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Molecular Genetics of Adult Acute Myeloid Leukemia: Prognostic and Therapeutic Implications

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ABSTRACT

Molecular analyses of leukemic blasts from patients with acute myeloid leukemia (AML) have revealed a striking heterogeneity with regard to the presence of acquired gene mutations and changes in gene and microRNA expression. Multiple submicroscopic genetic alterations with prognostic significance have been discovered. Application of gene- and microRNA profiling has identified genome-wide expression signatures that separate cytogenetic and molecular subsets of patients with AML into previously unrecognized biologic and/or prognostic subgroups. These and similar future findings are likely to have a major impact on the clinical management of AML because many of the identified genetic alterations not only represent independent prognosticators, but also may constitute targets for specific therapeutic intervention. In this report, we review genetic findings in AML and discuss their clinical implications.

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INTRODUCTION

Acute myeloid leukemia (AML) is a genetically heterogeneous clonal disorder characterized by the accumulation of somatically acquired genetic alterations in hematopoietic progenitor cells that alter normal mechanisms of self-renewal, proliferation, and differentiation.[1] In recent years, gene mutations and deregulated expression of genes and noncoding RNAs (ie, microRNAs) have been identified, providing insights into the mechanisms of leukemogenesis and unraveling the enormous molecular genetic heterogeneity within distinct cytogenetically defined subsets of AML, in particular the large group of cytogenetically normal (CN) AML.[2-5] With progress in genomics technology, such as gene- and microRNA-expression profiling, genome-wide single nucleotide polymorphism–based mapping arrays, next-generation sequence techniques, and functional genomics, systematic characterization of cancer genomes has now become feasible, and the identification of disease-relevant mutations and related gene and microRNA profiles will be largely facilitated.

From a clinical perspective, there are at least three important aspects with respect to the genetic changes in AML.[6] First, the current WHO classification reflects the fact that an increasing number of cases of AML can be categorized on the basis of their underlying genetic defects that define distinct clinicopathologic entities.[7] Second, it has become clear that specific chromosome abnormalities and molecular genetic changes are among the most important prognostic markers and therefore may be used for stratification of patients with AML to risk-adapted therapeutic strategies. Finally, novel therapies are being developed that target some of the identified genetic defects. It is therefore anticipated that these genetic markers will acquire a predictive value, that is, the ability to predict differential efficacy of a therapy. Thus it is hoped that genetic markers will allow us to optimize the treatment of distinct subtypes of AML.

RECURRENT GENE MUTATIONS IN AML

Somatically acquired mutations have been identified in several genes (Table 1). In general, these mutations are found most frequently in CN-AML but are associated with other cytogenetic subgroups as well.

GENE MUTATIONS IN AML: CLINICAL PRACTICE

To date, only diagnosis of *NPM1*, *CEBPA*, and *FLT3* mutations has entered clinical practice and affects diagnosis, risk assessment, and also guidance of therapy.[6,7] On the basis of characteristic clinical, pathologic, and biologic features, AML with *NPM1* mutation and AML with *CEBPA* mutation have been incorporated as provisional entities in the 2008 WHO classification of AML.[7] Although *FLT3* mutations are not considered to define a distinct entity,

Table 1. Recurrent Molecular Genetic Abnormalities in Adult AML: Biologic Features and Clinical Significance

Gene	Biologic Features	Clinical Significance
Mutated genes		
NPM1	Nuclear-cytoplasmic shuttling phosphoprotein with pleiotropic functions Mutations lead to abnormal cytoplasmic localization of the protein that can be diagnosed by immunohistochemistry on bone marrow sections Mutations found in 25%-35% of AML (CN-AML, 45%-64%; del(9q) outside a complex karyotype, 35%-40%; trisomy 8, approximately 15%) Associated with *FLT3*-ITD (approximately 40%), *FLT3* TKD (10%-15%), and *IDH* mutations (approximately 25%) Leukemic blasts show high CD33 and absent to low CD34 expression Associated with myelomonocytic or monocytic morphology Higher prevalence in female sex	Provisional disease entity (WHO 2008) In younger adult patients, mutated *NPM1* without *FLT3*-ITD predicts for achievement of CR and favorable RFS and OS In younger adults with mutated *NPM1* without *FLT3*-ITD, standard induction therapy followed by repetitive cycles of HiDAC is a reasonable treatment option; patients may not benefit from allogeneic HSCT in first CR Favorable impact of *NPM1* mutations in older patients Older patients with *NPM1*-mutated AML may benefit from intensive conventional chemotherapy
CEBPA	Master regulatory transcription factor in hematopoiesis Mutations predominantly in CN-AML (10%-18%), and AML with del(9q) occurring outside a complex karyotype (approximately 40%) Mutations mostly biallelic (N-terminal and C-terminal); only double-mutant cases may define the entity	Provisional disease entity (WHO 2008) Mutations associated with higher CR rate and favorable RFS and OS Only double, not single, *CEBPA* mutations predict for favorable outcome In younger adults, standard induction therapy followed by repetitive cycles of HiDAC is a reasonable treatment option for AML with *CEBPA* mutation
FLT3	Member of the class III receptor tyrosine kinase family; *FLT3* and its ligand play an important role in proliferation, survival, and differentiation of hematopoietic progenitor cells	
ITD	*FLT3*-ITD found in approximately 20% of all AML (normal karyotype: 28%-34%) Insertion site of the ITD commonly in the JM domain; in approximately 30% in the tyrosine kinase 1 domain Homozygous mutations result from mitotic recombination leading to partial UPD	*FLT3*-ITD associated with inferior outcome, in particular those cases with high mutant to wild-type allelic ratio; non-JM *FLT3*-ITD possibly associated with dismal outcome Allogeneic HSCT should be considered in AML with *FLT3*-ITD Phase II and III clinical trials evaluating *FLT3* tyrosine kinase inhibitors underway
TKD	*FLT3* TKD point mutations found in 5%-10% of all AML [CN, 11%-14%; inv(16)/t(16;16), 14%-24%]	Prognostic significance controversial Phase II and III clinical trials evaluating *FLT3* tyrosine kinase inhibitors underway
IDH1, *IDH2*	Cytosolic (*IDH1*) and mitochondrial (*IDH2*) metabolic enzymes catalyzing oxidative decarboxylation of isocitrate to α-ketoglutarate; involved in cellular defense of oxidative damage Mutations commonly located at residue 132 (*IDH1*) and residues 140 and 172 (*IDH2*); lead to a neomorphic enzyme activity and to accumulation of a putative oncogenic metabolite, R(2)-2-hydroxyglutarate (2HG) Mutations found in approximately 16% of unselected AML (*IDH1*, 8%; *IDH2*, 8%); association with CN-AML (*IDH1*, 10%-16%; *IDH2*, 10%-19%) *IDH1* R132 (and possibly also *IDH2* R140) mutation associated with molecular low-risk AML (mutated *NPM1* without *FLT3*-ITD) *IDH2* R172 mutation rarely found in combination with other known gene mutations and associated with distinctive microarray gene- and microRNA-expression profiles	*IDH2* mutations found more frequently in older patients *IDH1* and possibly also *IDH2* mutations confer higher risk of relapse and inferior OS in molecular low-risk CN-AML (mutated *NPM1* without *FLT3*-ITD) *IDH2* R172 mutation associated with lower CR rate and possibly with inferior outcome *IDH1* SNP rs11554137 (located in the same exon as the R132 mutation) in one study found to be associated with inferior outcome in molecular high-risk CN-AML (either *NPM1* wild-type or *FLT3*-ITD positive)
KIT	Member of the class III receptor tyrosine kinase family; *KIT* and its ligand stem cell factor have a key role in survival, proliferation, differentiation, and functional activation of hematopoietic progenitor cells Mutations mostly found in CBF-AML (25%-30%)	Mutations associated with inferior outcome in CBF-AML Currently no data available supporting the use of *KIT* mutational status to guide therapy Clinical trials evaluating *KIT* inhibitors underway
WT1	Transcription factor implicated in regulation of apoptosis, proliferation, and differentiation of hematopoietic progenitor cells Mutations found in 10%-13% of CN-AML	Prognostic significance somewhat controversial; most studies report a negative prognostic impact Postremission therapy with HiDAC may abrogate poor prognosis *WT1* SNP rs16754 located in the proximity of the *WT1* mutational hotspot in one study found to be associated with inferior outcome in CN-AML
RUNX1	Transcription factor required for definitive hematopoiesis Mutations found in 5%-13% of AML; association with trisomy 13, trisomy 21, and normal karyotype Mutations associated with *MLL*-PTD; inverse correlation with presence of *CEBPA* and *NPM1* mutations Association with undifferentiated morphology (FAB M0)	Prognostic significance under investigation; first data indicate association with lower CR rate and shorter RFS and OS
MLL-PTD	DNA binding protein that regulates gene expression in hematopoiesis possibly through epigenetic mechanisms *MLL*-PTD found in 5%-11% of normal karyotype AML, and up to 90% of AML with trisomy 11	In initial studies associated with shorter CR duration, inferior RFS and EFS, but not OS In multivariable analyses in general not an independent prognostic factor

