

Acute myeloid leukemia: recommendations for next-generation sequencing

Leucemia mieloide aguda: 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 still lacks standardized, and experts have yet to agree on the best methods or gene targets for each disease. **Material and method:** The Molecular Biology Group in Hematology (GBMH), in collaboration with a panel of experts, developed this consensus guide.

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Date of reception: 29-01-2025

Date of acceptance: 14-07-2025

DOI: 10.24875/SANGREE.M25000045

Available online: 18-06-2026

Sangre (Eng). 2026;45(2):89-99

www.revistasangre.com

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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 acute myeloid leukemia.

Keywords: Acute myeloid leukemia. 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 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 la leucemia mieloide aguda.

Palabras clave: Leucemia mieloide aguda. Secuenciación masiva (NGS). Guía de consenso.

Introduction

Acute myeloid leukemia (AML) is a malignant process characterized by very rapid proliferation of immature myeloid hematopoietic cells. AML is the most common subtype of acute leukemia in adult patients, with an incidence of 3 to 4 new cases per year per 100,000 inhabitants (approximately 2,300 cases per year in Spain).¹ The median age at presentation is approximately 70 years, making it a disease of older patients. Despite decades of research, AML remains one of the most severe malignant hematologic diseases, with survival rates in older patients not exceeding 20-40%, although they can reach 70% in pediatric patients.

Over the past decade, driven by next-generation sequencing (NGS) techniques, thousands of variants have been identified in several dozen genes involved in AML. Although most are sporadically acquired alterations, in an increasing number of cases, NGS has allowed the identification of variants of germline origin, making myeloid neoplasms with germline predisposition a new category in current diagnostic classifications.^{2,3} Recognizing this new category is important because of its impact on the clinical management of these patients and the adoption of genetic counseling strategies.

Similarly, the identification of somatic alterations and their involvement in leukemogenesis mechanisms has allowed progress toward more precise classifications and clinical algorithms in AML. Recent revisions published in 2022 by different expert groups (World Health Organization

[WHO],² International Consensus Classification [ICC],³ and European LeukemiaNet [ELN]⁴) emphasize the importance of molecular studies as a fundamental tool for establishing diagnosis, stratifying risk, guiding therapy, and defining measurable residual disease (MRD) follow-up markers.

The increasing number of alterations, combined with the need to obtain rapid information to identify the subset of patients who can benefit from targeted therapies, has created major challenges in managing AML, turning molecular diagnosis into a complex task that requires multiple techniques. There is currently a demand for standardization of new molecular methods that optimize diagnosis and allow reproducible results to be obtained in the clinical context. The use of NGS enables broader and more precise genetic characterization in patients, facilitating a more robust diagnosis of the disease, firmer prognostic typing, and/or unequivocal prediction of patient resistance to drugs, before or during their treatment, thus offering possible therapeutic alternatives in an individualized manner.

Methodologically relevant aspects in acute myeloid leukemia

Samples

The study is usually performed on bone marrow, but other types of samples showing at least 5% tumor infiltration (e.g., peripheral blood) can also be included.

Timing of study

It should be performed at diagnosis and in case of treatment failure (whether due to relapse or refractoriness).

Studies to be performed

The presence of alterations of different nature implies that most NGS methodological approaches in AML will require two studies in parallel: one on DNA to detect single-nucleotide variants (SNVs), indels, and copy number variations (CNVs), and another on RNA to detect fusion transcripts and alterations in gene expression. Currently, there are DNA panels designed using probe capture that allow the detection of point mutations, indels, and rearrangements.

DNA-BASED PANELS

The study of point variants or small indels by NGS has been extensively developed in recent years, with different gene panels offering a very extensive study of alterations in genes relevant to AML. The incorporation of specific analysis programs and increased reading depth of complex genes have overcome limitations associated with the detection of variants in genes considered “difficult,” such as NGS detection of *FLT3*-ITD mutations or variants in *CEBPA*.

Furthermore, current evidence suggesting germline predisposition in some AML cases suggests that the NGS panel should include genes listed under the myeloid neoplasms with germline predisposition category in current classifications. If there is a family history of myeloid neoplasms or suspected predisposition, the patient should be referred to a specialized center for study using an expanded germline neoplasm panel and genetic counseling. Additionally, panels are being validated for the detection of DNA rearrangements, whose advantage is their potential ability to capture translocated chromosomal regions where one of the two parts is unknown, allowing the identification of new variants of some translocations.

The study of relative quantification of the number of reads in the test sample compared with a baseline generated from control samples would allow CNV detection with the same NGS panel. This application is the least developed to date. However, those NGS approaches that include it can be interesting as a complementary technique to karyotyping or fluorescence in situ hybridization (FISH), as well as for detecting small-sized CNVs whose study by cytogenetic techniques is

compromised.⁵ For this application, it is advisable to include bioinformatic algorithms for their analysis in AML panels.

