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REVIEW

# Identifying high-risk adult AML patients: epigenetic and genetic risk factors and their implications for therapy

Caroline Bret[a,b,c], Elena Viziteu[b], Alboukadel Kassambara[a,b] and Jerome Moreaux [a,b,c]

[a]Department of Biological Hematology, CHU Montpellier, Montpellier, France; [b]Institute of Human Genetics, CNRS-UPR1142, Montpellier F-34396, France; [c]University of Montpellier 1, UFR de Médecine, Montpellier, France

**ABSTRACT**

Acute myeloid leukemia (AML) is a heterogeneous disease at molecular level, in response to therapy and prognosis. The molecular landscape of AML is evolving with new technologies revealing complex panorama of genetic abnormalities where genomic instability and aberrations of epigenetic regulators play a key role in pathogenesis. The characterization of AML diversity has led to development of new personalized therapeutic strategies to improve outcome of the patients.

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## Introduction

Acute myeloid leukemias (AMLs) are the most frequent acute leukemias in adult patients. They constitute a heterogeneous group of hematopoietic malignancies with distinct cytogenetic, molecular, epigenetic, phenotypic, and morphological features [1]. In addition to the diversity of the biological aspects, these malignancies display variable responses to treatment [2].

AML is characterized by recurrent genetic alterations, including amplifications, deletions, rearrangements, and mutations [3]. Molecular abnormalities, in AML, have been studied using cytogenetics since decades. Therapeutic choices are usually determined by cytogenetic profiles allowing the identification of different subgroups of patients (favorable, intermediate, and unfavorable) [4]. On the basis of these karyotype stratification, patients with relatively good outcomes will receive conventional chemotherapy, whereas patients classified within unfavorable groups will be treated by allogeneic transplantation-based regimens [5]. However, the majority of patients have an intermediate cytogenetic risk, commonly a normal cytogenetic (CN-AML), with patients responding to chemotherapeutic consolidation and others with a very poor prognosis. A better stratification within the intermediate-risk group allowed by the description of recurrent mutations in Fms-like tyrosine kinase 3 (*FLT3*), Nucleoplasmin family member 1 (*NPM1*), CCAAT/enhancer-binding protein alpha (*CEBPA*), tet methylcytosine dioxygenase 2 (*TET2*), DNA (Cytosine-5-)-Methyltransferase 3 Alpha (*DNMT3A*), and isocitrate dehydrogenase 1/2 (*IDH1/2*) has been described using sequencing strategies [6].

Despite treatments, relapse is unfortunately frequent and is linked to the emergence of a clonal complexity during progression. The global outcome of AML patients remains poor, with the exception of acute promyelocytic leukemia (APL), which is characterized by a remission in about 75% of cases [7].

Innovative genomic technologies, with next-generation or whole-sequencing approaches, have provided the description of new molecular abnormalities including new recurrent mutations, at the coding, noncoding RNA, and epigenetic levels. These data will lead to a better understanding of AML pathogenesis and progression, to an alternative stratification of AML patients and to the perspective of a better clinical management using novel targeted strategies.

## Classical diagnosis and prognostic markers

Recurrent karyotypic alterations and their molecular counterparts have been identified as diagnosis markers with prognostic significance for the last three decades. They constitute the basis of the definition of AML in the WHO classification of hematological malignancies, underlying their impact in the patient management. The cytogenetic classification of AML is important for risk-adapted therapy of patients. According to cytogenetics, patients could be classified in 'favorable', 'intermediate risk', and 'adverse risk' [4] (Table 1). Two major classifications are used to classify AML patients in prognostic subsets: the United Kingdom Medical Research Council (MRC-C) and the European Leukemia Net (ELN-C) [2,8]. Patients with favorable prognosis include APL with t(15;17), AML with t(8;21) or inv(16). Those with adverse prognosis include patients with 11q23 abnormalities excluding t(9;11), t(11;19), and t(9;22), abnormal 3q, complex karyotype, −17/abn(17p), −5, del(5q), −7, del(7q), t(6;11), t(10;11), t(6;9), and monosomal karyotype [2,4,9]. Monosomal karyotype is defined by the presence of one monosomy and one additional structure aberration or monosomy and was described as an adverse prognosis factor independent of complex cytogenetic abnormalities [10–14]. The beneficial effect of allogeneic hematopoietic stem cell transplantation in patients with monosomal karyotype was reported to be marginal [10,13]. The prognostic

