

Large B-cell lymphoma: recommendations for next-generation sequencing

Linfoma B de células grandes: recomendaciones para el estudio de secuenciación masiva

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Abstract

Introduction: The integration of molecular technologies into clinical practice is essential for the diagnosis and risk stratification of hematologic neoplasms. Next-generation sequencing (NGS) has enabled the genomic characterization of large B-cell lymphomas (LBCL), identifying mutations, copy number variations, and recurrent fusion genes. This advance have motivated the development of specific recommendations for its clinical application. **Objective:** To develop a consensus guideline defining the priority genes and genetic alterations to be assessed by NGS in LBCL. **Material and method:** The Molecular Biology Group in Hematology (GBMH), in collaboration with a panel of experts, reviewed recent literature and the main international classifications (World Health Organization, International Consensus Classification y European LeukemiaNet). Through structured discussion and consensus, genes and chromosomal regions of greatest clinical relevance were selected and categorized into levels of evidence (mandatory, recommended, and optional) based on their diagnostic, prognostic, and therapeutic utility. **Results:** A panel of 60 genes and several structural alterations relevant to LBCL was agreed upon. The genomic associations with the described molecular subtypes are detailed, as well as their clinical value. The guideline provides practical recommendations for the implementation of targeted NGS panels in route clinical practice, highlighting where their use may provide significant diagnostic and prognostic benefit. **Conclusions:** These recommendations constitute the first national consensus guideline for the application of targeted NGS panels in LBCL. Their adoption will promote the standardization of diagnostic practice and contribute to improved classification and management of patients.

Keywords: Large B-cell lymphoma. Next generation sequencing. Consensus guidelines.

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Resumen

Introducción: La integración de tecnologías moleculares en la práctica clínica es fundamental para el diagnóstico y la estratificación del riesgo en las neoplasias hematológicas. La secuenciación masiva (NGS, next-generation sequencing) ha permitido caracterizar el perfil genómico de los linfomas B de células grandes (LBCG), identificando mutaciones, variaciones en el número de copias y genes de fusión recurrentes. Este avance ha motivado la elaboración de recomendaciones específicas para su aplicación clínica. **Objetivo:** Desarrollar una guía de consenso que defina los genes y las alteraciones genéticas prioritarias que deben evaluarse mediante NGS en los LBCG. **Material y método:** El Grupo de Biología Molecular en Hematología (GBMH), en colaboración con un panel de expertos, revisó la bibliografía reciente y las principales clasificaciones internacionales (Organización Mundial de la Salud, International Consensus Classification y European LeukemiaNet). Mediante discusión estructurada y consenso, se seleccionaron los genes y las regiones cromosómicas de mayor relevancia clínica, clasificándolos en niveles de evidencia (obligatorios, recomendables y opcionales) en función de su utilidad en el diagnóstico, el pronóstico y el tratamiento. **Resultados:** Se consensuó un panel de 60 genes y diversas alteraciones estructurales relevantes para los LBCG. Se detallan las asociaciones genómicas con los subtipos moleculares descritos, así como su valor clínico. La guía proporciona recomendaciones prácticas para la implementación de paneles dirigidos de NGS en la práctica clínica donde su uso puede aportar un beneficio diagnóstico y pronóstico significativo. **Conclusiones:** Estas recomendaciones constituyen la primera guía de consenso nacional para la aplicación de paneles dirigidos de NGS en los LBCG. Su adopción favorecerá la estandarización de la práctica diagnóstica y contribuirá a una mejor clasificación y un adecuado manejo de los pacientes.

Palabras clave: Linfoma B de células grandes. Secuenciación masiva. Guías de consenso.

Introduction

In these recommendations, we have included all conditions that are mainly encompassed within large B-cell lymphoma (LBCL) and that are included in the new classifications of the World Health Organization (WHO) and the International Consensus Classification (ICC) (Table 1).^{1,2}

Below, we discuss some of the characteristics of the most relevant entities.

