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CLIA #: 05D2111917 CAP #: 9441547

Solid Tumor Profile Plus

Patient Name:		Ordering Physician:	
Date of Birth:		Physician ID:	
Gender (M/F):		Accession #:	
Client:		Specimen Type:	
Case #:		Specimen ID:	
Body Site:			

MRN:		Tumor Type:	
Collected Date:			
Received Date:			
Reported Date:			

Detected Genomic Alterations				
Level 1 (FDA-Approved)	Level 2 (Standard of Care)	Level 3 (Clinical Evidence)	Level 4 (Biological Evidence)	Other
t(9;22)(q31.1;q12.2) EWSR1-NR4A3 mRNA fusion	-Homologous recombination deficiency (HRD): Positive-High -No evidence of microsatellite instability -Tumor Mutation Burden Low: 7 Mut/Mb	EPHA7, FBXW7	RTEL1, FANCI, NOTCH4, SDHA	Chromosomal structural analysis shows 1q-, 3p- (proximal), 7p-, 8q-, 14q+ (distal), 16q- (distal), and 20p-

Results Summary

- **-t(9;22)(q31.1;q12.2) EWSR1-NR4A3 mRNA fusion**
  -Mutations in EPHA7, FBXW7, RTEL1, FANCI, NOTCH4, and SDHA genes
  -Homologous recombination deficiency (HRD): Positive-High
  -Increased MYC mRNA
  -Chromosomal structural analysis shows 1q-, 3p- (proximal), 7p-, 8q-, 14q+ (distal), 16q- (distal), and 20p-
  -No evidence of microsatellite instability
  -Tumor Mutation Burden Low: 7 Mut/Mb
  -EBV viral RNA: Not detected
  -HPV viral RNA : Not detected
  -HLA Genotyping:
  -HLA-A: A*26:01-A*23:01
  -HLA-B: B*50:01-B*38:01
  -HLA-C: C*12:292-C*06:03

-The EWSR1-NR4A3 fusion is consistent with extraskeletal myxoid chondrosarcoma.

-Positive homologous recombination deficiency (HRD) suggests response to platinum-based chemotherapy and PARP inhibitors.

-EPHA7 mutation suggests possible response to EPHA inhibitors (Vandetanib).

-FBXW7 mutation suggests possible response to ubiquitin-proteosome system inhibitors as well as mTOR inhibitors.

Tumor Heterogeneity
There is a dominant abnormal clone with EPHA7 and FBXW7 mutations. The RTEL1, FANCI, NOTCH4, and SDHA mutations are detected in subclones.

Diagnostic Implications
The EWSR1-NR4A3 fusion is consistent with extraskeletal myxoid chondrosarcoma

FDA-Approved Therapeutics in Other Tumor Types	
HRD Positive	Niraparib + platinum-based chemotherapy

Levels 2, 3 & 4 (Standard of Care and Clinical/Biological Evidence)	
EPHA7	EPHA7 inhibitors
FBXW7	MTOR inhibitors or Tubulins

Relevant Genes with NO Alteration		
-No evidence of mutation in KRAS, NRAS, EGFR, BRAF, TP53, or BRCA 1/2 -No specific mutation in DPYD gene, associated with enzymatic deficiency	No evidence of fusion mRNA involving ALK, RET, ROS1, or NTRK	-No evidence of MET14 deletion or EGFR Viii -No evidence of ERBB2 (HER2) amplification

Test Description:

This is a comprehensive molecular profile which uses next generation sequencing (NGS), fragment length analysis and Sanger Sequencing testing to identify molecular abnormalities (including SNVs, INDELS, CNVs, Fusions, TMB, MSI, HRD, EBV, and HPV) in DNA of 434 genes and RNA in 1600 genes implicated in solid tumors. Whenever possible, clinical relevance and implications of detected abnormalities are described below.

