Cairo Diagnostics

Medical Director
Sherif Ibrahim, M.D. Ph.D.

NY State PFI# 8707
NY State License# 224332-1
NJ State License# 25MA08078000
CLIA# 33D2047314

244 Westchester Ave
White Plains, NY 10604

T. (914) 339 5000
F. (914) 881 3561

cairodiagnostics.com

Specimen	PERIPHERAL BLOOD	**Final Report**	08/30/2025, 3:56pm
Your No	123456 **Requisition**	**Case No.**	**Z25-00179**
Collected	08/27/25, 9:00pm	**Received**	08/27/25, 1:00pm

Patient			
Name	**Doe, Jane**		
DOB	01/01/46 (79 yrs)	**Sex**	Female
ID#		**Tel.**	

Physician					
Facility	Location123			**Account No.**	10604-test
Attending:	John Smith, M.D.	**Tel.**		**Fax**	
Corresponding		**Tel.**		**Fax**	

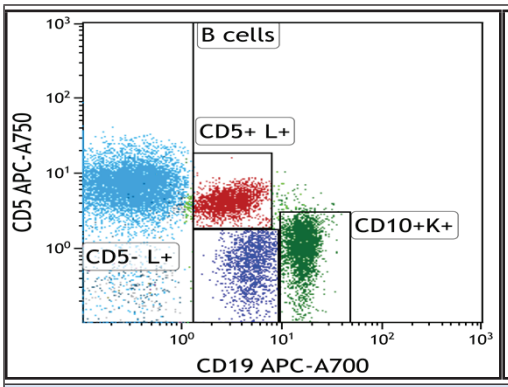
DIAGNOSIS

PERIPHERAL BLOOD

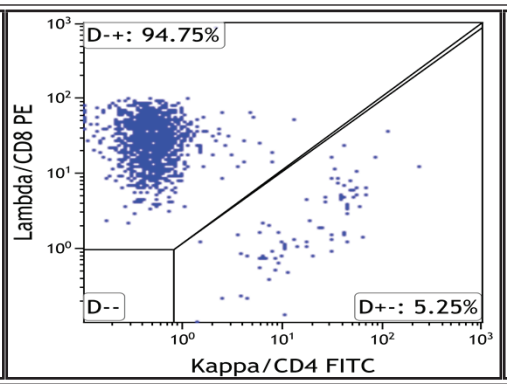
- FLOW CYTOMETRY: THREE POPULATIONS OF CLONAL B CELLS ARE DETECTED:

A) CD5 POSITIVE LAMBDA RESTRICTED B CELLS WITH CLL PHENOTYPE (5%)
B) CD5/CD10 NEGATIVE LAMDA RESTRICTED B CELLS (3%)
C) CD10 POSITIVE KAPPA RESTRICTED B CELLS WITH FOLLICULAR LYMPHOMA PHENOTYPE (7%)

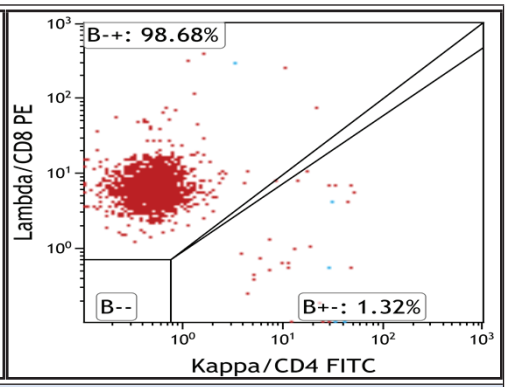
- POSITIVE FISH STUDIES FOR LOW-LEVEL TRISOMY 12 AND IGH/BCL2 TRANSLOCATION.
- IGVH MUTATION STATUS: MUTATED (MUTATION RATE: 5%).
- POSITIVE NGS STUDY FOR EZH2 C.1936T>C (AF: 4.22%, TIER II) MUTATION.



