

Myeloproliferative neoplasms: recommendations for next-generation sequencing

Neoplasias mieloproliferativas: 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:** Establish consensus-based recommendations for the standardized application of NGS studies in the molecular diagnosis of myeloproliferative neoplasms, defining the most relevant genes and alterations according to the available scientific evidence. **Material and method:** The Molecular Biology in Hematology Group (GBMH), in collaboration with a multidisciplinary panel of experts, reviewed recent scientific literature and the main international classifications (WHO, ICC, ELN). Based on this review, a structured consensus was developed through discussion meetings

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and peer validation. **Results:** Genes of interest were compiled based on their clinical relevance and the scientific evidence. **Conclusions:** This guide aims to assist professionals in the use of targeted NGS panels in myeloproliferative neoplasms.

Keywords: Chronic myeloid leukemia. Myeloproliferative neoplasms. Myelofibrosis. Next generation sequencing. 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, next-generation sequencing) 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 el manejo de los pacientes con neoplasias hematológicas. Las clasificaciones recientes de la Organización Mundial de la Salud (OMS), la International 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:** Establecer recomendaciones consensuadas para la aplicación estandarizada de estudios de NGS en el diagnóstico molecular de las neoplasias mieloproliferativas, definiendo los genes y alteraciones más relevantes según la evidencia científica disponible. **Material y método:** El Grupo de Biología Molecular en Hematología (GBMH), en colaboración con un panel multidisciplinar de expertos, revisó la literatura científica reciente y las principales clasificaciones internacionales (WHO, ICC, ELN). A partir de esta revisión, se elaboró un consenso estructurado mediante reuniones de discusión y validación por pares. **Resultados:** Se recogen 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 las neoplasias mieloproliferativas.

Palabras clave: Leucemia mieloide crónica. Neoplasias mieloproliferativas. Mielofibrosis. Secuenciación masiva. Guías de consenso.

Introduction

Chronic myeloproliferative neoplasms (MPNs) are a group of clonal disorders of the hematopoietic stem cell characterized by the proliferation of mature cells from one or more myeloid lineages. The most clinically relevant chronic MPNs are chronic myeloid leukemia (CML) and the classical Philadelphia (Ph)-negative MPNs, which include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). Other less frequent disorders, but also recognized by the World Health Organization (WHO) and the International Consensus Classification (ICC), include chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia (CEL), and MPN, not otherwise specified (MPN-NOS), according to WHO, and unclassifiable MPN according to ICC.^{1,2}

Of note, some myeloid/lymphoid neoplasms with eosinophilia associated with tyrosine kinase gene rearrangements (M/LN-eo) may present clinically as chronic MPNs with persistent eosinophilia, mimicking diseases such as CEL and even MPN-NOS. Therefore, it is essential to consider them in the differential diagnosis, although they are not formally included within the group of classical MPNs.

Molecular diagnosis

Detection of the *BCR::ABL1* fusion gene (Ph-positive) is a major diagnostic criterion in CML. Meanwhile, regarding classical Ph-negative MPNs, the presence of mutations in the driver genes *JAK2*, *CALR*, and *MPL* is a major diagnostic criterion in PMF and ET, as is the detection of *JAK2* p.V617F or mutations in exon 12 of *JAK2* in PV. Although the *JAK2* p.V617F mutation is characteristic of classical Ph-negative MPNs, it is important to note that it is not specific to these neoplasms and may also be detected in patients with acute myeloid leukemia, myelodysplastic syndromes, or chronic myelomonocytic leukemia, and even in healthy individuals (although at low allelic frequency).³⁻⁵ In addition to mutations in *JAK2*, *CALR*, or *MPL*, a large proportion of patients present alterations in other genes that are also involved in other myeloid neoplasms and contribute to the marked biological and clinical heterogeneity of these diseases.

These mutations affect genes involved in epigenetic regulation (*DNMT3A*, *TET2*, *ASXL1*...), mRNA splicing (*U2AF1*, *SRSF2*, *SF3B1*...), or DNA repair (*TP53*).

