Patient: John A. Doe

DOB/Gender: xx/xx/xxxx (85 yrs) - Male
SSN: xxx-xx-xxxx
MRN/ID: xxxx

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

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Case No: GS11-00125

Collected: xx/xx/xx
Received: xx/xx/xx
Reported: xx/xx/xx

Alert Status: Routine
Report Status: Final
Report Category: Detected

Clinical information: Hepatomegaly, not elsewhere classified. Personal history of malignant neoplasm of prostate. Skin cancer.

Specimens received: 2 Green-top tubes, 1 lavender-top tube **Specimen analyzed:** Peripheral blood **Tests ordered:** NGS-68 Panel

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RESULT:

Peripheral blood:

DISEASE ASSOCIATED MUTATIONS (SEE INTERPRETATION AND COMMENTS):

GENE	PROTEIN CHANGE	cDNA CHANGE
TET2	p.T1122Sfs*13	c.3364_3370del
SRSF2	p.P95H	c.284C>A

VARIANTS OF UNCERTAIN SIGNIFICANCE (SEE INTERPRETATION AND COMMENTS):

GENE	PROTEIN CHANGE	cDNA CHANGE
PHF6	p.F263S	c.788T>C

TOXICITY RISK VARIANTS (SEE INTERPRETATION AND COMMENTS):

GENE	PROTEIN CHANGE	cDNA CHANGE
TPMT	p.Y240C	c.719A>G
TPMT	p.A154Y	c.460A>G

PLEASE NOTETesting cannot distinguish between *1/*3A (i.e. wt/460A>G,719A>G) which would be intermediate risk with one wild-type and one variant allele (wt/v) with the alterations in cis and *3B/*3C (460A>G/719A>G), with the alterations in trans, and would be high risk (variant/variant, with two variant alleles) individuals. However since the *3B allele is exceedingly rare (~ 0.16% per PMID 11681423, vs 2-5% for *3C and *3A), it is typically assumed when both variants are detected that the patient is *1/*3A, intermediate risk.

COMMENT:

The sequencing test reported above was performed and solely interpreted at the Department of Pathology and Lab Medicine at the Penn Clinical CytoGenomics Lab 3020 Market Street, Philadelphia, PA 19104, by Rachel L. Sargent, MD (Scientific Director, Clinical Cancer Cytogenetics/Clinical Director, Clinical Genomics: Jennifer J.D. Morrissette, PhD, FACMG; CLIA #39D2060198).

Electronically Signed By: S. David Hudnall, MD, FCAP

INTERPRETATION:

This is an Abnormal sequencing study that identified the following disease associated mutations:
1. A deletion of 7 nucleotides resulting in a frameshift in TET2 in 1837 reads out of a total 4995 sequence reads for an allele frequency of **36.78%.** (Transcript ID NM_001127208)
2. A missense mutation in SRSF2 at amino acid 95 converting the wild type residue, Proline, to Histidine in 194 reads out of a total 870 sequence reads for an allele frequency of **22.30%.** (Transcript ID NM_001195427)

The following variants of uncertain significance were also identified in this patient's sample:
1. A missense variant in PHF6 at amino acid 263 converting the wild type residue, Phenylalanine, to Serine in 950 reads out of a total 4411 sequence reads for an allele frequency of **21.54%.** (Transcript ID NM_001015877)

TOXICITY RISK VARIANTS:
This sequencing study identified the following Toxicity Risk Variant:
A missense mutation in TPMT at amino acid 240 converting the wild type residue, Tyrosine, to Cysteine (Transcript ID NM_000367)

A missense mutation in TPMT at amino acid 154 converting the wild type residue, Alanine, to Threonine (Transcript ID NM_000367)

GENE INFORMATION
SRSF2: The serine/arginine-rich splicing factor 2 (SRSF2) gene is located on chromosome 17q25.1. It encodes a pre-mRNA splicing factor, a component of the mRNA spliceosome. SRSF2 contains an RNA-binding domain as well as a protein-binding domain. In addition to its role in splicing, SRSF2 plays a role in mRNA nuclear export and translation.

Mutations in SRSF2 have been identified in MDS, CMML, myelofibrosis, and AML. The frequency of mutations varies, from 15% of MDS patients up to almost half of patients with CMML. The most frequently mutated hotspot in SRSF2 is a proline at the 95th codon (P95), which may be mutated to another amino acid (most frequently leucine, histidine, or arginine), or be the site of a deletion or amino acid insertion. The mechanisms by which these mutations exert their effects are unclear.

