

Molecular cytogenetics in acute myeloid leukemia in adult patients: practical implications

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KEY WORDS

acute myeloid leukemia, chromosome abnormality, karyotype, mutation, prognostication

ABSTRACT

Throughout the last 50 years, cytogenetic analyses of pretreatment bone marrow and/or blood samples from patients diagnosed with acute myeloid leukemia (AML) revealed a large number of recurring chromosome aberrations, both structural and numerical. Using standard banding methods and, more recently, molecular cytogenetic techniques, such as fluorescence in situ hybridization, spectral karyotyping, multiplex fluorescence in situ hybridization and comparative genomic hybridization, cytogenetic investigations detect acquired abnormalities that, together with submicroscopic gene mutations and changes in gene expression, strongly influence the clinical features and prognosis of patients with AML. Selected reciprocal translocations and inversions and their molecular counterparts, as well as a number of unbalanced chromosome abnormalities are used, together with bone marrow morphology, immunophenotype, and clinical characteristics, to define separate AML entities in the World Health Organization Classification of Haematolymphoid Tumours. Moreover, cytogenetic findings (and specific gene mutations) are being used in genetic risk classifications, such as the 2022 European LeukemiaNet classification. Such classifications divide patients into broad prognostic categories: favorable, intermediate, and adverse, which are useful in the management of adults with AML. In this article, I review the present data on recurrent chromosome rearrangements in AML and on correlations between cytogenetic findings and clinical features and treatment outcomes of adult patients diagnosed with AML.

Introduction During the last 50 years, cytogenetic investigations of acute myeloid leukemia (AML), a neoplastic disease characterized by deregulation of proliferation, differentiation, and/or programmed cell death (apoptosis) of hematopoietic progenitor cells, brought about major advances in our understanding of the genetic basis of this disorder and its considerable histopathologic, immunophenotypic, and clinical heterogeneity. As early as in 1972, de la Chapelle et al¹ reported a sole trisomy of chromosome 8, and in 1973, Janet D. Rowley² described a reciprocal translocation t(8;21)(q22;q22), which are, respectively, numerical and structural abnormalities that were later shown to be among the most frequent and clinically relevant recurrent chromosome aberrations in AML.^{3,4} To date, over 300 recurrent chromosome abnormalities have been reported in patients with AML.⁵ Molecular

investigation of breakpoints in a large number of AML-associated translocations, insertions, inversions and, occasionally, deletions has resulted in identification of genes involved in leukemogenesis, many of which encode transcription factors whose disruption constitutes a disease-initiating event. However, in most instances, the presence of a single chromosomal abnormality is not sufficient for the development of an overt AML, which is caused by acquisition of several (on average 13) somatic mutations affecting different pathways. These include, in addition to the aforementioned transcription-factor fusions, mutations in the *NPM1* gene, and mutations in genes belonging to such functional groups as tumor-suppressors as well as DNA methylation-related, signaling, chromatin-modifying, myeloid transcription-factor, cohesin-complex, and spliceosome-complex genes.⁶

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TABLE 1 Genetic abnormalities used to define specific acute myeloid leukemia entities in the 5th edition of the World Health Organization Classification of Haematolymphoid Tumours

AML with defining genetic abnormalities	Most frequent cytogenetic findings
APL with <i>PML::RARA</i> fusion	t(15;17)(q22–24;q12–21)
AML with <i>RUNX1::RUNX1T1</i> fusion	t(8;21)(q22;q22.1)
AML with <i>CBFB::MYH11</i> fusion	inv(16)(p13.1q22) or t(16;16)(p13.1;q22)
AML with <i>DEK::NUP214</i> fusion	t(6;9)(p23;q34.1)
AML with <i>RBM15::MRTFA</i> fusion	t(1;22)(p13.3;q13.3)
AML with <i>BCR::ABL1</i> fusion ^a	t(9;22)(q34;q11.2)
AML with <i>KMT2A</i> rearrangement	t(9;11)(p21.3–22;q23.3) ^b
AML with <i>MECOM</i> rearrangement	inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)
AML with <i>NUP98</i> rearrangement	t(7;11)(p15;p15) ^c
AML with <i>NPM1</i> mutation	Normal karyotype
AML with <i>CEBPA</i> mutation ^a	Normal karyotype
AML with other defined genetic alterations	Abnormalities creating “rare genetic fusions”
AML, myelodysplasia-related ^d	Defining cytogenetic abnormalities
	Complex karyotype (≥3 abnormalities)
	5q deletion or loss of 5q due to unbalanced translocation
	Monosomy 7, 7q deletion, or loss of 7q due to unbalanced translocation
	11q deletion
	12p deletion or loss of 12p due to unbalanced translocation
	Monosomy 13 or 13q deletion
	17p deletion or loss of 17p due to unbalanced translocation
	Isochromosome 17q
	idic(X)(q13)
	Defining somatic mutations in the genes:
	<i>ASXL1</i> , <i>BCOR</i> , <i>EZH2</i> , <i>SF3B1</i> , <i>SRSF2</i> , <i>STAG2</i> , <i>U2AF1</i> and/or <i>ZRSR2</i>

Data from Khoury et al⁷

- a** At least 20% of blasts are required for diagnosis.
- b** Other recurrent chromosome rearrangements involving 11q23/*KMT2A* are listed in [TABLE 3](#).
- c** Other recurrent chromosome rearrangements involving 11p15/*NUP98* are listed in [TABLE 2](#).
- d** To diagnose AML, myelodysplasia-related in patients with 20% of blasts in their bone marrow or blood, the presence of at least 1 cytogenetic or molecular abnormality listed in the second column of the Table and/or history of myelodysplastic or myeloproliferative neoplasm are required.

Abbreviations: AML, acute myeloid leukemia; APL, acute promyelocytic leukemia

It has been now well established that alterations detectable using cytogenetic methods comprise only a fraction of somatic mutations in patients with AML. However, chromosome aberrations, regardless of whether they are characterized molecularly or not, serve as tumor markers with diagnostic and prognostic significance. A number of more frequent genetic abnormalities, many of which are a result of recurring chromosome

abnormalities, are used to define distinct disease entities in the World Health Organization (WHO) Classification of Haematolymphoid Tumours.⁷ Furthermore, cytogenetic findings at diagnosis have been repeatedly demonstrated to be among the most important, independent prognostic factors for attainment of a complete remission (CR), as well as the disease-free (DFS) and overall (OS) survival in both younger adults (ie, those younger than 55–60 years) and among older patients (aged 55–60 years and older).^{3,4,8–18} Also, pretreatment karyotypic data together with molecular genetic findings are used to determine the choice of therapy in these patients.^{19,20} Consequently, cytogenetic analysis is a mandatory part of the diagnostic work-up of patients with AML according to the Clinical Practice Guidelines in Oncology of the National Comprehensive Cancer Network¹⁹ and the recommendations of an international expert panel working on behalf of the European LeukemiaNet (ELN).²⁰

Disease entities recognized in the World Health Organization classification of acute myeloid leukemia that are defined by gene fusions created by recurrent chromosome aberrations For the first time, the WHO classification used selected reciprocal translocations and inversions and their molecular counterparts to identify distinct disease entities of AML in 2001. The most recent, 5th edition of this classification⁷ has increased the role of genetic markers and recognized 11 subgroups within the “AML with defining genetic abnormalities” category, 9 of which are directly associated with chromosome aberrations ([TABLE 1](#)). One of these categories, namely “AML with *RBM15::MRTFA*,” has been hitherto diagnosed exclusively in children, most of whom were younger than 2 years,⁵ and will not be described here in more detail.