(continued on following page)

Table 1. Recurrent Molecular Genetic Abnormalities in Adult AML: Biologic Features and Clinical Significance (continued)

Gene	Biologic Features	Clinical Significance
NRAS	Membrane-associated proteins regulating mechanism of proliferation, differentiation, and apoptosis Mutations found in 9%-14% of CN-AML, in up to 40% of CBF-AML (in particular inv[16]), and in 25%-30% of AML with inv(3)	No prognostic significance shown Mutant *NRAS* may predict sensitivity to cytarabine
KRAS	Mutations found in 5%-17% of CBF-AML	No prognostic significance shown
TP53	Encodes tumor suppressor protein p53, which responds to diverse cellular stresses to regulate target genes that induce cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism Mutations/deletions mostly in AML with complex karyotype (56%-78%)	Associated with inferior outcome (overlap with complex karyotypes)
TET2	Belongs to a 3-member family of highly conserved genes; protein function unknown, may be involved in epigenetic regulation Mutations found in a wide spectrum of myeloid neoplasms; MDS (20%), MPN (12%), secondary AML (25%); frequency in de novo AML under investigation	Clinical significance under investigation
ASXL1	Putative polycomb group protein; possibly involved in chromatin remodeling Mutations found in MDS, MPN, AML	Clinical significance under investigation
JAK2	Protein tyrosine kinase, involved in a specific subset of cytokine receptor signaling pathways Mutations common in MPN, rare in AML (mostly in CBF-AML)	Likely not of major clinical significance
CBL	Adaptor protein for receptor protein-tyrosine kinases, positive regulation of receptor protein-tyrosine kinase ubiquitination Mutations rare in AML (mostly in CBF-AML)	Likely not of major clinical significance
Deregulated genes		
BAALC	Biologic function unknown High *BAALC* expression correlates with that of genes expressed in hematopoietic progenitors and those involved in chemoresistance	High *BAALC* expression associated with inferior CR rate, DFS, and OS
ERG	Member of the ETS family of transcription factors *ERG* rearrangements involved in other cancers (eg, prostate cancer) Highly expressed in CN-, complex karyotype, and megakaryoblastic AML	High *ERG* expression associated with inferior CR rate and EFS High *ERG* expression mostly impacts prognosis in CN-AML with molecular low risk (*NPM1* mutated without *FLT3*-ITD)
EVI1	EVI1 protein involved in regulation of transcription factors critical for hematopoiesis (eg, GATA 1, GATA2) and in epigenetic regulation Deregulated expression of *EVI1* found in AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *EVI1-RPN1* *EVI1* overexpression also found in approximately 10% of unselected AML; association with −7 and t(11q23)/*MLL* rearrangements	Deregulated *EVI1* expression associated with low CR rate and inferior survival; effect most pronounced in cytogenetic intermediate-risk AML Allogeneic HSCT may improve survival of AML with high *EVI1*
MN1	*MN1* gene has the role of a transcription coregulator Associated with cytogenetic abnormalities in other type of cancer High expression associated with *NPM1* wild-type and *BAALC* and *MN1* upregulation In CN-AML associated with *miR-126*	High *MN1* expression correlates with lower CR, shorter DFS, and OS Low *MN1* expression in one study has been shown to predict response to therapy with ATRA in elderly AML patients

Abbreviations: AML, acute myeloid leukemia; ITD, internal tandem duplication; CR, complete remission; RFS, relapse-free survival; OS, overall survival; CN, cytogenetically normal; TKD, tyrosine kinase domain; HiDAC, high-dose cytarabine; HSCT, hematopoietic stem-cell transplantation; JM, juxtamembrane domain; UPD, uniparental disomy; SNP, single nucleotide polymorphism; CBF, core-binding factor; PTD, partial tandem duplication; EFS, event-free survival; FAB, French-American-British; EFS, event-free survival; MDS, myelodysplastic syndromes; MPN, myeloproliferative neoplasms; DFS, disease-free survival; ATRA, all-*trans*-retinoic acid.

they provide important prognostic information (Fig 1). Furthermore, they are now being targeted in clinical trials with tyrosine kinase inhibitors. Therefore, *NPM1*, *CEBPA*, and *FLT3* mutations are recommended to be analyzed in clinical trials and in routine practice at least in patients with CN-AML who will receive treatment other than low-dose chemotherapy or best supportive care.[6]

