24. Campbell PJ, Baxter EJ, Beer PA, Scott LM, Bench AJ, Huntly BJ, et al. Mutation of JAK2 in the myeloproliferative disorders: timing, clonality studies, cytogenetic associations, and role in leukemic transformation. Blood 2006;108:3548-55.
25. Xing S, Ho WT, Zhao W, Ma J, Wang S, Xu X, et al. Transgenic expression of JAK2V617F causes myeloproliferative disorders in mice. Blood 2008. In press.
26. Wernig G, Mercher T, Okabe R, Levine RL, Lee BH, Gilliland DG. Expression of Jak2V617F causes a polycythemia vera-like disease with associated myelofibrosis in a murine bone marrow transplant model. Blood 2006;107: 4274-81.
27. Pardanani A, Fridley BL, Lasho TL, Gilliland DG, Tefferi A. Host genetic variation contributes to phenotypic diversity in myeloproliferative disorders. Blood 2008;111:2785-9.
28. Spivak JL. Polycythemia vera: myths, mechanisms, and management. Blood 2002;100:4272-90.
29. Campbell PJ, Scott LM, Buck G, Wheatley K, East CL, Marsden JT, et al. Definition of subtypes of essential thrombocythaemia and relation to polycythaemia vera based on JAK2 V617F mutation status: a prospective study. Lancet 2005;366:1945-53.
30. Borze I, Mustiojki S, Juvonen E, Knuutila S. Oligoarray CGH in polycythemia vera and essential thrombocythemia. Haematologica 2008.
31. Bruchova H, Merkerova M, Prchal JT. Aberrant expression of microRNAs in polycythemia vera. Haematologica 2008.
32. Guglielmelli P, Tozzi L, Pancrazzi A, Bogani C, Antonioli E, Ponziani V, et al. MicroRNA expression profile in granulocytes from primary myelofibrosis patients. Exp Hematol 2007;35:1708-18.
33. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, et al. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A 2002;99:15524-9.

Molecular characterization of acute myeloid leukemia

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Acute myeloid leukemia (AML) is a genetically heterogeneous clonal disorder characterized by the accumulation of acquired somatic genetic alterations in hematopoietic progenitor cells that alter normal mechanisms of self-renewal, proliferation and differentiation.[1] Non-random clonal chromosome aberrations (i.e., balanced translocations, inversions, deletions, monosomies, and trisomies) are detectable in the leukemic blasts of approximately 55% of adults with AML. These chromosome changes have contributed to the classification of the disease and in the past they have been recognized as the most important prognostic factor for achievement of complete remission, risk of relapse, and overall survival.[2,3] In recent years, a number of gene mutations as well as deregulated expression of genes have been identified, illustrating the enormous heterogeneity of cytogenetically defined AML subsets, in particular in the large subset of AML exhibiting a normal karyotype (Table 1).[4,5] The characterization of the gene mutations has provided insights into the mechanisms of leukemogenesis. From a clinical point of view there are two important aspects. First, some of these gene mutations have emerged as important prognostic and predictive markers. Second, novel therapies are now being developed that target these molecular changes. Based on these findings, it is now recommended to include molecular genetic diagnosis of several of these markers in the initial work-up of a patient with newly diagnosed AML. The importance of a comprehensive genetic diagnostic work-up is highlighted in a study by Lo-Coco *et al.* from the Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA) which appears in this issue of the journal.

Multi-step leukemogenesis

There are several lines of evidence showing that different genetic changes cooperate in leukemogenesis.[1,6] First, data from murine models suggest that a single mutation is not sufficient to cause AML. For example, the *RUNX-RUNX1T1* and *CBFB-MYH11* fusion genes, resulting from t(8;21) and inv(16)t(16;16) respectively, impair myeloid differentiation in these models but they do not cause an overt leukemic phenotype. Second, rare germline mutations have been described in *RUNX1* and *CEBPA* that predispose affected individuals to the development of AML. Constitutional heterozygous loss-of-function mutations in the transcription factor RUNX1 have been associated with familial platelet disorder with propensity to AML. In these individuals, overt leukemia likely develops upon the somatic acquisition of further mutations in hematopoietic progenitor cells. Finally, evidence comes from human disease, since in the majority of AML cases more than one genetic change can be detected (Figures 1 and 2).

There are different types of mutations which appear to fall into broadly defined complementation groups and cooperate in leukemogenesis.[6] One group (class I) comprises mutations which activate signal transduction pathways resulting in enhanced proliferation and/or survival of leukemic progenitor cells such as mutations leading to activation of the receptor tyrosine kinase FLT3 or the RAS signaling pathway. The second complementation group (class II) comprises mutations that affect transcription factors or components of the transcriptional co-activation complex, resulting in impaired differentiation and/or aberrant acquisition of self renewal properties by hematopoietic progenitors. Prominent examples are the recurring gene fusions resulting from t(8;21), inv(16)/t(16;16), t(15;17), as well as mutations in *CEBPA*, *MLL*, and possibly also *NPM1*.

