MAYO CLINIC LABORATORIES

Test Definition: CXLPL

CXCR4 Mutation in B-cell Lymphoma

Overview

Useful For

Aiding in the prognostication and clinical management of lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia

Genetics Test Information

This test detects gene variants within the C-terminal end of the *CXCR4* gene that are commonly found in association with *MYD88* L265P variants in cases of lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia (LPL/WM).

Highlights

This test offers highly sensitive detection of the well-characterized hotspot variants c.1013C->G/A, p.S338X and routine Sanger sequencing for other variant in the C-terminus region. It is strongly recommended that this test be used in the context of the MYD88 / *MYD88,* L265P, Somatic Gene Mutation, DNA Allele-Specific PCR assay during evaluation of lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia (LPL/WM).

Special Instructions

- Hematopathology Patient Information

Method Name

Mutation Detection in DNA using BNAClamp Sanger Sequencing Technology and Routine Sanger Sequencing

(BNAClamp is utilized pursuant to a license agreement with BNA Inc)

NY State Available

Yes

Specimen

Specimen Type

Varies

Shipping Instructions

Specimen must arrive within 10 days of collection.

Necessary Information

The following information is required:

1. Pertinent clinical history

2. Clinical or morphologic suspicion

3. Date and time of collection

4. Specimen source

Specimen Required

Submit only 1 of the following specimens:

Specimen Type: Blood

Container/Tube:

Preferred: Lavender top (EDTA)

Acceptable: Yellow top (ACD)

Specimen Volume: 3 mL

Collection Instructions:

1. Invert several times to mix blood.

2. Send specimen in original tube.

3. Label specimen as blood.

Specimen Stability Information: Ambient (preferred)/Refrigerated

Specimen Type: Bone marrow aspirate

Container/Tube:

Preferred: Lavender top (EDTA)

Acceptable: Yellow top (ACD)

Specimen Volume: 2 mL

Collection Instructions:

1. Invert several times to mix bone marrow.

2. Send specimen in original tube.

3. Label specimen as bone marrow.

Specimen Stability Information: Ambient (preferred)/Refrigerated

Specimen Type: Extracted DNA from blood or bone marrow

Container/Tube: 1.5- to 2-mL tube

Specimen Volume: Entire specimen

Collection Instructions: Label specimen as extracted DNA from blood or bone marrow and provide indication of volume and concentration of the DNA

Specimen Stability Information: Frozen (preferred)/Refrigerated/Ambient

Specimen Type: Paraffin-embedded tissue

Container/Tube: Paraffin block

Specimen Stability Information: Ambient

Specimen Type: Tissue

Slides: Unstained slides

Specimen Volume: 10 slides

Additional Information: Tissue must demonstrate involvement by a hematologic neoplasm (eg, acute myelocytic leukemia), not solid tumors.

Specimen Stability Information: Ambient

Forms

1. Hematopathology Patient Information (T676) in Special Instructions

2. If not ordering electronically, complete, print, and send a Hematopathology/Cytogenetics Test Request (T726) with the specimen.

Specimen Minimum Volume

Blood, Bone marrow: 1 mL
Extracted DNA: at least 20 mcL with a concentration of at least 10 nanograms per mcL

Reject Due To

Gross hemolysis	Reject
Other	B5 fixed tissues, Decalcified bone marrow core biopsies Paraffin shavings Frozen tissue Methanol acetic acid (MAA) fixed pellets Moderately to severely clotted

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies	10 days	

Clinical and Interpretive

Clinical Information

Lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia (LPL/WM) is a B-cell lymphoma characterized by an aberrant accumulation of malignant lymphoplasmacytic cells in the bone marrow, lymph nodes, and spleen. It is a B-cell neoplasm that can exhibit excess production of serum IgM symptoms related to hyperviscosity, tissue filtration, and autoimmune-related pathology. *CXCR4* variants are identified in approximately 30% to 40% of LPL/WM patients and are almost always associated with *MYD88* L265P, which is highly prevalent in this neoplasm. The status of *CXCR4* variants in the context of *MYD88* L265P is clinically relevant as important determinants of clinical

presentation, overall survival, and therapeutic response to ibrutinib. A *MYD88*-L265P/*CXCR4*-WHIM (C-terminus nonsense/frameshift variants) molecular signature is associated with intermediate to high bone marrow disease burden and serum IgM levels, less adenopathy, and intermediate response to ibrutinib in previously treated patients. A *MYD88*-L265P/*CXCR4*-WT (wild type) molecular signature is associated with intermediate bone marrow disease burden and serum IgM levels, more adenopathy, and highest response to ibrutinib in previously treated patients. A *MYD88*-WT/*CXCR4*-WT molecular signature is associated with inferior overall survival, lower response to ibrutinib therapy in previously treated patients, and lower bone marrow disease burden in comparison to those harboring a *MYD88*-L265 variant.