NPM1 *Mutations*

Abnormal cytoplasmic localization of the NPM1 protein shown by immunohistochemical analysis led to the discovery of *NPM1* mutations in AML.[8] This mislocalization is caused by mutations in exon 12 of the gene. *NPM1* mutations are found in approximately one third of adult cases of AML, making it the most frequent mutation known in this disease to date. *NPM1* mutations are associated with other recurrent genetic changes, secondary chromosome abnormalities such as +8, +4, del(9q),[9] and additional gene mutations, most frequently in *FLT3*[2,3,10] and *IDH1*.[11-15]

Prognostic significance. *NPM1* mutations, in particular the genotype "mutated *NPM1* without concurrent *FLT3* internal tandem duplication (ITD)," has been associated with achievement of complete remission (CR) and favorable outcome.[2,3,10] On the basis of this observation, AML with mutated *NPM1* without *FLT3*-ITD has then recently been allocated to the genetic favorable-risk category of AML, together with core-binding factor (CBF) AML.[6]

Therapeutic implications. Similar to CBF-AML, standard induction chemotherapy followed by three to four cycles of high-dose cytarabine is a recommended treatment approach for AML with mutated *NPM1* without *FLT3*-ITD.[6] In studies from Cancer and Leukemia Group B (CALGB), patients with this genotype also had favorable

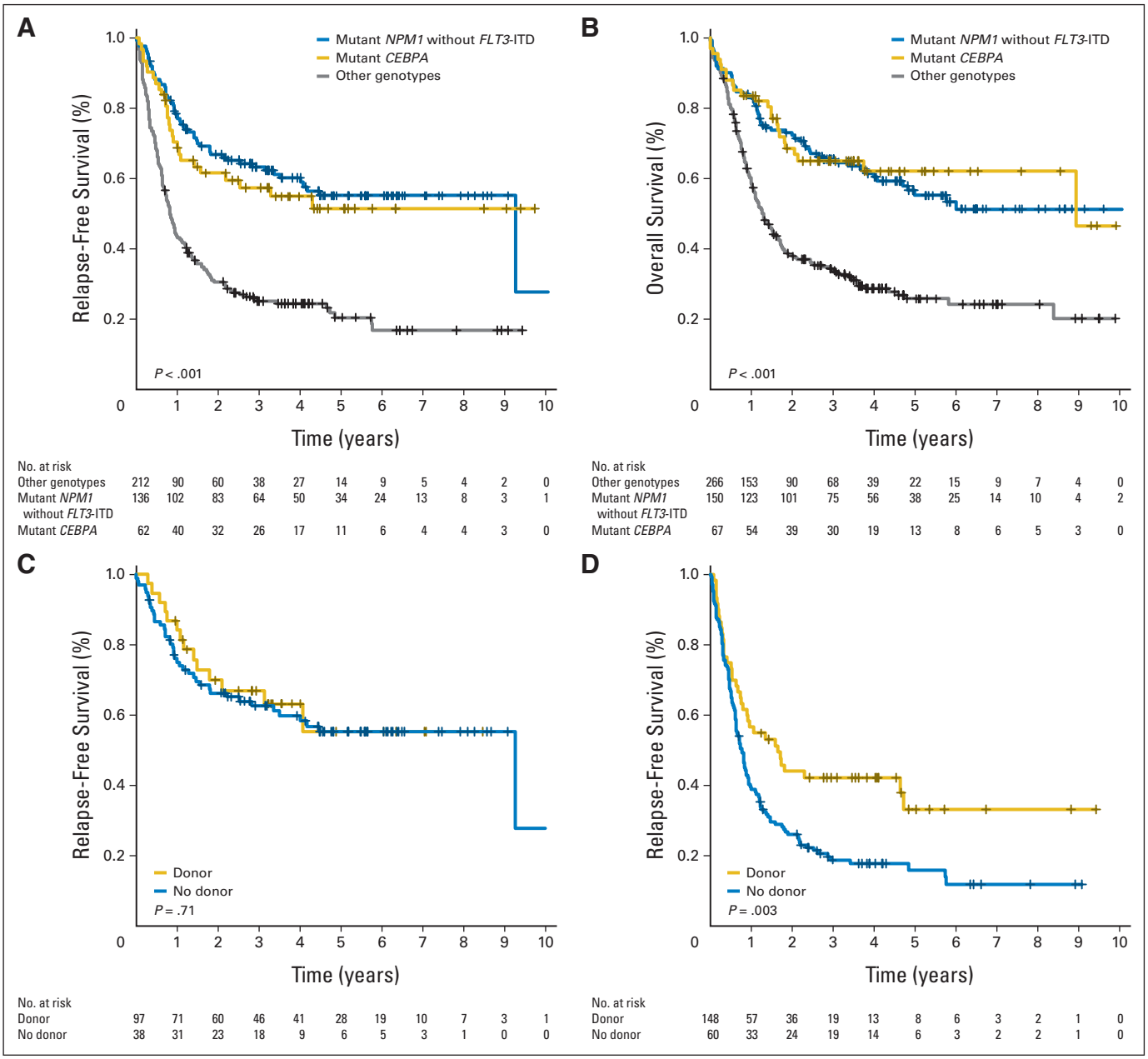

Fig 1. Clinical outcome in cytogenetically normal acute myeloid leukemia (AML). (A) Relapse-free survival according to genotype; (B) overall survival according to genotype; (C) relapse-free survival of patients with mutant *NPM1* without *FLT3* internal tandem duplication (ITD) according to the availability of an HLA-matched related donor; (D) relapse-free survival of patients with other genotypes, excluding the mutant *CEBPA* genotype, according to the availability of an HLA-matched related donor. "Other genotypes" is defined as the *FLT3*-ITD genotype and the triple-negative genotype consisting of wild-type *NPM1* and *CEBPA* without *FLT3*-ITD. The data in (D) suggest that molecular high-risk AML (eg, AML with *FLT3*-ITD) may benefit from allogeneic hematopoietic stem-cell transplantation in first complete remission. Tick marks represent patients whose data were censored at the last time they were known to be alive and in complete remission (A, C, D) or whose data were censored at the last time they were known to be alive (B). Adapted with permission from Schlenk et al.[10]

outcome when treated with autologous hematopoietic stem-cell transplantation (HSCT).[3] Patients with mutated *NPM1* without *FLT3*-ITD may not be considered candidates for allogeneic HSCT in first CR, unless they have a very low risk for transplant-related morbidity and mortality or new transplantation strategies are evaluated within a clinical trial.[6,10] Of note, *NPM1* mutations also seem to predict better outcome in older patients,[16-18] even extending to patients older than 70 years.[17] Thus it is also important to assess for *NPM1* mutations in older patients to identify those individuals who are likely to benefit from intensive conventional chemotherapy.