**CONTACT** Jérôme Moreaux jerome.moreaux@igh.cnrs.fr Laboratory for Monitoring Innovative Therapies, Department of Biological Hematology, Hôpital Saint-Eloi - CHRU de Montpellier, 80, av. Augustin Fliche, 34295 Montpellier Cedex 5, IGH - Institute of Human Genetics, CNRS UPR 1142, France

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Table 1. Prognostic subgroups of acute myeloid leukemia based in cytogenetics.

| Risk status | Cytogenetics |
|---|---|
| Favorable | t(15;17)(q22;q21) |
| | t(8;21)(q22;q22) |
| | inv(16)(p13q22)/t(16;16)(p13;q22) |
| Intermediate | Normal cytogenetics |
| | +8 |
| | t(3;5) |
| | t(9;11)(p22;q23) |
| | Entities not classified as favorable or adverse |
| Adverse | Complex karyotype |
| | −17/abn(17p) |
| | abn(3q) excluding t(3;5)(q21–25;q31–q35) |
| | inv(3)(q21q26)/t(3;3)(q21;q26) |
| | del(5q), −5, add(5q) |
| | −7, del(7q), add(7q) |
| | t(6;11)(q27;q23) |
| | t(10;11)(p11–13;q23) |
| | 11q23 abnormalities excluding t(9;11), t(11;19), and t(9;22) |
| | t(6;9) |
| | Monosomal karyotype |

Adapted from [2,8,17–19]; United Kingdom Medical Research Council (MRC-C)

impact of monosomal karyotype was confirmed in secondary AML [15] and elderly patients [16].

Nevertheless, up to 45% of all AML patients have a normal karyotype and the majority of patients fall into an intermediate group risk category, making cytogenetics alone limited to accurately assess prognosis for all AML patients.

The ELN-C classification includes the prognostic value of somatic mutations in AML including *NPM1, FLT3,* and *CEBPA*. ELN-C classifies AML into four prognostic categories: favorable (t(8;21)(q22;q22); inv(16)/t(16;16)(p13;q22); *NMP1*(+) and *FLT3* internal tandem duplication (ITD) WT with normal karyotype; mutated *CEBPA* with normal karyotype), intermediate-1 (*NPM1* (+) and *FLT3* ITD(+) with normal karyotype; *NPM1*WT and *FLT3* ITD(+) with normal karyotype; *NPM1*WT and *FLT3* ITD(+) with normal karyotype), intermediate-2 (t(9;11)(p22;q23) and cytogenetic abnormalities not classified as favorable or adverse) and adverse (inv(3)/t(3;3)(q21;q26); t(6;9)(p23,q34); t(v;11)(v;23) MLL rearranged and −5 or del(5q), −7, abnormal (17p), complex karyotype)[2,6,20].

## Genomic instability in AML: link with pathogenesis, prognosis, and drug resistance

Recurrent nonrandom chromosomal translocations result in generation of chimeric oncoproteins that are found in 30% of AML patients. Complex karyotype AMLs are defined by three or more cytogenetic abnormalities and represent 20% and 10% of AML cases [5,21].

Biological analyses of fusion proteins provided significant improvements in the molecular mechanisms involved in leukemogenesis and led to potent therapeutic strategies including arsenic trioxide and all-trans-retinoic acid for t(15;17) patients characterized by the promyelocytic leukemia (*PML*)-retinoic acid receptor alpha (*RARA*) fusion [22,23].

