

## Hematology Profile

Patient Name:		Ordering Physician:	
Date of Birth:		Accession #:	
Gender (M/F):	M	Specimen Type:	Bone Marrow
Client:		Specimen ID:	
Case #:			
Body Site:	NOT SPECIFIED		

Collected Date:	06/12/2020	Time:	12:00 AM
Received Date:	06/16/2020	Time:	12:04 PM
Reported Date:	06/22/2020	Time:	05:03 PM

### 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 in 177 genes implicated in hematologic neoplasms, including leukemia, lymphoma and MDS. Whenever possible, clinical relevance and implications of detected abnormalities are described below.

Detected Genomic Alterations				
TP53	NTRK2	GRIN2A	TERT	Chromosomal structural abnormalities including: 5q-, 11p-, 11q+, 16q-, 17p-, +18 and others.

Heterogeneity
There is a dominant abnormal clone with TP53 mutation. The NTRK2, GRIN2A, and TERT mutations are detected in subclones.

Diagnostic Implications	
TP53, NTRK2, GRIN2A, TERT	These abnormalities are consistent with myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML)

Therapeutic Implications	
TP53	Aurora kinase A inhibitors, Wee1 inhibitors, Chk1 inhibitors, kevetrin, APR-246, nutlins, gene therapy
NTRK2	TRK inhibitors (Larotrectinib, Entrectinib)

Prognostic Implications	
TP53	Poor
NTRK2	Poor
GRIN2A	Poor
TERT	Poor

Relevant Genes with NO Alteration
No evidence of mutation in: FLT3, NPM1, IDH1 and IDH2

## Results Summary

- Mutations in TP53, NTRK2, GRIN2A, and TERT genes**  
**-Chromosomal structural abnormalities including: 5q-, 11p-, 11q+, 16q-, 17p-, +18 and others.**  
  
**-These findings are consistent with highly aggressive MDS/AML with complex cytogenetics and TP53 mutation.**

## Biological relevance of detected Alterations

- TP53 mutations are universal across cancer types. The loss of a tumor suppressor is most often through large deleterious events, such as frameshift mutations, or premature stop codons. In TP53 however, many of the observed mutations in cancer are found to be single nucleotide missense variants. These variants are broadly distributed throughout the gene, but with the majority localizing in the DNA binding domain. There is no single hotspot in the DNA binding domain, but a majority of mutations occur in amino acid positions 175, 245, 248, 273, and 282 (NM\_000546) (Olivier et al., 2010). To fulfill its proper biological function four TP53 polypeptides must form a tetramer which functions as a transcription factor, therefore even if one out of four polypeptides has inactivating mutation it may lead to dominant negative phenotype of variable degree. While a large proportion of cancer genomics research is focused on somatic variants, TP53 is also of note in the germline. Germline TP53 mutations are the hallmark of Li-Fraumeni syndrome, and many (both germline and somatic) variants have been found to have a prognostic impact on patient outcomes. The significance of many polymorphisms for susceptibility and prognosis of disease is still very much up for debate.

TP53, a tumor suppressor in the DNA damage pathway, is the most frequently mutated gene in cancer.

TP53 encodes the p53 tumor suppressor protein, a transcription factor that responds to cellular stresses, including DNA damage and oncogenic activation, by inducing downstream anti-tumor responses such as DNA repair and apoptosis (PMID: 11099028). TP53 is the most commonly mutated gene in human cancers, and germline mutations occur in the cancer predisposition syndrome Li-Fraumeni (PMID: 22713868, 21765642). The p53 protein consists of an N-terminal transactivation domain, a central DNA-binding domain, an oligomerization domain and a C-terminal regulatory domain (PMID: 22713868).

- NTRK2, a receptor tyrosine kinase, is altered by mutation or chromosomal rearrangement in a diverse range of cancers.  
  
