

Genetic Characterization and Risk Stratification of Acute Myeloid Leukemia

This article was published in the following Dove Press journal:
Cancer Management and Research

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Abstract: The most common acute leukemia in adults is acute myeloid leukemia (AML). The pathophysiology of the disease associates with cytogenetic abnormalities, gene mutations and aberrant gene expressions. At the molecular level, the disease manifests as changes in both epigenetic and genetic signatures. At the clinical level, two aspects of AML should be taken into account. First, the molecular changes occurring in the disease are important prognostic and predictive markers of AML. Second, use of novel therapies targeting these molecular changes. Currently, cytogenetic abnormalities and molecular alterations are the common biomarkers for the prognosis and choice of treatment for AML. Finding a panel of multiple biomarkers is a crucial diagnostic step for patient classification and serves as a prerequisite for individualized treatment strategies. Furthermore, the most important way of identifying relevant targets for new treatment approaches is defining specific patterns or a spectrum of driver gene mutations occurring in AML. Then, an algorithm can be established by the use of several biomarkers, to be used for personalized medicine. This review deals with molecular alterations, risk stratification, and relevant therapeutic decision-making in AML.

Keywords: acute myeloid leukemia, AML, genetic characterization, risk stratification

Acute Myeloid Leukemia

Acute myeloid leukemia (AML) results from clonal expansion of transformed hematopoietic stem cells (HSCs) through the acquisition of genetic abnormalities including chromosomal rearrangements and multiple gene mutations whereby HSCs are conferred a proliferative and survival advantage and impaired hematopoietic differentiation. AML is a phenotypically and genetically heterogeneous disease. Biologically, different types of mutations which complement each other and cooperate in leukemogenesis should occur in an HSC. Acquired mutations enhance self-renewal and proliferation in an HSC wherein normal mechanisms of differentiation are impaired and eventually lead to a clonal expansion of abnormal immature leukemic blasts which accumulate in the bone marrow. They gradually replace normal hematopoietic tissue and interfere with the production of normal blood cells, resulting in impaired hematopoiesis and bone marrow failure^{1,2} manifested as cytopenia. Blasts then frequently spread to other parts of the body such as the lymph nodes, spleen, liver, testes and central nervous system.³ Different subtypes of AML are each associated with distinct genetic and molecular abnormalities. Due to heterogeneity in the genomic landscape of leukemia, there are great efforts to devise new-targeted therapies to personalize medicine in AML therapy.^{4,5}

This is evident by the increasing number of compounds have been made available and combined with conventional chemotherapy during induction or

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consolidation therapy, novel interventions that are personalized to the host and tumor genotype.^{6,7}

A well-known procedure to distinguishing tumor genotypes and classify different subgroups of AML was proposed by the World Health Organization (WHO), through using morphologic, immunologic, cytogenetic and molecular biologic classification techniques (MICM). The classification scheme was proposed to distinguish different genetic and molecular abnormalities in the diagnosis of AML and provides a framework for clinical management. It especially emphasizes the importance of genetic test results to define clinically relevant disease entities in conjunction with morphology, immunophenotype, and other clinicopathologic features.⁷⁻⁸

However, for any samples suspected for AML, karyotyping is first required; if it is normal, then the fluorescent in situ hybridization (FISH) or reverse transcriptase-polymerase chain reaction (RT-PCR) technique is essential to detect cryptic rearrangement of the relevant locus (Table 1). This genetic approach seems to be used in clinics for initial diagnosis and decision made on therapeutic choices. Then, at the time of first remission or relapse, additional testing is ordered to refine prognosis.⁹⁻¹¹

Genetic Abnormalities in AML (a Collaboration of at Least Three Types of Mutations)

Based on the cytogenetic and molecular analysis and according to the risk stratification, AML genetic aberrations are placed into three categories; 1) Non-random chromosomal aberrations including balanced translocations, inversions, deletions, monosomies, and trisomies (Tables 2 and 3), 2) Multiple gene mutations (Table 4) and 3) Epigenetic alterations (Table 5).^{6,12-14}

Actually, aberrations should occur in genes relevant for pathogenesis, ie, master transcription factor fusions (18% of cases), NPM1 (27% of cases), tumor-suppressor genes (16%), DNA-methylation-related genes (44%), signaling genes (59%), chromatin-modifying genes (30%), myeloid-transcription genes (22%), cohesion complex genes (13%), and spliceosome complex genes (14%).^{1,3,7}

At the molecular level, AML is the consequence of collaboration between at least three broad classes of gene alterations (Table 6 & Figure 1). Class I gene alterations are those aberrations that activating signal transduction pathways and enhancing proliferation with survival advantages of hematopoietic stem cells. This class of gene aberrations can involve

and activate the receptor tyrosine kinase FLT3 and Kit or the RAS-associated signaling pathway. Class II gene alterations affect a master transcription factor or a protein involved in hematopoietic differentiation. This second aberration would impair differentiation of a hematopoietic progenitor cell (HPC) and aberrant acquisition of self-renewal properties whereby increase the likeliness of malignant transformation. Outstanding examples for this class of aberrations are the recurring gene fusions *RUNX1-RUNX1T1* and *PML-RARA* resulting from *t(8;21)*, *inv(16)/t(16;16)* and *t(15;17)* rearrangements, as well as mutations in *CCAAT enhancer-binding protein A (CEBPA)* and *nucleophosmin 1 (NPM1)* genes.^{15,16,17}

Class 0/III are those alterations that promote epigenetic modifications of chromatin in a large area and affect further transcription factors or components of the transcriptional co-activation complexes whereby would confer malignant transformation to the HPCs and lead to overt AML (eg, *DNMT3A* and *IDH1/2*, involved in epigenetic regulation of chromatin and cellular processes). Class 0/III alterations, however, can also be happened before class I.^{2,6,12,13}

Cytogenetic and Chromosomal Rearrangements

Cytogenetic abnormalities, detectable in approximately 50–60% of newly diagnosed AML patients, are categorized as non-random chromosomal rearrangements. These aberrations are further classified into three categories; AML with balanced translocations/inversions (Table 2), AML with various cytogenetic abnormalities (un-balanced translocations, eg deletions, monosomies, and trisomies) and AML with complex karyotype (representing at least 3 acquired chromosomal aberrations) (Table 3).

Cytogenetically, AML has three prognostic categories: favorable, intermediate and poor-risk group. The first group includes balanced translocations with a favorable outcome. The poor risk group has a complex aberrant karyotype that confers a poor clinical outcome. The intermediate prognosis group includes normal karyotype and other karyotypic abnormalities (Table 1).^{6,7}

Balanced Chromosomal Rearrangements (Translocations/Inversions)

Balanced translocations and inversions include *inv(16) (p13.1q22)/t(16;16) (p13.1;q22)*, *t(8;21)(q22;q22)*, *inv(3) (q21q26.2)/t(3;3)(q21;q26.2)*, *t(6;9)(p23;q34)*, *t(inv(11q23) and t(15;17)(q24;q21)*. These lesions on their own are not sufficient to induce leukemia, and there is a requirement for

Table 1 Summary of Major Cytogenetic Abnormalities Observed in AML and Related Methods of Detection

Abnormality	Cytogenetic Biomarkers	Prognosis	Methods for Detection
<i>t(8;21)(q22;q22)</i>	<i>AML1-ETO</i> or <i>RUNX1-CBFA2T1</i> (AML M4)	Favorable	FISH, RT-PCR
<i>Inv(16)(p13;q22)</i>	<i>CBFB-MYH11</i> (AML M2)	Favorable	FISH, RT-PCR, RQ-PCR
<i>t(9;22)(q43;q11)</i>	<i>BCR-ABL1</i> (MDS)	Unfavorable	FISH, RT-PCR, RQ-PCR
<i>t(15;17)(q22;q21)</i>	<i>PML-RARA</i> (APL, AML)	Favorable	FISH, RT-PCR
<i>11q23</i>	<i>MLL</i> or <i>LEK1</i> , <i>HXR</i> , <i>HTRX1</i>	Intermediate or adverse risk in AML	FISH, RT-PCR, Southern blot
<i>t(11;19)(q23;p13.3)</i>	<i>MLL-ENL</i> (AML M4, M5)	Unfavorable in AML	FISH, RT-PCR, Southern blot
<i>t(6;11)(q27;q23)</i>	<i>MLL-AF6</i> (AML M4, M5)	Unfavorable in AML	FISH, RT-PCR, Southern blot
<i>t(9;11)(p22;q23)</i>	<i>MLL-AF9</i>	Favorable in AML (patients with no additional abnormalities or M5 FAB)	FISH, RT-PCR, Southern blot
<i>t(10;11)(p12;q23)</i>	<i>MLL-AF10</i> or <i>MLLT10</i> (AML M4, M5)	Unfavorable in AML	FISH, RT-PCR, Southern blot
<i>9q</i>	Unknown	Unfavorable	FISH
<i>t(1;22)(p13;q13)</i>	<i>OTT-MAL</i> (AML M7)	Unfavorable	Standard cytogenetic analysis
<i>t(6;9)(p23;q34)</i>	<i>DEK-NUP214</i>	Unfavorable	

Note: Data from Prada-Arisemendy et al³ and Mrozek et al.⁸

Abbreviations: AML, acute myeloid leukemia; FISH, fluorescence in situ hybridization; RQ-PCR, real-time quantitative polymerase chain reaction; RT-PCR, reverse transcriptase-polymerase chain reaction; APL, Acute promyelocytic leukemia; MDS, myelodysplastic syndrome; FAB, the French-American-British group.

additional secondary genetic lesions to accompany the balanced translocation/inversion. For example, *trisomy 22* in AML with *inv(16)/t(16;16)*, *deletion 9q* in AML with *t(8;21)*, or *monosomy 7* in AML with *inv(3)/t(3;3)* (Table 2).^{2,15}

The results of *t(8;21)* and *inv(16)/t(16;16)* are, respectively, fusion proteins RUNX1-RUNX1T1 (AML1-ETO) and CBFB-MYH11 which block the transactivation of tumor-suppressor genes, and associate with favorable outcome in AML. Accordingly, the fusion proteins RUNX1/AML1 and CBFB-MYH11 impair myeloid differentiation and induce self-renewal properties of HPCs but on their own do not cause an overt leukemic phenotype. Rather, there should be further rare mutations in the germline of affected patients to predispose these persons for developing AML. More than one genetic change should occur in HPCs to show overt leukemia phenotype (Figures 2 and 3).¹⁵⁻⁹

T(8;21)(q22;q22) (AML1/RUNX1-ETO/RUNX1T1, CBF)

The reciprocal *t(8;21)(q22;q22)* between the chromosomes 8 and 21 results in the fusion gene *AML1-ETO* (*RUNX1-RUNX1T1*) whose product is the fusion protein AML1-ETO. The RUNX protein family or core-binding factors (CBFs) constitutes of a group of heterodimeric transcription factors that are composed of an α -subunit (CBF α ; encoded by 3 distinct genes: *Runx1/Runx2/Runx3*) and a β -subunit (CBF β ; encoded by *CBFB*). This family is also known as the polyomavirus enhancer-binding protein-2 (PEB-2).¹⁰ At the molecular level, RUNX1 (AML1) is a DNA-binding factor, and a master regulator of HPCs, while RUNX1T1 (ETO) is a transcription factor with the repressor activity (an oncogene). The

N-terminal domain of AML1 fuses to the 577 residues from the ETO C-terminal domain. The fusion protein AML1-ETO recognizes AML1 consensus binding sites and heterodimerizes with CBF β , wherein harboring transcriptional repressor activities.^{1,2} The fusion protein acts as a transcriptional repressor to block AML1-dependent transactivation and transcription of tumor suppressors. In fact, the translocation interrupts AML1 protein between the N-terminal and transactivation domains, while leaving the transactivation domain of ETO protein intact.^{5,6} This type of translocation has the highest incidence in childhood AML (~12% of AML cases in children) (Figure 4), and profoundly associates with M2 FAB subtype (AML with maturation), but rarely seen with AML M1 or M4 subtypes. This kind of cytogenetic aberration is highly significant for diagnosis and therapy management, since on its own associates with favorable outcomes, high remission rates and long median survival, whereas *c-Kit* mutations occurring concurrently with *t(8;21)(q22;q22)*, compose an independent adverse prognostic biomarker (Table 2, Figure 2). In addition, in pediatric AML M2 subtype with *t(8;21)*, the X-chromosome loss may occur as the secondary cytogenetic events with no clinical significance, whereas the loss of the Y chromosome, as the secondary genetic event, forms a critical mutational event. Generally, conventional cytogenetic analysis FISH, or RT-PCR can readily predict AML1-ETO outcomes (Table 1).⁴⁻⁶ Patients with *t(8;21)* have a favorable prognosis with good response to conventional chemotherapy (the combination of an anthracycline and cytarabine, the “3 + 7” regimen) and show complete remissions (Tables 1 and 2). At least three or four cycles of intensive