Approximately 5% to 15% of ET and PMF cases do not present mutations in the driver genes and are

known as triple-negative. In the absence of mutations in *JAK2*, *CALR*, and *MPL*, the search for somatic mutations in other genes (for example, *ASXL1*, *EZH2*, *TET2*, *SRSF2*, or *SF3B1*) as markers of clonality is included as a major diagnostic criterion for PMF and as a minor criterion for the diagnosis of ET,¹ as well as in the recommendations of the European LeukemiaNet (ELN).⁶

On the other hand, the presence of activating mutations in the *CSF3R* gene (mainly T618I) is a major diagnostic criterion in CNL.⁶ Other genes that may be affected in CNL include *SETBP1*, *SRSF2*, *U2AF1*, and *ASXL1*.^{7,8}

Unlike other MPNs, in CEL there is no recurrent molecular or cytogenetic alteration. However, genetic testing may aid in the differential diagnosis with other diseases that may also present with eosinophilia, since the detection of genetic rearrangements involving *PDGFRA*, *PDGFRB*, *FGFR1*, *JAK2*, *FLT3*, and *ETV6* genes would indicate the presence of a myeloid/lymphoid neoplasm with eosinophilia (M/LN-eo), which may clinically mimic a chronic MPN but represents a distinct disease with specific therapeutic and prognostic implications. For example, an M/LN-eo with the translocation t(9;12)(q34.1;p13.2)/*ETV6:ABL1* may share clinical and laboratory features with CML presenting with eosinophilia.² Activating mutations in *KIT* (for example, p. D816V and p. M541L) are present in some patients with eosinophilia associated with systemic mastocytosis.

Although mastocytosis is no longer considered a subgroup of MPNs, but rather an independent condition,¹ it will be addressed in these guideline recommendations. Detection of an activating mutation at codon 816 of the *KIT* gene is a minor diagnostic criterion for systemic and cutaneous mastocytosis. Recently, additional mutations have been identified in mastocytosis involving the genes *TET2*, *ASXL1*, *SRSF2*, *RUNX1*, *CBL*, and *NRAS*.^{9,10}

Hereditary myeloproliferative neoplasm

It has been described that the probability of developing an MPN is 5-7 times higher in individuals with a first-degree relative affected by MPN,¹¹ although the causative mutation is often unknown. Germline mutations in *JAK2* and *MPL* have been identified in hereditary thrombocytosis and thrombocytopenia, *LNK* mutations in familial erythrocytosis, and *CSF3R* mutations in hereditary neutrophilia, which can be differentiated from MPNs as they are non-clonal disorders.

Method

In MPNs, in clinical practice, analysis by next-generation sequencing (NGS) is recommended in patients with a high suspicion of MPN who have tested negative in the initial screening for driver mutations. Following gene selection and literature review, the genomic alterations of interest agreed upon by the MPN experts of the *Grupo de Biología Molecular en Hematología* (GBMH) are summarized in **table 1**, which compiles recommendations for mutation detection according to their level of evidence. Mandatory genes have been selected based on their diagnostic, prognostic, or therapeutic value in international classification and stratification systems, as well as by unanimous consensus of the GBMH.

It is recommended that the design of the NGS panel includes the complete coding regions of the driver genes *JAK2* and *MPL* for the detection of non-canonical mutations. Analysis of the *SH2B3/LNK* gene may also be useful in *JAK2*-negative cases with suspected PV, as well as analysis of exons 4 and 10 of the *MPL* gene if ET is suspected. NGS studies may also provide useful information in cases of ET and PV that show progression to PMF and are candidates for transplantation, as well as in those who develop acute leukemia. Regarding patients with PMF who are eligible for transplantation, it is recommended to perform an NGS study at the time of diagnosis, as well as to repeat the study when disease progression is suspected. With respect to NGS analysis of non-driver genes, it is recommended to include in the panel the genes indicated in **table 1** (genes with prognostic value or potential therapeutic targets).

