

Lymphoplasmacytic lymphoma: recommendations for next-generation sequencing

Linfoma linfoplasmocítico: recomendaciones sobre el estudio de secuenciación masiva

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Abstract

Introduction: The integration of laboratory and clinical technologies and knowledge is key to an accurate diagnosis of hematologic neoplasms. Over the past decade, next-generation sequencing (NGS) has enabled the molecular characterization of these diseases, identifying mutations, copy number variations, and recurrent fusion genes. This advancement has driven the update of recommendations for the diagnosis and management of patients with hematologic neoplasms. Recent classifications by the World Health Organization (WHO), the International Consensus Classification (ICC), and the European LeukemiaNet (ELN) highlight the relevance of genetic data, incorporating them into their diagnostic and risk stratification algorithms. In this context, the implementation of targeted NGS panels is recommended for their ability to simultaneously analyze multiple genetic alterations. **Objective:** Although NGS is available in many laboratories, its use is not yet standardized, and there is no clear consensus on the methodology or the genes to be evaluated for each pathology. **Material and method:** The Molecular Biology Group in Hematology (GBMH), in collaboration with a panel of experts, developed this consensus guide. **Results:** Genes of interest were compiled based on their clinical relevance and scientific evidence. **Conclusions:** This guide aims to assist professionals in the use of targeted NGS panels in lymphoplasmacytic lymphoma.

Keywords: Lymphoplasmacytic lymphoma. Next-generation sequencing (NGS). Consensus guidelines.

Resumen

Introducción: La integración de tecnologías y conocimientos del laboratorio y la clínica es clave para un diagnóstico preciso de las neoplasias hematológicas. En la última década, la secuenciación masiva (NGS) ha permitido caracterizar el perfil molecular de estas enfermedades, identificando mutaciones, variaciones en el número de copias y genes de fusión recurrentes. Este avance ha impulsado la actualización de las recomendaciones para el diagnóstico y manejo de los pacientes con neoplasias hematológicas. Las clasificaciones recientes de la World Health Organization (WHO), la International

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*Consensus Classification (ICC) y la European LeukemiaNet (ELN) destacan la relevancia de los datos genéticos, incorporándolos en sus algoritmos diagnósticos y de estratificación del riesgo. En este contexto, la implementación de paneles dirigidos de NGS se recomienda por su capacidad para analizar múltiples alteraciones genéticas. **Objetivo:** Aunque la NGS está disponible en muchos laboratorios, su uso no está estandarizado y no hay consenso sobre la metodología ni los genes que evaluar en cada afección. **Material y método:** El Grupo de Biología Molecular en Hematología (GBMH), en colaboración con un panel de expertos, elaboró esta guía consensuada. **Resultados:** Recoge los genes de interés según su relevancia clínica y evidencia científica. **Conclusiones:** Esta guía busca orientar a los profesionales sobre el uso de paneles dirigidos de NGS en el linfoma linfoplasmocítico.*

Palabras clave: Linfoma linfoplasmocítico. Secuenciación masiva (NGS). Guías de consenso.

Introduction

Lymphoplasmacytic lymphoma (LPL) of IgM type, more widely known as Waldenström macroglobulinemia (WM), is an uncommon B-cell lymphoproliferative neoplasm characterized by the infiltration of the bone marrow by a LPL and the presence of a monoclonal immunoglobulin (Ig) of the IgM type of any degree^{1,2}. It represents 2% of lymphomas, with an incidence rate of 3.1 cases per million inhabitants per year in Spain, although this incidence rate may be underestimated. It mainly affects elderly people (median age at diagnosis > 70 years)³. It is an indolent disease with a median survival of 11 years. Clinical presentation is variable, with symptoms related to medullary tumor and or to the monoclonal component⁴.

At genomic level, it is characterized by the presence of the *MYD88* L265P hotspot mutation in virtually all patients (> 90%)⁵, which allows for the differential diagnosis against other lymphoproliferative syndromes, such as marginal zone lymphoma, where its frequency is much lower (5-10%). It is also present in most (~50-60%) patients with IgM monoclonal gammopathy of undetermined significance (IgM-MGUS), a condition that precedes WM⁶. In addition to the L265P mutation, other variants in *MYD88* (such as S219C, M232T, and S243N) have been identified may have diagnostic relevance in certain cases⁷.

The determination of the *MYD88* L265P mutation can be performed using allele-specific quantitative polymerase chain reaction (PCR), digital PCR, or by next-generation sequencing (NGS)⁸. In patients with low medullary infiltration, the separation of CD19-positive cells can improve diagnostic accuracy. The isolation of the tumor B-cell population reduces the interference from other cells, thus optimizing the evaluation of relevant mutations.

Digital PCR offers advantages in these cases, as it allows for a highly sensitive and precise detection of mutations such as *MYD88* L265P and *CXCR4* S338X.

International clinical practice guidelines on the management of WM consider the detection of the *MYD88* mutation in peripheral blood (PB) as a valid alternative when bone marrow is not available. The detection of *MYD88* in PB allows for a less invasive diagnosis and an accurate evaluation if techniques such as digital PCR are used.

Studies to be performed using NGS panels in lymphoplasmacytic lymphoma

NGS studies can complement the diagnosis and can also be important for prognosis, follow-up, and treatment selection. Table 1 illustrates the 5 selected genes in this pathology.

