Chapter 13
Chromosome Abnormalities in Acute Myeloid Leukaemia and Their Clinical Importance

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Contents

Abstract Acute myeloid leukaemia (AML) is a neoplastic disease developing as a result of accumulation of somatic genetic alterations, both detectable microscopically as structural and numerical chromosome abnormalities and submicroscopic, such as gene mutations and changes in gene and microRNA expression, in haematopoietic progenitor cells. The cytogenetic landscape of AML is very heterogeneous with more than 300 recurrent abnormalities identified to date. Several of these abnormalities are now used to delineate separate disease entities in the World Health Organization Classification of AML. Moreover, pretreatment cytogenetic findings are among the most important, independent prognostic factors in both adults and children with AML.

Keywords Acute myeloid leukaemia • Cytogenetics • Mutation • Leukaemia diagnosis • Prognostication

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J.D. Rowley et al. (eds.), *Chromosomal Translocations and Genome Rearrangements in Cancer*, DOI 10.1007/978-3-319-19983-2_13

13.1 Introduction

Cytogenetic analyses of leukaemic blasts from patients diagnosed with acute myeloid leukaemia (AML) have been instrumental in determining the genetic basis of this disease, and greatly increased our understanding of its extraordinary histopathologic, immunophenotypic and clinical heterogeneity. Since 1973, when Janet D. Rowley employed the then newly introduced chromosome Q-banding technique [1] to describe the t(8;21) (q22;q22) [2], the translocation that turned out to be one of the more frequent and clinically important chromosome abnormalities in AML, more than 300 numerical and structural abnormalities, both balanced and unbalanced, have been identified as recurring [3–5]. Importantly, molecular dissection of breakpoints in numerous AML-associated translocations and inversions has led to cloning of genes that play a pivotal role in leukaemogenesis through deregulation of differentiation, proliferation and/or apoptosis (programmed cell death) of haematopoietic progenitor cells [6, 7]. However, it has become clear that a single cytogenetic abnormality is usually not sufficient to cause overt AML, whose development is usually a result of acquisition of multiple somatic alterations affecting different pathways within the same cell [8]. These include mutations activating genes involved in signal transduction of proliferation pathways that bestow a survival advantage and cause increased rate of cell proliferation. The other kind of leukaemia-associated rearrangements, often occurring in the form of intragenic mutations or gene fusions generated by reciprocal chromosome translocations or inversions, disrupt genes involved in the process of normal cell differentiation [9].

Even though abnormalities detectable using cytogenetic methodology constitute only a fraction of acquired genetic alterations in AML, chromosome abnormalities, both those that have been characterized molecularly and those that have not, constitute tumour markers with diagnostic and prognostic significance. Thus, several chromosome abnormalities and their molecular counterparts are now used to delineate separate disease entities in the World Health Organization (WHO) Classification of Tumours of Haematopoietic and Lymphoid Tissues [10]. Moreover, pretreatment karyotype has been repeatedly shown to be among the most important, independent prognostic factors for achievement of complete remission (CR), and duration of disease-free (DFS) and overall (OS) survival in both adults [11–23] and children with AML [24–26].

In this chapter, after a brief overview of general cytogenetic features of AML, we will present the role that cytogenetic findings play in establishing diagnosis of distinct disease subsets, and then discuss correlations between karyotype and clinical outcome of patients with AML.

13.2 General Cytogenetic Characteristics of AML

Following short-term, i.e., 24- or 48-h, unstimulated in vitro culture of bone marrow (or, less preferably, blood) samples aspirated before the start of therapy, sufficient numbers of good quality metaphase cells are obtained in a vast majority of patients,

with failure rates usually below 10 % [3, 12, 14, 23, 27]. Unsuccessful pretreatment cytogenetic investigation has been reported to portend an adverse prognosis in a recent study, in which 6 % of almost 1,500 AML patients whose samples were subjected to standard chromosome analysis had results deemed to be unacceptable [28]. Similarly, cytogenetic analysis failed in 7 % of over 700 children treated on the United Kingdom Medical Research Council (MRC) protocols [25].

