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JAX SomaticSeq

Patient Name: Jane Doe
Specimen ID: ADM52A5772
Order ID: LP1000473
Report Date: 2024-11-22 (UTC)

Demographics

PATIENT
Patient Name: Jane Doe
Patient ID: JL1000420
Source Patient ID: 9999999
D.O.B: 1950-10-01
Sex: Female
Diagnosis: Glioblastoma

SPECIMEN
Specimen ID: ADM52A5772
Source Specimen ID: VA07A
1° Tumor Site: Unknown
Specimen Site: Brain
Neoplastic Content: 80%
Collection Date: 2024-10-01
Received Date: 2024-11-08

PHYSICIAN
Physician Name: Bart Bass
Physician Affiliation: JAX-Lab-Internal-Quality

Interpretation Summary

The following clinically significant variants were detected in this specimen: FGFR1 p.(Asn546Lys), SETD2 p.(Arg400Ter), SETD2 p.(Arg1598Ter), TP53 p.(Arg248Gln), PTPN11 p.(Gly503Ala), BAP1 p.(Arg385Ter), and NCOR1 p.(Arg414Ter). No fusions of clinical significance were detected in this specimen.

This specimen has a TMB of 54.71 mutations/Mb. Pembrolizumab is FDA-approved for the treatment of adult and pediatric patients with unresectable or metastatic solid tumors with tumor mutational burdens >= 10 mutations/Mb as determined by an FDA approved test (FDA.gov).

Germline variants in the following genes are associated with cancer pre-disposing syndromes: TP53 (MIM: 202300, 614740, 114500, 137800, 618165, 151623), BAP1 (MIM: 614327, 606661). This assay does not distinguish between somatic and germline variation; therefore, the variant was interpreted in the somatic context. Additional testing and genetic counseling may be indicated.

Clinical correlation is REQUIRED.

Genomic Biomarkers

Findings	**TMB** *54.7 mut/MB*	
	FGFR1 *p.(Asn546Lys)*	Likely Oncogenic
	SETD2 *p.(Arg400Ter)*	Likely Oncogenic
	SETD2 *p.(Arg1598Ter)*	Likely Oncogenic
	TP53 *p.(Arg248Gln)*	Likely Oncogenic
	PTPN11 *p.(Gly503Ala)*	Likely Oncogenic
	BAP1 *p.(Arg385Ter)*	Likely Oncogenic
	NCOR1 *p.(Arg414Ter)*	Likely Oncogenic
Pertinent Negatives*	**ATRX, BRAF, CDKN2A, EGFR, H3-3A, H3C2, IDH1, IDH2, MGMT, NTRK1, NTRK2, NTRK3, PTCH1, PTEN, TERT**	

*Contains disease related genes only.

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

Immunotherapy Biomarkers

Tumor Mutation Burden (TMB) - 54.71 mut/MB | **TMB-High** | See "Biomarker Details" section for assertion details
Microsatellite Instability (MSI) -3.20% unstable sites | **MS-Stable**

Therapies

Biomarker	Therapy in Patient's Disease	Therapy in Other Diseases	Clinical Trials
TMB *54.7 mut/MB* **TMB-High**	Tier 1A • Sensitive Nivolumab Pembrolizumab	Nothing reported	Nothing reported
TP53 *p.(Arg248Gln)*	Tier 2D • No Benefit AZ31 + Radiotherapy	Nothing reported	Nothing reported

Therapies

Biomarker	Therapy in Patient's Disease	Therapy in Other Diseases	Clinical Trials
Likely Oncogenic	Tier 2D • Predicted - sensitive AZ32 + Radiotherapy KU-60019 + Radiotherapy Tier 2D • Sensitive Adavosertib Adavosertib + Radiotherapy AZD1390		

Genomic Biomarker Details

TMB *54.7 mut/MB* Tier 1A **TMB-High**

Tier 1A - **Therapeutic** - **Pembrolizumab (Sensitive)**

Keytruda (pembrolizumab) is included in guidelines as adjuvant therapy for pediatric patients with diffuse high-grade gliomas with high tumor mutational burden (TMB), or as a preferred regimen for patients with recurrent or progressive disease (NCCN.org).