Reference Values

Variants present or absent in the test region of the *CXCR4* gene (NCBI NM_003467.2, GRCh37).

Interpretation

Variants detected or not detected. An interpretive report will be issued.

Cautions

This test is a targeted assay for the C-terminal end of the *CXCR4* gene only. It examines c.898-1059 of the *CXCR4* gene (NCBI NM_003467.2 GRCh37) and does not detect variants outside this region. A 1% analytical sensitivity was established at 50 ng DNA input for the hotspot variants c.1013C->G/A only, which uses BNA-clamped Sanger sequencing and DNA that does not meet the established criteria can lead to false-negative results. In the extremely rare event that a rare polymorphism, insertion, or deletion may occur at the Sanger sequencing primer binding sites, in cis, with a c.1013C->G/A, data can yield a failed result. Routine Sanger sequencing is used to interrogate other variants in the tested region with a 15% to 20% analytical sensitivity. The analytical sensitivity of the assay can be affected by a variety of factors, including biologic availability (ie, tumor burden), fixation of paraffin-embedded specimens, rare polymorphisms, insertions or deletions at the primer binding sites, or nonspecific PCR interferences.

Clinical Reference

1. Hunter ZR, Xu L, Yang G, et al: The genomic landscape of Waldenstrom macroglobulinemia is characterized by highly recurring MYD88 and WHIM-like CXCR4 mutations, and small somatic deletions associated with B-cell Lymphomagenesis. Blood 2014;123(11):1637-1646

2. Landgren O, Tageja N: MYD88 and beyond: novel opportunities for diagnosis, prognosis and treatment in Waldenstromâ€™s Macroglobulinemia. Leukemia 2014;28:1799-1803

3. Poulain S, Roumier C, Venet-Caillault A, et al: Genomic Landscape of *CXCR4* Mutations in Waldenstrom Macroglobulinemia. Clin Cancer Res 2016;22(6):1480-1488

4. Roccaro A, Sacco A, Jimenez C, et al: C1013G/CXCR4 acts as a driver mutation of tumor progression and modulator of drug resistance in lymphoplasmacytic lymphoma. Blood 2014;123(26):4120-4131

5. Schmidt J, Federmann B, Schindler N, et al: *MYD88* L265P and *CXCR4* mutations in lymphoplasmacytic lymphoma identify cases with high disease activity. Br J Haematol 2015;169:795-803

6. Treon S, Cao Y, Xu L, et al: Somatic mutations in MYD88 and CXCR4 are determinants of clinical presentation and overall survival in Waldenstrom macroglobulinemia. Blood 2014;123(18):2791-2796

7. Treon S, Tripsas C, Meid K, et al: Ibrutinib in previously treated Waldenstromâ€™s Macroglobulinemia. NEJM 2015;372(15):1430-1440

8. Xu L, Hunter ZR, Tsakmaklis N, et al: Clonal architecture of *CXCR4* WHIM-like mutations in Waldenstrom Macroglobulinaemia. Br J Haematol 2016;172:735-744

Performance

Method Description

The C-terminal end of *CXCR4 (*NM_003467.2, c.898-1059) is amplified from extracted genomic DNA by polymerase chain reaction, followed by Sanger sequencing and capillary electrophoresis analysis. Review of the sequence data is performed using a combination of automated calls and manual inspection.(Unpublished Mayo method)

The hotspot mutations c.1013C->G/A (p.S338X) are examined using bridged nucleic acids (BNA) clamped Sanger sequencing with an analytic sensitivity of 1%. All other genetic variants in the test region are examined by routine Sanger sequencing with an analytic sensitivity of 15% to 20%.(Unpublished Mayo method)

PDF Report

No

Day(s) and Time(s) Test Performed

Monday through Friday

Analytic Time

7 days

Maximum Laboratory Time

10 days

Specimen Retention Time

DNA: 3 months

Performing Laboratory Location

Rochester

Fees and Codes

Fees

- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact Customer Service.

Test Classification

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information

81479-Unlisted molecular pathology procedure

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
CXLPL	CXCR4 Mutation in B-cell Lymphoma	In Process

Result ID	Test Result Name	Result LOINC Value
MP032	Specimen Type	31208-2
113436	CXLPL Result	59465-5
38287	Final Diagnosis	50398-7