Diffuse large B-cell lymphoma

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma in Spain; according to the Spanish Lymphoma Registry, it accounts for 30-40% of cases.³ It comprises a heterogeneous group of biologically distinct conditions resulting from the clonal proliferation of a malignant germinal or postgerminal B cell. The disease is typically aggressive, and diagnosis is usually established through biopsy of a suspicious lymph node or extranodal tumor in which normal architecture is replaced by sheets of large cells demonstrating positive staining for pan-B-cell antigens, such as CD20 and CD79a. The standard treatment for DLBCL remains chemotherapy with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone). Although this treatment approach is safe and effective, up to 45%-50% of patients experience relapse.

Genomic and transcriptomic studies of DLBCL have identified patient subgroups with poor prognosis when treated with chemoimmunotherapy.

CLASSIFICATION ACCORDING TO EXPRESSION OR GENETIC BIOMARKERS

Over the past 2 decades, multidisciplinary efforts by pathologists, molecular scientists, and clinicians have enabled the identification of unique DLBCL subtypes based on cell of origin (COO) or molecular characteristics. These classification systems are now routinely used to identify patients with high-risk disease and poorer outcomes with frontline treatments, traditionally R-CHOP and more recently Pola-R-CHP. Despite these advances, in our setting the relapse or refractory rate after R-CHOP remains approximately 40%.⁴

Cell of origin

Gene expression profiling was performed on 96 normal lymphocytes and DLBCL lymphocytes, identifying 3 unique genetic signatures with distinct patterns of somatic mutations.⁵ Germinal center B-cell-like DLBCL (GCB), which accounts for 40%-50% of cases, displays a gene expression profile characteristic of normal germinal center B cells with intraclonal heterogeneity, somatic hypermutation, and expression of CD10 and BCL6 proteins. The activated B-cell (ABC) subtype, which represents 30%-40% of cases, exhibits gene

Table 1. World Health Organization (WHO) and International Consensus Classification (ICC) classifications of large B-cell lymphomas

WHO Classification, 5 th Edition	ICC 2022
DLBCL, NOS	DLBCL, NOS (ABC/GCB)
High-grade B-cell lymphoma (HGBCL) with 11q alterations	LBCL with 11q alterations
LBCL with <i>IRF4</i> rearrangement	LBCL with <i>IRF4</i> rearrangement
T-cell/histiocyte-rich LBCL	T-cell/histiocyte-rich LBCL
LBCL associated with immune-privileged “sanctuary” sites. Includes CNS, testicular, and ocular lymphoma	CNS and ocular DLBCL; testicular DLBCL
Primary cutaneous DLBCL, leg type	Primary cutaneous DLBCL, leg type
Intravascular LBCL	Intravascular LBCL
Fluid overload-associated DLBCL	EBV- and HHV8-negative effusion lymphoma
EBV+LBCL	EBV + LBCL
Chronic inflammation-associated DLBCL	Chronic inflammation-associated DLBCL
Fibrin-associated LBCL	Fibrin-associated LBCL
Lymphomatoid granulomatosis	Lymphomatoid granulomatosis
ALK+LBCL	ALK + LBCL
Plasmablastic lymphoma	Plasmablastic lymphoma
DLBCL/HGBCL with <i>MYC/BCL2</i> rearrangement HGBCL with <i>MYC/BCL2</i> rearrangement	HGBCL with <i>MYC/BCL6</i> rearrangement
HGBCL, NOS	HGBCL, NOS
Primary mediastinal B-cell lymphoma (PMBL)	Primary mediastinal B-cell lymphoma (PMBL)
Mediastinal gray zone lymphoma (MGZL)	Mediastinal gray zone lymphoma (MGZL)

HHV8: human herpesvirus 8; HGBCL: high-grade B-cell lymphoma; LBCL: large B-cell lymphoma; DLBCL: diffuse large B-cell lymphoma; MGZL: mediastinal gray zone lymphoma; NOS: not otherwise specified; PMBL: primary mediastinal B-cell lymphoma; CNS: central nervous system; EBV: Epstein-Barr virus.