RNA-BASED PANELS

The study of rearrangements using NGS has not been as widespread as the study of point variants or indels. However, an increasing number of NGS strategies are addressing their study. Amplicon-based panels include the study of a large number of fusion transcripts, considering different breakpoints. However, this strategy requires knowledge of the genes involved in the fusion and their breakpoints, which limits the detection capacity of other fusions not included in the panel design. However, there are methodological alternatives that overcome this limitation: a) some panels allow indirect detection of fusion genes through an imbalance in the number of reads from the 5' and 3' ends of the driver gene; in these cases, it is not possible to characterize the rearrangement, since the gene involved in the fusion is unknown; and b) capture-based panels. In any case, the criteria and cutoff points of the commercial panel should be followed, or proprietary ones established in customized panels, and their detection validated by alternative methods whenever possible.

Furthermore, some clinically relevant translocations do not result in the transcription of chimeric genes. However, the oncogenes involved are generally positioned under the control of potent promoters, leading to their overexpression. An example of this mechanism would be *MECOM* alterations. In these cases, RNA panels can be used to quantify the expression of dysregulated genes, using the number of target reads relative to the total number of RNA reads.

Studies to be performed using NGS panels in acute myeloid leukemia

Following gene selection and literature review, the Molecular Biology Group in Hematology (GBMH) identified the key genomic alterations to be analyzed in all AML patients at diagnosis or during relapse/refractory stages, as listed in **tables 1** and **2**.

The required genes were selected by GBMH consensus due to their recommendation by international expert groups for diagnosis, risk stratification, and treatment: mutations in *ANKRD26*, *ASXL1*, *BCOR*, *CEBPA*, *DDX41*, *ETV6*, *EZH2*, *FLT3-ITD*, *FLT3-TKD*, *GATA2*, *IDH1*, *IDH2*, *NPM1*, *RUNX1*, *SAMD9*, *SAMD9L*, *SF3B1*, *SRSF2*, *STAG2*, *TP53*, *U2AF1*, and *ZRSR2*; fusions *BCR::ABL1*,

CBFA2T3:GLIS2, *CBFB::MYH11*, *DEK::NUP214*, *MECOM::X*, *ETV6::X*, *FUS::ERG*, *KAT6A::CREBBP*, *KMT2A::X*, *NUP98::X*, *RBM15:MRTFA*, *RUNX1:RUNX1T1*, *NPM1:MLF1*, *PICALM::MLLT10*, *RUNX1:CBFA2T3*, *PRDM16:RPN1*, *PML::RARA*, and other *X::RARA* fusions.

The recommended genes were selected by GBMH consensus based on literature due to their involvement in AML, though their clinical utility remains debated: mutations in *ATG2B*, *GSKIP*, *BRAF*, *CALR*, *CBL*, *CSF3R*, *CUX1*, *DNMT3A*, *GATA1*, *JAK2*, *KIT*, *KRAS*, *KMT2A-PTD*, *MBD4*, *MPL*, *NF1*, *NRAS*, *PHF6*, *PPM1D*, *PTPN11*, *RAD21*, *SETBP1*, *SH2B3*, *SMC1A*, *SMC3*, *TERC*, *TERT*, *TET2*, and *WT1* (Supplementary Table 1); regarding fusions, the study of rearrangements is recommended using NGS approaches that allow detection of the greatest number of rearrangements currently recognized as specific entities in AML (Supplementary Table 2).

Clinical utility of NGS panel studies in acute myeloid leukemia

Diagnosis

Recently, the 2022 update of the WHO diagnostic classification has been published.² Additionally, another new diagnostic classification, the ICC, has emerged, developed by an international group of experts.³ Both classifications have greater emphasis on genetic alterations and have lowered the blast threshold required for diagnosing AML in certain cases. Specifically, the ICC establishes the category of AML with recurrent genetic alterations when the alteration is detected and they present > 10% blasts (except *BCR::ABL1* AML). For the remaining subtypes, the threshold of > 20% blasts continues to be considered, but a new category is introduced: myelodysplastic syndrome (MDS)/AML directly associated with genetic alterations related to myelodysplasia and 10-19% blasts. The WHO has gone further and does not establish a required blast threshold for the diagnosis of AML with recurrent genetic alterations (except *BCR::ABL1* AML and AML with mutated *CEBPA*). Table 3 summarizes both diagnostic classifications, highlighting their similarities and differences.

Likewise, both classifications have included myeloid neoplasms with a germline predisposition. These germline neoplasms occur in association with inherited germline mutations and specific clinical features. The most established forms (e.g., Fanconi anemia or dyskeratosis congenita) occur as inherited syndromes that present predominantly non-hematological findings in

children. However, today we know of the existence of autosomal dominant diseases with predisposition to hematologic neoplasms. Some of these patients present as a hematologic neoplasm, while others have a preexisting disease or some organ dysfunction. Both classifications are summarized in table 4.

The 2022 WHO classification maintains the subclassification of this entity into three subgroups, and they are now classified using the combination of disease phenotype (AML, MDS...) with genotype (mutation); for example, AML with a germline pathogenic variant in *RUNX1*.

In addition to this terminological novelty, *TP53* mutations (Li-Fraumeni syndrome) have been incorporated, as well as *SAMD9/SAMD9L* and *BLM* mutations present in MIRAGE and Bloom syndrome, respectively. On the other hand, the ICC, unlike the WHO classification, subclassifies the entity into four subgroups, as it incorporates acute lymphoblastic leukemias with germline predisposition with mutations in the *PAX5* and *IKZF1* genes.