Acute promyelocytic leukemia with *PML::RARA* fusion Acute promyelocytic leukemia (APL) is an AML subtype characterized by unique bone marrow (BM) morphology and biological features, which make it a distinctive disease entity that was the first to become curable by therapy targeting causative genetic alterations. Patients with APL constitute 8% to 12% of adults with AML ([FIGURE 1](#)).^{4,12} These patients invariably harbor a gene fusion between *RARA*, a gene encoding the retinoic acid receptor α , and a partner gene, which in most cases (99%) is the *PML* gene. The *PML::RARA* fusion is generated most often by a reciprocal translocation t(15;17)(q22–24;q12–21) or its infrequent 3- or 4-way variants that involve, respectively, 1 or 2 additional chromosomes. In a recent study of over 800 patients with *PML::RARA* fusion,²¹ 96.6% of all cases had a reciprocal t(15;17), including 3.4% of patients with an additional abnormality of either der(17)t(15;17) or der(15)t(15;17). Three- and four-way variant translocations were detected in 2.2% and 0.1% of cases, respectively, and cryptic *PML::RARA*

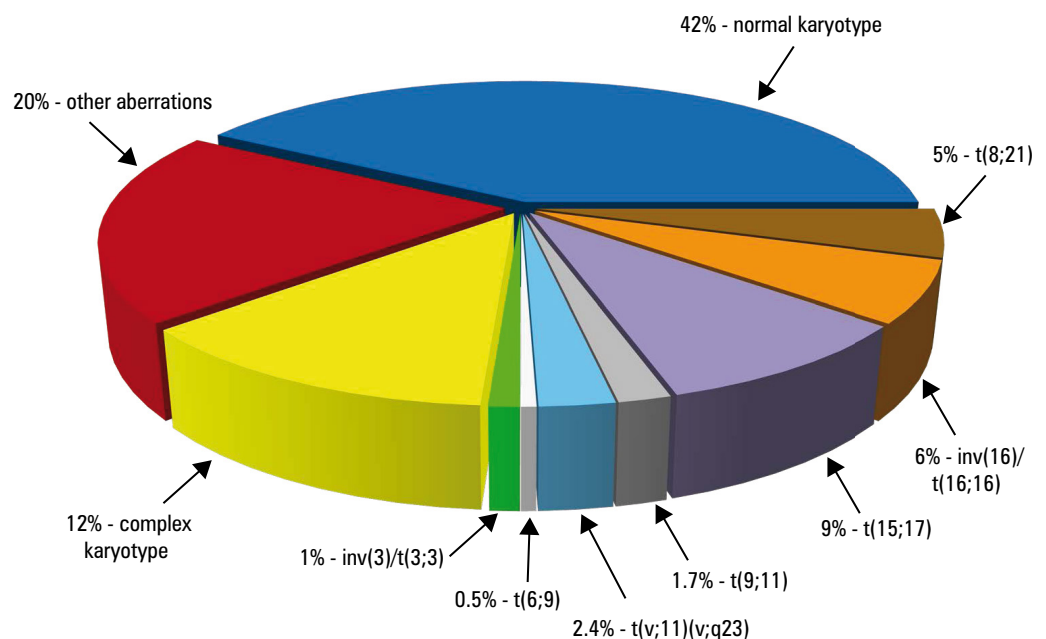


FIGURE 1 Distribution of more frequent pretreatment cytogenetic findings among 4246 adult patients with acute myeloid leukemia enrolled into the Cancer and Leukemia Group B (CALGB) companion protocol 8461. Complex karyotype is defined as 3 or more chromosome abnormalities other than t(15;17), t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23), t(6;9), or inv(3), or t(3;3). The t(v;11)(v;q23) denotes balanced rearrangements involving band 11q23 other than t(9;11). Reprinted from Mrózek and Bloomfield⁶⁹ with permission.

fusions with morphologically normal chromosomes 15 and 17 were found in 0.7% of the patients.²¹ In the latter subgroup, which in an earlier study was estimated to constitute as many as 4% of APL patients,²² the *PML::RARA* fusion is created by an insertion of a tiny segment containing the *RARA* gene into the *PML* locus (or, less frequently, vice versa). Such cryptic rearrangements can be identified using fluorescence in situ hybridization (FISH) or reverse transcription–polymerase chain reaction (RT-PCR). Importantly, the patients with these hidden alterations do not differ from those harboring standard t(15;17) with regard to clinical features or response to treatment.²²

In a small fraction (<1%) of APL patients, *RARA* is fused with genes other than *PML* as a result of variant rearrangements that include 7 recurring translocations between chromosome 17 and such partners as chromosomes 3, 4, 5, 11 (twice, with different breakpoints in 11q), 17, and X (TABLE 2). Determination of the exact gene fusion is vital because this informs the treating physician if the patient can respond to a therapy with all-trans-retinoic acid (ATRA) and/or arsenic trioxide (ATO). ATRA has been effective in the patients with the classic *PML::RARA* fusion and in those with variant fusions between *RARA* and the *BCOR*, *NPM1*, *NUMA1*, *FIP1L1*, *IRF2BP2*, *PRKARIA*, and *FNDC3B* genes.²³ In contrast, APL with t(11;17)(q23;q21)/*ZBTB16::RARA*, t(3;17)(q26;q21)/*TBL1XR1::RARA*, and t(17;17)(q21;q21)/*STAT5B::RARA* are resistant to ATRA and associated with an inferior prognosis. Additionally, only APL with *PML::RARA*, *TBL1XR1::RARA*, *IRF2BP2::RARA*, *PRKARIA::RARA*, and

FNDC3B::RARA has been thus far responsive to treatment with ATO.²³

The prognosis of patients with APL and t(15;17)/*PML::RARA*, which historically had been the worst among the subtypes of AML, has become the most favorable when treatment regimens containing ATRA and/or ATO became available, with CR rates of 85% to 100% and a cure rate of up to 97% in recent studies.²⁴ Patients with therapy-related APL receiving adequate treatment with ATRA-containing regimens have an outcome comparable to that of patients with de novo disease.²⁵ However, APL patients are prone to coagulopathy and hyperfibrinolysis that may lead to gastrointestinal bleeding and intrapulmonary and/or intracranial hemorrhages, thrombotic events, and early mortality. Therefore, prompt diagnosis and early detection of APL-associated rearrangements followed by ATRA treatment is vital. The use of RT-PCR and/or FISH offers potentially quicker results²²; however, these assays should not replace but rather supplement conventional cytogenetic analysis. The latter can identify rare (and potentially novel) variant translocations as well as detect secondary cytogenetic changes, which accompany t(15;17) in approximately one-third of APL patients at diagnosis. These secondary abnormalities most often include +8 or +8q, followed by del(7q)/add(7q) and del(9q).^{21,26} Complex karyotype [ie, the presence of ≥2 aberrations in addition to t(15;17)] is detected in 8% to 12% of the patients.^{21,27,28} Although individual secondary abnormalities do not appear to influence patients' prognosis,^{22,27,28} a complex karyotype conferred a lower CR rate,²⁷ shorter event-free survival,²⁸ and shorter OS²⁷ than those in

TABLE 2 Recurrent balanced chromosome abnormalities in acute myeloid leukemia other than those involving band 11q23 and the *KMT2A* gene^a (continued on the next page)