expression patterns of postgerminal or activated B cells with high expression and constitutive activity of the nuclear factor kappa B (NF- κ B) complex and expression of IRF4 and BCL2 proteins. The third subtype is unclassified and accounts for approximately 10%-15% of cases. The ability to systematically perform gene expression profiling on fresh frozen samples is limited, and immunohistochemical algorithms have become the most widely used method to determine COO in clinical

practice. The immunohistochemical algorithm developed by Hans et al.⁶ and the Tally algorithm⁷ are the most frequently used. More recently, novel platforms such as Lymph2Cx have enabled gene expression profiling in formalin-fixed paraffin-embedded tissue. Although their use remains primarily restricted to research, several studies have demonstrated better concordance than immunohistochemistry.^{8,9}

Molecular characteristics

Approximately 10-15% of patients exhibit *MYC* gene rearrangement (a proto-oncogene located on chromosome 8q24),¹⁰ and approximately half of these cases also harbor rearrangement of the antiapoptotic proto-oncogene *BCL2* or its transcriptional repressor *BCL6*. These genetic rearrangements are typically identified by fluorescence in situ hybridization (FISH). The concomitant presence of *MYC* and *BCL2* rearrangements is referred to as double-hit (DH), recognized in the most recent WHO classification as HGBCL-DH (high-grade B-cell lymphoma-double hit),¹ and usually affects 8-10% of cases, generally with a poorer prognosis. Additionally, cases may exhibit double expression (DEL), characterized by overexpression of the *MYC* oncogene and *BCL2* detected by immunohistochemistry ($\geq 40\%$ and $> 50\%$, respectively). DEL cases account for approximately one-third of de novo cases and are associated with an intermediate prognosis with frontline R-CHOP therapy. DEL can also be detected in up to 50% of relapsed/refractory DLBCL cases, which are likewise associated with poorer outcomes following salvage chemotherapy.¹¹ Of note, GCB is enriched for DH subtypes, whereas ABC is enriched for DEL subtypes. Although the concept of “double expresser” (DEL) has historically been used, neither the WHO nor the ICC currently includes or recommends its use in contemporary classification systems. Regarding *BCL6* rearrangements, there is a discrepancy between classification systems: WHO does not consider these within the double-hit lymphoma category, whereas the ICC provisionally includes them as “HGBCL with *MYC* and *BCL6* rearrangements.”^{1,2} In summary, these data highlight the need to systematically perform both immunohistochemical and FISH studies at diagnosis and, preferably, also at relapse.

Genetic subtype

The use of whole-exome sequencing has also identified novel genetic disease subtypes characterized by frequently recurrent mutations. Schmitz et al.¹²

Table 2. Genetic subtypes in diffuse large B-cell lymphoma

Genetic subtype according to Schmitz et al.	Included genes	Genetic subtype according to Chapuy et al.	Included genes
EZB (associated with GCB-DLBCL)	<i>BCL2</i> translocation; <i>EZH2</i> mut; <i>TNFRSF14</i> mut/del; <i>CREBBP</i> mut; <i>EP300</i> mut; <i>REL</i> ampl	C3	<i>BCL2</i> translocation; <i>BCL2</i> mut; <i>EZH2</i> mut; <i>CREBBP</i> mut; <i>KMT2D</i> mut; <i>TNFRSF14</i> mut/del
BN2	<i>NOTCH2</i> mut/ampl; <i>SPEN</i> mut; <i>BCL6</i> translocation; <i>TNFAIP3</i> mut; <i>BCL10</i> mut/ampl; <i>PRKCB</i> mut/ampl	C1 (associated with ABC-DLBCL)	<i>BCL6</i> translocation; <i>BCL10</i> mut/ampl; <i>TNFAIP3</i> mut; <i>UBE2A</i> mut; <i>CD70</i> mut; <i>B2M</i> mut; <i>NOTCH2</i> mut; <i>MYD88</i> mut (non-L265P)
MCD (associated with ABC-DLBCL)	<i>MYD88</i> (L265P) mut; <i>CD79A</i> or <i>CD79B</i> mut/ampl; <i>PIM1</i> mut; <i>CDKN2A</i> mut/del; <i>HLA</i> mut/del	C5	18q/18p ampl; 3q ampl; <i>CD79B</i> mut; <i>MYD88</i> mut; <i>PIM1</i> mut
N1 (associated with ABC-DLBCL)	<i>NOTCH1</i> mut; <i>IRF4</i> mut; <i>ID3</i> mut		
A53		C2	<i>TP53</i> mut/del
ST2		C4	<i>SGK1</i> mut; <i>HIST1H1A-E</i> mut; <i>NFKB1E</i> mut; <i>BRAF</i> mut; <i>CD83</i> mut; <i>CD58</i> mut