Biological relevance of detected Alterations

- EPHA7. This gene belongs to the ephrin receptor subfamily of the protein-tyrosine kinase family. EPH and EPH-related receptors have been implicated in mediating developmental events, particularly in the nervous system. Receptors in the EPH subfamily typically have a single kinase domain and an extracellular region containing a Cys-rich domain and 2 fibronectin type III repeats. The ephrin receptors are divided into 2 groups based on the similarity of their extracellular domain sequences and their affinities for binding ephrin-A and ephrin-B ligands.

Increased expression of this gene is associated with multiple forms of carcinoma. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Dec 2013]

- FBXW7. This gene encodes a member of the F-box protein family which is characterized by an approximately 40 amino acid motif, the F-box. The F-box proteins constitute one of the four subunits of ubiquitin protein ligase complex called SCFs (SKP1-cullin-F-box), which function in phosphorylation-dependent ubiquitination. The F-box proteins are divided into 3 classes: Fbws containing WD-40 domains, Fbls containing leucine-rich repeats, and Fbxs containing either different protein-protein interaction modules or no recognizable motifs. The protein encoded by this gene was previously referred to as FBX30, and belongs to the Fbws class; in addition to an F-box, this protein contains 7 tandem WD40 repeats. This protein binds directly to cyclin E and probably targets cyclin E for ubiquitin-mediated degradation. Mutations in this gene are detected in ovarian and breast cancer cell lines, implicating the gene's potential role in the pathogenesis of human cancers. Multiple transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Mar 2012]

- RTEL1. This gene encodes a DNA helicase which functions in the stability, protection and elongation of telomeres and interacts with proteins in the shelterin complex known to protect telomeres during DNA replication. Mutations in this gene have been associated with dyskeratosis congenita and Hoyerall-Hreidarsson syndrome. Read-through transcription of this gene into the neighboring downstream gene, which encodes tumor necrosis factor receptor superfamily, member 6b, generates a non-coding transcript. Alternative splicing results in multiple transcript variants encoding different isoforms. [provided by RefSeq, Sep 2013]

- FANCI. The Fanconi anemia complementation group (FANC) currently includes FANCA, FANCB, FANCC, FANCD1 (also called BRCA2), FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ (also called BRIP1), FANCL, FANCM and FANCN (also called PALB2). The previously defined group FANCH is the same as FANCA. Fanconi anemia is a genetically heterogeneous recessive disorder characterized by cytogenetic instability, hypersensitivity to DNA crosslinking agents, increased chromosomal breakage, and defective DNA repair. The members of the Fanconi anemia complementation group do not share sequence similarity; they are related by their assembly into a common nuclear protein complex. This gene encodes the protein for complementation group I. Alternative splicing results in two transcript variants encoding different isoforms. [provided by RefSeq, Jul 2008]

- NOTCH4. This gene encodes a member of the NOTCH family of proteins. Members of this Type I transmembrane protein family share structural characteristics including an extracellular domain consisting of multiple epidermal growth factor-like (EGF) repeats, and an intracellular domain consisting of multiple different domain types. Notch signaling is an evolutionarily conserved intercellular signaling pathway that regulates interactions between physically adjacent cells through binding of Notch family receptors to their cognate ligands. The encoded preproprotein is proteolytically processed in the trans-Golgi network to generate two polypeptide chains that heterodimerize to form the mature cell-surface receptor. This receptor may play a role in vascular, renal and hepatic development. Mutations in this gene may be associated with schizophrenia. Alternative splicing results in multiple transcript variants, at least one of which encodes an isoform that is proteolytically processed. [provided by RefSeq, Jan 2016]

- SDHA. This gene encodes a major catalytic subunit of succinate-ubiquinone oxidoreductase, a complex of the mitochondrial respiratory chain. The complex is composed of four nuclear-encoded subunits and is localized in the mitochondrial inner membrane. Mutations in this gene have been associated with a form of mitochondrial respiratory chain deficiency known as Leigh Syndrome. A pseudogene has been identified on chromosome 3q29. Alternatively spliced transcript variants encoding different isoforms have been found for this gene. [RefSeq, Jun 2014]