Cytogenetic abnormality	Cases, n	% sole	Gene fusion	Cytogenetic abnormality	Cases, n	% sole	Gene fusion
Rearrangement involving band 1p36 and the <i>PRDM16</i> gene				Rearrangement involving band 11q14 and the <i>PICALM</i> gene			
t(1;3)(p36.3;q21.1)	56	61	<i>RPN1::PRDM16</i>	t(10;11)(p12–13;q14–21)	34	35	<i>MLLT10::PICALM</i>
Rearrangement involving band 1p13 and the <i>RBM15</i> gene				Rearrangements involving band 12p13 and the <i>ETV6</i> gene			
t(1;22)(p13;q13)	64	63	<i>RBM15::MRTFA</i>	t(1;12)(q21;p13)	2	50	<i>ETV6::ARNT</i>
Rearrangement involving band 2p23 and the <i>ALK</i> gene				t(1;12)(q25;p13)	2	50	<i>ETV6::ABL2</i>
inv(2)(p23q13)	4	25	<i>RANBP2::ALK</i>	t(3;12)(q26;p13)	45	62	<i>ETV6::MECOM</i>
Rearrangements involving band 3q26 and the <i>MECOM</i> (<i>EVI1</i>) gene				t(4;12)(q11–12;p13)	31	71	<i>CHIC2::ETV6</i>
t(2;3)(p15–21;q26–27)	21	38	<i>MECOM</i>	t(5;12)(q31;p13)	5	40	<i>ETV6::ACSL6</i>
inv(3)(q21q26.2)	391	35	<i>RPN1::MECOM</i> <i>GATA2::MECOM</i>	t(7;12)(q36;p13)	29	7	<i>MXN1::ETV6</i>
t(3;3)(q21;q26.2) ^b	170	41	<i>RPN1::MECOM</i> <i>GATA2::MECOM</i>	t(8;12)(q12;p13)	3	0	<i>ETV6::LYN</i>
t(3;5)(q26–27;q31)	2	50	<i>MACROH2A1::MECOM</i>	t(10;12)(q24;p13)	7	29	<i>ETV6::GOT1</i>
t(3;8)(q26;q24)	18	56	<i>MECOM</i>	t(12;13)(p13;q12)	4	50	<i>ETV6::CDX2</i> or <i>FLT3::ETV6</i>
Rearrangement involving band 5q34 and the <i>NPM1</i> gene				t(12;22)(p12–13;q11–13)	29	21	<i>ETV6::MN1</i>
t(3;5)(q25;q34) ^c	78	80	<i>MLF1::NPM1</i>	Rearrangements involving band 12q13 and the <i>RARG</i> gene			
Rearrangement involving band 6q23 and the <i>MYB</i> gene				t(11;12)(p15;q13)	3	100	<i>NUP98::RARG</i>
t(X;6)(p11;q23)	5	100	<i>MYB::GATA1</i>	Rearrangements involving band 16q22 and the <i>CBFB</i> gene			
Rearrangements involving band 8p11 and the <i>KAT6A</i> gene				inv(16)(p13.1q22)	1003	68	<i>MYH11::CBFB</i>
inv(8)(p11q13)	7	86	<i>KAT6A::NCOA2</i>	t(16;16)(p13.1;q22)	52	83	<i>MYH11::CBFB</i>
t(8;16)(p11;p13)	148	58	<i>KAT6A::CREBBP</i>	Rearrangements involving band 16q24 and the <i>CBFA2T3</i> gene			
t(8;19)(p11;q13.3)	6	100	<i>KAT6A::LEUTX</i>	t(1;16)(p31;q24)	4	0	<i>NFIA::CBFA2T3</i>
t(8;22)(p11;q13)	5	60	<i>KAT6A::EP300</i>	inv(16)(p13q24) ^d	32	6	<i>CBFA2T3::GLIS2</i>
Rearrangements involving band 8p11 and the <i>FGFR1</i> gene				t(16;21)(q24;q22)	31	29	<i>CBFA2T3::RUNX1</i>
t(3;8)(q12;p11)	2	0	<i>TFG::FGFR1</i>	Rearrangement involving band 17q11 and the <i>TAF15</i> gene			
t(6;8)(q27;p11)	5	60	<i>CEP43::FGFR1</i>	t(12;17)(p13;q11–12)	6	100	<i>TAF15::ZNF384</i>
t(8;9)(p11;q33)	2	0	<i>CNTRL::FGFR1</i>	Rearrangements involving bands 17q12–21 and the <i>RARA</i> gene			
t(8;13)(p11;q12)	4	25	<i>ZMYM2::FGFR1</i>	t(1;17)(q42;q21)	2	0	<i>IRF2BP2::RARA</i>
t(8;22)(p11;q11)	4	75	<i>BCR::FGFR1</i>	t(3;17)(q26;q21)	3	0	<i>TBL1XR1::RARA</i> or <i>FNDC3B::RARA</i>
Rearrangement involving band 9p24 and the <i>JAK2</i> gene				t(4;17)(q12;q21)	2	0	<i>FIP1L1::RARA</i>
t(8;9)(p22;p24)	6	50	<i>PCM1::JAK2</i>	t(5;17)(q35;q12–21)	4	25	<i>NPM1::RARA</i>
Rearrangement involving band 9q34 and the <i>NUP214</i> gene				t(11;17)(q13;q12–21)	2	50	<i>NUMA1::RARA</i>
t(6;9)(p23;q34)	116	72	<i>DEK::NUP214</i>	t(11;17)(q23;q21)	27	78	<i>ZBTB16::RARA</i>
Rearrangement involving band 9q34 and the <i>ABL1</i> gene				t(15;17)(q22–24;q12–21) ^e	1380	70	<i>PML::RARA</i>
t(9;22)(q34;q11.2)	302	44	<i>BCR::ABL1</i>	t(17;17)(q21;q21)	3	67	<i>STAT5B::RARA</i>
Rearrangement involving band 10q22 and the <i>KAT6B</i> gene				t(X;17)(p11.4;q21)	2	0	<i>BCOR::RARA</i>
t(10;16)(q22;p13)	4	50	<i>KAT6B::CREBBP</i>	Rearrangements involving band 21q22 and the <i>RUNX1</i> gene			
Rearrangement involving band 10p15 and the <i>ZMYND11</i> gene				t(1;21)(p36;q22)	6	83	<i>RUNX1::PRDM16</i>
t(10;17)(p15;q21)	12	75	<i>MBTD1::ZMYND11</i>	t(1;21)(p32;q22)	6	33	<i>RUNX1::PRPF38A</i> or <i>RUNX1::NDC1</i>
Rearrangements involving band 11p15 and the <i>NUP98</i> gene				t(1;21)(p22;q22)	2	100	<i>RUNX1::CLCA2</i>
t(1;11)(q23;p15)	5	40	<i>NUP98::PRRX1</i>	t(3;21)(q26;q22)	70	44	<i>MECOM::RUNX1</i>
t(2;11)(q31;p15)	7	86	<i>NUP98::HOXD11</i> or <i>NUP98::HOXD13</i>	t(4;21)(q31;q22)	2	50	<i>RUNX1::SH3D19</i>
t(3;11)(p11;p15)	2	100	<i>NUP98::POU1F1</i>	t(5;21)(q23.3–31;q22)	3	67	<i>RUNX1::ADAMTS19</i>
t(3;11)(q12;p15)	5	80	<i>NUP98::LNP1</i>	t(7;21)(p22;q22)	11	36	<i>RUNX1::USP42</i>
t(4;11)(q21–23;p15)	5	40	<i>NUP98::RAP1GDS1</i>	t(8;21)(p11;q22)	3	33	<i>RUNX1::TACC1</i>
t(5;11)(q35;p15)	42	69	<i>NUP98::NSD1</i> or <i>STIM1::NSD1</i>	t(8;21)(q22;q22)	1768	42	<i>RUNX1T1::RUNX1</i>
t(7;11)(p15;p15)	79	78	<i>NUP98::HOXA9</i> or <i>NUP98::HOXA11</i> or <i>NUP98::HOXA13</i>	t(8;21)(q23–24;q22)	3	67	<i>RUNX1::TRPS1</i>

TABLE 2 Recurrent balanced chromosome abnormalities in acute myeloid leukemia other than those involving band 11q23 and the *KMT2A* gene^a (continued from the previous page)

Cytogenetic abnormality	Cases, n	% sole	Gene fusion	Cytogenetic abnormality	Cases, n	% sole	Gene fusion
t(8;11)(p11;p15)	5	40	<i>NUP98::NSD3</i>	t(11;21)(p13–4;q22)	4	50	<i>RUNX1::KIAA1549L</i>
t(9;11)(p22;p15)	7	86	<i>NUP98::PSIP1</i>	t(11;21)(q13;q22)	2	50	<i>RUNX1::MACROD1</i>
t(9;11)(q34;p15)	2	50	<i>NUP98::PRRX2</i>	t(15;21)(q21;q22)	3	33	<i>RUNX1::TCF12</i>
t(10;11)(q23;p15)	4	50	<i>NUP98::HHEX</i>	t(20;21)(q11;q22)	2	0	<i>RUNX1::NOL4L</i> or <i>RUNX1::CBFA2T2</i>
inv(11)(p15q22)	17	65	<i>NUP98::DDX10</i>	Rearrangement involving band 21q22 and the <i>ERG</i> gene			
t(11;12)(p15;p13) ^f	6	17	<i>NUP98::KDM5A</i>	t(16;21)(p11;q22)	63	67	<i>FUS::ERG</i>
t(11;12)(p15;q13)	14	86	<i>NUP98::HOXC11</i> or <i>NUP98::HOXC13</i>				
t(11;17)(p15;p13)	5	100	<i>NUP98::PHF23</i>				
t(11;20)(p15;q11–12)	18	78	<i>NUP98::TOP1</i>				