The German-Austrian AML Study Group previously reported that AML with mutated *NPM1* without *FLT3*-ITD benefit from all-*trans*-retinoic acid (ATRA) given in combination with conventional chemotherapy.[16] This finding was not confirmed in a study by the British Medical Research Council.[19] Differences in the trial designs may account for these discrepant data. Results from ongoing trials

need to be awaited to determine whether ATRA will have a role in the molecular therapy of AML with *NPM1* mutation.

CEBPA *Mutations*

Two major types of heterozygous *CEBPA* mutations have been identified in AML.[20] Nonsense mutations affecting the N-terminal region result in a truncated CEBPA isoform with dominant-negative properties, and in-frame mutations in the C-terminal basic region-leucine zipper domain result in CEBPA proteins with decreased DNA-binding or dimerization activity. In approximately two thirds of cases, C- and N-terminal mutations are biallelic mutations (also called double mutations), with the majority (approximately 90%) of them being compound heterozygous (C-terminal on one allele and N-terminal on the other) and the rest being homozygous (either both C- or N-terminal), likely through the mechanism of uniparental disomy. *CEBPA* mutations are predominantly found in CN-AML and in cases with 9q deletion (Table 1).[2]

Prognostic significance. Among CN-AML, *CEBPA* mutations have consistently been associated with a relatively favorable outcome, similar to that of AML with mutated *NPM1* without *FLT3*-ITD.[2,3,10] Importantly, recent studies show that only double, but not single, *CEBPA* mutations predict for this favorable outcome, whereas AML with a single *CEBPA* mutation is associated with survival similar to that of AML with wild-type *CEBPA*.[20] This finding is biologically substantiated by a discrete gene-expression signature of double-mutated cases[21] and by mouse experiments modeling the mutations by knock-in mutagenesis.[22] Therefore, with regard to disease classification and risk stratification, only AML with double *CEBPA* mutations likely define a distinct entity with favorable prognosis. However, whether the biologic and clinical significance of double *CEBPA* mutations related to lack of at least one normally functioning allele remains to be elucidated.

Therapeutic implications. Therapeutic recommendations are similar to those for AML with mutated *NPM1* without *FLT3*-ITD, that is, standard induction chemotherapy followed by three to four cycles of high-dose cytarabine.[6] Also, AML with double *CEBPA* mutations may not benefit from allogeneic HSCT; however, this statement is currently not substantiated by data, but rather by the assumption that in general, patients with favorable-risk AML do not seem to benefit from this approach in first CR. Because of the low incidence of the mutation, effects of novel antileukemic agents and of allogeneic HSCT in this subset of AML can only be evaluated in intergroup trials or in retrospective large meta-analyses.

FLT3 *Mutations*

Mutations that result in the constitutive activation of *FLT3* have been identified in two functional domains of the receptor, the juxtamembrane (JM) domain and the tyrosine kinase domain (TKD).[1] *FLT3*-ITD are found in approximately 20% of unselected cases of AML and mainly cluster in the JM domain; however, recently it was shown that approximately 30% of ITDs do not insert in the JM but in the TK1 domain of the receptor.[23] The activation loop in the carboxy-terminal lobe of the TKD is affected by point mutations, small insertions, or deletions, mainly at codons 835 and 836, in 5% to 10% of cases of AML.[1,2] Rare point mutations or insertions have been reported at other codons in the TKD. In vitro studies and results from global gene-expression profiling (GEP) revealed that there are similarities but also important differences in signal transduction properties between *FLT3*-ITD and *FLT3* TKD mutations that may explain differences in clinical phenotypes.[1]

Prognostic significance. Prognosis of CN-AML with *FLT3*-ITD is significantly inferior compared with CN-AML without the mutation when treated with current standard chemotherapy.[2,3,10] There is evidence that outcome is related to the ratio of mutated versus wild-type allele in that a high burden of mutated allele predicts for inferior survival.[3] Furthermore, a recent study suggests that patients with AML with non-JM ITD do significantly worse than those with AML with JM domain ITD.[24] The prognostic relevance of *FLT3* TKD mutations remains controversial.[2,3]

Therapeutic implications. Therapy of AML with *FLT3*-ITD is a clinical dilemma. Given the poor results after standard chemotherapy, new modalities need to be evaluated. There is accumulating evidence that allogeneic HSCT in general is an attractive option for patients who are at high risk of relapse. A beneficial effect of allogeneic HSCT has been shown for the entire group of patients with intermediate-risk cytogenetics and for CN-AML with unfavorable genotypes that includes most AML with *FLT3*-ITD.[6,10] Thus, although evidence from prospective trials is lacking (Fig 1), allogeneic HSCT should be considered in patients with *FLT3*-ITD-positive AML. Randomized phase III trials evaluating *FLT3* inhibitors in combination with chemotherapy as a frontline approach to patients with AML with activating *FLT3* mutations are underway.

GENE MUTATIONS IN AML: INVESTIGATIONAL STUDIES

All other gene mutations identified in AML have not yet entered clinical practice and remain investigational.

KIT *Mutations*

KIT mutations are found in 25% to 30% of cases of CBF-AML and are rare in other AML subsets.[25] In most studies, *KIT* mutations have been associated with inferior outcome. Of note, *KIT* is not only mutated, but is also expressed at significantly higher levels in CBF-AML compared with other AML subsets.[26,27] At present, there are no data supporting the use of *KIT* mutational status to guide therapy. Clinical trials are currently underway evaluating *KIT* inhibitors in CBF-AML.

IDH1/IDH2 *Mutations*

Mutations of *IDH1* and *IDH2* were first reported in gliomas and were reported only more recently in AML.[5,11-15] Interestingly, of the three types of mutations that have been hitherto reported, those affecting the arginine residues on *IDH1* codon 132 and *IDH2* codon 172 have been found both in brain tumors and AML, whereas those affecting the arginine residue on *IDH2* codon 172 are private to AML. The functional differences in the mutant proteins remain to be fully elucidated. The aggregate frequency of these two mutations in AML is relatively high, with approximately 15% to 20% of all patients with AML and 25% to 30% of patients with CN-AML harboring either *IDH1* or *IDH2* mutations.[11,12] Of note, IDH proteins bring a new class of mutated proteins in leukemogenesis, that is, metabolic enzymes. Both IDH1 and IDH2 mutants cause loss of the physiologic enzyme function and create a novel ability of the enzymes to convert α-ketoglutarate into 2-hydroxyglutarate, a putative oncogenic metabolite.[28] Initial studies from larger and homogeneous cohorts of patients indicate that *IDH1* and possibly also *IDH2* R140 mutations are

