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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:	THIGH		

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

Detected Genomic Alterations				
Level 1	Level 2 (Standard of Care)	Level 3 (Clinical Evidence)	Level 4 (Biological Evidence)	Other
t(12;16)(q13;p11) FUS-DDIT3 mRNA fusion	-Tumor Mutation Burden Low: 2 Mut/Mb -No evidence of microsatellite instability	Homologous recombination deficiency (HRD): Negative	LYN	Chromosomal structural analysis shows 12p+, 16p+ and +20

Results Summary

- **-t(12;16)(q13;p11) FUS-DDIT3 mRNA fusion**
  -Mutation in LYN gene
  -PD-L1 and MDM2 mRNA: low
  -No evidence of microsatellite instability
  -Tumor Mutation Burden Low: 2 Mut/Mb
  -Chromosomal structural analysis shows 12p+, 16p+ and +20
  -Homologous recombination deficiency (HRD): Negative
  -No evidence of MDM2 amplification
  -No evidence of fusion mRNA involving ALK, RET, ROS1, or NTRK
  -EBV viral RNA: Not detected
  -HPV viral RNA: Not detected
  -TTV viral RNA: Not detected
  -HLA Genotyping:
  -HLA-A: A*24:02-A*02:06
  -HLA-B: B*59:01-B*40:02
  -HLA-C: C*01:02-C*14:02

  -The FUS-DDIT3 fusion is consistent with myxoid liposarcoma.

Tumor Heterogeneity
There is an abnormal low-level clone with LYN mutation.

Expression	
PD-L1 mRNA: low	MDM2 mRNA: low

Diagnostic Implications	
FUS-DDIT3 fusion	This finding is consistent with myxoid liposarcoma

Levels 2, 3 & 4 (Standard of Care and Clinical/Biological Evidence)	
LYN	Src family kinase inhibitors

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) to identify molecular abnormalities, including single nucleotide variants (SNVs), insertions/deletions (indels), copy number variants (CNVs), fusions, tumor mutational burden (TMB), microsatellite instability (MSI), homologous recombination deficiency (HRD), B- and T-cell clonality, and viruses (HPV, EBV, and TTV), in DNA of 434 genes and RNA in greater than 1600 genes implicated in solid tumors. Whenever possible, clinical relevance and implications of detected abnormalities are described below.

Biological relevance of detected Alterations

- LYN. This gene encodes a tyrosine protein kinase, which maybe involved in the regulation of mast cell degranulation, and erythroid differentiation. Alternatively spliced transcript variants encoding different isoforms have been found for this gene. [RefSeq, Jul 2011]

Drug Information

Imatinib

Imatinib is indicated treatment of Philadelphia chromosome positive chronic myeloid leukemia (Ph+ CML), Ph+ acute lymphoblastic leukaemia, myelodysplastic/myeloproliferative diseases, aggressive systemic mastocytosis, hypereosinophilic syndrome and/or chronic eosinophilic leukemia (CEL), dermatofibrosarcoma protuberans, and malignant gastrointestinal stromal tumors (GIST). It inhibits proliferation and induces apoptosis in Bcr-Abl positive cell lines as well as fresh leukemic cells from Philadelphia chromosome positive chronic myeloid leukemia. Imatinib also inhibits the receptor tyrosine kinases for platelet derived growth factor (PDGF) and stem cell factor (SCF) - called c-kit.

Dasatinib

Dasatinib inhibits the following kinases: BCR-ABL, SRC family (SRC, LCK, YES, FYN), c-KIT, EPHA2, and PDGFRß.

Based on modeling studies, dasatinib is predicted to bind to multiple conformations of the ABL kinase. In vitro, dasatinib was active in leukemic cell

lines representing variants of imatinib mesylate sensitive and resistant disease. Dasatinib inhibited the growth of chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL) cell lines overexpressing BCR-ABL. Under the conditions of the assays, dasatinib was able to overcome imatinib resistance resulting from BCR-ABL kinase domain mutations, activation of alternate signaling pathways involving the SRC family kinases (LYN, HCK), and multi-drug resistance gene overexpression.

Nilotinib

Nilotinib is a transduction inhibitor that targets BCR-ABL, c-kit and PDGF, for the potential treatment of various leukemias, including chronic myeloid leukemia (CML).

Bosutinib

Bosutinib is a tyrosine kinase inhibitor. Although it is able to inhibit several tyrosine kinases such as Src, Lyn, and Hck, which are members of the Src-family of kinases, its primary target is the Bcr-Abl kinase.

The Bcr-Abl gene is a chimeric oncogene created from the fusion of the breakpoint-cluster (Bcr) gene and Abelson (Abl) tyrosine gene. This chromosomal abnormality results in the formation of what is commonly known as the Philadelphia chromosome or Philadelphia translocation. The Bcr-Abl gene expresses a particular kinase that promotes the progression of CML. A decrease in the growth and size of the CML tumour has been observed following administration of bosutinib. Bosutinib did not inhibit the T315I and V299L mutant cells.

