PRECIPIO EXPECT MORE.
203-787-1717
203-901-1289

MOLECULAR

**Patient:** John A. Doe
**DOB/Gender:** 10/10/44 (75 yrs) - Male
**Patient ID/MRN:** 123456
**Date Collected:** 03/09/2020

**Case#/Status:** X20-00633 - Final

**Report Category:**
**Detected**

**Provider:** Jane Smith, M.D.
Hematology Oncology Associates
Tel: 800-123-4567
Fax: 800-765-4321

## DIAGNOSIS:

Peripheral blood:
Detected Genomic Alterations
TET2 (2 mutations)  SF3B1

Heterogeneity
There is an abnormal clone with TET2 (2 mutations) and SF3B1 mutations.

Diagnostic Implications
TET2 (2 mutations), SF3B1: These abnormalities are consistent with very early myelodysplastic syndrome: refractory anemia with ring sideroblasts.

Therapeutic Implications
SF3B1: Spliceosome modifiers (H3B-8800, sudemycins, spliceostatin A, and meayamycin)

Prognostic Implications
TET2 (2 mutations): Poor
SF3B1: Favorable

## COMMENT

- Mutations in TET2 (2 mutations) and SF3B1 genes detected at extremely low level.

- Despite the extremely low levels, the presence of three different mutations is suggestive of very early low grade MDS, refractory anemia with ring sideroblasts.

- Relevant Genes with NO Alteration: FLT3, NPM1, IDH1 and IDH2

## INTERPRETATION

Biological relevance of detected Alterations

**TET2**, a tumor suppressor and DNA demethylase, is frequently mutated in hematologic malignancies.
BACKGROUND
TET2 belongs to a family of alpha-ketoglutarate and iron-dependent enzymes involved in converting 5-methylcytosine to 5-hydroxymethylcytosine (PMID: 19372391). This modification is implicated in active DNA demethylation, a process that is important for cellular reprogramming and gene regulation (PMID: 20639862). TET2 has been shown to function as a tumor suppressor with mutations leading to loss-of-function, particularly those affecting the C-terminal catalytic domain (PMID: 21057493). In animal models, TET2 loss cooperates with other mutations such as JAK2 and FLT3-ITD mutations to promote cancer progression and can induce genomic hypermethylation and increase stem cell self-renewal (PMID:21723200, 25281607,

25873173, 25886910). TET2 mutations are most often found in hematologic malignancies (PMID: 24220273). Isolated mutations in TET2 have also been found in individuals with clonal hematopoiesis but with no apparent hematologic disease (PMID: 23001125). However, these patients are at a higher risk of developing hematologic cancer with aging (PMID: 25426837, 25426838). TET family enzyme activity is also inhibited by specific mutations in IDH1 and IDH2 that produce an inhibitory co-factor, 2-hydroxyglutarate (PMID: 21130701). Mutations in WT1 may also affect TET2 function as an associated co-factor (PMID: 25482556, 25601757).