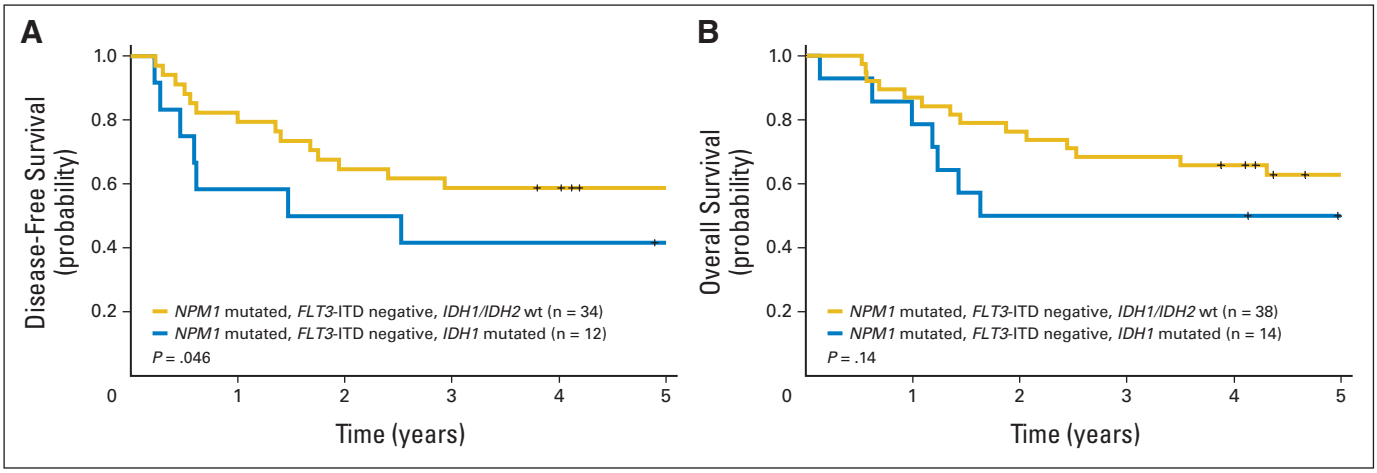

Fig 2. Clinical outcome in cytogenetically normal acute myeloid leukemia (CN-AML) according to *IDH1* and *IDH2* mutational status. (A) Outcome of the CN-AML subset with *NPM1* mutation and without *FLT3* internal tandem duplication (ITD) according to *IDH1* mutational status; (B) outcome of patients with CN-AML according to *IDH2* R172 mutational status. wt, wild type. Adapted with permission from Marcucci et al.[11]

significantly associated with *NPM1* mutations and predict worse outcome for patients with mutated *NPM1* without *FLT3*-ITD.[11-14] Interestingly, the distinct R172 *IDH2* mutation is rarely associated (Fig 2) with any of the other known prognostic mutations and seems to confer lower probability of achieving CR and possibly also inferior outcome.[11-13]

WT1 *Mutations*

WT1 mutations are found in 10% to 13% of CN-AML.[29-31] In some studies, *WT1* mutations have been associated with inferior outcome,[29,30] whereas in the largest study reported by the German-Austrian AML Study Group,[31] *WT1* mutations did not affect outcome. Differences in postremission therapy may account for these discrepant results. In one study, *WT1* single nucleotide polymorphism rs16754 located in the proximity of the *WT1* mutational hotspot was found to be associated with inferior outcome in CN-AML.[32]

RUNX1 *Mutations*

RUNX1 is deregulated in AML by chromosomal translocations and by mutations clustering in the Runt domain of the gene.[2] *RUNX1* mutations have been associated with undifferentiated (M0) morphology and with specific chromosome aberrations, such as trisomy 21 and trisomy 13. In a study of 470 Chinese adult patients with AML, *RUNX1* mutations were found in 13.2% of cases.[33] Mutations were associated with lower CR rate and with inferior survival.

MLL *Mutations*

Partial tandem duplications (PTD) of *MLL* are found in 5% to 11% of patients with CN-AML and frequently in those with AML with trisomy 11.[2,3] *MLL*-PTD have been shown to contribute to leukemogenesis through DNA hypermethylation and epigenetic silencing of tumor suppressor genes, pointing to a potential role of DNA methyltransferase and/or histone deacetylase inhibitors in the treatment of this AML subset.[3] *MLL*-PTD have been associated with inferior CR duration and relapse-free survival, although more recent studies show no prognostic impact in patients with CN-AML intensively treated with autologous HSCT or four cycles of consolidations.[2,3]

Other Gene Mutations

Selected biologic and clinical features of other mutations (eg, *NRAS*,[34] *TP53*,[35] *TET2*,[36,37] and *ASXL1*,[38]) are summarized in Table 1. These mutations either occur at very low frequency, do not seem to significantly contribute to risk stratification, or have so far been less well studied and their prognostic significance remains to be fully defined.[39,40] Nonetheless, it will be necessary to evaluate these biomarkers in future clinical trials to study potential differential treatment effects of new antileukemic agents in subsets of AML defined by the various gene mutations.

EXPRESSION OF SINGLE GENES WITH PROGNOSTIC RELEVANCE

In addition to structural genetic aberrations, changes in expression of specific genes seem to impact prognosis of molecular subsets of patients with AML. The *BAALC* (brain and acute leukemia, cytoplasmic) gene, localized on chromosome band 8q22.3, was first identified to have higher expression in AML with trisomy 8; however, a wide range of expression was described in CN-AML.[3] Baldus et al[41] initially demonstrated that higher *BAALC* expression levels predicted lower CR rates as well as inferior disease-free and overall survival (OS), which was confirmed by subsequent studies.[42,43]

The *MN1* (meningioma [disrupted in balanced translocation] 1) gene was first identified as a fusion partner of the *TEL* gene in reciprocal translocations in AML. Recent studies have shown that *MN1* overexpression is associated with poor response to induction chemotherapy and higher relapse rate and worse OS in AML.[44,45] Interestingly, in one study, low *MN1* expression was correlated with better response to ATRA in elderly patients with non–acute promyelocytic leukemia (APL) AML, thereby suggesting that *MN1* expression is not only a prognostic but also a predictive marker for response to treatment.[44] In patients with CN-AML younger than 60 years, higher *MN1* expression was significantly correlated with *NPM1* wild-type status and increased *BAALC* expression.[45]

ERG (v-ets erythroblastosis virus E26 oncogene homolog, avian) mapping to band 21q22 is involved in various chromosomal rearrangements. With gene-expression analysis based on oligonucleotide arrays, amplification of the *ERG* locus with consecutive gene overexpression was observed in AML with complex aberrant karyotypes.[3] Subsequently, the adverse prognostic significance of

high blood *ERG* levels was established in CN-AML.[3,46] Interestingly, high *ERG* expression levels mostly impacted outcome of low molecular risk CN-AML (mutated *NPM1* without *FLT3*-ITD).[46] In a subsequent GEP study investigating *ERG*, *MN1*, and *BAALC* transcript levels, *ERG* expression was the strongest negative prognostic factor and provided prognostic information in addition to established parameters (eg, *FLT3*-ITD).[40]

