PATIENT	SPECIMEN	PHYSICIAN
Patient Name:	Specimen ID:	Physician Name:
Patient ID:	Source Specimen ID:	Physician Affiliation:
Source Patient ID:	1° Tumor Site: Liver	
D.O.B:	Specimen Site: Liver	
Sex:	Neoplastic Content: 100%	
Diagnosis: Malignant neoplastic disease	Collection Date:	
	Received Date:	

Interpretation Summary

The following clinically significant variants were detected in this specimen: TP53 p.(Arg273Cys), EP300 p.(Ser531TyrfsTer15), NSD1 p.(Glu2214Ter), and MYC amplification.

Germline variants in TP53 are associated with cancer predisposition syndromes (MIM: 202300, 614740, 114500, 137800, 618165, 151623). This assay does not distinguish between somatic and germline variation; therefore, the variants were interpreted in the somatic context. Additional testing and genetic counseling may be indicated.

Clinical correlation is REQUIRED.

Immunotherapy Biomarkers

Tumor Mutation Burden (TMB) - 7.86 mut/MB | **TMB-Low**
Microsatellite Instability (MSI) - 1.04% unstable sites | **MS-Stable**

Genomic Biomarkers

Findings	**MYC** *Copy Number Gain*	Oncogenic
	TP53 *p.(Arg273Cys)*	Oncogenic
	EP300 *p.(Ser531TyrfsTer15)*	Likely Oncogenic
	NSD1 *p.(Glu2214Ter)*	Likely Oncogenic
Pertinent Negatives*	**ALK, APC, BCOR, BRAF, BRCA1, BRCA2, CDK4, CIC, CTNNB1, DNAJB1, EPCAM, ERBB2, ERG, ETV1, ETV4, ETV6, EWSR1, FGFR2, FGFR3, FOXO3, GLI1, IDH1, IDH2, KIT, KRAS, MDM2, MET, MLH1, MSH2, MSH6, MYOD1, NAB2, NTRK1, NTRK2, NTRK3, PAX3, PAX7, PDGFRA, PMS2, RANBP2, RET, ROS1, SDHB, SMARCB1, TFE3, WT1, YAP1**	

*Contains disease related genes only.

Variants of uncertain significance are detected and listed at the end of the report.

Therapies

Biomarker	Therapy in Patient's Disease	Therapy in Other Diseases	Clinical Trials
TP53 *p.(Arg273Cys)* Oncogenic	Tier 1B • Responsive Pazopanib + Vorinostat Tier 2C • Predicted - sensitive Adavosertib Tier 2C • Sensitive Bevacizumab Pazopanib + Vorinostat Unspecified VEGFR inhibitor Tier 2D • Resistant KRT-232 Tier 2D • Sensitive	Tier 2C • Predicted - sensitive Zanubrutinib Tier 2C • Sensitive Acalabrutinib Acalabrutinib + Cyclophosphamide + Cytarabine + Dexamethasone + Doxorubicin + Prednisone + Rituximab + Vincristine Sulfate Acalabrutinib + Obinutuzumab Acalabrutinib + Obinutuzumab + Venetoclax Alemtuzumab + Rituximab	Nothing reported

Therapies

Biomarker	Therapy in Patient's Disease	Therapy in Other Diseases	Clinical Trials
	CTX-1	Cyclophosphamide + Cytarabine + Dexamethasone + Doxorubicin + Ibrutinib + Prednisone + Rituximab + Vincristine Sulfate Cyclophosphamide + Cytarabine + Dexamethasone + Doxorubicin + Prednisone + Rituximab + Vincristine Sulfate + Zanubrutinib Decitabine Duvelisib Ibrutinib Ibrutinib + Venetoclax Idelalisib Idelalisib + Rituximab Lenalidomide + Rituximab Lisocabtagene maraleucel Methylprednisolone + Obinutuzumab Methylprednisolone + Rituximab Obinutuzumab Obinutuzumab + Venetoclax Obinutuzumab + Venetoclax + Zanubrutinib Pirtobrutinib Rituximab + Venetoclax Venetoclax	

Potential Clinical Trials

Biomarker	Phase	NCT ID	Title
MYC *Copy Number Gain* Oncogenic	I	NCT04693468	Talazoparib and Palbociclib, Axitinib, or Crizotinib for the Treatment of Advanced or Metastatic Solid Tumors, TalaCom Trial

Genomic Biomarker Details

MYC *Copy Number Gain* Fold change: 4.8345 Oncogenic Copy number: 9.7

Gene Information

MYC, MYC proto-oncogene, bHLH transcription factor, is a transcription factor that regulates expression of genes involved in cell cycle progression, apoptosis, cellular transformation (PMID: 25038584, PMID: 32071436) and the immune system (PMID: 29514782). Amplification, overexpression, and rearrangement of MYC is commonly observed in solid and hematological tumors (PMID: 28587062, PMID: 32203465), such as lung cancer (PMID: 32014901) and diffuse large B-Cell lymphoma (PMID: 32074595).

Oncogenic · **Biological**

Genomic Biomarker Details

MYC gene amplification leads to overexpression of MYC protein (PMID: 22464321,15083194). MYC amplification has been characterized in the scientific literature to result in aberrant transcriptional activation of MYC target genes (PMID: 22464321,15083194), which regulate cell proliferation, cell cycle exit, vascularization, and genomic instability (PMID: 22464321,10378696,16934487,19029958).