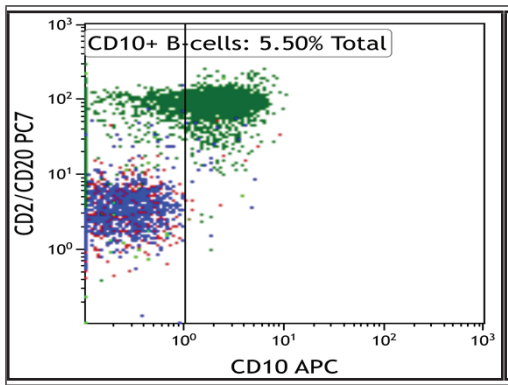
CD5 Positive B Cells (Red)



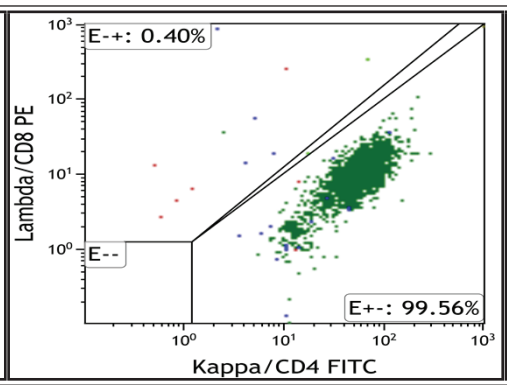
CD5-Negative Lambda Restricted B Cells



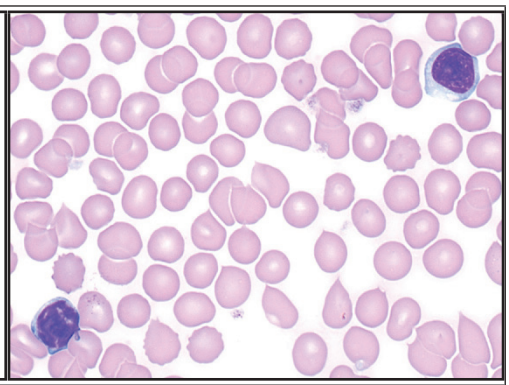
CD5-Positive Lambda Restricted B Cells



CD10 Positive B Cells



CD10-Positive Kappa Restricted B Cells



Lymphocytosis

Cairo Diagnostics

Medical Director
Sherif Ibrahim, M.D. Ph.D.

NY State PFI# 8707
NY State License# 224332-1
NJ State License# 25MA08078000
CLIA# 33D2047314

244 Westchester Ave
White Plains, NY 10604

T. (914) 339 5000
F. (914) 881 3561

cairodiagnostics.com

Patient Name Doe, Jane **Case No. Z25-00179**

COMMENT

The findings are supportive of at least two different subtypes of B cell lymphoma (CD5+), CLL and follicular lymphoma(CD10+). Correlation with relevant clinical data (lymph nodes and spleen status) is recommended for complete interpretation.
The case was discussed with Dr. Smith on 8/27/25.

CLINICAL HISTORY

79 year old female with lymphocytosis, rule out leukemia/lymphoma. ICD-10: C91.10, D72.820, C95.90, C85.90.

VIABILITY AND ANALYSIS

The specimen has viability of ~92%. The above data were generated on a population of cells that cytometrically correspond to lymphoid cells. The gated lymphoid population comprises ~42% of the total cells.

PHENOTYPE

The B cells represent ~15% of the total. Three aberrant subsets of B cells are noted. One of the subsets of B cells (~7% of the total) expresses the pan B-cell markers CD19, CD20 (bright), and CD22 (dim). These co-express CD10 and CD23, lack CD5, and show kappa light chain restriction. A second subset of B cells (~5% of the total) express CD19, CD20 (dim), and CD22 (dim), co-express CD5 (dim) and CD23, and shows lambda light chain restriction. A third subset of B cells (~3% of the total) express CD19, CD20 (dim), and CD22 (dim), are negative for CD5 and CD10, and show lambda restriction. All B cells are negative for CD11c, CD25, and CD103. The T-cell population (~23% of the total) expresses the pan T-cell antigens (CD2, CD3, CD5, and CD7) in a non-aberrant fashion. The CD4/CD8 ratio is normal, at ~3/2. The myeloid elements (~51% of the total) express maturation markers and show normal forward and side scatter properties with no significant increase in blasts.

PERIPHERAL BLOOD

A CBC study (performed on 08/27/2025) was submitted, and a peripheral blood smear preparation was performed at Cairo Diagnostics: WBCs 9.99, Hgb 12.9, MCV 89.4, Platelets 322.
Manual differential.
Neutrophils: 47, Bands: 2, Lymphocytes: 44, Monocytes: 5, Eosinophils: 2.