Deregulated expression of *EVI1* (ecotropic viral integration site 1) is found in virtually all AML, with inv(3)(q21q26.2/t(3,3)(q21; q26.2) leading to rearrangement of the *EVI1* and *RPN1* genes. *EVI1* overexpression is not restricted to these cases but was found as well in approximately 8% of unselected AML and has been shown to predict poor outcome.[47] A subsequent validation study confirmed that high *EVI1* expression predicts poor outcome, particularly in the cytogenetic intermediate-risk group. Patients with high *EVI1* expression who received allogeneic HSCT in first CR had significantly better 5-year relapse-free survival and OS.[48]

GENOME-WIDE GEP

A decade ago, Golub et al[49] demonstrated that acute leukemia subtypes can be classified on the basis of distinctive signatures identified by genome-wide GEP. Since then, multiple studies confirmed that GEP has the potential to predict specific cytogenetic or molecular alterations (Table 2) in AML and to diagnose specific subtypes by unique patterns of expressed genes ("class prediction").[54,55] Beyond that, this technology is able to add new prognostic information to established cytogenetic or single gene molecular prognosticators ("class discovery"; Table 3).[56,57] The robustness of gene-expression signatures derived from patients with AML at diagnosis is supported by reasonably comparable results attained across different laboratories, the use of different microarray profiling platforms, and validation analyses using different techniques such as quantitative reverse-transcriptase polymerase chain reaction.[59-61] However, at this time, GEP remains an investigational tool for research studies in AML that so far has not found entrance in clinical practice and decision making.

Prediction of Cytogenetic Alterations

AML with t(15;17)/*PML-RARA* and CBF-AML with t(8;21)/*RUNX1-RUNX1T1* and inv(16)/*CBFB-MYH11* were clearly discriminated from other cytogenetic subgroups and were predictable with gene-expression analysis by independent research groups.[27,62] Discrimination accuracies up to 100% were achieved, which emphasizes the biologic uniqueness of the respective subtypes in concordance with the distinct morphologic and genetic features.[62] Rearrangements of the *MLL* gene on 11q23 occur both in AML and acute lymphoblastic leukemia (ALL) and are generally correlated with an adverse prognosis. Kohlmann et al[63] performed unsupervised and supervised data analysis algorithms in ALL and AML cases with 11q23/*MLL* rearrangements and demonstrated the segregation of cases according to myeloid or lymphoid lineage.

Complex karyotype cases were clearly separated from other cytogenetic AML subtypes by distinct gene-expression signatures characterized by upregulation of genes with a role in DNA repair such as *RAD21*.[64,65]

Prediction of Molecular Alterations by Specific Patterns of Genes Expressed

NPM1-mutated AML was found to be associated with highly specific underlying gene-expression signatures characterized by activation of distinct *HOX* cluster genes (a homeodomain-containing family of transcription factors) and by involvement of genes with a function in signaling and apoptosis (Table 2).[50] The signatures for *NPM1* mutations allowed the prediction of this molecular alteration with the highest accuracy and thus clearly defined the biologic singularity of this AML subtype.[51]

Similarly, *CEBPA* mutations have been associated with distinct GEPs. In a recent study by Wouters et al,[21] a 19-probe set signature predictive of *CEBPA* mutations was derived. This classifier showed a high specificity (99%) but a limited sensitivity (67%) in cross-validation. Misclassification resulted almost entirely from *CEBPA* single-mutated cases, whereas *CEBPA* double-mutated cases were predictable with the highest accuracy. Furthermore, gene expression was variable depending on the occurrence of a double- or single-mutation status. These data reinforce the hypothesis that double-mutated *CEBPA* likely defines a distinct biologic and clinical AML entity. Another study on CN-AML compared gene-expression signatures of *CEBPA*-mutated and unmutated cases and reported more than 93% prediction accuracy for predicting *CEBPA* status.[51] Frequently selected genes for classification included *HOXA* and *HOXB* cluster genes, which were downregulated in patients with *CEBPA* mutatation. *CEBPA*-mutated AML were further characterized by distinct gene-expression signatures comprising upregulation of erythroid-specific genes, including *GATA1* and *EPOR*.[52]

Bullinger et al[53] revealed a novel gene signature that showed overlaps to *FLT3*-ITD positive AML and strong correlations to FLT3 pathway activation. When compared with so-called classical *FLT3*-ITD, this *FLT3*-ITD derived signature revealed an even stronger impact on clinical outcomes.[53]

Beyond that, gene-expression signatures are linked to expression levels of distinct genes that are prognostically relevant in CN-AML. Langer et al[42] identified a specific gene-expression signature in association with high *BAALC* expression that included overexpression of genes involved in drug resistance (*MDR1*) and stem cell markers (eg, *CD133*, *CD34*, *KIT*). The same group derived a gene profile associated with *MN1* high expression levels, which was positively correlated with expression of *BAALC*, *CD200* (associated with inferior prognosis in AML), and *ABCB1* (involved in chemoresistance).[45]

Identification of Novel Prognostic Classifiers

Multiple study groups have focused on the identification of prognostic gene-expression signatures, especially in CN-AML (Table 3).[26,53,56,58] Bullinger et al[26] initially subdivided CN-AML cases into two clusters corresponding to different prognostic profiles. One cluster was characterized by overexpression of several transcriptional regulators such as *GATA2*, and the second one was characterized by involvement of genes playing a role for leukocyte differentiation and immune response. Radmacher et al[56] validated the prognostic power of this gene signature in an independent cohort, thereby underlining the reproducibility and utility of GEP classifiers across distinct study groups and laboratories. It should be underscored that most of the reported signatures were derived using unselected bone marrow or blood mononuclear cell samples

Table 2. Specific Patterns of Gene-Expression Signatures Associated With Recurrent Mutated Genes in AML