Among patients with de novo AML, an abnormal karyotype containing one or more clonal chromosomal aberrations, namely, the same structural alteration or an extra copy of the same chromosome (trisomy) present in ≥2 metaphases or a loss of the same chromosome (monosomy) seen in ≥3 metaphases, is detected in 55–61 % of adult [14–16, 22, 23] and 76–78 % of paediatric cases [25, 26, 29, 30]. Although the reasons for this age discrepancy are unknown, they likely reflect biologic differences between adult and paediatric disease, as exemplified by age-related disparities in the incidence of specific chromosome abnormalities. For instance, reciprocal translocations, insertions and inversions involving chromosome band 11q23 that lead to rearrangements of the *KMT2A* gene (formerly known as *MLL* [31]) are approximately four times more frequent in children than in adults [17]. Moreover, their frequency diminishes substantially with age, being the highest, 51–58 %, in infants with AML below the age of 12 months [32–34], followed by 39 % of children aged from 13 to 24 months [35], 8–9 % of children older than 24 months [30, 35], and 4–7 % of adults [15, 16, 22], among whom only ≤3 % of those aged 60 years or older harbour 11q23/*KMT2A(MLL)* alterations [19, 20]. Likewise, a cryptic, i.e., not detectable by a routine cytogenetic study, translocation (5;11)(q35.2;p15.4), creating the *NUP98-NSD1* fusion gene, is found seven times more often in paediatric as opposed to adult AML patients [36]. Additionally, there are rare abnormalities that have been hitherto detected in children only. These include another cryptic translocation, t(7;12)(q36;p13), resulting in the *MNX1-ETV6* fusion gene [37, 38], and the t(1;22)(p13;q13), creating the *RBM15-MKL1* fusion gene [39]. The latter translocation has been hitherto found almost exclusively in young children under the age of 24 months [5, 39].

Conversely, t(8;21) and t(15;17)(q22-24;q12-21), the two most frequent translocations in both older children and adults diagnosed with AML [17], have not been detected in infants younger than 12 months [26, 35], although the incidence of t(8;21) is twice as high in older children as it is in adults. In contrast, inv(3)(q21;q26.2) and t(3;3)(q21;q26.2), as well as del(5q) and other unbalanced structural abnormalities leading to loss of material from the long arm of chromosome 5 (5q), are more frequent in adult rather than paediatric AML, as is complex karyotype with five or more chromosome aberrations. Finally, the incidence of certain abnormalities, such as inv(16)(p13.1q22) or t(16;16)(p13.1;q22), or trisomy of chromosome 8 (+8) is comparable in childhood and adult AML [5, 17].

Based on presumed significance, recurrent chromosome aberrations have been separated into primary and secondary ones. The former are considered to be most important and deemed to play an essential role in the early stages of leukaemogenesis. Primary abnormalities are quite specific for AML; that is, they are seldom (or never) found in other types of haematologic neoplasms or solid tumours, and can be found as the only microscopically detectable rearrangements in some patients [40].

They may have a profound influence on clinical characteristics of patients that harbour them, and thus several primary abnormalities have been chosen to denote distinct disease categories of AML (please see Sect. 13.3 below). Balanced rearrangements, such as reciprocal translocations, insertions and inversions, with presumed primary significance, which are currently known to be recurrent, are presented in Table 13.1 together with the genes they alter (whenever known) and the numbers of patients reported to carry these abnormalities in the literature and indexed in the Mitelman Database [5].

In addition to balanced abnormalities, a number of unbalanced aberrations, e.g., deletions, unbalanced translocations and isochromosomes, have been detected recurrently as a sole abnormality in AML patients, and consequently might represent rearrangements of primary importance in these patients. The most frequent of those are del(5q), del(7q), del(9q), del(12p), del(20q) and del(13q) [3, 5]. In contrast to reciprocal translocations or inversions, which result in rearrangements of specific genes, the molecular consequences of recurrent deletions do not seem to be restricted to a loss of and/or mutation in a single tumour suppressor gene, but rather to haploinsufficiency of multiple genes located in a deleted chromosome segment, i.e., diminished gene expression caused by the presence of only one functional allele remaining after a deletion of the second allele. However, it is possible that some of the deleted and/or underexpressed genes may still be more important than others, and recent studies have identified *CTNNA1* [41] and *EGR1* [42] as candidate tumour suppressor genes (TSGs) in AML with del(5q), and *CUX1* [43] and *KMT2C* (*MLL3*) [44] as TSGs in AML with del(7q).