Recently, specific recurrent chromosomal translocations, including *PML-RARA* and *AML1-ETO*, have been associated with DNA repair deficiencies. Several studies have shown that *AML1-ETO* represses DDR (DNA damage response) genes and especially genes involved in base excision repair (BER) including *OGG1, FEN1, MPG, POLD2, POLD3, POLE,* and *ATM* [24–26]. This may be involved in DNA damage accumulation in *AML1-ETO* AML cells. *PML-RARA* defines a genetically and clinically distinct AML subtype named APL. Studies indicated that *PML-RARA* could repress DDR genes, such as BER genes (*FEN1, LIG3, MPG, OGG1, POLD2, POLD3,* and *POLE*), homologous recombination repair (HR) genes (*RPA1, RECQL4, RECQL5, BRCA1,* and *RAD51C*), mismatch repair (MMR) genes (*MSH6* and *MLH1*), and nonhomologous end joining (NHEJ) genes (*ku80* and *DNA-PK*) [24,27]. PML is critical for formation of nuclear bodies performing important functions in DNA repair [28–30]. Colocalization of PML and BLM in nuclear bodies has been shown [30]. In APL cells, PML and BLM are delocalized from the nuclear bodies into microspeckled nuclear regions [30]. *PML*-depleted cells are characterized by a significant increase in sister chromatid exchanges and genomic instability characteristics similar to Fanconi anemia and Bloom's syndromes predisposed to cancers including AML [31,32]. ATRA treatment of APL patients leads to degradation of *PML-RARA* and relocalization of BLM to nuclear bodies [30], suggesting that *PML-RARA* are involved in genomic instability in APL through disruption of BLM and PML localization and activity.

AMLs with a complex karyotype are associated with a poor prognosis. This subgroup is characterized by increased expression of DNA repair and cell cycle checkpoint genes including *RAD1, RAD9, RAD21,* and *MSH6* that could be involved in chemoresistance [33]. Furthermore, this subgroup was also distinguished by high level of genomic instability and replication stress identified by γH2AX and CHK1 staining [34]. DDR activation in these patients may explain the chemoresistance and represent a potent therapeutic target for synthetic lethality approaches [34].

Furthermore, single-nucleotide polymorphisms or mutations in genes belonging to HR, BER, nucleotide excision repair (NER), and MMR pathways have been associated with leukemia susceptibility [35].

Polymorphic variants of genes involved in NER have been described in AML patients including *XPD Lys751Gln, XPC Ala499Val,* and *XPA UTR 5'A>G. XPD Lys751Gln* was described to be associated with increased risk of t-AML development but not de novo AML [36–38]. Furthermore, XPD Lys751Gln could be an adverse prognostic factor in elderly patients [37]. *XPD Lys751Gln* combined with *XPC Ala499Val* polymorphisms are linked with a poor prognosis in AML patients [38]. *XPA UTR 5'A>G* was also reported to be associated with drug resistance and shorter overall survival (OS) in AML [39]. Several studies reported a correlation between the presence of polymorphic mutations of *RAD51-G135C* with increased risk of t-AML [36,40]. This *RAD51* variant results in RAD51 upregulation [41]. High RAD51 levels could be associated with an increased susceptibility of cancer cells to survive to replication stress and chemotherapy [42]. Furthermore, the increased risk of t-AML was shown to be higher when *RAD51-G135C* is merged with *XRCC3-Thr241Met* polymorphic variant [40]. This polymorphism combination has been also linked with an increased risk of de novo AML development [40,43]. Two polymorphic variants of *XRCC1* (*XRCC1 Arg399Gln* and *XRCC1 Arg194Trp*) have also been described in AML patients [44] without a clear association with risk of AML [45]. A reduced DNA repair capacity has been described for these polymorphisms [46]. Therefore, these variants are associated with a

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significant better OS in AML [44]. However, another study did not found a significant link between *XRCC1* polymorphisms and risk of AML [45].

Microsatellite instability has been reported in 50% of t-AML [47,48] and in elderly patients [49], suggesting that MMR defects could be involved in t-AML development. Mutations or promoter methylation of *MSH2* and *MLH1*, two genes involved in MMR, have been identified in AML [27,48–51].

Generation of chromosomal translocations has also been linked to aberrant NHEJ [52–54]. In patients developing t-AML after topoisomerase II inhibitor treatment (mitoxantrone and etoposide), microhomologous sequences have been identified in *PML-RARA, MLL,* or *AML1* oncofusion genes supporting a link between aberrant NHEJ and chromosomal translocations in AML [55,56].