GCB: germinal center B-cell subtype; ABC: activated B-cell subtype.

analyzed 574 pretreatment DLBCL biopsy samples and identified 4 distinct genetic disease subtypes with different high-frequency recurrent mutations. These categories include the MCD, BN2, N1, and EZB subtypes.

The MCD subtype is characterized by the co-occurrence of *MYD88* (L265P) and *CD79* mutations; the BN2 subtype by *BCL6* fusions and *NOTCH2* mutations; the N1 subtype by frequent *NOTCH1* mutations; and the EZB subtype by the presence of *EZH2* and *BCL2* translocations. The MCD and N1 subtypes correspond to ABC-type cases, whereas the BN2 and EZB subtypes correspond to the GCB type. These groups show differential responses to frontline therapy. The BN2 and EZB subtypes correlate with a favorable prognosis,

whereas the remaining subtypes are associated with a poorer prognosis. Chapuy et al.¹³ classified 304 previously untreated primary DLBCL cases into 5 distinct groups. These included 2 different subtypes: low-risk ABC-DLBCL (C1 associated with *MYD88* mutations), poor-prognosis ABC-DLBCL (C5 resembling the MCD subtype with *MYD88*-L265P and *CD79* mutations), an independent ABC/GCB group (C2 characterized by chromosome 17p mutations and deletions), and finally poor- and favorable-prognosis GCB-DLBCL (C3 and C4, respectively). Subsequently, the National Cancer Institute (NCI) group refined the classification proposed by Schmitz et al.¹² by incorporating 2 new categories, ST2 and A53, corresponding to groups C4 and C2 described by Chapuy et al.,¹³ respectively. Furthermore, this study highlighted the existence of cases that do not clearly fit into any category, emphasizing the biological heterogeneity of DLBCL.¹⁴

There are 2 main methodological approaches for genomic subtype classification: hierarchical clustering analysis (Harvard model), which allows classification of most samples, and probabilistic analysis (NCI model), in which approximately one-third of cases remain without definitive assignment. To facilitate implementation in clinical practice, the LymphGen algorithm was developed, allowing probabilistic assignment of individual cases to different genetic subtypes.¹⁴

- Primary central nervous system and primary testicular DLBCL: these entities share several characteristics, including their frequent ABC phenotype and a high frequency of *MYD88* and *CD79B* alterations.
- High-grade B-cell lymphoma (HGBCL) with 11q alterations: this entity is characterized by morphological, clinical, and immunophenotypic features similar to Burkitt lymphoma, but without *MYC* rearrangement. These cases exhibit 11q alterations (11q23.2-23.3 gains and 11q24.1-qter losses) and frequently harbor associated mutations (*GNAI3*).
- LBCL with *IRF4* rearrangement: this entity commonly affects children and adolescents and generally follows a favorable clinical course. Detection of the rearrangement is usually performed using FISH techniques. Moreover, cases harboring *MYC* or *BCL2* rearrangements are excluded.
- HGBCL: morphologically, it displays more blastoid features and frequently demonstrates *MYC*, *BCL2*, and *BCL6* rearrangements. Patients with *MYC/BCL2* rearrangements have a molecular profile more similar to follicular lymphoma (*CREBBP*, *BCL2*, *KMT2D*, *MYC*, *EZH2*, and *FOXO*). Patients with *MYC/BCL6* rearrangements often exhibit extranodal involvement

and worse prognosis. Finally, HGBCL-NOS (not otherwise specified) cases are uncommon, display substantial molecular heterogeneity, and approximately half harbor *MYC* rearrangements.