Molecular alterations in cytogenetically normal acute myeloid leukemia

In the last decade, somatically acquired mutations have been identified in several genes in cytogenetically normal (CN) AML: 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

Table 1. Biological and clinical features associated with gene mutations in acute myeloid leukemia.

Gene	*Biological / Clinical Features*
NPM1	Encodes a phosphoprotein with pleiotropic functions.
	NPM1 mutations found in 25-35% of all adult AML, in 45-64% of CN-AML, in 35-40% of AML with 9q deletion, and in about 15% of AML with trisomy 8; *NPM1* mutations associated with *FLT3*-ITD (~40%) and *FLT3* TKD mutations.
	Association with female sex, higher BM blast counts and serum LDH levels, myelomonocytic or monocytic morphology, and high CD33 and absent CD34 expression.
	NPM1 mutation in general associated with better response to induction chemotherapy; genotype "mutant *NPM1* without *FLT3*-ITD" associated with favorable RFS and OS; patients with "mutant *NPM1* without *FLT3*-ITD" may not benefit from MRD transplantation in first CR.
CEBPA	Encodes a master regulatory transcription factor in hematopoiesis.
	CEBPA mutations found in 10-18% of CN-AML, and in about 40% of AML with 9q deletion occurring within a non-complex karyotype.
	CEBPA mutations associated with higher CR rate and better RFS and OS.
MLL	Encodes a DNA binding protein which regulates gene expression in hematopoiesis possibly through epigenetic mechanisms.
	MLL-PTD found in 5-11% of CN-AML, and up to 90% of AML with trisomy 11.
	Associated with shorter CR duration and inferior RFS and EFS; autologous transplantation may improve outcome.
RUNX1	Encodes a transcription factor involved in normal hematopoietic differentiation.
	RUNX1 mutations found in about 10% of CN-AML, nearly all AML with trisomy 13, and about half of AML with trisomy 21.
	Data on prognostic significance not yet available.
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 about 20% of all AML, and in 28-34% of CN-AML.
	Association with inferior outcome; level of mutant allele likely of importance; homozygous *FLT3* mutations as a result of mitotic recombination leading to partial UPD.
TKD	*FLT3* TKD point mutations found in 5-10% of all AML, in 11-14% of CN-AML, and in 14-24% of AML with inv(16).
	Prognostic significance controversial.
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.
	KIT mutations found in about 30% of CBF AML, and in rare cases of other AML types.
	KIT mutations, in particular in exon 17, associated with inferior outcome in many but not all studies.
RAS	Encodes membrane-associated proteins regulating mechanism of proliferation, differentiation and apoptosis.
	NRAS mutations found in 9-14% of CN-AML, in up to 40% of CBF AML [in particular inv(16)], and in 25% of AML with inv(3); *KRAS* mutations found in 5-17% of CBF AML.
	So far, no prognostic significance found; mutant *NRAS* may confer sensitivity to cytarabine.
WT1	Encodes a transcription factor implicated in regulation of apoptosis, proliferation, and differentiation of hematopoietic progenitor cells.
	Mutations found in about 10% of CN-AML.
	Initial studies suggest association with induction failure, in particular in patients with *WT1* mutation and concurrent FLT3-ITD; prognostic impact currently under investigation.

CN: cytogenetically normal; ITD: internal tandem duplication; TKD: tyrosine kinase domain; BM: bone marrow; LDH: lactate dehydrogenase; RFS: relapse-free survival; OS: overall survival; MRD: matched related donor; PTD: partial tandem duplication; CR: complete remission; EFS: event-free survival (EFS); JM: juxtamembrane domain; UPD: uniparental disomy; CBF: core-binding-factor.

oncogene homolog (*NRAS*) gene, the Wilms tumor 1 (*WT1*) gene, and the runt-related transcription factor 1 (*RUNX1*) gene.[4,5] These gene mutations are most prevalent in CN-AML; however, they also occur in AML with abnormal karyotypes (Figure 1).

Mutations in NPM1

In 2005, Falini and colleagues made the important discovery that in a substantial proportion of AML cases, there is abnormal cytoplasmic localization of the NPM1 protein.[7] This mislocalization is caused by mutations in exon 12 of the gene which result in loss of tryptophan residues normally required for NPM1 binding to the nucleoli and in the generation of an additional nuclear export signal motif at the C-terminus. *NPM1* mutations are the most frequent genetic alteration in adult AML. They are found in 45-64% of CN-AML. *NPM1* mutations occur less frequently in pediatric AML (2-8%).[4,5,8]

NPM1 mutations have been associated with several presenting clinical and biological features (Table 1). In univariate analysis, data on the prognostic impact of *NPM1* mutations have been somewhat controversial with some studies showing a significant effect on complete remission (CR) rate (as in the study by Lo-Coco *et al.* in this issue of the journal), relapse-free survival (RFS), and event-free survival (EFS), while other studies did not reveal significant differences in these parameters. *NPM1* mutations cooperate with other gene mutations in leukemogenesis (Figure 2). Approximately 40% of patients with *NPM1* mutations also carry *FLT3* internal tandem duplications (*FLT3*-ITD), and several studies unanimously showed that the genotype *mutant NPM1 without FLT3-ITD* represents a favorable prognostic marker.[4,5,8] Figure 3 shows just such favorable results from the largest series of patients published by the German-Austrian AML Study Group (AMLSG).[8] The data from this study also suggested that patients with the genotype *mutant NPM1 without FLT3-ITD* may be exempted from allogeneic hematopoietic stem cell transplantation during the first complete remission, since the outcome following allogeneic transplantation was not superior to that after conventional consolidation chemotherapy.

