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*****Example Report*****

Patient Age/Gender: Unknown Unknown
Printed: 10-Mar-20 08:44:43

Procedure	Result	Units	Ref Interval	Accession	Collected	Received	Reported/ Verified
Chronic Lymphocytic Leukemia Specimen	Bone Marrow			20-070-900037	10-Mar-20 08:09:00	10-Mar-20 08:29:00	10-Mar-20 08:37:48
Chronic Lymphocytic Leukemia Interp	See Note f			20-070-900037	10-Mar-20 08:09:00	10-Mar-20 08:29:00	10-Mar-20 08:37:48
EER, CLL Panel by NGS	EERUnavailable			20-070-900037	10-Mar-20 08:09:00	10-Mar-20 08:29:00	10-Mar-20 08:37:48

***** Abnormal, **#** = Corrected, **C** = Critical, **f** = Footnote, **H** = High, **L** = Low, **t** = Interpretive Text, **@** = Reference Lab

10-Mar-20 08:09:00 Chronic Lymphocytic Leukemia Interp:

Section 79-1 of New York State Civil Rights Law requires informed consent be obtained from patients (or their legal guardians) prior to pursuing genetic testing. These forms must be kept on file by the ordering physician. Consent forms for genetic testing are available at www.aruplab.com. Incidental findings are not reported unless clinically significant but are available upon request.

Submitted diagnosis or diagnosis under consideration for variant interpretation:
Chronic lymphocytic leukemia (CLL)

Result:
I. Tier 1 (Variants of known significance in myeloid malignancies):

1. SF3B1 c.2098A>G, p.Lys700Glu (NM_012433.3)
Variant Frequency: 5.4%

Interpretation: SF3B1 encodes a component of the RNA splicing machinery known as the spliceosome (1, 2). SF3B1 mutations have been reported in 6-21% of patients with chronic lymphocytic leukemia (CLL) (3-7).This particular missense mutation is a recurrent mutation within the HEAT repeat domain reported in CLL (3, 5, 7, 8). SF3B1 mutations predict poor survival in CLL patients (3, 4, 8-10). In addition, patients with both SF3B1 mutations and del(13q) have worse prognosis compared to the favorable prognosis seen in patients with only del(13q) (3). The prognostic significance of SF3B1 mutations in relapsed and refractory CLL patients may depend on the patient's specific treatment regimens. While an earlier study suggested SF3B1 mutations do not affect survival in fludarabine-refractory patients (11), a more recent study suggests patients with SF3B1 mutations refractory to lenalidomide-based therapies and treated with alemtuzumab have shorter overall survival and event-free survival (6).

II. Tier 2 (Variants of unknown significance in myeloid malignancies):

NONE DETECTED

References:
1. T. J. Carrocci et al., SF3b1 mutations associated with myelodysplastic syndromes alter the fidelity of branchsite selection in yeast. Nucleic Acids Res 2017. PMID: 28062854.
2. Y. Wan, C. J. Wu, SF3B1 mutations in chronic lymphocytic leukemia. Blood 2013. PMID: 23568491.
3. S. Jeromin et al., SF3B1 mutations correlated to cytogenetics and mutations in NOTCH1, FBXW7, MYD88, XPO1 and TP53 in 1160 untreated CLL patients. Leukemia 2014. PMID: 24113472.
4. D. A. Landau et al., Mutations driving CLL and their evolution in progression and relapse. Nature 2015. PMID: 26466571.
5. F. Nadeu et al., Clinical impact of clonal and subclonal TP53, SF3B1, BIRC3, NOTCH1, and ATM mutations in chronic lymphocytic leukemia. Blood 2016. PMID: 26837699.
6. K. Takahashi et al., Clinical implications of cancer gene mutations in patients with chronic lymphocytic leukemia treated with lenalidomide. Blood 2018. PMID: 29358183.
7. D. Rossi et al., Mutations of the SF3B1 splicing factor in chronic lymphocytic leukemia: association with progression and fludarabine-refractoriness. Blood 2011. PMID: 22039264.
8. D. G. Oscier et al., The clinical significance of NOTCH1 and SF3B1 mutations in the UK LRF CLL4 trial. Blood 2013. PMID: 23086750.
9. S. Stilgenbauer et al., Gene mutations and treatment outcome in chronic lymphocytic leukemia: results from the CLL8 trial. Blood 2014. PMID: 24652989.
10. Z. Zhang et al., SF3B1 mutation is a prognostic factor in chronic lymphocytic leukemia: a meta-analysis. Oncotarget 2017. PMID: 29050251.
11. A. Schnaiter et al., NOTCH1, SF3B1, and TP53 mutations in fludarabine-refractory CLL patients treated with alemtuzumab: results from the CLL2H trial of the GCLLSG. Blood 2013. PMID: 23821658.