 The NTRK2 gene (neurotrophic receptor tyrosine kinase 2) encodes a transmembrane neurotrophic receptor involved in signaling that is important for normal neurologic development (PMID: 8402890, 8145823). NTRK2 consists of an extracellular ligand-binding domain, a transmembrane domain and an intracellular region harboring the tyrosine kinase domain. Normal activation in neural cells occurs upon binding one of its three ligands, the nerve growth factor (NGF), the brain-derived neurotrophic factor (BDNF) or neurotrophin-3 (NT-3), leading to autophosphorylation and activation of downstream signaling pathways controlling and promoting cell proliferation, survival and differentiation via MAPK, PI3K and PLC- (PMID: 1649702, 1649703, 10851172). NTRK2 alterations, especially fusions, are found in several human cancers, such as lung cancer, pilocytic astrocytoma, and neuroblastoma (PMID: 25204415, 21242122, 23817572, 8264643, 9049830).
- GRIN2A, a subunit of the NMDA glutamate receptor, is recurrently altered by mutation in various cancer types, most frequently in melanoma.  
 GRIN2A, also known as GluN2A or NR2A, is a regulatory subunit of the glutamate-gated N-methyl-d-aspartate receptor (NMDAR), which plays an important role in cell death, survival, and migration in cancer cells (PMID: 23540692). NMDARs are best known for their roles in the brain, and high expression is found in neurons in the brain and the spinal cord where they play an important role in controlling cation flow through the receptor (PMID: 7512349). Normal NMDAR activity can promote cell survival in neurons through the PI3K and ERK signaling pathways (PMID: 11902114). Studies have shown a high prevalence of somatic mutations in GRIN2A in malignant melanoma (PMID: 21499247, 22197930), although the mechanism or function of these mutations is still unknown.

- TERT is an enzyme that functions to maintain telomere length and genomic stability. The TERT promoter is frequently mutated in various cancer types.

The TERT gene encodes the catalytic subunit of telomerase, an enzyme that maintains telomere length and genomic integrity. TERT expression is low or absent in somatic cells; however, telomerase activity is upregulated in a vast majority of tumors and likely contributes to cancer cell immortality (PMID: 24657534, 9282118). Sequencing of the TERT promoter identified activating mutations in a number of cancer types including melanoma, hepatocellular carcinoma, urothelial carcinoma, medulloblastoma and glioma (PMID: 23348506, 23530248). Tumors with highly recurrent TERT promoter mutations tend to originate from tissues with lower rates of self-renewal (PMID: 23530248). TERT promoter mutations, C228T and C250T, account for the majority of the somatic TERT promoter alterations and occur 124 and 146 base pairs upstream of the ATG start codon of TERT, respectively. Both promoter mutations create binding motifs for erythroblast transformation-specific (ETS)/ T-cell factor (TCF) transcription factors and enhance telomerase activity (PMID: 23348503, 23348506, 26194807). In addition to promoter mutations, TERT, located on chromosome 5p, is amplified across many cancer types (PMID: 20164920).

## Drug Information

### CENISERTIB

Cenisetib is an aurora kinase inhibitor (targeting Aurora kinase A) that is being investigated for use/treatment in solid tumors, leukemia (myeloid), myelodysplastic syndrome, and cancer/tumors (unspecified). Cenisetib (R763) is a highly potent and specific inhibitor of Aurora kinase, which has been shown to block proliferation and trigger apoptosis (cell death) in several tumor cell lines including cervical, colon, lung, pancreas and prostate. The over-expression of Aurora kinase can cause cells to rapidly develop an abnormal number of chromosomes. Elevated levels of Aurora kinase are frequently associated with various human cancers and inhibition of this enzyme disrupts cell division and promotes apoptosis.

### APR-246

APR-246 is a first-in-class agent targeting mutant p53. In vitro and in vivo preclinical models have demonstrated that APR-246 has excellent efficacy in OC (both adenocarcinoma and squamous cell carcinoma) and potently synergises with chemotherapies used in the treatment of OC, restoring sensitivity to chemotherapy-resistant tumours. An initial phase I clinical trial has shown APR-246 to be safe in humans and early results from a currently running phase Ib/II trial of APR-246 with carboplatin and liposomal doxorubicin in ovarian cancer have been promising. Together, these data provide a strong rationale for investigating the efficacy of APR-246 in OC. APR-246 has been used in trials studying the treatment of Prostatic Neoplasms, Hematologic Neoplasms, and Platinum Sensitive Recurrent High-grade Serous Ovarian Cancer With Mutated p53. APR-246 is an analogue of PRIMA-1, which modifies the core domain of mutant p53, resulting in restoration of wild-type p53 conformation and reactivation of normal p53 function, leading to increased cell cycle arrest and tumor cell death (PMID: 20498645).