Mutations in FLT3

In AML, somatic mutations resulting in constitutive activation of FLT3 have been identified in two functional domains of the receptor, the juxtamembrane (JM) domain and the tyrosine kinase domain (TKD). Physiologically, the JM domain is essential for kinase autoinhibition. Disruption of this domain by ITDs of various sizes and insertion sites is detectable in 28-34% of CN-AML, whereas JM point mutations are rare.[4,8] Mutations in the TKD mostly affect the activation loop in the carboxy-terminal lobe. These point mutations, small insertions, or deletions mainly involve codons 835 and 836 in 11%-14% of CN-AML.[4,8] Point mutations or insertions located at other codons in the TKD are rare. Based on *in vitro* and *in vivo* studies there are similarities but also important differences in signal transduction properties between *FLT3*-ITDs and *FLT3* TKD mutations which may explain differences in clinical phenotypes.

From a clinical perspective, *FLT3* mutations are of major relevance because of their prognostic impact and because constitutively active FLT3 is an attractive target for molecular therapy. Numerous studies have shown that CN-AMLs harboring *FLT3*-ITD have a significantly inferior outcome compared to patients without *FLT3*-ITD. More recent data provide evidence that outcome is also related to the level of the mutant allele, and not just its mere presence. First evidence came from a study by Whitman *et al.* (2001) showing that only patients with *FLT3*-ITD and loss of wild-type *FLT3*, but not patients with heterozygous *FLT3*-ITD had inferior outcome.[9] Thiede *et al.* (2002) used DNA fragment analysis to quantify the relative level of the mutant allele and were able to define a cut-off value that distinguished between prognostic subgroups.[10] The prognostic relevance of *FLT3* TKD mutations remains controversial.[4,5] While a previous meta-analysis on more than 1,000 unselected AML cases (including 84 cases with FLT3 TKD mutations) showed a negative prognostic impact, another study even suggested a positive impact of TKD mutations on survival.

At present, several FLT3 inhibitors, including midostaurin (PKC412), lestaurtinib (CEP-701), sorafenib (BAY 43-9006), and sunitinib (SU11248), are under clinical investigation. Initial studies have shown that, when used as single agents, these compounds have limited activity in AML with mutant FLT3. However, the data look more promising when these inhibitors are combined with conventional chemotherapy. Results from phase III clinical trials, which are about to start, will answer the question whether inhibition of FLT3 will be a successful targeted approach for these patients.

Mutations in CEBPA

Two major types of heterozygous *CEBPA* mutations have been identified in sporadic and familial AML. Nonsense mutations affecting the N-terminal region of the molecule prevent expression of the full-length CEBPA protein, thereby up-regulating the formation of a truncated isoform with dominant-negative properties; and in-frame mutations in the C-terminal basic region-leucine zipper domain resulting in CEBPA proteins with decreased DNA-binding or dimerization activity. N- and C-terminal mutations often occur simultaneously. Interestingly, by using gene expression profiling, a subgroup of AML could be defined that exhibits a transcriptional signature which resembles that of AMLs with *CEBPA* mutations, while lacking such mutations.[11] In most, but not all, of these AMLs, the *CEBPA* gene was silenced by promoter hypermethylation. In addition, this subset of AML showed a strong association with putatively activating mutations in the *NOTCH1* gene.

Several studies have shown that CN-AMLs with *CEBPA* mutations are associated with favorable outcome (Figure 3).[4,8] The survival data reported are very similar to those of CN-AML patients with mutant *NPM1* without *FLT3*-ITD. It remains an open question whether the presence of a *FLT3*-ITD impacts on prognosis in patients with mutant *CEBPA*.

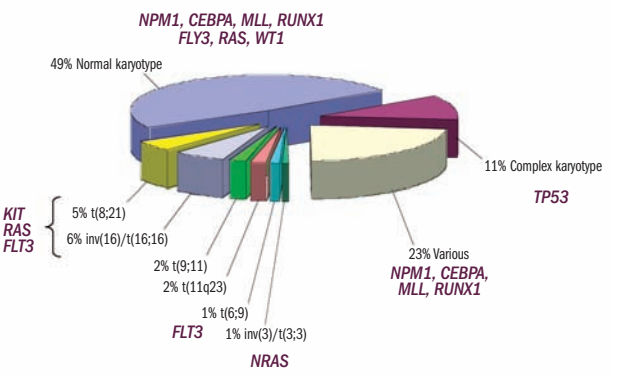

Figure 1. **Major cytogenetic subgroups of acute myeloid leukemia (AML) (excluding acute promyelocytic leukemia) and associated gene mutations. In the subgroup *various*, *NPM1* mutations are frequently found in AML with 9q deletion and trisomy 8, *CEBPA* mutations in AML with 9q deletion, *MLL* mutations in AML with trisomy 11, and *RUNX1* mutations in AML with trisomy 13 and trisomy 21. Frequencies of the cytogenetic subgroups are derived from 2,654 cytogenetically characterized adult (≥18 years) patients with *de novo* or secondary AML entered on five AMLSG treatment trials.**