For other less frequent diseases, such as CNL and CEL, whenever there is a clear clinical suspicion of MPN, it is recommended to perform NGS studies at the time of diagnosis to correctly classify the disease. Regarding mastocytosis, although NGS is not the technique of choice for initial screening of *KIT* mutations due to the lower sensitivity of panels vs quantitative polymerase chain reaction, in aggressive forms it is recommended to perform mutational analysis by NGS due to the prognostic value of other genes, ensuring a minimum pathological cell population to allow the detection of additional mutations.¹² The starting sample for NGS library preparation in MPNs will be tumor DNA extracted from whole peripheral blood or from purified granulocytes. To detect rearrangements or fusion genes, as well as to quantify expression levels of specific targets, the starting sample will generally be cDNA,

Table 1. List of genes recommended for mutation detection by next-generation sequencing (NGS); variants in these genes may have diagnostic, prognostic, or therapeutic value, guide the selection of targeted treatments, or predict response or resistance to drugs

Gene	Reference sequence	Regions of study	Clinical value			
			Diagnosis	Prognosis	Therapy	Hereditary predisposition
Mandatory genes						
ASXL1	NM_015338.6	Exon 13	X	X		
CALR	NM_004343.4	Exon 9	X	X		
CBL	NM_005188.4	Exons 8, 9	X	X		
CSF3R	NM_000760.4	Exons 14-17	X		X	
DNMT3A	NM_022552.5	Full coding region	X	X		
EZH2	NM_004456.5	Full coding region	X	X	X	
FLT3	NM_004119.3	Exons 14-17, 19-23				X
IDH1	NM_005896.4	Exon 4	X	X	X	
IDH2	NM_002168.4	Exon 4	X	X	X	
JAK2	NM_004972.4	Full coding region	X	X	X	X
KIT	NM_000222.3	Exons 2, 8-11, 13, 17	X	X	X	
MPL	NM_005373.3	Full coding region	X	X		X
PPM1D	NM_003620.4	Full coding region		X		
RUNX1	NM_001754.5	Full coding region	X	X		X
SETBP1	NM_015559.3	Exon 4	X	X		
SF3B1	NM_012433.4	Exons 13-16	X	X		
SH2B3/LNK	NM_005475.3	Full coding region	X	X		X
SRSF2	NM_001195427.2	Exon 1	X	X		
TET2	NM_001127208.3	Full coding region	X			
TP53	NM_000546.6	Full coding region	X	X		X
U2AF1	NM_006758.3	Exons 2, 6, 7	X	X		
ZRSR2	NM_005089.4	Full coding region	X	X		
Recommended genes						
BCOR	NM_001123385.2	Full coding region	X	X		
CUX1	NM_181552.4	Full coding region	X	X		
GNAS	NM_000516.7	Full coding region	X	X		
KRAS	NM_004985.5	Full coding region	X	X	X	
NFE2	NM_001136023.3	Full coding region		X		
NRAS	NM_002524.5	Exons 2-6	X	X	X	
PHF6	NM_001015877.2	Full coding region	X	X		
STAG2	NM_001042750.2	Full coding region	X	X		

although panels capable of analyzing rearrangements from genomic DNA are already commercially available.

One of the main challenges in the study of MPNs by NGS has been the detection of *CALR* type-1/type-1-like mutations (52 base pair deletion), as these are long deletions that may complicate analysis, since NGS generally requires sequencing of short fragments. Currently, commercial panels already include validated bioinformatic tools for detecting these alterations. However, when using custom panels, it is recommended to optimize and validate the sequencing length required for variant detection and to analyze these variants “raw” using software such as Integrative Genomics Viewer (IGV, Broad Institute, Cambridge, MA, United States) for confirmation, as well as subsequent validation using other technologies (Sanger sequencing, fragment analysis, etc.).

Unlike the interpretation of germline variants, which focuses on pathogenicity and causal relationships with hereditary diseases, the evaluation of somatic variants should be oriented toward their clinical impact, considering their diagnostic, prognostic, or therapeutic relevance. It is recommended to follow the 2017 joint guidelines of the Association for Molecular Pathology (AMP), the American Society of Clinical Oncology (ASCO), and the College of American Pathologists (CAP), which propose a four-tier classification (I-IV) based on clinical and experimental evidence.¹³ In general, only pathogenic or likely pathogenic variants (I-II) should be considered clinically, avoiding clinical decisions based on variants of uncertain significance, although it should be taken into consideration that these may be reclassified over time as more data become available.