Potential Clinical Trials

Trial URL	Status	Title	Disease	Drug	Sites
https://classic.clinicaltrials.gov/show/NCT04037527	Recruiting	Gemcitabine and Docetaxel With Radiation in Adults With Soft Tissue Sarcoma of the Extremities	Soft Tissue Sarcoma	Gemcitabine Docetaxel Radiation Surgical Resection Blood draws	Wake Forest Baptist Comprehensive Cancer Center, Winston-Salem, North Carolina, United States
https://classic.clinicaltrials.gov/show/NCT04577014	Recruiting	Retifanlimab (Anti-PD-1 Antibody) With Gemcitabine and Docetaxel in Patients With Advanced Soft Tissue Sarcoma	Soft Tissue Sarcoma	Retifanlimab Gemcitabine Docetaxel	Memorial Sloan-Kettering Cancer Center, New York, New York, United States
https://classic.clinicaltrials.gov/show/NCT05301283	Recruiting	Habitat Escalated Adaptive Therapy (HEAT), With Neoadjuvant Radiation for Soft Tissue Sarcoma	Soft Tissue Sarcoma	Intensity Modulated Radiation Therapy (IMRT) MRI	Moffitt Cancer Center, Tampa, Florida, United States

https://classic.clinicaltrials.gov/show/NCT04784247	Recruiting	Lenvatinib and Pembrolizumab in People With Advanced Soft Tissue Sarcoma	Soft Tissue Sarcoma	Lenvatinib Pembrolizumab	Memorial Sloan Kettering Basking Ridge (Limited Protocol Activities), Basking Ridge, New Jersey, United States Memorial Sloan Kettering Monmouth (Limited Protocol Activities), Middletown, New Jersey, United States Memorial Sloan Kettering Bergen (Limited Protocol Activities), Montvale, New Jersey, United States
https://classic.clinicaltrials.gov/show/NCT05879185	Recruiting	A Study of XmAb23104 in People With Sarcoma	Soft Tissue Sarcoma	XmAb23104	Memorial Sloan Kettering at Basking Ridge (Limited Protocol Activities), Basking Ridge, New Jersey, United States Memorial Sloan Kettering Monmouth (Limited Protocol Activities), Middletown, New Jersey, United States Memorial Sloan Kettering Bergen (Limited Protocol Activities), Montvale, New Jersey, United States

Detailed Results

Single Nucleotide Variant (SNV) and Insertions-Deletions (INDELS)

Gene name	Hgvsp	Hgvsc	Aminoacids	Codons	Consequence	Allele frequency	Read depth	Predicted effect on protein
EPHA7	NP_004431.1:p.Ala520Gly	NM_004440.3:c.1559C>G	A/G	gCt/gGt	missense_variant	44.36	133	deleterious (0.03)
FBXW7	NP_361014.1:p.Glu529ThrfsTer8	NM_033632.3:c.1584_1600delAGAGACTGAAACCTGTC	PETETCL/PX	ccAGAGACTGAAACCTGTCta/ccta	frameshift_variant	40.68	118	0
RTEL1	NP_116575.3:p.Gln994SerfsTer2	NM_032957.4:c.2980delC	I/X	atC/at	frameshift_variant	22.81	228	0
FANCI	NP_001106849.1:p.Glu707Gly	NM_001113378.1:c.2120A>G	E/G	gAg/gGg	missense_variant	4.51	133	deleterious (0)
NOTCH4 (RNA)	NP_004548.3:p.Arg547Ter	NM_004557.3:c.1639C>T	R/*	Cga/Tga	stop_gained	41.67	132	0
SDHA (RNA)	NP_004159.2:p.Leu649GlufsTer4	NM_004168.2:c.1945_1946delTT	TL/TX	acTTtg/actg	frameshift_variant	10.03	788	0