Peripheral blood smear examination shows normocytic normochromic red cells with mild anisocytosis. Platelets are adequate and are morphologically unremarkable. Neutrophils are adequate and show complete maturation with no left shift, nuclear, or cytoplasmic abnormalities. Lymphocytes are mildly increased and most are small to intermediate in size, with scant cytoplasm. A few lymphocytes show deeply cleaved nuclei, and others have prominent nucleoli.

Markers Tested				
B-Cell Markers	**T-Cell Markers**	**Myeloid Markers**	**Plasma Cell Markers**	**Miscellaneous Markers**
CD19	**CD4**	**CD11b**		**CD38**
CD10	**CD5**	**CD33**		**CD117**
CD20	**CD2**	**CD13**		**CD34**
CD22	**CD3**	**CD14**		**CD45**
Lambda	**CD8**	**CD16**		**CD56**
Kappa	**CD7**			**HLA-DR**
CD11c				**CD25**
CD103				
CD23				
CD79b				
CD49d				
CD200				

Electronically Signed and Reported by: Sherif Ibrahim, M.D. , PhD

Cairo Diagnostics

Medical Director
Sherif Ibrahim, M.D. Ph.D.

NY State PFI# 8707
NY State License# 224332-1
NJ State License# 25MA08078000
CLIA# 33D2047314