Data from Mitelman et al⁵

- a** Only the abnormalities for which fusion genes have been identified are included (except for rearrangements involving the *MECOM* gene).
- b** Also interpreted as ins(3;3)(q21;q21q26)
- c** Also interpreted as t(3;5)(q21;q31)
- d** This inversion is cryptic; there are no individual cases listed in the Mitelman database.⁵ The numbers of cases listed in the Table are based on data from Gruber et al (doi:10.1016/j.ccr.2012.10.007) and Masetti et al (doi:10.1182/blood-2012–11–469825).
- e** In the literature, the breakpoints in t(15;17) have been variously assigned to 15q22 or 15q24, and to 17q11, 17q12, 17q21, or 17q22.
- f** This translocation is cryptic; there are no individual cases listed in the Mitelman database.⁵ The numbers of cases listed in the Table are based on data from van Zutven et al (doi:10.1002/gcc.20308), Hollink et al (doi:10.1182/blood-2011–04–346643), and Gruber et al (doi:10.1016/j.ccr.2012.10.007).

APL patients with a noncomplex karyotype who were treated with regimens containing ATO. This indicates a need for an alternative, more intense treatment when a complex karyotype is detected in an APL patient.

Internal tandem duplications of the *FLT3* gene (*FLT3*-ITD), which confer adverse prognosis in cytogenetically normal AML (CN-AML), are detected in 20% to approximately 40% of APL patients and have been associated with variant microgranular (or hypogranular) BM morphology and higher white blood cell (WBC) counts at diagnosis.²⁹ Although *FLT3*-ITD has been associated with higher frequency of induction death, CR rates have not been affected by its presence and the adverse impact of *FLT3*-ITD on survival, shown in some but not all studies, remains debatable.²⁹ Moreover, treatment with ATO appears to overcome the potentially negative influence of *FLT3*-ITD on patients' survival.^{24,27}

Acute myeloid leukemia with *RUNX1::RUNX1T1* fusion

The presence of t(8;21)/*RUNX1::RUNX1T1* denotes one of the 2 AML subtypes that constitute the so-called core-binding factor AML (CBF-AML), the second being AML with inv(16)(p13.1q22). Both chromosome aberrations result in rearrangements of genes encoding the α and β subunits of the CBF complex, respectively, the *RUNX1* gene, located at 21q22.1 and disrupted by t(8;21) and the *CBFB* gene, located at 16q22 and disrupted by inv(16)/t(16;16). The CBF complex is a heterodimeric transcription factor regulating

transcription of genes encoding proteins involved in hematopoietic differentiation. A protein encoded by a chimeric gene *RUNX1::RUNX1T1* created by t(8;21) inhibits the wild-type *RUNX1* in a dominant-negative fashion and leads to impairment of normal hematopoiesis and predisposition to develop leukemia.³⁰

Translocation t(8;21) or its infrequent variants [eg, ins(8;21)(q22;q22q22), ins(21;8)(q22;q21q22), t(8;10;21)(q22;q26;q22), t(8;20;21)(q22;p11.2;q22), etc] are found in 5% to 8% of adults with AML (FIGURE 1).^{12,31} Only roughly 30% of the patients harbor t(8;21) as the only chromosome abnormality. The most frequent secondary aberration is –Y, detected in about 60% of male patients, followed by –X, found in 33% to 40% of women with t(8;21), and by del(9q) (17%), +8 (5%–7%), and +4 (4%).^{32,33} Evidently, additional genetic alterations cooperating with *RUNX1::RUNX1T1* are required because the presence of this gene fusion alone has not been sufficient to induce leukemia.³⁴ Potential genetic rearrangements that cooperate with t(8;21)/*RUNX1::RUNX1T1* include *KIT* mutations (found in ca 25% of patients), *NRAS* and *KRAS* mutations (10%–20%), *FLT3*-ITD (7%), and point mutations in the tyrosine kinase domain of *FLT3* (*FLT3*-TKD; 4%).^{34–36}

Treatment outcome of patients with t(8;21) receiving postremission therapy with repetitive cycles of high-dose cytarabine (HiDAC) is relatively favorable,^{32–34,37} albeit OS and survival after the first relapse of patients with t(8;21) are

shorter than those of patients with inv(16).³³ Since secondary aberrations are present in most patients, their prognostic significance has been of major interest and was assessed by many studies with conflicting results. Loss of the Y chromosome was reported as having adverse,^{32,38} favorable,³⁹ or no^{33,40} significant impact on the outcome of men with t(8;21). Likewise, loss of the X chromosome was recently associated with better outcome in a large cohort of female patients with t(8;21) in China,⁴¹ but this has not been observed in European or American studies.^{32,33,39} Deletion of 9q was found to confer favorable prognosis in 2 studies,^{33,40} but this effect was limited to non-White patients in one of them.³³ On the other hand, worse outcome has been repeatedly associated with *KIT* mutations.^{34,35} Moreover, high cumulative incidence of relapse (CIR), but not shorter OS, was associated with higher ($\geq 25\%$) relative *KIT* mutant levels only.⁴² Likewise, high levels of *FLT3*-ITD resulted in shorter OS, but not higher CIR, in younger adults with t(8;21).⁴² These results should be further corroborated.

Acute myeloid leukemia with *CBFB::MYH11* fusion

The second subset of CBF-AML is defined by the presence of *CBFB::MYH11* gene fusion created by either inv(16)(p13.1;q22) or, less commonly, t(16;16)(p13.1;q22). These abnormalities are seen in 5% to 6% of adults with AML (FIGURE 1), and are closely associated with myelomonocytic BM morphology and abnormal eosinophils, which are pathognomonic for this type of AML.¹² The *CBFB::MYH11* fusion gene encodes a chimeric protein that retains the ability to interact with *RUNX1* and block CBF-dependent transcription. Due to the marked variability of genomic breakpoints within the *CBFB* and *MYH11* genes, over 10 fusion transcript variants of different sizes have been reported.⁴³ The most frequent type A fusion is detected in 85%, and type D and type E fusions are each found in 5% to 10% of the patients. For still unclear reasons, the patients harboring type A fusions differ from those with non-type A fusions with regard to the occurrence of additional genetic alterations. In addition to higher WBC counts, the patients with type A fusions had less often secondary +8 or +21, but only they carried +22 and the *KIT* mutations, which were not detected in patients with non-type A fusions. Mutations in the *KIT* gene bestowed adverse prognosis among type A fusion-positive patients, whereas treatment outcomes of the type A and non-type A patients with wild-type *KIT* were not significantly different.⁴³

Prognosis of patients with inv(16)/t(16;16) is relatively favorable, especially if they are treated with 3 to 4 cycles of HiDAC postremission.⁴⁴ Recent studies demonstrated that clinical outcome of CBF-AML patients with inv(16)/t(16;16) and those with t(8;21) can be further improved by the addition of dasatinib, a multi-kinase inhibitor, to intensive chemotherapy.^{45,46} Prognosis for

therapy-related CBF-AML with inv(16)/t(16;16) or t(8;21) seems to be worse than for de novo CBF-AML, but still better than for therapy-related non-CBF-AML.²⁵

The presence of secondary +22 has been repeatedly found to reduce the patients' risk of relapse,³²⁻³⁴ lengthen their OS duration^{13,34} and, in patients who also carry *FLT3*-ITD, extend relapse-free survival.⁴⁷ Among molecular markers, mutations in *KIT*^{34,35} and *FLT3*, mainly *FLT3*-TKD,³⁴ adversely affect OS. Although *NRAS* and *KRAS* mutations are frequent (seen in >50% of patients), they do not influence prognosis.^{34,42,48} However, *RAS* mutations seem to sensitize AML blasts to postremission therapy with HiDAC.⁴⁹