## Molecular genomics and risk stratification

Next-generation or whole-sequencing approaches have revealed several recurrent somatic mutations that allow to progress in the understanding of AML genomic landscape [1]. AML genomes were reported to present a limited number of mutations with an average of 13 mutated genes per patient [1]. The most frequently mutated genes include *FLT3, NPM1, DNMT3a, IDH1, IDH2, TET2, RUNX1, WT1, p53, NRAS,* and *CEBPA* [1]. The development of next-generation sequencing in routine will extend the information on the mutational profile of AML patients and affect clinical decisions. Several molecular markers have been reported for AML risk stratification. Gene mutations such as ITD of the *FLT3* gene, mutations in the *NPM1* gene, partial tandem duplication of the *MLL* gene, *RAS* mutations, mutations in the *CEBPA* gene, and changes in gene expression, such as overexpression of *BAALC, ERG, EVI1, MN1,* and *CDKN1B*, have been discovered to strongly affect clinical outcome of CN-AML patients [57,58]. Twenty-four percent of CN-AML patients show none of the aforementioned mutations, underlining the biological and clinical heterogeneity of this disease [59].

Mutation of *FLT3* receptor is a common event in CN-AML, occurring in 30% of the patients [60,61]. Also, 20–25% of the patients have ITD on the juxtamembrane domain, whereas 7% of the patients present mutations affecting the tyrosine kinase domain (TKD). These abnormalities are associated with constitutive activation of *FLT3* conferring a growth advantage and playing a role in leukemogenesis [60]. *FLT3-ITD* mutated AMLs are associated with a poor prognosis, whereas the prognostic significance of TKD mutations is less clear [62–66]. In addition, the allelic ratio of *FLT3* mutant allele to wild-type *FLT3* allele was associated with a prognostic value [65,66]. High *FLT3*-mutant allelic ratio have been reported to be more sensitive to FLT3 inhibitor therapy [67].

*NPM1* is a nuclear phosphoprotein mutated in 50% of CN-AML patients and 60% of patients with *FLT3-ITD* mutations [59,68]. *NPM1* mutations lead to aberrant cytoplasmic localization of the protein and confer a favorable prognosis in the absence of *FLT3-ITD* mutations [59,62,68,69]. CN-AML with *NPM1* and *IDH1* or *IDH2* mutations in the absence of *FLT3-ITD* mutations are associated with a favorable prognosis [70]. However, patients with *FLT3-ITD* and *NPM1* mutations have a poor prognosis [59,62,68,69].

The *CEBPA* is a transcription factor with critical roles in tissue-specific gene expression and proliferation arrest [71]. Also, 10–18% of CN-AML display loss of function mutation of *CEBPA* [72]. Whereas single mutation in CEBPA was not associated with a prognostic value, biallelic mutation confers a favorable prognosis [62,73–76].

Intragenic mutations of *RUNX1* (runt-related transcription factor 1) were reported in 6–26% of AML and were linked with an adverse prognostic [77,78].

*RAS* mutations have been identified in 10–25% of AML patients with a significant enrichment in patients with inv (16) karyotype[79,80]. *RAS* mutations were not reported to be associated with a prognostic value in AML, but these patients may benefit from postremission consolidation with high-dose ara-C [79,80].

*EVI1* gene encodes a transcription factor with important role in normal hematopoiesis and leukemogenesis [81]. *EVI1* upregulates cell proliferation through the activation of AP1 and by repression of transforming growth factor β [82]. Moreover, high *EVI1* blocks differentiation through its interaction with transcription factors essential in hematopoiesis such *GATA1* [83], *SPI1* [84], and *RUNX1* [85]. The prognostic impact of *EVI1* expression has been a subject of debate since many years. A study has demonstrated that *EVI1* deregulation is a relatively frequent event in AML, with no predictive impact on patients' outcome[86]. On the contrary, other groups showed that high *EVI1* levels predict adverse outcome among intermediate cytogenetic risk AML [87,88]. Brain and acute leukemia cytoplasmic (*BAALC*), ETS-related gene (*ERG*), and meningioma 1 (*MN1*) overexpression have also been identified to strongly affect clinical outcome of CN-AML patients [57,58].