Tier 1A - **Therapeutic** - **Nivolumab (Sensitive)**

Opdivo (nivolumab) is included in guidelines as adjuvant therapy for pediatric patients with diffuse high-grade gliomas with high tumor mutational burden (TMB), or as a preferred regimen for patients with recurrent or progressive disease (NCCN.org).

FGFR1 *p.(Asn546Lys)* Likely Oncogenic Tier 2C VAF 28.8 %
Missense Variant, Gain of Function Variant NM_023110.3 c.1638C>A chr8:38274849

Gene Information

FGFR1, fibroblast growth factor receptor 1, is a receptor tyrosine kinase activated upon binding of the FGF ligand, which activates RAS-MAPK and PI3K-AKT pathways (PMID: 22508544). Altered function of Fgfr1 may lead to increased cell proliferation and decreased apoptosis (PMID: 22508544) and amplification and/or overexpression has been identified in colorectal cancer (PMID: 30181810), gastric cancer (PMID: 29976636), and breast cancer (PMID: 30119151).

Likely Oncogenic - **Biological**

FGFR1 p.(Asn546Lys) is a hotspot variant that lies within the protein kinase domain of the FGFR1 protein (UniProt.org). FGFR1 p.(Asn546Lys) does not confer a growth advantage in a competition assay (PMID:34272467), but results in increased FGFR1 protein nuclear localization, ERK, AKT, and STAT3 phosphorylation (PMID: 35488346), and kinase activity, and is transforming in cultured cells (PMID: 26179511, PMID: 23817572, PMID: 29533785).

Tier 2C - **Diagnostic**

FGFR1 variants aid in the diagnosis of low grade gliomas (PMID: 33433639, 23817572, 27984673).

SETD2 *p.(Arg400Ter)* Likely Oncogenic Tier 2C VAF 20.42 %
Stop Gained, Loss of Function Variant NM_014159.7 c.1198C>T chr3:47164928

Genomic Biomarker Details

Gene Information

SETD2, histone-lysine N-methyltransferase SETD2, regulates chromatin structure through H3K36 to influence gene transcription (PMID: 28159833; PMID: 28386724) and plays a role in genomic stability (PMID: 31747945). Loss of function and decreased expression of Setd2 has been demonstrated in clear cell renal cell carcinoma (PMID: 28260718), breast cancer (PMID: 20501857) and lung cancer (PMID: 28202515).

Likely Oncogenic · **Biological**

SETD2 p.(Arg400Ter) is a nonsense variant predicted to result in premature truncation of the SETD2 protein at residue 400 of 2564 (UniProt.org). This variant has not been characterized in the scientific literature, however due to the effect of downstream truncations (PMID: 23417712, 24509477), is predicted to lead to a loss of protein function. C-terminal truncating variants lead to loss of the SRI domain and reduced H3K36 trimethyltransferase activity (PMID: 23417712,24509477). SETD2 LOF variants are associated with enhanced leukemia development in mouse models (PMID:24509477). Truncating variants in renal cell carcinomas are associated with replication stress and impaired DNA repair (PMID: 25728682).

Tier 2C · **Diagnostic**

SETD2-inactivating variants occur in ~15% and ~8% of pediatric and adult high grade gliomas, respectively, and are not found in other gliomas (PMID: 23417712).

SETD2 *p.(Arg1598Ter)* Likely Oncogenic Tier 2C VAF 7.91 %
Stop Gained, Loss of Function Variant NM_014159.7 c.4792C>T chr3:47147534

Gene Information

SETD2, histone-lysine N-methyltransferase SETD2, regulates chromatin structure through H3K36 to influence gene transcription (PMID: 28159833; PMID: 28386724) and plays a role in genomic stability (PMID: 31747945). Loss of function and decreased expression of Setd2 has been demonstrated in clear cell renal cell carcinoma (PMID: 28260718), breast cancer (PMID: 20501857) and lung cancer (PMID: 28202515).

Likely Oncogenic · **Biological**

SETD2 p.(Arg1598Ter) is a nonsense variant predicted to result in premature truncation of the SETD2 protein at residue 1598 of 2564 (UniProt.org). This variant has not been characterized in the scientific literature, however due to the effect of downstream truncations (PMID: 23417712, 24509477), is predicted to lead to a loss of protein function. C-terminal truncating variants lead to loss of the SRI domain and reduced H3K36 trimethyltransferase activity (PMID: 23417712, 24509477). SETD2 LOF variants are associated with enhanced leukemia development in mouse models (PMID:24509477). Truncating variants in renal cell carcinomas are associated with replication stress and impaired DNA repair (PMID: 25728682).