Numerical chromosome changes can also be considered of potential primary import when they are found as the only cytogenetic alteration. The most frequent isolated numerical aberration in AML is +8, detected in ~4 % of adults with de novo AML, followed by recurrent, albeit less frequent, monosomy 7 (−7; 1 %), +11 (1 %), +13 (1 %), loss of chromosome Y (−Y; 1 %), +21 (0.5 %), and +4 (0.3 %) [45]. The molecular mechanisms whereby recurrent trisomies contribute to leukaemogenesis are mostly unknown. To date, only isolated +11 and +13 have been linked with particular molecular defects occurring in a high proportion of patients with these trisomies, namely, a partial tandem duplication of the *KMT2A(MLL)* gene [*KMT2A(MLL)*-PTD] associated with +11 [46] and *RUNX1* mutations associated with +13 [47]. Recently patients with +8 were shown to harbour recurrent gene mutations but the most frequent of them were each detected in ~30 % of patients [mutations in *RUNX1* and *ASXL1*, and the internal tandem duplication of the *FLT3* gene (*FLT3*-ITD)]; also common were *IDH2*, *DNMT3A* and *NPM1* mutations found in around 25 % of the patients [48].

Secondary abnormalities, which can accompany a primary abnormality either in all cells or be present in only a fraction of cells that harbour a primary aberration, are predominantly unbalanced, and include both numerical (trisomy, monosomy) and structural alterations (deletion, unbalanced translocation) [49]. Secondary abnormalities are generally less specific, and the same one can be recurrently found together with diverse primary aberrations in AML as well as in other types of leukaemia or even in non-haematologic malignancies. A prime example of such an

Table 13.1 Chromosome aberrations of presumed primary significance in AML

Cytogenetic abnormality[a]	Gene(s) involved	Number of AML cases with the abnormality[b]	No. (%) of AML cases with the abnormality occurring as a sole alteration[b]
Rearrangements involving band 1p36 and the *PRDM16* gene			
t(1;3)(p36.3;q21.3)	*RPN1-PRDM16*	49	32 (**65**%)
t(1;17)(p36;q21)	*PRDM16*	3	2 (**67**%)
Rearrangement involving band 1p36			
t(1;7)(p36;q34)	–	3	2 (**67**%)
Rearrangement involving band 1p13 and the *RBM15* gene			
t(1;22)(p13;q13)	*RBM15-MKL1*	41	33 (**80**%)
Rearrangement involving band 2p23			
t(2;4)(p23;q25)	–	2	2 (**100**%)
Rearrangement involving band 2p13			
t(2;12)(p13;p13)	–	3	1 (**33**%)
Rearrangement involving band 3p21			
t(3;7)(p21;q35)	–	4	2 (**50**%)
Rearrangements involving band 3q26 and the *MECOM* (*EVI1*) gene			
t(2;3)(p15-21;q26-27)	*MECOM*	22	10 (**45**%)
inv(3)(q21q26.2)	*RPN1-MECOM*	306	111 (**36**%)
t(3;3)(q21;q26.2)[c]	*RPN1-MECOM*	139	62 (**45**%)
t(3;8)(q26;q24)	*MECOM*	9	4 (**44**%)
Rearrangements involving band 5q31			
t(5;6)(q31;q21)	–	3	3 (**100**%)
t(5;21)(q31;q22)	–	2	1 (**50**%)
Rearrangement involving band 5q35 and the *NPM1* gene			
t(3;5)(q25;q35)[d]	*MLF1-NPM1*	71	58 (**82**%)
Rearrangement involving band 6q23 and the *MYB* gene			
t(X;6)(p11;q23)	*MYB-GATA1*	4	4 (**100**%)
Rearrangements involving band 8p11 and the *KAT6A* gene			
inv(8)(p11q13)	*KAT6A-NCOA2*	7	6 (**86**%)
t(8;16)(p11;p13)	*KAT6A-CREBBP*	115	68 (**59**%)
t(8;19)(p11;q13.3)	*KAT6A*	3	3 (**100**%)
t(8;22)(p11;q13)	*KAT6A-EP300*	4	3 (**75**%)
Rearrangements involving band 8p11 and the *FGFR1*gene			
t(6;8)(q27;p11)	*FGFR1OP-FGFR1*	4	3 (**75**%)
t(8;9)(p11;q33)	*CNTRL-FGFR1*	2	0
Rearrangement involving band 8q24			
t(8;14)(q24;q32)	–	5	1 (**20**%)
Rearrangement involving band 9p24 and the *JAK2* gene			
t(8;9)(p22;p24)	*PCM1-JAK2*	4	2 (**50**%)
Rearrangement involving band 9q34 and the *NUP214* gene			
t(6;9)(p23;q34)	*DEK-NUP214*	94	78 (**83**%)

(continued)

Table 13.1 (continued)