- Primary mediastinal B-cell lymphoma (*PMBL*, primary mediastinal B-cell lymphoma): this is a rare and aggressive B-cell lymphoma arising from thymic B cells. It accounts for approximately 2%-3% of diffuse large B-cell non-Hodgkin lymphomas, although it shares clinical, morphological, and molecular features with the nodular sclerosis subtype of classical Hodgkin lymphoma. *BCL2*, *BCL6*, and *MYC* rearrangements are uncommon. Of note, *CIITA* rearrangements or mutations occur in up to 53% of cases. Translocations involving *CIITA* occur with *CD274* (also known as *PDCD1LG1* or *PDL1*) and *PDCD1LG2* (also known as *PDL2*). *CIITA* alterations and PDL locus copy number gains and amplifications occur in 29%-70% of PMBL cases. The genomic profile also includes gains on chromosome 2p16.1 (approximately 50% of cases). Gains involving chromosomes Xp11.4-21, Xq24-26, 7q22, 12q31, and 9q34 are also observed in approximately one-third of cases.

Clinical utility of next-generation sequencing (NGS) panels in diffuse large B-cell lymphoma

Genetic classification of patients based on published studies^{12,13}

Patients are classified according to genetic alterations with prognostic implications. **Table 2** shows the most relevant genes within each group.

Patients belonging to the C1 and C4 subgroups have a better prognosis than those classified as C2, C3, and C5. Conversely, patients classified as N1 and MCD have a poorer prognosis than those classified as EZB or BN2.

Below are the biological and therapeutic characteristics of each subgroup.

C3/EZB

Most cases exhibit *BCL2* translocations or mutations. Moreover, they show alterations in chromatin modifiers, transcription and B-cell differentiation factors, and indirect modifiers of B-cell receptor (BCR) and PI3K signaling, suggesting potential benefit from EZH2, BCL2, PI3K, and BCR inhibitors. As mentioned in the

introduction, there are 2 relevant publications in which patients are grouped according to genetic alterations with prognostic implications.

BN2/C1

The most frequent alteration is *BCL6* rearrangement in combination with *NOTCH2* alterations. In addition, abnormalities in the NF- κ B signaling pathway are observed (*BCL10*, *TNFAIP3*, and *FAS*). Genetic alterations associated with immune evasion are also present, including *B2M*, *CD70*, *FAS*, and *PD-L1* or *PD-L2* genes. Most cases are categorized as ABC-DLBCL according to transcriptional profiling; approximately 25% of cases harbor non-L265P *MYD88* mutations.

Unlike C5, this genetic subtype lacks cytidine deaminase activity, confirming its extrafollicular origin and a lower *IGH* somatic hypermutation ratio. Therefore, it may benefit from therapies targeting alterations in *NOTCH* and *BCL6* signaling pathways, in addition to strategies targeting immune evasion.

MCD/C5

This group frequently demonstrates gains in 18q leading to increased expression of *BCL2* or *MALT1*. In addition, mutations in *CD79B* and *MYD88* (L265P), alterations associated with ABC-DLBCL (COO), are commonly observed. The genetic features of this group are often associated with primary central nervous system and testicular lymphoma. This may suggest that this genetic subtype is associated with extranodal tropism. On the other hand, these tumors exhibit high cytidine deaminase activity and *IGH* somatic hypermutation, indicating prior germinal center transit.

N1

This subtype is characterized by alterations in *NOTCH1* and genes regulating B-cell transcription (*IRF4*, *ID3*, or *BCOR*), which may contribute to a plasmacytoid phenotype.

C2

This subtype commonly exhibits TP53 inactivation due to biallelic alterations (*TP53* mutations and 17p deletion). In addition, loss of 9p21.13/*CDKN2A* and 13q14.2/*RB1* is frequently observed, disrupting chromosomal stability and cell-cycle regulation. This subtype includes both ABC and GCB patients.