Gene/Mutation of Interest	No. of Patients	Microarray Platform	Results	Impact on Prognosis	Reference
NPM1	148 de novo CN-AML, ≥ 60 years; CALGB	U133 2.0 plus	*NPM1* mutation associated with specific gene-expression and miRNA signatures, comparable in patients > and < 60 years of age, characterized by upregulation of *HOX* genes and downregulation of *MN1*, *BAALC*, and *ERG*. *NPM1* mutation status was predicted in patients ≥ 60 years of age with 89.4% accuracy, 91.9% sensitivity, and 86.2% specificity.	*NPM1* mutation constitutes a marker defining a biologically homogeneous entity in CN-AML; might be treated with specific and/or targeted therapies across age groups.	17
CEBPA, comparison of double- *v* single-mutated cases	Analysis of a cohort of 41 *CEBPA* mutation cases of 598 AML	U133 plus 2.0	28 *CEBPA* double- and 13 *CEBPA* single-mutated cases. *CEBPA* double-mutated cases were associated with a unique gene-expression profile (sensitivity 100%, specificity 98%) as well as favorable OS and EFS. In contrast, *CEBPA* single-mutated AML did not express a discriminating signature and could not be distinguished from wild-type cases regarding clinical outcome.	*CEBPA* double-mutated cases have specific gene-expression patterns and distinct clinical outcomes. *CEBPA*-mutated AML is outlined by significant heterogeneity with prognostic significance (double- *v* single-mutated status).	21
CEBPA	285 AML	U133 A	*CEBPA* had specific gene-expression pattern, predictable with 98% accuracy in a validation set.	No data available.	27
FLT3-ITD			For *FLT3*-ITD no signature possible.		
RAS (N-RAS; K-RAS)			For *RAS* no signature possible.		
NPM1	275 AML	U133 A	Mutations in *NPM1* had 100% positive predictive value and 80.3% negative predictive value by gene-expression analysis. Presence of the mutation was associated with revealed a strong *HOX* and *TALE* gene–specific signature.	Confirmation of favorable prognosis of the *NPM1*mut/*FLT3*-ITD negative subgroup.	50
NPM1	251 CN-AML	U133 2.0 plus	Prediction of underlying mutation status with high accuracy for *NPM1* (95.6%) and *CEBPA* (93.6%) mutated cases.	No data available.	51
CEBPA			For *NPM1*-mutated CN-AML, a consensus 301-probe sets signature was observed, including expression of members of the *HOXA* and *HOXB* gene families.		
CEBPA	175 CN-AML	U133 2.0 plus	*CEBPA*-mutated patients had unique GEP and miRNA expression profiles; specific genes (eg, *GATA1*) were upregulated, associated with erythroid differentiation, and others downregulated (eg, homeobox genes).	The favorable prognostic impact of *CEBPA* mutation (as determined by PCR analysis) was confirmed. No new data based on GEP only.	52
FLT3-ITD	Training set, 65 CN-AML; test set, 72 CN-AML	cDNA Stanford	An "*FLT3* signature" composed by 20 genes predicted *FLT3*-ITD status with 73% sensitivity and 85% specificity. The new *FLT3* signature was a significant predictor of relapse ($P = .003$) and unfavorable DFS ($P < .001$) and OS ($P = .001$) and outperformed the *FLT3*-ITD status.	New *FLT3*-ITD signature could better define clinical outcome than *FLT3*-ITD status as measured by standard PCR and may better define *FLT3* pathway activation.	53

Abbreviations: AML, acute myeloid leukemia; CN, cytogenetically normal; CALGB, Cancer and Leukemia Group B; miRNA, microRNA; OS, overall survival; EFS, event-free survival; ITD, internal tandem duplication; GEP, gene-expression profile; PCR, polymerase chain reaction; DFS, disease-free survival.

comprising subpopulations of nonleukemic cells. This approach is of a relatively simple and immediate application and has provided strong and reproducible signatures. However, to improve accuracy of diagnosis and molecular risk assessment, it is likely that future studies will capitalize on improving techniques to sort for minute populations of malignant blasts and/or leukemia initiating cells and will report only gene-expression signatures that are specific of these malignant cell compartments.[66]

DEREGULATED MICRORNA EXPRESSION IN AML

MicroRNAs are naturally occurring noncoding RNAs that are cleaved from hairpin precursors and hybridize to imprecisely complementary mRNA of protein-coding genes, thereby leading to downregulation of the encoded proteins by RNA degradation or translation inhibition.[67] Deregulation of microRNAs and in turn of their target genes have

Table 3. Gene-Expression Profiles Associated With Prognosis in AML

Genes of Interest As Revealed by Gene-Expression Analysis	No. of Patients	Microarray Platform	Results	Impact on Prognosis	Reference
Unsupervised analysis identified clinical outcome predictor based on a 133-gene probe set; validation of the novel gene classifier in training and test group	116 AML (including 45 CN-AML)	cDNA Stanford platform	Identification of new subgroups in AML based on GEP, including two prognostically different groups in CN-AML	Novel gene expression–based prognostic predictor with a strong influence in CN-AML; prognostic impact as well in multivariate analysis, including parameters such as s-AML, cytogenetics, and *FLT3* mutation status	26
Unsupervised approach identified 16 subgroups based on molecular signatures. Clustering was driven by chromosomal lesions (eg, reciprocal rearrangements), molecular mutations (eg, *CEBPA*), or aberrant gene-expression (eg, *EVI1*)	285 AML	U133A	Clusters driven by cytogenetics and molecular genetics including *EVI1* expression; identification of novel clusters in normal karyotype; identification of a new cluster with poor outcome	Diagnosis of AML and of several subtypes; prediction of prognosis based on GEP; definition of new clusters with a prognostic impact	27
Supervised clustering, using genes depicted by Bullinger et al[25]	64 CN-AML < 60 years; CALGB	U133 plus 2.0	Confirmation of the discriminative gene signature from Bullinger et al[25] with respect to OS ($P = .001$) and DFS ($P = .001$); correlation of the respective gene signature to the *FLT3*-ITD status, but moderate differences in survival also in *FLT3* wild-type patients	Confirmation of the Bullinger signature by an independent study group; should be further explored for improved prognostic predictions in CN-AML	56
Supervised approach, followed by validation group	Cohort of 525 adult AML patients (one third youngest, median 31 years, n = 175; compared with one third oldest, median 59 years, n = 175)	U133A	Identification of 41 probe sets regulating aging, including downregulation of tumor-suppressor gene *CDKN2A* ($p16^{INK4A}$); confirmation of the prognostic impact only in the intermediate- and adverse-risk group, but not in favorable cytogenetic risk group and in *NPM1*mut/*FLT3*-ITD negative group	Older AML patients have different GEP; downregulation of *CDKN2A* ($p16^{INK4A}$) is an independent prognostic parameter in AML besides cytogenetic/molecular genetic risk groups	57
Identification of a new prognostically relevant gene signature in CN-AML by unsupervised principal component analysis, followed by independent test set and validation set	163 CN-AML plus independent cohort of 79 CN-AML (both AMLCG), and validation in 64 CN-AML (CALGB)	U133 A+B and U113 plus 2.0	A new score based on 86-probe sets was predictive for OS, EFS, and RFS in the test cohort. Confirmation of prognostic significance in multivariate analysis adjusted for age, *FLT3*-ITD, and *NPM1* mutation status	Definition of a new 86-probe-set gene-expression signature predictive for prognosis in CN-AML	58

Abbreviations: AML, acute myeloid leukemia; CN, cytogenetically normal; GEP, gene-expression profile; s-AML, secondary AML; CALGB, Cancer and Leukemia group B; OS, overall survival; DFS, disease-free survival; ITD, internal tandem duplication; EFS, event-free survival; AMLCG, German-Austrian AML Study Group.

been found to contribute to malignant transformation in several human solid tumors and hematologic malignancies by interfering with critical steps of cell development, differentiation, proliferation, and apoptosis processes.[68] Aberrant expression of multiple microRNAs have recently been reported in AML.[4]

Correlations With Morphologic, Cytogenetic, and Molecular Features

Similar to the initial studies showing the GEP capability of distinguishing different types of acute leukemia, Mi et al[69] showed that microRNA-expression profiling can separate patients with AML from those with ALL on the basis of a signature that included 21 upregulated and six downregulated microRNAs. Of these microRNAs, four—*let-7b*, *miR-128a*, *miR-128b*, and *miR-223*—were the most informative in separating patients with AML from those with ALL.