The recommendation is to include in somatic mutation panels the genes included in the WHO 2022 and ICC classifications related to germline hematologic neoplasm predisposition. However, due to the specific needs of these studies (expanded gene panels, specific sample, and genetic counseling), if there is a family history of myeloid neoplasms or suspected predisposition, the patient should be referred to a specialized center. A specific working group for this type of neoplasms has been established within the Spanish Society of Hematology and Hemotherapy (SEHH) to develop a guideline aimed at the diagnosis and follow-up of these patients.

Prognosis

In addition to cytogenetic analysis, the identification of mutations with prognostic and therapeutic impact can help refine prognostic groups in AML, especially in patients with a normal karyotype, potentially influencing subsequent treatment decisions.

In 2017, the ELN published recommendations on the management of AML treated with intensive chemotherapy, establishing risk stratification based on genetic and molecular alterations.⁶ These recommendations, updated in 2022, maintain this stratification into three risk groups: favorable, intermediate, and adverse, introducing the following changes:

- The *FLT3*-ITD allelic ratio is no longer considered in risk stratification; all AML with *FLT3*-ITD are classified

Table 1. Study of single-nucleotide variants (SNV)/indels using next-generation sequencing (NGS) techniques. Genes, regions of interest, and clinical utility in acute myeloid leukemia (AML)

Gene	Reference sequence	Study regions (hotspots)	Diagnosis	Prognosis	Treatment	Hereditary predisposition
Mandatory genes						
<i>ANKRD26</i>	NM_014915.2	5' UTR + exons 1-2	X			X
<i>ASXL1</i>	NM_015338.6	Full coding region (exon 13)		X		
<i>BCOR</i>	NM_001123385.2	Full coding region		X		
<i>CEBPA</i>	NM_004364.5	Full coding region	X	X		X
<i>DDX41</i>	NM_016222.4	Full coding region	X			X
<i>ETV6</i>	NM_001987.5	Full coding region	X			X
<i>EZH2</i>	NM_004456.5	Full coding region		X		
<i>FLT3-ITD</i>	NM_004119.3	Exons 11-20 (exons 14-15)		X	X	
<i>FLT3-TKD</i>	NM_004119.3	Exons 14-23 (exon 20)			X	
<i>GATA2</i>	NM_032638.5	Full coding region + intron 4	X			X
<i>IDH1</i>	NM_005896.4	Exon 4			X	
<i>IDH2</i>	NM_002168.2	Exon 4			X	
<i>NPM1</i>	NM_002520.6	Exons 10, 11	X	X	X	
<i>RUNX1</i>	NM_001754.5	Full coding region	X	X		X
<i>SAMD9</i>	NM_017654.4	Full coding region	X			X
<i>SAMD9L</i>	NM_152703.5	Full coding region	X			X
<i>SF3B1</i>	NM_012433.4	Exons 11-16	X	X		
<i>SRSF2</i>	NM_001195427.2	Full coding region		X		
<i>STAG2</i>	NM_001042749.2	Full coding region		X		
<i>TP53</i>	NM_000546.6	Full coding region	X	X	X	X
<i>U2AF1</i>	NM_006758	Exons 2, 6-8		X		
<i>ZRSR2</i>	NM_005089.4	Full coding region		X		
Recommended genes						
<i>ATG2B</i>	NM_018036.7	Exons 28-33	X			X
<i>BRAF</i>	NM_004333.6	Full coding region	X			X
<i>CALR</i>	NM_004343.3	Exon 9	X			
<i>CBL</i>	NM_005188.4	Exons 8-9	X			X
<i>CSF3R</i>	NM_156039.3	Exons 6-10 and 14-18	X			
<i>CUX1</i>	NM_001202543.2	Full coding region	X			
<i>DNMT3A</i>	NM_175629.2	Full coding region (exon 23)		X		
<i>GATA1</i>	NM_002049.4	Full coding region (exon 2)	X			

(Continues)

Table 1. Study of single-nucleotide variants (SNV)/indels using next-generation sequencing (NGS) techniques. Genes, regions of interest, and clinical utility in acute myeloid leukemia (AML) (*continued*)