Table 2 Cytogenetic Abnormalities (Non-Random Chromosomal Rearrangements) Found in the Leukemic Blasts of 55% of Adults with AML

Chromosomal Abnormalities	Gene Product	Secondary Genetic Alteration	Clinical Relevance	Chemotherapy Recommended
I. Balanced chromosomal rearrangements plus gene mutations (ie, balanced translocations, inversions)				
(a) Core-binding-factor acute myeloid leukemia (10–15% of AML, a good response to treatment)				
<i>inv(16)(p13.1;q22)/t(16;16)(p13.1;q22)</i>	<i>CBFβ-MYH11</i>	–	Favorable outcome, cytarabine-anthracycline responders in induction, obtaining CR	Intensive post-remission CC of HDAC [36, 44].
		Trisomy 22	?	?
		<i>KIT(exon 17)</i> , <i>RAS</i> , <i>FLT3</i>	Inferior & relapsing outcomes (eg, young adults), increases the risk of relapse, decreases duration of remission, adverse prognostic factor for OS	Intensive CC + dual TKi (Midostaurin), dual SRC/ABLKi (Dasatinib), post-remission of HDAC
<i>t(8;21)(q22;q22)</i>	<i>AML1-ETO/RUNX1-RUNX1T1</i>	–	Favorable outcome, cytarabine-anthracycline responders in induction, obtaining CR	Intensive post-remission CC of HDAC [36, 44]
		Deletion 9q	?	?
		<i>KIT(exon 17)</i> , <i>RAS</i> , <i>FLT3</i>	Inferior & relapsing outcomes (eg, young adults), increases the risk of relapse adverse, decreased duration of remission, adverse prognostic factor for OS	Intensive CC + dual TKi (Midostaurin), dual SRC/ABLKi (Dasatinib), post-remission of HDAC
(b) Other molecular alterations in AML with balanced translocations/inversions				
<i>t(6;9)(p23;q34)</i>	<i>DEK-NUP214</i>	–	Poor treatment outcome	?
		<i>FLT3</i>	Lower CR, inferior DFS and OS	?
<i>inv(3)(q21;q26.2)/t(3;3)(q21;q26.2)</i>	<i>MDS1/EVI1-RPN1</i>	–	Over-expressed <i>EVI1</i> , Independent predictor of poor survival	?
		Monosomy 7, 5q deletions	?	?
		<i>NRAS</i>	?	?
<i>t(inv(11q23) [43]</i>	<i>MLL, LEK1, HXR, HTRX1</i>	–	Poor prognosis, treated according to high-risk protocols	?
<i>t(11;19)(q23;p13.3)</i>	<i>MLL-ENL</i>	–	Unfavorable in AML, M4 or M5 subtypes	?
<i>t(6;11)(q27;q23)</i>	<i>MLL-AF6</i>	–	Unfavorable in AML, M4 or M5 subtypes	?
<i>t(9;11)(p22;q23)</i>	<i>MLL-AF9</i>	–	Unfavorable in AML but favorable in AML patients with no additional abnormalities or M5 FAB	?
<i>t(10;11)(p12;q23)</i>	<i>MLL-AF10 or MLLT10</i>	–	Unfavorable in AML, M4 or M5 subtypes	?
<i>t(15;17)(q24;q21)</i>	<i>PML-RARA</i>	–	Favorable response to treatment (ATRA + arsenitrioxid), 100% CR and a 97% 2-year EFS in low-risk APL patients	ATRA + arsenitrioxid as a remission induction

Notes: ?Data are not available. Data from references 2, 3, 5, 15, and 10.

Abbreviations: HDAC, high-doses of cytarabine; ATRA, all-trans retinoic acid; EFS, event-free survival; CC, conventional chemotherapy; RFS, relapse-free survival; OS, overall survival; RTK, receptor tyrosine kinase; TF, transcription factor; ITD, internal tandem duplication; TKD, tyrosine kinase domain; JM, juxtamembrane domain; MRD, matched related donor; PTD, partial tandem duplication; CR, complete remission; EFS, event-free survival; HDACi, histone deacetylase inhibitor; MYH11, smooth muscle myosin heavy chain 11; CBF, core-binding-factor; BAALC, brain and acute leukemia cytoplasmic gene; ERG, ETS-related genes; MNI, meningioma.

Table 3 Other Cytogenetic Abnormalities (Non-Random Chromosomal Rearrangements) Found in the Leukemic Blasts of 55% of Adults with AML

Chromosomal Abnormalities	Gene Product	Secondary Genetic Alteration	Clinical Relevance	Chemotherapy Recommended
1. Molecular alterations in AML with various cytogenetic abnormalities				?
Deletion 9q	Class I mutation	–	Intermediate risk group/unfavorable outcome	?
		<i>CEBPA/NPM1</i>	Class II mutations, mutually exclusive	?
Trisomy 8	Class I mutation	–	Intermediate risk group	?
		<i>NPM1</i>	?	?
Trisomy 11	Class I mutation	–	Intermediate risk group/unfavorable outcome	?
		<i>MLL</i>	?	?
Trisomy 13, 21, monosomy 7	Class I mutation	–	Intermediate risk group	?
		<i>RUNX1</i>	Oncogene, unfavorable/adverse outcome	?
2. AML with complex karyotype (≥3 acquired chromosomal aberrations) (10-12% of all AML)				
Deletions of: –5/5q, –17/17p, –7/7q, –18/18q, 16q, 17q, 12p, –20/20q, 18p, 3p	Genome instability	<i>TP53</i> (it is lost with high frequency)	Unfavorable; lower CR and OS rates and shorter DFS	?
Genomic gains to: 8q, 11q, 21q, 22q, 1p, 9p, 21q, 13q	Oncogene activation	<i>ETS</i> family (21q and 11q23-24)	Unfavorable; lower CR and OS rates and shorter DFS	?
	<i>BAALC</i> (8q22.3) Over-expression	–	Unfavorable; lower CR and OS rates and shorter DFS	?
	<i>ERG</i> (21q22.3) Over-expression	–	Unfavorable; lower CR and OS rates, higher relapse	?
	<i>MNI</i> (22 q11) Over-expression	–	Unfavorable; shorter RFS, ATRA resistance in elderly	?
Recurrent amplifications: 8q24 (<i>MYC</i> , <i>TRIB1</i>), 9p24 (<i>JAK2</i>), 11q13.5 (<i>GAB2</i>), 11q23.3 (<i>DDX6</i> , <i>MLL</i>), 13q12 (<i>CDX2</i> , <i>FLT3</i> , <i>FOXO1</i>), 11q23.3-q24.1. (<i>ETSI</i> , <i>FLI1</i>), 21q (<i>ERG</i> , <i>ETS2</i>)	Oncogene activation	–	?	?

Notes: The intermediate-risk group: any abnormality that is not classified as a favorable or adverse outcome. ?Data are not available. Data from references 2, 3, 5, 15, and 10.

Abbreviations: HDAC, high-doses of cytarabine; ATRA, all-trans retinoic acid; RFS, relapse-free survival; CC, conventional chemotherapy; RFS, relapse-free survival; OS, overall survival; RTK, receptor tyrosine kinase; TF, transcription factor; ITD, internal tandem duplication; TKD, tyrosine kinase domain; JM, juxtamembrane domain; MRD, matched related donor; PTD, partial tandem duplication; CR, complete remission; EFS, event-free survival (EFS); DNMTi, DNA methyltransferase inhibitor; AT, autologous transplantation; UPD, uniparental disomy; CBF, core-binding-factor; BAALC, brain and acute leukemia cytoplasmic gene; ERG, ETS-related genes; MNI, meningioma I.

post-remission therapy with high doses of cytarabine (HDAC) are recommended for adults, improving substantially the outcome and maximizing the gain of chemotherapy (Table 2).^{6,15}

However, in de novo AML cases, loss of function mutations (missense, nonsense or frameshift mutations) in *RUNX1* gene are classified as intermediate-risk AML, rather than as the favorable risk group, and highly

associate with AML M0. The *RUNX1* mutations associate with higher chemotherapy resistance (where refractory rates are about 30%), and lower event-free, relapse-free, and overall survival rates.³

Inv(16)(p13.1q22)/t(16;16)(p13.1;q22) (*CBFβ-MYH11*, *CBF* *Inv(16)(p13;q22)* leads to the formation of oncogene *CBFβ-*

Table 4 Prototype of Gene Mutations Occurred in Cytogenetically Normal AML or in Cytogenetically Defined AML Subsets (~40–50% of Adult and 25% of Pediatric AML)

Gene Mutations (Locations)	Biological and Clinical Features	Recommended Chemotherapy	Outcomes
<i>NPM1</i> (5q35) mutations	The most frequent genetic alteration in adult AML, mutated transcripts as MRD associated with a relapse and a lower rate of survival	Better response to induction & consolidation CC	Favorable outcome: (increased DFR, RFS and OS), achievement of CR
<i>FLT3-ITD</i> (13q12) mutations	A class III RTK, ITD in JM domain, constitutive activation of MAPK, STAT, and AKT/PI3K pathways, uncontrolled proliferation/survival of leukemic HPCs	CC + double TKi is recommended & promising	Inferior outcome/poor prognosis, especially depends on the high allelic ratio (the <i>FLT3</i> mutant allele/ <i>FLT3</i> wild-type allele >0.5); which show shorter CR duration, DFS and OS
<i>FLT3-TKD</i> (13q12) mutations	Point mutations in TK domain, constitutive activation of the receptor	CC + double TKi eg midostaurin, crenolanib, gilteritinib	Negative/positive prognostic impact if being with <i>NPM1</i> mutation
<i>CEBPA</i> (19q13) mutations	A master TF in hematopoiesis, mutations/its promoter hypermethylation decrease DNA-binding (leucine zipper domain) activity/its expression, mutually exclusive with <i>NPM1</i> mutations	–	Double-mutations have a favorable outcome: higher CR duration, better RFS, OS, similar to those of mutant <i>NPM1</i>
<i>MLL-PTD</i> (11q23) mutations	A DNA binding protein regulates hematopoiesis by epigenetics, cooperating with epigenetic factors (DNMTs & HDACs)	CC + HDACi (depsipeptide) + DNMTi (decitabine) reactivate the <i>MLL</i> wild-type allele & induce cell death of the blasts	Unfavorable outcome: shorter CR duration, inferior RFS & EFS, No effect on OS
<i>RUNX1/AML1</i> (21q22.3) mutations	A TF makes dimers with CBF- β for hematopoietic differentiation	–	Unfavorable outcome
<i>KIT</i> mutations	A class III RTK, a key role in proliferation & survival of hematopoietic progenitor cells, gain of function mutations, high frequency in t(8; 21), detected by allele specific PCR	CC + double TKi is recommended & promising	Inferior outcome, in particular in mutations of exon 17
<i>RAS</i> (<i>NRAS</i> , <i>KRAS</i>) (1p13) mutations	Membrane-associated G proteins, transforming oncogene, high frequency in the favorable risk inv(16) or inv(3) group, <i>NRAS/KRAS</i> the most frequent	Sensitive to HDCA (post-remission HDAC) + farnesyl transferase inhibitor (tipifarnib, shuts down RAS)	Poor outcome
<i>BAALC</i> (8q22.3) over-expression	Associated with high percentage of blood blasts, immature subtypes M0/M1, monocytic differentiation, accompanied by <i>FLT3-ITD</i> , <i>CEBPA</i> , <i>MLL-PTD</i> mutations, high <i>ERG</i> expression, a marker of MDR	Induction failure, modulation of induction + intensification of post-remission + consolidation with allogeneic SCT	An adverse risk factor; unfavorable outcome: (low CR rates, high CIR, inferior OS (3 years))
<i>WT1</i> (11p13) mutations	A TF is related to proliferation in hematopoietic progenitor cells, concurrent of <i>FLT3-ITD</i> , a marker of MRD,	Induction failure, modulation of induction + intensification of post-remission	Unfavorable; associated with induction failure
<i>MNI</i> (22 q11) over-expression	Low <i>MNI</i> expression responds to ATRA, high <i>MNI</i> expression resistant to ATRA	Poor response to the first induction treatment, ATRA resistance in elderly	Unfavorable outcome: (short RFS)

Note: Data from references 1–3,8, and 13.