Prognostic (Favorable)

MYC overexpression is suggested to be a predictor of adverse prognosis in hepatocellular carcinoma (PMID:36303795,10461064,7511953,15668888).

TP53 *p.(Arg273Cys)* Oncogenic Tier 1B VAF 100 %
Missense Variant, Loss of Function Variant NM_000546.6 c.817C>T chr17:7577121 G→A

Gene Information

TP53, tumor protein p53, is a tumor suppressor (PMID: 30562755) and oncogene (PMID: 30577483) involved in cell cycle arrest and apoptosis, and is the most frequently mutated gene in cancer (PMID: 10065147, PMID: 22713868). TP53 germline mutations are common in Li-Fraumeni syndrome (PMID: 30239254) and somatic missense mutations are frequent in almost all cancer types (PMID: 30224644) and are also implicated in chemoresistance (PMID: 9927204, PMID: 24065105, PMID: 27066457).

Oncogenic - Biological

TP53 p.(Arg273Cys) is a hotspot variant that lies within the DNA binding domain (DBD) of the TP53 protein (PMID: 22713868). This variant does not affect TP53 protein stability; however, leads to a loss of function as demonstrated by decreased activation of target genes due to reduction in TP53 DNA binding affinity (PMID: 23863845, 11900253). In addition, TP53 p.(Arg273Cys) leads to increased cellular proliferation, migration, invasion, and altered subcellular location in culture (PMID: 37030635, 23264849, 3612969, 16861262).

Tier 1B - Therapeutic - Pazopanib, Vorinostat (Responsive)

In a phase I trial, Pazopanib and Vorinostat treatment resulted in a 45% rate of stable disease (SD) ≥6 months or partial response (PR) (1 PR and 3 SD ≥6 months), a median progression-free survival of 3.5 months, and a median overall survival of 12.7 months in individuals with solid tumors harboring hotspot TP53 variants, comparing favorably relative to patients without TP53 variants (n = 25): 16% (1 PR and 3 SD ≥6 months, P = 0.096), 2.0 months (P = 0.042), and 7.4 months (P = 0.1), respectively.

Tier 2C - Therapeutic - Pazopanib, Vorinostat (Sensitive)

In a Phase I trial, the combination of Votrient (pazopanib) and Zolinza (vorinostat) improved progression-free survival and overall survival in advanced solid tumor patients harboring TP53 hotspot mutations, and resulted in an increased stable disease rate of 45% (5/11), compared to a stable disease rate of 16% (4/25) in patients without detected TP53 mutations (PMID: 25669829).

Tier 2C - Therapeutic - Unspecified VEGFR inhibitor (Sensitive)

In a clinical study, VEGF/VEGFR inhibitor treatment resulted in improved rates of response (stable disease over 6 months/partial/complete response, 31% vs 7%), time-to-treatment failure, and overall survival (both p<0.01) compared to control in patients with TP53 mutant advanced solid tumors (n=106), but not in patients with TP53 wild-type tumors (n=82) (PMID: 27466356).

Tier 2C - Therapeutic - Bevacizumab (Sensitive)

In a retrospective study, Avastin (bevacizumab) treatment was associated with increased progression-free survival in cancer patients carrying TP53 mutations (PMID: 23670029).

Tier 2C - Therapeutic - Adavosertib (Predicted - sensitive)

In a retrospective analysis of a Phase I trial, Adavosertib (MK-1775) combined with a chemotherapy resulted in a 21% (4/19) response rate in advanced solid tumor patients harboring a TP53 mutation and in those without a TP53 mutation, a 12% (4/33) response rate was observed (PMID: 27601554; NCT00648648).

Genomic Biomarker Details

Tier 2C - Therapeutic - Adavosertib (Predicted - sensitive)

In a Phase I trial, Adavosertib (MK-1775) treatment resulted in a prtial response in 3 and progressive disease in 2 of 6 patients with advanced solid tumors harboring TP53 mutations (J Clin Oncol 38: 2020 (suppl; abstr 3624); NCT01748825).

Tier 2C - Therapeutic - Zanubrutinib, Vincristine Sulfate, Cytarabine, Cyclophosphamide, Doxorubicin, Rituximab, Prednisone, Dexamethasone (Sensitive)

The combination of Brukinsa (zanubrutinib) plus RCHOP alternating with Rituxan (rituximab), Adexone (dexamethasone), Cytosar-U (cytarabine) plus a platinum is included in guidelines as induction therapy for patients with mantle cell lymphoma harboring a TP53 mutation (NCCN.org).

Tier 2C - Therapeutic - Alemtuzumab, Rituximab (Sensitive)

Campath (alemtuzumab) combined with Rituxan (rituximab) is included in guidelines for patients with relapsed or refractory chronic lymphocytic leukemia/small lymphocytic lymphoma with a TP53 mutation after prior BTK inhibitor-based and BCL2 inhibitor-containing regimens (NCCN.org).

Tier 2C - Therapeutic - Lenalidomide, Rituximab (Sensitive)

Revlimid (lenalidomide) combined with Rituxan (rituximab) is included in guidelines for patients with relapsed or refractory chronic lymphocytic leukemia/small lymphocytic lymphoma with a TP53 mutation after prior BTK inhibitor-based and BCL2 inhibitor-containing regimens (NCCN.org).

Tier 2C - Therapeutic - Venetoclax, Rituximab (Sensitive)

Venclexta (venetoclax) combined with Rituxan (rituximab) is included in guidelines as second-line or subsequent therapy (category 1) for chronic lymphocytic leukemia/small lymphocytic lymphoma patients with a TP53 mutation (NCCN.org).