CLINICAL UTILITY OF NGS PANEL STUDIES IN LYMPHOPLASMACYTIC LYMPHOMA

The detection of *MYD88* L265P can be useful to confirm the diagnosis of WM and IgM-MGUS and to support the diagnosis of a small group of patients with LPL who do not secrete IgM⁹. At a functional level, the mutated *MYD88* protein is constitutively activated, promoting cell survival pathways through the positive regulation of Bruton's tyrosine kinase (BTK). This finding drove the research of BTK inhibitors for the treatment of patients with WM¹⁰. *MYD88* mutations condition the quality and duration of responses to BTK inhibitors such as ibrutinib and zanubrutinib, both of which are currently approved and available in Spain for the treatment of WM. Thus, patients without *MYD88* mutations do not usually achieve objective responses with these drugs in monotherapy, especially with ibrutinib, the first to be approved for this indication^{11,12}.

Other frequent alterations are mutations in the *CXCR4* gene (> 30% of patients)¹³. Same as with *MYD88*, *CXCR4* mutations cause constant activation and perpetual signaling of the chemokine receptor. Patients with *CXCR4* mutations have a reduced

Table 1. Most relevant genes related to lymphoplasmacytic lymphoma included in the next-generation sequencing (ngs) panel

Gene	Clinical value	Level of evidence
<i>MYD88</i>	Diagnosis, treatment	Mandatory
<i>BTK</i>	Treatment	Recommended
<i>CXCR4</i>	Diagnosis, treatment	Recommended
<i>PLCG2</i>	Treatment	Recommended
<i>TP53</i>	Prognosis, treatment	Recommended

sensitivity to BTK inhibitors (ibrutinib, zanubrutinib) with less depth and duration of response^{7,13,14}. Specifically, nonsense mutations (grouped around the 338 hotspot, codons 318-246) vs frameshift mutations, were associated with a slower and less profound response and a shorter progression-free survival after monotherapy treatment with ibrutinib^{12,15-18}. *CXCR4* mutations can, therefore, be predictive of the duration of response to BTK inhibitor therapy¹⁴⁻¹⁷, which is why their study is highly recommended by the European Consortium for Waldenström's Macroglobulinemia (ECWM) if the patient is to be treated with these drugs⁸.

Furthermore, mutations in *BTK* at the ibrutinib binding site (Cys481) and in *PLCG2*, such as R665W and L845F, are common in patients who progress during treatment with this drug¹⁹. In patients with resistance or relapse after treatment with BTK inhibitors, the analysis of mutations associated with resistance becomes especially relevant to guide a change in inhibitor or to consider alternative therapies.

Finally, the detection of alterations in *TP53* can be valuable for prognostic evaluation and therapeutic decision-making in patients with WM. Recent studies have shown that *TP53* alterations, including mutations and deletions, were present in 11% of newly diagnosed WM patients and in > 25% at relapse^{18,20}. In addition, they were associated with a significantly shorter overall survival, regardless of the score on the International Prognostic Scoring System for WM (IPSSWM)²⁰.

Furthermore, there are consensus guidelines that recommend the study of these alterations. Thus, the National Comprehensive Cancer Network (NCCN) in its clinical guidelines on the management of WM/LPL version 2.2024, suggests the evaluation of genetic mutations, including *TP53*, as part of the prognostic evaluation and for the selection of therapy²¹. Similarly, the 3rd consensus panel of the 11th International Workshop on

Table 2. Most relevant structural alterations related to lymphoplasmacytic lymphoma included in the next-generation sequencing (ngs) panel

Structural alteration	Clinical value	Level of evidence
6q (deletion)	Diagnosis	Recommended
17p (deletion)	Prognosis, treatment	Recommended

Waldenström's Macroglobulinemia also recommends its study in all patients in whom treatment is planned²². Finally, the ECWM guidelines recommend the evaluation of *TP53* as part of diagnosis and risk stratification, highlighting its importance in treatment selection⁸.

In cases of small B-cell lymphomas with plasmacytic differentiation (LPL, marginal zone lymphomas, both nodal and splenic and extranodal, and follicular lymphoma [FL]), the detection of the *MYD88* L265P mutation is highly suggestive of LPL, but not absolutely specific, since it can be found in other lymphomas. The presence of the *CXCR4* mutation concomitantly with *MYD88* L265P does greatly increase the specificity for LPL²³. The deletion of the long arm of chromosome 6 – del(6q) – constitutes the most frequent chromosomal abnormality in WM, affecting 40-50% of patients²⁴ (Table 2). In addition, its frequency increases from IgM-MGUS (4% of patients) to asymptomatic WM (9% of patients) and symptomatic WM (30% of patients), and it is associated with an adverse prognosis. Asymptomatic patients with del(6q) required therapy more frequently and had a shorter time to transformation to symptomatic disease. Treated patients with del(6q) had a shorter progression-free survival (median of 20 vs 47 months in patients without deletion) and lower overall survival²⁵.

For the study of cytogenetic alterations such as deletion of 6q and 17p (*TP53*), techniques such as FISH or SNP arrays are usually used. However, currently, commercial NGS panels are available that allow the detection of both somatic point variants and copy number alterations, such as del(6q) and del(17p), and they include adequate bioinformatics tools for this analysis. Therefore, the study of the 6q and 17p deletions can be integrated as part of NGS in clinical practice.

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Conflicts of interest

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Ethical considerations

Protection of people and animals. The authors declare that no experiments on human beings or animals have been performed for this research.

Confidentiality, informed consent, and ethical approval. The study does not involve personal patient data and does not require ethical approval. The SAGER guidelines do not apply.

Statement on the use of artificial intelligence. The authors declare that they did not use any type of generative artificial intelligence for the writing of this manuscript.

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