NIPD GENETICS

NIPD GENETICS ForeSENTIA tumor profile

PATIENT INFORMATION		REFERRAL INFORMATION	
NAME **Jannis Doe**		CLINIC NAME **Oncology Center**	
Gender **Male**		CLINIC ID **Clinic XXX**	
DATE OF BIRTH **01/01/1965**		REFERRING CLINICIAN **John Smith, MD**	
TEST INDICATIONS **Lung Adenocarcinoma**		CLINIC EMAIL **Clinicxxx@clinic.com**	
SAMPLE INFORMATION			
ORDER NUMBER **NIPD1234**	LAB NUMBER **1234TS**	DATE OF COLLECTION **03/02/020**	DATE RECEIVED **04/02/2020**

CANCER TEST SELECTED

ForeSENTIA Pan-Cancer
A list of the genes tested is available at the end of the report

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 indications) *	FDA/EMA Approved Therapies (In other indications) **	Clinical Trials
EGFR	c.2239_2256del (p.Leu747_Ser752del)	25.3%	✓ NCCN Afatinib ✓ NCCN Dacomitinib ✓ NCCN Erlotinib ✓ NCCN Gefitinib ✓ NCCN Osimertinib	None	401
TP53	c.893G>A (p.Arg298His)	12%	None	None	8

✓ Approved in indication ✗ Lack of response 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 active enrolling by invitation and recruiting trials for the indicated 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.

INTERPRETATION

Variant summary:

EGFR c.2239_2256del (p.Leu747_Ser752del)

The EGFR variant c.2239_2256del is classified as Tier 1 variant with strong clinical significance. This variant is an in-frame deletion on exon 19 of the EGFR gene (NM_005228.4). This deletion results in a deletion of 6 amino acids at position 747-752 of the protein sequence. It has previously been identified in tissues derived from lung and submitted in the COSMIC database (COSMIC ID: COSM6255)[5]. EGFR encodes for the epidermal growth factor receptor with an important role in cell growth, proliferation and survival[6]. Somatic mutations in the tyrosine kinase domain of the (EGFR) gene are present in approximately 80% of the lung adenocarcinomas that respond to first and second generation EGFR inhibitors (eg, gefitinib, erlotinib and afatinib)[7].
Several approved targeted therapies are associated with this alteration. An indicative list of available therapies approved for lung adenocarcinoma or in other cancer types is available in the results summary section. Please refer to the appropriate regulatory authority and guidelines for the selection of the appropriate therapy.
Several clinical trials are active or currently recruiting patients diagnosed with non-small cell lung cancer with an EGFR mutation. Please refer to Clinicaltrials.gov for more information.
Guidelines from the National Comprehensive Cancer Network are available for patients diagnosed with non-small cell lung cancer with an EGFR mutation. Please refer to the NCCN guidelines for Non-small cell lung cancer for more information[8].

TP53 c.893G>A (p.Arg298His)

The TP53 variant c.893G>A is classified as Tier 1 variant with strong clinical significance. This variant is a missence G>A substitution on exon 10 pf the TP53 gene (NM_001276760.1). This missense variant results in an arginine to Histidine amino acid substitution at position 298 of the protein sequence. This variant has been identified in tissues derived from liver, adrenal gland, skin, pancreas and cervix and submitted in the COSMIC database (COSMIC ID: COSM43882). TP53 encodes p53, a tumor suppressor protein that consists of transactivation domain, proline-rich domain, DNA-binding domain, oligomerization domain, and regulatory domain. p53 responds to diverse cellular stresses to maintain genomic stability and to induce cell cycle arrest, apoptosis, DNA repair and metabolic changes. TP53 mutations represent an important mechanism of resistance to DNA-damaging chemotherapeutic agents. Somatic TP53 mutations are found in a variety of cancers with various frequencies depending on cancer type; overall, TP53 is mutated in over one-half of human cancers. Missense mutations were the most frequent (~70-80%), followed by frameshift and nonsense mutations.

At the moment there are no approved therapies targeting tumors with TP53 alterations. TP53 mutations may be potential prognostic and predictive markers in some tumor types, as well as targets for pharmacological intervention in some clinical settings[7]. Please refer to Clinicaltrials.gov for more information.