Low coverage regions:
This list contains exons where the average sequencing depth (number of times a particular position is sequenced) for 20% or more of the region is below our stringent cutoff of 300. Sensitivity for detection of low allelic frequency mutations may be reduced in areas with low depth of coverage. The sequencing reads from these exons were manually reviewed. If high quality variants are detected in these regions, they will be listed above in Tier 1 or Tier 2.

NONE

This result has been reviewed and approved by Jay Patel, M.D.

10-Mar-20 08:09:00 Chronic Lymphocytic Leukemia Interp:

BACKGROUND INFORMATION: Chronic Lymphocytic Leukemia (CLL) Mutation Panel by Next Generation Sequencing

CHARACTERISTICS: Chronic lymphocytic leukemia (CLL) is a hematopoietic disorder characterized by monoclonal B cell proliferation. Recent studies have identified recurrently mutated genes with diagnostic and/or prognostic impact in CLL and other lymphoid malignancies. The presence of certain mutations may inform clinical management. This multi-gene panel by massively parallel sequencing (next generation sequencing) is a more cost-effective approach when compared to the cost of multiple single-gene tests. This test can be used to complement the morphologic and cytogenetic workup of CLL and other lymphoid malignancies.

GENES TESTED: ATM, BCL2, BIRC3*, BRAF, BTG1, BTK, CARD11, CD79B, CXCR4, DDX3X, FBXW7, IKZF3, KRAS, MAP2K1, MED12, MGA, MYD88, NOTCH1, NRAS, PLCG2, POT1, RPS15*, SAMHD1, SF3B1, TP53, XPO1, ZMYM3

* - One or more exons of the preferred transcript were not covered by sequencing for the indicated gene; see limitations section below.

METHODOLOGY: Genomic DNA was isolated from peripheral blood or bone marrow then enriched for the targeted exonic regions of the tested genes. The variant status of the targeted genes was determined by massively parallel sequencing. The hg19 (GRCh37) human genome assembly was used as a reference for identifying genetic variants.

LIMITATIONS: Variants outside the targeted regions or below the limit of detection are not identified. Variants in regions that are not included in the preferred transcript for the targeted genes are not detected. In some cases, variants may not be identified due to technical limitations in the presence of pseudogenes or in repetitive or homologous regions. It is also possible some insertion/deletion variants may not be identified.

The following regions were not sequenced due to technical limitations of the assay:

BIRC3 (NM_001165) exon 5

RPS15 (NM_001018) exon 3

LIMIT OF DETECTION (LOD): 5 percent variant allele fraction (VAF) for single nucleotide variants (SNV) and small variants less than 24 base pairs (bp). Variants greater than 24bp may be detected at LOD, but the analytical sensitivity may be reduced.

ANALYTICAL SENSITIVITY: The positive percent agreement (PPA) estimate for the respective variant classes (with 95 percent credibility region) are listed below. Genes included on this test are a subset of a larger methods-based validation from which the PPA values are derived.

Single nucleotide variants (SNVs): 96.9 percent (95.1 – 98.1 percent)

Insertions/Duplications (1-24bp): 98.1 percent (95.5 – 99.3 percent)

Insertions/Duplications (greater than 24bp): Greater than 99 percent (92.9 - 100.0 percent)

Deletions (1-24bp): 96.7 percent (92.8 - 98.7 percent)

Deletions (greater than 24bp): 90 percent (79.5 - 96.1 percent)

Multi-nucleotide variants (MNVs): 97 percent (93.0 – 99.0 percent)

CLINICAL DISCLAIMER: Results of this test must always be interpreted within the context of clinical findings and other relevant data and should not be used alone for a diagnosis of malignancy. This test is not intended to detect minimal residual disease.

Test developed and characteristics determined by ARUP Laboratories. See Compliance

Statement B: aruplab.com/CS