The development of high-throughput gene expression profiling (GEP) is of interest to improve risk classification of patients with CN-AML. By combining supervised and unsupervised data analysis from microarrays, Bullinger et al. [89] reported a 133-gene signature that split CN-AML patients into two groups with different outcomes. The prognostic significance of this signature was confirmed using an independent CN-AML cohort, using Affymetrix U133plus2.0 microarrays [90]. Metzeler et al. identified 66 genes, whose expression was prognostic for OS, and defined a prognostic score based on this signature [91]. More recently, starting from 22 genes whose expression is associated with a bad prognosis on CN-AML, a new GEP-based risk score was reported [30]. This GE-based risk score allowed identifying a high-risk group of patients (53.4%) in two independent cohorts of CN-AML patients. GE-based risk score and EVI1 gene expression remained independent prognostic factors using multivariate Cox analyses. Combining GE-based risk score with EVI1 gene expression allowed the identification of three clinically different groups of patients in two independent cohorts of CN-AML patients [88]. Altogether, these studies emphasized the power of GEP data to predict outcome of CN-AML patients.

## Epigenetic landscape of AML

Epigenetics designate modifications of gene expression without alteration of DNA sequences. Epigenetics is characterized by a wide range of changes that are reversible and orchestrate

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gene expression. Epigenetic modifications include methylation of DNA cytosine residues and histone modifications and are critical in the initiation and progression of many cancers [92]. The identification of abnormalities in epigenetic mediators and epigenetic landscape gives access to the development of novel targeted therapeutic strategies. Several genes involved in DNA methylation and histone post-transcriptional modifications have been reported to be mutated in AML, including *DNMT3A, TET2, EZH2, IDH1,* and *IDH2* [6,20].

DNMT3A is a DNA methyltransferase family member. *DNMT3A* was described as one of the most frequently mutated genes in AML in independent cohorts of patients. *DNMT3A* mutations were identified in 4–22% of adult AML and in 36% of CN-AML. *DNMT3A* mutations are enriched in patients with intermediate-risk karyotype [93]. Furthermore, *DNMT3A* mutations are associated with an adverse prognosis in AML patients [93,94]. Different *DNMT3A* mutations have been identified including nonsense, frameshift, and missense mutations. Among them, the most recurrent alteration is a missense substitution at codon R882 of *DNMT3A* [93]. In vitro assays reported a possible loss of methyltransferase activity in AML cells with R882 mutation [95]. Another study has shown no significant difference in DNA methylation comparing *DNMT3A* wild-type and mutant patients [93]. Furthermore, methylation analysis using HELP assay failed to identify a clear specific signature of *DNMT3A* mutant compared to wild-type patients [96].

Ten-eleven-translocation gene 2 (TET2) alterations have been identified in 8–23% of AML patients [70,97,98]. *TET2* mutations are enriched in intermediate-risk AML with a frequency of 18–23% [6,99]. TET2 plays a role in conversion of 5-methylcytosine to 5-hydroxymethylcytosine with a function in DNA methylation and epigenetic transcription regulation [100,101]. AML patients harboring *TET2* mutations exhibit a unique methylation signature with a propensity for hypermethylation [102]. TET2 depletion in mice results in inhibition of hematopoietic differentiation [101], suggesting that *TET2* mutation in AML could reactivate a stem cell state [101]. The link between *TET2* mutations and prognosis remains uncertain [103,104]. *TET2* mutations can coincide with alterations in *NPM1, RAS, FLT3, CEBPA*, and *RUNX1*, but are exclusive to mutations in *IDH1* and *IDH2* [104,105].

