ANYLAB, INC. MRN: XXXXXXXXX Report Status: FINAL

MOLECULAR BIOMARKER REPORT

Patient Name: XXXXXXXXX	Test: Lung Cancer Testing by NGS	Collection date: XX/XX/XXXX
Medical record number (MRN): XXXXXXXXX	Tumor Type: Lung	Received date: XX/XX/XXXX
Date of birth (DOB): XX/XX/XXXX	Specimen Type: FFPE	Report date: XX/XX/XXXX
Sex: XXXXXXXXX Gender: XXXXXXXXX	Specimen No.: XXXX-XXX	Report status: XX/XX/XXXX
	Percentage neoplastic cells: XX %	

MOLECULAR BIOMARKER RESULT SUMMARY

Tier*	Variant Detected	Alteration Type	Allele Frequency (VAF)† / Copy Number‡	Level of Evidence	Targeted Therapy
I	EGFR p.E746_A750del c.2236_2250del (Exon 19 deletion; NM_002524.4)	Inframe deletion	50%	Therapeutic A	Afatinib, erlotinib, gefitinib, osimertinib
II	*CDKN2A* Deletion	Copy number variant	Ratio 0.25X≠	Therapeutic A	Not available
III	*TP53* p.R248L, c.734G>T (NM_000546)	Missense	45%	Therapeutic A	Not available
IV	*EML4::ALK EML4 exon 13 (NM_019063.4) :: ALK exon 20 (NM_004304.4)* *(See note below)*	Gene Fusion	N/A	Therapeutic A	Alectinib, brigatinib, lorlatinib, ceritinib, crizotinib

*Tier and Level of Evidence based on *AMP/ASCO/CAP Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer* (PMID: 27993330)
†VAF = Variant Allele Frequency or Variant Allele Fraction. VAF is defined in Test Description section below. ‡More information available in the Interpretative Summary.
NOTE: EML4::ALK example was provided as example of reporting for a gene fusion (not to be considered part of this NSCLC sample report)

GENOMIC SIGNATURES RESULT SUMMARY*

Biomarker	Sample	Result
Microsatellite instability (MSI)	DNA; Tumor / Germline Pair	LOW
Tumor Mutational Burden (TMB)	DNA; Tumor / Germline Pair	HIGH (18 muts / MB)
Homologous recombination deficiency (HRD) status	Not available	Testing not performed

*Inclusion of genomic signatures results will depend upon the test(s) being ordered / assay(s) being performed.

INTERPRETATIVE SUMMARY

HISTOLOGICAL DIAGNOSIS

Non-small cell lung cancer (NSCLC)

TEST RESULT INTERPRETATION

Three clinically relevant variants were detected in this case:

1. The *EGFR* p.E746_A750del (Exon 19 deletion) variant is known to be oncogenic. Afatinib, erlotinib, gefitinib are FDA approved for Non-Small Cell Lung Carcinoma. Erlotinib, gefitinib, afatinib are included in the NCCN-Compendium for this indication. There is clinical evidence that the 746_750 variant confers sensitivity to dacomitinib (PF-00299804).
2. The *CDKN2A* Homozygous Loss is known to be oncogenic. There is clinical evidence that the Homozygous Loss confers sensitivity to palbociclib (PD-0332991).
3. The *TP53* R248L variant is known to be oncogenic, resulting in loss of TP53 protein function. *TP53* variants are well-described in NSCLC.

Microsatellite instability (MSI):
Review of 227 microsatellite repeat regions included in this test shows alterations in 1.3% of examined sites, which is below the 5% threshold for microsatellite instability. No pathogenic alterations are noted in the genes typically associated with MSI-High status (*MLH1*, *MSH2*, *MSH6*, and *PMS2*). Of note, *MLH1* promoter methylation was not assessed by this testing.

Tumor Mutational Burden (TMB):
TMB for this case is 18 non-synonymous variants / MB coding sequence (MSS median 12, 95% Confidence Interval 12-18 MSI-High median 48, 95% CI 42-66).
Higher TMB can help predict response to immunotherapies in certain cancer indications.