Tier 2C - Therapeutic - Acalabrutinib, Vincristine Sulfate, Cytarabine, Cyclophosphamide, Doxorubicin, Rituximab, Prednisone, Dexamethasone (Sensitive)

The combination of Calquence (acalabrutinib) plus RCHOP alternating with Rituxan (rituximab), Adexone (dexamethasone), Cytosar-U (cytarabine) plus a platinum is included in guidelines as induction therapy for patients with mantle cell lymphoma harboring a TP53 mutation (NCCN.org).

Tier 2C - Therapeutic - Venetoclax, Obinutuzumab (Sensitive)

The combination of Venclexta (venetoclax) and Gazyva (obinutuzumab) is included in guidelines as first-line therapy for chronic lymphocytic leukemia/small lymphocytic lymphoma patients with a TP53 mutation (NCCN.org).

Tier 2C - Therapeutic - Methylprednisolone, Obinutuzumab (Sensitive)

Artisone-Wyeth (methylprednisolone) combined with Gazyva (obinutuzumab) is included in guidelines as first-line therapy for chronic lymphocytic leukemia/small lymphocytic lymphoma patients with a TP53 mutation and for patients with relapsed or refractory disease after prior BTK inhibitor and BLC2 inhibitor-based regimens (NCCN.org).

Tier 2C - Therapeutic - Obinutuzumab (Sensitive)

Gazyva (obinutuzumab) is included in guidelines as first-line therapy for chronic lymphocytic leukemia/small lymphocytic lymphoma patients with a TP53 mutation (NCCN.org).

Tier 2C - Therapeutic - Acalabrutinib (Sensitive)

Calquence (acalabrutinib) is included in guidelines as first-line (category 2A) and second-line or subsequent therapy (category 1) for chronic lymphocytic leukemia/small lymphocytic lymphoma patients with a TP53 mutation (NCCN.org).

Genomic Biomarker Details

Tier 2C - Therapeutic - Acalabrutinib, Obinutuzumab (Sensitive)

Calquence (acalabrutinib) combined with Gazyva (obinutuzumab) is included in guidelines as first-line therapy for chronic lymphocytic leukemia/small lymphocytic lymphoma patients with a TP53 mutation (NCCN.org).

Tier 2C - Therapeutic - Methylprednisolone, Rituximab (Sensitive)

Artisone-Wyeth (methylprednisolone) combined with Rituxan (rituximab) is included in guidelines as first-line therapy for chronic lymphocytic leukemia/small lymphocytic lymphoma patients with a TP53 mutation and for patients with relapsed or refractory chronic lymphocytic leukemia/small lymphocytic lymphoma with a TP53 mutation after prior BTK inhibitor-based and BCL2 inhibitor-containing regimens (NCCN.org).

Tier 2C - Therapeutic - Pirtobrutinib (Sensitive)

Jaypirca (pirtobrutinib) is included in guidelines (category 1) as second-line or subsequent therapy for patients with chronic lymphocytic leukemia/small lymphocytic lymphoma with a TP53 mutation and for patients with relapsed or refractory chronic lymphocytic leukemia/small lymphocytic lymphoma with a TP53 mutation after prior BTK inhibitor-based and BCL2 inhibitor-containing regimens (NCCN.org).

Tier 2C - Therapeutic - Zanubrutinib (Predicted - sensitive)

Brukinsa (zanubrutinib) is included in guidelines as first-line therapy (category 2A) and second line therapy or subsequent therapy (category 1) for chronic lymphocytic leukemia/small lymphocytic lymphoma patients with a TP53 mutation (NCCN.org).

Tier 2C - Therapeutic - Duvelisib (Sensitive)

Copiktra (duvelisib) is included in guidelines for patients with relapsed or refractory chronic lymphocytic leukemia/small lymphocytic lymphoma with a TP53 mutation after prior BTK inhibitor-based and BCL2 inhibitor-containing regimens (NCCN.org).

Tier 2C - Therapeutic - Ibrutinib, Vincristine Sulfate, Cytarabine, Cyclophosphamide, Doxorubicin, Rituximab, Prednisone, Dexamethasone (Sensitive)

The combination of Imbruvica (ibrutinib) plus RCHOP alternating with Rituxan (rituximab), Adexone (dexamethasone), Cytosar-U (cytarabine) plus a platinum is included in guidelines as induction therapy for patients with mantle cell lymphoma harboring a TP53 mutation (NCCN.org).

Tier 2C - Therapeutic - Acalabrutinib, Venetoclax, Obinutuzumab (Sensitive)

The combination of Venclexta (venetoclax), Gazyva (obinutuzumab), and Calquence (acalabrutinib) is included in guidelines as first-line therapy for chronic lymphocytic leukemia/small lymphocytic lymphoma patients with a TP53 mutation (NCCN.org).

Tier 2C - Therapeutic - Ibrutinib (Sensitive)

Imbruvica (ibrutinib) is included in guidelines as first-line therapy (category 2A) and second-line or subsequent therapy (category 1) for chronic lymphocytic leukemia/small lymphocytic lymphoma patients with a TP53 mutation (NCCN.org).

Tier 2C - Therapeutic - Ibrutinib, Venetoclax (Sensitive)

Venclexta (venetoclax) combined with Imbruvica (ibrutinib) is included in guidelines as first-line (category 2A) and second-line or subsequent therapy (category 2B) for chronic lymphocytic leukemia/small lymphocytic lymphoma patients with a TP53 mutation (NCCN.org).