### KEVETRIN

Kevetrin (thioureidobutyronitrile) leads to stabilization of wild-type p53 and degradation of mutant p53, resulting in apoptosis and decreased growth of tumors with wild-type and mutant p53 (J Clin Oncol 31, 2013 (suppl; abstr TPS2627)).

### LAROTRECTINIB

Larotrectinib is a tyrosine kinase inhibitor that is currently indicated for the treatment of adult and pediatric patients with solid tumors that either a) have a neurotrophic receptor tyrosine kinase (NTRK) gene fusion without a known acquired resistance mutation, b) are metastatic or where surgical resection is likely to result in severe morbidity, and c) have no satisfactory alternative treatments or that have progressed following treatment. Larotrectinib functions as an inhibitor of TRKs including TRKA, B, and C. In in vitro and in vivo tumor models, larotrectinib demonstrated anti-tumor activity in cells with constitutive activation of TRK proteins resulting from gene fusions, deletion of a protein regulatory domain, or in cells with TRK protein overexpression.

### ENTRECTINIB

Entrectinib is a tyrosine kinase inhibitor which acts on several receptors. It functions as an ATP competitor to inhibit tropomyosin receptor tyrosine kinases (TRK) TRKA, TRKB, TRKC, as well as proto-oncogene tyrosine-protein kinase ROS1 and anaplastic lymphoma kinase (ALK).

## Potential Clinical Trials

Trial URL	Status	Title	Disease	Drug	Sites
<a href="https://ClinicalTrials.gov/show/NCT03593915">https://ClinicalTrials.gov/show/NCT03593915</a>	Recruiting	A Phase 1b/2 Study of Alvocidib Plus Decitabine or Azacitidine in	Myelodysplastic Syndromes (MDS)	Alvocidib Plus Decitabine or Azacitidine	University of Chicago, Chicago, Illinois, United States University of

		Patients With MDS			Iowa, Iowa City, Iowa, United States Johns Hopkins, Baltimore, Maryland, United States (and 2 more sites)
<a href="https://ClinicalTrials.gov/show/NCT03957876">https://ClinicalTrials.gov/show/NCT03957876</a>	Recruiting	CPX-351 Therapy for MDS After Hypomethylating Agent Failure	Myelodysplastic Syndrome (MDS)	CPX-351	Cleveland Clinic, Case Comprehensive Cancer Center, Cleveland, Ohio, United States
<a href="https://ClinicalTrials.gov/show/NCT03647800">https://ClinicalTrials.gov/show/NCT03647800</a>	Recruiting	Study of APVO436 in Patients With AML or MDS	Myelodysplastic Syndrome	APVO436	Sylvester Comprehensive Cancer Center/UMHC, Miami, Florida, United States The University of Kansas Clinical Research Center, Westwood, Kansas, United States Roswell Park Cancer Institute, Buffalo, New York, United States (and 2 more sites)
<a href="https://ClinicalTrials.gov/show/NCT03459859">https://ClinicalTrials.gov/show/NCT03459859</a>	Recruiting	Pevonedistat and Low Dose Cytarabine in Adult Patients With AML and MDS	Myelodysplastic Syndrome	Pevonedistat Cytarabine	University of Miami, Miami, Florida, United States
<a href="https://ClinicalTrials.gov/show/NCT03931291">https://ClinicalTrials.gov/show/NCT03931291</a>	Recruiting	APR-246 in Combination With Azacitidine for TP53 Mutated AML (Acute Myeloid Leukemia) or MDS (Myelodysplastic Syndromes) Following Allogeneic Stem Cell Transplant	Myelodysplastic Syndrome	APR-246	H. Lee Moffitt Cancer Center & Research Institute, Tampa, Florida, United States Johns Hopkins, Sidney Kimmel Comprehensive Cancer Center, Baltimore, Maryland, United States Massachusetts General Hospital, Boston, Massachusetts, United States (and 2 more sites)
<a href="https://ClinicalTrials.gov/show/NCT03770429">https://ClinicalTrials.gov/show/NCT03770429</a>	Recruiting	AZD6738 for Patients With Progressive MDS or CMML	Myelodysplastic Syndrome	AZD6738	BIDMC, Boston, Massachusetts, United States Dana-Farber