**SF3B1** mutations have been described in several myeloid malignancies, predominantly myelodysplastic syndromes (MDS), as well as other hematologic malignancies, breast cancer, and uveal melanoma (UM). SF3B1 is one of several genes involved in RNA splicing that has been identified as recurrently mutated in MDS and other malignanices. The mutations affecting SF3B1 are typically heterozygous, point mutations suspected to be functionally deleterious with R625 and K700E described as a major mutation hotspots. MDS patients with SF3B1 mutations have been reported to have better overall and event-free survival than their wildtype counterparts. Additionally, these mutations are highly associated with subtypes of MDS characterized by ringed sideroblasts (refractory anemia with ringed sideroblasts and refractory cytopenia with multilineage dysplasia and ring sideroblasts). In UM patients, SF3B1 mutations have been reported to be associated with chromosome 3 disomy, which defines a subgroup with low risk of metastasis.
SUMMARY
SF3B1, a component of the spliceosome complex, is frequently mutated in hematologic malignancies.
BACKGROUND
SF3B1 (splicing factor 3b subunit 1) is a component of the spliceosome complex that regulates the removal of introns from messenger RNA (PMID: 28958291). SF3B1 binds to nucleosomes to identify exon and intron junctions of coding genes (PMID: 25892229). Importantly, SF3B1 preferentially regulates alternative splicing and 3 splice site selection (PMID: 28445500). In addition, SF3B1 plays a role in the maintenance of genomic integrity due to contributions to sister chromatid cohesion and chromosome segregation (PMID: 25257310). Somatic mutations in SF3B1 are recurrent in uveal melanoma (PMID: 26842708) and myelodysplastic syndromes (MDS) (PMID: 21909114, 21995386), especially those with refractory anemia with ring sideroblasts (RARS) and refractory cytopenia with multilineage dysplasia and ring sideroblasts (RCMDRS) (PMID: 21995386, 21998214). Mutations in SF3B1 lead to altered gene expression and aberrant alternative splicing (PMID: 25428262) and tend to be missense mutations rather than nonsense or frameshift mutations, suggesting either gain-of-function or dominant negative activity (PMID: 22150006, 21909114). The SF3B spliceosome complex can be inhibited by naturally occurring compounds, including spliceostatin A (PMID: 17643111), and SF3B1-mutant cells are preferentially sensitive to spliceosome inhibitors (PMID: 29457796).

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.

**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. .MYCL.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, AKT1, AKT2, AKT3, ALK, AMER1, APC, ARID1A, ARID1B, ARID2, ASXL1, ATM, ATRX, B2M, BCL2, BCL2L1, BCL6, BCOR, BCORL1, BCR, BIRC3, BLM, BRAF, BRCA1, BRCA2, BTK, CALR, CARD11, CBL, CBLB, CBLC, CCND1, CCND3, CD274, CD79A, CD79B, CDH1, CDK12, CDK4, CDK6. CDKN2A, CDKN2B, CDKN2C, CEBPA, CHEK1, CHEK2, CIC, CREBBP, CRLF2, CSF1R, CSF3R, CTNNA1, CTNNB1, CUX1, CXCR4, DDR2, DICER1, DNMT3A, EP300, ERG, ETV6, EZH2, FAM175A, FAM46C, FANCA, FANCC, FANCD2, FANCE, FANCF, FANCG, FAS, FBXW7, FLT3, GATA1, GATA2, GATA3, GEN1, GNAQ, GNAS, H3F3A, HNF1A, HOXB13, HSP90AA1, IDH1, 1DH2, IGF1R, IKZF1, IKZF3, IRF4, JAK1, JAK2, JAK3, KAT6A, KDM5C, KDM6A, KDR, KEAP1, KIT, KMT2A, KMT2B, KMT2C, KMT2D, KRAS, MAP2K1, MAP2K2, MAP2K4, MAP3K1, MAP3K14, MAPK1, MCL1, MDM2, MDM4, MEF2B, MPL, MRE11A, MTOR, MUTYH, MYC, MYD88, NFKBIA, NOTCH1, NOTCH2, NOTCH3, NPM1, NRAS, NSD1, PALB2, PAX5, PBRM1, PDGFRA, PDGFRB, PHF6, PIK3CA, PIK3R1, PIK3R2, PIM1, PLCG1, POLD1, POLE, PPM1D, PPP2R1A, PTCH1, PTEN, PTPN11, RAD21, RAD50, RAD51, RB1, RHOA, RNF43, RUNX1, SDHB, SETBP1, SETD2, SF3B1, SMAD2, SMAD4, SMARCA4, SMARCB1, SMC1A, SMO, SOCS1, SRC, SRSF2, STAG2, STAT3, STK11, TERT, TET2, TGFBR2, TP53, TSC1, TSC2, TSHR, WT1, ZNF217, ZRSR2

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**Disclaimer:**
The sequencing test reported above was performed and solely interpreted at Genomic Testing Cooperative, 175 Technology Drive, Suite 100, Irvine, CA 92618, by Maher Albitar, MD (CLIA # 05D2111917). 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, MD, (CLIA # 05D2111917). 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.