Tier 2C · **Diagnostic**

SETD2-inactivating variants occur in ~15% and ~8% of pediatric and adult high grade gliomas, respectively, and are not found in other gliomas (PMID: 23417712).

TP53 *p.(Arg248Gln)* Likely Oncogenic Tier 2D VAF 7.03 %
Missense Variant, Loss of Function Variant NM_000546.6 c.743G>A chr17:7577538

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).

Genomic Biomarker Details

Likely Oncogenic - **Biological**

TP53 p.(Arg248Gln) is a hotspot mutation that lies within the DNA-binding domain of the TP53 protein (PMID: 22713868). TP53 p.(Arg248Gln) results in increased proliferation, migration, invasion, and protein stability and altered subcellular localization in culture (PMID: 37030635), loss of DNA binding and decreased transactivation of TP53 targets and interference with wild-type TP53 transactivation, leads to resistance to apoptosis and failure of G1 arrest in cell culture (PMID: 23538418, PMID: 16861262, PMID:31395785), as well as increased AKT activation, STAT3 dependent migration, and enhanced tumor onset and growth in mouse models (PMID: 30107178).

Tier 2D - **Therapeutic** - **AZD1390 (Sensitive)**

In a preclinical study, TP53 mutant glioblastoma cells demonstrated increased sensitivity to radiosensitization by AZD1390 treatment compared to TP53 wild-type cells in culture (Mol Cancer Ther 2018;17(1 Suppl):Abstract nr A104).

Tier 2D - **Therapeutic** - **Radiotherapy, AZ31 (No Benefit)**

In a preclinical study, AZ31 treatment increased sensitivity to radiotherapy in TP53-mutant mouse glioma cells in culture, but failed to radiosensitize tumors in syngeneic mouse models due to poor blood-brain barrier permeability (PMID: 29769307).

Tier 2D - **Therapeutic** - **Radiotherapy, KU-60019 (Predicted - sensitive)**

In a preclinical study, KU-60019 treatment increased sensitivity to radiotherapy in TP53-mutant mouse glioma cells in culture, and the combination prolonged survival in syngeneic intracranial tumor models compared to radiation alone (PMID: 29769307).

Tier 2D - **Therapeutic** - **Adavosertib (Sensitive)**

In a preclinical study, treatment with Adavosertib (MK-1775) decreased CDK1 phosphorylation and reduced tumor growth in patient-derived xenograft models of glioblastoma multiforme that harbor TP53 mutations (PMID: 27196784).

Tier 2D - **Therapeutic** - **AZ32, Radiotherapy (Predicted - sensitive)**

In a preclinical study, AZ32 treatment increased sensitivity to radiotherapy in TP53 mutant mouse glioma cells, resulting in reduced cell survival and impaired DNA damage response in culture, and prolonged survival and significantly increased apoptosis of tumor cells compared to normal brain cells ($p<0.01$) in syngeneic intracranial tumor models (PMID: 29769307).

Tier 2D - **Therapeutic** - **Radiotherapy, Adavosertib (Sensitive)**

In a preclinical study, the combination of Adavosertib (MK-1775) and radiation therapy synergized to inhibit tumor growth in a human glioblastoma multiforme cell line xenograft model harboring mutant TP53 (PMID: 21992793).

PTPN11 *p.(Gly503Ala)* Likely Oncogenic Tier 2D VAF 27.08 %
Missense Variant, Gain of Function Variant NM_002834.5 c.1508G>C chr12:112926888

Gene Information

PTPN11, protein tyrosine phosphatase non-receptor type 11, is a member of the PTP Src homology-2 (SH2) domain-containing phosphatases that induces the MAPK signal transduction pathway to regulate growth factor signaling and has been characterized as both an oncogene and tumor suppressor (PMID: 20337577, PMID: 28074573). PTPN11 activating mutations have been identified in a variety of tumors including, lung, colon, brain, thyroid, skin and heme (PMID: 18286234, PMID: 21407260), PTPN11 hypomethylation has been observed in gastric cancer (PMID: 32194661), and germline mutations are implicated in Noonan syndrome (PMID: 28328117, PMID: 32233106).