244 Westchester Ave
White Plains, NY 10604

T. (914) 339 5000
F. (914) 881 3561

cairodiagnostics.com

Patient Name Doe, Jane **Case No. Z25-00179**

FISH DIAGNOSIS

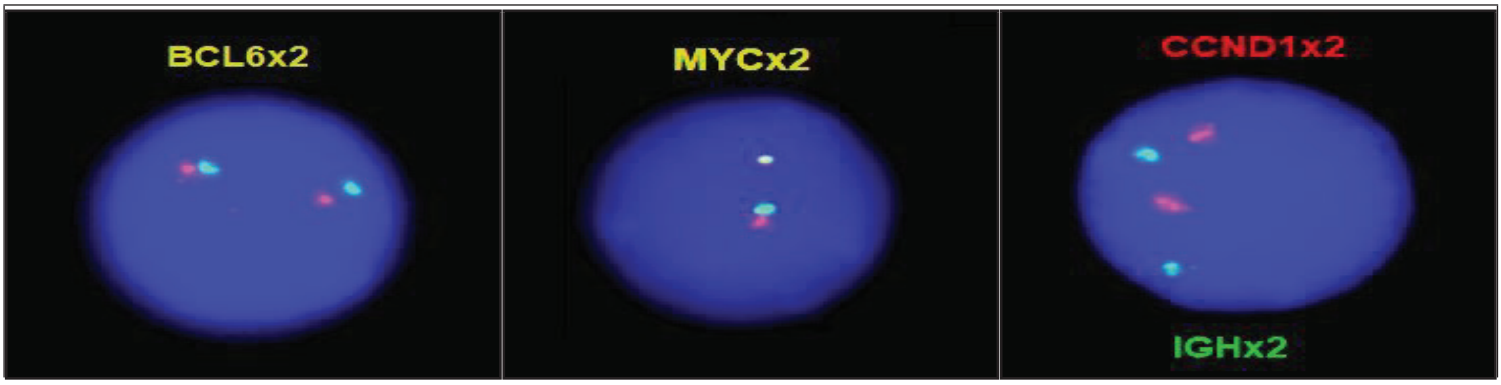
PERIPHERAL BLOOD

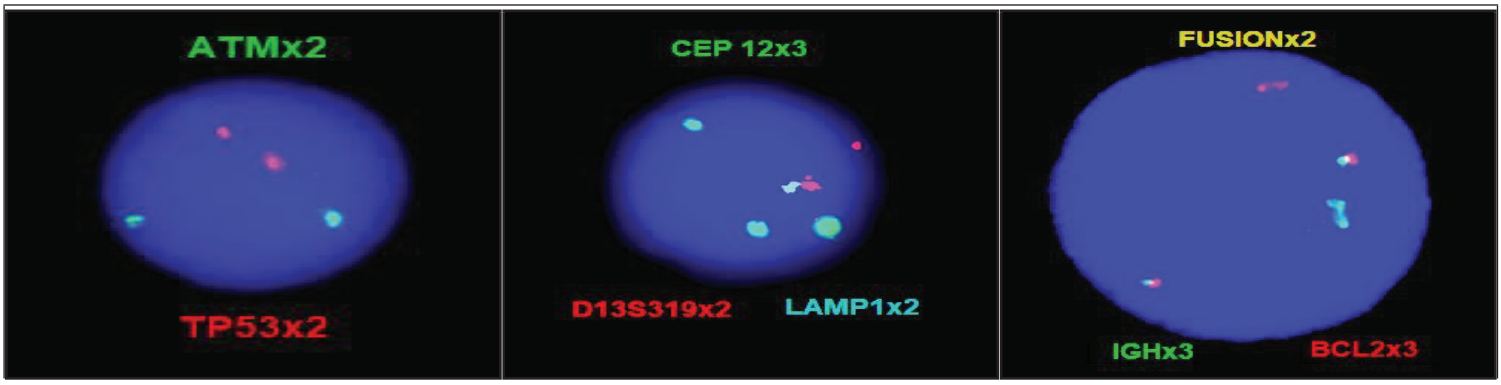
- CLL FISH study is positive for Low-Level Trisomy 12.
- Follicular Lymphoma FISH study is positive for Low-Level IGH/BCL2 translocation t(14;18).

Director of Cytogenetics: Karim Ouahchi, MD **Hematopathologist:** Sherif Ibrahim, MD, PhD

RESULT nuc ish (BCL6)x2,(MYC)x2,(CCND1,IGH)x2,(ATM,TP53)x2,(D12Z3x3,D13S319x2,LAMP1x2),(IGH,BCL2)x3(IGH con BCL2)x2; Abnormal FISH Pattern

Locus	Probe	Result	Cells Counted
11q13.3/14q32.3	CCND1/IGH	Negative/ (98%)	200
11q22.3/17p13.1	ATM/TP53	Negative/ (96%)	200
12p11.1-q11	D12Z3	Positive/ Trisomy 12 (5.5%)	200
13q14.3/13q34	D13S319/LAMP1 (CLL)	Negative/ (96.5%)	200
3q27	BCL6	Negative/ (100%)	200
8q24.21	MYC	Negative/ (99.5%)	200
14q32.3/18q21	IGH/BCL2	Positive/ IGH/BCL2 Translocation (4.0%)	200





Cairo Diagnostics

Medical Director
Sherif Ibrahim, M.D. Ph.D.

NY State PFI# 8707
NY State License# 224332-1
NJ State License# 25MA08078000
CLIA# 33D2047314

244 Westchester Ave
White Plains, NY 10604

T. (914) 339 5000
F. (914) 881 3561

cairodiagnostics.com

Patient Name Doe, Jane **Case No. Z25-00179**

INTERPRETATION

FISH study is positive for gain of an additional copy of chromosome 12 (trisomy 12) in 8.5% of nuclei. FISH study is positive for BCL2/IGH translocation t(14;18) in 8.5% of nuclei.

The finding of trisomy 12 is consistent with chronic lymphocytic leukemia (CLL) / small lymphocytic lymphoma (SLL). This abnormality occurs in 14% of patients with B-CLL and portends an average prognosis (median survival times and treatment-free intervals of 114 and 33 months, respectively).

IGH/BCL2 gene rearrangement is associated with translocation t(14;18) and is seen in up to 90% of grade 1-2 follicular lymphomas and 15% of diffuse large B cell lymphomas. Correlation with hematopathology and flow-cytometry is suggested. FISH study is negative for CCND1/IGH translocation t(11;14). The lack of CCND1/IGH translocation excludes mantle cell lymphoma.

FISH study is negative for 13q deletion, negative for ATM/11q deletion, and negative for TP53/17p13 deletion. FISH study is negative for MYC (8q24) translocation or amplification, and negative for gain of BCL6 (3q27) or BCL6 (3q27) translocation. Limitations: Please note that this FISH test detects only the loci specifically targeted by these probes. It does not detect any other numerical nor structural abnormalities of the chromosomes that are not tested. Correlation with other clinical and laboratory findings is recommended.

For any probes tested, cutoff values were determined by analyzing normal cases. If the percentage of abnormal cells is at or below the cutoff values, the probe is considered normal. If the percentage is above the cutoff values, the probe is considered abnormal.