Cytogenetic abnormality[a]	Gene(s) involved	Number of AML cases with the abnormality[b]	No. (%) of AML cases with the abnormality occurring as a sole alteration[b]
Rearrangement involving band 9q34 and the *ABL1* gene			
t(9;22)(q34;q11.2)	*BCR-ABL1*	230	94 (**41**%)
Rearrangement involving band 9q34			
t(9;13)(q34;q12)	–	2	2 (**100**%)
Rearrangement involving band 10q22 and the *KAT6B* gene			
t(10;16)(q22;p13)	*KAT6B-CREBBP*	2	1 (**50**%)
Rearrangements involving band 11p15 and the *NUP98* gene			
t(1;11)(q24;p15)	*NUP98-PRRX1*	3	1 (**33**%)
t(2;11)(q31;p15)	*NUP98-HOXD11* or *NUP98-HOXD13*	6	5 (**83**%)
t(3;11)(p11;p15)	*NUP98-POU1F1*	2	2 (**100**%)
t(3;11)(q12;p15)	*NUP98-LNP1*	3	3 (**100**%)
t(4;11)(q23;p15)	*NUP98-RAP1GDS1*	4	2 (**50**%)
t(5;11)(q35.2;p15.4)	*NUP98-NSD1* or *STIM1-NSD1*	34	26 (**77**%)
t(7;11)(p15;p15)	*HOXA9-NUP98* or *HOXA11-NUP98* or *HOXA13-NUP98*	67	58 (**87**%)
t(7;11)(q22;p15)	*NUP98*	3	2 (**67**%)
t(8;11)(p11;p15)	*NUP98-WHSC1L1*	5	2 (**40**%)
t(9;11)(p22;p15)	*NUP98-PSIP1*	5	5 (**100**%)
t(9;11)(q34;p15)	*NUP98-PRRX2*	2	2 (**50**%)
t(10;11)(q23;p15)	*NUP98-HHEX*	4	2 (**50**%)
inv(11)(p15q22)	*NUP98-DDX10*	10	7 (**70**%)
t(11;11)(p15;q22)	*NUP98-DDX10*	2	2 (**100**%)
inv(11)(p15q23)	*NUP98-KMT2A(MLL)*	13	9 (**69**%)
t(11;12)(p15;p13)[e]	*NUP98-KDM5A*	6	1 (**17**%)
t(11;12)(p15;q13)	*NUP98-HOXC11* or *NUP98-HOXC13*	13	12 (**92**%)
t(11;17)(p15;q21)	*NUP98*	3	3 (**100**%)
t(11;17)(p15;q23)	*NUP98*	3	0
t(11;20)(p15;q12)	*NUP98-TOP1*	13	10 (**77**%)
Rearrangement involving band 11p15			
t(X;11)(q13;p15)	–	3	1 (**33**%)
Rearrangements involving band 11q13			
t(9;11)(p22;q13)	–	6	6 (**100**%)
t(11;12)(q13;p13)	–	4	1 (**25**%)
Rearrangement involving band 11q14 and the *PICALM* gene			
t(10;11)(p12;q14)	*MLLT10-PICALM*	72	36 (**50**%)
Rearrangements involving band 11q23 and the *KMT2A(MLL)* gene			
t(X;11)(q13;q23)	*KMT2A(MLL)-FOXO4*	3	3 (**100**%)

(continued)

Table 13.1 (continued)

