ANTHOLOGY DIAGNOSTICS

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CLIA – 31D0124320 | CAP – 1215701

Anthology Diagnostics
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Hematology Fusion/Expression Profile

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

Ethnicity:				Family History:	
MRN:				Indication for Testing:	
Collected Date:		Time :		Reason for Referral:	Malignant Neoplasm of Lung
Received Date:		Time :		Tumor Type:	Lung
Reported Date:		Time :		Stage:	T2B

Test Description:

This is a next generation sequencing (NGS) test to identify mutations, levels of RNA and fusion RNA in 68 genes implicated in hematologic neoplasms, including leukemia, lymphoma and myeloma. Whenever possible, clinical relevance and implications of detected abnormalities are described.

Detected Genomic Alterations				
JAK2	FIP1L1	CHD2	KDM6A	
PAX5-ZCCHC7, t(9;9)(p13.2;p13.2)				

Heterogeneity
CD2 and FI1L1 mutations are detected in a small subclone

Expression	
CD19	High
CD22	High
CD79A	High
CD79B	High
CRLF2	High
PAX5	High
CD274	Low

Diagnostic Implications	
Acute Leukemia	The Findings are consistent with Ph-Like acute lymphoblastic leukemia
MDS	N/A
Lymphoma	N/A
Myeloma	N/A
Other	N/A

The professional and technical components of this assay were performed at Genomic Testing Cooperative, LCA, 27 Technology Drive, Suite 100, Irvine, CA 92618 (CLIA

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Therapeutic Implications	
JAK2	Momelotinib and ruxolitinib

Prognostic Implications	
JAK2	Poor
CRLF2	Poor
Overall	Poor

Relevant Genes with No Alteration
None

Results Summary

- Fusion (PAX5-ZCCHC7) transcript involving PAX5 and ZCCHC7 genes resulting from t(9;9)(p13.2;p13.2) translocation is detected. PAX5 gene is frequently translocated in ALL, but the partner gene (SEC31A) is unusual and rare. However, this fusion has been reported in large B-cell lymphoma and non-small cell lung cancer.

- The expression profile shows high levels of CD19, CD22, CD79A, CD79B and significantly high levels of CRLF2, consistent with B-cell neoplasm.

- Mutations in JAK2, CHD2, FIP1L1, and KDM6A are detected.

- THESE FINDINGS ARE CONSISTENT WITH Ph-LIKE ACUTE LYMPHOBLASTIC LEUKEMIA.

Biological Relevance of Detected Alterations

- JAK2 (janus kinase 2) is a gene encodes for a tyrosine kinase directly involved in activating the STAT pathway. It is mutated or translocated in acute lymphoblastic leukemia, myeloproliferative disease. JAK2/STAT inhibitors are currently available in clinical trials for the treatment of certain types of cancer.
- PAX5 (paired box 5/B-cell lineage specific activator) gene encodes a member of the paired box (PAX) family of transcription factors. The central feature of this gene family is a novel, highly conserved DNA-binding motif, known as the paired box. Paired box transcription factors are important regulators in early development, and alterations in the expression of their genes are thought to contribute to neoplastic transformation. This gene encodes the B-cell lineage specific activator protein that is expressed at early, but not late stages of B-cell differentiation. Its expression has also been detected in developing CNS and testis and so the encoded protein may also play a role in neural development and spermatogenesis. This gene is located at 9p13, which is involved in t(9;14)(p13;q32) translocations recurring in small lymphocytic lymphomas of the plasmacytoid subtype, and in derived large-cell

lymphomas. Mutations in this gene have been reported lymphomas and melanoma and rarely in other types of tumors.