Tier 2C - Therapeutic - Lisocabtagene maraleucel (Sensitive)

Breyanzi (lisocabtagene maraleucel) is included in guidelines for patients with relapsed or refractory chronic lymphocytic leukemia/small lymphocytic lymphoma with a TP53 mutation after prior BTK inhibitor-based and BCL2 inhibitor-containing regimens (NCCN.org).

Tier 2C - Therapeutic - Zanubrutinib, Venetoclax, Obinutuzumab (Sensitive)

Genomic Biomarker Details

The combination of Brukinsa (zanubrutinib), Gazyva (obinutuzumab), and Venclexta (venetoclax) is included in guidelines as induction therapy for patients with mantle cell lymphoma harboring a TP53 mutation (NCCN.org).

Tier 2C - Therapeutic - Idelalisib, Rituximab (Sensitive)

Zydelig (idelalisib) combined with Rituxan (rituximab) is included in guidelines for patients with relapsed or refractory chronic lymphocytic leukemia/small lymphocytic lymphoma with a TP53 mutation after prior BTK inhibitor-based and BCL2 inhibitor-containing regimens (NCCN.org).

Tier 2C - Therapeutic - Decitabine (Sensitive)

Dacogen (decitabine) is included in guidelines for adult patients with acute myeloid leukemia harboring a TP53 mutation (NCCN.org).

Tier 2C - Therapeutic - Venetoclax (Sensitive)

Venclexta (venetoclax) is included in guidelines as second-line or subsequent therapy for chronic lymphocytic leukemia/small lymphocytic lymphoma patients with a TP53 mutation (NCCN.org).

Tier 2C - Therapeutic - Idelalisib (Sensitive)

Zydelig (idelalisib) is included in the guidelines for patients with relapsed or refractory chronic lymphocytic leukemia/small lymphocytic lymphoma with a TP53 mutation after prior BTK inhibitor-based and BCL2 inhibitor-containing regimens (NCCN.org).

Tier 2D - Therapeutic - KRT-232 (Resistant)

In a preclinical study, KRT-232 (AMG 232) did not inhibit growth of human tumor cell lines harboring TP53 mutations in culture (PMID: 25567130).

Tier 2D - Therapeutic - CTX-1 (Sensitive)

In a preclinical study, CTX-1 induced increased Tp53 protein levels and cell death in human tumor cell lines with mutant Tp53 in culture (PMID: 26883273).

EP300 *p.(Ser531TyrfsTer15)* Likely Oncogenic VAF 42.07 %

Frameshift Variant, Loss of Function Variant NM_001429.4 c.1592_1593del chr22:41531880 CA→-

Gene Information

EP300, E1A binding protein p300, is a lysine acetyltransferase that regulates gene transcription through acetylation of histones and other proteins, as well as acting as a transcriptional co-activator in the regulation of the cell cycle (PMID: 27881443). EP300 is frequently mutated in various tumor types (PMID: 25111821, PMID: 24622842), including skin squamous cell carcinoma, marginal zone B-cell lymphoma (PMID: 27881443), and bladder cancer (PMID: 32012118), and may be oncogenic in esophageal squamous carcinoma (PMID: 31632486).

Likely Oncogenic - Biological

EP300 p.(Ser531TyrfsTer15) introduces a frameshift and premature stop in exon 7 of 31, likely resulting in truncation of the functional protein (UniProt.org). This truncation leads to loss of key domains, including the HAT, bromodomain, and transcription factor binding regions, and is predicted to loss of acetyltransferase and tumor suppressor function (PMID: 10700188, 15156177, 12402157).

NSD1 *p.(Glu2214Ter)* Likely Oncogenic VAF 56.49 %

Stop Gained, Loss of Function Variant NM_022455.5 c.6640G>T chr5:176721009 G→T

Genomic Biomarker Details

Gene Information

NSD1, nuclear receptor binding SET domain protein 1, is a histone methyltransferase that regulates gene expression through methylation of histone H3 lysine 36 (PMID: 21196496) and lysine 37 (H3K27me3) (PMID: 31727171) and is also a coregulator of steroid receptors (PMID: 11733144, PMID: 11509567). Overexpression of Nsd1 has been observed in metastatic pancreatic ductal adenocarcinoma (PMID: 31060750) and in hepatocellular carcinoma patients with poor prognosis (PMID: 31727171), while loss of function mutations in NSD1 may increase sensitivity to cisplatin in HPV-negative head and neck squamous cell carcinoma (PMID: 29636367).

Likely Oncogenic · Biological

NSD1 p.(Glu2214Ter) is a nonsense variant that introduces a premature termination in the last exon of NSD1. While it is not predicted to result in nonsense mediated decay, truncating variants downstream of the NSD1 p.(Glu2214Ter) variant have been reported as pathogenic.