Cytogenetic abnormality[a]	Gene(s) involved	Number of AML cases with the abnormality[b]	No. (%) of AML cases with the abnormality occurring as a sole alteration[b]
t(X;11)(q24;q23)	*KMT2A(MLL)-SEPT6*	4	4 (**100**%)
ins(11;X)(q23;q28q12)	*KMT2A(MLL)-FLNA*	2	2 (**100**%)
t(1;11)(p32;q23)	*KMT2A(MLL)-EPS15*	11	6 (**55**%)
t(1;11)(q21;q23)	*KMT2A(MLL)-MLLT11*	26	20 (**77**%)
t(2;11)(p21;q23)	*KMT2A(MLL)*	22	11 (**50**%)
t(2;11)(q37;q23)	*KMT2A(MLL)-SEPT2*	6	3 (**50**%)
t(4;11)(q21;q23)	*KMT2A(MLL)-AFF1*	29	18 (**62**%)
t(5;11)(q31;q23)	*KMT2A(MLL)-ARHGAP26*	5	5 (**100**%)
t(6;11)(q15;q23)	*KMT2A(MLL)-CASP8AP2*	3	2 (**67**%)
t(6;11)(q21;q23)	*KMT2A(MLL)-FOXO3*	5	2 (**40**%)
t(6;11)(q27;q23)	*KMT2A(MLL)-MLLT4*	93	83 (**89**%)
t(9;11)(p22;q23)	*KMT2A(MLL)-MLLT3*	264	177 (**67**%)
t(9;11)(q21;q23)	*KMT2A(MLL)*	3	3 (**100**%)
t(9;11)(q22;q23)	*KMT2A(MLL)*	6	5 (**83**%)
ins(10;11)(p11-13;q23q13-25)	*KMT2A(MLL)-MLLT10*	35	19 (**54**%)
ins(11;10)(q23;p12-13p11-15)	*KMT2A(MLL)-MLLT10*	4	2 (**50**%)
t(10;11)(p12;q13-23)	*KMT2A(MLL)-MLLT10*	47	28 (**60**%)
t(10;11)(p12;q23)	*KMT2A(MLL)-ABI1*	3	3 (**100**%)
t(10;11)(q21;q23)	*KMT2A(MLL)-TET1*	5	2 (**40**%)
t(11;11)(q13;q23)	*KMT2A(MLL)-ARHGEF17*	3	2 (**67**%)
del(11)(q23q23)[f]	*KMT2A(MLL)-ARHGEF12*	2	0
t(11;12)(q23;q13)	*KMT2A(MLL)-SARPN*	2	2 (**100**%)
t(11;14)(q23;q23)	*KMT2A(MLL)-GPHN*	3	3 (**100**%)
t(11;14)(q23;q32)	*KMT2A(MLL)-CEP170B*	3	3 (**100**%)
t(11;15)(q23;q15)	*KMT2A(MLL)-CASC5* or *KMT2A(MLL)-ZFYVE19*	8	1 (**13**%)
t(11;16)(q23;p13.3)	*KMT2A(MLL)-CREBBP*	10	5 (**50**%)
t(11;17)(q23;p13.1)	*KMT2A(MLL)-GAS7*	2	?1 (?**50**%)[g]
t(11;17)(q23;q12-21)	*KMT2A(MLL)-MLLT6*	42	34 (**81**%)
t(11;17)(q23;q23)	*KMT2A(MLL)*	4	2 (**50**%)
t(11;17)(q23;q25)	*KMT2A(MLL)-SEPT9*	34	22 (**65**%)[h]
t(11;19)(q23;p13.1)	*KMT2A(MLL)-ELL*	69	58 (**85**%)[h]
t(11;19)(q23;p13.3)	*KMT2A(MLL)-MLLT1*	47	19 (**41**%)
t(11;19)(q23;p13.2-13.3)	*KMT2A(MLL)-MYO1F*	4	1 (**25**%)
t(11;22)(q23;q11)	*KMT2A(MLL)-SEPT5*	8	5 (**63**%)
t(11;22)(q23;q13)	*KMT2A(MLL)-EP300*	6	4 (**67**%)

(continued)

Table 13.1 (continued)

Cytogenetic abnormality[a]	Gene(s) involved	Number of AML cases with the abnormality[b]	No. (%) of AML cases with the abnormality occurring as a sole alteration[b]
Rearrangements involving band 12p13 and the *ETV6* gene			
t(1;12)(q25;p13)	*ETV6-ABL2*	2	1 (**50**%)[i]
t(3;12)(q26;p13)	*ETV6-MECOM*	36	23 (**64**%)
t(4;12)(q12;p13)	*CHIC2-ETV6*	25	17 (**68**%)
t(5;12)(q13;p13)	*ETV6*	3	2 (**67**%)
t(5;12)(q31;p13)	*ACSL6-ETV6*	4	2 (**50**%)
t(7;12)(p15;p13)	*ETV6*	7	1 (**14**%)
t(7;12)(q36;p13)	*MNX1-ETV6*	20	2 (**10**%)
t(10;12)(q24;p13)	*ETV6-GOT1*	6	2 (**33**%)
inv(12)(p13q24)	*ETV6*	2	0
t(12;12)(p13;q13)	*ETV6*	3	0
t(12;13)(p13;q12)	*ETV6-CDX2* or *FLT3-ETV6*	4	2 (**50**%)
t(12;22)(p12-13;q11-13)	*ETV6-MN1*	22	5 (**23**%)
Rearrangements involving band 16q22 and the *CBFB* gene			
inv(16)(p13.1q22)	*MYH11-CBFB*	848	581 (**69**%)
t(16;16)(p13.1;q22)	*MYH11-CBFB*	45	37 (**82**%)
Rearrangements involving band 16q24 and the *CBFA2T3* gene			
inv(16)(p13.3q24.3)[j]	*CBFA2T3-GLIS2*	32	22 (**69**%)
t(16;21)(q24.3;q22)	*CBFA2T3-RUNX1*	23	6 (**26**%)
Rearrangement involving band 17q11 and the *TAF15* gene			
t(12;17)(p13;q11-12)	*TAF15-ZNF384*	4	4 (**100**%)
Rearrangements involving bands 17q12-21 and the *RARA* gene			
t(4;17)(q12;q21)	*FIP1L1-RARA*	2	0
t(5;17)(q35;q12-21)	*NPM1-RARA*	4	1 (**25**%)
t(11;15;17)(q13;q22;q12)	*PML-RARA*	2	2 (**100**%)
t(11;17)(q23;q21)	*ZBTB16-RARA*	11	7 (**64**%)
t(15;17)(q22-24;q12-21)[k]	*PML-RARA*	1171	843 (**72**%)
ins(15;17)(q22;q21q21)	*PML-RARA*	5	0
Rearrangements involving band 21q22 and the *RUNX1* gene			
t(1;21)(p36;q22)	*RUNX1-PRDM16*	2	2 (**100**%)
t(1;21)(p22;q22)	*RUNX1-CLCA2*	2	2 (**100**%)
t(3;21)(q26.2;q22.1)	*MECOM-RUNX1* or *RUNX1-RPL22P1*	63	30 (**48**%)
t(7;21)(p22;q22)	*RUNX1-USP42*	5	3 (**60**%)
t(8;21)(q22;q22)	*RUNX1-RUNX1T1*	1569	676 (**43**%)
ins(8;21)(q22;q22q22)	*RUNX1-RUNX1T1*	7	3 (**43**%)
ins(21;8)(q22;q13q22)	*RUNX1-RUNX1T1*	2	1 (**50**%)