FISH PROBE: NORMAL CUTOFF
BCL6: BCL6 Rearrangement: >2.3% / Loss of BCL6: >4.4% / Extra Copy of BCL6: >2.3% / Others: >4.4%
MYC: MYC Rearrangement: >4.4% / Loss of MYC: >3.8% / Extra Copy of MYC: >4.4% / Others: >3.8%
IGH;BCL2: IGH;BCL2 Translocation: >1.5% / Single Fusion: >5.1% / Extra Copy of IGH: >3.8% / Extra Copy of BCL2: >1.5% / Others: >3.8%
CCND1;IGH: CCND1;IGH Translocation: >1.5% / Single Fusion: >5.1% / Extra Copy of CCND1: >1.5% / Extra Copy of IGH: >1.5% / Others: >3.8%
ATM: ATM Deletion: >3.8% / Others: >1.5%
CEP 12: Trisomy 12: >2.3% / Others: >2.3%
D13S319: D13S319 Deletion: >5.1% / Biallelic D13S319 Deletion: >2.3% / Others: >3.8%
TP53: TP53 Deletion: >5.1% / Others: >1.5%

Imaging analysis for all FISH probes are performed manually. No automated imaging for FISH is performed by Cairo Diagnostics, LLC.

The following probes contain 2 or more separate fluorochromes and are considered multiplex probes: ABBOTT MOLECULAR PROBES: 1PTEL/P58/1Q25, CEP 3/MYB, BCL6, FGFR3/IGH, D5S23;D5S721/EGR1, D7Z1/D7S522, CEP 8 (D8Z2)/D20S108, RUNX1T1/RUNX1, MYC, MYC/IGH/CEP 8, ABL1/BCR, CEP 9/CEP 15/TP53, CCND1/IGH, BIRC3/MALT1, ATM/TP53, MLL, CEP 12/D13S319/LAMP1, ETV6, D13S319/LAMP1, IGH, IGH/MAF, IGH/BCL2, PML/RARA, CBFB,TP53/CEP 17 and CYTOCELL FISH PROBES: CHIC2/FIP1L1/PDGFRA, PDGFRB, PCM1/JAK2, FGFR1/CEP 8, IGH/MAFB.

Cairo Diagnostics

Medical Director
Sherif Ibrahim, M.D. Ph.D.

NY State PFI# 9615
NY State License# 224332-1
NJ State License# 25MA08078000
CLIA# 31D2213643

50 Tice Blvd. Ste 183
Woodcliff Lake, NJ 07677

T. (888) 530 2239
F. (201) 573 0719

cairodiagnostics.com

Specimen	PERIPHERAL BLOOD	**Final Report**	08/30/2025, 3:56pm
Your No	123456 **Requisition**	**Case No.**	**Z25-00179**
Collected	08/27/25, 9:00pm	**Received**	08/27/25, 1:00pm

Patient			
Name	Doe, Jane		
DOB	01/01/46 (79 yrs)	**Sex**	Female
ID#		**Tel.**	

Physician					
Facility	Location123			**Account No.**	10604-test
Attending:	John Smith, M.D.	**Tel.**		**Fax**	
Corresponding		**Tel.**		**Fax**	

MOLECULAR STUDIES

PERIPHERAL BLOOD

RESULTS: POSITIVE for NGS lymphoid panel

1. EZH2 c.1936T>C (p.Tyr646His, Tier II) mutation was detected with 4.22% allele frequency.

Interpretation:

The molecular study is positive for EZH2 (c.1936T>C, p.Tyr646His, 4.22%) mutation. The specific variant has been detected in lymphoid neoplasms (COSMIC, cBioPortal, cBioPortal GENIE). EZH2, a chromatin-modifying gene (CMG) and a key component of the polycomb repressive complex 2 (PRC2), is frequently mutated in B-cell lymphomas. Alongside other CMGs such as KMT2D, CREBBP, and EP300, EZH2 mutations are observed in approximately 40% of high-grade B-cell lymphomas, 20% of follicular lymphomas (FL), and 10% of marginal zone lymphomas, while being rare in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and mantle cell lymphoma (MCL). Tazemetostat, a selective EZH2 inhibitor, has received FDA approval for the treatment of relapsed or refractory FL in appropriate clinical settings (NCCN Guidelines, B-Cell Lymphomas, Version 2.2025). According to the NCCN Guidelines (Version 3.2024), Tazemetostat is listed as an option for third-line and subsequent therapy in relapsed/refractory FL, regardless of EZH2 mutation status.