PERTINENT NEGATIVES

No other clinically relevant molecular alterations detectable by this assay were identified. Pertinent negatives include but are not limited to the absence of *KRAS* mutation, *ALK* rearrangement, *ROS1* rearrangement, *RET* rearrangement, *NTRK1/2/3* rearrangement, *MET* exon 14 skipping mutation, *MET* amplification or *ERBB2* mutation

CLINICAL CORRELATION

Clinical correlation of these results in the patient is required.
Findings are a snapshot based on currently available information, therefore subject to change.

CLINICAL TRIALS

Several clinical trials are shown later in the report.

DETAILED INTERPRETATION

SPECIFIC VARIANT IDENTIFIED	
EGFR p.E746_A750del chr7:g.55242466_55242480del NM_005228.3:c.2236_2250del	**BACKGROUND** *EGFR* is a transmembrane receptor tyrosine kinase of the ErbB family. EGFR signaling is initiated by ligand binding to the extracellular ligand-binding domain. This initiates receptor homo-/hetero-dimerization and autophosphorylation by the intracellular kinase domain, resulting in receptor activation and the initiation of downstream signaling cascades that regulate growth, survival proliferation, and differentiation (PMID: 16729045). EGFR is involved by increased expression, amplification and/or expression of an aberrant protein in a high proportion of GBM, NSCLC, HNSCC, bladder and GI cancers. Numerous variants of the gene have been identified and investigated for their role in oncogenesis, role in sensitivity/resistance to targeted therapy. **VARIANT PREVALENCE** This *EGFR* variant has been reported in numerous lung cancers multiple tumor types, and several times in other types of solid tumors (COSMIC). **VARIANT EFFECT** NCCN Version: 3.2017. Cancer type: Non-Small Cell Lung Cancer. Recommendation category 1: Erlotinib, gefitinib or afatinib are used as first-line therapy in patients with NSCLC whose tumors harbor *EGFR* inhibitors-sensitive variants. For patients in whom sensitizing variants are discovered during firstline chemotherapy, erlotinib, gefitinib or afatinib may be used either in combination with, in place of, or after the current chemotherapy. (PMID: 25589191, 21783417, 23816960, 22285168, 20022809, 19692680, 20573926). **PRACTICE GUIDELINES** NCCN Version: 3.2017. Cancer type: Non-Small Cell Lung Cancer. Recommendation category 1: Erlotinib, Gefitinib or Afatinib are used as first-line therapy in patients with NSCLC whose tumors harbor *EGFR* inhibitors-sensitive variants. For patients in whom sensitizing variants are discovered during firstline chemotherapy, Erlotinib, Gefitinib or Afatinib may be used either in combination with, in place of, or after the current chemotherapy. (PMID: 29398453, 25589191, 21783417, 23816960, 22285168, 20022809, 19692680, 20573926). **THERAPEUTIC IMPLICATIONS** (incorporates predictive) TUMOR TYPE: Afatinib, erlotinib, gefitinib are FDA approved for Non-Small Cell Lung Carcinoma. Erlotinib, gefitinib, afatinib are included in the NCCN-Compendium for this indication. There is clinical evidence that the 745-750del variant confers sensitivity to dacomitinib (PF-00299804). NON-TUMOR TYPE: No therapeutics available at the time of report. **PROGNOSTIC IMPLICATIONS** Unknown

DETAILED INTERPRETATION

TP53 p.R248L c.734G>T NM_000546.5	**BACKGROUND** The *TP53* gene encodes a tumor suppressor protein most frequently mutated within the transcriptional activation, DNA binding, and oligomerization domains. The p53 protein is involved in cell cycle arrest, apoptosis, senescence, DNA repair and change in metabolism. **VARIANT PREVALENCE** *TP53* R248L has been reported in multiple tumor types including lung cancer (COSMIC). **VARIANT EFFECT** *TP53* R248L is a hotspot variant that lies in exon 7 encoding the DNA binding domain of TP53 (Uniprot.org) and multiple preclinical in vitro studies, including those in human lung cancer cell lines, indicate it leads to a loss of protein function (PMID: 12826609, PMID: 30224644, PMID: 29979965). **PRACTICE GUIDELINES** None **THERAPEUTIC IMPLICATIONS** TUMOR TYPE: No therapeutics available at the time of report. NON-TUMOR TYPE: No therapeutics available at the time of report. **PROGNOSTIC IMPLICATIONS** The presence of a *TP53* variant is reported as a negative predictor of outcome in non-small cell lung cancer (NSCLC) patients (PMID 31986371; 28101350; 30885352; 33233456; 33777783).

