level) and 100% (93-100% at 0.05 sign. level), respectively. MSI status is reported when VAF of insertions and/or deletions at selected microsatellite regions is greater than or equal to 0.25% with an estimated sensitivity and specificity of 100% (98.8-100% at 0.05 sign. level) and 100% (92.9-100% at 0.05 sign. level), respectively. Variants are classified according to the criteria set by the American College of Medical Genetics and Genomics[16]. Classification and interpretation of variants is performed using the Varsome Clinical platform and is according to published knowledge at the time of testing. Variants which are classified as variants of strong clinical significance (Tier I) or variants of potential clinical significance (Tier II) are reported. Variants of unknown significance (Tier III) are also reported. Variants which have been detected and are classified as benign or likely benign (Tier IV) are not reported. Genetic counselling for the clinical interpretation and significance of the results is recommended. A 'no clinically significant variant' result reduces the chance of presence but does not guarantee the absence of a somatic variant in the patient's tumor. Genomic findings from cfDNA may originate from circulating tumor DNA (ctDNA), germline alterations, or non-tumor somatic alterations, namely clonal hematopoiesis of indeterminate potential (CHIP). Mutations in genes covered by the test that may be derived from CHIP include, but are not limited to ATM, JAK2, and TP53.

The test does not determine whether a variant is somatic or germline or a result of CHIP. Patients with an alteration identified in genes that are also associated with cancer predisposition might benefit from additional germline testing.

ADDITIONAL INFORMATION / DISCLOSURE

Test performance is valid only for the presence or absence of the tested cancer-associated variants in the genes included in the test. Therefore, a negative result indicates the absence of a cancer variant out of all the targeted variants included in the test and does not eliminate the possibility of a variant in a genomic position not tested by this assay. A positive result indicates the presence of a clinically relevant alteration. The results are interpreted

based on information provided on the sample information form. Misinterpretation of results may occur if insufficient or inaccurate information is provided. A positive finding does not guarantee association with a certain treatment or drug. Drugs or treatments mentioned in this report may not necessarily be suitable for the patient. Decisions on medical management must be based on the clinician's judgment taking into consideration all available information such as the patient's medical history, family history and other medical tests and examinations performed.

Although this test is highly accurate, there is still a small possibility for false positive or false negative results. This may be caused by technical and/or biological limitations, including but not limited to poor sample quality, bone marrow transplants or other rare molecular events. Other reasons for false positive or false negative results include, but are not limited to: mislabeled samples, inaccurate reporting of clinical/medical information and rare technical errors.

Genetic testing is an important part of the diagnostic process. However, genetic tests may not always give a definitive answer. In some cases, testing may not identify a genetic variant even though one exists. This may be due to limitations in current medical knowledge or testing technology. Clinical correlation with other clinical data and tests is recommended. Results should always be considered in the context of other clinical criteria. The analysis is specific only for the test ordered. The referral clinician is responsible for counselling before and after the test; including the provision of advice regarding the need for additional genetic testing. Other diagnostic tests may still be necessary.

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