(continued)

Table 13.1 (continued)

Cytogenetic abnormality[a]	Gene(s) involved	Number of AML cases with the abnormality[b]	No. (%) of AML cases with the abnormality occurring as a sole alteration[b]
ins(21;8)(q22;q21q22)	*RUNX1-RUNX1T1*	4	4 (**100**%)
ins(21;8)(q22;q22q22)	*RUNX1-RUNX1T1*	5	2 (**40**%)
t(1;8;21)(p13;q22;q22)	*RUNX1-RUNX1T1*	2	1 (**50**%)
t(5;8;21)(q31;q22;q22)	*RUNX1-RUNX1T1*	2	2 (**100**%)
t(8;10;21)(q22;q24;q22)	*RUNX1-RUNX1T1*	2	1 (**50**%)
t(8;12;21)(q22;p13;q22)	*RUNX1-RUNX1T1*	2	1 (**50**%)
t(8;12;21)(q22;q13;q22)	*RUNX1-RUNX1T1*	3	3 (**100**%)
t(8;15;21)(q22;q21;q22)	*RUNX1-RUNX1T1*	2	1 (**50**%)
t(8;17;21)(q22;q23;q22)	*RUNX1-RUNX1T1*	3	1 (**33**%)
t(8;20;21)(q22;q13;q22)	*RUNX1-RUNX1T1*	2	1 (**50**%)
t(11;21)(q13;q22)	*RUNX1-MACROD1*	2	1 (**50**%)
t(17;21)(q11.2;q22)	*RUNX1*	3	0
t(18;21)(q21;q22)	*RUNX1*	3	1 (**33**%)
Rearrangement involving band 21q22 and the *ERG* gene			
t(16;21)(p11;q22)	*FUS-ERG*	58	39 (**67**%)
Rearrangements involving band 21q22			
t(5;21)(q13;q22)	–	3	2 (**67**%)
t(6;21)(q13;q22)	–	4	1 (**25**%)
Rearrangement involving band Xp11			
t(X;10)(p11;p11)	–	4	1 (**25**%)

Data from Mitelman et al. [5]

[a]Chromosome aberrations disrupting the same chromosome band and the same gene, if known, are grouped together. Within a given group, aberrations are arranged according to the numerical order of the first chromosome involved. Each aberration is presented only once in the Table

[b]Number of cases with a given abnormality indexed in the Mitelman Database [5]

[c]Also interpreted as ins(3;3)(q21;q21q26)

[d]Also interpreted as t(3;5)(q21;q31)

[e]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. [183], Hollink et al. [36], and Gruber et al. [128]

[f]This deletion 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 Kourlas et al. [184] and Shih et al. [185]

[g]For one patient with *KMT2A(MLL)-GAS7* fusion, the karyotype description was not available [88]

[h]In one case, it is unknown whether t(11;19) was a sole abnormality or not. This case was not included in calculation of a percentage of cases with sole t(11;19)