Gene	Reference sequence	Study regions (hotspots)	Diagnosis	Prognosis	Treatment	Hereditary predisposition
Recommended genes						
<i>GSKIP</i>	NM_016472.5	Full coding region	X			X
<i>JAK2</i>	NM_004972.3	Full coding region (exons 12, 14)	X			
<i>KIT</i>	NM_000222.2	Exons 2-18		X		
<i>KRAS</i>	NM_033360.4	Exons 2-4		X		
<i>KMT2A-PTD</i> *	NM_001197104.2	Exons 2-11		X		
<i>MBD4</i>	NM_003925.3	Full coding region	X			X
<i>MPL</i>	NM_005373.2	Exons 3-6, 10, 12	X			
<i>NF1</i>	NM_001042492.3	Full coding region	X	X		X
<i>NRAS</i>	NM_002524.4	Exons 2-4		X		
<i>PHF6</i>	NM_032458.3	Full coding region		X		
<i>PPM1D</i>	NM_003620.4	Full coding region	X			
<i>PTPN11</i>	NM_002834.5	Full coding region	X	X		X
<i>RAD21</i>	NM_006265.3	Full coding region		X		
<i>SETBP1</i>	NM_015559.3	Exon 4	X	X		
<i>SH2B3</i>	NM_005475.3	Full coding region	X			X
<i>SMC1A</i>	NM_006306.4	Full coding region		X		
<i>SMC3</i>	NM_005445.4	Full coding region		X		
<i>TERC</i>	NR_001566.1	Full coding region	X			X
<i>TERT</i>	NM_198253.3	Full coding region	X			X
<i>TET2</i>	NM_001127208.3	Full coding region		X		
<i>WT1</i>	NM_024426.6	Exons 7, 9		X		

These duplications cannot be detected by conventional cytogenetics and/or fluorescence *in situ* hybridization (FISH). NGS techniques can explore these duplications in DNA or RNA.

**KMT2A-PTD*: partial tandem duplications of variable size involving between 2 and 3 exons of the *KMT2A* gene.

- as intermediate risk, regardless of the ratio or the simultaneous presence of *NPM1* mutation.⁷
 - In addition to the *ASXL1* and/or *RUNX1* genes considered in the previous classification, the “AML with myelodysplasia–related mutations” category is expanded to include the genes *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, or *ZRSR2* and they are classified in the adverse risk group, even in the absence of cytogenetic abnormalities related to myelodysplasia.^{8,9}
 - The presence of adverse risk cytogenetic abnormalities in AML with *NPM1* mutations is now defined as adverse risk.¹⁰
 - Only in-frame mutations in the *bZIP* region of the *CEBPA* gene are considered favorable risk, regardless of their occurrence as biallelic or monoallelic mutations.¹¹
 - Other recurrent cytogenetic abnormalities that define the disease are included in the adverse risk group, such as t(3q26.2;v) affecting the *MECOM* gene¹² or t(8;16)(p11;p13) associated with the *KAT6A:CREBBP* fusion.¹³
 - Finally, hyperdiploid karyotypes with multiple trisomies (or polysomies) are no longer considered complex karyotypes and adverse risk.¹⁴
- Thus, the groups are as follows:

Table 2. Study of rearrangements using next-generation sequencing (NGS) techniques. Fusion genes and clinical utility in acute myeloid leukemia (AML)

Fusion genes	Diagnosis	Prognosis	Treatment
Mandatory rearrangements			
<i>BCR::ABL1</i>	X	X	X
<i>CBFA2T3::GLIS2*</i>	X		
<i>CBFB::MYH11</i>	X	X	X
<i>DEK::NUP214</i>	X	X	
<i>MECOM::X[‡]§</i> (<i>MYC</i> , <i>ETV6</i> , <i>RUNX1</i> , <i>RPN1</i>)	X	X	
<i>ETV6::X</i> (<i>MXN1</i>) [§]	X		
<i>FUS::ERG</i>	X		
<i>KAT6A::CREBBP</i>	X	X	
<i>KMT2A::X</i> (<i>AFF1*</i> , <i>AFDN</i> , <i>MLLT1*</i> , <i>MLLT3</i> , <i>MLLT4</i> , <i>MLLT6</i> , <i>MLLT10</i> , <i>MLLT11</i> , <i>ELL</i> , <i>TET1</i>) [§]	X	X	
<i>KMT2A-PTD[†]</i>	X		
<i>NUP98::X</i> (<i>NSD1*</i> , <i>KMD5A*</i>) [§]	X		
<i>RBM15::MRTFA</i> (<i>MKL1</i>)	X		
<i>RUNX1::RUNX1T1</i>	X	X	X
<i>PML::RARA</i>	X	X	X
<i>NPM1::MLF1</i>	X		
<i>PICALM::MLLT10</i>	X		
<i>RUNX1::CBFA2T3</i>	X		
<i>PRDM16::RPN1</i>	X	X	
<i>X::RARA</i> (<i>IRF2BP2</i> , <i>NPM1</i> , <i>ZBTB16</i> , <i>STAT5B</i> , <i>STAT3</i> , <i>TBL1XR1</i> , <i>FIP1L1</i> , <i>BCOR</i>) [§]	X		X
Recommended rearrangements			
Current diagnostic and prognostic classifications of AML include a large number of rearrangements. Their study is recommended using NGS approaches that allow for the detection and characterization of the greatest number of possible rearrangements, including those with high incidence in AML, as well as other less frequent but recurrent rearrangements in AML.			

*Rearrangements described predominantly in pediatric AML.

[†]*KMT2A-PTD*: partial tandem duplications of variable size involving between 2 and 3 exons of the *KMT2A* gene. These duplications cannot be detected by conventional cytogenetics and/or fluorescence *in situ* hybridization (FISH). NGS techniques can explore these duplications in DNA or RNA.

