FOCUS::CLL™

Next-Generation Sequencing Panel

for **Chronic Lymphocytic Leukemia**

Access the Next-Generation of Cancer Diagnostics

Finding the mutation that matters can make all the difference.

One of the major challenges in cancer diagnostics is tumor heterogeneity. Mutations that have critical clinical implications may only be present at very low levels, making detection of these mutations difficult. Detection of such mutations is especially important in hematological malignancies, where tumors show a great deal of heterogeneity and accurate prognosis is essential to identifying patients with more aggressive disease. Next-generation sequencing (NGS) provides a comprehensive view of the tumor's genomic profile by detecting multiple mutations present at very low levels.

CGI's Unique Targeted NGS Panel

Indicated for CLL/SLL, Focus::CLL™ is a unique NGS panel with 7 actionable biomarkers that have value for prognosis and treatment selection. Based on the Focus::CLL™ result, each patient will receive the most suitable treatment tailored to their unique cancer. By individualizing diagnosis and treatment selection, Focus::CLL™ helps deliver the promise of personalized medicine.

A Reliable Solution for Your Patients

With an analytical sensitivity of 5%, Focus::CLL™ surpasses other sequencing methodologies and offers robust specificity (>99%). Focus::CLL™ is a valuable tool to help monitor patients and guide clinical management throughout the course of the disease.

Gene	Exons	Clinical Relevance
TP53	2-11	Indicates an overall poor outcome and reduced time to treatment
NOTCH1	25-28 & 34	Indicates an overall poor outcome and an increased risk of transformation
SF3B1	13-19	Indicates an overall poor prognosis
BIRC3	6-8	Indicates an overall poor prognosis
ATM	2-63	Indicates an overall poor prognosis
MYD88	3-5	Indicates a favorable outcome
CARD11	2-8	Used to assist in treatment selection

Methodology and Interpretation

After extraction, regions of interest relative to the 7 target genes are amplified using specific primers. Multiplexed sequencing by synthesis is performed using the MiSeq System (Illumina©). Sequencing reads are aligned and annotated. Variants identified in each gene are confirmed and reported as pathogenic or uncertain with reference to the predicted functional effect of the variant.

Reporting

Non-synonymous single nucleotide variants and short insertions/deletions in covered exons and splice junctions are reported and confirmed by conventional Sanger sequencing, Pyro-sequencing, or by independent NGS analysis. The results of the assay should be interpreted in the context of available clinical, pathologic, and laboratory information. Identification of mutation should not be used alone for the prognosis of CLL/SLL. A negative finding cannot exclude the possibility of CLL/SLL diagnosis.

Specimen Requirements

- One Lavender (EDTA) tube of peripheral blood or bone marrow aspirate. Minimum: 2-3 mL.
- Shipped at room temperature.

TAT 10-14 days

CPT Codes 81405; 81408; 81479(x5)

CAP ACCREDITED™

Focus::CLL™ Sample Report

Results: **Pathogenic mutations are detected in the TP53 and SF3B1 genes.**

GENE	REFERENCE SEQUENCE	EXONS TESTED	MUTATION(S) DETECTED	FUNCTIONAL IMPACT
TP53	NM_000546.5	2-11	**c.481G>A;p.A161T**	**Pathogenic**
NOTCH1	NM_017617.3	25-28 & 34	None	Not Applicable
SF3B1	NM_012433.2	13-19	**c.2098A>G;p.K700E**	**Pathogenic**
BIRC3	NM_182962.2	6-8	None	Not Applicable
ATM	NM_000051.3	2-63	None	Not Applicable
MYD88	NM_001172567.1	3-5	None	Not Applicable
CARD11	NM_032415.4	2-8	None	Not Applicable

Interpretation: **A single nucleotide variant was detected in the TP53 gene and is expected to impact the function of the protein.**

A single nucleotide variant was detected in the SF3B1 gene and is expected to impact the function of the protein.

TP53 (Tumor Protein 53) is located at chromosome 17p13 and encodes for a transcription factor that responds to diverse cellular stresses to regulate target genes that induce cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. Somatic TP53 gene alterations including mutations and deletions are frequent in most human cancers and in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) occurs in approximately 10-12% of cases at diagnosis.[1] While up to two-thirds of CLL/SLL with del(17p13) also harbor TP53 mutations, only a fraction carry TP53 mutations without del(17p13) (3-5%).[2] More than 90% of mutations in CLL are in the DNA-binding domain of TP53, with most being missense mutations.[3] CLL patients with TP53 abnormalities have an aggressive clinical course, require earlier intervention because of progressive disease and clinical symptoms and respond poorly to conventional DNA-damaging chemotherapy but also to its combination with rituximab.[3-8] Recently, two studies have suggested a limited number of cases with TP53 abnormality and mutated IGHV genes may have stable disease for many years, never requiring therapy.[9,10]

The Splicing factor 3B subunit 1 (SF3B1) gene product is part of the U2 snRNP complex involved in anchoring pre-mRNA for splicing. Mutations have been suggested to lead to defective spliceosome assembly, deregulated global mRNA splicing and nuclear-cytoplasm export, and altered expression of multiple genes. More than 95% of SF3B1 mutations reported to date in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) are in exons 14-16 (assessed in this assay), being mostly missense mutations with the following hotspots: codons 662, 666, 700 and 742.[11-13] SF3B1 mutations occur in about 4% of unselected previously untreated CLL/SLL patients and higher in selected patients (9-17%).[11-18] The presence of SF3B1 somatic mutations in CLL/SLL has been associated with overall poor outcome (shorter time to disease progression and poor overall survival).[14-17] A recent integrated mutational and cytogenetic model for CLL/SLL prognostication suggested that cases with SF3B1 or NOTCH1 mutations be classified into intermediate risk group with or without del(11q22-q23).[18]

Description: Risk stratification in Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL) currently comprises the use of a combination of clinical and molecular features.[19] Molecular analyses generally include assessment of the mutation status of the clonal IGHV rearrangement, gain or loss of specific genomic loci, and more recently somatic mutations in relevant genes: TP53, NOTCH1, SF3B1, BIRC3, ATM, and MYD88.[19] For untreated CLL/SLL patients, 7-10% exhibit mutations in TP53, 5-12% in NOTCH1, 4-10% in SF3B1, 2-5% in BIRC3, 4-10% in ATM, and 1-5% in MYD88, often with exclusivity.[11, 20-21] Mutations in TP53, NOTCH1, SF3B1, BIRC3, and ATM are found to be associated with more aggressive disease and unfavorable outcome.[6,8,16,17,22-26] On the other hand, MYD88 mutations are predominantly found in cases with mutated clonal IGHV rearrangement, associated with favorable outcome.[11,24] CARD11 mutations are suggested to have prognostic value in mature B-cell lymphomas in response to certain treatments that are also indicated for CLL/SLL.[25-26]

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