In FL, EZH2 and other CMGs (KMT2D, CREBBP, EP300) play a major role in epigenetic dysregulation. To enhance prognostication, the m7-Follicular Lymphoma International Prognostic Index (m7-FLIPI) was developed. This model integrates the mutational status of seven genes (EZH2, ARID1A, MEF2B, EP300, FOXO1, CREBBP, and CARD11), the traditional FLIPI score, and the Eastern Cooperative Oncology Group (ECOG) performance status to improve risk stratification in patients with advanced-stage FL receiving first-line chemoimmunotherapy. Incorporating these molecular and clinical parameters has been shown to better predict 5-year failure-free survival (PMID: 31814159; PMID: 26256760; Blood 2019, 134(Suppl 1):122).
At present, the therapeutic and prognostic implications of EZH2 and related CMG mutations in FL are not fully established and are not yet formally integrated into routine clinical decision-making algorithms (NCCN Guidelines, B-Cell Lymphomas, Version 3.2024).

Director of Molecular: Sherif Ibrahim, M.D. , PhD **Hematopathologist:** Sherif Ibrahim, M.D. , PhD

Cairo Diagnostics

Medical Director
Sherif Ibrahim, M.D. Ph.D.

NY State PFI# 9615
NY State License# 224332-1
NJ State License# 25MA08078000
CLIA# 31D2213643

50 Tice Blvd. Ste 183
Woodcliff Lake, NJ 07677

T. (888) 530 2239
F. (201) 573 0719

cairodiagnostics.com

Patient Name Doe, Jane **Case No. Z25-00179**

COMMENT

Test Information

The NGS Lymphoid Assay is a multi-biomarker assay that covers the major lymphoid neoplasms, from blood, bone marrow and or tissue FFPE specimens including: CLL/SLL, DLBCL, and FL.

This assay is designed to detect specific single nucleotide variants (SNV) and small InDels (Sensitivity: 3%) within defined regions. It detects variants in 66 DNA target genes. The assay might not detect very large deletions or insertions. The minimum sample coverage is 500x.

A negative test does not exclude the presence of mutated cells.

The variant tier categorization is based on AMP/ASCO/CAP consensus recommendations by using Ion Reporter, which is four tiers evidence-based classification. (Li et al., JMD. 2017, 19 (1): 4-23; PMID: 27993330).

The variant has not been reported in publicly available databases (COSMIC; gnomAD) or in the literature as either a somatic mutation in tumors or as a population variant. Due to the lack of clinical and functional evidence, its clinical significance is currently considered as “Unknown”.

Results of this test must always be interpreted in the context of clinical, morphologic and immunophenotypic data.

Methods:

Genomic DNA, extracted from peripheral blood, bone marrow aspirate or tissue FFPE specimens, is subjected to a PCR-based library amplification by using the Comprehensive Lymphoid Neoplasms NGS Panel (Designed and developed by Cairo Diagnotics). Coding and non-coding regions of the selected genes are enriched and sequenced on an Ion Torrent S5 utilizing the ion torrent superconductor technology. The sequencing data are mapped to the human genome (reference build GRCh37/hg19). The following genes including 66 key DNA target genes are interrogated by this test:

5 Full Genes: ATM, CREBBP, KMT2D, NOTCH1, TP53.

61 Hotspot Genes: ARID1A, B2M, BCL2, BIRC3, BRAF, BTK, CARD11, CD79B, CDKN1B, CDKN2A, CIITA, CXCR4, DDX3X, DNMT3A, EP300, EZH2, FBXW7, FOXO1, GNA13, ID3, IDH1, IDH2, IRF4, JAK1, JAK2, JAK3, KLF2, KMT2C, KRAS, MAP2K1, MEF2B, MTOR, MYC, MYD88, NF1, NOTCH2, NRAS, PAX5, PHF6, PIK3CA, PIM1, PLCG2, PRDM1, PTEN, PTPN11, PTPRD, RB1, RHOA, SETD2, SF3B1, SGK1, SOCS1, SPEN, STAT3, STAT5B, STAT6, TCF3, TET2, TNFAIP3, NFRSF14, XPO1.