This evaluation should be supported by multiple sources, including bioinformatic tools (Franklin, Varsome, ClinVar, COSMIC, etc.), MPN-specific guidelines or recommendations, population databases (such as gnomAD), or gene-specific databases.

In MPNs, some polymorphisms may be annotated, mainly in the *JAK2* and *MPL* genes, as several studies suggest their potential association with patients' clinical parameters. However, the evidence is still very limited, and they should only be reported for purely informational purposes.^{14,15}

Study of mutations in the kinase domain of *BCR::ABL1* by NGS

The study of *BCR::ABL1* mutations by NGS is indicated in patients with CML and Philadelphia-positive

acute lymphoblastic leukemia who do not show an optimal response to treatment with *ABL1* tyrosine kinase inhibitors.¹⁶ NGS must be performed exclusively on the *ABL1* fragment fused to *BCR*; therefore, after cDNA synthesis, an initial polymerase chain reaction should be carried out using a forward primer targeting *BCR* and a reverse primer targeting *ABL1*. This first step is essential to enable detection of *ABL1* mutations in samples with low *BCR::ABL1* levels. After this step, the rest of the methodology will be similar to that described for other genes or gene panels. In the case of *ABL1*, amplicons will be generated to include exons where clinically relevant mutations are located (exons 4 to 9 of *ABL1*).¹⁷

Clinical utility

NGS studies provide information on mutations incorporated into the latest prognostic models for patients with MPNs and are very useful for establishing clonality in patients with triple-negative MPNs. In this regard, one-third of patients with MPNs present at least one additional somatic mutation in other genes (increasing to 50-60% in patients with myelofibrosis), which may modulate the disease phenotype and therefore have prognostic impact in terms of disease progression to MF or leukemia, as well as adverse complications such as thrombotic events¹⁸⁻²² (Table 2 and Supplementary Table 1).

Recently, a molecular classification of MPNs has been proposed with the ability to predict clinical evolution and risk of transformation to leukemia, establishing eight genomic groups:

- 1) MPN with loss of function in *TP53*/aneuploidy (*TP53* mutation, del17p or chromosome 5 loss, -5/5q-), associated with worse prognosis, high risk of transformation to acute leukemia, and early death.
- 2) MPN with chromatin/splicing mutations (with > 1 mutations in *EZH2*, *IDH1*, *IDH2*, *ASXL1*, *PHF6*, *CUX1*, *ZRSR2*, *SRSF2*, *U2AF1*, *KRAS*, *NRAS*, *GNAS*, *CBL*, *RUNX1*, *STAG2*, and *BCOR*), associated with a higher risk of transformation to myelofibrosis and shorter event-free survival.
- 3) MPN with *CALR*/20q mutations, without significant differences in prognosis.
- 4) MPN with *MPL* mutations, associated with a higher risk of transformation to leukemia.
- 5) MPN with homozygous *JAK2* p.V617F mutation, with concurrent mutations in *NFE2*, associated with a higher risk of transformation to myelofibrosis after PV.

Table 2. Clinical impact of mutations in genes recommended for study by next-generation sequencing (NGS)

