

Template for Reporting Results of Biomarker Testing of Specimens From Patients With Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

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For the Members of the Cancer Biomarker Reporting Committee, College of American Pathologists

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CAP Chronic Lymphocytic Leukemia Biomarker Template Revision History

Version Code

The definition of version control and an explanation of version codes can be found at www.cap.org (search: cancer protocol terms).

Version: CLL_Biomarkers 1.0.0.1

Summary of Changes

RESULTS

The following note was added:

Note: If a marker is tested by more than one method (eg, polymerase chain reaction and immunohistochemistry), please document the additional result(s) and method(s) in the Comments section of the report.

The following data elements were changed to "select all that apply":

Chromosomal Abnormalities

Protein Expression.

Sequence Based Testing

Somatic gene mutations

Added: Other gene mutation(s) (specify): _____

Not detected

Detected (specify variant): _____

METHODS

Added unit of measure (kbp) to:

Molecular Testing

Array platform: _____

Minimum size of detected copy number variation (CNV): _____ kbp

Added the following data element and notes:

+ COMMENT(S)

Gene names should follow recommendations of The Human Genome Organisation (HUGO) Nomenclature Committee (www.genenames.org; accessed February 10, 2015).

All reported gene sequence variations should be identified following the recommendations of the Human Genome Variation Society (www.hgvs.org/mutnomen/; accessed February 10, 2015).

Chronic Lymphocytic Leukemia Biomarker Reporting Template

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Completion of the template is the responsibility of the laboratory performing the biomarker testing and/or providing the interpretation. When both testing and interpretation are performed elsewhere (eg, a reference laboratory), synoptic reporting of the results by the laboratory submitting the tissue for testing is also encouraged to ensure that all information is included in the patient's medical record and thus readily available to the treating clinical team.

CHRONIC LYMPHOCYTIC LEUKEMIA/SMALL LYMPHOCYTIC LYMPHOMA (CLL)

Select a single response unless otherwise indicated.

Note: Use of this template is optional.

+ SPECIMEN TYPE

- + ___ Peripheral blood
- + ___ Bone marrow
- + ___ Lymph node (specify site): _____
- + ___ Other (specify): _____

+ RESULTS

Note: If a marker is tested by more than one method (eg, polymerase chain reaction and immunohistochemistry), please document the additional result(s) and method(s) in the Comments section of the report.

+ Chromosomal Abnormalities (Note B) (select all that apply)

- + ___ 13q deletion
 - + ___ Not detected
 - + ___ Detected
 - + ___ Other abnormal signal patterns (specify): _____
- + ___ Trisomy 12
 - + ___ Not detected
 - + ___ Detected
 - + ___ Other abnormal signal patterns (specify): _____
- + ___ 11q deletion
 - + ___ Not detected
 - + ___ Detected
 - + ___ Other abnormal signal patterns (specify): _____
- + ___ 17p deletion
 - + ___ Not detected
 - + ___ Detected
 - + ___ Other abnormal signal patterns (specify): _____
- + Other probes tested
 - + Specify probe: _____
 - + Specify results: _____
- + Additional copy number variations noted
 - + Gains (specify regions): _____
 - + Losses (specify regions): _____

+ Data elements preceded by this symbol are not required.

- + Loss of heterozygosity
 - + ___ Not identified
 - + ___ Identified (specify regions): _____
- + Cytogenetic testing complete karyotype (specify): _____

+ Protein Expression (Notes C and D) (select all that apply)

- + ___ ZAP-70
 - + ___ Not expressed (percent of CLL cells positive): _____
 - + ___ Expressed (percent of CLL cells positive): _____
- + ___ CD38
 - + ___ Not expressed (percent of CLL cells positive): _____
 - + ___ Expressed (percent of CLL cells positive): _____