Methodology and Test Background

This is a next generation sequencing (NGS) test that analyzes DNA for abnormalities in 434 genes that are reported to

be altered in various types of tumors. Nucleic acid is isolated from paraffin-embedded tissue. Testing is performed using massive parallel sequencing of the coding DNA of the listed genes. This includes sequencing of all the exons as well as 50 nucleotides at the 5' and 3' ends of each coding exon. Our sequencing method has a typical sensitivity of 3% for detecting common specific mutations and 5% for other mutations. MSI status is inferred by interrogating all available genomic microsatellites covered. Tumor mutational burden (TMB) is measured by counting all non-synonymous variants and filter settings as follows: (A) Pass all filters; (B) inside genes; (C) had a mutant allele frequency >5%; (D) not found in the dbSNP (to exclude germline variations). The median for TMB is 10 based on lung carcinoma analysis. The cut off for other types of tumors is not well established at this time. Performance of the assay may vary dependent on the quantity and quality of nucleic acid, sample preparation and sample age. The assay is designed to detect significant gene amplification and deletion in addition to various single nucleotide variations (SNV) and indels.

In addition to DNA analysis, targeted RNA NGS analysis is performed. This is a next generation sequencing (NGS) test that analyzes targeted RNA on 1,600 genes implicated in solid tumors. It is based on hybrid capture of targeted RNA. Duplicates are excluded for levels measurements. While the major focus of the analysis is the detection of fusion mRNA, mutations in the expressed RNA of the analyzed genes are also analyzed and reported. mRNA expression levels are evaluated, and only significant high expression of specific genes are relatively reported. CD274 (PD-L1) mRNA levels are reported when they are significantly elevated. All detect fusion transcripts are reported. This test specifically covers translocations that lead to the expression of fusion RNA. Translocations that lead to deregulation of expression can be addressed by this test if compared to the expression proper normal control. The sensitivity of this assay in detecting fusion mRNA is between 1% and 5%. This assay is not designed to detect minimal residual disease and should be used for diagnosis. For optimal results neoplastic cells should be >30% of the analyzed cells. The Universal Human Reference (UHR) RNA is used as control.

Based on our validation study, the following exonic regions of the genes listed below are not covered appropriately <100 X coverage and sequencing by NGS may not be reliable in these regions. This poor coverage is mainly due to high GC content with inherited problem in obtaining adequate coverage. ASXL1 NM_001164603 20:30946620-30946635, ATM NM_000051 11:108186550-108186638, BAP1 NM_004656 3:52443858-52443894, BCR NM_004327 22:23652510-23652620, BRD4 NM_058243 19:15353808-15354193,5355041-15355411, CCNE1 NM_001238 19:30303463-30303485, CD274 NM_001267706 9:5456109-5456165, CD79A NM_001783 19:42384736-42384805, CSF3R NM_000760 1:36937667-36937740, DDX11 NM_001257144 12:31240872-31240917, ERBB3 NM_001982 12:56492284-56492359, FANCI NM_001113378 15:89835919-89836052, FLT3 NM_004119 13:28674605-28674652, FLT4 NM_002020 5:180035281-180035284, GEN1 NM_001130009 2:17954486-17954525, H3-3A NM_002107 1:226259140-226259180, IRS2 NM_003749 13:110437126-110437363, 110437805-110437899, 110438359-110438400, JAK1 NM_002227 1:65309747-65309771, MAGI2 NM_012301 7:77648719-77649044, MITF NM_000248 3:70005606-70005681, MYCL NM_001033081 1:40367518-40367565, NF1 NM_000267 17:29664837-29664898, NOTCH2 NM_001200001 1:120572528-120572610, PBRM1 NM_018313 3:52677264-52677322, PIK3R2 NM_005027 19:18272089-18272305, PMS2 NM_000535 7:6013024-6013173, RANBP2 NM_006267 2:109363166-109363254, 109367779-109367838, 109367984-109368069, 109369453-109369497, 109378578-109378651, RHEB NM_005614 7:151216546-151216597, SUFU NM_001178133 10:104263911-104264039, TNFRSF14 NM_003820 1:2494304-2494335

The table below contains a partial list of the tested DNA genes. For a complete list, please go to:
https://genomictestingcooperative.com/genomic-tests/solid-tumor-profile-plus/ *(click the DNA tab)*

The table below contains a partial list of the tested RNA genes (Fusions/Expression). For a complete list, please go to: https://genomictestingcooperative.com/genomic-tests/solid-tumor-profile-plus/ *(click the RNA tab)*