Gene	Type of mutation	Frequency (%)			Clinical impact		
		PV	ET	MF	PV	ET	MF
<i>JAK2</i>	V617F activating	95-97	50-60	50-60	Risk of thrombosis, transformation to MF/AML, and shorter OS		Intermediate prognosis; higher risk of thrombosis than CALR (+) patients
	Exon 12 activating	1-3	0	0	Higher risk of thrombosis, transformation to MF/AML, and shorter OS		
<i>CALR</i>	Exon 9 activating <i>indels</i>	Infrequent	15-30	23-35		Lower risk of thrombosis than JAK2 (–) patients	Lower risk of thrombosis and better survival than JAK2 (+) or triple-negative patients. Better OS than CALR type 2/type 2-like (+) and JAK2 patients
<i>MPL</i>	S505N, W515L/K activating	Infrequent	3-5	5-10			Intermediate prognosis and higher risk of thrombosis than patients with CALR mutation
<i>SH2B3</i>	CDS inactivating	1	3-6	3-6		Shorter OS	
<i>CBL</i>	Exons 8, 9	Infrequent		5-10			Shorter OS after allogeneic HSCT
<i>KRAS/ NRAS</i>	Activating	Infrequent		6			Shorter OS
<i>PTPN11</i>	Activating	Infrequent		5-10	Fibrotic transformation, leukemic transformation, and shorter OS		
<i>GNAS</i>	Exons 8, 9 (R201, Q227)	Infrequent		5-10	Fibrotic transformation, leukemic transformation, and shorter OS		
<i>TET2</i>	CDS inactivating	10-20	10-15	10-20	Inconclusive		
<i>IDH1/ IDH2</i>	<i>IDH1</i> -R132, <i>IDH2</i> -R140/ R172	Infrequent		3-6	Shorter OS; <i>IDH2</i> leukemic transformation	<i>IDH2</i> : shorter OS	Shorter leukemia-free survival; shorter leukemia-free survival after HSCT
<i>DNMT3A</i>	CDS inactivating	1-5	1-5	5-15	Prognostic value to be determined	Prognostic value to be determined	Prognostic value to be determined; shorter OS after allogeneic HSCT
<i>ASXL1</i>	Exon 13 inactivating	2-5	2-5	13-25	Shorter OS; transformation to MF		Shorter OS, shorter leukemia-free survival, and shorter leukemia-free survival after HSCT
<i>EZH2</i>	CDS inactivating	1-3	1	5-10		Shorter OS; leukemic transformation	Shorter OS
<i>SF3B166</i>	Exons 13-16 inactivating	Infrequent	3	4-7		Shorter OS; transformation to MF	
<i>SRSF2</i>	Exon 1 (P95) inactivating	Infrequent	Infrequent	9-17	Shorter OS	Shorter OS	Shorter OS and shorter leukemia-free survival

(Continues)

Table 2. Clinical impact of mutations in genes recommended for study by next-generation sequencing (NGS) (continued)

Gene	Type of mutation	Frequency (%)			Clinical impact		
		PV	ET	MF	PV	ET	MF
<i>U2AF1</i>	Q157 inactivating	Infrequent	Infrequent	15		Shorter OS; transformation to MF	Shorter OS than patients with <i>U2AF1</i> -S34/WT; shorter OS after allogeneic HSCT
<i>ZRSR2</i>	CDS inactivating	Infrequent	3	5-10	Fibrotic and leukemic transformation, and shorter OS		
<i>RUNX1</i>	CDS inactivating	3-5	3-5	5	Shorter OS; leukemic transformation		
<i>NFE2</i>	CDS inactivating	2	Infrequent	2-3	Associated with homozygous <i>JAK2</i> , PV, and fibrosis		
<i>PHF6</i>	CDS inactivating	Infrequent			Fibrotic and leukemic transformation, and shorter OS		
<i>CUX1</i>	CDS inactivating	Infrequent			Fibrotic and leukemic transformation, and shorter OS		
<i>BCOR</i>	CDS inactivating	Infrequent			Fibrotic and leukemic transformation, and shorter OS		
<i>TP53</i>	Exons 2-11 inactivating	1	1	< 5		Shorter progression-free survival and OS	Leukemic transformation
<i>PPM1D</i>	Exon 6	2			Chemotherapy resistance		
<i>STAG2</i>	Inactivating	Infrequent			Fibrotic and leukemic transformation, and shorter OS		

CDS: coding sequence; AML: acute myeloid leukemia; MF: myelofibrosis; PV: polycythemia vera; OS: overall survival; PFS: progression-free survival; ET: essential thrombocythemia; HSCT: hematopoietic stem cell transplantation.