Variants of Unknown Significance

Gene	Variant	VAF	Consequence	Relevant Evidence
ATRX	NM_000489.6 c.1489G>A	49.54 %	Missense Variant	N/A
NRAS	Copy Number: 5.9		Copy Number Increase	NRAS amplification results in overexpression of the protein. This mutation has been found in ovarian cancer and amplification of hot-spot mutated NRAS has been found in melanoma (PMID: 26266759, 25263998; Abstract: Hélias-Rodzewicz et al. Abstract# 9578, ASCO 2016. http://meetinglibrary.asco.org/content/169489-176; Abstract: Zehir A et al. Nature Medicine, 2017. http://doi:10.1038/nm.4333). Overexpression of NRAS in a knock-in mouse model demonstrated that it is activating as measured by T-cell expansion and decreased survival compared to wildtype (PMID: 22876308), but functional studies are limited; therefore, the functional impact of NRAS amplification are unknown.
EGFR	NM_005228.5 c.3005T>C	31.94 %	Missense Variant	N/A
EP300	NM_001429.4 c.3469A>G	14.53 %	Missense Variant	N/A
IL7R	NM_002185.5 c.214G>C	57.04 %	Missense Variant	N/A
INPP4A	NM_001134225.2 c.1134G>T	35.86 %	Missense Variant	N/A
MED12	NM_005120.3 c.1001C>T	43.24 %	Missense Variant	N/A
PDK1	NM_002610.5 c.1108C>T	30.36 %	Stop Gained	PDK1 p.(Gln370Ter) is a nonsense variant that introduces a premature termination in exon 10 of 11 (UniProt.org). While the variant is expected to result in nonsense mediated decay, the effect of the oncogene PDK1 loss-of-function is unknown.
PREX2	NM_024870.4 c.2388C>A	17.4 %	Missense Variant	N/A

PTPRD	NM_002839.4 c.739A>G	100 %	Missense Variant	N/A
PTPRT	NM_007050.6 c.2435G>T	30.16 %	Missense Variant	N/A
SHQ1	NM_018130.3 c.1331G>T	12.24 %	Missense Variant	N/A
SOX10	NM_006941.4 c.781C>A	49.78 %	Missense Variant	N/A

Regions of Low Coverage

HIST2H3A Exon1 (chr1:149812316-149812731)
HIST2H3A Exon1 (chr1:149824214-149824629)
REL Exon9 (chr2:61147515-61147615)
RANBP2 Exon8 (chr2:109363164-109363256)
RANBP2 Exon13 (chr2:109369451-109369617)
BCL2L11 Exon3 (chr2:111887706-111887814)
PAX8 Exon8 (chr2:113994175-113994300)
LRP1B Exon18 (chr2:141739726-141739847)
LRP1B Exon17 (chr2:141747098-141747228)
LRP1B Exon16 (chr2:141751561-141751706)
LRP1B Exon15 (chr2:141762901-141763028)
LRP1B Exon14 (chr2:141771122-141771316)
LRP1B Exon13 (chr2:141773262-141773486)
LRP1B Exon12 (chr2:141777488-141777673)
MYB Exon1 (chr6:135502649-135502676)
ETV1 Exon5 (chr7:14026260-14026312)
PTEN Exon1 (chr10:89624224-89624307)
PTEN Exon2 (chr10:89653779-89653868)
FGF8 Exon1 (chr10:103535623-103535659)
SDHD Exon4 (chr11:111963801-111963923)
RB1 Exon15 (chr13:48954186-48954222)
RB1 Exon24 (chr13:49047493-49047528)
PDPK1 Exon1 (chr16:2588111-2588139)
PDPK1 Exon3 (chr16:2611478-2611525)
PDPK1 Exon4 (chr16:2611769-2611911)
PDPK1 Exon5 (chr16:2615551-2615700)
PDPK1 Exon6 (chr16:2616354-2616456)
PDPK1 Exon8 (chr16:2631293-2631366)
PDPK1 Exon9 (chr16:2631605-2631706)
PDPK1 Exon10 (chr16:2633410-2633735)
CYLD Exon7 (chr16:50788369-50788378)
SUZ12 Exon3 (chr17:30267438-30267507)
SUZ12 Exon6 (chr17:30300162-30300252)
STAT5B Exon7 (chr17:40371327-40371483)
STAT5A Exon8 (chr17:40452145-40452301)
DNMT1 Exon5 (chr19:10290860-10290912)

Test Methods & Limitations

The JAX SomaticSeq™ is a somatic-only test that incorporates two targeted enrichment sequencing assays: a DNA based panel comprising 517 cancer related genes and an RNA based panel evaluating 55 genes known to form fusions in solid tumors. Clinically significant small nucleotide variants (SNVs) and insertion-deletions (indels) are reported across the 517 gene panel. Copy number variants (CNVs) and fusions are reported in 60 and 55 genes, respectively. Additionally, MET exon 14 and EGFR exons 2-7 splicing (EGFRvIII) events are covered.

As necessary (for FFPE blocks or unstained slides), specimens are sectioned and stained using Fisher Chemical Eosin Y and Richard-Allan Scientific™ Hematoxylin Stain (Modified Mayer). Slides are digitally scanned on the Leica Aperio CS2 Scanner for remote pathologist review of neoplastic content, tissue type, tumor area, and specimen quality (Remote Testing Site: LBH07).

The JAX SomaticSeq™ uses genomic DNA and RNA extracted from macro dissection enriched FFPE tissue sections (30% neoplastic content), followed by enrichment of target exons and introns by hybrid capture (Illumina). The Illumina NextSeq 2000 generates 101bp paired end sequence reads with a median exon coverage of greater than or equal to 150X. Mutational analysis is performed using the DRAGEN TSO500 Tissue HT v2.6.2.4 bioinformatic pipeline within the Illumina Connected Analytics platform. Variants are called against human genome build GRCh37. A minimum coverage of 100X is required for reporting SNVs (single nucleotide variants) and indels (insertions and deletions up to 50 bp in length). Variants within regions that do not meet our coverage thresholds are not reported. The LOD (limit of detection) for SNVs and indels was determined as 5% during the analytical validation; however, sensitivity and specificity may be reduced for variants with allele fraction <10%. The LOD for copy number variants (CNVs) was 5 copies for amplifications and 1 copy for deletions; however, chromosome/arm-level copy number changes may mask gene-level deletions/amplifications. Copy number alterations at the chromosome and arm levels are not assessed by this test.