Abbreviations: CC, conventional chemotherapy; MRD, minimal residual disease; RFS, relapse-free survival; OS, overall survival; CR, complete remission; EFS, event-free survival; CIR, cumulative incidence of relapse; DFS, disease-free survival; HPCs, hematopoietic progenitor cells; RTK, receptor tyrosine kinase; TF, transcription factor; FLT3, FMS-related tyrosine kinase 3; FLT3-ITD, internal tandem duplication of FLT3; TKD, tyrosine kinase domain; JM, juxtamembrane domain; MRD, matched related donor; PTD, partial tandem duplication; DNMTi, DNA methyltransferase inhibitor; CEBPA, CCAAT enhancer-binding protein gene; WT1, Wilms tumor gene; HDACi, histone deacetylase inhibitor; AT, transcription factor; CBF, core-binding-factor; BAALC, brain and acute leukemia cytoplasmic gene; MNI, meningioma; SCT, stem-cell transplantation; MDR, multi-drug resistance; RUNX1, Runt-related transcription factor 1, HDAC, high-doses of cytarabine; HDACi, histone deacetylase inhibitor.

Table 5 Summary of the Most Common Epigenetic Mutations Occurred in AML

Mutation Biomarkers	Clinical/Biological Features	Prognosis and Therapy
<i>DNMT3A</i>	The earliest & recurrent alteration in pre-leukemic HSCs, limiting myeloid differentiation and promoting HSC expansion, associated with enzyme loss-of-function & hypomethylation of Runx1, Erg, Myc, Smad3, mutation persists in HSCs after remission, whereby consequently impairing HSC differentiation	Unfavorable outcome and poor prognosis, or favorable response (higher CR & a superior OS) in DNMTi treatments (Dec, Aza)
<i>TP53</i>	Rare in hematological malignancies, very common in complex karyotypes, the most important prognostic factor in AML	Poor prognosis: (lower CR, IEF, RFS, RFR, OS)
<i>IDH 1/2</i>	DNA hypermethylation phenotype, blocking histone demethylation, neomorphic function, adverse clinical outcome & poor prognosis when accompany <i>NPM1</i> and <i>FLT3</i> mutations,	Adverse prognostic factor for overall survival, favorable response (higher CR and a superior OS) in DNMTi treatments (Dec, Aza)
<i>αKGDD TET</i>	DNA hypermethylation phenotype, blocking histone demethylation, <i>TET1</i> mutation (oncogenic role, reduce DNA methylation); <i>TET2</i> mutation (mutually exclusive with <i>IDH1/2</i> mutations) tumor suppressor role, induce high DNA methylation mainly in enhancer regions of <i>TS</i> genes	A higher clinical remission rate & favorable response (higher CR and a superior OS) in DNMTi treatments (Dec, Aza)

Note: Data from references 1–3, and 12.

Abbreviations: HSCs, hematopoietic stem cells; CR, complete remission rates; IEF, inferior event-free, RFR, relapse-free survival; OS, overall survival; CC, conventional chemotherapy; TKi, tyrosine kinase inhibitor; DNMTi, DNA methyltransferase inhibitor; Dec, decitabine; Aza, azacitidine; TS, tumor-suppressor genes; αKGDD, alpha-ketoglutarate-dependent dioxygenase; 2HG, 2-hydroxyglutarate

Table 6 Prototype of the Collaboration Between at Least Three Classes of Mutations (Class I, II, III) Associated with Appearance of AML

Class I Mutations (Confer Proliferation & Survival)	Class II Mutations (Confer Differentiation and Apoptosis)	Class 0/III Mutations (Confer Epigenetic Alteration)
<i>FLT3</i> <i>KIT</i> <i>NRAS</i> <i>BAALC</i> <i>ERG</i> <i>TEL-MNI</i> <i>MDS-EVII</i>	<i>PML-RARA</i> <i>NPM1</i> <i>CEBPA</i> <i>RUNX1-RUNX1T1</i> <i>CBFB-MYH11</i> <i>WT1</i> <i>P53</i>	<i>MLL-PTD</i> <i>DNMT3A</i> <i>IDH 1</i> <i>IDH 2</i> <i>TET (αKG-DD)</i> <i>ASXL1</i> -

Note: Data from Chiaretti et al.¹

Abbreviations: *FLT3*, FMS-like tyrosine kinase 3 gene; *CEBPA*, CCAAT/enhancer-binding protein alpha gene; *MLL-AML/PTD*, Partial tandem duplication (PTD) of the mixed-lineage leukemia (MLL) gene; *BAALC*, brain and acute leukemia, cytoplasmic gene; *ERG*, ETS-related gene; *WT1*, Wilms tumor gene; *MNI*, Meningioma 1 gene; *DNMT3A*, DNA methyltransferase 3A gene; *IDH 1/2*, isocitrate dehydrogenase gene; *TET*, (Ten-Eleven translocation) proteins; *αKG-DD*, α-Ketoglutarate-dependent dioxygenase; *NPM1*, nucleophosmin 1 gene; *NRAS*, neuroblastoma RAS viral oncogene homolog gene; *RUNX1*, the runt-related transcription factor 1 gene.

MYH11 whose product is the oncoprotein CBFβ-MYH11. The fusion protein acts as a transcriptional repressor cooperating with AML1 to repress transcription of tumor-suppressor genes *PTEN*, *Bcl-2*, *CEBPA*, *ARF* and *PSGL-1*. In the

rearrangement, the C-terminal coiled-coil region of the smooth muscle myosin heavy chain 11 (*MYH11*) fuses with the first 165 residues of CBFβ.^{1,15} *CBFβ-MYH11* fusion gene forms at least eight different transcripts in progenitor cells wherein fusion protein heterodimerizes with RUNX1 (CBFα).

In progenitor cells, RUNX1 (CBFα) and CBFβ form a complex regulating gene expression, at the Runx-binding sites on DNA. In a dominant negative manner by competing for heterodimerization and/or for DNA binding at Runx-binding sites, CBFβ-MYH11 and RUNX1-ETO fusion proteins inhibit the function of normal RUNX1 (CBFα)/CBFβ complex. CBFs are required in the hematopoietic ontogeny as the key regulators of different steps of hematopoiesis; therefore, the abnormal fused products disrupt their function and inhibit hematopoietic differentiation.^{3,4} The balanced rearrangements *t(8;21)(q22;q22)* and *inv(16)(p13.1q22)/t(16;16)(p13.1;q22)* are assigned as core-binding transcription factor leukemia (CBF-AML), and are the most common events found in AML, representing approximately 15–25% of all AML cases.^{3,6}

Now, RQ-PCR and nested PCR techniques are sensitive and efficient approaches for the detection of these rearrangements (sensitivities ranging from 10⁻⁴–10⁻⁶).^{5,10} *CBFβ-MYH11* has been linked with the AML M4Eo and a higher incidence in adults (Figure 4).^{3,4}

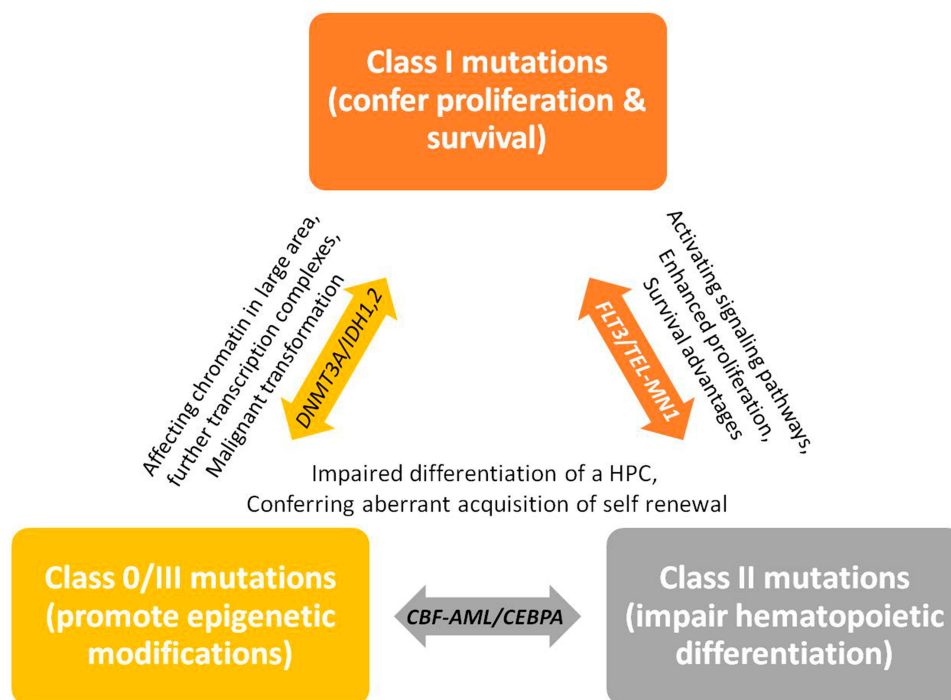


Figure 1 A schematic representation of the collaboration between at least three classes of gene alterations (class I, II, III), that happen in a hematopoietic progenitor cell (HPC) and are associated with the appearance of AML. Class I alterations enhance proliferative signaling pathways and confer survival advantages. Class II alterations impair the processes of cell differentiation and apoptosis. Class 0/III, however, can also be an early event before class I, promotes epigenetic modifications which finally confer malignant transformation to the HPCs and lead to overt leukemia. Data from references 2, 6, 12, 13, 16, and 17.

Patients with *inv(16)/t(16;16)* have a favorable prognosis, good response to conventional chemotherapy (the combination of an anthracycline and cytarabine, the “3 + 7” regimen) and complete remissions (Tables 1 and 2). Likewise, *t(8;21)*, receiving 3–4 cycles of HDAC is efficient and recommended for these patients (Table 2). The improved outcome with repeated therapy with cytarabine is addressed to the incorporation of cytarabine into the genomic DNA where increased sensitivity of the leukemic cells to cytarabine and inducing apoptosis.^{6,15}

Patients with CBF-AML are consistently cytarabine-anthracycline responders with favorable outcomes (Table 2). The substantial heterogeneities observed in the outcome of CBF-AML patients are associated with the co-occurrence of other mutations occurred in specific genes, eg, *FLT3*, *KIT* and *RAS* (Figure 2 & Table 2). Therefore, for right diagnosis and proper management of the cancer, specific mutations occurred in *FLT3*, *NPM1*, *CEBPA*, *RAN*, and *KIT* genes should be taken into account, besides the cytogenetic alterations.^{3,15,10}

T(15;17)(q24;q21)(PML-RARA)

The *t(15;17)(q22;q12)* is classified as a balanced translocation which fusing the *retinoic acid receptor-α (RARA)* gene on chromosome 17(*q21*) with the *PML* gene on chromosome

15(*q24*) and leading to the expression of oncogene *PML-RARA* in hematopoietic myeloid cells. In patients with acute promyelocytic leukemia (APL), the presence of the *PML-RARA* rearrangement predicts a favorable response to treatment with retinoic acid (RA). APL accounts for about 10–15% of AML cases. The *t(15;17)(q24;q21)* and its fusion protein PML-RARα are present in about 98% of APL cases, accounted as the biomarker of APL.^{1–3}

Notably, low-risk APL patients can attain 100% complete remission (CR) and 2-year event-free survival (EFS) of 97%, just by a combination of arsenic trioxide and all-*trans* retinoic acid (ATRA), with no additional chemotherapy. Now, ATRA is considered as a proper treatment of leukemia or as a part of remission induction therapy, both in adults and in children.^{8,10} RARα is a member of the nuclear hormone-receptor superfamily, which activating transcription in the presence of retinoic acids (RAs) (ATRA or 9-*cis* retinoic acid, CTRA) and inducing many target genes required for the hematopoietic differentiation.^{5,6} At the molecular level, RARα and retinoid X receptor-α (RXRα) form the heterodimer RAR-RXR that binds DNA at retinoic acid-response elements (RARE). RAR-RXR forms a transcription activator complex required for promyelocytic differentiation. In the

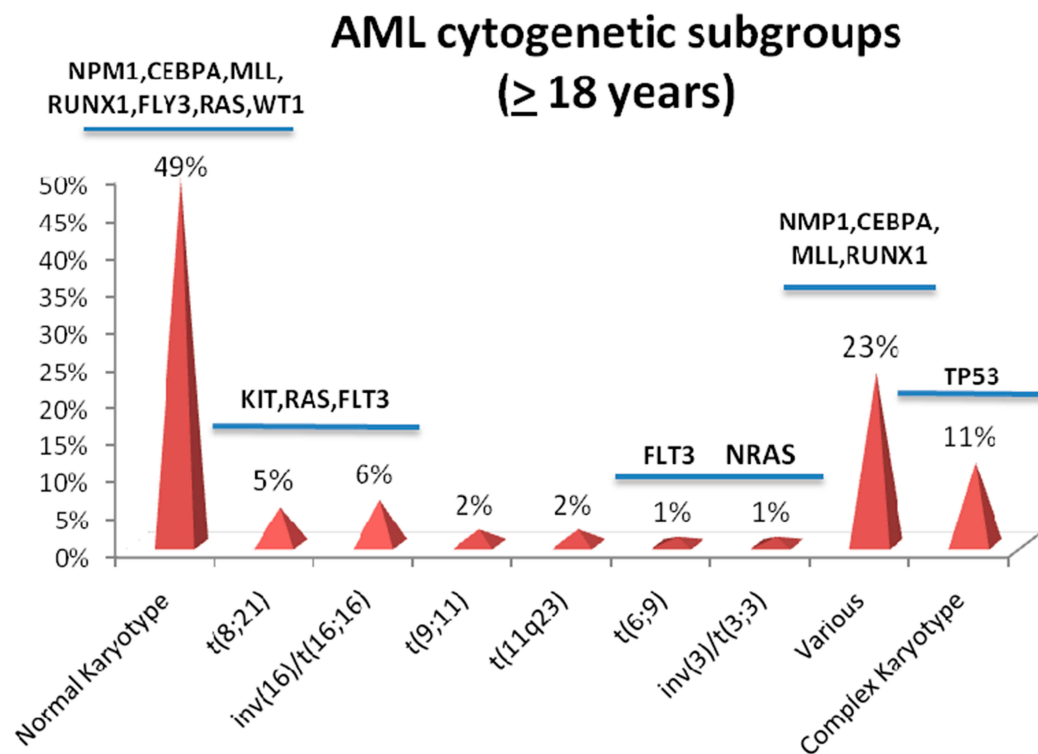


Figure 2 Percentage of the major cytogenetic subgroups of acute myeloid leukemia (AML) (excluding acute promyelocytic leukemia), and associated gene mutations. In the subgroup with various karyotypes, NPM1 mutations are frequently found in AML with 9q deletion and trisomy 8, CEBPA mutations in AML with 9q deletion, MLL mutations in AML with trisomy 11, and RUNX1 mutations in AML with trisomy 13 and trisomy 21 (frequencies of the cytogenetic subgroups are taken from Reference 36, derived from 2654 cytogenetically characterized adults (≥18 years) with de novo or secondary AML entered on AMLSG treatment trials).