Table 3. Most relevant genes associated with diffuse large B-cell lymphoma recommended for inclusion in next-generation sequencing (NGS) panels for the evaluation of single nucleotide variants and small indels in coding regions and splice sites

Alteration	Clinical value	Level of evidence
<i>B2M</i>	Diagnosis (C1), prognosis	Recommended
<i>BCL2</i>	Diagnosis (GCB), treatment	Recommended
<i>BCL10</i>	Diagnosis (C1), prognosis	Recommended
<i>BRAF</i>	Diagnosis (C4), prognosis	Recommended
<i>CARD11</i>	Diagnosis (ABC), prognosis	Recommended
<i>CD58</i>	Diagnosis (C4), prognosis	Recommended
<i>CD70</i>	Diagnosis (X1), prognosis	Recommended
<i>CD79A</i>	Diagnosis (MCD/C5), prognosis	Recommended
<i>CD79B</i>	Diagnosis (MCD/C5), prognosis	Recommended
<i>CD83</i>	Diagnosis (C4), prognosis	Recommended
<i>CDKN2A</i>	Diagnosis (MCD/C5), prognosis	Recommended
<i>CREBBP</i>	Diagnosis (EZB/C3), prognosis	Recommended
<i>EP300</i>	Diagnosis (C3/EZB), prognosis	Recommended
<i>EZH2</i>	Diagnosis (C3/EZB), prognosis, treatment	Recommended
<i>GNA13</i>	Diagnosis (PMBL)	Recommended
<i>HIST1H1 family (B-E)</i>	Diagnosis (C4), prognosis	Recommended
<i>ID3</i>	Diagnosis (N1), prognosis	Recommended
<i>IL4R</i>	Diagnosis	Recommended
<i>IRF4</i>	Diagnosis (N1), prognosis	Recommended
<i>ITPKB</i>	Diagnosis (CNS)	Recommended
<i>KMT2D</i>	Diagnosis (EZB/C3), prognosis	Recommended
<i>MYD88 L265P</i>	Diagnosis (C1), prognosis, treatment	Recommended
<i>MYD88</i>	Diagnosis (C5/MCD), prognosis, treatment	Recommended
<i>NFKBIE</i>	Diagnosis (C4), prognosis	Recommended
<i>NOTCH1</i>	Diagnosis (N1), prognosis	Recommended
<i>DDX3X</i>	Diagnosis	Optional
<i>DTX1</i>	Prognosis	Optional

(Continues)

Table 3. Most relevant genes associated with diffuse large B-cell lymphoma recommended for inclusion in next-generation sequencing (NGS) panels for the evaluation of single nucleotide variants and small indels in coding regions and splice sites (*continued*)

Alteration	Clinical value	Level of evidence
<i>DUSP2</i>	Diagnosis	Optional
<i>EBF1</i>	Prognosis	Optional
<i>ETS1</i>	Diagnosis, prognosis	Optional
<i>ETV6</i>	Prognosis	Optional
<i>FAS</i>	Prognosis	Optional
<i>FOXO1</i>	Diagnosis, prognosis, and treatment	Optional
<i>GNAI2</i>	Diagnosis (GCB), prognosis	Optional
<i>HVCN1</i>	Diagnosis, prognosis	Optional
<i>IGLL5</i>	Diagnosis (DH)	Optional
<i>IRF8</i>	Diagnosis	Optional
<i>JUNB</i>	Diagnosis	Optional
<i>KLF2</i>	Diagnosis, prognosis	Optional
<i>KLHL6</i>	Prognosis	Optional
<i>KLHL14</i>	Prognosis	Optional
<i>KRAS</i>	Prognosis	Optional
<i>MEF2B</i>	Diagnosis, prognosis	Optional
<i>MYC</i>	Diagnosis (DH)	Optional
<i>NFKBIA</i>	Diagnosis, prognosis	Optional
<i>PTEN</i>	Diagnosis, prognosis	Optional
<i>STAT3</i>	Prognosis	Optional
<i>TBL1XR1</i>	Prognosis	Optional
<i>TMEM30A</i>	Prognosis	Optional
<i>ZNF217</i>	Diagnosis	Optional

ABC: activated B-cell subtype; DH: double-hit; GCB: germinal center B-cell subtype; PMBL: primary mediastinal B-cell lymphoma; CNS: central nervous system.