Potential Clinical Trials

Trial URL	Status	Title	Disease	Drug	Sites
https://classic.clinicaltrials.gov/show/NCT05580588	Recruiting	Open-Label Study of the CDK4/6 Inhibitor SPH4336 in Subjects With Locally Advanced or Metastatic Liposarcomas	Liposarcoma	SPH4336	Mayo Clinic Hospital, Phoenix, Arizona, United States City of Hope, Duarte, California, United States University of Miami, Miami, Florida, United States
https://classic.clinicaltrials.gov/show/NCT04438824	Recruiting	Palbociclib and INCMGA00012 in People With Advanced Liposarcoma	Liposarcoma	INCMGA00012 Palbociclib	Memorial Sloan Kettering at Basking Ridge, New Jersey, United States Memorial Sloan Kettering Bergen, Montvale, New Jersey, United States Memorial Sloan Kettering Westchester, Harrison, New York, United States
https://classic.clinicaltrials.gov/show/NCT04044768	Recruiting	Spearhead 1 Study in Subjects With Advanced Synovial Sarcoma or Myxoid/Round Cell Liposarcoma	Myxoid Liposarcoma	afamitresgene autoleucel (previously ADP-A2M4)	City of Hope, Duarte, California, United States Stanford Cancer Center, Palo Alto, California, United States University of Colorado, Aurora, Colorado, United States

https://classic.clinicaltrials.gov/show/NCT03425279	Recruiting	CAB-AXL-ADC Safety and Efficacy Study in Adult and Adolescent Patients With Sarcoma	Liposarcoma	CAB-AXL-ADC PD-1 inhibitor	The University of Arizona Cancer Center, Tucson, Arizona, United States Children's Hospital Los Angeles, Los Angeles, California, United States USC Norris Comprehensive Cancer Center, Los Angeles, California, 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
LYN	NP_002341.1:p.Val465Leu	NM_002350.3:c.1393G>C	V/L	Gtg/Ctg	missense_variant	8.04	174	deleterious (0.02)

Methodology and Test Background

This is a next generation sequencing (NGS) test that analyzes DNA for abnormalities in 434 genes and RNA of >1600 genes that are reported to be altered in various types of solid tumors. The assay also detects several viruses that are important in oncology, including EBV, HPV and TTV. TTV (torque teno virus) was first discovered in a patient with non-A-E hepatitis and is now regarded as a part of the human virome. In general, TTV does not cause pathology in immunocompetent individuals. TTV is considered as a marker of immune competence in patients with immunological impairment and inflammatory disorders. High TTV load is associated with increased risk of infection. In patients with organ transplant, low TTV load is associated with an increased risk of rejection.

Nucleic acid is isolated from paraffin-embedded tissue. For optimal results neoplastic cells should be greater than 30% of the analyzed cells. H&E-sections are reviewed by a pathologist and tumor-enrichment is performed by macrodissection when possible. 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 approximately 50 nucleotides at the 5' and 3' ends of each coding exon to detect splice site abnormalities. The TERT promoter region, including the hotspots at -124 and -146 bp, is also covered. Our DNA sequencing method has a sensitivity of 3% for detecting hotspot mutations and 5% for detecting single nucleotide variants (SNVs) and small (<60 bp) insertions/ deletions (indels). MSI status is inferred by interrogating all available genomic microsatellites covered. Tumor mutational burden (TMB) is measured by counting all nonsynonymous 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 mutations/Mb based on lung carcinoma analysis. The cut off for other types of tumors is not well-established at this time. Significant gene amplification and deletion (copy number variants) are also reported. Targeted RNA NGS is performed by hybrid capture and duplicates are excluded for levels measurements. The Universal Human Reference (UHR) RNA is used as control. All detected fusion transcripts are reported. While the major focus of the RNA analysis is the detection of fusion mRNA, mutations in the expressed RNA of the analyzed genes, HLA class I genotyping, and Epstein-Barr virus (EBV), human papillomavirus (HPV) and torque teno virus (TTV) viral RNA are also analyzed and reported. B- and T-cell clonality will be reported, if clonal or clinically relevant. The sensitivity of this assay in detecting fusion mRNA is between 5% and 10%. 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. Since the clinical relevance of the RNA expression level of most of the genes is not characterized at this time, only a few specific genes will be commented on when abnormalities are detected. CD274 (PD-L1) mRNA levels are reported when they are significantly elevated. This assay is not designed to detect minimal residual disease and should be used for diagnosis. Performance of the assay may vary dependent on the quantity and

quality of nucleic acid, sample preparation and sample age. Decalcified specimens have not been validated. Decalcification with strong acids is not recommended and may lead to poor nucleic acid quality and suboptimal results.

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. Since the clinical relevance of the RNA expression level of most of the genes is not characterized at this time, only a few specific genes will be commented on when abnormalities are detected. CD274 (PD-L1) mRNA levels are reported when they are significantly elevated.

Based on our validation study, the following exonic regions of the genes listed below are not covered appropriately <100X coverage and sequencing by NGS may not be reliable in these regions. This poor coverage is mainly due to high GC content and inherent 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

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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.