*EZH2* mutations have also been reported in myeloid malignancies. EZH2, one of the most studied histone-modifying enzymes, is the catalytic subunit of the polycomb repressive complex 2 (PRC2) polycomb complex. EZH2 induces transcriptional repression of target genes by trimethylating lysine 27 residue of histone H3 (H3K27me3) [106]. The other members of PRC2 complex are proteins EED, SUZ12, RbAp46/48, and AEBP2. EZH2 requires at least EED and SUZ12 to be catalytically active in vitro, whereas RbAp46/48 and AEBP2 have been shown to stimulate EZH2 activity [106]. EZH2, EED, or SUZ12 loss-of-function mutation increases hematopoietic stem cells (HSC) and progenitors self-renewal activity [107]. EZH2 overexpression in HSCs prevents exhaustion of their long-term repopulating potential during serial transplantation [108–110]. EZH2 has been proposed to be a gene preventing stem cell senescence [108]. EZH2 also affects adult HSC differentiation but not their self-renewal capacity [111–113]. A correlation between *EZH2* overexpression and myeloid malignancy development has also been described [114]. *EZH2* is highly expressed in high-risk myelodysplastic syndrome (MDS) and in AML arising from preexisting MDS. Indeed, *EZH2* is significantly overexpressed in MDS and AML primary tumor cells displaying aberrant DNA methylation of the tumor suppressor p15INK4B gene compared with patients without p15INK4B methylation [115]. More recently, a model was proposed in which *EZH2*-inactivating mutations would be part of cancer stem cells development through the induction of *HOXA9* expression, supporting myeloid progenitor self-renewal [116]. In MDS, *EZH2*-inactivating mutations are frequently associated with *RUNX1* mutations. In a MDS mouse model induced by *RUNX1* mutation in HSCs, *EZH2* loss promotes disease development but decreases its propensity to evolve to AML [117].

*IDH* 1 and 2 mutations have been identified in genome-wide studies of AML [118]. IDH1 and IDH2 are important players in normal citrate metabolism catalyzing the decarboxylation of isocitrate to α-ketoglutarate in the Krebs cycle [119]. *IDH1* and *IDH2* mutations have been reported in 15–33% of AML patients [70,120,121]. *IDH1* and *IDH2* mutations are more frequent in intermediate-risk AML, including normal karyotype AML [120,122]. These mutations are heterozygous and occur at arginine 132 or 170 in *IDH1* and at Arg172 or Arg140 in *IDH2* [118,123–125] conferring to these enzymes a new function to convert α-ketoglutarate to 2-hydryglutarate [126,127]. The increase in 2-hydryglutarate production will interfere with α-ketoglutarate-dependent enzymes including TET enzymes, Jumonji-C domain-containing histone lysine demethylases, and prolyl hydroxylases, and affect epigenetic regulation [119,128,129]. *IDH1* or *2* mutated AML display a specific methylation pattern with global hypermethylation and aberrant hypermethylation of genes important in myeloid differentiation and in leukemogenesis [1,119,130]. Moreover, the increased level of 2-hydryglutarate will lead to ROS-mediated DNA damages [130,131]. *IDH2* R140 mutation was reported to be associated with *NPM1* mutations and a favorable prognosis in one study [70]. The prognostic impact of *IDH1* or *IDH2* mutations is not clear with conflicting results from different studies [70,120,124].

## Therapeutic approaches emerging from new molecular markers

There is an ardent activity in the development of novel therapeutic approaches for AML. Identification of recurrent mutations in AML has led to development of targeted treatments (Table 2). *RAS* mutations have been shown to be associated to PI3K-AKT and MAPK pathways upregulation [79]. Dual-pathway inhibition clinical trial combining Mek and PI3K-AKT inhibitors is in progress (NCT01907815).

Several FLT3 inhibitors are tested in AML, alone or in combination with chemotherapy. Studies suggest that FLT3 inhibitors are tolerated [132,133]. However, a higher toxicity was reported in older patients [134]. Lower intensity therapy combined with FLT3 inhibitor is investigated in older patients with *FLT3-ITD* mutations like sorafenib and azacytidine combination [135]. Treatment with sorafenib did not significantly improved event

Table 2. Prospective targets in acute myeloid leukemia with prognostic implications and potential targeted therapies.