Likely Oncogenic - **Biological**

Genomic Biomarker Details

PTPN11 p.(Gly503Ala) lies within the tyrosine-protein phosphatase domain of the PTPN11 protein (UniProt.org). PTPN11 p.(Gly503Ala), in the context of KMT2A-MLLT10, leads to increased colony size and cell cycle alterations in response to growth factor in culture, macrophage differentiation, and leukemogenesis induction in a shorter time period in mouse models compared to the KMT2A-MLLT10 fusion alone, and individually, results in increased phosphatase activity in an in vitro assay (PMID: 27859216), and therefore, is predicted to lead to a gain of PTPN11 protein function.

Tier 2D - Prognostic (Unfavorable)

PTPN11 variants are suggested to be a predictor of poorer prognosis in gliomas (PMID:37552362).

BAP1 *p.(Arg385Ter)* Likely Oncogenic Tier 2D VAF 22.61 %
Stop Gained, Loss of Function Variant NM_004656.4 c.1153C>T chr3:52438566

Gene Information

BAP1, BRCA1 associated protein 1, belongs to the ubiquitin C-terminal hydrolase subfamily and interacts with the RING finger domain of the BRCA1 protein, resulting in deubiquitylation of BARD1 (PMID: 23550303) and is associated with genomic stability (PMID: 31747945). BAP1 germline mutations are associated with mesothelioma and melanoma (PMID: 23550303) and somatic mutations are highest in renal and endometrial cancers (PMID: 27283171).

Likely Oncogenic - Biological

BAP1 p.(Arg385Ter) results in a premature truncation of the BAP1 protein at amino acid 385 of 729 (UniProt.org). BAP1 p.(Arg385Ter) has not been characterized; however, due to the effects of other downstream nonsense and frameshift variants (PMID: 26011428, PMID: 18757409), is predicted to lead to a loss of BAP1 protein function. These BAP1 truncating variants are predicted to produce several forms of C-terminally truncated BAP1 proteins with loss of the nuclear localization signal (PMID: 21874000). Expression of a BAP1 truncating variant in lung cancer cells demonstrated that it is unable to localize to the nucleus and this nuclear loss of BAP1 is oncogenic, as measured by the reduced cell cycle suppression (PMID: 18757409).

Tier 2D - Prognostic (Unfavorable)

Loss-of-function variants in BAP1 that the disrupt nuclear localization of BAP1 protein and result in aberrant accumulation in the cytoplasm may be associated with adverse prognosis in individuals with gliomas (PMID:26191197). BAP1 LOF is associated with poorer prognosis in meningiomas (PMID:34504799,28482042)

NCOR1 *p.(Arg414Ter)* Likely Oncogenic VAF 25.26 %
Stop Gained, Loss of Function Variant NM_006311.4 c.1240C>T chr17:16042434

Gene Information

NCOR1, nuclear receptor corepressor 1, is a transcriptional co-repressor, with diverse binding partners, that functions in differentiation, homeostasis, and metabolism (PMID: 23630073, PMID: 20091860). Overexpression of Ncor1 has been observed in gastric cancer (PMID: 26589942) and a NCOR1-MAP2K1 fusion has been reported in undifferentiated pleomorphic sarcoma (PMID: 31467233).

Likely Oncogenic - Biological

NCOR1 p.(Arg414Ter) introduces a premature stop codon in exon 12 of 46 and is predicted to result in a loss of function of the tumor suppressor. Knockdown of NCOR in a glioblastoma cell line resulted in a significant increases in anchorage-independent growth and proliferation, as well as tumor formation capacity and invasiveness (PMID: 24335696).