Note: Data from Chiaretti et al.¹

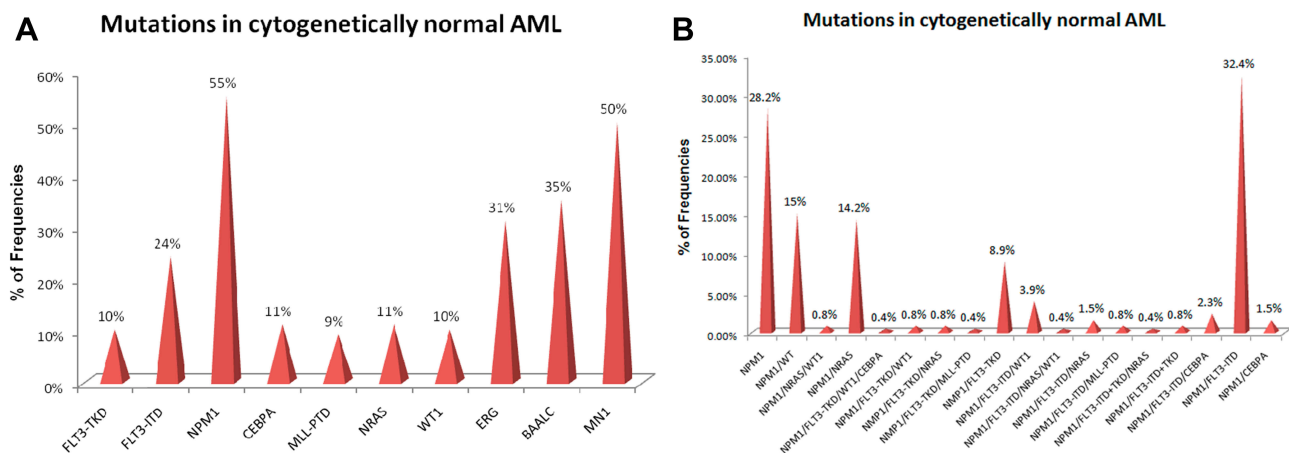


Figure 3 (A) Approximate distribution of gene mutations in cytogenetically normal acute myeloid leukemia (CN-AML) in all patients (class I & II gene mutations).⁸ Adapted from Zaidi SZ, Owaidah T, Al Sharif F, Ahmed SY, Chaudhri N, Aljurf M. The challenge of risk stratification in acute myeloid leukemia with normal karyotype. Data from Zaidi et al.⁹ **(B):** Distribution of class I/II gene mutations among CN-AML patients with NPM1 mutation. In about 28% of cases, NPM1 mutation is the only detectable genetic change, whereas in the majority of cases additional mutations are found in genes such as FLT3, NRAS and WT1 (in approximately 40%, 21%, 17% of NPM1 mutations, respectively). A small number of AML with NPM1 mutations have an additional hypothetical class II mutation, eg, NPM1 and concurrent CEBPA mutation. Data from Döhner and Döhner.²

absence of RAs, RAR-RXR heterodimer acts as a transcription repressor by recruiting corepressors DNMT1, DNMT3A, histone deacetylases (HDACs) and histone

methyltransferases, all of which remodeling chromatin. In the presence of RAs, conformational changes occur in RAR-RXR, whereby causing dissociation of co-repressor

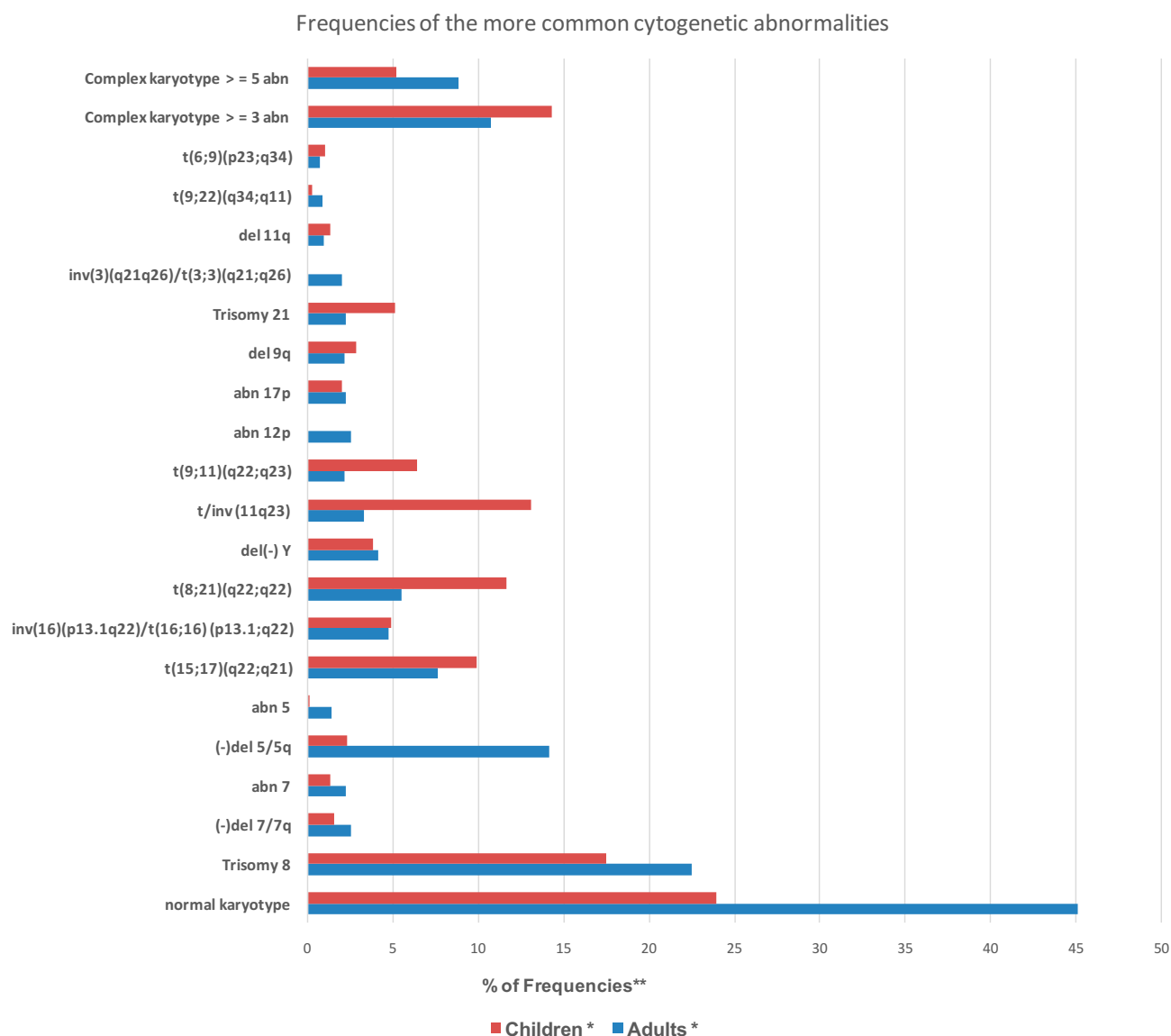


Figure 4 Frequencies of the more common cytogenetic abnormalities in adult and childhood AML. *Percentages for particular abnormalities were calculated using only those studies that provided relevant data where AML children aged from 2 months to 18 years and AML adults aged 18–86 years, diagnosed with de novo AML; data are derived from Mrozek et al⁶ and according to Cooperative Group Study of SWOG/ECOG, CALGB and MRC.

Abbreviations: CALGB, Cancer and Leukemia Group B; MRC, United Kingdom Medical Research Council; SWOG/ECOG, Southwest Oncology Group/Eastern Cooperative Oncology Group; abn, abnormality.

complexes from RAR-RXR, and transcriptional de-repression and activation of genes required for the differentiation of promyelocytes.^{6,8}

PML-RARA competes with RAR α to form a heterodimer with RXR α , then represses target promoters of the signaling chain in the same manner as RARA when it is not bound to its ligand. However, unlike the wild variant, it requires a greater concentration of ligand to eliminate the repression because it maintains a more stable interaction with the corepressor complex and some methylases such as DNMT1 and DNMT3A. Furthermore, PML-RARA has

important effects on apoptosis because in a negatively dominant manner it interferes with the function of wild PML and its regulation of p53.^{15,10} The pharmacological concentrations of ATRA can activate transcription by inducing dissociation of corepressors from PML-RAR α which is then degraded by a proteasome-dependent manner. Immature leukemic promyelocytes require ATRA to differentiate into mature granulocytes wherein bypassing the co-repressor activity of PML-RAR α and differentiating into the myeloid lineage.^{1,3} There are *t(15;17)* variants that all lead to the formation of *PML-RARA* fusion genes and occur

in AML, but are absent in patients with other leukemia types. To detecting this translocation successfully, the RT-PCR and FISH are reliable techniques that link approaches to a favorable prognosis (Table 1). Notably, the *PML* locus in *15(q24)* encodes transcripts through alternative splicing generating more than 20 different tumor-suppressor isoforms. These tumor suppressors participate in nuclear structures called the PML-nuclear bodies (PML-NBs) which sequesters and release proteins from the nucleus. PML-NBs mediate posttranslational modifications and promote nuclear events in response to cellular stressors.^{5,6}

T(9;22)(q34;q11)(BCR-ABL1)

The balanced translocation; *t*(9;22)(*q34;q11*) occurs between chromosomes 9 and 22, and produces the fusion oncogene *BCR-ABL1*. The 3'-sequences of proto-oncogene *ABL*, a tyrosine kinase on chromosome 9, is fused to the 5'-sequences of the *BCR*, a gene on chromosome 22. This translocation leads to the formation of Philadelphia chromosome (Ph) generally occurring in childhood ALL and chronic myelogenous leukemia (CML). Predominantly, the Ph⁺ chromosome causes an early B-cell phenotype. The Ph⁺ patients are mostly adolescence or young adulthood, usually older than 10 years, who show elevated leukocyte counts and frequently central nervous system (CNS) disease. The Ph⁺ chromosome may occur late as a secondary aberration in CML, B-ALL, T-ALL, and primarily AML. There are two variants of the *BCR-ABL1* oncogene, "minor" (*m-bcr*, encoding p190 kDa protein) and "major" (*M-bcr*, encoding p210 kDa protein), as a result of two different breakpoint in cluster regions of the *BCR* gene. The interphase FISH, RT-PCR, or RQ-PCR methods are reliable techniques to detect this rearrangement (Table 1).^{6,15,10}

11q23 Translocations, Deletions, and Duplications (MLL Mutations)

The *mixed-lineage myeloid/lymphoid leukemia (MLL)* gene is mapped to *11q23* covering a genomic DNA region of approximately 100 kb. The chromosomal rearrangements at *11q23* include translocations, deletions, and duplications. The *11q23* rearrangement most often results in gene hybridization between the 5'-sequences of the *MLL* gene and the 3'-sequences of the other partner gene. Exons 5–11 on *11q23*, cluster the majority of breakpoints, in a region extended about 8.3 kb long.^{8,10}