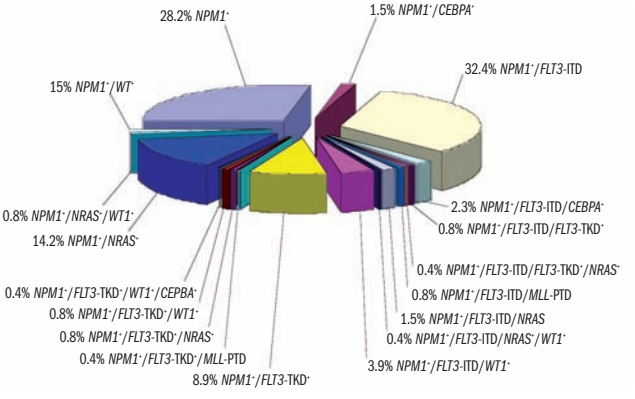

Figure 2. **Distribution of class I gene mutations among 259 cytogenetically normal acute myeloid leukemia (AML) with *NPM1* mutation. In about 28% of cases, *NPM1* mutation is the only detectable genetic change, whereas in the majority of cases additional mutations are found in genes such as *FLT3*, *NRAS* and *WT1*. A small number of AML with *NPM1* mutations have an additional hypothetical class II mutation, e.g., *NPM1* and concurrent *CEBPA* mutation.**

Mutations in MLL

Partial tandem duplications (PTD) of the *MLL* are detectable in 5%-11% of patients with CN-AML.[4,8] At the molecular level, *MLL*-PTDs most commonly involve duplication of a genomic region spanning exons 5 through 11 and insertion of the duplicated segment into intron 4 of the gene. In contrast to MLL chimeric fusion proteins, *MLL*-PTDs retain all functional protein domains. *MLL*-PTDs have been found to cooperate with silencing of the *MLL* wild-type allele, which appears to be mediated through epigenetic mechanisms. Whitman *et al.* (2005) showed that the combination of decitabine, a DNA methyltransferase inhibitor, and depsipeptide, a histone deacetylase inhibitor, can reactivate transcription of the wild-type allele in *MLL*-PTD-positive blasts.[12] The induction of MLL wild-type expression was associated with enhanced cell death of the blasts. These data may provide a rationale for evaluating DNA methyltransferase and/or histone decetylase inhibitors in AML with *MLL*-PTD.

Clinically, *MLL*-PTD has been associated with shorter CR duration and worse RFS and EFS; however, in these studies *MLL*-PTDs had no effect on OS.

Mutations in NRAS

NRAS mutations are found in 9-14% of younger adults with CN-AML.[4,8] Mutations are almost exclusively located at codons 12, 13 and 61, resulting in loss of intrinsic GTPase activity and constitutive activation of the RAS protein. None of the larger studies has found an impact on prognosis, neither in the CN-AML subgroup nor in AML with other intermediate-risk karyotypes. Nevertheless, these mutations may represent a target for molecular therapy.

Mutations in WT1

Mutations in the *WT1* gene in AML were first reported by King-Underwood and Pritchard-Jones in 1998.[13] In a recent study of 70 patients with CN-AML by Summers *et al.* (2007),[14] *WT1* mutations were detected in 10% of cases. Mutations consisted of insertions or deletions that mainly clustered in exons 7 and 9. Preliminary data resulting from these two small studies on heterogeneous patient populations suggest that *WT1* mutations may be associated with induction failure. However, the prognostic impact of *WT1* mutations needs to be evaluated in larger patient cohorts and within the context of other molecular markers.

Mutations in RUNX1 (AML1)

RUNX1 encodes a transcription factor that is involved in regulation of normal hematopoietic differentiation through dimerization with the CBFB transcription factor. Notably, both RUNX1 and CBFB are involved in chromosomal translocations associated with AML. Mutations in *RUNX1* have recently been shown to occur in approximately 10% of CN-AML.[15] At present, no data are available regarding the clinical relevance of *RUNX1* mutations in CN-AML.