Evidence of association between genomic variants and potential therapeutic (including clinical trials), prognostic and/or diagnostic outcomes is obtained from peer reviewed literature, clinical practice guidelines, FDA labels, publicly available databases, and other resources. Information from these sources is curated into the Illumina Connected Insights platform and clinical significance of genomic variants interpreted in the context of each patient's molecular/disease profile. The JAX SomaticSeq™ report reflects the variants determined to be clinically relevant at the time of reporting. Variants are classified into four tiers based on the joint consensus guidelines published by AMP/ASCO/CAP on interpretation of sequence variants in cancer (PMID: 27993330). The four tiers include strong clinical significance (Tier I), potential clinical significance (Tier II), unknown clinical significance (Tier III) and benign or likely benign variants (Tier IV). The patient's complete molecular profile is available to the ordering clinician(s) upon request, up to 18 months after the date of report, including variants of uncertain significance (VUS) and variants with no current therapeutic correlation.

Tumor mutation burden (TMB) is calculated as the mutations per megabase (mut/Mb) across the ~1.94Mb of coding DNA captured by the JAX SomaticSeq™ panel. Variant types included in the calculation are synonymous and nonsynonymous SNVs/indels at 5% limit of detection within high-confidence, coding regions with a minimum of 50X coverage. Germline estimation is used for TMB calculation and leverages the latest publicly available population data. The impact of rare germline mutations is expected to be limited for the TMB estimation. TMB number may be inflated in samples with >5% supplementary (chimeric) alignments due to the larger number of false positive indels. Tumors containing 10 mut/Mb are classified as TMB high and may respond to immunotherapy treatment (PMID: 28835386, PMID: 29658845). Microsatellite instability (MSI) status is determined through analysis of 130 MSI marker sites to calculate the percentage of unstable sites. A minimum of 40 analyzed MSI sites are required for classification. Specimens with 10% unstable MSI sites are reported as microsatellite instability high (MSI-H) and specimens with <10% unstable MSI sites are reported as microsatellite stable (MSS).

Review of digital data, results, and/or clinical report was performed at the following remote testing sites: LWH25, MKH11.

System Version: 5.2.3

Data Source Versions: Illumina Connected Annotations 3.26.0-0-g9a48876a+9a48876ad0c4171484f5d5b0622a957ebd3a6ff2 (Ensembl: 110; RefSeq: 105.20220307; ClinVar: 20241201; ClinVarPreview: 20241201; dbSNP: 156; dbSNP: 151; GME: 20160618; gnomAD: 2.1; MITOMAP: 20200819; 1000 Genomes Project: Phase 3 v5a; REVEL: 20200205; TOPMed: freeze_5; COSMIC: 99; PrimateAI: 0.2; PrimateAI-3D: 1.0; SpliceAI: 1.3; ClinGen: 20160414; ClinGen Dosage Sensitivity Map: 20241218; DECIPHER: 201509; gnomAD_SV: 2.1; MITOMAP_SV: 20200819; 1000 Genomes Project (SV): Phase 3 v5a; COSMIC gene fusions: 99; FusionCatcher: 1.33; DANN: 20200205; Gerp: 20110522; ClinGen disease validity curations: 20241218; gnomAD_gene_scores: 4.1; Cosmic Cancer Gene Census: 99; OMIM: 20241218; phyloP: hg19; gnomAD_LCR: 2.1; MitochondrialHeteroplasmy: 20180410; CancerHotspots: 2017; CIViC: 01-Jan-2026; OncoKB: v6_0; CKB: 2026_01_02)

Disclaimer

Decisions on patient care must be based on the independent medical judgment of the treating physician, taking into consideration all relevant information about the patient's condition, including patient medical and family history, physical examinations, information from other diagnostic tests, and patient preferences. A treating physician's decisions should not be based on a single test, such as this test, or the information contained in this report alone. Results of this test must always be interpreted in the context of all relevant clinical and pathological data and should not be used alone for diagnosis or patient care decisions. Genetic counseling is recommended to discuss the implications of these test results.

The JAX SomaticSeq™ uses high throughput sequencing to identify clinically significant variants (SNVs and indels) within 517 cancer related genes including the TERT promoter, CNVs of 60 cancer related genes, fusions across 55 gene partners, and splicing events in MET and EGFR as listed in the appendix of this report. The assay may not detect all potentially relevant variants. Tumor tissue is not homogenous, and its characteristics may differ from sample to sample for the same tumor. Sample neoplastic content levels near the required minimum (30%) may have decreased sensitivity for copy number alterations, and chromosomal copy number alterations can mask gene-level events. It may be possible for a biomarker variant to be present yet go undetected by our assay either due to the heterogeneous nature of the tumor tissue or the limit of detection of our assay (please see “test methods and limitations” section). Therefore, to the extent a particular biomarker variant is not reported, we cannot guarantee that the variant does not exist.

The JAX SomaticSeq™ examines tumor tissue only and does not examine normal tissue (such as tissue adjacent to the tumor). Thus, the origin of a mutation detected by our assay may be a somatic (not inherited) or a germline mutation (inherited) and will not be distinguishable by this assay. If a germline inheritance pattern is suspected, then counseling by a genetic counselor is recommended.