VARIANTS OF UNKNOWN SIGNIFICANCE

MET(NM_001127500.3):c.3029C>T (p.Thr1010Ile)

Note: One or more variants of unknown significance (VUS) have been detected in this patient's tumor sample. These variants are known as VUS due to their limited characterization and clinical evidence in the scientific literature at the time of writing of this report, making their significance unclear. However, we do include them here for reference in case they become clinically important in the future.

METHODOLOGY

ForeSENTIA is a Laboratory Developed Test (LTD) from NIPD Genetics Public Company Ltd for tumor molecular testing. Genomic deoxyribonucleic acid (gDNA) is extracted using a standardized methodology and subjected to enzymatic fragmentation and 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. ForeSENTIA can be used for the identification of selected single nucleotide variants (SNVs), small insertions and deletions (Indels, ≤30bp), translocations 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 ForeSENTIA 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. ForeSENTIA is intended for clinical purposes and should not be regarded as investigational or for research. 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

This test aims to detect selected targeted variants, CNAs and translocations relevant to cancer development in the genes described above, by targeting the exons and hotspot regions in the genes described above. Variants that fall outside of the targeted bases are not intended to be detected by this assay. Sequence specific alterations such as SNVs and Indels are evaluated when the variant allele frequency exceeds 5% and 10% respectively. Translocations are reported when the frequency exceeds 20% and CNAs are evaluated when the copy number amplification is either ≥4-fold with tumor DNA purity being >30% or ≥6-fold with tumor DNA purity at 20%. Biallelic deletions in the genes tested for CNAs are evaluated when the tumor fraction is higher than 50%. The overall sensitivity and specificity of the assay is ≥97% and 99.9%, respectively. Reduced sensitivity for the detection of the targeted genetic alterations may be due to low quality of the sample because of the procedure of tissue formalin-fixation or other factors that include, but are not limited to, low DNA yield, insufficient tumor DNA content and high intratumor heterogeneity in the specimen provided.

The test does not determine whether a variant is somatic or germline. 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.

REFERENCES

1. Whirl-Carrillo, M. *et al.* Pharmacogenomics knowledge for personalized medicine. *Clinical Pharmacology and Therapeutics* (2012). doi:10.1038/clpt.2012.96
2. Griffith, M. *et al.* DGIdb: Mining the druggable genome. *Nat. Methods* (2013). doi:10.1038/nmeth.2689
3. Varsome Clinical. Available at: https://saphetor.com/varsome-editions/varsome-clinical/.
4. Clinical trials.gov. *U.S. National Institutes of Health* (2015).
5. Li, M. M. *et al.* Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *Journal of Molecular Diagnostics* (2017). doi:10.1016/j.jmoldx.2016.10.002
6. Huang, L. *et al.* The cancer precision medicine knowledge base for structured clinical-grade mutations and interpretations. *J. Am. Med. Informatics Assoc.* (2017). doi:10.1093/jamia/ocw148
7. Forbes, S. *et al.* COSMIC 2005. *British Journal of Cancer* **94**, 318–322 (2006).
8. Zhang, J. *et al.* International cancer genome consortium data portal-a one-stop shop for cancer genomics data. *Database* (2011). doi:10.1093/database/bar026
9. Wang, Y. *et al.* Rapamycin inhibits FBXW7 loss-induced epithelial-mesenchymal transition and cancer stem cell-like characteristics in colorectal cancer cells. *Biochem. Biophys. Res. Commun.* (2013). doi:10.1016/j.bbrc.2013.03.077
10. Mao, J. H. *et al.* FBXW7 targets mTOR for degradation and cooperates with PTEN in tumor suppression. *Science (80).* (2008). doi:10.1126/science.1162981
11. Jardim, D. L. *et al.* FBXW7 mutations in patients with advanced cancers: Clinical and molecular characteristics and outcomes with mTOR inhibitors. *PLoS One* (2014). doi:10.1371/journal.pone.0089388
12. U.S Department of Health and Human Services. Genetics Home Reference. *U.S Natl. Libr. Med.* (2017).
13. Xiao, P. *et al.* LKB1 gene inactivation does not sensitize non-small cell lung cancer cells to mTOR inhibitors in vitro. *Acta Pharmacol. Sin.* (2015). doi:10.1038/aps.2015.19
14. Mahoney, C. L. *et al.* LKB1/KRAS mutant lung cancers constitute a genetic subset of NSCLC with increased sensitivity to MAPK and mTOR signalling inhibition. *Br. J. Cancer* (2009). doi:10.1038/sj.bjc.6604886
15. Choi, M., Kipps, T. & Kurzrock, R. ATM mutations in cancer: Therapeutic implications. *Molecular Cancer Therapeutics* (2016). doi:10.1158/1535-7163.MCT-15-0945