- 6) MPN with heterozygous *JAK2* p.V617F mutation; this was the reference group.
- 7) Triple-negative patients, mainly with ET of good prognosis, in whom progression to leukemia or myelofibrosis is rare.
- 8) MPN with another driver mutation, without any of the previously described alterations; these may present mutations in *TET2*, *DNMT3A*, and *KIT*.²⁰

Myelofibrosis

The presence of *CALR* mutations is associated with better overall survival vs *JAK2* p.V617F or *MPL* mutations.²³ Patients with *CALR* (+)/*ASXL1* (-) have longer survival (10.4 years) than those with *CALR* (-)/*ASXL1* (+) (2.3 years), while *CALR* (+)/*ASXL1* (+) and *CALR* (-)/*ASXL1* (-) show intermediate survival (5.8 years). The absence of driver gene mutations is associated with shorter leukemia-free survival and worse prognosis in patients with myelofibrosis.^{15,24} Mutations in *ASXL1*, *EZH2*, *SRSF2*, or *TP53*, among others, have been

identified as high-risk markers.²⁵⁻²⁹ In addition, mutations in *U2AF1* and *RAS* have also been associated with poorer overall prognosis.^{20,30-33} In the case of genes in the *RAS* pathway, their analysis is recommended because it may provide relevant information for the differential diagnosis between myelodysplastic/myeloproliferative neoplasms (such as chronic myelomonocytic leukemia or atypical CML) and triple-negative PMF.

Regarding prognostic scoring systems for myelofibrosis, the following are available:

- DIPSS (Dynamic International Prognostic Scoring System), which does not include cytogenetic data.
- DIPSS-plus, which incorporates karyotype but not molecular data.
- MIPSS-70 (Mutation-Enhanced International Prognostic Score System)-70, for patients with PMF ≤ 70 years, which includes the presence of variants in *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, and *IDH2* as high molecular risk.

– MIPSS-70+ version 2.0, which adds the *U2AF1* p.Q157 variant.

It is estimated that > 40% of patients with myelofibrosis present at least one high molecular risk variant.^{25,28} Furthermore, the presence of > 2 high-risk molecular variants is an adverse prognostic factor due to its association with shorter overall survival and leukemia-free survival. Recently, the AIPSS-MF (Artificial Intelligence Prognostic Scoring System for Myelofibrosis), based on data from the Spanish myelofibrosis registry, has been developed, integrating clinical variables with molecular information obtained by NGS and variant allele frequency (VAF). In an improved version (AIPSS-MF molecular), greater discriminatory capacity has been demonstrated for predicting leukemic transformation and superiority compared to MIPSS.³⁴ For risk stratification in secondary myelofibrosis, the MYSEC-PM model considers the absence of *CALR* type 1 variant.

Of note, the adverse impact of *ASXL1* mutations in myelofibrosis has recently been questioned, especially at low VAF.^{35,36} To date, *TP53* mutations are not included in any prognostic model for myelofibrosis. However, there is strong evidence of their association with shorter overall survival and reduced leukemia-free survival. Moreover, multi-hit *TP53* mutations, defined as the presence of multiple alterations in the *TP53* gene (which may include multiple gene mutations, a combination of mutation with deletion or loss of heterozygosity in the 17p region, or a mutation with VAF > 50%), are associated with worse prognosis.³⁷

Polycythemia vera

JAK2 mutations, both V617F and exon 12 mutations, are associated with similar rates of thrombosis, transformation to myelofibrosis, and transformation to leukemia.^{38,39}

A study by the *Spanish MPN group* demonstrated that patients with resistance or intolerance to hydroxyurea frequently present mutations in *TP53* or in chromatin/splicing genes.⁴⁰ Regarding prognostic models for PV, MIPSS-PV takes into consideration the presence of variants in the *SRSF2* gene.