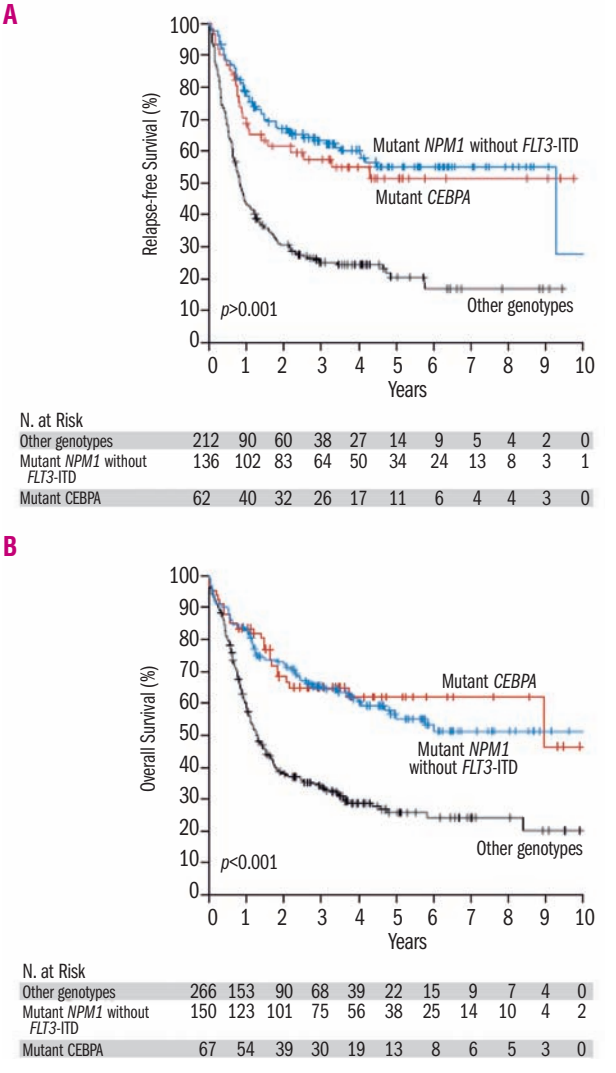

Figure 3. **Kaplan–Meier survival estimates, according to genotype, in younger adult patients with cytogenetically normal AML. Data are shown for relapse-free survival (Panel A) and overall survival (Panel B). *Other genotypes* is defined as the *FLT3*-ITD genotype and the triple-negative genotype consisting of wild-type *NPM1* and *CEBPA* without *FLT3*-ITD. Tick marks represent patients whose data were censored at the last time they were known to be alive and in complete remission (Panel A) or whose data were censored at the last time they were known to be alive (Panel B) (Reprinted by permission of Schlenk RF, Döhner K, *et al.*, N Engl J Med 2008;358:1909-18).**

Molecular alterations in acute myeloid leukemia with balanced translocations/inversions

As has been shown in murine models of leukemogenesis, at least some of the recurrent gene fusions that are generated by balanced translocations and inversions are not sufficient to induce leukemia, suggesting a requirement for secondary genetic lesions. Consistent with these models, additional genetic lesions are found in many human AML with balanced translocations or inversions. For example, these lesions may include secondary chromosome abnormalities such as 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). The mechanisms underlying the formation of these secondary chromosome abnormalities remain elusive. Furthermore, several gene mutations have been identified in cases of AML with balanced translocations or inversions.

Gene mutations in core-binding-factor acute myeloid leukemia

CBF leukemias defined by inv(16)(p13.1q22)/t(16;16)(p13.1;q22) or by t(8;21)(q22;q22) account for about 10-15% of AML. At the molecular level, inv(16)/t(16;16) result in the generation of a CBFB-MYH11 fusion protein, whereas t(8;21) creates a RUNX1-RUNX1T1 fusion product. CBF AML are associated with favorable outcome; nevertheless, there is substantial heterogeneity with approximately 30-40% of younger adults relapsing. Over recent years, mutations have been identified in several genes in CBF AML, i.e. the *KIT* gene, the *NRAS* and *KRAS* genes, and the *FLT3* gene.

KIT exon 8 mutations located in the extracellular portion of the receptor or mutations in the activation loop at codon 816 (exon 17) are detectable in about 30% of CBF AML. A number of studies have evaluated the prognostic significance of *KIT* mutations in CBF AML.[5,16,17] In t(8;21) AML, mutant *KIT*, and in some studies specifically mutations at codon 816, have been associated with inferior outcome.[16] In inv(16)/t(16;16), a study by the Cancer and Leukemia Group B (CALGB) on a larger patient cohort showed that *KIT* mutations are associated with a higher cumulative incidence of relapse; this difference was mainly due to the effect of *KIT* exon 17 mutations.[17] In multivariable analysis, *KIT* mutation was an adverse prognostic factor for OS. These results await independent confirmation in large patient cohorts that have received uniform treatment. The prognostic significance of the other gene mutations is less well defined.

Irrespective of their prognostic significance, mutant *KIT* alleles represent a potential target for molecular therapies. Compounds such as the tyrosine kinase inhibitor midostaurin or the dual SRC/ABL kinase inhibitor dasatinib (BMS-354825) have significant inhibitory efficacy against mutant and wild-type KIT. The efficacy of tyrosine kinase inhibitors combined with conventional chemotherapy needs to be tested in future clinical trials of CBF AML.