					Cancer Institute, Boston, Massachusetts, United States Massachusetts General Hospital Cancer Center, Boston, Massachusetts, United States (and 1 more sites)
<a href="https://ClinicalTrials.gov/show/NCT03094637">https://ClinicalTrials.gov/show/NCT03094637</a>	Recruiting	Azacitidine and Pembrolizumab in Treating Patients With Myelodysplastic Syndrome	Myelodysplastic Syndrome	Azacitidine Pembrolizumab	M D Anderson Cancer Center, Houston, Texas, United States
<a href="https://ClinicalTrials.gov/show/NCT04256317">https://ClinicalTrials.gov/show/NCT04256317</a>	Recruiting	A Study of ASTX030 (Cedazuridine in Combination With Azacitidine) in MDS, CMML, or AML	Myelodysplastic Syndromes	Azacitidine ASTX030 (cedazuridine + azacitidine) Cedazuridine	Vanderbilt University Medical Center, Nashville, Tennessee, United States MD Anderson Cancer Center, Houston, Texas, United States
<a href="https://ClinicalTrials.gov/show/NCT01211457">https://ClinicalTrials.gov/show/NCT01211457</a>	Recruiting	Study of Sapacitabine in Acute Myeloid Leukemia (AML) or Myelodysplastic Syndromes (MDS)	Myelodysplastic Syndrome	sapacitabine and decitabine (Part 1 - completed) sapacitabine and venetoclax (Part 2 - recruiting)	Rush University Medical Center, Chicago, Illinois, United States Roswell Park Cancer Institute, Buffalo, New York, United States MD Anderson Cancer Center, Houston, Texas, United States
<a href="https://ClinicalTrials.gov/show/NCT03359460">https://ClinicalTrials.gov/show/NCT03359460</a>	Recruiting	Ibrutinib and Lenalidomide in Treating Patients With Myelodysplastic Syndrome	Myelodysplastic Syndrome	Ibrutinib Lenalidomide	University of California Davis Comprehensive Cancer Center, Sacramento, California, United States
<a href="https://ClinicalTrials.gov/show/NCT03746041">https://ClinicalTrials.gov/show/NCT03746041</a>	Recruiting	A Phase I Pilot Study of Abaloparatide + Bevacizumab in Myelodysplastic Syndromes	Myelodysplastic Syndromes	abaloparatide bevacizumab	University of Rochester, Rochester, New York, United States
<a href="https://ClinicalTrials.gov/show/NCT04278768">https://ClinicalTrials.gov/show/NCT04278768</a>	Recruiting	An Open Label Dose Escalation Trial of CA-4948 in Patients With Acute Myelogenous Leukemia or Myelodysplastic Syndrome	Myelodysplastic Syndrome	CA-4948	Oncology Hematology West, PC dba Nebraska Cancer Specialists, Omaha, Nebraska, United States The University of Texas MD

					Anderson Cancer Center, Houston, Texas, United States
<a href="https://ClinicalTrials.gov/show/NCT04402541">https://ClinicalTrials.gov/show/NCT04402541</a>	Recruiting	Study of CB-5339 in Acute Myeloid Leukemia or Myelodysplastic Syndrome	Myelodysplastic Syndrome	CB-5339	HonorHealth Research Institute, Scottsdale, Arizona, United States
<a href="https://ClinicalTrials.gov/show/NCT03175978">https://ClinicalTrials.gov/show/NCT03175978</a>	Recruiting	IGF-MTX Conjugate in the Treatment of Myelodysplastic Syndrome	Myelodysplastic Syndromes	IGF/MTX	Mayo Clinic, Rochester, Minnesota, United States Regions Cancer Care Center, Saint Paul, Minnesota, United States
<a href="https://ClinicalTrials.gov/show/NCT04022785">https://ClinicalTrials.gov/show/NCT04022785</a>	Recruiting	PLX51107 and Azacitidine in Treating Patients With Acute Myeloid Leukemia or Myelodysplastic Syndrome	Myelodysplastic Syndrome	Azacitidine BRD4 Inhibitor PLX51107	M D Anderson Cancer Center, Houston, Texas, United States