Approved by:  Elena Kypri, Ph.D, ASCP

Approved by:  Philippos Patsalis, Ph.D, HCLD, Laboratory Director

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ForeSENTIA
tumor profile

ForeSENTIA PAN CANCER Gene List

AKT1 Exon 4 (NM_001014432.1)	**ALK** Exons 18-20, 22-23 (NM_004304.5) *Selected non-coding regions covered*	**APC** Full exonic coverage (NM_000038.6) *Selected non-coding regions covered*	**AR** Full exonic coverage (NM_000044.6) Exon 2 (NM_001011645.3)	**ARAF** Exon 7 (NM_001654.5)	**ATM** Full exonic coverage (NM_000051.3) *Selected non-coding regions covered*
ATRX Exons 5, 7, 9, 11, 13-14, 17, 20-22, 29-31, 35 (NM_000489.5)	**BARD1** Full exonic coverage (NM_00465.4)	**BRAF** Exons 9-12, 15 (NM_001354609.2) *Selected non-coding regions covered*	**BRCA1** Full exonic coverage (NM_007294.4) *Selected non-coding regions covered*	**BRCA2** Full exonic coverage (NM_000059.3) *Selected non-coding regions covered*	**BRIP1** Full exonic coverage (NM_032043.3) *Selected non-coding regions covered*
CDH1 Full exonic coverage (NM_004360.5)	**CDKN2A** Full exonic coverage (NM_058195.3) *Selected non-coding regions covered*	**CHEK2** Exons 1-4, 6-11 (NM_001257387.2) *Selected non-coding regions covered*	**CIC** Exon 19 (NM_015125.4)	**CTNNB1** Exon 3 (NM_001904.4)	**DDR2** Exon 17 (NM_006182.4)
DICER1 Exons 2-26 (NM_177438.2) Exons 1-3 (NM_030621.4) Exon 1 (NM_001271282.3) *Selected non-coding regions covered*	**EGFR** Exons 2-5, 8-10, 12, 14-15, 17-24, 27-28 (NM_005228.5) *Selected non-coding regions covered*	**ERBB2** Exons 7-8, 11-12, 14, 23-28, 31 (NM_001289936.1) *Selected non-coding regions covered*	**ERBB3** Exons 3, 6-11, 20, 21 (NM_001982.3)	**ERBB4** Exons 7, 15, 19, 23 (NM_005235.3)	**ESR1** Exons 1, 3-7, 9-10 (NM_001122742.1) Exon 1 (NM_001291230.1) *Selected non-coding regions covered*
FBXW7 Exons 6-7, 9-14 (NM_001349798.2)	**FGFR1** Exons 3, 6-10, 13-14, 17, 19 (NM_001174064.2) *Selected non-coding regions covered*	**FGFR2** Exons 2-9, 11, 13-18 (NM_000141.4) *Selected non-coding regions covered*	**FGFR3** Exons 4, 7-8, 11-12, 16-18 (NM_000142.4) *Selected non-coding regions covered*	**FLT3** Exon 20 (NM_004119.3)	**FOXA1** Exon 2 (NM_004496.5)
FOXL2 Exon 1 (NM_023067.4)	**FUBP1** Exon 14 (NM_003902.5) *Selected non-coding regions covered*	**GATA3** Exons 5-6 (NM_001002295.2)	**GNA11** Exons 4-5 (NM_002067.5)	**GNAQ** Exons 5, 7 (NM_002072.5)	**GNAS** Exon 8 (NM_000516.6)
H3F3A Exon 2 (NM_002107.6)	**IDH1** Exon 4 (NM_005896.3)	**IDH2** Exon 4 (NM_002168.3)	**JAK2** Exon 14 (NM_004972.3)	**KEAP1** Exons 2-4 (NM_203500.2)	**KIT** Exons 2-21 (NM_000222.2) *Selected non-coding regions covered*
KRAS Exons 2-4 (NM_004985.5) Exon 5 (NM_001369786.1) *Selected non-coding regions covered*	**MAP2K1** Exons 2-3, 6-7 (NM_002755.3)	**MAP3K1** Exons 4-6, 16-18 (NM_5921.2)	**MET** Exons 2-6, 8-21 (NM_000245.4) *Selected non-coding regions covered*	**MLH1** Full exonic coverage (NM_000249.3) *Selected non-coding regions covered*	**MRE11A** Exons 1-18, 20 (NM_005591.4) *Selected non-coding regions covered*
MSH2 Full exonic coverage (NM_000251.3) *Selected non-coding regions covered*	**MSH6** Full exonic coverage (NM_000179.2) *Selected non-coding regions covered*	**MTOR** Exons 43, 47, 53, 56 (NM_004958.4)	**MYC** Exons 1, 3 (NM_002467.6) *Selected non-coding regions covered*	**MYCN** Exon 3 (NM_005378.6) *Selected non-coding regions covered*	**NBN** Full exonic coverage (NM_002485.5) *Selected non-coding regions covered*
NF1 Exons 6, 12, 17-18, 21-22, 25, 27-28, 34, 37, 40, 44-47, 49, 53 (NM_001042492.3) *Selected non-coding regions covered*	**NPM1** Exon 11 (NM_002520.6)	**NRAS** Exons 2-4 (NM_002524.5)	**NTRK1** Exons 7-15 (NM_002529.3) *Selected non-coding regions covered*	**NTRK2** Exons 12-13, 15-16 (NM_006180.4) *Selected non-coding regions covered*	**NTRK3** Exons 14-15 (NM_002530.4) *Selected non-coding regions covered*