SRSF2 mutations are generally poor prognosis markers. They portend a poor prognosis in MDS and myelofibrosis and are associated with reduced overall survival and leukemia-free survival in these diseases. SRSF2 mutations are associated with inferior outcomes in AML, though this may reflect the evolution of AML from an underlying MDS. On the other hand, there is no prognostic implication in CMML.

TET2: Tet methylcytosine dioxygenase 2 (TET2) is located on chromosome 4q24, and encodes a tumor suppressor, and acts as an epigenetic modifier, preventing transcriptional silencing. TET2 mutations occur in 10-20% of cases of MPN and MDS and in 7-23% of cases of AML. Most TET2 mutations are heterozygous in leukemia; with the expression of the wild-type allele is retained, which suggests that TET2 can function as a haploinsufficient tumor suppressor. Biallelic TET2 inactivation occurs in <10% of patients with leukemia (Delhommeau, 2009).

Somatic mutations in TET2 are thought to lead to loss of function, with nonsense and frameshift mutations (approximately 50% of mutations) and missense mutations accounting for the remainder (Gaidzik, 2012). Mutations are distributed throughout the gene and most commonly affect exons 10 and 11.

PHF6: The plant homeodomain finger 6 (PHF6) gene is located on Xq26.3 and is thought to be a tumor suppressor. Mutations in PHF6 have been described in T-cell acute lymphoblastic leukemia and in acute myeloid leukemia.

Somatic mutations in PHF6 have been described in 3% of adult AML and are typically nonsense and frameshift mutations that lead to loss of function. The mutations are distributed throughout the gene. Mutations in PHF6 were predominantly identified in immature acute myeloid leukemias and were associated with reduced overall survival (Patel 2012).

TPMT: The thiopurine S-methyltransferase *(TPMT)* gene is located on chromosome 6p22.3. It encodes an enzyme that metabolizes thiopurine drugs, including azathioprine (AZA), 6-mercaptopurine (6-MP), and 6-thioguanine (6-TG) in the treatment of acute lymphoblastic leukemia, autoimmune disorders and organ transplant recipients.

Up to 1/300 individuals are homozygous for an allele that causes complete deficiency of TPMT activity. Identification of these individuals is important to reduce therapy-related toxicity, including prolonged myelosuppression.

Reduced TPMT activity can be caused by polymorphisms in the *TPMT* gene. Molecular studies have identified 4 variant alleles that together account for >95% of reduced TPMT activity: *TPMT*2* (c.G238C/p.A80P), *TPMT*3A* (cis c.A460G/p.A154T and c.A719G/p.Y240C), *TPMT*3B* (c.A460G/p.A154T), and *TPMT*3C* (c.A719G/p.Y240C). Individuals with 2 variant alleles have low or no TPMT activity, while those with 1 variant allele have intermediate TPMT activity. Wild-type (*TPMT*1*) homozygotes, on the other hand, have normal enzyme activity.

This assay cannot distinguish the *TPMT*1* (wild type)/*TPMT*3A* genotype (intermediate enzyme activity) from *the TPMT*3B/TPMT*3C* genotype (low or no enzyme activity). However, the *TPMT*3B/TPMT*3A* genotype is rare.

*TPMT*2* allele has only been observed in the Caucasian population. *TPMT*3C* has been observed in Caucasian, African (14.8%, predominantly from Ghana), Southwest Asian and Chinese populations. *TPMT*3A* is the most common allele among Caucasians which is likely a product of recent genetic acquirement of *TPMT*3B* allele, while TPMT*3B by itself is rare.

The alleles are:
TPMT*1 is wild type
TPMT*2 is 238G>C: p.A80P
TPMT*3A is both 460A>G and 719A>G in cis
TPMT*3B is just 460A>G
TPMT*3C is just 719A>G p.Y240C
There are other alleles, but the alleles listed above account for >95% of alleles.

HEMATOLOGIC MALIGNANCY SEQUENCING PANEL: Sequence analysis of 68 genes (*ABL1, ASXL1, ATM, BCOR, BCORL1, BIRC3, BRAF, CALR, CBL, CDKN2A, CEBPA**, CSF1R, CSF3R, DDX3X, DNMT3A, ETV6, EZH2, FAM5C, FBXW7, FLT3, GATA2, GNAS, HNRNPK, IDH1, IDH2, IL7R, JAK2, KIT, KLHL6, KRAS, MAP2K1, MAPK1, MIR142, MPL, MYC, MYCN, MYD88, NF1, NOTCH1, NOTCH2, NPM1, NRAS, PDGFRA, PHF6, POT1, PRPF40B, PTEN, PTPN11, RAD21, RIT1, RUNX1, SETBP1, SF1, SF3A1, SF3B1, SMC1A, SRSF2, STAG2, TBL1XR1, TET2, TP53, TPMT, U2AF1, U2AF2, WT1, XPO1, ZMYM3, ZRSR2*).