| Target | Prognostic value | Potential targeted therapy |
|---|---|---|
| *FLT3* | Unfavorable prognosis for FLT3-ITD | FLT3 inhibitors: sorafenib, midostaurin, quizartinib, crenolanib |
| RAS | No prognostic value | Mek inhibitor: trametinib |
| *IDH1 and 2* | Not clear with conflicting results from different results | AG221 IDH2 inhibitor, AG120 IDH1 inhibitor, ABT-199 BH3-mimetic |
| *TET2* | Remain uncertain | DNMTi |
| DNMT3A | Unfavorable prognosis | DNMTi |
| *MLL* | Adverse prognosis | *DOT1L* inhibitor |
| CD200 | Adverse prognosis | Anti-CD200 MoAb |
| CD33 | No prognostic value | Gemtuzumab ozogamicin, SGN-33a |
| MLL or p53 | Adverse prognosis | *BET* inhibitors |

FLT3: Fms-like tyrosine kinase 3; TET2: tet methylcytosine dioxygenase 2; DNMT: DNA methyltransferase; IDH1/2: isocitrate dehydrogenase 1/2; MLL: mixed-lineage leukemia; MoAb: Monoclonal antibody; DOT1L: DOT1-like histone H3K79 methyltransferase; BET: bromodomain and extra terminal protein.

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free survival (EFS) or OS of patients with AML [134]. Another trial in younger patients reported no difference in the complete remission rate, whereas EFS was significantly improved in sorafenib-treated patients [133]. More selective FLT3 inhibitors are currently evaluated [136–138].

AML is characterized by epigenetics abnormalities. DNA methyltransferase inhibitors (DNMTi) have shown activity in AML and represent a valuable option for older patients that could not benefit from intensive chemotherapy. *DNMT3A* mutations are associated with an adverse prognosis in AML patients [93,94], and an improved response rate to decitabine treatment was recently reported in patients with *DNMT3A* mutations [139]. However, these data should be validated. It has also been hypothesized that patients with *TET2* loss-of-function mutations, in association with increase in DNA methylation, could be targeted by DNMTi [140].

*IDH1* and *IDH2* mutations represent attractive therapeutic targets. Small molecules to target *IDH1/2* mutants and demethylating agents are tested in clinical trials [141,142] (clinical trials NCT02074839 and NCT01915498). Furthermore, BCL-2 inhibition has been proposed as a synthetic lethal approach in AML patients with *IDH2* mutations [143].

Another emerging target for treatment is aberrant methylation of histone lysines by histone methyltransferases involved in AML pathogenesis like MLL or EZH2. Translocations involving MLL will lead to fusion proteins where MLL retains its DNA-binding activity, loses its histone 3 lysine 4 methyltransferase activity but gains the ability to recruit DOT1L histone 3 lysine 79 methyltransferase. Studies have demonstrated the role of DOT1L in pathogenesis of AML induced by MLL-fusion proteins [1,144]. DOT1L inhibitors are currently in clinical trials in AML (NCT01684150) [1,144].

Development of synthetic lethality approaches in AML, exploiting DNA repair defects or addiction, represents another interesting strategy. AML with complex karyotype being characterized by high genomic instability, CHK1 inhibition was associated with sensitization of complex karyotype AML cells to Ara-C treatment in vitro [34,145]. The therapeutic potential to combine temozolomide with PARP inhibitors (PARPi) has been demonstrated in vitro in MMR-deficient AML [146–148]. PARPi will block BER pathway and overcome resistance to temozolomide. Furthermore, PARPi could also be useful to target the function of PARP1 in restart of stalled replication forks to sensitize AML cells to genotoxic agents [149–151]. PARP1 is also involved in alternative NHEJ involved in chromosomal translocation process, and combination of PARPi with chemotherapy could represent an interesting strategy to reduce the risk of secondary AML [152,153]. Recently, it was shown that DDR gene expression could be targeted by histone deacetylases inhibitors sensitizing AML cells to chemotherapeutic agents [154,155].

Specific immunotherapy using anti-CD33 antibody-drug conjugate gemtuzumab ozogamicin [156]. Several studies analyzing the combination of gemtuzumab ozogamicin to intensive chemotherapy have been performed [157–160]. Gemtuzumab ozogamicin addition was associated with a significant reduced risk of relapse and improved survival especially in patients with favorable but also intermediate cytogenetic characteristics [161]. More recently, the expression of CD200, a protein delivering an immunosuppressive signal, was described as a poor prognosis factor in AML in association with other molecular prognostic factors [162]. Interestingly, CD200 appears as a potent therapeutic target in AML for antibody-based therapy [162].