Gene mutations in acute myeloid leukemia with t(6;9)(p23;q34)

The translocation t(6;9) is found in about 0.5%-1.0% of adult patients with AML, and the presence of this abnormality has been associated with poor treatment outcome. In a recent international meta-analysis, 55 patients (AML, n=50; MDS, n=5) were studied for activating *FLT3* mutations.[18] *FLT3*-ITDs were found in 42 of the 55 (76.4%) patients. Notably, compared to patients with wild-type *FLT3*, patients with *FLT3*-ITD had a significantly lower probability of achieving a CR (75% *vs.* 36%, p=0.042). In addition, patients with *FLT3*-ITD had significantly inferior DFS and OS. These data provide evidence that the poor prognosis of patients with t(6;9) may not only be due to the *DEK-NUP214* gene fusion resulting from this chromosomal translocation, but also to the presence of activating *FLT3* mutations. These data provide a rationale for the evaluation of FLT3 inhibitors in this subtype of AML.

Molecular alterations in acute myeloid leukemia with inv(3)(q21q26.2)/ t(3;3)(q21;q26.2):

inv(3)/t(3;3) are detected in about 1-2% of all AML and result in fusion of the ecotropic virus integration-1 (*EVI1*) gene or the related *MDS-EVI1* gene at 3q26.2 with the ribophorin 1 gene (*RPN1*) at 3q21. These rearrangements lead to increased *EVI1* gene expression. inv(3)/t(3;3) have been consistently associated with a dismal outcome. Monosomy 7 and 5q deletions are recurrent secondary chromosome aberrations, and *NRAS* gene mutations have been found in about 25% of cases with inv(3)/t(3;3). Notably, deregulated expression of *EVI1* has previously also been identified in a subset of AML without 3q rearrangements. In a recent study of 534 untreated adult patients with de novo AML, deregulated expression of various *EVI1* splice variants and of the related *MDS1/EVI1* (*ME*) gene was found in 7.8% of the cases, and high *EVI1* expression was an independent predictor of poor survival.[19] Some of these cases lacked cytogenetically visible 3q rearrangements, but using fluorescence *in situ* hybridization, additional cryptic 3q26 breakpoints were identified. In addition, there was a correlation between *EVI1/ME* overexpression and the presence of 11q23 chromosome rearrangements, suggesting a role for MLL fusion proteins in the regulation of *EVI1* and *ME* expression. The authors argued that quantitative *EVI1/ME* expression analysis should be added to the molecular diagnostic procedures in AML.

Molecular alterations in acute myeloid leukemia with various cytogenetic abnormalities

Most of the gene mutations described for CN-AML are not restricted to this cytogenetic subset of AML but occur with varying frequencies in AML with abnormal karyotypes.

In an AMLSG-CALGB intergroup study,[20] AMLs with deletion 9q occurring in the context of a non-complex karyotype were shown to be associated with either *CEBPA* or *NPM1* mutations; the prevalence of *CEBPA* and *NPM1* mutations was 43% and 37% respectively. Notably, *CEBPA* and *NPM1* mutations occurred mutually exclusive, a finding that supports the hypothesis that both *CEBPA* and *NPM1* mutations act as class II mutations which cooperate with a class I mutation affecting an as yet unknown gene on 9q. *NPM1* mutations have also been found in up to 20% of cases with trisomy 8. Large patient numbers need to be studied to evaluate the exact prevalence of *NPM1* mutations in this cytogenetic subgroup. Such studies may eventually help clarify the enormous clinical heterogeneity seen among patients whose leukemic cells exhibit trisomy 8 as a single abnormality. Finally, in a recent study, *RUNX1* gene mutations have been shown to be associated with specific cytogenetic abnormalities.[15] Interestingly, in this study 14 out of 14 cases with trisomy 13, 5 out of 9 (56%) cases with trisomy 21, and 10 out of 32 (31%) cases with monosomy 7 exhibited *RUNX1* mutations.

Molecular alterations in acute myeloid leukemia with complex karyotype

AML with complex karyotype is commonly defined by the presence of at least 3 acquired chromosome aberrations in the absence of t(8;21), inv(16)/t(16;16), and t(15;17). Complex karyotype AML constitutes 10-12% of all AML, with its incidence increasing with age. So far, little is known about the molecular pathogenesis of these leukemias.[21] Notably, complex karyotype AML is characterized by the rare occurrence of balanced chromosomal rearrangements, and also by a low frequency of mutations in *NPM1*, *FLT3*, *CEBPA*, *RAS* or *KIT*, which are frequently mutated in other types of AML, suggesting deregulation of other molecular pathways in these cases. In AML with complex karyotype, there is a predominance of chromosomal imbalances, with chromosomal losses occurring more frequently than chromosomal gains.

Over recent years, molecular cytogenetic and array-based techniques have contributed to an improved characterization of these genetic changes.[21,22,23] Deletions have been mapped, in order of decreasing frequency, to 5q, 17p, 7q, 18q, 16q, 17q, 12p, 20q, 18p, and 3p, and genomic gains to 8q, 11q, 21q, 22q, 1p, 9p, and 13q. Efforts to identify the target genes of these recurrent genomic losses have been complicated by the fact that these tumors are mostly characterized by large genomic deletions. The *TP53* gene is the likely target for losses occurring on 17p; in fact, *TP53* gene mutations have been identified in about two-thirds of AML with complex karyotype. Loss of p53 protein function by *TP53* mutation is known to result in genetic instability and *TP53* inactivation by gene loss or gene mutation may therefore represent one molecular pathway leading to complex genetic changes. Another difficulty in the analysis of chromosomal deletions is that of identifying genes that contribute to tumorigenesis by haploinsufficiency, i.e. the inactivation of a single allele. The value of functional genetic techniques in this regard is illustrated by a recent study in which down-regulation of candidate genes by RNAi was used to identify *RPS14* on 5q32 as a causal gene for the 5q$^-$ syndrome, a subtype of the myelodysplastic syndromes.[24] It is conceivable that other genes located within critical regions of recurrent genomic losses may contribute to leukemogenesis through haploinsufficiency.