## Detailed Results

Single Nucleotide Variant (SNV)								
Gene name	Hgvsp	Hgvsc	Aminoacids	Codons	Consequence	Allele frequency	Read depth	Predicted effect on protein
TP53	NP_000537.3:p.Val173Met	NM_000546.5:c.517G>A	V/M	Gtg/Atg	missense_variant	74.11	224	deleterious (0.02)
NTRK2	NP_006171.2:p.Thr340Met	NM_006180.3:c.1019C>T	T/M	aCg/aTg	missense_variant	32.88	365	deleterious (0.04)
GRIN2A	NP_000824.1:p.Thr265Lys	NM_000833.3:c.794C>A	T/K	aCg/aAg	missense_variant	17.56	279	tolerated (0.3)
TERT	NP_937983.2:p.Ala401Val	NM_198253.2:c.1202C>T	A/V	gCg/gTg	missense_variant	17.42	356	tolerated (0.12)

## Methodology and Test Background

This is a next generation sequencing (NGS) test that analyzes DNA for abnormalities in 177 genes that are reported to be altered in various types of hematologic neoplasms. Nucleic acid is isolated from plasma, fresh cells peripheral blood cells or bone marrow), or 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. Fragment length analysis is also performed for CALR, FLT3 and NPM1 to enhance the detection of large duplication. The DNA assay is optimized to be run using 50 ng from fresh cells, 100 ng from FFPE, and 20 ng from cfDNA. Extraction of DNA and RNA from various tissue type is automated. Library for targeted DNA sequencing is based on Single Primer Extension (SPE) chemistry. The DNA sequencing includes all coding exons of 177 genes. The GTC-Hematology assay is a qualitative in vitro diagnostic test that uses targeted next generation sequencing of formalin-fixed paraffin-embedded (FFPE), bone marrow cells, peripheral blood cells, and peripheral blood plasma cell-free DNA (cfDNA) from patients with hematologic neoplasms to detect genomic alterations using a multigene panel. The test is intended

to provide information on somatic mutations (point mutations as well as small insertions and deletions) for use by qualified health care professionals in accordance with professional guidelines. This assay is not conclusive or prescriptive for labeled use of any specific therapeutic product. This Assay is a single-site assay performed at Genomic Testing Cooperative. Specifically, the test is indicated for: -Molecular profiling of genomic abnormalities (SNV and indels) in DNA from patients with hematologic neoplasms using bone marrow fresh cells, peripheral blood fresh cells, peripheral blood cfDNA and non-decalcified lymphoid tissue in formalin-fixed paraffin-embedded (FFPE). -cfDNA testing is to be used only for detecting abnormalities in myeloid neoplasms (AML, MDS, MPN and aplastic anemia) and not validated for non-myeloid neoplasms. This test is for in vitro complementary diagnosis and classification. It should not be used as the primary diagnosis of hematologic neoplasm or for managing therapy in patients with hematologic neoplasms. Our sequencing method has a typical sensitivity of 3% for detecting hot-spots specific mutations and 5% for other mutations. Known hot spots in specific genes such as IDH1/2, NRAS, and KRAS are reported at levels of 3% and higher. The FLT3-ITD fragment analysis assay has a sensitivity of 2%-5% for detecting FLT3-ITD in wildtype background. The NPM1 fragment analysis assay has a sensitivity of 2%-5% for detecting mutations in wildtype background. The assay is not designed to detect gene amplification. Based on our validation study, the following 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 due to high GC content with inherited problem in obtaining adequate coverage. Region; Transcript; Exon; AA Range; Promoter Range. TNFRSF14.8; NM\_003820; 7; 232 to 242. MYCL1.117; NM\_001033082; 1; 1 to 27. AXIN1.1161; NM\_003502; 1; NC. PIK3R2.1897; NM\_005027; 6; 200 to 272. KMT2B.1928; NM\_014727; 1; 1 to 121. CD79A.1981; NM\_001783; 4; 167 to 189. ASXL1.2390; NM\_015338; 1; 1 to 19. BCR.2530; NM\_021574; 17; 981 to 1017. TERT.3105;;; -59 to -72. TERT.3106;;; -81 to -94. PMS2.3489; NM\_001322008; 13; 710 to 757. RHEB.3700; NM\_005614; 1; 1 to 18. Variant calling is based on DRAGEN somatic pipeline using tumor-only analysis against the GRCh37 reference genome.