Microsatellite instability (MSI) LOW DNA; Tumor / Germline Pair	Short repeat sequences included in the panel are analyzed using a custom algorithm to assess accumulation of DNA replication errors. This tumor has a score of 1.3%. A score >5% is required for MSI-high. MSI Total Sites = 227 MSI Somatic Sites = 3 MSI Percent Somatic = 1.3%

DETAILED INTERPRETATION

Tumor Mutational Burden (TMB) HIGH (18 muts / MB) DNA; Tumor / Germline Pair	This specimen has a calculated tumor mutational burden (TMB) of 31.8 with 95% confidence that the TMB is greater than 24.8. As such, most of the individual variants in the specimen are likely secondary to the disease process, or 'passenger variants.' The clinical value of review for each individual variant is low, so for this case, specific variant review has been limited to known disease-associated variants, loss-of-function variants in tumor suppressor genes, and review of variants that may drive high TMB (*BRCA1*, *BRCA2*, *MLH1*, *MSH2*, and *MSH6*). The variants evaluated in this case are reported above. Variants remain unevaluated, but can be selectively reviewed upon request. Please contact the signatory of the case or the laboratory with any questions or to request additional review. The estimated tumor mutational burden for this specimen is approximately 31.8 mutations per megabase, with a 95% confidence interval (CI) from 24.8 to 40.2. This CI reflects the range of values expected if this assay covered the entire exome, and does not account for other technical or biological variability. The true TMB may lie outside this range.

Homologous recombination deficiency (HRD) status	Not available—Testing not performed

VARIANTS OF UNKNOWN CLINICAL SIGNIFICANCE (VUS)

The variant(s) below were detected in this sample. The significance of these variant(s) has not been adequately characterized in the scientific literature at the time of this report and/or the context makes the significance of these variant(s) unclear. They are included here in the event that they become clinically meaningful in the future.

VUS DETECTED:

EGFR: c.88+6G>C (NM_005228.5), EGFR: c.474 C>G (NM_005228.5), (Source: https://www.ncbi.nlm.nih.gov/clinvar-, accessed 7/3/2023)

TEST DESCRIPTION

This ANYLAB test is designed to detect variants present in any FFPE tissue from solid tumors. The test is designed to detect single nucleotide variants (SNVs) and small insertions/deletions (In/Dels) as well as whole gene copy number alterations and translocations in a select group of genes. Results of the test should be correlated with clinical findings. The genes (listed below) were selected based on the clinical significance of variants identified in those genes using currently available evidence from national and international guidelines and literature. Clinical relevance is defined as information a clinician might find useful to aid in diagnosis, prognosis and/or treatment strategy for a patient. Results of the test should be correlated with clinical findings. Clinical trial information provided in this report is solely for informational purposes for the physician and does not constitute any endorsement or a recommendation for enrollment of patients in any trial by ANYLAB, its affiliates, or its employees.

This test has an analytical sensitivity for detecting 5% SNVs and 5% INDEL mutated sequences in a background of non-mutated DNA sequence, two-fold or higher gene amplifications, and homozygous gene deletions. Translocation detection is limited to a set of specified acceptor genes at 20% analytical sensitivity. The performance characteristics of the test can change based on the adequacy of tumor tissue or pre-analytical variables. The genes tested include: *AKT1, AKT2, ALK, AR, AURKA, BAP1, BRAF, BRCA1, BRCA2, CDKN2A, CDKN2B, CTNNB1, DDR2, EGFR, EP300, ERBB2, ERBB3, ERBB4, ESR1, FGFR1, FGFR2, FGFR3, FGFR4, FLT3, HRAS, IDH1, JAK2, KDR, KIT, KRAS, MAP2K1, MET, MTOR, MYC, MYCN, NRAS, NTRK1, PDGFRA, PDGFRB, PIK3CA, PTCH1, PTEN, RET, ROS1, TERT, TMPRSS2, TP53, TSC1, VHL*. The genes tested for translocations include *ALK, BRAF, EGFR, FGFR2, FGFR3, NTRK1, RET, ROS1*, and *TMPRSS2*.