[‡]*MECOM::X* rearrangements generally involve the 3q26 region and result in overexpression of the *MECOM* gene. *inv* (3)(q21;q26)/*t* (3;3)(q21;q26) are the most frequent chromosomal alterations in patients with 3q26 alterations. These alterations do not result in the transcription of a chimeric gene; however, overexpression of the *MECOM* gene occurs.

[§]It is recommended to study the greatest number of partner genes possible.

FAVORABLE RISK

– Mutations:

- Mutated *NPM1* in the absence of *FLT3* internal tandem duplications (*FLT3*-ITD) and adverse risk cytogenetic alterations. The risk may change during treatment according to MRD results.
- Presence of in-frame mutation in the bZIP region of *CEBPA*.

– Rearrangements:

- *t*(8;21)(q22;q22.1); *RUNX1::RUNX1T1*.

- *inv*(16)(p13.1;q22) or *t*(16;16)(p13.1;q22); *CBFB::MYH11*.

The initial risk assigned to these rearrangements may change during treatment according to MRD results. The co-occurrence of mutations in *KIT* and/or *FLT3* does not alter risk categorization.

INTERMEDIATE RISK

– Mutations:

- Mutated *NPM1* with *FLT3*-ITD mutation. The risk may change according to MRD results.

Table 3. World Health Organization (WHO) and International Consensus Classification (ICC) 2022 classifications for the diagnosis of acute myeloid leukemia (AML)

WHO	ICC*
AML with recurrent genetic alterations (no minimum blast threshold required except for indicated entities)	AML with recurrent genetic alterations (requires 10% blasts for diagnosis)
APL with <i>PML::RARA</i> rearrangement	APL with t (15;17)(q24.1;q21.2)/ <i>PML::RARA</i> [†]
AML with <i>RUNX1::RUNX1T1</i> rearrangement	AML with t (8;21)(q22;q22.1)/ <i>RUNX1::RUNX1T1</i>
AML with <i>CBFB::MYH11</i> rearrangement	AML with inv (16)(p13.1q22) or t (16;16)(p13.1;q22)/ <i>CBFB::MYH11</i>
AML with <i>DEK::NUP214</i> rearrangement	AML with t (6;9)(p22.3;q34.1)/ <i>DEK::NUP214</i>
AML with <i>KMT2A</i> rearrangements	AML with t (9;11)(p21.3;q23.3)/ <i>MLLT3::KMT2A</i> [‡]
AML with <i>MECOM</i> rearrangements	AML with inv (3)(q21.3q26.2) or t (3;3)(q21.3;q26.2)/ <i>GATA2, MECOM (EVI1)</i> [§]
AML with <i>BCR::ABL1</i> rearrangements (≥ 20% blasts)**	AML with t (9;22)(q34.1;q11.2)/ <i>BCR::ABL1</i> ^{†**} (≥ 20% blasts)**
AML with <i>NPM1</i> mutation	AML with <i>NPM1</i> mutation AML with <i>CEBPA</i> mutation (≥ 20% blasts) ^{††}
AML with in-frame mutation in bZIP in <i>CEBPA</i>	AML with <i>NUP98</i> rearrangements
AML with <i>RBM15::MRTFA</i> rearrangement	AML with other rare recurrent translocations
AML with other defined genetic alterations ^{‡‡}	
Categories considered AML (> 20% blasts) or MDS/AML (10-19% blasts)	
AML related to myelodysplasia (≥ 20% blasts) ^{§§}	AML with gene mutations associated with myelodysplasia ^{¶¶}
	AML with cytogenetic alterations associated with myelodysplasia ^{***}
	AML with mutated <i>TP53</i>
AML defined by differentiation (> 20% blasts) ^{†††}	Category without recurrent genetic alterations AML
	AML not otherwise specified (NOS) (> 20% blasts) or MDS/AML (10-19% blasts)

*The diagnostic categories indicated in the top take precedence over those located lower in the classification.

[†]Other recurrent translocations affecting *RARA* should be reported, including: *IRF2BP2::RARA*, *NPM1::RARA*, *ZBTB16::RARA*, *STAT5B::RARA*, *STAT3::RARA*; other rarely rearranged *RARA* genes are *TBL1XR1*, *FIP1L1*, *BCOR*.

[‡]Other recurrent translocations affecting *KMT2A* should be reported, including *AFF1::KMT2A*[§], *AFDN::KMT2A*, *MLLT10::KMT2A*, *TET1::KMT2A*, *KMT2A::ELL*, *KMT2A::MLLT1*[§] (occur predominantly in children).

[§]Other recurrent translocations affecting *MECOM* should be reported, including *MECOM::?*, *MECOM::MYC*, *ETV6::MECOM*, *MECOM::RUNX1*.

^{**}Both classifications require 20% blasts to adequately differentiate it from chronic myeloid leukemia.

^{††}Includes both in-frame mutation in bZIP in *CEBPA* and biallelic mutations and requires 20% blasts for diagnosis.

^{†††}Includes rare but recurrent gene fusions in AML.