Genetic subtypes C1-C5 according to Schmitz et al.¹² and subtypes BN2, EZB, N1, ST2, A53, X1, or MCD according to Chapuy et al.¹³

C4

C4 is associated with genetic alterations involving histone genes, chromatin modifiers, immune-evasion molecules (*CD83*, *CD58*, and *CD70*), BCR/PI3K signaling pathways (*RHOA*, *GNA13*, and *SGK1*), NF-κB

Table 4. Most relevant structural alterations associated with diffuse large B-cell lymphoma recommended for inclusion in next-generation sequencing (NGS) panels

Alteration	Clinical value	Level of evidence
<i>NOTCH2</i>	Diagnosis (BN2/C1), prognosis	Recommended
<i>PIM1</i>	Diagnosis (C5/MCD), prognosis	Recommended
<i>PTPN1</i>	Diagnosis (PMBL)	Recommended
<i>PRDM1</i>	Diagnosis (ABC), prognosis	Recommended
<i>PRKCB</i>	Diagnosis (BN2), prognosis	Recommended
<i>RHOA</i>	Diagnosis (GCB), prognosis	Recommended
<i>SGK1</i>	Diagnosis (C4), prognosis	Recommended
<i>SOCS1</i>	Diagnosis (PMBL), prognosis	Recommended
<i>SPEN</i>	Diagnosis (BN2/C1), prognosis	Recommended
<i>STAT6</i>	Diagnosis (PMBL), prognosis	Recommended
<i>TNFAIP3</i>	Diagnosis (C1), diagnosis (PMBL), prognosis, treatment	Recommended
<i>TNFRSF14</i>	Diagnosis (C3/EZB), prognosis	Recommended
<i>TP53</i>	Diagnosis (C2), prognosis, treatment	Recommended
<i>UBE2A</i>	Diagnosis (C1), prognosis	Recommended
<i>XP01</i>	Diagnosis (PMBL), prognosis	Recommended
<i>ARID1A</i>	Prognosis	Optional
<i>BCL6</i>	Diagnosis	Optional
<i>BCL11A</i> (amplification)	Diagnosis (PMBL)	Optional
<i>BCOR</i>	Prognosis	Optional
<i>BTG1</i>	Prognosis	Optional
<i>BTG2</i>	Diagnosis, prognosis	Optional
<i>CCND1</i>	Diagnosis (CNS)	Optional
<i>CCND3</i>	Diagnosis (DH)	Optional
<i>CIITA</i>	Prognosis	Optional

(Continues)

Table 4. Most relevant structural alterations associated with diffuse large B-cell lymphoma recommended for inclusion in next-generation sequencing (NGS) panels (*continued*)

Alteration	Clinical value	Level of evidence
Fusions <i>BCL2</i> translocation <i>BCL6</i> translocation <i>MYC</i> translocation <i>ALK</i> <i>CIITA</i> <i>IRF4</i> <i>PDL1</i> , <i>PDL2</i> <i>PDCD1LG2</i> / <i>CD274</i> <i>PAX5</i> CNVs	Diagnosis (C3/EZB), prognosis, treatment Diagnosis (C1/BN2) Diagnosis, prognosis Diagnosis Diagnosis (PMBL) Diagnosis Diagnosis Diagnosis, prognosis Diagnosis	Mandatory Mandatory Mandatory Mandatory Recommended Recommended Recommended Optional Optional
Amplifications Chromosome 3 trisomy or 3q amplification 4q22-24q (<i>ALK</i> insertion) 9p24 (<i>PDL1</i> , <i>PDL2</i>) 11q Chromosome 18 trisomy, 18q amplification 4q amplification Chromosome 5 trisomy Chromosome 6 trisomy 12p Chromosome 21 trisomy	Diagnosis (C5), prognosis Diagnosis Diagnosis, prognosis Diagnosis Diagnosis (C5), prognosis Diagnosis, prognosis Prognosis Prognosis Diagnosis, prognosis Diagnosis, prognosis	Recommended Recommended Recommended Recommended Recommended Optional Optional Optional Optional Optional
Deletions 11q 6q (<i>BLIMP1</i>) 7q 8p 9p21.3 (<i>CDKN2A/B</i>) 13q 17p deletion	Diagnosis Diagnosis, prognosis Prognosis Prognosis Prognosis Diagnosis, prognosis Diagnosis, prognosis	Recommended Optional Optional Optional Optional Optional Optional