## Expert commentary

AML is a highly heterogeneous disease with a wide diversity in molecular alterations explaining why AML treatment remains challenging. However, advances made to progress in the understanding of the AML genetic and epigenetic landscape lead to the emergence of novel treatments to develop tailored therapies and improve patient outcome. Several targets have been identified, and clinical trials investigating targeted therapies are ongoing in AML. However, some limitations in the success of these clinical trials could come from the selection of patients included in targeted therapy trials. These trials are mainly limited to patients with relapsed or refractory AML where the advanced genomic instability of tumor cells and the toxicity of previous treatments could lead to false negative results. Extension to younger high-risk newly diagnosed patients and fit newly diagnosed older patients could improve the results of this approach. Furthermore, identification of the most efficient drug combination with chemotherapy based on biological rationale is also needed. Another requirement is the inclusion of prospective studies with detailed genomic and epigenetic profiling specified in advance and performed routinely to distinguish responder from nonresponder patients. Furthermore, mutation characterization and identification of aberrant proteins could not always be druggable. Synthetic lethal or RNA interference screens may help to identify vulnerabilities that could be exploited through targeted therapies [163]. RNA screen recently identified the protein bromodomain-containing 4 (Brd4) as being critically required for disease maintenance [163]. Brd4 inhibitor (JQ1) demonstrated robust antileukemic activity *in vitro* and *in vivo* targeting Myc expression [163]. Interestingly, Brd4 inhibitors were efficient to target AML cell lines with unfavorable aberrations as well as primary tumor cells from relapsed/refractory AML patients [163,164]. According to these data, Brd4 inhibitors are currently in clinical trials in AML

(NCT01943851 and NCT01713582). Currently, most drug development strategies using next-generation sequencing for patient stratification do not consider clonal heterogeneity and patterns of temporal acquisition of mutations. A better understanding of clonal heterogeneity and clonal evolution will be important to improve the treatment of AML patients. Treatment may act as a source of genomic instability with a significant increase in genomic abnormalities, in AML patients, at relapse following cytotoxic therapy compared with primary samples [165]. Development of functional model to study tumor evolution will have to be integrated to develop efficient therapeutic strategies [166]. Progresses are needed to understand the biology associated with cytotoxic agent response and the DNA damage pathways involved in the context of the interactions between tumor cells and the microenvironment to address this therapeutic challenge.

## Five-year view

The increased understanding of the pathophysiology of AML associated with genetic and epigenetic deregulations, aberrant signaling responses, and interactions with the microenvironment might be used to design and implement targeted strategies with a markedly improved therapeutic index. These aberrations are constantly evolving due to several selective pressures induced by molecular alterations, replicative stress, the microenvironment, and the different treatments. In the complex scenario of AML progression, it is essential to recognize the possible pitfalls of continuous therapy incorporating agents with a known mutagenic potential. It is important to manage the use of chemotherapeutic agents with a known mutagenic potential in order to reduce the risk of generating mutant clones. According to this, targeted treatment of AML represents a significant way forward and is aimed at increasing survival rates. Progress in computational and mathematical models will help to develop predictive biomarkers to optimize targeted treatment strategies with the most efficient drug combination in AML patients.

## Financial & competing interests disclosure

*The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.*

## ORCID

*Jerome Moreaux* http://orcid.org/0000-0002-7271-9570

Key issues

- Acute myeloid leukemia (AML) is a heterogeneous disease at molecular level, in response to therapy and prognosis.
- Genomic instability in AML is linked with pathogenesis, prognosis, and drug resistance.
- The genetic and epigenetic landscape of AML is evolving in association with the development of new personalized therapeutic strategies to improve outcome of the patients.
- Clinical trials investigating targeted therapies are ongoing in AML.
- Earlier inclusion of combinations associating targeted treatment and chemotherapy and of newly diagnosed patients into clinical trials is recommended.
- Progress in computational and mathematical models will help to develop predictive biomarkers to optimize targeted treatment strategies in AML.

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