^{§§}Can be defined by gene mutations or cytogenetic alterations. Gene mutations are *ASXL1*, *BCOR*, *EZH2*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, or *ZRSR2*. Cytogenetic alterations are complex karyotype (≥ 3 alterations), 5q deletion or loss of 5q due to unbalanced translocations, monosomy 7, 7q deletion or loss of 7q due to unbalanced translocations, 11q deletion, 12p deletion or loss of 12p due to unbalanced translocations, monosomy 13, 13q deletion, 17p deletion or loss of 17p due to unbalanced translocations, isochromosome 17q, isodicentric idic (X)(q13).

^{¶¶}Defined by the presence of mutations in the genes *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, or *ZRSR2*. It is noteworthy that they are the same mutations included by the WHO adding the *RUNX1* mutation.

^{***}The cytogenetic alterations defining this entity are complex karyotype (≥ 3 alterations without the presence of other recurrent genetic alterations collected in the classification and excludes hyperdiploid karyotypes with three or more trisomies or polysomies without structural alterations) and different unbalanced alterations: del (5q)/t (5q)/add (5q); -7/del (7q); +8; del (12p)/t (12p)/add (12p); i (17q), -17/add (17p) or del (17p); del (20q); and/or idic (X)(q13).

^{††††}Includes cases of AML without specific genetic alterations.

APL: acute promyelocytic leukemia.

- Non-mutated *NPM1* with *FLT3*-ITD mutation.
- Rearrangements:
 - t(9;11)(p21.3;q23.3); *MLLT3::KMT2A*. The presence of this alteration takes precedence over the co-occurrence of rare adverse risk mutations. Additionally, initial risk may change according to MRD results.
 - Presence of cytogenetic alterations is not considered favorable or adverse risk.

ADVERSE RISK

- Mutations:
 - Mutations in *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, or *ZRSR2*, although they should not be considered adverse prognostic markers if they coexist with a favorable prognostic marker.

Table 4. World Health Organization (WHO) and International Consensus Classification (ICC) 2022 classifications of hematologic neoplasms with germline predisposition

WHO	ICC
Myeloid neoplasms with germline predisposition without preexisting platelet abnormalities or organ dysfunction	Hematological neoplasms with germline predisposition without constitutional disease affecting multiple organ systems
Germline P/LP variants of <i>CEBPA</i> (familial AML associated with <i>CEBPA</i>)	Myeloid neoplasms with germline mutation of <i>CEBPA</i>
Germline P/LP variants of <i>DDX41</i>	Myeloid or lymphoid neoplasms with germline mutation of <i>DDX41</i>
Germline P/LP variants of <i>TP53</i> (Li-Fraumeni syndrome)	Myeloid or lymphoid neoplasms with germline mutation of <i>TP53</i>
Myeloid neoplasms with germline predisposition and preexisting platelet abnormalities	Hematological neoplasms with germline predisposition associated with constitutional platelet abnormalities
Germline P/LP variants of <i>RUNX1</i> (familial platelet disorder with associated myeloid neoplasm, FPD-MM)	Myeloid or lymphoid neoplasms with germline mutation of <i>RUNX1</i>
Germline P/LP variants of <i>ANKRD26</i> (thrombocytopenia 2)	Myeloid neoplasms with germline mutation of <i>ANKRD26</i>
Germline P/LP variants of <i>ETV6</i> (thrombocytopenia 5)	Myeloid or lymphoid neoplasms with germline mutation of <i>ETV6</i>
Myeloid neoplasms with germline predisposition and potential organ dysfunction	Hematological neoplasms with germline predisposition with constitutional disease affecting multiple organ systems
Germline P/LP variants of <i>GATA2</i> (<i>GATA2</i> deficiency)	Myeloid neoplasms with germline mutation of <i>GATA2</i>
Bone marrow failure syndromes: Severe congenital neutropenia Shwachman-Diamond syndrome Fanconi anemia	Myeloid neoplasms with germline mutation of <i>SAMD9</i> Myeloid neoplasms with germline mutation of <i>SAMD9L</i>
Telomeropathies	Myeloid neoplasms associated with bone marrow failure syndromes Fanconi anemia Shwachman-Diamond syndrome Telomeropathies including dyskeratosis congenital Severe congenital neutropenia Diamond-Blackfan anemia
RASopathies (neurofibromatosis type 1, <i>CBL</i> syndrome, Noonan syndrome or Noonan syndrome-like diseases)	
Down syndrome	
Germline P/LP variants of <i>SAMD9</i> (MIRAGE syndrome)	
Germline P/LP variants of <i>SAMD9L</i> (pancytopenia syndrome with <i>SAMD9L</i> mutations)	Juvenile myelomonocytic leukemia associated with neurofibromatosis
Germline biallelic P/LP variants of <i>BLM</i> (Bloom syndrome)	Juvenile myelomonocytic leukemia associated with Noonan syndrome
	Myeloid or lymphoid neoplasms associated with Down syndrome
	Acute lymphoblastic leukemia with germline predisposition*
	Acute lymphoblastic leukemia with germline mutation of <i>PAX5</i>
	Acute lymphoblastic leukemia with germline mutation of <i>IKZF1</i>

*Down syndrome, with germline mutations in *ETV6* and *TP53*, also predisposes to acute lymphoblastic leukemia.
P: pathogenic; LP: likely pathogenic.