Variants of Unknown Significance

Gene	Variant	VAF	Consequence	Relevant Evidence
GATA4	NM_001308093.3 c.912+5G>A	8.84 %	Splice Region Variant	N/A
GNA11	NM_002067.5 c.358_360del	13.53 %	Inframe Deletion	N/A
FBXW7	NM_033632.3 c.2066G>A	19.54 %	Missense Variant	FBXW7 p.(Arg689Gln) does not lie within any known functional domains of the FBXW7 protein (UniProt.org). FBXW7 p.(Arg689Gln) is predicted to have activity similar to wild-type FBXW7 based on comparison and correlation analysis of induced gene expression signatures (PMID: 27147599), but has not been biochemically characterized and therefore, its effect on FBXW7 protein function is unknown.
NOTCH4	NM_004557.4 c.5944C>T	16.37 %	Stop Gained	NOTCH4 p.(Gln1982Ter) is a nonsense variant predicted to result in premature truncation of the NOTCH4 protein at residue 1928 of 2003 (UniProt.org). This variant has not been characterized in the scientific literature. While upstream truncating variants within the ankyrin repeats domain have been shown to impair NOTCH4 inhibition endothelial sprouting and angiogenesis, leading to VEGF-mediated angiogenesis (PMID: 15187023), it is unknown if the C-terminal location of the truncation of p.(Gln1982Ter) confers a LOF effect.
GABRA6	NM_000811.3 c.1004C>T	15.86 %	Missense Variant	N/A
SPTA1	NM_003126.4 c.5497G>A	8.06 %	Missense Variant	N/A
FGFR1	NM_023110.3 c.621+4C>T	13.21 %	Splice Region Variant	N/A
BRCA2	NM_000059.4 c.2776A>G	10.53 %	Missense Variant	N/A
APC	NM_000038.6 c.3164T>G	8.56 %	Missense Variant	N/A
DNMT3A	NM_022552.5 c.2374C>T	13.53 %	Missense Variant	N/A
PTPRD	NM_002839.4 c.5188C>T	10.5 %	Missense Variant	N/A
TAF1	NM_004606.5 c.2563C>T	12 %	Missense Variant	N/A
NRG1	NM_013964.5 c.818del	6.01 %	Frameshift Variant	N/A
ADGRA2	NM_032777.10 c.1976A>G	7 %	Missense Variant	N/A
RB1	NM_000321.3 c.2105A>G	11.81 %	Missense Variant	N/A
NOTCH1	NM_017617.5 c.4015-7C>T	10.06 %	Splice Region Variant Intron Variant	N/A
CDH1	NM_004360.5 c.2644G>A	12.76 %	Missense Variant	N/A
KMT2A	NM_001197104.2 c.2020G>A	7.79 %	Missense Variant	N/A
SPEN	NM_015001.3 c.8276C>T	8.47 %	Missense Variant	N/A
PLCG2	NM_002661.5 c.398C>T	11.83 %	Missense Variant	N/A