- KDM6A: (LYSINE (K)-SPECIFIC DEMETHYLASE 6A) gene is located on the X chromosome and encodes a tetratricopeptide repeat (tpr) protein. This protein catalyzes the demethylation of tri/dimethylated histone h3 and involved in chromatine remodeling. This gene belong to a family of genes that were found to be mutated in both solid and liquid tumors, including aml, chronic myelogenous leukemia (cml), t-all, mm, hodgkin's lymphoma (hl), tcc, breast, prostate, colon, esophageal, pancreas, endometrial, gbm, small cell lung cancer (sclc), non-small cell lung cancer (nsclc), and rcc. KDM6A mutations in bladder carcinoma are quite common (20%–29%). Patients with activating mutation in kdm6a might be good candidate for therapy with kdm inhibitors..
- CHD2 (chromodomain helicase DNA binding protein 2) gene encodes a protein involved in altering expression by modification of chromatin structure. Currently there is no targeting therapy for this abnormality.
- FIP1L1 (Factor Interacting With PAPOLA And CPSF1) gene is located on 4q12 and encodes a subunit of the CPSF (cleavage and polyadenylation specificity factor) complex that polyadenylates the 3' end of mRNA precursors. This gene, the homolog of yeast Fip1 (factor interacting with PAP), binds to U-rich sequences of pre-mRNA and stimulates poly(A) polymerase activity. Its N-terminus contains a PAP-binding site and its C-terminus an RNA-binding domain. An interstitial chromosomal deletion on 4q12 creates an in-frame fusion of human genes FIP1L1 and PDGFRA (platelet-derived growth factor receptor, alpha). The FIP1L1-PDGFRA fusion gene encodes a constitutively activated tyrosine kinase that joins the first 233 amino acids of FIP1L1 to the last 523 amino acids of PDGFRA. This gene fusion and chromosomal deletion is the cause of some forms of idiopathic hypereosinophilic syndrome (HES), also called chronic eosinophilic leukemia (CEL), is responsive to treatment with tyrosine kinase inhibitors. Mutations in this genes are rarely reported.

Drug Information

Ruxolitinib

Ruxolitinib phosphate/ The phosphate salt form of ruxolitinib, an orally bioavailable Janus-associated kinase (JAK) inhibitor with potential antineoplastic and immunomodulating activities. Ruxolitinib specifically binds to and inhibits protein tyrosine kinases JAK 1 and 2, which may lead to a reduction in inflammation and an inhibition of cellular proliferation. The JAK-STAT (signal transducer and activator of transcription) pathway plays a key role in the signaling of many cytokines and growth factors and is involved in cellular proliferation, growth, hematopoiesis, and the immune response; JAK kinases may be upregulated in inflammatory diseases, myeloproliferative disorders, and various malignancies.

Momelotinib

Momelotinib is an orally bioavailable small-molecule inhibitor of Janus kinases 1 and 2 (JAK1/2) with potential antineoplastic activity. JAK1/2 inhibitor CYT387 competes with JAK1/2 for ATP binding, which may result in inhibition of JAK1/2 activation, inhibition of the JAK-STAT signaling pathway, and so the induction of apoptosis and a reduction of tumor cell proliferation in JAK1/2-expressing tumor cells. JAK2 is the most common mutated gene in bcr-abl-negative myeloproliferative disorders; the JAK2V617F gain-of-function mutation involves a valine-to-

phenylalanine modification at position 617. The JAK-STAT signaling pathway is a major mediator of cytokine activity and is often dysregulated in a variety of tumor cell types.

Potential Clinical Trials

Title	Conditions	Interventions	Locations	URL
A Phase 2 Study of Ruxolitinib With Chemotherapy in Children With Acute Lymphoblastic Leukemia	ALL	Drug: Ruxolitinib Drug: Asparaginase Erwinia Chrysanthemi Drug: Cyclophosphamide (and 10 more...)	Children's Hospital of Alabama Birmingham, Alabama, United States Phoenix Childrens Hospital Phoenix, Arizona, United States Arkansas Children's Hospital Little Rock, Arkansas, United States (and 111 more...)	https://clinicaltrials.gov/ct2/results?cond=Acute+Lymphoid+Leukemia&term=JAK2&cntry=US&state=&city=&dist=

Detailed Results

Single Nucleotide Variant (SNV)

Gene_0	HGVSc_43	HGVSp_44	Alt Variant Freq_13
FIP1L1	NM_030917.3:c.1701dupA	NP_112179.2:p.Ser568IlefsTer2	10.43
JAK2	NM_004972.3:c.2624C>A	***NP_004963.1:p.Thr875Asn (somatic, 0.95)***	35.71
CHD2	NM_001271.3:c.522dupA	NP_001262.3:p.Val175SerfsTer18	14.96
KDM6A	NM_021140.2:c.1834C>T	NP_066963.2:p.Arg612Ter	32.26

Fusion	
PAX5-ZCCHC7, t(9;9)(p13.2;p13.2)	51%

Methodology and Test Background

This is a next generation sequencing (NGS) test that analyzes targeted RNA with a focus on 68 genes. 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, mainly to distinguish B-cell neoplasms from myeloid. CRLF2 mRNA levels are reported in acute lymphoblastic leukemia. CD274 (PD-L1) mRNA levels are reported when they are significantly elevated. If requested, detailed expression levels will be provided as a research data and not for clinical use. 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. Since the clinical relevance of the expression level of most of these genes is not characterized at this time, only few specific genes (MYC, BCL2, CD274, CD19, CD22, CD79A, CD79B) will be commented on. 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 when neoplastic cells are >10% of the analyzed cells. The Universal Human Reference (UHR) RNA is used as control.