The detection of recurrent genomic amplifications by modern techniques, such as array-based comparative genomic hybridization, has led to the identification of genes that are potentially involved in the pathogenesis of cytogenetically complex AML as well as other cytogenetic AML subtypes.[21,22] Genes that have been shown to be frequently amplified include members of the *ETS* gene family, specifically *ERG* and *ETS2* on chromosome 21q, and *ETS1* and *FLI1* on chromosome band 11q23.3-q24.1. Other recurrent amplifications (candidate genes within amplicons) have been mapped to 8q24 (*MYC, TRIB1*), 9p24 (*JAK2*), 11q13.5 (*GAB2*), 11q23.3 (*DDX6, MLL*), and 13q12 (*CDX2, FLT3, FOXOA1*). The frequent finding of chromosomal amplification suggests that oncogene activation through genomic gain is more common in this type of AML than previously thought.

Conclusions

Important discoveries have been made over recent years that have contributed to a better understanding of the molecular pathogenesis and to a refinement of the classification of AML. Furthermore, mutations in genes such as *NPM1*, *FLT3* or *CEBPA* have been shown to provide important prognostic information, and it is now recommended to include mutation analysis of these genes in the initial diagnostic work-up of a patient with AML, at least in the context of a clinical trial.

Nevertheless, we have only just begun to unravel the enormous genetic diversity of AML. The constant improvements in genomics technology will lead to the identification of additional genetic changes and novel mechanisms of leukemogenesis. The introduction of genome-wide single nucleotide polymorphism based mapping arrays has led to the identification of a novel mechanism of leukemogenesis involved in AML, partial uniparental disomy, that has been shown to occur in up to 20% of AML cases at the time of diagnosis. In addition, high-throughput DNA sequence analysis in large numbers of primary patient samples will become possible at an affordable cost, which may ultimately result in the development of comprehensive, disease and allele-specific gene mutation profiling strategies. Finally, innovative functional genetic approaches, such as large-scale RNA interference screens, have great potential for the identification of novel cancer genes. It is hoped that the data resulting from these studies will also eventually lead to the development of successful molecular targeted therapies.

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Key words: acute myeloid leukemia somatic mutation, chromosomal abnormality, NPM1, FLT3, CEBPA

References

1. Fröhling S, Scholl C, Gilliland DG, Levine RL. Genetics of myeloid malignancies: pathogenetic and clinical implications. J Clin Oncol 2005;23:6285-95.
2. Mrózek K, Heerema NA, Bloomfield CD. Cytogenetics in acute leukemia. Blood Rev 2004;18:115-36.
3. Estey E, Döhner H. Acute myeloid leukaemia: Lancet 2006; 368:1894-907.
4. Mrózek K, Marcucci G, Paschka P, Whitman SP, Bloomfield CD. Clinical relevance of mutations and gene-expression changes in adult acute myeloid leukemia with normal cytogenetics: are we ready for a prognostically prioritized molecular classification? Blood 2007;109:431-48.
5. Döhner H. Implication of the molecular characterization of acute myeloid leukemia. Hematology Am Soc Hematol