The information presented in the clinical trials section of this report is compiled from public sources believed to be reliable and current. However, the information available in the public domain is continuously updated. While we endeavor to make this information accurate and complete, we cannot guarantee the accuracy or completeness of this information. Accordingly, the patient's physician or research staff should independently investigate the clinical trials information. The clinical trials information was compiled from www.clinicaltrials.gov. The clinical trials are not ranked in order of potential or predicted efficacy. The clinical trial information is to be used for clinical trial guidance and may not include all relevant trials. The clinical trials listed in this report were enrolling at the time of report generation, but the status may change at any time. Specific entrance criteria for each clinical trial should be reviewed as additional inclusion criteria may apply. The clinical trials identified may or may not be suitable for a particular patient and we do not guarantee or suggest that any particular trial will be effective with the treatment of any particular condition. Health care providers should employ independent clinical judgment in interpreting this

information for their patients.

This report includes information about therapeutic agents that appear to be associated with clinical benefit based on National Comprehensive Cancer Network (NCCN) Compendium guidelines, relevance of tumor lineage, and published evidence, as available and compiled by The Jackson Laboratory. The Jackson Laboratory expressly disclaims and makes no representation or warranty relating to the published evidence and scientific literature identified in this report, or any of the conclusions and information set forth in this report that is derived from a review thereof, including information and conclusions relating to therapeutic agents that are included or omitted from this report. The therapeutic agents included in this report are not ranked in order of potential or predicted efficacy. Agents with potential clinical benefit (or lack of clinical benefit) are not evaluated for source or level of published evidence and are identified based on the information available at the time of the test. The agents identified may or may not be suitable for use on a particular patient and we do not guarantee or suggest that any particular agent will be effective with the treatment of any particular condition. The selection of any, all or none of the agents associated with potential clinical benefit (or lack of clinical benefit) resides solely within the discretion of the treating physician.

This report includes some clinically relevant interpretation of next generation sequencing data powered by external resources. This information may include associations between a biomarker variant (or lack of a variant) and one or more therapeutic agents with potential clinical benefit (or lack of clinical benefit), including agents that are being studied in clinical research. A finding of a biomarker variant does not necessarily indicate pharmacologic effectiveness (or lack thereof) of any agent or treatment regimen. A finding of "no biomarker variant" does not necessarily indicate lack of pharmacologic effectiveness (or lack of effectiveness) of any agent or treatment regimen. The Jackson Laboratory expressly disclaims, and makes no representation or warranty of, the accuracy or completeness with respect to the publicly available information included herein or compiled in creating this report.

This test was developed and its performance characteristics determined by The Jackson Laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration (FDA). This test may be used for clinical purposes and should not be regarded as purely investigational or for research only. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA 88) as qualified to perform high complexity clinical testing. The Jackson Laboratory 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 this test.