CNV: copy number variation; DH: double-hit; PMBL: *primary* mediastinal B-cell lymphoma.
Genetic subtypes C1-C5 according to Schmitz et al.¹² and subtypes BN2, EZB, N1, ST2, A53, X1, or MCD according to Chapuy et al.¹³

modifiers (*RHOA*, *GNA13*, and *SGK1*), and the RAS/JAK/STAT signaling pathway (*BRAF* and *STAT3*). Most patients belong to the GCB-DLBCL subtype. Because these tumors harbor histone mutations, mutation rates

may increase due to chromatin opening and consequently greater DNA exposure.

As a recent example of the clinical application of individualized treatment based on molecular characterization, the GUIDANCE-01 trial should be highlighted, in which treatment assignment was performed according to the DLBCL genetic subtype identified through NGS, demonstrating the feasibility of this approach in clinical practice.¹⁵

Genomic profile according to the described conditions¹⁶

Supplementary table 1 illustrates the most frequent genomic alterations among the different DLBCL conditions. Identification of these genetic alterations may improve patient classification.

Relevant methodological aspects in the genetic study of diffuse large B-cell lymphoma

Timing of evaluation

The evaluation should be performed at the time of diagnosis and in the event of failure of different treatment lines (primary or secondary relapse or refractoriness to treatment).

Studies to be performed

Assessment of *MYC*, *BCL2*, *BCL6*, and *IRF4* rearrangements is traditionally carried out using FISH. At present, evaluation of these rearrangements using NGS has not yet been standardized because, although some recent publications suggest that it appears technically feasible,¹² further validation studies are required to confirm these findings and extend their use into clinical practice. Evaluation with an NGS panel including the most relevant genes may facilitate diagnosis of different diseases and help establish prognosis.

A practical strategy consists of combining single nucleotide variant (SNV) panels with FISH studies for the detection of relevant rearrangements. Alternatively, the use of capture panels enables simultaneous analysis of SNVs, translocations, and copy number variations,¹⁷ thereby optimizing the clinical performance of NGS platforms.

Of note, according to the WHO and ICC classifications, genetic characterization by subtype is not yet

considered a diagnostic requirement for DLBCL. Nevertheless, the availability of broader and more detailed molecular studies using NGS platforms is highly recommended because it provides prognostic information and may guide future therapeutic strategies.

Recommended studies using NGS panels in diffuse large B-cell lymphoma

After conducting a literature review, the DLBCL working group reached a consensus on a list of 60 genes (Table 3) and chromosomal regions (Table 4). The supplementary table includes mutations – SNVs and small insertions and deletions (indels) – within coding regions and splice-site regions across 60 genes previously described in DLBCL.

Conclusions

The development of novel genomic technologies such as NGS is helping us better understand lymphoma biology. In recent years, several studies have shown that identifying genetic alterations in DLBCL is becoming increasingly important for diagnosis, prognosis, and patient treatment. Gradually, gene panels for this disease will become incorporated into routine clinical practice. Therefore, promoting implementation of NGS in everyday clinical practice is essential so that different centers may have access to this tool and contribute to its consolidation and integration into oncohematologic care.

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Conflicts of interest

D. Colomer-Pujol has received honoraria from ThermoFisher, SOPHiA Genetics, and AstraZeneca. M.T. Gómez-Casares received honoraria for participation as a speaker, consultant, or advisor for Novartis, Roche, GSK, Pfizer, and BMS, as well as research

grants from Novartis, Astellas, and Incyte. The remaining authors declare no conflicts of interest.

Ethical considerations

Protection of humans and animals. The authors declare that no experiments involving humans or animals were performed for this research.

Confidentiality, informed consent, and ethical approval. This study does not involve patient personal data and does not require ethical approval. SAGER guidelines were not applied.

Declaration on the use of artificial intelligence. The authors declare that no type of generative artificial intelligence was used in the preparation of this manuscript.

Supplementary data

Supplementary data is available at DOI: 10.24875/SANGRE.25000014. This material is provided by the corresponding author and published online for the benefit of readers. The content of the supplementary material is the sole responsibility of the authors.

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