Cairo Diagnostics

Medical Director
Sherif Ibrahim, M.D. Ph.D.

NY State PFI# 9615
NY State License# 224332-1
NJ State License# 25MA08078000
CLIA# 31D2213643

50 Tice Blvd. Ste 183
Woodcliff Lake, NJ 07677

T. (888) 530 2239
F. (201) 573 0719

cairodiagnostics.com

Specimen	PERIPHERAL BLOOD	**Final Report**	08/30/2025, 3:56pm
Your No	123456 **Requisition**	**Case No.**	**Z25-00179**
Collected	08/27/25, 9:00pm	**Received**	08/27/25, 1:00pm

Patient			
Name	Doe, Jane		
DOB	01/01/46 (79 yrs)	**Sex**	Female
ID#		**Tel.**	

Physician					
Facility	Location123			**Account No.**	10604-test
Attending:	John Smith, M.D.	**Tel.**		**Fax**	
Corresponding		**Tel.**		**Fax**	

MOLECULAR STUDIES

PERIPHERAL BLOOD

IgVH RESULTS:
IgVH Mutation Status: Mutated
Mutation Rate: 5%
IgVH Family: IGHV3-23*01

INTERPRETATION
- Reference Range: < 2%: unmutated, ≥ 2%: mutated.
- The clonal B-cell population detected in this sample is greater than or equal to 2% mutated compared with germline sequence. This is a favorable prognostic indicator in chronic lymphocytic leukemia.

Director of Molecular: Sherif Ibrahim, M.D. , PhD **Hematopathologist:** Sherif Ibrahim, M.D. , PhD

Cairo Diagnostics

Medical Director
Sherif Ibrahim, M.D. Ph.D.

NY State PFI# 9615
NY State License# 224332-1
NJ State License# 25MA08078000
CLIA# 31D2213643

50 Tice Blvd. Ste 183
Woodcliff Lake, NJ 07677

T. (888) 530 2239
F. (201) 573 0719

cairodiagnostics.com

Patient Name Doe, Jane **Case No. Z25-00179**

COMMENT

Test Information (IgVH):

B-cell CLL patients can be stratified into prognostically indolent and more aggressive disease types by assessment of the presence or absence of somatic mutations in the variable region of the immunoglobulin heavy chain gene locus (IgVH). Patients with unmutated IgVH (< 2% mutated) have a shorter median survival than those with mutated IgVH (> 2% mutated).

Other adverse independent prognostic factors in CLL, according to multivariate analysis, include clinical-stage, loss or mutation of p53 (17p deletion), and loss of ATM (11q deletion). Patients with a normal karyotype or deletion of 13q14 (RB1) have a better prognosis than those with a complex karyotype. CD38 and ZAP-70, commonly assessed by flow cytometry, correlate with IgVH mutational status, in that high CD38 and/or ZAP-70 expression is overrepresented in the unmutated (germline or naïve) group of
patients.
In mantle cell lymphoma (MCL) patients, classical mantle is usually composed of IGHV-unmutated or minimally mutated B cells and typically involves lymph nodes and other extranodal sites. Acquisition of additional molecular/cytogenetic abnormalities can lead to even more aggressive blastoid or pleomorphic MCL. Other MCL develop from IGHV-mutated B cells which leads to leukemic non-nodal MCL, usually involving the PB, bone marrow, and often spleen. These cases are frequently clinically indolent; however, secondary abnormalities, often involving TP53, may occur and lead to very aggressive disease.

The sensitivity of this assay is 10%; sequencing may not be possible for peripheral blood or bone marrow specimens with < 10% clonal B-CLL cells.

Methodology

Genomic DNA was extracted and amplified by polymerase chain reaction (PCR) using primer sets for leader-constant and framework-joining regions. The amplicons were detected by gel electrophoresis. PCR products meeting pre-specified criteria for a clonal population are cleaned up and analyzed by direct DNA sequencing using capillary electrophoresis. The resultant DNA sequence of the IgH V region was then compared and matched to the most homologous germline V sequence within the genomic database of the NCBI (National Center for Biotechnology Information) using the analysis tool IgBLAST (Basic Local Alignment Search Tool). False positive or negative results may occur for reasons that include genetic variants, blood transfusions, or somatic heterogeneity of the tissue.