[i]inv(16) was present in one case and a cryptic rearrangement resulting in the *CBFB-MYH11* gene fusion was detected in the other

[j]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. [128] and Masetti et al. [129]

[k]In the literature, the breakpoints in t(15;17) have been variously assigned to 15q22 or 15q24, and to 17q11, 17q12, 17q21 or 17q22. Based on the human genome sequence, the breakpoints are currently defined as 15q24.1 and 17q21.1

ubiquitous secondary aberration is trisomy of chromosome 8, which can be found not only in AML patients with such primary aberrations as t(6;9)(p23;q34), t(9;11)(p22;q23), t(9;22)(q34;q11.2), t(15;17) or inv(16)/t(16;16) but also in patients diagnosed with myelodysplastic syndromes (MDS), acute lymphoblastic leukaemia (ALL), non-Hodgkin lymphoma and several types of solid tumours, including Ewing sarcoma, myxoid liposarcoma, synovial sarcoma or clear cell sarcoma of tendons and aponeuroses [5]. Similarly widespread among various types of neoplasia, albeit less frequent, is an unbalanced structural abnormality, der(16)t(1;16)(q12-23;q12-24) [50]. Conversely, other secondary aberrations, e.g., loss of the entire chromosome X (–X), del(5q) or del(9q), seem to be much more specific for AML [49]. Notably, some primary abnormalities are predisposed to occur together with secondary changes more often than others. For instance, one or more secondary aberrations are detected in ~70 % of patients with either t(8;21) or inv(3)/t(3;3), whereas this is the case in only one-third of patients with t(15;17) or those with inv(16) and ~10 % of t(6;9)-positive patients [5, 51–55].

13.3 Chromosome Abnormalities Denoting Separate Entities in the WHO Classification of AML

Specific chromosome abnormalities together with their molecular counterparts were first used to identify separate disease entities in the 2001 revision of the WHO classification [56]. The latest 2008 revision increased the role of the cytogenetic findings and molecular genetics for disease categorization, and recognized seven separate groups within the "AML with recurrent genetic abnormalities" category (Table 13.2 and Fig. 13.1) [10]. Below, we will briefly review each of these groups.

13.3.1 AML with t(8;21)(q22;q22)/RUNX1-RUNX1T1

This is one of the two AML entities that together constitute core binding factor (CBF) AML. CBF-AML is so named because the genes rearranged by chromosome aberrations, i.e., *RUNX1*, located at 21q22 and disrupted by t(8;21)(q22;q22), and *CBFB*, located at 16q22 and disrupted by inv(16)(p13.1q22) or t(16;16)(p13.1;q22), encode, respectively, the alpha and beta subunits of the CBF complex, which is a heterodimeric transcription factor regulating transcription of genes encoding proteins involved in haematopoietic differentiation, such as interleukin-3, neutrophil elastase, macrophage colony-stimulating factor receptor or granulocyte-macrophage colony-stimulating factor [57]. Juxtaposition of 8q22 and 21q22 loci by the t(8;21) or its variants creates a chimeric *RUNX1-RUNX1T1* gene whose protein product is capable of a dominant-negative inhibition of the wild-type RUNX1 and impairment of normal haematopoiesis.

Table 13.2 Specific chromosomal alterations used to define AML entities in the WHO classification of tumours of haematopoietic and lymphoid tissues

Chromosome abnormalities and the related gene rearrangements denoting specific entities within the WHO category of AML entitled "AML with recurrent genetic abnormalities"
t(8;21)(q22;q22); *RUNX1-RUNX1T1*
inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
t(15;17)(q22;q12); *PML-RARA*
t(9;11)(p22;q23); *MLLT3-MLL*
t(6;9)(p23;q34); *DEK-NUP214*
inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*
t(1;22)(p13;q13); *RBM15-MKL1*
Chromosome abnormalities whose detection allows diagnosis of "AML with myelodysplasia-related changes"
Complex karyotype[a]
Unbalanced abnormalities
−7 or del(7q)
−5 or del(5q)
i(17q) or t(17p)
−13 or del(13q)
del(11q)
del(12p) or t(12p)
del(9q)
idic(X)(q13)
Balanced abnormalities
t(11;16)(q23;p13.3)[b]
t(3;21)(q26.2;q22.1)[b]
t(1;3)(p36.3;q21.1)
t(2;11)(p21;q23)[b]
t(5;12)(q32;p12)
t(5;7)(q32;q11.2)
t(5;17)(q32;p13)
t(5;10)(q32;q21)
t(3;5)(q25;q35)[b]

Data from Vardiman et al. [186]