Microsatellite instability (MSI) and/or hypermutated phenotype can be reported if identified. If there is an established association in the literature for the patient's tumor type and MSI-H status with Lynch syndrome, or for the hypermutable phenotype and POLE/POLD1 mutations, this will be noted in the report. In this case, clinical correlation and additional germline testing may be warranted, if appropriate. Tumor mutation burden (TMB) or the tumor mutational load was calculated as an index of the number of variants per megabase (muts/Mb) harbored by tumor cells from this neoplasm. TMB is considered high if it exceeds a threshold of 17 muts/Mb (PMID: 34206554).

The Variant Allele Fraction or Variant Allele Frequency (VAF) is the frequency at which the variant is detected in a specimen. It is often used as an indicator of somatic versus germline status and to estimate disease burden (e.g., a VAF of 50% may suggest a germline variant, whereas a VAF of 15% may suggest a neoplastic disease burden of 30%). VAF information should be interpreted with caution as it can be affected by many factors, including assay variance, sampling, assay design, copy number changes, loss of heterozygosity (LOH) and subclonal variants. For additional assistance, please contact the laboratory or the molecular professional who issued the report.

TEST LIMITATIONS

Only variants present in the interrogated regions of the genes are reported. The test does not identify variants present outside the interrogated regions. Normal population variations, promoter and intronic variations (with the exception of the TERT promoter and splice variants), single nucleotide polymorphisms (SNPs), as well as benign variants are not included in this report. This test is not designed for circulating tumor DNA variant/mutation analysis. This test was designed for detection and annotation of somatic tumor variants and is not intended to be a germline test. When patient consent is provided, and where warranted, limited information is included about whether a germline or possible germline alteration was detected in certain genes related to hereditary cancer predisposition. This information should be confirmed with germline testing in consultation with a clinician and genetic counselor, if appropriate, given that variants reported do not undergo germline annotation.

TESTING LABORATORY

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This test was developed and its performance characteristics determined by ANYLAB, INC. It has not been cleared or approved by the U.S. Food and Drug Administration.

CLINICAL TRIALS

EGFR 745-750del	**CLINICAL TRIALS MATCHED FOR VARIANT AND DISEASE** NCT02511106, Phase 3 TITLE: A Phase III, Double-blind, Randomized, Placebo-controlled Multi-centre, Study to Assess the Efficacy and Safety of AZD9291 Versus Placebo, in Patients With Epidermal Growth Factor Receptor Mutation Positive Stage IBIIIA Non-small Cell Lung Carcinoma, Following Complete Tumour Resection With or Without Adjuvant Chemotherapy (ADAURA) NCT01582191, Phase 1 TITLE: A Phase 1 Trial of Vandetanib (a Multi-kinase Inhibitor of EGFR, VEGFR and RET Inhibitor) in Combination With Everolimus (an mTOR Inhibitor) in Advanced Cancer
	CLINICAL TRIALS MATCHED FOR VARIANT ONLY None provided

CDKN2A Deletion	**CLINICAL TRIALS MATCHED FOR VARIANT AND DISEASE** NCT01037790, Phase 2 TITLE: Phase II Trial of the Cyclin-Dependent Kinase Inhibitor PD 0332991 in Patients With Cancer NCT02308020, Phase 2 TITLE: A Phase 2 Study of Abemaciclib in Patients With Brain Metastases Secondary to Hormone Receptor Positive Breast Cancer, Non-small Cell Lung Cancer, or Melanoma NCT02450539, Phase 2 TITLE: A Randomized Phase 2 Study of Abemaciclib (LY2835219) Versus Docetaxel in Patients With Stage IV Squamous Non-Small Cell Lung Cancer Previously Treated With Platinum-Based Chemotherapy
	CLINICAL TRIALS MATCHED FOR VARIANT ONLY None provided

GENOMIC SIGNATURE Tumor Mutational Burden (TMB) HIGH (18 muts / MB)	**CLINICAL TRIALS MATCHED FOR VARIANT AND DISEASE** NCT03178552, Phase II/III TITLE: A Study to Evaluate the Efficacy and Safety of Multiple Targeted Therapies as Treatments for Participants With Non-Small Cell Lung Cancer (NSCLC) (B-FAST)
	CLINICAL TRIALS MATCHED FOR VARIANT ONLY None provided

DISCLAIMER
Availability of clinical trials depends on many factors. Whether any specific trial is appropriate for an individual patient should be discussed with the care team.