- Mutated *TP53* with an allelic fraction of at least 10% and regardless of whether it is mono- or biallelic. Frequently, mutated *TP53* is associated with complex and monosomic karyotype.
- Rearrangements:
 - t(6;9)(p23;q34.1); *DEK::NUP214*.
 - t(v;11q23.3); *KMT2A* rearrangements excluding partial tandem duplications (PTD).
 - t(9;22)(q34.1;q11.2); *BCR::ABL1*.
 - t(8;16)(p11;p13); *KAT6A::CREBBP*.
 - inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2*, *MECOM(EVI1)*.
 - t(3q26.2;v); *MECOM(EVI1)*-rearranged.
 - -5 or del(5q); -7.
 - -17/abn(17p).

- Complex or monosomic karyotype. A complex karyotype is defined by the presence of three or more unrelated chromosomal abnormalities in the absence of other defining recurrent genetic abnormalities of class; hyperdiploid karyotypes with three or more trisomies (or polysomies) without structural abnormalities are excluded. On the other hand, a monosomic karyotype is defined by the presence of two or more distinct monosomies (excluding loss of X or Y), or a single autosomal monosomy in combination with at least one structural chromosomal abnormality (excluding CBF-AML).

In addition to genetic stratification at diagnosis, experts highlight early MRD assessment¹⁵ and the use of targeted therapies as key factors in individual risk evaluation. Thus, in clinical practice, a patient with *NPM1* mutations or *CBFB::MYH11*, *RUNX1::RUNX1T1*, and *MLLT3::KMT2A* rearrangements can transition groups according to the presence or absence of MRD.

Regarding follow-up markers, currently flow cytometry and quantitative polymerase chain reaction (PCR) are the two standard techniques for MRD follow-up. However, new approaches are being explored, such as digital PCR (dPCR), limited by sensitivity and the need for a specific design for each alteration, or error-corrected NGS, which allows the detection threshold to be increased to 0.01-0.001%. This latter possibility is very attractive, as it would allow the identification of MRD molecular markers in almost all AMLs, while excluding germline variants and premalignant alterations associated with clonal hematopoiesis.

In addition to the ELN prognostic classification, the extensive molecular characterization of AML that NGS has provided has enabled new classification proposals that aim to integrate the classification and stratification of AML, such as that proposed by Papaemmanuil et al. in 2016¹⁶ and revised in 2022.¹⁷ This genomic classification performed in 3,653 patients eligible for intensive treatment includes 16 genetic-molecular classes. Each class represents a biological subgroup of AML associated with clinical-biological characteristics and a different response to induction treatment with chemotherapy.

Treatment

Currently, apart from the relevance of correct characterization of mutations at diagnosis of all AMLs due to their impact on prognosis, we must bear in mind that it can also have an impact on treatment, which constitutes an important advance beyond schemes based on standard chemotherapy.

At present, the only drug targeting mutations that is approved for use in first-line treatment is midostaurin, an FLT3 inhibitor that can be administered in combination with chemotherapy to those patients presenting an internal tandem duplication (*FLT3*-ITD) or a point mutation in the tyrosine kinase domain (TKD) (*FLT3*-TKD), both of which lead to constitutive receptor activation. In relapsed or refractory AML patients with *FLT3* mutations, both ITD and TKD, the use of the FLT3 inhibitor gilteritinib as monotherapy has been approved. These inhibitors have received approval for all patients carrying *FLT3* mutations, regardless of cytogenetic characteristics. Additionally, there are other drugs targeting these mutations that are in late phases of clinical trials and showing very promising results (quizartinib, sorafenib, crenolanib, etc.) not only in first line but also in more advanced phases or as maintenance treatment.

Similar to *FLT3*, the detection of mutations in *IDH1* and *IDH2* by NGS may also have therapeutic relevance. In this regard, clinical trials are being developed for the approval of IDH1 inhibitors (enasidenib) and IDH2 (ivosidenib), which are already in very advanced phases and could also be administered in first line. In earlier phases of evaluation are other targeted treatments such as menin inhibitors for patients with *KMT2A* rearrangement or *NPM1* mutation¹⁸ and another group of drugs targeting *TP53* mutations.¹⁹

As a consequence of all the innovation occurring in the field of drugs targeting molecular targets, the treatment of AML, which until recently was uniform for all patients, will become increasingly personalized. Both in the case of new diagnoses and in patients presenting relapse, it will be necessary to perform mutational analysis rapidly, as treatment initiation should wait for these results.

Acknowledgments

To the Spanish Society of Hematology and Hemotherapy for their support in the publication of these guidelines.

Funding

Longwood Diagnostics supported the production of the guidelines.

Conflicts of interest

J.M. Alonso-Domínguez received honoraria for participation as speaker, consultant, or advisor from Astellas

and Servier. B. Bellosillo-Paricio received honoraria for participation as speaker, consultant, or advisor from AstraZeneca, Janssen, Merck-Serono, Novartis, Roche, ThermoFisher, Pfizer, and BMS, as well as research grants from AstraZeneca, ThermoFisher, Roche Diagnostics, and Roche Farma. CBS received honoraria as speaker from Astellas and ThermoFisher. M.T. Gómez-Casares received honoraria for participation as speaker, consultant, or advisor from Novartis, Roche, GSK, Pfizer, and BMS, as well as research grants from Novartis, Astellas, and Incyte. The remaining authors have no conflicts of interest.