The hybrid proteins harbor the N-terminal residues of MLL and the C-terminal residues of the partner protein. The hybrid proteins localize in the nucleus of the

hematopoietic stem cell wherein exhibiting transforming activity. MLL is a master regulator of hematopoiesis in hematopoietic stem cells wherein regulating the *homeobox* genes (*HOX*). This group of genes consecutively influences hematopoietic stem-cell renewal and leukemogenesis. In addition to mixed-lineage leukemia, the rearrangement also occurs in myeloid and lymphoid leukemic cells, thereby exhibiting its origination from a stem cell or an early progenitor cell. The prognostic significance of *11q23* abnormalities has been variably assigned between intermediate-risk and adverse-risk groups (Tables 1 and 7).^{1,2}

The most common genetic events occurring in ALL or AML children aged <12 months are the *MLL* rearrangements. It is dominant in 43–58% of infants aged less than 12 months, in 39% of children aged between 13 and 24 months, and in 8–9% of children who older than 24 months. Generally, the chance of *MLL* rearrangement decreases with age where it has 4 times more incidence in children than in adults (Figure 4). Approximately 5–10% of *MLL* rearrangements occur as a secondary event or therapy-related leukemia that arise in patients treated with topoisomerase II inhibitors for other malignancy.³

MLL is a large protein with multi-domains and DNA-binding activity interacting directly with DNA and other DNA-binding proteins. As a histone methyltransferase belonging to the trithorax-group family, hematopoietic cells including stem and progenitor populations ubiquitously express MLL. The fusion protein binds DNA constitutively and sequentially activates *HOX* genes such as *HoxA9* and *HoxA10* which commonly up-regulated in leukemia. At the molecular level, MLL participates to methylate histone H3 on lysine residue 4 (H3K4), which positively regulates gene expression in embryogenesis and hematopoiesis.⁶

Profoundly, *MLL* gene fusion to a wide array of partner genes, including *AF4*, *AF9*, *ENL*, *AF10* and *ELL* has leukemogenic effects. *MLL* rearrangements involve several partner genes including *t*(6;11)(*q27;q23*), in which *MLL* is fused to *AF6*; *t*(9;11)(*p22;q23*), in which *MLL* is fused with *AF9*; and *t*(10;11)(*p12;q23*), in which *MLL* (on *11q*, the long arm of chromosome 11) is fused to *AF10* or *MLLT10* (on *10p*, the short arm of chromosome 10). *MLL-AF10*, *MLL-AB11*, and *CALM-AF10* are fusion genes identified in leukemic patients with *t*(10;11)(*p12;q23*). The *MLL* fusion proteins attain a dominant gain-of-function trait enhancing the transcriptional activity and giving rise to

Table 7 Risk Stratification of the Most Common Chromosomal Aberrations Detected in AML

Recurrent Chromosomal Aberrations	Outcomes ^a	Chemotherapy Responses ^b		
		CR Rate	CIR	OS
<i>inv(16)(p13.1;q22)</i>	Favorable	Favorable	Favorable	Favorable
<i>t(16;16)(p13.1;q22)</i>	Favorable	Favorable	Favorable	Favorable
<i>t(8;21)(q22;q22)</i>	Favorable	Favorable	Favorable	Favorable
<i>del(16q)</i>	Favorable	NA	NA	NA
<i>t(15;17)(q22;q21)</i>	Favorable	NA	NA	NA
<i>t(6;9)(p23;q34)</i>	Adverse	Intermediate	NA	Adverse
<i>t(6;11)(q27;q23)</i>	Adverse	Intermediate	NA	Adverse
<i>t(11;19)(q23;p13)</i>	Adverse	Intermediate	NA	Adverse
<i>t(9;22)(q34;p11)</i>	Adverse	NA	NA	NA
<i>t(9;11)(p22;q23)</i>	Adverse	Intermediate	Intermediate	Intermediate
<i>del 5q, 9q, 11q, 20q</i>	Adverse	Intermediate	Intermediate	Intermediate
<i>del Y</i>	Intermediate	Intermediate	Intermediate	Intermediate
Trisomy 8	Intermediate	Intermediate	Intermediate	Adverse
Trisomy 11, 13	NA	Intermediate	Intermediate	Intermediate
Trisomy 21	NA	Intermediate	Adverse	Intermediate
Trisomy 6	Intermediate	NA	NA	NA
Trisomy 22	NA	NA	NA	NA
Monosomy 7	Adverse	Intermediate	Adverse	Adverse
Monosomy 5	Adverse	NA	NA	NA
<i>del 7q</i>	Adverse	NA	NA	NA
<i>inv(3)t(3;3)</i>	Adverse	Adverse	Adverse	Adverse
Complex ≥ 3	Adverse	Adverse	Adverse	Adverse
Complex ≥ 5	Adverse	Adverse	Adverse	Adverse
<i>abn(21q)</i>	Adverse	NA	NA	NA
<i>abn(17p)</i>	Adverse	NA	NA	NA
<i>abn(20q)</i>	Adverse	NA	NA	NA
<i>Abn(12)</i>	Intermediate	Adverse	Intermediate	Intermediate

Notes: ^aAccording to SWOG/ECOG, Southwest Oncology Group/Eastern Cooperative Oncology Group, a Cooperative Group Study. ^bAccording to CALGB, Cancer and Leukemia Group B, Cooperative Group Study, three large collaborative studies proposed prioritization schemata that assign AML patients to one of the three risk groups, favorable, intermediate or adverse, based on pretreatment cytogenetic findings. Data from Mrozek et al.¹⁵

Abbreviations: CR, complete remission; CIR, cumulative incidence of relapse; OS, 5-year overall survival; abn, abnormality; NA, not available.

the poor prognosis of patients with *MLL* translocations. Herein, RT-PCR method is a reliable technique to identify these subtle translocations, besides cytogenetic analysis. The fusion transcripts are readily detectable by RT-PCR, which is especially useful in tracking the minimal residual disease. Southern blotting with cytogenetic analysis is able to detect the majority of *MLL* rearrangements and iFISH in a complementary fashion for the accurate identification of *MLL* status.^{15,10}

Patients with *11q23* rearrangements (*MLL* mutations) show intermediate to the adverse prognosis that depends on the second or partner genetic aberrations (Table 1). The *11q23* rearrangement with the partner *t(9;11)(p22;q23)* shows a more favorable prognosis that places it in the intermediate group (Tables 1 and 7). Patients with *11q23* rearrangements have an intermediate response to

conventional chemotherapy (the combination of an anthracycline and HDAC for induction, then 1–2 cycles of HDAC-based consolidation followed by HSCT) (Tables 1 and 2).^{6,15,10}

T(1;22)(p13;q13) (OTT-MAL)

The hybrid gene *OTT-MAL* is a result of *t(1;22)(p13;q13)* translocation occurring mainly in young children, especially in those younger than 24 months, and is highly correlated with acute megakaryoblastic leukemia (AML M7). The strong correlation existing between *t(1;22)* and AML M7 implies that there is an existence of prenatal genetic factors involved in the incidence of this particular form of disease. Specifically, this translocation occurs primarily in girls and exhibits an inferior outcome.^{6,15}

Balanced t(12;22)(TEL-MN1)

The *meningioma 1 (MN1)* gene, localized on human chromosome 22, is disrupted by the balanced translocation *t*(12;22) which results in the fusion protein TEL-MN1. In cooperation with *HOXA9*, *TEL-MN1* promotes the growth of primitive HPCs. *HOXA9* is over-expressed in AML. *MN1* as a unique oncogene involving in hematopoiesis participates in both aspects of self-renewal; by promoting proliferation and blocking differentiation. The *MN1* expression level is a predictive marker in AML treatment, wherein *MN1* high-expression levels correlate with resistance to ATRA treatment. These predictive levels are particularly important in elderly patients.⁸

AML with Complex Karyotype (Secondary Cytogenetic Aberrations)

AML without the balanced chromosomal rearrangements (eg, *t*(8;21), *inv*(16)/*t*(16;16), and *t*(15;17)), but with at least 3 acquired chromosomal aberrations is defined as complex karyotype AML. There may be a low frequency of *NPM1*, *FLT3*, *CEBPA*, *RAS* and *KIT* gene mutations. There is an occurrence of chromosomal imbalances, with chromosomal losses occurring more frequently than chromosomal gains, and regarded as the worst prognostic group (Table 3). Patients with monosomal karyotype (MSK) abnormalities show an inferior OS. Incidence of AML with complex karyotype is increasing with age and constitutes 10–12% of all AML. This kind of AML seems to be induced by the deregulation of molecular pathways involved in genome stability.^{6,7,9} For instance, two-thirds of complex karyotype AML are identified with *TP53* mutations and loss of p53 function which resulting in genetic instability (Table 3). Another contributing mechanism is chromosomal deletions and inactivation of a single allele, eg, deletion of *5q32* (*RPS14* gene) identified for the -5q myelodysplastic syndromes. Genomic gain or amplifications associated with oncogene activation is another mechanism common in this type of AML. This mechanism includes members of the *ETS* gene family: eg, *ERG/ETS2* located on 21q, and *ETS1/FLI1* mapped to band 11q23.3-q24.1 (Table 3).^{2,6}

Inv(11)(p15.5;q31)/t(7;11)(p15;p15.5)/t(5;11)(q35;p15.5) (Nup98)

The rearrangements of *Nucleoporin 98 (NUP98)* occur in a wide range of hematopoietic malignancies which including both ALL and AML. *NUP98* has several different partners comprising two groups: the *HOX* partner genes (*HOXA9*,

HOXA11, *HOXA13*, *HOXC11*, *HOXC13*, *HOXD11*, *HOXD13*, *PRRX1/PMX1*, and *PRRX2*) and the non-*HOX* partner genes (*FNI*, *RAP1GDS1*, *NSD1*, *WHSC1L1/NSD3*, *PSIP2/LEDGF*, *ADD3*, *DDX10*, and *TOP1*). The rare but recurrent translocation *t*(7;11)(p15;p15.5) causes fusion of *NUP98* gene to the *HOXA9* gene. The fusion occurs at the gly-leu-phe-gly (GLFG)-repeated domains of *NUP98*. In *inv*(11)(p15.5;q31), the acidic domain of a putative heli-case encoded by the *DDX10* gene fuses to the *NUP98*-GLFG repeat domains. This inversion frequently occurs in therapy-related AML (t-AML). Moreover, 5q deletion associated with the recurrent *t*(5;11)(q35;p15.5), frequently occurs in pediatric AML and disrupt *NUP98* at 11p15.5. In translocation *t*(5;11), the conserved finger domains SET, SAC, and PHD of the *NSD1* gene can fuse to the *NUP98*-GLFG repeat domains. Clinically, the incidence of *NUP98* rearrangements is quite low in childhood AML, which appear with an aggressive clinical course and unfavorable therapeutic outcomes.^{5,6,10}

Recurrent Amplification of 21q (EST2/ERG Over-Expression)

ETS-related gene (*ERG*) at 21q22 is a cryptic amplification and frequently amplified in AML patients with a complex karyotype. *ERG* is a member of the *ETS* gene family (which has more than 30 members). Generally, the *ETS* gene family members are downstream nuclear targets of signal transduction pathways promoting cell proliferation and tissue invasion. Herein, recurrent amplification of *ERG* leads to its over-expression in complex karyotype AML, wherein contributing to an aggressive malignant phenotype. Additionally, *ERG* over-expression increases RR and short survival in AML with normal karyotype. High *ERG* expression in presence of *MLL-PTD* predicts a worse cumulative incidence of relapse (CIR).¹⁵

Monosomy 7 and Deletion of 7q

The incidence of AML with monosomy 7 and deletion of 7q [(*del*(7q))] is approximately 5% to 7%. AML patients exhibit *del*(7q) would experience an unfavorable outcome. Additionally, myelodysplastic syndrome (MDS), Fanconi anemia, congenital neutropenia, and neurofibromatosis are conditions characterized by *del*(7q) as a sole cytogenetic abnormality which are frequently followed by secondary AML. Individuals with these constitutional disorders are predisposed to myeloid leukemia. The balanced rearrangement of 7q22 or 7q32-q35 leads to the loss of chromosome 7 and AML. These regions contain the gene of a putative tumor

suppressor that controls myeloid growth and regulates differentiation, whose loss of function leads to leukemic transformation. The deletion of a single allele leads to abnormal growth due to the reduced level of the tumor-suppressor protein, while inactivation of both alleles transformed immature myeloid cells to the leukemic phenotype.^{9,10}

Generally, the incidence of chromosomal aberrations is higher in pediatric patients than in adults (approximately 76% of childhood AML, compared to 55% of adult AML) (Figure 4). For example, balanced rearrangements at *11q23* are on average four times more common in children than in adults. The frequency of *11q23* rearrangements in AML decreases appreciably with age. Likewise, the incidence of *t(8;21)* is twice as common in pediatric as in adult AML. In contrast, -5 , *del(5q)* and other unbalanced structural abnormalities resulting in loss of material from *5q* are much more frequent in adult than in childhood AML. Likewise, both *inv(3)(q21q26)/t(3;3)(q21;q26)*, which are found in 2% of adults, are extremely rare in children and have so far never been detected in a patient with de novo AML younger than 12 years (Figure 4).^{8,16} The varying proportions of specific chromosomal aberrations existing between pediatric and adult AML can be attributed to the molecular reason and biological differences.