Acute myeloid leukemia with *DEK::NUP214* fusion

The t(6;9)(p23;q34.1), which generates the *DEK::NUP214* fusion, is fairly rare, being detected in 0.5% to 0.7% of adults with AML (FIGURE 1). In approximately 90% of cases, it is the sole chromosome abnormality,^{5,50} indicating that t(6;9)/*DEK::NUP214* represents the main driver event in leukemogenesis. Although secondary chromosome aberrations are rare, patients with t(6;9) or its infrequent 3-way variants harbor *FLT3*-ITD more often than individuals with any other AML subtype, with 73% to 85% of t(6;9)-positive adults acquiring this mutation.^{50,51}

The clinical outcome of adult patients receiving chemotherapy is very poor irrespective of the presence or absence of *FLT3*-ITD.⁵⁰ In contrast, allogeneic hematopoietic stem cell transplantation (alloHSCT) performed in patients in CR has been reported to substantially improve their prognosis.^{51,52}

Acute myeloid leukemia with *KMT2A* rearrangement

Band 11q23.3 is a locus of the *KMT2A* gene (formerly *MLL*), which is fused with the highest number of partner genes in human leukemia as a result of balanced chromosome abnormalities occurring both in AML and acute lymphoblastic leukemia. In AML, at least 77 different fusion partners of 11q23/*KMT2A* have been reported, some in single patients.⁵³ It is likely that further rearrangements involving 11q23/*KMT2A* and novel partner genes will be discovered using targeted next-generation sequencing (NGS) in addition to the conventional cytogenetic, FISH, and long-distance inverse (LDI)-PCR assays.⁵⁴ TABLE 3 lists 43 reciprocal translocations, insertions, inversions, and deletions involving 11q23/*KMT2A*, together with gene fusions they created. Only recurrent abnormalities found in at least 2 cases of AML are displayed.

Translocation t(9;11) is the most frequent among 11q23/*KMT2A* rearrangements, being detected in approximately 2% of adults with AML (FIGURE 1). This translocation fuses *KMT2A*, the gene encoding a DNA-binding protein methylating histone H3 lysine 4 (H3K4) and positively regulating the expression of multiple genes

TABLE 3 Recurrent balanced chromosome abnormalities involving band 11q23 and the *KMT2A* gene in acute myeloid leukemia^a

Cytogenetic abnormality	Cases, n	% sole	Gene fusion	Cytogenetic abnormality	Cases, n	% sole	Gene fusion
t(1;11)(p32;q23)	16	56	<i>KMT2A::EPS15</i>	t(11;11)(q13;q23)	3	67	<i>KMT2A::C2CD3</i> or <i>KMT2A::ARHGEF17</i>
t(1;11)(q21;q23)	31	71	<i>KMT2A::MLLT11</i>	inv(11)(q21q23)	7	43	<i>KMT2A::MAML2</i>
t(2;11)(q37;q23)	8	63	<i>KMT2A::SEPTIN2</i>	t(11;12)(q23;q13)	2	100	<i>KMT2A::SARNP</i>
t(3;11)(p21;q23)	2	50	<i>KMT2A::NCKIPSD</i>	t(11;14)(q23;q23–24)	3	100	<i>KMT2A::GPHN</i>
t(4;11)(q21;q23)	39	49	<i>KMT2A::AFF1</i>	t(11;14)(q23;q32)	3	100	<i>KMT2A::CEP170B</i>
t(5;11)(q31;q23)	6	100	<i>KMT2A::ARHGAP26</i>	t(11;15)(q23;q14–15)	11	18	<i>KMT2A::KNL1</i> or <i>KMT2A::ZFYVE19</i>
t(6;11)(q15;q23)	3	67	<i>KMT2A::CASP8AP2</i>	t(11;16)(q23;p13)	14	50	<i>KMT2A::CREBBP</i> or <i>KMT2A::MYH11</i>
t(6;11)(q21;q23)	6	33	<i>KMT2A::FOXO3</i>	t(11;16)(q23;q24)	3	67	<i>KMT2A::USP10</i>
t(6;11)(q25;q23)	2	100	<i>KMT2A::SNX9</i>	t(11;17)(q23;p13)	2	100	<i>KMT2A::GAS7</i>
t(6;11)(q27;q23)	118	81	<i>KMT2A::AFDN</i>	t(11;17)(q23;q12–21)	67	81	<i>KMT2A::MLLT6</i> or <i>KMT2A::RARA</i> or <i>KMT2A::ACACA</i>
t(9;11)(p21–22;q23)	462	67	<i>KMT2A::MLLT3</i>	t(11;17)(q23;q23)	4	50	<i>KMT2A::CLTC</i>
t(9;11)(q33–34;q23)	2	100	<i>KMT2A::DAB2IP</i>	t(11;17)(q23;q25)	45	67	<i>KMT2A::SEPTIN9</i>
ins(10;11)(p11–13;q23q13–25)	33	45	<i>KMT2A::MLLT10</i>	t(11;18)(q23;q21)	3	33	<i>KMT2A::ME2</i>
ins(11;10)(q23;p12–13p11–15)	8	75	<i>KMT2A::MLLT10</i>	t(11;19)(q23;p13.1)	69	85	<i>KMT2A::ELL</i>
t(10;11)(p12;q23)	3	100	<i>KMT2A::ABI1</i> or <i>KMT2A::NEBL</i>	t(11;19)(q23;p13.3)	47	41	<i>KMT2A::MLLT1</i> or <i>KMT2A::VAV1</i>
t(10;11)(p11–13;q13–23)	60	48	<i>KMT2A::MLLT10</i>	t(11;19)(q23;p13.2–13.3)	4	25	<i>KMT2A::MYO1F</i>
t(10;11)(q21–22;q23)	9	67	<i>KMT2A::TET1</i>	t(11;22)(q23;q11)	13	62	<i>KMT2A::SEPTIN5</i>
del(11)(q23q23) ^b	5	20	<i>KMT2A::ARHGEF12</i>	t(11;22)(q23;q13)	6	67	<i>KMT2A::EP300</i>
del(11)(q23q24)	4	50	<i>KMT2A::DCPS</i> or <i>KMT2A::TIRAP</i>	t(X;11)(q13;q23)	3	100	<i>KMT2A::FOXO4</i>
inv(11)(p15q23)	13	69	<i>KMT2A::NRIP3</i> or <i>NUP98::KMT2A</i>	t(X;11)(q24;q23)	4	100	<i>KMT2A::SEPTIN6</i>
t(11;11)(p15;q23)	3	100	<i>KMT2A::NRIP3</i> or <i>KMT2A::AP2A2</i>	ins(11;X)(q23;q28q12)	3	67	<i>KMT2A::FLNA</i>
inv(11)(q13q23)	3	0	<i>KMT2A::C2CD3</i>				

Data from Mitelman et al¹⁵**a** Only the abnormalities for which fusion genes have been identified are included.**b** The numbers of cases listed in the Table include data from Kourlas et al (doi:10.1073/pnas.040569197) and Shih et al (doi:10.1038/sj.leu.2404024)

including the *HOX* genes, with *MLLT3*, a gene encoding a nuclear protein containing serine-rich and proline-rich regions, which appear to be important for leukemogenesis.⁵⁵ The t(9;11) is the sole chromosome change in nearly two-thirds of patients; around 20% of cases have +8, and less frequent are secondary +19 and +21.⁵

Several studies reported that the patients with t(9;11) had better treatment outcome than those with other rearrangements involving 11q23/*KMT2A*,^{11,13,56–59} which placed the patients with t(9;11) in the intermediate-risk category in both prognostic categorizations based solely on cytogenetic data^{11,13} and in classifications combining cytogenetics with selected molecular genetic findings, such as the 2010⁶⁰ and 2022²⁰ ELN classifications. However, a more favorable prognosis of the patients with t(9;11) was observed in

studies analyzing exclusively^{13,57} or mostly^{11,56,58} adults younger than 60 years. When the analysis was restricted to patients aged 60 years or older, the outcome of the t(9;11)-positive patients was very poor and comparable to that of individuals with other 11q23/*KMT2A* alterations,^{59,61} whose prognosis is invariably adverse regardless of the age group.^{11–13,56,59,61} The reasons for such age difference are presently unknown.