Tested genes

Genes Tested for Abnormalities in Coding Sequence																
ABCB7	AURKB	C15ORF41	CEBPA	DNMT3A	FANCC	FLT3	GRIN2A	IRF2	LMO1	MSH6	NTRK2	POT1	RARA	SF3B1	STAT6	TSHR
ABL1	AURKC	CALR	CHD2	DOT1L	FANCD2	FLT4	GRM3	IRF4	LPIN2	MTOR	NTRK3	PPM1D	RB1	SLIT2	STK11	U2AF1
ABL2	AXIN1	CARD11	CHD4	EED	FANCE	FOXL2	GSK3B	IRS2	LRP1B	MUTYH	NUP93	PPP2R1A	RBBP6	SLX4	SUFU	U2AF2
ACD	AXIN2	CBFB	CHEK1	EGFR	FANCF	FOXP1	GSKIP	JAGN1	LYN	MVK	PAK3	PRDM1	RBM10	SMAD2	SUZ12	VEGFA
ACVR1B	AXL	CBL	CHEK2	EGLN1	FANCG	FRS2	H3F3A	JAK1	LYST	MYC	PALB2	PREX2	RBM8A	SMAD3	SYK	VHL
ADA	B2M	CBLB	CIC	ELANE	FANCI	FUBP1	HAX1	JAK2	LZTR1	MYCL	PARK2	PRKAR1A	RET	SMAD4	TAF1	WAS
AK2	BAP1	CBLC	CREBBP	EP300	FANCL	G6PC3	HGF	JAK3	MAGI2	MYCN	PAX5	PRKCI	RHEB	SMAD9	TAL1	WHSC1
AKT1	BARD1	CCND1	CRKL	EPAS1	FANCM	GABRA6	HIST1H3B	JUN	MAP2K1	MYD88	PBRM1	PRKDC	RHOA	SMAD9L	TBX3	WISP3
AKT2	BCL2	CCND2	CRLF2	EPCAM	FAS	GALNT12	HNF1A	KAT6A	MAP2K2	NBN	PDCD1LG2	PRSS1	RICTOR	SMARCA4	TCF3	WT1
AKT3	BCL2L1	CCND3	CSF1R	EPHA3	FAT1	GATA1	HOXA11	KDM5A	MAP2K4	NF1	PDGFRA	PRSS8	RIT1	SMARCB1	TCIRG1	XPO1
ALK	BCL2L2	CCNE1	CSF3R	EPHA5	FBXW7	GATA2	HOXB13	KDM5C	MAP3K1	NF2	PDGFRB	PSTPIP1	RNF168	SMC1A	TERC	XRCC2
AMER1	BCL6	CD274	CTC1	EPHA7	FGF10	GATA3	HRAS	KDM6A	MAP3K14	NFE2L2	PDK1	PTCH1	RNF43	SMC3	TERF1	XRCC3
ANKRD26	BCOR	CD79A	CTCF	EPHB1	FGF14	GATA4	HSD3B1	KDR	MAPK1	NFKBIA	PHF6	PTEN	ROS1	SMO	TERF2	ZBTB2
APC	BCORL1	CD79B	CTNNA1	ERBB2	FGF19	GATA6	HSP90AA1	KEAP1	MCL1	NHP2	PIK3C2B	PTPN11	RPTOR	SNCAIP	TERF2IP	ZNF217
AR	BCR	CDAN1	CTNNB1	ERBB3	FGF23	GEN1	ID3	KEL	MDM2	NKX2-1	PIK3CA	QKI	RTEL1	SOCS1	TERT	ZNF703
ARAF	BIRC3	CDC73	CUL3	ERBB4	FGF3	GFI1	IDH1	KIF23	MDM4	NLRP3	PIK3CB	RAB27A	RUNX1	SOX10	TET2	ZRSR2
ARFRP1	BLM	CDH1	CUX1	ERCC4	FGF4	GFI1B	IDH2	KIT	MED12	NME1	PIK3CG	RAC1	RUNX1T1	SOX2	TGFBR2	-
ARID1A	BMPR1A	CDK12	CXCR4	ERG	FGF6	GID4	IGF1R	KLF1	MEF2B	NOP10	PIK3R1	RAD21	SBDS	SOX9	TNFAIP3	-
ARID1B	BRAF	CDK4	CYLD	ERRFI1	FGFR1	GLI1	IGF2	KLHL6	MEFV	NOTCH1	PIK3R2	RAD50	SBF2	SPEN	TNFRSF14	-
ARID2	BRCA1	CDK6	DAXX	ESR1	FGFR2	GLI2	IKBKE	KLLN	MEN1	NOTCH2	PIM1	RAD51	SDHA	SPOP	TNFRSF1A	-
ASXL1	BRCA2	CDK8	DDR2	ETV6	FGFR3	GNA11	IKZF1	KMT2A	MET	NOTCH3	PLCG1	RAD51B	SDHB	SPTA1	TOP1	-
ATG2B	BRD4	CDKN1A	DDX11	EXO1	FGFR4	GNA13	IKZF3	KMT2B	MITF	NPM1	PLCG2	RAD51C	SDHC	SRC	TOP2A	-
ATM	BRIP1	CDKN1B	DDX41	EZH2	FH	GNAQ	IL2RG	KMT2C	MLH1	NRAS	PMS1	RAD51D	SDHD	SRSF2	TP53	-
ATR	BTG1	CDKN2A	DICER1	FAM175A	FLCN	GNAS	IL7R	KMT2D	MPL	NROB1	PMS2	RAD54L	SEC23B	STAG2	TRAF3	-
ATRX	BTK	CDKN2B	DKC1	FAM46C	FLI1	GPR124	INHBA	KRAS	MRE11A	NSD1	POLD1	RAF1	SETBP1	STAT3	TSC1	-
AURKA	C11orf40	CDKN2C	DNM2	FANCA	FLT1	GREM1	INPP4B	LIG4	MSH2	NTRK1	POLE	RANBP2	SETD2	STAT4	TSC2	-