## Tested genes

Genes Tested for Abnormalities in Coding Sequence												
ABL1	BCL2	CBL	CDKN2C	DICER1	FAS	IDH2	KMT2A	MEF2B	PALB2	PPP2R1A	SF3B1	TET2
AKT1	BCL2L1	CBLB	CEBPA	DNMT3A	FBXW7	IGF1R	KMT2B	MPL	PAX5	PTCH1	SMAD2	TGFBR2
AKT2	BCL6	CBLC	CHEK1	EP300	FLT3	IKZF1	KMT2C	MRE11A	PBRM1	PTEN	SMAD4	TP53
AKT3	BCOR	CCND1	CHEK2	ERG	GATA1	IKZF3	KMT2D	MTOR	PDGFRA	PTPN11	SMARCA4	TSC1
ALK	BCORL1	CCND3	CIC	ETV6	GATA2	IRF4	KRAS	MUTYH	PDGFRB	RAD21	SMARCB1	TSC2
AMER1	BCR	CD274	CREBBP	EZH2	GATA3	JAK1	MAP2K1	MYC	PHF6	RAD50	SMC1A	TSHR
APC	BIRC3	CD79A	CRLF2	FAM175A	GEN1	JAK2	MAP2K2	MYD88	PIK3CA	RAD51	SMO	U2AF1
ARID1A	BLM	CD79B	CSF1R	FAM46C	GNAQ	JAK3	MAP2K4	NFKBIA	PIK3R1	RB1	SOCS1	WT1
ARID1B	BRAF	CDH1	CSF3R	FANCA	GNAS	KAT6A	MAP3K1	NOTCH1	PIK3R2	RHOA	SRC	ZNF217
ARID2	BRCA1	CDK12	CTNNA1	FANCC	H3F3A	KDM5C	MAP3K14	NOTCH2	PIM1	RNF43	SRSF2	ZRSR2
ASXL1	BRCA2	CDK4	CTNNA1	FANCD2	HNF1A	KDM6A	MAPK1	NOTCH3	PLCG1	RUNX1	STAG2	
ATM	BTK	CDK6	CUX1	FANCE	HOXB13	KDR	MCL1	NPM1	POLD1	SDHB	STAT3	
ATRX	CALR	CDKN2A	CXCR4	FANCF	HSP90AA1	KEAP1	MDM2	NRAS	POLE	SETBP1	STK11	
B2M	CARD11	CDKN2B	DDR2	FANCG	IDH1	KIT	MDM4	NSD1	PPM1D	SETD2	TERT	

## Reference

- Hosono N. Genetic abnormalities and pathophysiology of MDS. *Int J Clin Oncol*. 2019;24(8):885-892. doi:10.1007/s10147-019-01462-6
- Welch JS, Petti AA, Miller CA, et al. TP53 and Decitabine in Acute Myeloid Leukemia and Myelodysplastic Syndromes. *N Engl J Med*. 2016;375(21):2023-2036. doi:10.1056/NEJMoa1605949
- Haase D, Stevenson KE, Neuberg D, et al. TP53 mutation status divides myelodysplastic syndromes with complex karyotypes into distinct prognostic subgroups. *Leukemia*. 2019;33(7):1747-1758. doi:10.1038/s41375-018-0351-2



## Electronic Signature

Maher Albitar, M.D.

The Technical Component Processing, Analysis and Professional Component of this test was completed at Genomic Testing Cooperative, LCA, 175 Technology Drive, Suite 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.