Melissa Kelly, PhD, HCLD/CC(ABB)
Clinical Laboratory Director — 

Report Date — 2026-03-09

Appendix I – Genes Sequenced

Small Variants Only		Small Variants & CNVs		Small Variants & Fusions		Small Variants, CNVs, & Fusions	
ABL1	CD274	EPHB1	GATA6	KDM5A	NFE2L2	PRKN (PARK2)	SOX10
ABL2	CD276	ERBB2 (HER2)	GEN1	KDM5C	NFKBIA	PRSS8	SOX17
ABRAXAS1 (FAM175A)	CD74	ERBB3	GID4	KDM6A	NKX2-1	PTCH1	SOX2
ACVR1	CD79A	ERBB4	GLI1	KDR	NKX3-1	PTEN	SOX9
ACVR1B	CD79B	ERCC1	GNA11	KEAP1	NOTCH1	PTPN11	SPEN
ADGRA2 (GPR124)	CDC73	ERCC2	GNA13	KEL	NOTCH2	PTPRD	SPOP
AKT1	CDH1	ERCC3	GNAQ	KIF5B	NOTCH3	PTPRS	SPTA1
AKT2	CDK12	ERCC4	GNAS	KIT	NOTCH4	PTPRT	SRC
AKT3	CDK4	ERCC5	GPS2	KLF4	NPM1	QKI	SRSF2
ALK	CDK6	ERG	GREM1	KLHL6	NRAS	RAB35	STAG1
ALOX12B	CDK8	ERRFI1	GRIN2A	KMT2A (MLL)	NRG1	RAC1	STAG2
AMER1 (FAM123B)	CDKN1A	ESR1	GRM3	KRAS	NSD1	RAD21	STAT3
ANKRD11	CDKN1B	ETS1	GSK3B	LAMP1	NTRK1	RAD50	STAT4
ANKRD26	CDKN2A	ETV1	H1-2 (HIST1H1C)	LATS1	NTRK2	RAD51	STAT5A
APC	CDKN2B	ETV4	H2BC5 (HIST1H2BD)	LATS2	NTRK3	RAD51B	STAT5B
AR	CDKN2C	ETV5	H3-3A (H3F3A)	LMO1	NUP93	RAD51C	STK11
ARAF	CEBPA	ETV6	H3-3B (H3F3B)	LRP1B	NUTM1	RAD51D	STK40
ARFRP1	CENPA	EWSR1	H3-4 (HIST3H3)	LYN	PAK1	RAD52	SUFU
ARID1A	CHD2	EZH2	H3-5 (H3F3C)	LZTR1	PAK3	RAD54L	SUZ12
ARID1B	CHD4	FANCA	H3C1 (HIST1H3A)	MAGI2	PAK5 (PAK7)	RAF1	SYK
ARID2	CHEK1	FANCC	H3C10 (HIST1H3H)	MALT1	PALB2	RANBP2	TAF1
ARID5B	CHEK2	FANCD2	H3C11 (HIST1H3I)	MAP2K1	PARP1	RARA	TBX3
ASXL1	CIC	FANCE	H3C12 (HIST1H3J)	MAP2K2	PAX3	RASA1	TCF3
ASXL2	COP1 (RFWD2)	FANCF	H3C13 (HIST2H3D)	MAP2K4	PAX5	RB1	TCF7L2
ATM	CREBBP	FANCG	H3C14 (HIST2H3C)	MAP3K1	PAX7	RBM10	TENT5C (FAM46C)
ATR	CRKL	FANCI	H3C15 (HIST2H3A)	MAP3K13	PAX8	RECQL4	TERC
ATRX	CRLF2	FANCL	H3C2 (HIST1H3B)	MAP3K14	PBRM1	REL	TERT
AURKA	CSF1R	FAS	H3C3 (HIST1H3C)	MAP3K4	PDCD1	RET	TET1
AURKB	CSF3R	FAT1	H3C4 (HIST1H3D)	MAPK1	PDCD1LG2	RHEB	TET2
AXIN1	CSNK1A1	FBXW7	H3C6 (HIST1H3E)	MAPK3	PDGFRA	RHOA	TFE3
AXIN2	CTCF	FGF1	H3C7 (HIST1H3F)	MAX	PDGFRB	RICTOR	TFRC
AXL	CTLA4	FGF10	H3C8 (HIST1H3G)	MCL1	PDK1	RIT1	TGFBR1
B2M	CTNNA1	FGF14	HGF	MDC1	PDPK1	RNF43	TGFBR2
BAP1	CTNNB1	FGF19	HNF1A	MDM2	PGR	ROS1	TMEM127
BARD1	CUL3	FGF2	HNRNPK	MDM4	PHF6	RPS6KA4	TMPRSS2
BBC3	CUX1	FGF23	HOXB13	MED12	PHOX2B	RPS6KB1	TNFAIP3
BCL10	CXCR4	FGF3	HRAS	MEF2B	PIK3C2B	RPS6KB2	TNFRSF14
BCL2	CYLD	FGF4	HSD3B1	MEN1	PIK3C2G	RPTOR	TOP1
BCL2L1	DAXX	FGF5	HSP90AA1	*MET	PIK3C3	RUNX1	TOP2A
BCL2L11	DCUN1D1	FGF6	ICOSLG	MGA	PIK3CA	RUNX1T1	TP53
BCL2L2	DDR2	FGF7	ID3	MITF	PIK3CB	RYBP	TP63
BCL6	DDX41	FGF8	IDH1	MLH1	PIK3CD	SDHA	TRAF2
BCOR	DHX15	FGF9	IDH2	MLLT3	PIK3CG	SDHAF2	TRAF7
BCORL1	DICER1	FGFR1	IFNGR1	MPL	PIK3R1	SDHB	TSC1
BCR	DIS3	FGFR2	IGF1	MRE11 (MRE11A)	PIK3R2	SDHC	TSC2
BIRC3	DNAJB1	FGFR3	IGF1R	MSH2	PIK3R3	SDHD	TSHR
BLM	DNMT1	FGFR4	IGF2	MSH3	PIM1	SETBP1	U2AF1
BMPR1A	DNMT3A	FH	IKBKE	MSH6	PLCG2	SETD2	VEGFA
BRAF	DNMT3B	FLCN	IKZF1	MST1	PLK2	SF3B1	VHL
BRCA1	DOT1L	FLI1	IL10	MST1R	PMAIP1	SH2B3	VTCN1
BRCA2	E2F3	FLT1	IL7R	MTOR	PMS1	SH2D1A	WT1
BRD4	EED	FLT3	INHA	MUTYH	PMS2	SHQ1	XIAP
BRIP1	EGFL7	FLT4	INHBA	MYB	PNRC1	SLIT2	XPO1
BTG1	*EGFR	FOXA1	INPP4A	MYC	POLD1	SLX4	XRCC2
BTK	EIF1AX	FOXL2	INPP4B	MYCL (MYCL1)	POLE	SMAD2	YAP1
CALR	EIF4A2	FOXO1	INSR	MYCN	PPARG	SMAD3	YES1
CARD11	EIF4E	FOXP1	IRF2	MYD88	PPM1D	SMAD4	ZBTB2
CASP8	ELOC (TCEB1)	FRS2	IRF4	MYOD1	PPP2R1A	SMARCA4	ZBTB7A
CBFB	EML4	FUBP1	IRS1	NAB2	PPP2R2A	SMARCB1	ZFHX3
CBL	EMSY (C11orf30)	FYN	IRS2	NBN	PPP6C	SMARCD1	ZNF217
CCN6 (WISP3)	EP300	GABRA6	JAK1	NCOA3	PRDM1	SMC1A	ZNF703
CCND1	EPCAM	GATA1	JAK2	NCOR1	PREX2	SMC3	ZRSR2
CCND2	EPHA3	GATA2	JAK3	NEGR1	PRKAR1A	SMO	*EGFR exons 2-7 (vIII) & MET exon 14 skipping events also reported
CCND3	EPHA5	GATA3	JUN	NF1	PRKCI	SNCAIP	
CCNE1	EPHA7	GATA4	KAT6A	NF2	PRKDC	SOCS1	