## CLINICAL DATA

ICD-10: D53.9. Nutritional anemia, unspecified.

Received CBC, reported on 03/09/2020: WBC 8.8; RBC 2.66; HGB 8.5; HCT 25.4; MCV 96; MCH 32.0; MCHC 33.4; RDW 13.3%; PLT 306; MPV 6.7; LYM 18.0%; GRAN 76.2%; MON 5.8%

**Electronically Signed By:** Frank Bauer, MD (03/20/20 13:00)

## Potential Clinical Trials

| Trial URL | Status | Title | Disease | Drug | Sites |
|---|---|---|---|---|---|
| https://ClinicalTrials.gov/show/NCT03593915 | Recruiting | A Phase 1b/2 Study of Alvocidib Plus Decitabine in Patients With MDS | Myelodysplastic Syndromes (MDS) | Alvocidib Plus Decitabine | University of Iowa, Iowa City, Iowa, United States<br>Johns Hopkins, Baltimore, Maryland, United States<br>Columbia University, New York, New York, United States<br>(and 8 more sites |
| https://ClinicalTrials.gov/show/NCT03502668 | Recruiting | Phase 1-2 Study of Low Dose ASTX727 (ASTX727 LD) in Lower Risk MDS | Myelodysplastic Syndromes | ASTX727 LD<br>ASTX727 SD | The University of Alabama at Birmingham, Birmingham, Alabama, United States<br>University of Colorado, Anschutz Cancer Pavilion, Aurora, Colorado, United States<br>BRCR Medical Center Inc., Plantation, Florida, United States<br>(and 12 more sites |
| https://ClinicalTrials.gov/show/NCT02452983 | Recruiting | Sertraline in Treatment of Low-Risk Myelodysplastic Syndrome | Myelodysplastic Syndromes | Sertraline<br>Bone Marrow Aspirate/Biopsy | Baylor College of Medicine, Houston, Texas, United States<br>Michael E. DeBakey VA Medical Center, Houston, Texas, United States |
| https://ClinicalTrials.gov/show/NCT03770429 | Recruiting | AZD6738 for Patients With Progressive MDS or CMML | MDS/CML | AZD6738 | BIDMC, Boston, Massachusetts, United States<br>Dana-Farber Cancer Institute, Boston, Massachusetts, United States<br>Massachusetts General Hospital Cancer Center, Boston, Massachusetts, United States<br>(and 1 more sites |
| https://ClinicalTrials.gov/show/NCT02530463 | Recruiting | Nivolumab and/or Ipilimumab With or Without Azacitidine in Treating Patients With Myelodysplastic Syndrome | Leukemia | Azacitidine<br>Ipilimumab<br>Laboratory Biomarker Analysis<br>Nivolumab | 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 |
| TET2 | NP_001120680.1:p.Thr1883Ile | NM_001127208.2:c.5648C>T | T/I | aCa/aTa | missense_variant | 8.57 | 245 | deleterious (0) |
| TET2 | NP_001120680.1:p.Gln831ThrfsTer15 | NM_001127208.2:c.2490dupA | I/IX | ata/atAa | frameshift_variant | 3.65 | 219 | 0 |
| SF3B1 | NP_036565.2:p.His662Gln | NM_012433.2:c.1986C>A | H/Q | caC/caA | missense_variant | 3.47 | 202 | deleterious (0.02) |

**Patient:** John A. Doe
**Case #:** X20-00633
**Received Information:** 1 lavender-top tube
**Received:** 03/10/20 09:30
**Reported:** 03/20/20 13:00