RNA Fusions/Expression

Fusion/Expression													
ABL1	BCL2	CBFB	ERG	FGFR2	FOXO1	IKZF3	MAP3K1	NTRK1	NUP98	PICALM	RHOA	SS18	TCF3
AKT3	BCL6	CIC	ETV6	FGFR3	FUS	JAK2	MECOM	NTRK2	PDGFRA	PML	ROS2	STAT6	TFG
ALK	BRAF	CREBBP	EWSR1	FIP1L1	GLI1	KIAA1549	MYC	NTRK3	PDGFRB	RARA	RUNX1	TAFG	YWHAE
BCL1	CAMTA1	EGFR	FGFR1	FLAG1	HMGA2	KMT2A	NOTCH1	NUP214	PD-L1	RET	RUNX1T1	TAL1	

Reference

6. Filion C, Motoi T, Olshen AB, Laé M, Emnett RJ, Gutmann DH, Perry A, Ladanyi M, Labelle Y. The EWSR1/NR4A3 fusion protein of extraskeletal myxoid chondrosarcoma activates the PPARG nuclear receptor gene. J Pathol. 2009 Jan;217(1):83-93. doi: 10.1002/path.2445. PMID: 18855877; PMCID: PMC4429309.

Electronic Signature

Ahmad Charifa, M.D.

The test (sample processing, sequencing and data generation) was performed at Genomic Testing Cooperative, LCA, Genomic Testing Cooperative, LCA, 175 Technology Drive, Suite 100, Irvine, CA 92618. Medical Director Maher Albitar, M.D. Analysis of the data was performed by Genomic Testing Cooperative, LCA, 175 Technology Drive, Suite 100, Irvine, CA 92618. Medical Director: Maher Albitar, M.D.

The test was developed and its performance characteristics have been determined by Genomic Testing Cooperative, LCA. This test has not been approved by the FDA. The FDA has determined such clearance or approval is not necessary. This laboratory is CLIA certified to perform high complexity clinical testing.