Acute myeloid leukemia with *MECOM* rearrangement The *MECOM* rearrangements are caused most often by inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2). Both inv(3) and the less frequent t(3;3) cause relocation of a distal enhancer of the *GATA2* gene that leads to simultaneous deregulated expression of the *MECOM* gene (formerly known as *EVII*) and haploinsufficiency of

GATA2. The inv(3)/t(3;3) are detected in approximately 1% of patients with AML (FIGURE 1), both de novo and evolving from preceding myelodysplastic syndrome, and are associated with multilineage dysplasia, abnormal megakaryopoiesis with micromegakaryocytes in the BM, normal or increased platelet counts, and higher WBC counts at diagnosis.⁶² The majority of patients have secondary abnormalities, with -7 being found in approximately 50% of cases.⁵ Since inv(3) is a subtle rearrangement, which can be illustrated by the fact that it was occasionally missed by a cytogenetic laboratory to be later recognized on central karyotype review performed by the Cancer and Leukemia Group B/Alliance for Clinical Trial in Oncology (CALGB/Alliance),⁶³ detection of an apparently sole -7 should always prompt cytogeneticists to closely inspect chromosomes 3 in such patients.

The treatment outcome of the patients with inv(3)/t(3;3) is invariably dismal regardless of the presence or absence of -7 in most studies,^{11,13} although Lugthart et al⁶² reported that patients with -7 fared even worse than those without -7. To date, alloHSCT, especially if performed in CR, appears to be the only option to improve the very poor prognosis of patients with inv(3)/t(3;3).⁶⁴

Acute myeloid leukemia with *NUP98* rearrangement *NUP98* participates in over 30 gene fusions with different partner genes that are generated by reciprocal translocations or inversions, the recurrent of which are listed in TABLE 2. Because *NUP98* is located near the terminus of 11p, at 11p15.4, some 11p15.4/*NUP98* alterations may be undetectable by conventional karyotyping.⁷ AML with *NUP98* rearrangement is usually characterized by deregulation of the *HOXA* gene cluster and decreased terminal differentiation of hematopoietic cells.⁶⁵ The majority of specific abnormalities involving 11p15.4/*NUP98* are quite rare and occur considerably more often in children than adults.⁶⁶ Although these abnormalities bestow poor prognosis in children, their prognostic significance is yet to be conclusively established in large cohorts of adult patients.

Acute myeloid leukemia, myelodysplasia-related

This is another WHO category of AML that employs cytogenetic information to classify patients lacking any of the abnormalities described above.⁷ To be assigned to this AML subtype, it is sufficient for the patient whose BM or blood contains at least 20% of blasts to harbor a complex karyotype with 3 or more chromosome aberrations, and/or one of 8 unbalanced abnormalities or a somatic mutation in one of the following genes: *ASXL1*, *BCOR*, *EZH2*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, and *ZRSR2* (TABLE 1). Most of the unbalanced aberrations, except for idic(X)(q13) or del(11q), occur frequently as part of a complex karyotype and their presence negatively affects patient prognosis.⁶⁷

Patients who do not carry any of the aforementioned defining genetic alterations or whose

pretreatment cytogenetic investigation fails are categorized into AML subtypes defined by differentiation.⁷ Notably, unsuccessful cytogenetic assays are rare, occurring in 2% to 6% of patients in large studies.^{68,69}

Complex karyotype and its clinical significance in acute myeloid leukemia

Most studies define complex karyotype as the one encompassing at least 3 chromosome aberrations,^{9,11,15-17,70} although definitions of 5 or more unrelated cytogenetic abnormalities^{14,16} and 4 or more abnormalities (excluding the chromosome changes that confer a favorable or adverse prognosis)¹³ have also been used. The complex karyotype subset usually does not include patients with t(15;17), t(8;21), inv(16)/t(16;16),^{11,15} t(9;11),^{11,16} or other balanced rearrangements affecting band 11q23.^{15,16} Recently, the Francophone Group of Hematological Cytogenetics suggested⁷¹ that to standardize the cytogenetic practice, a complex karyotype with 3 chromosome abnormalities should be defined as a low, one with 4 aberrations as an intermediate, and one with 5 or more abnormalities as a highly complex karyotype. The most recent definition proposed by the ELN denotes a complex karyotype as containing at least 3 abnormalities in the absence of "other class-defining recurring genetic abnormalities" and does not consider karyotypes with 3 or more trisomies in the absence of structural abnormalities as complex.²⁰

Patients with AML and a complex karyotype defined as at least 3 chromosome aberrations constitute 10% to 12%, and those with 5 or more aberrations 8% to 9% of all AML patients.^{9,11,14} The incidence of AML with a complex karyotype increases with advancing age of the patients and is more than twice as high in patients older than 60 years (17%–19%) than in younger adults (6%–8%).⁷⁰ Although the lowest number of aberrations in a complex karyotype is 3, in most patients more aberrations are present, with the median numbers being 6,⁷² 8,⁷³ and 10,⁷⁴ respectively, in 3 studies analyzing complex karyotypes using multiplex FISH or spectral karyotyping. These molecular cytogenetic techniques were instrumental in a more precise delineation of chromosome abnormalities in complex karyotypes, including marker chromosomes, and revealed the nonrandom nature of the occurrence of particular structural and numerical abnormalities in complex karyotypes. Most recurring chromosome alterations are unbalanced (monosomies, deletions, and unbalanced translocations) and result in a loss of chromosome segments mainly from, in decreasing order, chromosome arms 5q, 17p, 7q, 18q, 16q, 17q, 12p, 20q, 18p, and 3p.^{70,72-75} The loss of the 17p13 locus has been correlated with a loss of and mutations in the *TP53* gene, which cause a loss of the p53 protein function. This results in increased cell survival that contributes to pronounced genomic instability and poor outcome of patients with a complex karyotype.^{76,77} Gains of chromosomal material occur less often and are frequently hidden in unbalanced translocations and

marker chromosomes. These gains involve most often 8q, 11q, 21q, 22q, 1p, 9p, and 13q,^{70,72-75} and their further analysis has led to identification of genes residing in amplified regions such as *MYC* in 8q, *DDX6*, *ETS1*, *FLI1*, and *KMT2A* in 11q, *ERG*, *ETS2*, and *APP* in 21q, and *CDX2* in 13q12.^{70,75,78-80}

Among unbalanced rearrangements, the most frequent are those leading to a loss of the 5q material that are found in approximately 80% of the patients, followed by losses of 7q and 17p, each detected in circa 50% of the patients. These abnormalities often coexist in the same individual, resulting in around 85% of all patients with complex karyotypes carrying at least one of them.⁷⁰ Recently, we designated such complex karyotypes with the loss of 5q, 7q, and/or 17p as typical, and complex karyotypes with at least 3 abnormalities that do not include 5q, 7q, and/or 17p loss as atypical.⁸¹ An example of a typical complex karyotype with abnormalities that caused the loss of segments from 5q and 17p, in addition to other chromosome rearrangements, is shown in [FIGURE 2](#). Our comparison of pretreatment characteristics and outcomes of patients with these 2 complex karyotype types revealed that AMLs with typical and atypical complex karyotypes constitute different disease subtypes. In comparison with patients with a typical complex karyotype, those with an atypical one were younger, had higher WBC counts and percentages of blood and BM blasts, harbored *TP53* mutations less frequently (10% vs 67%) and more frequently carried *FLT3*-TKD and *PHF6*, *MED12*, and *NPM1* mutations. Importantly, they had higher CR rates and longer OS.⁸¹ Similarly, in another study,⁸² the patients with a hyperdiploid complex karyotype (ie, with 49–65 chromosomes) that included exclusively numerical abnormalities that would be recognized as an atypical complex karyotype, had a relatively long OS comparable to the OS of the patients classified in the intermediate cytogenetic risk group as defined by the United Kingdom Medical Research Council trials.¹³