AML with Various Cytogenetic Abnormalities

Non-random chromosomal rearrangements with unbalanced translocations may result in monosomies (monosomy 7), deletions of part or all chromosome 5 or 7 ($-5/-7$ AML) and trisomies (eg 8, 11, 13 and 21 trisomies).^{9,10} The most common trisomies in de novo AML are, in decreasing order of frequency, +8, +22, +13, +21 and +11.¹⁵ Notably, in this cytogenetic subset of AML, gene mutations such as *CEBPA* and *NPM1* are occurring, which acting as class II mutations in the collaboration with class I alterations occurred in the chromosomal abnormality.^{1,2}

Trisomy 8 (One of the Most Common Cytogenetic Alterations in AML)

An enormous clinical heterogeneity is seen among patients whose leukemic cells exhibit trisomy 8 that can be associated with an intermediate or poor prognosis.^{1,2,19} The trisomy 8 is likely to be a primary event modulating underlying cryptic translocations, deletions, or mutations.¹⁹ This aberration represents a mechanism to achieve the amplification of oncogenes required for leukemogenesis. Trisomy 8 causes an increasing dosage of oncogenes, including *c-myc*

(*8q24*), *c-mos* (*8q22*), and *ETO* (*8q22*), significance in leukemogenesis.^{1,19}

Trisomy 11 (AML with Various Cytogenetic Abnormality)

Trisomy 11 is a rare event in AML associated with FAB-M1 morphologic features and shows an intermediate to poor prognosis. Trisomy 11 represents a class I aberration conferring proliferation and apparent cell survival advantages to the clone, whereas a class II mutation impairs cellular differentiation. Cases of MDS with +11 show a high risk of progression to AML. A quantitative increase in the production of oncogenes including *Cyclin D1* gene (*11q13*, regulating cellular transition from G 1 to S phase), and *MLL* gene (*11q23*, over-expressed in both myeloid and lymphoid leukemic cells, and in an elevated proportion of mixed-lineage leukemia) is observed. *MLL* plays a key role in hematopoiesis by regulating *HOX* genes, which sequentially involved in hematopoietic stem-cell renewal and leukemogenesis.^{15,10} The well-known proto-oncogene *WT1* expressed in various cell types to promoting proliferation is mapped to *11p13* band.^{2,8}

Trisomy 13 (Homogeneous AML Subtype with Various Cytogenetic Abnormality)

This trisomy is a rare chromosomal abnormality cooperated with a high frequency of mutations in *RUNX1* and spliceosome genes and characterized with poor prognosis. Trisomy 13 represents a class I alteration associated with the amplification of oncogenes and over-expression of *FOX1* and *FLT3* (*13q12*), a potential mechanism for leukemogenesis. The frequent morphology of AML+13 cells is associated with FAB M0 and showing a high frequency (80% to 100%) of *RUNX1* mutations. As the master regulator of hematopoietic differentiation, *AML1* (*RUNX1*) mutations function as a transcriptional repressor that blocks *AML1*-dependent HPC differentiation.^{2,20}

Monosomy 7 (AML with Various Cytogenetic Abnormality)

The pathogenesis of MDS/AML associated with monosomy 7 is mediated by gene dosage effects as class I alterations, identified by microarray analysis. Monosomy 7 is one of the most frequent chromosomal abnormalities observed in patients with MDS/AML and associated with poor clinical outcome, both in MDS and in AML. This aberration represents a primary event superimposed to the Philadelphia chromosome in chronic myelocytic

leukaemia (CML) and in a variety of Mendelian and non-Mendelian predisposing disorders wherein subjects developing MDS/AML. The methods to monitor the monoclonal clone and the parental origin of the chromosome 7 loss are FISH and QR-PCR.^{5,10,21}

Cytogenetically Normal (CN) AML with Gene Mutations

A large subset of AML (~40–50% of adult and 25% of pediatric AML) (Figure 4) is cytogenetically normal (CN-AML), but a number of gene mutations, as well as deregulated expression of genes, have been identified in this subset (Table 4). AML patients with a normal karyotype (CN-AML) are considered to be at intermediate risk associated with 5-year survival rates between 24% and 42%.^{3,8} In CN-AML, mutations in specific genes are identified: the *nucleophosmin 1* (*NPM1*) gene, the *fms-related tyrosine kinase 3* (*FLT3*) gene, the *CCAAT/enhancer-binding protein alpha* (*CEBPA*) gene, the *myeloid-lymphoid or mixed-lineage leukemia* (*MLL*) gene, the *neuroblastoma RAS viral oncogene homolog* (*NRAS*) gene, the *Wilms tumor 1* (*WT1*) gene, and the *runt-related transcription factor 1* (*RUNX1*) gene (Figure 3A). These gene mutations are the most prevalent in CN-AML; however, these also occur in AML with abnormal karyotypes as secondary abnormalities (Figure 2) (Tables 2 and 4).^{11,18}

Interestingly, class I mutations in *KIT* are exclusively associated with *inv(16)* and *t(8;21)*, being rare in other AML subtypes and are associated with unfavorable outcomes.^{1,2}

Mutations in *NPM1* Gene

NPM1 mutations (duplication and insertion) are the most frequent AML-related mutation occurred in adults and found in one third of all adult cases of AML (~30%) (Figures 2 and 3). *NPM1* mutations are enriched in CN-AML (about 45–64% of CN-AML cases) (Figure 3A), wherein exhibit good response to conventional induction chemotherapy (based on a combination of anthracycline and cytarabine) and favorable outcomes are achieving in CN-AML subtype without the *FLT3-ITD* mutation (high CR rate ~ 85%, EFS ~ 50–60%, OS rates ~ 50%).^{11,16,18}

In pediatric AML, however, findings indicate that *NPM1* mutations generally confer an independent favorable prognostic impact despite *FLT3-ITD* mutations. In addition, pediatric AML patients with both *NPM1* and *FLT3-ITD* mutations appear to have favorable prognoses and may not need hematopoietic stem-cell transplantations. Incidence of *NPM1* mutations cannot be found

below the age of 3 years, but above this age increases with age. *NPM1* mutations occur frequently in adult CN-AML and confer favorable outcome. The studies on CN-AML in adults have found that the prognosis of *NPM1* mutations positive for *FLT3-ITD* is unfavorable. The European LeukemiaNet (ELN) scheme proposes that *NPM1* mutations with *FLT3-ITD* allele ratio (AR) <0.5 (low AR) has a favorable prognosis, and allogeneic hematopoietic stem-cell transplant (HSCT) in the first complete remission (CR1) period is not actively recommended.^{22–24}

Then in adults, CN-AML with isolated *NPM1* mutations (ie the *NPM1+FLT3*-subgroup) exhibits a good response to chemotherapy, relapse-free survival (RFS), and improved OS. This favorable effect is however lost in the presence of an *FLT3-ITD*. The survival of double-positive subtype *NPM1+FLT3+* will be similar to the *NPM1-/FLT3-ITD+* subtype. *NPM1+FLT3+* mutations show an intermediate prognosis where there is no survival difference between *NPM1+FLT3+* and *NPM1-/FLT3+*. Postulating, treatment of *NPM1+FLT3+* patients with recently developed *FLT3* inhibitors would be effective by converting *NPM1+FLT3+* to a more chemotherapy-sensitive status (Table 4).^{11,16,18}

NPM1 mutation is the only detectable genetic mutation in approximately 28% of CN-AML cases, whereas in the majority of cases, additional mutations exist in class I genes such as *FLT3*, *NRAS* and *WT1*, (approximately in 40%, 21% and 17.5% of cases, respectively) (Figure 3B).^{2,11} Additionally, *NPM1* mutation can also coincide with secondary chromosomal abnormalities, such as *trisomy 8* and *4* and *del(9q)*.

Generally, there are two prognoses for the outcome of *NPM1* mutation without *FLT3-ITD*; 1) patients do not necessarily benefit from allogeneic stem-cell transplant following conventional induction chemotherapy (based on anthracycline and cytarabine), and 2) elderly patients might benefit from combination of ATRA to their chemotherapy regimen.^{1,3,5}

At the molecular level, the *nucleophosmin 1* (*NPM1*) gene encodes a multi-functional chaperone protein shuttling between nucleus and cytoplasm. *NPM1* seems to be mainly involved in ribosomal protein assembly and biogenesis, stabilization of the tumor-suppressor p19ARF and p53 pathway, DNA-repair process, genomic stability, and finally regulation of DNA transcription through modulation of chromatin structure. Any mutation that disrupts *NPM1* normal function as a tumor suppressor would lead to malignant transformation. Herein, *NPM1* mutations prevent proper folding and lead to loss-of-function. Moreover, *NPM1* single allele mutations perturb the function of WT

protein through cytoplasmic localization of both mutant and WT protein.^{8,18}

Other concordant conclusions are that *NPM1* mutations are mostly present in adult AML with more preference in females, associated with increased blood leukocyte count, myelomonocytic phenotype and low CD34 expression. This highly prevalent mutation provides a suitable marker for monitoring minimal residual disease (MRD) of AML.^{11,16} QRT-PCR is an implement to quantify mutated transcripts and to evaluate the presence of MDR. Herein, the persistence of mutated transcripts in blood of patients would associate with a greater risk of relapse after chemotherapy cycle, for example, NPM1-mutated transcripts detected in the blood of 15% of patients after the second chemotherapy cycle, associates with a greater risk of relapse after 3 years of follow-up as well as a lower rate of survival.^{3,5}

Mutations in *FLT3* Gene

FLT3 (FMS-like tyrosine kinase) is a member of class III of receptor tyrosine kinases (RTK, the same family of *FMS*, *KIT*, *PDGFR- α/β*), whereby ligand binding signals intracellular pro-proliferative pathways. *FLT3* mutations are the most common RTK mutation in AML (~30% of all cases). The most frequent *FLT3* mutations (~25%) are the internal tandem duplication (ITD) within the cytoplasmic-juxtamembrane (JM) region (*FLT3-ITD*). Less frequent (~7%) are point mutations in the activation loop of the tyrosine kinase domain (*FLT3-TKD* mutation), such as the D835Y mutation. *FLT3-ITD* usually occurs in CN-AML wherein is associated with a dismal outcome (Figure 3) (Table 4). Both types of mutations are classified as class I mutations which provide cells constitutive-proliferative and survival advantages. *FLT3* mutations result in ligand-independent kinase activation of *FLT3* and its downstream signaling transducers STAT5, RAS/MAPK, PI3K, src homologous and collagen gene (SHC), and cytoplasmic tyrosine phosphatase SH2 domains (SHP2).^{5,6,8}

Clinically, *FLT3-ITD* patients represent high blast counts and normal cytogenetics. The *t(15;17)* may coincide *FLT3-ITD* but rarely co-occurs in AML with complex karyotype or CBF-leukemia (*CBF-MYH11* and *AML1-ETO*). The *FLT3-ITD* presence is a negative risk factor for OS, EFS and prognoses (Tables 3 and 4).^{10,11,16}

Patients who are homozygous for *FLT3-ITD* or lose wild-type *FLT3* allele would experience inferior outcomes or poor prognosis. By using DNA fragment analysis (PCR products sized by capillary electrophoresis which detecting *ITD*

abnormally large amplicons), labs can quantify the relative level of the mutant allele and are able to define a cut-off value that distinguishes between prognostic subgroups.^{2,5}

Unfavorable prognosis and inferior outcome depend, especially, on a high ratio of the expression of *FLT3* mutant allele to *FLT3* wild-type allele (the allelic ratio >0.78). The high level of the mutant allele (some studies, however, mentioned a mutant allele/wt allele ratio >0.5), is likely of importance which leads to shorter CR duration, death-free survival (DFS) and OS.^{2,15,8}

Expression levels of *FLT3* (CD135) are associated with certain subtypes of AML; with a minimum level in FAB M3 subtype and a maximum level in FAB M5 subtype.^{3,8} Small molecule tyrosine kinase inhibitors are remarkable strategies to inhibit *FLT3* signaling and to specifically kill leukemic cells.^{4,8} From clinical trials, multi-targeted tyrosine kinase inhibitors showed a great benefit to the therapeutic anti-leukemia activity. Nevertheless, none of the designed inhibitors would lead to sustained therapeutic response on their own while in combination with other therapeutics make a preferred strategy for the treatment of AML patients. Some RTK inhibitors include midostaurin, lestaurtinib (CEP701) and sorafenib.³