Furthermore, microRNA-expression profiling has been shown to be able to distinguish distinct cytogenetic subtypes of AML. Although complete concordance could not be demonstrated among the microRNA signatures reported by different studies of favorable cytogenetic risk subsets, upregulation of microRNAs transcribed from genes localized at chromosome band 14q32 in APL with t(15;17) and the downregulation of *miR-133a* in patients with t(8;21) were shared by at least two studies.[69-73] Distinct patterns of microRNA expression have been correlated with trisomy 8, balanced chromosomal 11q23 rearrangements involving the *MLL* gene, and CN-AML.[70,73] In AML with aberrations of 11q23/*MLL* studied by Li et al,[70] microRNAs from a unique polycistronic microRNA cluster—*miR-17-92*—was found overexpressed, as well as *miR-196b*, located between the homeobox (*HOX*) *A9* and *HOXA10* genes at 7p15.

MicroRNA-expression signatures have also been correlated with recurrent molecular aberrations in AML. *NPM1* mutations associate with upregulation of *miR-10a*, *miR-10b*, and *miR-196a*, all of which reside in the genomic cluster of *HOX* genes that is found to be consistently overexpressed in this molecular subset.[17,74] *FLT3*-ITD has been reported to be associated with *miR-155* upregulation. Recent data from animal models showing that nuclear factor kappa B activation drives *miR-155* expression, thereby leading to granulocyte/monocyte expansion perhaps by interfering with the phosphatase activity SH2 domain–containing inositol phosphatase 1 (SHP1).[75] *CEBPA* mutations have been associated with an upregulation of members of the *miR-181* family in CN-AML.[52] Notably, *CEBPA* mutation-related GEP comprised several upregulated genes involved in erythroid differentiation,[65] in agreement with the results by Choong et al[76] reporting an increase of *miR-181a* and *miR-181b* levels during erythroid differentiation. Consequently, it is reasonable to hypothesize that high expression levels of the members of the *miR-181* family contribute to the partial erythroid differentiation of leukemic blasts harboring *CEBPA* mutations.[52]

Lastly, *miR-181* expression was correlated with French-American-British (FAB) morphologic groups in CN-AML, with their higher expression seen in FAB M1 and M2 in comparison with FAB M4 and M5. Also in CN-AML, *miR-10a*, *miR-10b*, and *miR-196a-1* were correlated with expression of *HOX* genes.[77] This is consistent with a high incidence of *NPM1* mutations in CN-AML and the reported *NPM1* mutation-associated gene-expression signature known to be related to *HOX* gene overexpression.[3,4]

Correlations With Clinical Outcome

Recent studies have also shown that changes in microRNA expression can affect clinical outcome in AML. Low expression of *miR-let7b* and *miR-9* was found in patients classified in the favorable-risk group, whereas higher expression of these microRNAs was detected in samples from patients with adverse- or intermediate-risk cytogenetic findings.[71] Garzon et al[73] reported that, across all cytogenetic subgroups, overexpressed *miR-20a, miR-25*, *miR-191*, *miR-199a*, and *miR-199b* adversely affected OS. A more recent study identified a microRNA-expression signature with prognostic significance in patients with CN-AML belonging to the molecular high-risk group, defined by the presence of *FLT3*-ITD, wild-type *NPM1* alleles, or both.[78] The signature comprised 12 microRNA probes and was associated with event-free survival. Five probes in the signature represented *miR-181a* and *miR-181b*; their increased expression was associated with a decreased risk of an event (failure to achieve CR, relapse, or death). Moreover, the genome-wide microRNA-expression profile was integrated with gene-expression signature with the goal of identifying genes regulated by microRNAs whose altered expression contributed to leukemogenesis in molecular high-risk CN-AML.[78] microRNA-derived gene-expression signature included, among others, genes encoding proteins involved in innate immunity that were previously shown to support proliferation and survival of malignant myeloid blasts.[78] These data support a functional relationship between microRNA and gene expression and suggest that down-regulation of the members of *miR-181* family may contribute to the aggressive leukemia phenotype. On the other hand, high levels of *miR-181* expression may reduce the aggressiveness of the disease. The latter was confirmed by a subsequent study showing that upregulated *miR-181a* predicted favorable outcome in CN-AML.[79]

In conclusion, it is clear that any complete prognostic assessment has to consider the aberrant genome and transcriptome changes occurring in AML. These results need also to be integrated with recurrent aberrant epigenetic changes (ie, DNA hypermethylation and histone posttranscriptional modifications) that deregulate the expression of genes involved in normal hematopoiesis and define biologic and prognostic epigenetic subsets of AML. Hypermethylated, silenced genes seem to play a relevant role in myeloid malignancies as both prognostic markers and therapeutic targets. Recently, Figueroa et al[80] examined the methylation status of a relatively large cohort of patients with AML and reported unique methylation profiles for distinct cytogenetic and molecular AML subsets. Furthermore, a 15-gene methylation classifier was found to be predictive for survival, even after controlling for other molecular prognosticators (*CEBPA*, *NPM1*, *FLT3* mutations).[80] These data, therefore, support the important prognostic role of genome-wide epigenetic profiling and underscore the need for standardized analyses that integrate both genetic and epigenetic information to accurately predict for clinical outcome. To pursue integrated information, however, it is imperative that multicenter studies will collaborate in developing and validating novel bioinformatic algorithms that will be readily applicable to routine diagnostics, prognostication, and risk-stratification of patients with AML. Advanced next-generation studies of nucleic acid sequencing technologies are being currently implemented to improve the amount of informative data that can be collected and analyzed in real time, thereby providing a powerful tool for the clinicians for stratifying individual patients to risk-adapted treatment. Hopefully, these approaches will lead to more effective strategies that will be part of novel individualized therapies for patients with AML. The proof of concept for these approaches is provided by implementation of rapid molecular screening tests such as those that have allowed identification and accrual of *FLT3*-mutated patients with AML to multi-institutional clinical trials testing the therapeutic value of tyrosine kinase inhibitors within less than 48 hours from their initial presentation (eg, CALGB 10603).

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