***CEBPA will be analyzed only when a diagnosis of AML is provided*

The design of the panel was based on the literature at the time of development, either the full length of a gene or the mutational hot spots of a gene are targeted. For a complete listing of genomic coordinates covered in this panel please contact the laboratory.

DISCLAIMERS

This report has been created based on various scientific manuscripts, references and publically available databases that describe correlations between certain genetic mutations and disease. This information which comes from numerous sources is subject to change over time in response to future scientific and medical findings and correlations. The University Of Pennsylvania Health System (UPHS) makes no representation or warranty of any kind regarding the accuracy of information provided or contained in these manuscripts, references or other sources of information. If any of the information provided by or contained in the referenced material is later deemed to be inaccurate, this may impact the accuracy of this report and interpretation of the findings. UPHS is not obligated to notify you of any impact that additional or modified information, or future scientific or medical research may have on this report.

This report must always be interpreted and considered within the clinical context, and the treating physician(s) should always consider other pertinent information and data that a physician would prudently consider, in addition to the mutations identified in this report.

The results in this report are not based on diagnostic or prognostic test, and therefore should be carefully considered within the context of clinical and other laboratory data. The genes tested in this assay have been found to be altered in the manifestation of many diseases. The manifestation of disease is commonly caused by many genes, in addition to other variables not addressed in this report, including but not limited to modifier genes, epigenetic factors, environmental factors, and factors that are not known at this time. This report is relevant only in its interpretation based on the context of the patient's clinical manifestations.

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This test was developed and its performance characteristics determined by the University of Pennsylvania Center for Personalized Diagnostics Laboratory as required by the CLIA 1988 regulations. It has not been cleared or approved for specific uses by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. Pursuant to the requirements of CLIA 1988, this laboratory has established and verified the test's accuracy and precision.
This test was performed in the Department of Pathology and Lab Medicine at the Penn Clinical CytoGenomics Lab 3020 Market Street, Philadelphia, PA 19104.

METHODOLOGY:
Genomic DNA was extracted from the submitted specimen according to manufacturer's instructions (Qiagen, Inc.). Targeted analysis for mutations in the regions specified in this testing panel was achieved by enrichment of those genomic loci using the Illumina Truseq Amplicon Assay. Sequencing of enriched libraries was performed on the Illumina MiSeq platform using multiplexed, paired end reads. Analysis and interpretation utilized a customized bioinformatics pipeline, v2.1.1. All variants listed are with reference to the hg19 Genome build. Variants are reported according to HGVS nomenclature and classified into 3 categories: Pathogenic, Variants of uncertain significance and Benign. Categorization of variants was dependent upon literature review, variant existence in a variety of publically available databases including dbSNP, COSMIC, and the 1000 genome project. Variants classified as Benign are not listed in this report.

This assay will detect single nucleotide variants and small insertions or deletions (indels). Large or complex indels, inversions, translocations, gene amplifications, copy number changes or other complex genomic mutations may not be detected by this assay. Variants existing outside the target regions will not be detected. Only variants in the exonic regions of the gene panel will be reported. This assay does not determine variant causality, or whether a variant is inherited or somatically acquired. This assay will detect variants representing at least 10% of the total sequence reads at a given genomic position. Note that it is possible that pathogenic variants present in the sample will not be detected by one of more of the informatics processing tools because of the parameters used. During test validation the choice of parameters was optimized to maximize sensitivity and specificity.

This assay cannot guarantee all regions will meet variant calling criteria due to the possibility of underlining genomic changes. Genomic regions not meeting criteria for variant calling include the genomic loci for which the depth of coverage did not meet our minimum criteria of 250 reads at all positions within that loci. Since the sensitivity and specificity of our assay was determined by achieving a minimum depth of coverage of 250 reads, regions not meeting this criterion cannot be guaranteed to be mutation negative. The depth of coverage is dependent upon the starting amount of DNA for a given region which could be affected by underlying chromosomal copy number changes. Since the genomic loci targeted in this assay are enriched using loci specific primers, patient variants existing within those primer sequences may also cause a region to fail enrichment and therefore not achieve 250xcoverage.

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END OF REPORT

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