Selected non-coding regions which are covered by the test are indicated above.

▲ Single Nucleotide Variant / Indels ● Copy Number Alterations ■ Rearrangements

31 Neas Engomis Str, 2409 Engomi, Nicosia, Cyprus.
Tel: + 357 22266888, Web Page: www.nipd.com

PALB2 Full exonic coverage (NM_024675.4) *Selected non-coding regions covered* ▲	**PDGFRA** Exon 18 (NM_006206.6) *Selected non-coding regions covered* ▲	**PIK3CA** Exons 2-6, 8, 10, 15-17, 19-21 (NM_006218.4) *Selected non-coding regions covered* ▲ ●	**PIK3CB** Exons 13, 15, 24 (NM_006219.3) *Selected non-coding regions covered* ▲	**PMS2** Exons 6-8, 10 (NM_000535.7) *Selected non-coding regions covered* ▲	**POLE** Exons 2-49 (NM_006231.4) *Selected non-coding regions covered* ▲
PTEN Full exonic coverage (NM_000314.8) *Selected non-coding regions covered* ▲ ●	**RAD51C** Full exonic coverage (NM_058216.3) *Selected non-coding regions covered* ▲	**RAD51D** Full exonic coverage (NM_002878.3) *Selected non-coding regions covered* ▲	**RAF1** Exon 7 (NM_001354689.3) *Selected non-coding regions covered* ▲	**RB1** Exons 1-13, 16-27 (NM_000321.2) *Selected non-coding regions covered* ●	**RET** Full exonic coverage (NM_020975.6) *Selected non-coding regions covered* ▲ ■
ROS1 Exons 31-36 (NM_002944.2) *Selected non-coding regions covered* ■	**RUNX1** Exon 5 (NM_001754.4) *Selected non-coding regions covered* ▲	**SMAD4** Full exonic coverage (NM_005359.6) *Selected non-coding regions covered* ▲	**SPOP** Exons 4-5 (NM_001007228.2) *Selected non-coding regions covered* ▲	**STK11** Full exonic coverage (NM_000455.5) *Selected non-coding regions covered* ▲	**TERT** Exon 1 (NM_198253.3) *Selected non-coding regions covered* ▲
TMPRSS2 Exons 1-4 (NM_005656.4) *Selected non-coding regions covered* ■	**TP53** Full exonic coverage (NM_000546.6) *Selected non-coding regions covered* ▲ ●				

The pan-cancer panel also screens for 1p/19q codeletion

Selected non-coding regions which are covered by the test are indicated above.

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