MITF	NM_001354604.2 c.1516G>A	8.72 %	Missense Variant	N/A
SETD2	NM_014159.7 c.4786T>C	7.8 %	Missense Variant	N/A
BRCA2	NM_000059.4 c.7336A>G	7.14 %	Missense Variant	N/A
SETD2	NM_014159.7 c.7528C>T	10 %	Missense Variant	N/A
GNAQ	NM_002072.5 c.578C>A	6.47 %	Missense Variant	N/A
MET	NM_000245.4 c.1171G>A	19.21 %	Missense Variant	N/A
TSC2	NM_000548.5 c.229G>A	8.33 %	Missense Variant	N/A
GRM3	NM_000840.3 c.1630T>C	6.94 %	Missense Variant	N/A
PMS2	NM_000535.7 c.401G>A	10.82 %	Missense Variant	N/A
SNCAIP	NM_005460.4 c.1496G>A	9.4 %	Missense Variant	N/A
CD276	NM_001024736.2 c.734-8G>A	6.82 %	Splice Region Variant Intron Variant	N/A
PTPN11	NM_002834.5 c.518G>A	17.67 %	Missense Variant	N/A
JAK1	NM_002227.4 c.1745A>T	5.74 %	Missense Variant	N/A
AKT1	NM_001382430.1 c.1099C>T	12.23 %	Missense Variant	N/A
LRP1B	NM_018557.3 c.6178C>T	10.23 %	Missense Variant	N/A
CDKN2B	NM_004936.4 c.377G>A	6.47 %	Missense Variant	N/A
TET1	NM_030625.3 c.6112C>T	9.29 %	Missense Variant	N/A
NF1	NM_001042492.3 c.4375_4377del	11.9 %	Inframe Deletion	N/A
TAF1	NM_004606.5 c.2759T>C	8.59 %	Missense Variant	N/A
FOXP1	NM_001349338.3 c.1138C>T	43.24 %	Missense Variant	N/A
PAK3	NM_001128168.3 c.302C>A	7.72 %	Missense Variant	N/A
MAP3K14	NM_003954.5 c.1867G>A	7 %	Missense Variant	N/A
RPS6KA4	NM_003942.3 c.1607T>C	7.02 %	Missense Variant	N/A
PIK3R3	NM_003629.4 c.734G>A	12.21 %	Missense Variant	N/A
JAK2	NM_004972.4 c.2515C>T	5.41 %	Missense Variant	N/A
ALOX12B	NM_001139.3 c.1406G>A	12.06 %	Missense Variant	N/A
VTCN1	NM_024626.4 c.445+2T>A	5.22 %	Splice Donor Variant	N/A
PIK3C2B	NM_001377334.1 c.4281-5G>A	8.71 %	Splice Region Variant	N/A
EPHA3	NM_005233.6 c.323G>A	14.34 %	Missense Variant	N/A
TET2	NM_001127208.3 c.5849A>G	7.14 %	Missense Variant	N/A
RET	NM_020975.6 c.2452G>A	19.7 %	Missense Variant	N/A
GSK3B	NM_001146156.2 c.779A>T	10.16 %	Missense Variant	N/A
ANKRD11	NM_013275.6 c.7855G>A	18.22 %	Missense Variant	N/A
EWSR1	NM_005243.4 c.1798C>T	11.79 %	Missense Variant	N/A
NOTCH3	NM_000435.3 c.4898C>T	8.91 %	Missense Variant	N/A
MTOR	NM_004958.4 c.7535A>G	7.32 %	Missense Variant	N/A
RET	NM_020975.6 c.607G>A	7.11 %	Missense Variant	N/A
BCL2L1	NM_138578.3 c.610C>T	6.99 %	Stop Gained	N/A

FAT1	NM_005245.4 c.1031C>T	9.3 %	Missense Variant	N/A
ADGRA2	NM_032777.10 c.2845C>T	19.38 %	Missense Variant	N/A
RAD21	NM_006265.3 c.503G>A	12.37 %	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)
MYB Exon1 (chr6:135502649-135502676)
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)
SUZ12 Exon3 (chr17:30267438-30267507)
DNMT1 Exon5 (chr19:10290860-10290912)
ICOSLG Exon1 (chr21:45660684-45660702)

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 61 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. 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. The LOD for copy number variants (CNVs) was 5 copies for amplifications and 1 copy for deletions. Mutational analysis is performed using the DRAGEN TSO500 Tissue HT v2.5.2 bioinformatic pipeline within the Illumina Connect Analytics platform. Variants are called against human genome build GRCh37.

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 per-

centage 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: 5.0.1

Data Source Versions: Illumina Connected Annotations 3.24.0 (Ensembl: 110; RefSeq: 105.20220307; ClinVar: 20231230; ClinVar: 20240301; dbSNP: 156; dbSNP: 151; GME: 20160618; gnomAD: 2.1; MITOMAP: 20200819; 1000 Genomes Project: Phase 3 v5a; PrimateAI: 0.2; REVEL: 20200205; TOPMed: freeze_5; COSMIC: 99; PrimateAI-3D: 1.0-ici; SpliceAI: 1.3; ClinGen: 20160414; ClinGen Dosage Sensitivity Map: 20240110; 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; gnomAD_gene_scores: 2.1; ClinGen disease validity curations: 20240110; Cosmic Cancer Gene Census: 99; OMIM: 20240110; phyloP: hg19; gnomAD_LCR: 2.1; MitochondrialHeteroplasmy: 20180410; CancerHotspots: 2017)

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.

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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 61 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. 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.

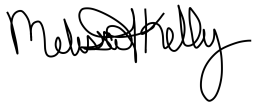
The information presented in the clinical trials section of this report is compiled from public sources believed to be reliable and current. However, the information available in the public domain is continuously updated. While we endeavor to make this information accurate and complete, we cannot guarantee 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 in- formation 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 pharmaco- logic 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 laborat-

ory 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 — 2024-11-22

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	