Cytogenetically normal acute myeloid leukemia

The largest cytogenetic subset of adult AML consists of 40% to 45% of patients with a normal karyotype ([FIGURE 1](#)).^{3,4,12} The proportion of patients with CN-AML recognized by standard G-banding analysis is likely overestimated because sometimes subtle aberrations such as inv(16), inv(3) or t(11;19)(q23;p13.1) might be overlooked in suboptimal-quality preparations. Thus, to eliminate this possibility, CALGB/Alliance has been conducting central karyotype reviews for almost 40 years.⁶³ Moreover, occasionally, CN-AML patients harbor the above-mentioned cryptic insertions producing AML-associated gene fusions, such as *PML::RARA* or *CBFB::MYH11*, albeit patients with these alterations constitute only a fraction of all CN-AML cases.^{21,22,31} However, there are also recurring cryptic rearrangements involving terminal

bands with similar morphology that are, by definition, undetectable on routine cytogenetic investigation (but detectable by FISH or NGS-based methods), such as a prognostically adverse t(5;11)(q35;p15)/*NUP98::NSD1*, detected in 2% of adults with CN-AML, or a rare t(11;12)(p15;p13)/*NUP98::KDM5A* ([TABLE 2](#)). Moreover, the genome-wide copy number analysis with paired normal and leukemic DNA using single nucleotide polymorphism (SNP) arrays and ultra-dense array comparative genomic hybridization (CGH) platform revealed acquired copy number alterations (CNAs) in 24% and uniparental disomy (UPD) segments in 14% of the study patients with CN-AML.⁸³ UPDs were found with even higher frequency (26%) in a subsequent study,⁸⁴ which demonstrated that the most common were UPDs affecting 13q with the locus of the *FLT3* gene (found in 7.5% of the patients), 6p (2.8%), and 11p (2.8%), and that their presence was associated with treatment outcome in patients with CN-AML aged under 60 years. Specifically, UPD of 11p conferred longer OS and UPD of 13q conferred shorter DFS and OS.⁸⁴ CNAs in AML can also be detected by the relatively fast and cost-effective multiplex ligation-dependent probe amplification (MLPA) assay, as shown recently.⁸⁵ Still, karyotypes of a relatively large proportion of AML patients are truly normal (within resolution of metaphase karyotyping), which, of course, does not preclude the existence of molecular genetic alterations.

In all major cytogenetic risk classifications, patients with CN-AML were classified as having intermediate risk, because their CR rates, DFS, and OS were inferior to those of adequately treated patients with favorable t(15;17), inv(16), and t(8;21), but better than the outcome of patients with adverse chromosome aberrations.⁹⁻¹³ However, CN-AML is very heterogeneous at the molecular level and consists of molecular genetic subsets with greatly differing prognoses.^{86,87} In most instances, *NPM1* mutations as well as *CEBPA* bZIP in-frame mutations and high expression of *miR-181a* bestow good prognosis, whereas poor treatment outcome is associated with *MLL*-PTD and *FLT3*-ITD, as well as mutations in the *ASXL1*, *BCOR*, *DNMT3A* (both R882 and non-R882 mutations), *IDH1*, *IDH2*, *RUNX1*, *TET2*, and *WT1* genes and high expression of *BAALC*, *DNMT3B*, *ERG*, *MN1*, *SPARC*, *miR-155*, and *miR-3151*.^{20,69,86,87} Because patients with CN-AML may harbor multiple mutations and/or gene expression changes with prognostic import, various combinations of molecular alterations might modify the prognostic impact of individual abnormalities. This makes research aimed at determination of how combinations of molecular genetic and cytogenetic alterations affect prognosis of both CN-AML and cytogenetically abnormal patients necessary. Additionally, it is important to underscore the fact that prognostic factors in AML depend on the types of induction