+ Sequence Based Testing

- + Immunoglobulin heavy chains (IgVH) hypermutation status
 - + ___ Mutated ($\leq 97\%$ identity to reference)
 - + ___ Unmutated ($\geq 98\%$ identity to reference)
 - + ___ Borderline ($>97\%$ and $<98\%$ identity to reference)
- + IGHV3-21 usage
 - + ___ Not detected
 - + ___ Detected
- + Somatic gene mutations
 - + ___ *TP53*
 - + ___ Not detected
 - + ___ Detected (specify variant): _____
 - + ___ Other gene mutation(s) (specify): _____
 - + ___ Not detected
 - + ___ Detected (specify variant): _____
- + Other markers tested
 - + Specify marker: _____
 - + Specify results: _____

+ METHODS

+ Chromosomal Abnormalities

- + ___ Chromosomal array
- + ___ Fluorescence in situ hybridization (FISH)
- + ___ Conventional karyotype

+ Molecular Testing

- + Array platform: _____
 - + Minimum size of detected copy number variation (CNV): _____ kbp
- + Gene sequencing platform: _____
 - + Maximum sensitivity (variant allele frequency): _____
 - + Genes/exons sequenced: _____

+ Protein Expression (Notes C and D)

- + ___ Flow cytometry
- + ___ Immunohistochemistry
- + ZAP-70 positive threshold: _____
- + CD38 positive threshold: _____

+ COMMENT(S)

Gene names should follow recommendations of The Human Genome Organisation (HUGO) Nomenclature Committee (www.genenames.org; accessed February 10, 2015).

All reported gene sequence variations should be identified following the recommendations of the Human Genome Variation Society (www.hgvs.org/mutnomen/; accessed February 10, 2015).

Explanatory Notes

A. Introduction

Somatic mutation in the rearranged variable regions of immunoglobulin heavy chains (IgVH) has been reported to be of prognostic importance since 1999.^{1,2} Patients with IgVH unmutated genes have a more aggressive disease and are more resistant to therapy than those with mutated IgVH genes. Most researchers defined unmutated IgVH based on 98% or more homology to reference and mutated IgVH with less than or equal to 97% homology to reference.³ Determining IgVH mutations requires specific equipment and is laborious, expensive, and time-consuming. Due to all these limitations, surrogate markers including CD38 and ZAP-70, with the similar prognostic value as IgVH mutation status are more widely used.

Detection of immunoglobulin VH3-21 usage by sequencing of IgH rearrangements has been associated with poor outcome in CLL and should be reported when detected by IgH sequencing.

B. Prognosis in CLL FISH

Del 11q contains several tumor suppressor genes including *ATM*.^{4,5} This gene is associated with cell cycle regulation and p53 pathway activation. *BIRC3*, which is also in the deleted region of interest, is a candidate gene that may also play a role in CLL pathobiology. Del 11q is associated with younger age and poor prognosis.

Del 13q is often seen as a sole abnormality in CLL.^{4,5} It is associated with a favorable prognosis. Several genes and micro-RNAs (mRNA) have been suggested as candidate genes in these cases of CLL.

Del 17p is thought to affect the *TP53* gene, a key regulator of cell cycle.^{4,5} Other deleted genes may also play a role. Patients with del17p will often have other genetic abnormalities and other poor prognosis markers.

Trisomy 12 (+12) affects CLL by an unknown mechanism.^{4,5} Patients with trisomy 12 have a good response to treatment. Some additional trisomies (+19, +19) are seen in association with trisomy 12.

Marker	Approx. Frequency	Prognosis	Notes
Del 13q14	35%-45%	Low risk	
Trisomy 12	11%-16%	Intermediate-High risk	
Del 11q22-23 (<i>ATM</i> ; <i>BIRC3</i>)	10%-17%	Intermediate-High risk	Bulky disease, aggressive clinical course, shorter survival
Del 17p (<i>TP53</i>)	3%-7%	High risk	Frequently no response to therapy or relapse after therapy
No abnormalities by FISH		Low-Intermediate risk	