Ethical considerations

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

Confidentiality, informed consent, and ethical approval. The study does not involve patient personal data nor require ethical approval. SAGER guidelines do not apply.

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

Supplementary material

Supplementary material is available at DOI: 10.24875/SANGRE.25000001. This material 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.

References

- Shallis RM, Wang R, Davidoff A, Ma X, Zeidan AM. Epidemiology of acute myeloid leukemia: Recent progress and enduring challenges. *Blood Rev.* 2019;36:70-87.
- Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. *Leukemia.* 2022;36(7):1703-19.
- Arber DA, Orazi A, Hasserjian RP, Borowitz MJ, Calvo KR, Kvasnicka HM, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. *Blood.* 2022;140(11):1200-28.
- Döhner H, Wei AH, Appelbaum FR, Craddock C, DiNardo CD, Dombret H, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. *Blood.* 2022;140(12):1345-77.
- Chicano M, Carbonell D, Suárez-González J, Lois S, Ballesteros-Culebras M, Andrés-Zayas C, et al. Next generation cytogenetics in myeloid hematological neoplasms: detection of CNVs and translocations. *Cancers (Basel).* 2021;13(12):3001.
- Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood.* 2017;129(4):424-47.
- Döhner K, Thiede C, Jahn N, Panina E, Gambietz A, Larson RA, et al. Impact of NPM1/FLT3-ITD genotypes defined by the 2017 European LeukemiaNet in patients with acute myeloid leukemia. *Blood.* 2020;135(5):371-80.
- van der Werf I, Wojtuszkiewicz A, Meggendorfer M, Hutter S, Baer C, Heymans M, et al. Splicing factor gene mutations in acute myeloid leukemia offer additive value if incorporated in current risk classification. *Blood Adv.* 2021;5(17):3254-65.
- Awada H, Durmaz A, Gurnari C, Kishtagari A, Meggendorfer M, Kerr CM, et al. Machine learning integrates genomic signatures for subclassification beyond primary and secondary acute myeloid leukemia. *Blood.* 2021;138(19):1885-95. Erratum in: *Blood.* 2022;139(9):1424-5.
- Angenendt L, Röhlig C, Montesinos P, Martínez-Cuadrón D, Barragán E, García R, et al. Chromosomal abnormalities and prognosis in NPM1-mutated acute myeloid leukemia: a pooled analysis of individual patient data from nine international cohorts. *J Clin Oncol.* 2019;37(29):2632-42.
- Taube F, Georgi JA, Kramer M, Stasik S, Middeke JM, Röhlig C, et al.; Study Alliance Leukemia (SAL). CEBPA mutations in 4708 patients with acute myeloid leukemia: differential impact of bZIP and TAD mutations on outcome. *Blood.* 2022;139(1):87-103.
- Ottma S, Mulet-Lázaro R, Beverloo HB, Erpelinck C, van Herk S, van der Helm R, et al. Atypical 3q26/MECOM rearrangements genocopy inv(3)/(t(3;3)) in acute myeloid leukemia. *Blood.* 2020;136(2):224-34.
- Kayser S, Hills RK, Langova R, Kramer M, Gujjarro F, Sustkova Z, et al. Characteristics and outcome of patients with acute myeloid leukaemia and t(8;16)(p11;p13): results from an International Collaborative Study. *Br J Haematol.* 2021;192(5):832-42.
- Chilton L, Hills RK, Harrison CJ, Burnett AK, Grimwade D, Moorman AV. Hyperdiploidy with 49-65 chromosomes represents a heterogeneous cytogenetic subgroup of acute myeloid leukemia with differential outcome. *Leukemia.* 2014;28(2):321-8.
- Heuser M, Freeman SD, Ossenkoppele GJ, Buccisano F, Hourigan CS, Ngai LL, et al. 2021 Update on MRD in acute myeloid leukemia: a consensus document from the European LeukemiaNet MRD Working Party. *Blood.* 2021;138(26):2753-67.
- Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic Classification and Prognosis in Acute Myeloid Leukemia. *N Engl J Med.* 2016;374(23):2209-21.
- Tazi Y, Arango-Ossa JE, Zhou Y, Bernard E, Thomas I, Gilkes A, et al. Unified classification and risk-stratification in Acute Myeloid Leukemia. *Nat Commun.* 2022;13(1):4622.
- Stein EM, Aldoss I, DiPersio JF, Stone RM, Arellano ML, Rosen G, et al. Safety and efficacy of menin inhibition in patients with MLL-rearranged and NPM1 mutant acute leukemia: a phase 1, first-in-human study of SNDX-5613 (AUGMENT 101). *Blood.* 2021;138(Suppl):699.
- Granowicz EM, Jonas BA. Targeting TP53-mutated acute myeloid leukemia: research and clinical developments. *Onco Targets Ther.* 2022;15:423-36.