Educ Program 2007;2007:412-9.
6. Kelly LM, Gilliland DG. Genetics of myeloid leukemias. Annu Rev Genomics Hum Genet 2002;3:179-98.
7. Falini B, Mecucci C, Tiacci E, Alcalay M, Rosati R, Pasqualucci L, et al. Gimema Acute Leukemia Working Party. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. N Engl J Med 2005; 352:254-66.
8. Schlenk RF, Döhner K, Krauter J, Fröhling S, Corbacioglu A, Bullinger L, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. N Engl J Med 2008;358:1909-18.
9. Whitman SP, Archer KJ, Feng L, Baldus C, Becknell B, Carlson BD, et al. Absence of the wild-type allele predicts poor prognosis in adult de novo acute myeloid leukemia with normal cytogenetics and the internal tandem duplication of FLT3: a cancer and leukemia group B study. Cancer Res 2001;61:7233-9.
10. Thiede C, Steudel C, Mohr B, Schaich M, Schäkel U, Platzbecker U, et al. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. Blood 2002;99:4326-35.
11. Wouters BJ, Alberich Jordà M, Keeshan K, Louwers I, Erpelinck-Verschueren CAJ, Tielemans D, et al. Distinct gene expression profiles of acute myeloid/T-lymphoid leukemia with silenced CEBPA and mutations in NOTCH1. Blood 2007;110:3706-14.
12. Whitman SP, Liu S, Vukosavljevic T, Rush LJ, Yu L, Liu C, et al. The MLL partial tandem duplication: evidence for recessive gain-of-function in acute myeloid leukemia identifies a novel patient subgroup for molecular-targeted therapy. Blood 2005;106:345-52.
13. King-Underwood L, Renshaw J, Pritchard-Jones K. Mutations in the Wilms' tumor gene WT1 in leukemias. Blood 1996;87:2171-9.
14. Summers K, Stevens J, Kakkas I, Smith M, Smith LL, Macdougall F, et al. Wilms' tumour 1 mutations are associated with FLT3-ITD and failure of standard induction chemotherapy in patients with normal karyotype AML. Leukemia 2007;21:550-1.
15. Schnittger S, Dicker F, Kern W, Haferlach T, Haferlach C. Cooperating molecular mutations in AML/RUNX1 mutated AML differ dependent on the cytogenetic subgroup. Blood 2007;110:114a (abstr).
16. Schnittger S, Kohl TM, Haferlach T, Kern W, Hiddemann W, Spiekermann K, et al. KIT-D816 mutations in AML1-ETO positive AML are associated with impaired event-free and overall survival. Blood 2006;107:1791-9.
17. Paschka P, Marcucci G, Ruppert AS, Mrózek K, Chen H, Kittles RA, et al. Adverse prognostic significance of KIT mutations in adult acute myeloid leukemia with inv(16) and t(8;21): a Cancer and Leukemia Group B Study. J Clin Oncol 2006;24:3904-11.
18. Thiede C, Bloomfield CD, Lo Coco F, Fröhling S, Hiddemann W, Krauter J, et al. The high prevalence of FLT3-ITD mutations is associated with the poor outcome in adult patients with t(6;9)(p23;q34) positive AML – Results of an international metaanalysis. Blood 2007; 110:234a (abstr).
19. Lugthart S, van Drunen E, van Norden Y, van Hoven A, Erpelinck CAJ, Valk PJM, et al. High EVI1 levels predict adverse outcome in acute myeloid leukemia: prevalence of EVI1 overexpression and chromosome 3q26 abnormalities underestimated. Blood 2008;111:4329-37.
20. Corbacioglu A, Fröhling S, Paschka P, Marcucci G, Anhalt A, Urlbauer K, et al. Acute myeloid leukemia (AML) with 9q aberrations occurring within a non-complex karyotype is highly associated with CEBPA and NPM1 mutations – a joint analysis of the German-Austrian AML Study Group (AMLSG) and Cancer and Leukemia Group B (CALGB). Blood 2007;110:234a (abstr).
21. Mrózek K. Acute myeloid leukemia with complex karyotype. Sem Oncol 2008, in press.
22. Rücker FG, Bullinger L, Schwaenen C, Lipka DB, Wessendorf S, Fröhling S, et al. Disclosure of candidate genes in acute myeloid leukemia with complex karyotypes using microarray-based molecular characterization. J Clin Oncol 2006;24:3887-94.
23. Schoch C, Haferlach T, Bursch S, Gerstner D, Schnittger S, Dugas M, et al. Loss of genetic material is more common than gain in acute myeloid leukemia with complex aberrant karyotype: a detailed analysis of 125 cases using conventional chromosome analysis and fluorescence in situ hybridization including 24-color FISH. Genes Chromosomes Cancer 2002;35:20-9.
24. Ebert BL, Pretz J, Bosco J, Chang CY, Tamayo P, Galili N, et al. Identification of RPS14 as a 5q- syndrome gene by RNA interference screen. Nature 2008;451:335-9.

Gene expression signatures in follicular lymphoma: are they ready for the clinic?

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The lymphoma field has fully embraced the molecular era for several years, the result of a number of studies utilizing genome-wide analysis platforms including gene expression profiling, array comparative genomic hybridization (aCGH), single nucleotide polymorphisms (SNP) arrays and several more novel technologies that provide information about methylation status and the epigenome.[1-4] As these new technologies became available, lymphoid cancers were a relatively early priority, in part because of the ease of sample acquisition and the availability of retrospective fresh-frozen biopsy material.

Heterogeneity of follicular lymphoma

Follicular lymphoma (FL) is the most common indolent non-Hodgkin's lymphoma (NHL). It typically presents as advanced-stage disease and in this setting is considered imminently treatable, but not curable with standard chemotherapy or immuno-chemotherapy regimens used today. There is growing evidence that the current era of therapy has produced improvements in overall survival.[5] Survival following diagnosis is markedly heterogeneous, with median survivals in the order of 8-10 years. Currently we have no reliable means to distinguish those patients who will die within three years of diagnosis from those who will be alive with little morbidity and requiring little if any therapy 25 years after diagnosis. These strikingly disparate outcomes in FL underlie the fury of activity surrounding the discovery of prognostic and predictive markers. The identification of molecular biomarkers that would help us better predict clinical outcome and risk of transformation would be a welcome addition, if indeed they could improve the prediction of prognosis beyond our current multi-parameter clinical indices.[6] An ideal biomarker would not only identify patients with inferior