The French study group has developed 2 new prognostic systems specific for thrombotic events in patients with MPNs. The VETS (Venous Thrombosis Score) identifies as the only risk factors a *JAK2* p.V617F mutation burden > 50% and a history of venous thrombosis. On the other hand, the ARTS (Arterial Thrombosis Score) includes, in addition to classical cardiovascular risk factors (age, history of arterial thrombosis, etc.), the

mutational status of *TET2* and *DNMT3A* as independent predictors of arterial thrombosis.⁴¹

Essential thrombocythemia

The presence of *CALR* mutations is associated with a lower risk of thrombosis vs *JAK2* variants; however, it has no impact on overall survival or transformation to myelofibrosis or leukemia.³⁹⁻⁴⁴ The presence of *MPL* mutations may be associated with a higher risk of transformation to myelofibrosis.^{40,43} In addition, mutations in *SH2B3*, *IDH2*, *U2AF1*, *SF3B1*, *EZH2*, and *TP53* have been associated with shorter overall survival and myelofibrosis-free survival, and a recent study has demonstrated a high frequency of *TP53* mutations in patients with ET resistant to hydroxyurea.⁴⁵ For ET prognosis, the MIPSS-ET system considers the presence of variants in the *SRSF2*, *SF3B1*, *U2AF1*, and *TP53* genes.

Chronic neutrophilic leukemia

CNL is a rare MPN recognized by the WHO and ICC. The presence of driver mutations in *CSF3R* constitutes the diagnostic genetic hallmark of CNL, with the p. T618I mutation being the most characteristic, although other activating mutations may also be found. However, the absence of a *CSF3R* mutation does not exclude the possibility of CNL. In most cases, additional mutations are also observed, such as in *SETBP1*, *ASXL1*, *SRSF2*, as well as in genes related to cell signaling.² An updated set of recommendations for the diagnosis and prognosis of this disease has recently been published.⁴⁶

Chronic myeloid leukemia

To establish risk in patients with CML at diagnosis, the 2020 recommendations of the ELN group are available, following the new EUTOS score.¹⁶ NGS is not necessary to establish the diagnosis of CML, and currently the ELN does not recommend its use in clinical practice, as its results do not modify therapeutic management.

However, approximately 30% of patients in the chronic phase present additional variants. The most frequently mutated gene is *ASXL1*, but mutations in *RUNX1*, *BCOR*, *TET2*, *DNMT3A*, *TP53*, and *IKZF1* are also common (deletions in the latter are associated with a lymphoid phenotype), and the incidence of mutations increases in advanced stages of the disease.¹⁶

Mastocytosis

Detection of an activating mutation at codon 816 of the *KIT* gene is a minor diagnostic criterion for systemic and cutaneous mastocytosis.⁴⁷ The allelic burden of this mutation is generally higher in more aggressive forms of systemic mastocytosis, as it is detected not only in mast cells but also in other hematopoietic cells.⁴⁸

Targeted therapies

In the case of CML, 6 ABL1 tyrosine kinase inhibitors have been approved.¹⁶ However, there are few targeted therapies available for MPNs. Ruxolitinib is a selective JAK1/2 inhibitor that has demonstrated efficacy in myelofibrosis and PV. The allelic burden of *JAK2* p.V617F decreases with ruxolitinib treatment, although this is not associated with improved clinical response.^{49,50} On the other hand, pegylated interferon has shown the ability to reduce the burden of driver mutations in patients with PV and ET.

Several clinical trials with targeted therapies and combinations are ongoing, particularly in patients with myelofibrosis. The molecular factors that predict response to JAK inhibitors and other targeted therapies remain to be determined. However, some variants have already been associated with resistance to ruxolitinib, such as mutations in the RAS pathway and in *CBL*,⁵¹ while mutations in *DNMT3A* have been associated with a lower response to pegylated interferon.⁵²

Future studies will need to establish treatment efficacy based on mutational profiles, which will enable more precise molecular stratification and more personalized therapeutic selection.

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Conflicts of interest

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Ethical considerations

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

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

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

Supplementary data

The supplementary data is available at DOI: 10.24875/SANGRE.25000003. This data is provided by the corresponding author and published online for the benefit of the reader. The content of the supplementary material is the sole responsibility of the authors.

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