Mutations in *CEBPA*

CEBPA encodes CCAAT-enhancer-binding protein- α (CEBP- α), a member of transcription factors with leucine-zipper motifs. Members of basic leucine-zipper (bZIP) transcription factors consist of C-terminal DNA-binding (basic region) and dimerization (leucine zipper) motifs and two less-conserved N-terminal transactivation domains.^{5,8} CEBP- α is a master transcription factor with a primary role in myeloid differentiation. About 10% of AML patients experience *CEBPA* mutations which mostly coincide with intermediate-risk karyotypes, and associate with a good prognosis.^{11,18} About 7-15% of CN-AML patients experience *CEBPA* mutations (Figure 3A) (Table 4), the majority of which being FAB-M2 subtype. *CEBPA* double-mutations show favorable outcomes (longer remission duration and higher OS ~ 8 years), and better prognostic impact (especially in patients aged 16–60 years).^{5,8} Importantly, aberrations such as *FLT3* mutations and *MLL-PTD* rearrangement have no significant influence on the prognosis outcome of *CEBPA* double-mutation. This benefit is lost in the presence of *CEBPA* wild-type allele or *CEBPA* single-mutation which is an independent adverse prognostic marker affecting remission duration and OS rates.^{11,16} The coincidence of *CEBPA* double-mutation

with *FLT3-ITD* mutation is rare, where *CEBPA* mutation is mutually exclusive with *NPM1* mutation.^{3,11,18} At the molecular level, *CEBPA* has a single exon mapped to band 19q13.1, with tumor-suppressor activity. CEBP- α induces a number of genes involved in granulocytic specific differentiation. Consecutive up-regulation of *CEBPA* gives rise to granulocytic differentiation, while its further conditional expression triggers neutrophil differentiation. *CEBPA* mutations result in loss-of-function trait contributing to an early block of granulocyte maturation. The *t(8;21)* is an alternative mechanism for *CEBPA* inactivation wherein the *AML1-ETO* fusion protein suppresses *CEBPA* transcription and hence blocks granulocyte differentiation.^{2,16,17}

Mutations in *CEBPA* have a favorable prognosis and good response to the conventional chemotherapy and are associated with higher CR rate and better RFS and OS, comparable with that of CBF-AML (Tables 1 and 2).^{2,6,8}

BAALC Over-Expression

The *brain and acute leukemia, cytoplasmic (BAALC)* gene is mapped to band 8q22.3. *BAALC* expression occurs in neural and hematopoietic stem cells. In HPCs, *BAALC* is a specific marker for the proliferation stage, as its down-regulation leads to cell differentiation. CD34+ progenitor cells from both normal and abnormal bone marrow express *BAALC*. Since all subtypes of CD34 + cells express *BAALC*, it would be present in early progenitor cells, as a novel marker common for the myeloid, lymphoid, and erythroid pathways. As patients with an elevated level of *BAALC* show a higher rate of primary resistant leukemia, a higher CIR and an inferior OS (3 years), its over-expression is an independent risk factor associated with chemotherapy resistance and unfavorable outcomes. To improve the unfavorable outcome for *BAALC* high-risk patients, modifying and adjusting induction therapy and intensifying post-remission therapy should be taken into account. Reports suggest that AML patients with a high level of *BAALC* would benefit from consolidation with allogeneic SCT, while therapy with autologous SCT seems unfavorable. Clinically, high-level *BAALC* patients exhibit an escalated percentage of blood blasts and immature FAB M0/M1 subtypes, while low *BAALC* expression associates with monocytic differentiation and FAB M5b subtype and gingival hyperplasia. *BAALC* over-expression may be accompanied by *FLT3-ITD*, *CEBPA*, *MLL-PTD*, and high *ERG* over-expression mutations. Importantly, over-expressed *BAALC* induces drug resistance gene (*MDR1*) and stem-cell markers (CD133, CD34, KIT).^{5,15,8}

RAS Mutations

RAS (*rat sarcoma*) oncogenes encode a family of membrane-associated G proteins that regulate signal transduction by binding to a variety of membrane receptors. The RAS G proteins are involved in signal transduction pathways that induce proliferation. The genome contains three functional *RAS* genes: *N*-(*neuroblastoma*), *K*-(*Kirsten*), and *H*-(*Harvey*) *RAS*. Each *RAS* gene (*H-RAS*, *K-ras*, *N-RAS*) contains 4 exons can carry transforming mutations exclusively in codons 12/13/61, which leads to constitutive activation of RAS protein identified in many cancer types.^{3,8}

Accordingly, *RAS* mutations are the most frequent mutations occurring in CBF-AML patients (45% *N-RAS*; 13% *K-ras*) (Figure 2) (Table 2) and *N-RAS* is prominent (11–30% in all AML) (Figures 2 and 3), especially in those under the 60 years of age. As mentioned, *RAS* mutations occur with a higher frequency in the favorable risk groups wherein lead to poor outcomes. Screening *RAS* mutations in AML patients are thereby proposed as a benefit guide for therapeutic decisions. AML patients with *RAS* mutations showed a good response to post-remission high-dose cytarabine therapy.^{8,16,18}

WT1 Mutations

Wilms tumor (WT1) mutations (insertions or deletions) are detectable in 10% of AML cases, mainly clustered in exons 7 and 9, and show induction failure. *WT1* mutations belong to the class I oncogene activating mutations which confer WT1 constitutive activation.^{2,16}

The *WT1* gene is mapped to band 11p13 which encodes the transcription factor WT1, an oncogene expressed in various cell types to promoting proliferation, as well as to blocking differentiation. At the molecular level, *WT1* mutations mostly occur with *FLT3-ITD* mutation and are failure to standard induction chemotherapy. *WT1* can be a reliable marker for MDR assessment in acute leukemia patients. After induction chemotherapy, the *WT1* levels in blood samples of treated patients would allow to distinguish those with continuous CR from those who obtain only an “apparent” CR or who relapse within a few months. The level of *WT1* helps identify patients at high risk of relapse soon after induction chemotherapy, those who need post-induction therapy being intensified. Moreover, the expression level of the *WT1* gene in normal HPCs is approximately 10 times less than those in leukemic cells.^{2,8}

Partial Tandem Duplication (PTD) of *MLL* Gene

Approximately 5–11% of CN-AML patients carry partial tandem duplications of the *MLL* gene (*MLL-PTD*). In *MLL-PTD*, duplication of genomic regions spanning exons 5–11 occurs where the duplicated segment is inserted into intron 4 of the gene. In contrast to *MLL* fusion protein, *MLL-PTD* retains all its functional domains. In the presence of *MLL-PTD*, the wild-type *MLL* allele is suppressed. This silencing is mediated through epigenetic mechanisms, wherein a combination of decitabine and depsipeptide, which inhibiting DNA methyltransferase and histone deacetylase, respectively, leads to transcriptional reactivation of the wild-type allele in *MLL-PTD*-positive blasts. Reactivation of wild-type *MLL* induces cell death in blasts. Clinically, *MLL-PTD* mutations associate with unfavorable outcomes (short-duration CR, high relapse rate and inferior EFS).^{6,16,18}

MNI Over-Expression

In CN-AML, high expression of *MNI* strongly occurs in un-mutated *NPM1* cases and is an independent prognostic marker for poor response to the first course of induction therapy, higher relapse rate, shorter RFS and less OS. In treatment with ATRA, patients who express low levels of *MNI* would have prolonged EFS and more OS, but patients with high-expression level of *MNI* would exhibit higher relapse rate, shorter RFS and less OS. HPCs express *MNI* in high levels while differentiated CD34+cells down-regulate it.^{15,8}

TP53 Mutations

Tumor-suppressor p53 is a DNA-binding protein and the master transcription factor activated in response to diverse cellular stresses to induce cell cycle arrest and DNA repair or apoptosis. *TP53* mutations involve single nucleotide changes which are prevailing in other types of cancer but not predominant in blood cancers. *TP53* mutations occur in 75–78% of AML patients with complex karyotype, but rare in other AML subtypes. *TP53* mutation is the most important marker of poor prognosis in both CK-AML and therapy-related AML. *TP53* alterations frequently occur in old patients who display decreases in CR, EFS, RFS, and OS.^{3,16}

CN-AML and cytogenetically abnormal AML patients have the chance to get more chromosomes in rearrangements in their leukemic karyotype. Late rearrangements or secondary aberrations are common in both ALL and AML, prevailing follow a primary change and lead to

substantial variability in patient outcome. These include *del(7q)* (–7), trisomy 8 (+8), *del(16)/t(1;16)(q12-23;q12-24)* and trisomy 21 (+21), which have been observed in both ALL and AML (Table 2)¹⁰

Mutations in Epigenetic Genes

Class 0/III mutations impairing epigenetic regulation in HPCs and defined in AML can be the earliest alteration occurring in an HSC. Importantly, mutations in the genes that encode DNA-methylation regulators (eg, *DNMT3A*, *IDH1/2*, *TET2*) are often acquired early and with a high recurrence rate with *NPM1* and *CEBPA* alterations. Particularly, 73% of the *NPM1*-mutated AML carry mutations in DNA-methylation genes (*DNMT3A*, *IDH1*, *IDH2*, and *TET2*). Furthermore, there is evidence confirming that *TET2* with *FLT3* mutations and *IDH1* with *NPM1* mutations cooperate to induce AML. Moreover, the epigenetic changes are reversible by small molecules and therapeutics that reactivate epigenetically silenced genes and improve outcomes of the disease (Table 5).^{12,13}

DNMT3A Mutations

DNMT3A mutations, one of the most important epigenetic-related alterations, are classified as the earliest and recurrent aberration in myeloid malignancies, occurring in 20–22% of adults with de novo AML (rare in children). Almost all CN-AML patients harbor at least a single point mutation in one of *DNMT3A* alleles and about 30–37% of them show *DNMT3A* loss-of-function mutations. In this regard, the CN-AML group is categorized into two subtypes, ie, with or without *DNMT3A* mutations with prognosis significance.^{12–14} Clinically, *DNMT3A* mutations frequently accompany intermediate-risk AML, where they may correlate with inferior outcomes.^{12,16} *DNMT3A* mutations have a negative impact on patient clinical outcome (a significant decrease in OS in comparison to those with wild-type *DNMT3A*). In particular, *DNMT3A*-mutation effects persist in HPCs and mature cells after remission (Table 5).³

At the molecular level, *DNA methyltransferase 3A* gene (*DNMT3A*) encodes DNMT3A that catalyzes the de novo addition of a methyl group to the cytosine residue of CpG dinucleotide. *DNMT3A* mutations have a severe impact on DNA-methylation patterns whereby give rise to global shifts in gene expression accompanied by increased self-renewal of hematopoietic cells at the blocking of normal differentiation.¹² Mutations mainly disrupt the catalytic domain of the enzyme, which are associated with a loss-of-function of the enzyme

activity.^{12–14} DNMT3A loss-of-function mutations result in hypomethylation of HSC specific genes commonly over-expressed in AML (eg *Runx1*, *Erg*, *Myc*, *Smad3*) which consequently impair HSC differentiation.^{1,3} In particular, DNMT3A function is required for HSC self-renewal and myeloid differentiation; its mutations have been detected in preleukemic HSCs and considered as an early event in AML. *DNMT3A* mutations lead to myeloid transformation in vivo and promote myeloid malignancies in impaired HSCs. Decitabine treatment caused an improved response in patients harboring mutated *DNMT3A*. Additionally, patients achieved a higher clinical remission rate and a superior OS when compared to those bearing the wild-type *DNMT3A* (75% vs 34% and 15.2 vs 11 months, respectively). High levels of *miR-29b* (which targets *DNMT3A*) would be a good marker for response to decitabine treatment. Importantly, patients with myeloid malignancies who are harboring *DNMT3A*, *IDH1/IDH2* mutations show a favorable response to decitabine and azacitidine, specific DNMT inhibitors and hypomethylating agents (HMAs).^{12–14}