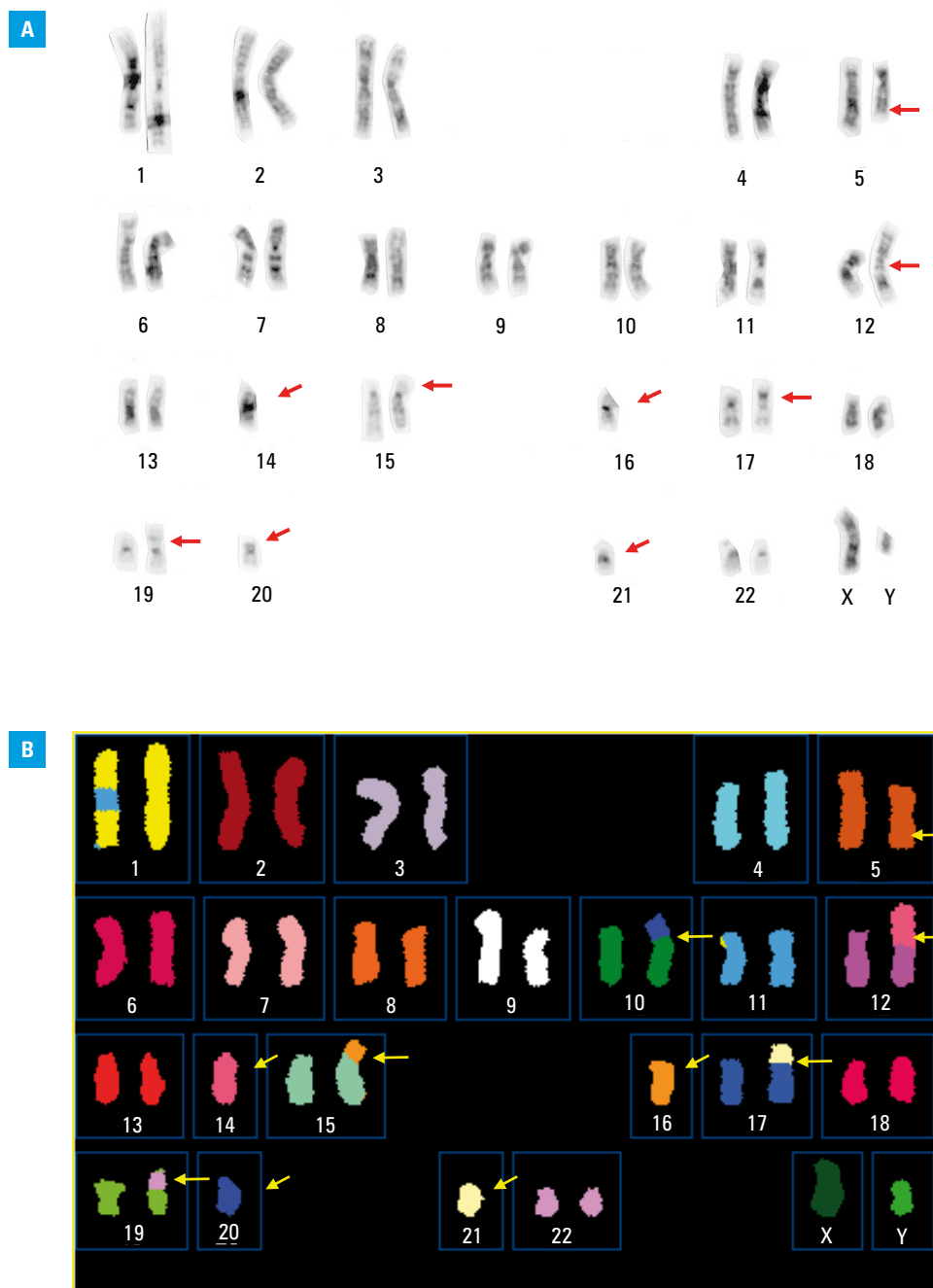


FIGURE 2 Standard cytogenetic and spectral karyotyping (SKY) analysis of a typical complex karyotype detected in a patient with de novo acute myeloid leukemia. **A** – G-banded karyotype interpreted as 42,XY,del(5)(q22q33 or q31q35),der(12)t(12;14)(p12;q11),–14,der(15;16)(q10;p10),–16, der(17)t(17;21)(p13;q11),add(19)(p13.3),–20,–21. Red arrows indicate chromosome abnormalities. **B** – spectral karyotype of the same patient shown in spectra-based classification colors. SKY allowed for determination of the origin of an unidentified material in add(19)(p13.3) as a segment from the long arm of chromosome 22, and revealed a cryptic rearrangement, dic(10;20)(p11;q11.2), that was not recognized in the G-banded preparation. The final karyotype interpretation was 42,XY,del(5)(q22q33 or q31q35),dic(10;20)(p11;q11.2),dic(12;14)(p11;p11),der(15;16)(q10;p10),–16,der(17)t(17;21)(p13;q11),der(19)t(19;22)(p13.3;q?),–21. This karyotype contains del(5q) and der(17)t(17;21) that result in a loss of material from 5q and 17p, respectively. Their presence makes this complex karyotype a typical one.⁸¹ Yellow arrows denote chromosome abnormalities. The blue segment in the middle of the left chromosome 1 does not represent a chromosome aberration, it is caused by an overlapping chromosome 11 in the depicted cell.

and postremission therapy. A prognostically adverse genetic alteration may portend a more favorable outcome when another treatment is given. This includes the use of compounds specifically targeting specific alterations, such as midostaurin and gilteritinib in patients with *FLT3*-ITD or

FLT3-TKD, ivosidenib in *IDH1*-mutated, or enasidenib in *IDH2*-mutated patients.⁸⁸

European LeukemiaNet genetic-risk classifications combining cytogenetic abnormalities with selected gene mutations In 2010, the ELN introduced

TABLE 4 The 2022 European LeukemiaNet genetic risk stratification at diagnosis

Risk category	Genetic abnormality
Favorable	t(8;21)(q22;q22.1)/ <i>RUNX1::RUNX1T1</i> ^a
	inv(16)(p13.1;q22) or t(16;16)(p13.1;q22)/ <i>CBFB::MYH11</i> ^a
	Mutated <i>NPM1</i> ^b without <i>FLT3</i> -ITD
	bZIP in-frame mutated <i>CEBPA</i> ^c
Intermediate	Mutated <i>NPM1</i> ^b with <i>FLT3</i> -ITD
	Wild-type <i>NPM1</i> with <i>FLT3</i> -ITD
	t(9;11)(p21.3;q23.3)/ <i>MLLT3::KMT2A</i> ^d
	Cytogenetic and/or molecular abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1)/ <i>DEK::NUP214</i>
	t(v;11)(v;q23.3); <i>KMT2A</i> -rearranged
	t(9;22)(q34.1;q11.2)/ <i>BCR::ABL1</i>
	t(8;16)(p11;p13)/ <i>KAT6A::CREBBP</i>
	inv(3)(q21.3;q26.2) or t(3;3)(q21.3;q26.2)/ <i>GATA2, MECOM (EVI1)</i>
	t(3;v)(q26.2;v)/ <i>MECOM (EVI1)</i> -rearranged
	−5 or del(5q); −7; −17/abn(17p)
	Complex karyotype, ^e monosomal karyotype ^f
	Mutated <i>ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, or ZRSR2</i> ^g
	Mutated <i>TP53</i> ^h

Data from Döhner et al²⁰

- a** Co-existing mutations in the *KIT* and/or *FLT3* genes do not change the risk classification.
- b** The presence of the *NPM1* mutation in patients with adverse-risk chromosome abnormalities does not change the patients' categorization in adverse-risk group.
- c** Only in-frame *CEBPA* mutations in the basic leucine zipper (bZIP) region are associated with good outcome.
- d** The presence of t(9;11) outweighs the presence of rare co-existing gene mutations denoting adverse risk.
- e** Complex karyotype is defined as ≥3 unrelated chromosome abnormalities, with the exception of karyotypes containing any of the class-defining recurring genetic abnormalities²⁰ or karyotypes with 3 or more trisomies in the absence of structural abnormalities.
- f** Monosomal karyotype is defined as a karyotype with ≥2 autosomal monosomies or 1 autosomal monosomy and ≥1 structural chromosome abnormality other than t(8;21) or inv(16) or t(16;16).
- g** For now, these gene mutations should not be used as adverse prognostic markers if they co-exist with abnormalities denoting favorable risk.
- h** At a variant allele fraction of ≥10%, regardless of whether the *TP53* mutation is mono- or biallelic.

a genetic risk classification, which divided patients with AML into 4 genetic groups: favorable, intermediate I, intermediate II, and adverse, based on a combination of cytogenetic findings and 3 molecular genetic markers, namely, *FLT3*-ITD as well as *NPM1* and *CEBPA* mutations, which were assessed only in patients with CN-AML.⁶⁰ Thereafter, 2 large studies, each analyzing over 1500 patients with AML,^{61,89} demonstrated that the 2010 ELN classification⁶⁰ was able to reliably separate the favorable and adverse genetic groups from each other and from both intermediate I and intermediate II groups for such outcome end points as CR rates,⁶¹ DFS,⁶¹ relapse probability,⁸⁹ and OS.^{61,89} Multivariable analyses showed that the association of the 2010 ELN genetic groups with clinical outcome was independent from other established prognostic factors.⁶¹

In 2017, the ELN classification⁹⁰ was modified by, among others, merging 2 intermediate groups into 1, removing the requirement for mutation

analysis to be performed only in CN-AML, substituting biallelic *CEBPA* mutations for any *CEBPA* mutation, requiring determination of low and high allelic ratios for *FLT3*-ITD, and adding *ASXL1*, *RUNX1*, and *TP53* mutations as further adverse markers to be evaluated. Subsequently, several studies undertook validation of this classification in large series of patients with AML and suggested its refinements.^{91–94} The recently pre-published 2022 ELN classification²⁰ further increased the role of gene mutations in prognostic stratification of AML. Details of the 2022 ELN classification are provided in TABLE 4. It remains to be established whether the 2022 changes have improved the usefulness of the ELN genetic risk classification.

Summary and future directions Cytogenetic research of AML identified many recurrent chromosomal aberrations with a diagnostic and prognostic value. Cytogenetic analysis of pretreatment samples

is now obligatory in the diagnostic workup of patients with AML. Karyotypic findings are being used together with the mutational status of several leukemia-associated genes in such genetic risk classifications as the ones published by the ELN^{20,60,90} to guide the selection of the most effective treatment. However, the prognostic significance of some less common chromosome abnormalities, many of which are categorized in the intermediate-risk category, has not yet been ascertained, thus supporting the need for large collaborative studies. Additionally, continuing prospective studies is necessary to correlate cytogenetic and molecular genetic alterations with clinical outcomes of patients treated with novel and/or experimental therapies targeting specific genetic rearrangements.⁹⁵

This makes correct detection of acquired genetic alterations of utmost importance. Recent years have seen an explosion of studies using high-throughput NGS technologies, including whole-genome sequencing (WGS), to analyze AML genomes and characterize complex interactions of genetic variations in leukemogenesis.^{6,66,96-99} Duncavage et al⁹⁹ demonstrated the clinical utility of WGS for the evaluation of patients with AML and suggested WGS to be an alternative to cytogenetic analysis of myeloid malignancies. The advantages and limitations of this and other novel technologies¹⁰⁰ in hematologic malignant disorders have been recently reviewed in depth by Akkari et al.⁶⁸ The authors discussed clinical, logistic, technical, and financial implications and concluded that although clinical usefulness of WGS is undisputable, the implementation of WGS technology by clinical laboratories worldwide is currently unlikely. At least in the near future, standard chromosome banding analysis, FISH, and SNP arrays will remain the gold standard of cytogenetic testing in AML and other hematologic neoplasms. It is hoped that further development of emerging technologies may soon enable the use of tools allowing simultaneous delineation of transcriptomic, epigenomic, and proteomic characteristics of leukemic blasts that have clinical significance in AML.

ARTICLE INFORMATION

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