C. CD38 Expression

CD38 is a 45 kDa transmembrane glycoprotein that was the first marker found to correlate with IgVH mutation.² Patients with CD38-positive cells have unmutated IgVH genes and higher need for chemotherapy as well as shorter overall survival. However, subsequent studies showed that association between mutation status and CD38 expression level was not absolute and that CD38 expression should be considered as an independent prognostic marker in CLL.⁶ CD38 expression is determined by flow cytometry. A 30% cutoff level is generally used empirically to classify CD38-positive and CD38-negative patients; individual laboratories should determine their own criteria for calling CD38-positive and CD38-negative cases, and specific laboratory cut-offs should be described in the methods section above.^{1,6} CD38 expression may vary over time and may show a bimodal expression profile.⁴

D. ZAP-70 Expression

Comparative microarray studies performed on cases of CLL with mutated and unmutated IgVH genes showed differential expression of gene encoding for zeta-associated protein of 70 kDa (ZAP-70).⁷ Zap-70 is normally

expressed in T cells and NK cells. The majority of the CLL cases with mutated IgVH are ZAP-70 negative, while cases with unmutated IgVH are ZAP-70 positive. ZAP-70 expression in CLL cells can be determined by various methods including western blotting, quantitative reverse transcription polymerase chain reaction (RT-PCR), immunohistochemistry, and flow cytometry. However, flow cytometry is the preferred technique for assessing ZAP-70 expression in CLL cells.⁸ Flow cytometry allows simultaneous evaluation of ZAP-70 protein expression in CLL cells and normal lymphocytes. A 20% cutoff threshold is commonly used to separate ZAP-70-negative from ZAP-70-positive CLL cases; however, this threshold may vary significantly from laboratory to laboratory depending on how negative controls are defined.

There is inherent laboratory-to-laboratory variability in ZAP-70 testing due to the following: different antibody clones used (variable antigen affinity), different conjugated fluorochromes (variable intensity), variable methods of cell permeabilization (for intracellular staining), variable staining procedures, variable gating procedures, and variable reporting methods. Moreover, ZAP-70 is a labile protein; most consensus guidelines recommend ZAP-70 testing within 24 hours of sample collection. Laboratories should establish firm gating criteria for sample collection and determine reference populations at the point of method validation of their assay to ensure optimal interassay precision. Different gating strategies are discussed extensively in a prior multicenter international harmonization study.⁹

References

1. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated IgV(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood*. 1999;94(6):1848-1854.
2. Damie RN, Wasil T, Fais F, et al. Ig VH gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. *Blood*. 1999;94(6):1840-1847.
3. Bockstaele FV, Verhasselt B, Philippe J. Prognostic markers in chronic lymphocytic leukemia: a comprehensive review. *Blood Reviews*. 2009;23:2-47.
4. Rosenquist R, Cortese D, Bhoi S, Gunnarsson R. Prognostic markers and their clinical applicability in chronic lymphocytic leukemia: where do we stand? *Leuk Lymphoma*. 2013;54(11):2351-2364.
5. Chiorazzi N. Implications of new prognostic markers in chronic lymphocytic leukemia. *Hematology Am Soc Hematol Educ Program*. 2012;2012:76-87.
6. Hamblin TJ, Orchard JA, Ibboston RE, et al. CD38 expression and immunoglobulin variable region mutations are independent prognostic variables in chronic lymphocytic leukemia, but CD38 expression may vary during the course of the disease. *Blood*. 2002;99(3):1023-1029.
7. Rosenwald A, Alizadeh AA, Widhopf G, et al. Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. *J Exp Med*. 2001;194(11):1639-1647.
8. Crespo M, Bosch F, Villamor N, et al. ZAP-70 expression as a surrogate for immunoglobulin-variable-region mutations in chronic lymphocytic leukemia. *N Engl J Med*. 2003;348(18):1764-1775.
9. Letestu R, Rawstron A, Ghia P, et al. Evaluation of ZAP-70 expression by flow cytometry in chronic lymphocytic leukemia: a multicentric international harmonization process. *Cytometry B Clin Cytom*. 2006;70(4):309-314.