CIRCULOGENE

PATIENT: GENDER: AGE: DOB:

CLIENT NAME: CLIENT NUMBER:

PHYSICIAN: SAMPLE ID: 015754

COLLECTION DATE: 10/24/2023 DATE RECEIVED: 10/25/2023 REPORT DATE: 10/30/2023

INDICATION: Lung Cancer SAMPLE TYPE: Blood

SOMATIC TESTING RESULTS

RNA Test(s) Ordered:	Alteration/Variant:	Results:
† # PD-L1 Expression		Not Detected
† ALK Fusion		Not Detected
† RET Fusion	KIF5B::RET	DETECTED
† ROS1 Fusion		Not Detected
† NTRK1/NTRK2/NTRK3 Fusion		Not Detected

Fragment Analysis Test(s) Ordered:	Alteration/Variant:	Results:
†† MSI Fragment Analysis Test		Not Detected

NGS 88 GENE Panel Ordered:	Alteration/Variant:	Results:	Mutant Fraction %
ARID1A	c.5839C>T p.Q1947*	DETECTED	1.5%
ATM	c.7307+1G>C	DETECTED	25%
RB1	c.2137A>T p.K713*	DETECTED	1.8%

The full gene list of the 88 Gene Panel by NGS is on page 2 of this report
† RNA is tested by RT-PCR
†† MSI is tested by fragment analysis
# If Detected, literature supports an association of PD-L1 mRNA expression with tissue PD-L1 protein of ≥ 1%[1]
An undetermined result cannot be resolved to definitively report as "detected" or "not detected."

DRUG	Drug/biomarker shown effective in patient's tumor type?	Gene/ALTERATION	MUTANT FRACTION	CLINICAL TRIALS‡
cabozantinib, pralsetinib, selpercatinib	Y	KIF5B::RET		39
		ARID1A	1.5%	31
olaparib	N	ATM	25%	30

‡ For current clinical trial information please visit www.clinicaltrials.gov

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GENES TESTED

Somatic DNA Genes: AKT1, ALK, AR, ARAF, ARID1A, ATM, ATR, AXL, BAP1, BARD1, BRAF, BRCA1, BRCA2, CCND1, CCNE1, CDH1, CDK12, CDK4, CDK6, CDKN2A, CHEK1, CHEK2, CRKL, CSF1R, CTNNB1, DDR2, EGFR, ERBB2, ERBB4, ESR1, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, FOXL2, GNA11 GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, IGF1R, JAK2, JAK3, KDR, KEAP1, KIT, KRAS, MAP2K1, MAP2K2, MAPK3, MET, MLH1, MTOR, MYC, NF1, NOTCH1, NRAS, NTRK1, NTRK2, NTRK3, PALB2, PDGFRA, PIK3CA, POLD1, POLE, PTEN, PTPN11, RAF1, RB1, RET, ROS1, SETD2, SMAD4, SMARCA4, SMARCB1, SMO, SRC, STAT3, STK11, TERT, TOP1, TP53, TSC1, TSC2, VHL

RNA tests:ALK GENE FUSION, RET, ROS1 GENE FUSION, PD-L1 EXPRESSION, NTRK1/NTRK2/NTRK3 GENE FUSIONS

MSI by fragment analysis

Methods and Limitations

Blood is collected in preservative containing tubes. This blood is centrifuged to separate plasma and white blood cells from other blood components. Nucleic acids are amplified via proprietary techniques. The DNA and RNA are then quantified and subjected to further analysis.

Such analysis may include any or all of: amplification and fragment analysis for presence of changes in length of microsatellites (Promega LMR MSI Analysis System), amplification and identification of fusions, amplification or expression (PD-L1), and amplification and sequencing for identification of sequence alterations.

The RT-PCR ALK and ROS1 fusion assays have an LOD95 (limit of detection/sensitivity) of 125 – 173 copies/uL based on 2ng input total RNA. NTRK fusion assays have an LOD95 of 71-123 copies/uL based on 2ng input RNA. RET fusion assays have an LOD of 6.8 copies per/uL. The PD-L1 assay has a LOD95 of 1ng of RNA. MSI has a limit of detection of 2.5% VAF.

The Circulogene NGS somatic NGS panel sequences 88 cancer-associated genes utilizing the Illumina platform. Mutations detected include single nucleotide variations, short insertions and deletions, and splice-site disrupting events in comparison to Hg38, as well as copy number alterations. This version of the Circulogene NGS test is not validated to detect other types of genomic alterations. The test cannot differentiate the source of detected variants, and for certain variants in the range of 40-60% or 90-100% mutant allele frequency the test cannot distinguish germline from somatic variants. This test is not validated to differentiate germline or de novo variants associated with hereditary cancer risk nor mutations arising from clonal hematopoesis.

CGT's tests target specific gene mutations and does not detect mutations that are outside of the targeted area. The limit of sensitivity is 2.5% and the limit of detection is 1% for SNV or INDEL and 2.5% for CNV. This technology cannot reliably detect mutations at coverage below 100X at 5% VAF or 500x at 1% VAF. Tissue testing should be considered when plasma testing is negative, if indicated clinically.

DISCLAIMER

All of the individual assays that are available through Circulogene Theranostics (CGT) were developed and their test performance characteristics were determined and validated by CGT pursuant to the Clinical Laboratory Improvements Amendments and accompanying regulations (CLIA). These tests have not been cleared or approved by the United States Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. CGT clinical laboratory is certified under CLIA to perform high-complexity testing.

The test report incorporates analyses of peer-reviewed studies and other publicly available information. Every effort is made to provide the most accurate and up-to-date information through FDA and PubMed, to exclude germline alterations via COSMIC and dbSNP. Identification of cancer associated mutations does not necessarily indicate good response to therapy; while absence of a cancer associated mutation does not necessarily indicate poor response to therapy. This report makes no promises or guarantees that a particular drug will be effective in the treatment of disease in any patient. This report also makes no promises or guarantees that a drug with potential lack of clinical benefit will in fact provide no clinical benefit.

Circulogene Theranostics makes no promises or guarantees that a healthcare provider, insurer or other third party payor, whether private or governmental, will reimburse a patient for the cost of our tests. Circulogene Theranostics does not make any endorsement, expressed nor implied, of any product, physician or procedure contained in this report.

Drugs referenced in this report may not be suitable for a particular patient. The selection of any, all or none of the drugs associated with potential clinical benefit (or potential lack of clinical benefit) resides entirely within the discretion of the treating physician. Indeed, the information in this report must be considered in conjunction with all other relevant information regarding a particular patient, before the patient's treating physician recommends a course of treatment. The treatments and trial information included in this report are based on the diagnosis written on the submitted test requisition form. Some drugs included in this report may not have FDA approval for use in the patient's disease.

Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care in a given community. A treating physician's decisions should not be entirely based on a single test.

[1]Additional information can be found in: DOI: 10.1016/j.anndiagpath.2022.151968

This test was performed by Circulogene Theranostics, technical and professional components of testing at 19 E Garden Street, Suite 300, Pensacola, FL 32502, CLIA#10D2224896.