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Tested genes

Genes Tested for Abnormalities in Coding Sequen												
ABL1	BCL2	CBL	CDKN2C	DICER1	FAS	IDH2	KMT2A	MPL	PAX5	PTCH1	SMAD2	TGFBR2
AKT1	BCL2L1	CBLB	CEBPA	DNMT3A	FBXW7	IGF1R	KMT2B	MRE11A	PBRM1	PTEN	SMAD4	TP53
AKT2	BCL6	CBLC	CHEK1	EP300	FLT3	IKZF1	KMT2C	MTOR	PDGFRA	PTPN11	SMARCA4	TSC1
AKT3	BCOR	CCND1	CHEK2	ERG	GATA1	IKZF3	KMT2D	MUTYH	PDGFRB	RAD21	SMARCB1	TSC2
ALK	BCORL1	CCND3	CIC	ETV6	GATA2	IRF4	KRAS	MYC	PHF6	RAD50	SMC1A	TSHR
AMER1	BCR	CD274	CREBBP	EZH2	GATA3	JAK1	MAP2K1	MYD88	PIK3CA	RAD51	SMO	WT1
APC	BIRC3	CD79A	CRLF2	FAM175A	GEN1	JAK2	MAP2K2	NFKBIA	PIK3R1	RB1	SOCS1	ZNF217
ARID1A	BLM	CD79B	CSF1R	FAM46C	GNAQ	JAK3	MAP2K4	NOTCH1	PIK3R2	RHOA	SRC	ZRSR2
ARID1B	BRAF	CDH1	CSF3R	FANCA	GNAS	KAT6A	MAP3K1	NOTCH2	PIM1	RNF43	SRSF2	MEF2B
ARID2	BRCA1	CDK12	CTNNA1	FANCC	H3F3A	KDM5C	MAP3K14	NOTCH3	PLCG1	RUNX1	STAG2	
ASXL1	BRCA2	CDK4	CTNNB1	FANCD2	HNF1A	KDM6A	MAPK1	NPM1	POLD1	SDHB	STAT3	
ATM	BTK	CDK6	CUX1	FANCE	HOXB13	KDR	MCL1	NRAS	POLE	SETBP1	STK11	
ATRX	CALR	CDKN2A	CXCR4	FANCF	HSP90AA1	KEAP1	MDM2	NSD1	PPM1D	SETD2	TERT	
B2M	CARD11	CDKN2B	DDR2	FANCG	IDH1	KIT	MDM4	PALB2	PPP2R1A	SF3B1	TET2	

Add-on RNA Fusions/Expression

Fusion/Expression																
ABL1	ALK	BRAF	CREBBP	EPOR	ETV5	FGFR2	FOXO1	*JAK2*	MAP3K1	NOTCH1	NUP214	PCM1	PICALM	RET	RUNX1T1	TCF3
ABL2	BCL1	CBFB	*CRLF2*	ERG	ETV6	FGFR3	FUS	KMT2A	MECOM	NTRK1	NUP98	PDGFRA	PML	RHOA	SS18	TCF3
AKT3	BCL2	*CBL*	*CSF1R*	ETV1	EWSR1	FIP1L1	GLI1	KRT18P6	MYC	NTRK2	P2RY8	PDGFRB	*PTK2B*	ROS2	STAT6	TFG
ALK	BCL6	CIC	EGFR	ETV4	*FGFR1*	*FLT3*	IKZF3	*LYN*	*MYH9*	*NTRK3*	PBX1	PD-L1	RARA	RUNX1	TAL1	*TYK2*

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Electronic Signature

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The Technical Component Processing, Analysis and Professional Component of this test was completed at GTC Laboratories, 21 Technology Dr. #100, Irvine, CA / 92618/ Medical Director: Maher Albitar, M.D. .

The performance characteristics of this test have been determined by GTC Laboratories. 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.