IDH1/2 (Isocitrate Dehydrogenase) Mutations

The *IDH1/2* genes encode tumor-suppressor proteins *IDH1/2* whose mutations appear to induce DNA hypermethylation at specific sequences. About 15–20% of all AML subtypes and 25–30% of CN-AML subtype harbor *IDH1/2* mutations. This frequency is similar in adults and children. *IDH1/2* mutations prevailingly accompany *NPM1* mutations but not *FLT3-ITD*.^{12,13} Epigenetic alterations occurring in *IDH1/2* mutations intensify HPC proliferation and increase its pool. *IDH1/2* impairment suppresses the histone demethylation process whereby associates with DNA hypermethylation, differentiation blocking and clonal expansion of HSCs.^{1,3} Biochemically, *IDH1/2* are, respectively, cytosolic and mitochondria NADP-dependent dehydrogenases, decarboxylating isocitrate into α KG. The produced molecule is used by TET enzyme when catalyzing histone demethylation. Mutations give rise to the new catalytic activity by the enzyme whereby converts α KG to 2-HG. The oncometabolite 2-HG is a putative inhibitor of histone demethylase TET2. Blocking histone demethylation is accompanied by DNA hypermethylation phenotype (Table 5). All *IDH1/2* mutations have been reported heterozygous and mutually exclusive to *TET2* mutations, possibly due to overlapping molecular effects.^{12,13} *IDH1/2* mutations are related to an adverse clinical outcome with poor prognosis, particularly when accompanying other

mutations like as *NMP1* and *FLT3-ITD*, however, compared to *DNMT3A*, *IDH1/2* mutation has a better prognosis, DFS and superior OS. In the favorable risk groups, the coincidence of *IDH1/2* mutations with other aberrations would lead to a lower rate of RFS and 5-year OS duration. Similar to *DNMT3A* mutations, *IDH 1/2s* show significantly higher remission rate and favorable response to anti-leukemia therapeutic decitabine and azacitidine. The *IDH1* and *IDH2* inhibitors are, respectively, AG-220 and AG-221, which reported to show a clinical response, with a significant reduction in 2-HG levels (~50–90%). It is reported that *IDH 1/2* inhibitors are being evaluated in Phase III clinical trials and show a prominent impact on AML prognosis.^{13,16}

The α -Ketoglutarate-Dependent Dioxygenase, Ten-Eleven Translocation (TET) Proteins

Approximately 10–20% of AMLs carry *TET2* mutations (deletions, nonsense and missense mutations). Clinically, *TET2* mutations associate with intermediated risk and short OS. Biologically, these loss-of-function mutations induce DNA hypermethylation, however, mutually exclusive with *IDH1/2* mutations. *TET2* mutations are prevalent in MDS and myeloproliferative disorders and are highly frequent in chronic myelomonocytic leukemia, wherein associated with monocytosis and poor outcomes.^{1,12} As an early event present in HSCs, *TET2* inactivation induces pre-leukemic HSCs, clonal expansion and leukemogenesis. *TET2* mutations may act in the same pathway as *IDH1/IDH2* (Table 5). Biochemically, TET activation leads to DNA demethylation in enhancer regions of tumor-suppressor genes through the sequential conversion of 5-methylcytosines (5mC) to 5-hydroxymethylcytosine (5hmC), then 5-formylcytosine to 5-carboxylcytosine which finally accompanied by DNA glycosylase and base excision repair system. Specifically, *TET2* mutations cause a reduction in 5hmC levels and an induction in DNA methylation mainly in enhancer regions of nearby tumor suppressors. *TET2* inactivation correlates with hypermethylation of tumor suppressors in AML.^{1,3,6}

Epigenetic Biomarkers in AML

The epigenetic regulators *DNMT3A*, *IDH1/IDH2* and *TET1/2* are epigenetic markers useful for risk stratification, therapy decision-making and clinical predicting of response to treatment. The epigenetic biomarkers can also include changes in DNA-methylation patterns or

expression profile of non-coding RNAs (miRNAs). These epigenome alterations can be a result of class 0/III mutations which contribute to the cancer molecular pathogenesis. It is also said that epigenetic changes can be relatively reversible in response to combinations of epigenetic small molecular agents such as inhibitors of methyltransferases (cytarabine, azacitidine and decitabine), in combination with other available inhibitors including histone deacetylase (HDAC) inhibitors, which are now tested in clinical trials.^{12,13}

Herein, purin and deoxynucleoside antimetabolites (eg thioguanine/mercaptopurine and cytarabine/azacytidine) are inhibitors of DNA methyltransferases (DNMTi) wherein their basic mechanism is quite similar. These compounds enter the cells and are converted into their respective nucleotide analogues and incorporated into the genomic DNA strands. Their incorporation into the DNA inhibits enzymes critical for DNA synthesis, repair, and methylation (acting as hypomethylating agents (HMAs)). Examples of HDAC inhibitors (HDACi) are vorinostat and valproic acid which numerous administrated in clinical trials in combination with sorafenib for treating patients with advanced/metastatic solid malignancies and refractory/relapsed AML. HDACi target directly the histone deacetylases. In clinical trials, rational combinations of HDACi and DNMTi result in the transcriptional activation of the corresponding genes including tumor-suppressor genes often silenced in cancers. Rational combinations and therapy duration of HDACi/DNMTi with DNA damage-inducing therapies (eg anthracyclines/TOP inhibitors) synergistically enhance the irradiation, growth inhibition and apoptotic effects of therapy. DNMT/HDAC inhibitors increase DNA accessibility by DNA damage-inducing therapies. Several cycles of therapeutics are required and the duration of inhibitors is emphasized for the manifestations of hematologic responses to improve survival in AML patients.^{25,26}

Alteration in the Expression Profile of Non-Coding RNA (miRNAs)

Gene expression profiling (GEP) of non-coding RNAs (miRNAs) can discriminate different cytogenetic subtypes or act as prognostic factors. These small RNAs have been proposed as potential biomarkers of integrated panels for prognosis and risk stratification of disease. For example, a panel of 12 miRNAs is proposed which can divide CN-AML into poor and intermediate-risk categories

independently of *FLT-ITD*.^{5,6} In particular, elevated expression of *miR-17-92*, *miR196*, *miR-29*, *miR-125*, *miR-142*, *miR-146* and *miR-155* is a characteristic of AML (Table 8), addressed as pathogenesis biomarkers. Generally, during normal myelopoiesis, there is a low expression of miRNA clusters whereas highly expressed in leukemic conditions. For instance, *miR-125* family, encoded by three conserved clusters mapped to chromosomes 19/21/11, is over-expressed in leukemic HSCs. Over-expression of *miR-21/10/196* is reported in *NPM1* mutants wherein associated with blockage of normal HSC differentiation and down-regulation of *PDCD4* (*Programmed Cell Death 4*).^{1,3} Moreover, *MLL* rearrangements are characterized by a high expression of *miR-17-92* cluster, as well as, *miR-196b* which is located at 7p15, between *HOXA9* and *HOXA10* genes. Also, *FLT3-ITD* is associated with increased expression of *miR-155/miR-181* family whereby leading to leukemic blast expansion. Moreover, *miR-181a/b* over-expression is a marker of unfavorable outcomes, in particular in CN-AML *CEBPA+/FLT3-IDT+/NPM1-.*^{11,16,18} Even more, expression of miRNAs changes during cytarabine therapy which correlates with outcomes and OS. For example, high expression of *miR-191* and *miR-199a* can be associated with worse OS while down-regulation of *miR29b* (targeting *DNMT3A* & *DNMT3B*) is a marker of a better response to treatment.^{1,3}

Changes in DNA-Methylation Patterns

Each cytogenetic or molecular subtype is supposed to have a distinct DNA-methylation profile. For example, cases with t(8;21), inv(16) or t(16;16), t(15;17) or t(v;11q23) exhibit unique DNA-methylation signatures whereby define AML subtypes. Methylome analysis has indicated that specific regions of the genome have lower methylation levels in AML compared to the normal, such as gene bodies and repetitive sequences. Hypomethylation of repetitive sequences with a high content of methylated CpGs (eg SINEs (short interspersed nuclear elements) and LINEs (long interspersed nuclear elements)) are associated with rearrangements.^{1,12} Additionally, in methylome analysis of CN-AML, the most pronounced DNA hypermethylation is detected in CpG islands representing tumor-suppressor promoters.³ Recurrent mutations (eg *NPM1*, *CEBPA*, *RUNX1*), can also be defined by distinct DNA-methylation patterns. For example, *NPM1* mutations can be defined by four distinct DNA-methylation clusters. AML subtypes can be further classified according to the methylation profiles related to prognosis and clinical

Table 8 Summary of Expression Profile of Prognostic miRNAs in AML

Prognostic Biomarker	Biological Features	Prognosis
<i>miR-20/21/10/25/19/1199</i> <i>Let7b, miR-9</i>	Down-regulated in normal myelopoiesis, over-expressed in AML Down-regulated in AML	Prognosis biomarker for unfavorable outcomes, expression may be changed during cytarabine therapy, Prognosis biomarker for favorable outcomes
<i>miR-125 (9, 21, 11)</i>	Located on chromosomes 19, 21, and 11, highly expressed in HSCs & myeloid transformation	Expression may be changed during cytarabine therapy
<i>miR-155/miR-181a/b</i>	Highly expressed in HSCs & myeloid transformation, associated with <i>FLT3-IDT</i> , leukemic blasts expansion, high sensitivity and specificity (90% and 96% respectively) for diagnosis	Associated with worse overall survival, expression may be changed during cytarabine therapy,
<i>miR-29b</i>	Targeting DNMT3A and DNMT3B, its down-regulation is an important biomarker of response to treatment	Associated with worse overall survival, expression is changed during cytarabine therapy
<i>miR-17-92/miR-196b (7p15)</i>	Highly expressed in <i>MLL</i> rearrangements	Prognosis biomarker
<i>miR-10/21/196a</i>	Highly expressed in <i>NPM1</i> mutations	Prognosis biomarker, expression may be changed during cytarabine therapy
<i>miR-181 family</i>	Highly expressed in <i>CEBPA</i> mutations in CN-AML,	Prognosis biomarker for unfavorable outcomes in <i>FLT3-IDT</i> & <i>WT-NPM1</i> concurrence

Note: Data from Chiaretti et al¹ and Prada-Arismendy et al.³

Abbreviations: miRNA, MicroRNA; HSCs, hematopoietic stem cells; WT, wild type.

outcomes. *CEBPA* double mutations show two distinct DNA-methylation signatures whereby patients could be split into two distinct prognosis subtypes: one hyper-methylated and one hypomethylated.^{12,13} Herein, initiation, progression and maintenance of the tumor phenotype associate with distinct DNA-methylation patterns.³

The underlying mechanism of aberrant DNA-methylation induction in these AML subsets is attributed to the fusion genes in recruiting DNMTs to their binding sites, or to a secondary epigenetic dysregulation including binding of PML-RAR α to genomic regions of epigenetic modifiers such as *DNMT3A* and/or DNA-methylation disruption of AML1-ETO target genes. For instance, methylation of target genes involved in the genome stability were found to be changed in APL patients at diagnosis time, which predispose them to a hypomethylation phenotype seen in *CBFB-MYH11* fusion gene, *CEBPA* promoter and in the regulatory regions of *MNI*.^{1,12} Methylation assays suggest that global DNA hypomethylation occurred in AML patients during treatment can be a marker associated with a successful response, an increased CR rate and an OS improvement. Methylome analyses indicate that hypomethylation of LINE-1 may also associate with

low blast counts (<45%), better CR rate during the first cycle of azacitidine, and hematological improvement.

According to the DNA methylation-shifted loci observed in AML, samples can be defined into three categories: shifted loci unique to diagnosis, loci unique to relapse, lastly loci can be seen at both diagnosis and relapse. Accordingly, dividing AML samples into these categories do not correlate with age, white blood cell count, or the French–American–British (FAB) classification. Finally, DNA methylation-shifted loci are expected to be an independent classification for AML. Therefore, identification of mutations associated with DNA methylation and evaluation of changes occurred in methylation signatures would contribute to individual therapy of AML patients.^{3,12,13}

Conclusion

The AML development is a consequence of an accompaniment between genetic, epigenetic and proteomic alterations, causes of specific molecular mechanisms involved in. Nowadays, genetic mutations and cytogenetic alterations are taken into account as markers of great importance for risk stratification and therapeutic decision-making in clinical management. Due to the heterogeneity of

mutations in AML, finding a panel of biomarkers would be of more importance for diagnosis, prognosis or monitoring of individual patients, in addition to facilitating individualized-therapeutic decision-making. For example, a panel of integrated biomarkers was recently proposed combining *FLT3*, *NPM1*, *ERG*, *CEBPA*, and *BAALC* mutations to place patients into one of the four categories respecting the risks and benefits of proper therapy. Other integrated panels combining *ERG*, *BAALC*, *WT1*, *EVII*, *MNI* mutations and *microRNA* expression profiles have been proposed which similarly respecting prognostic factors for risk stratification and therapeutic decision-making. Once an algorithm is established according to these panels, clinical practices can be much closer to achieving personalized medicine and the development of precision medicine. Therefore, identification of mutations and specific biomarkers in each patient and evaluation of the change in methylation signature would contribute to individualized-therapeutic decision-making in AML.^{5,12,16}

Disclosure

The authors report no conflicts of interest in this work.

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