[a]Defined as the presence of ≥3 unrelated abnormalities, none of which can be a balanced abnormality defining one of the entities within the WHO "AML with myelodysplasia-related changes" category

[b]Because this translocation can be often found in therapy-related AML, therapy-related disease should be excluded first for this translocation to support the diagnosis of AML with myelodysplasia-related changes

Translocation (8;21) is among the most frequent chromosomal aberrations in AML, occurring in 5–6 % of adults (Fig. 13.2 and data from reference no. [17]) and 12–14 % of children with AML [17, 25]. Much less often, cytogenetic variants of t(8;21) are encountered; these include recurrent insertions, i.e., ins(8;21) and ins(21;8), and three-way translocations, e.g., t(8;10;21), t(8;12;21), t(8;17;21) or t(8;20;21) (Table 13.1). Although the t(8;21) represents a primary chromosome

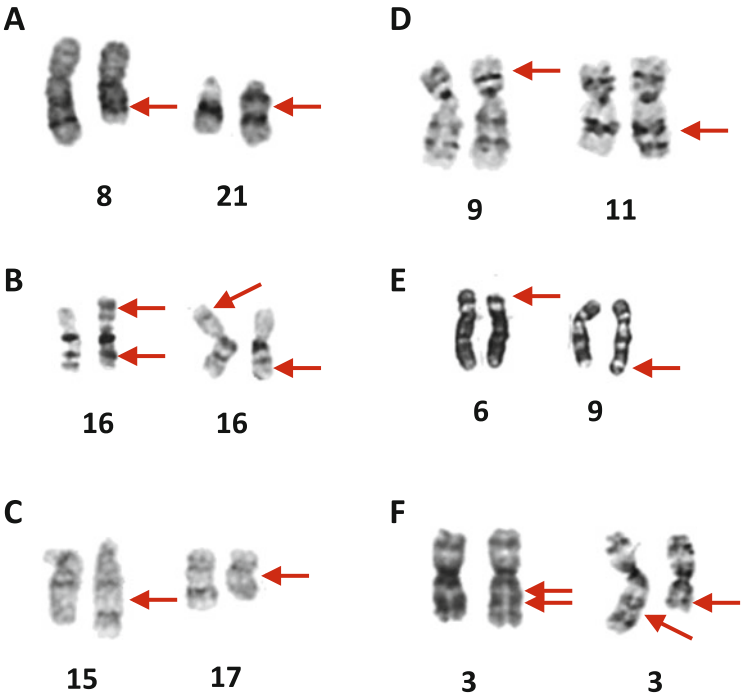

Fig. 13.1 G-banded partial karyotypes showing chromosome abnormalities used to denote specific entities in adults with AML within the WHO category entitled "AML with recurrent genetic abnormalities". (**a**) t(8;21)(q22;q22). (**b**) inv(16)(p13.1q22) (*left*) and t(16;16)(p13.1;q22) (*right*). (**c**) t(15;17)(q22;q12). (**d**) t(9;11)(p22;q23). (**e**) t(6;9)(p23;q34). (**f**) inv(3)(q21q26.2) (*left*) and t(3;3)(q21;q26.2) (*right*). *Arrows* indicate breakpoints in the rearranged chromosomes

aberration, it is detected as the sole cytogenetic abnormality in only ~30 % of patients. Among secondary chromosome abnormalities, the most frequent by far is −Y, found in roughly 60 % of male patients with t(8;21), followed by −X, seen in 33–40 % of female patients, and by del(9q) (17 %), +8 (5–7 %), and +4 (4 %) [52, 53]. The molecular consequences of these secondary aberrations are not yet well understood, although there are data indicating that del(9q) may act through loss of *TLE1* and *TLE4*, the putative tumour suppressor genes mapped to 9q21.3 [58]. Clearly, additional genetic rearrangements cooperating with *RUNX1-RUNX1T1* are required because the presence of *RUNX1-RUNX1T1* alone has been shown to be insufficient to induce leukaemia [59]. Recent studies revealed that such cooperating alterations include mutations in the *KIT* gene (detected in around 25 % of patients), *NRAS* and *KRAS* mutations (10–20 %), internal tandem duplications of the *FLT3* gene (*FLT3*-ITD; 7 %) and mutations in the *FLT3* tyrosine kinase domain (*FLT3*-TKD; 4 %) [59].

With regard to morphology, the presence of the t(8;21)/*RUNX1-RUNX1T1* is strongly, albeit not entirely, associated with AML with maturation in the neutrophil lineage. An increased number of eosinophil precursors, but without abnormalities

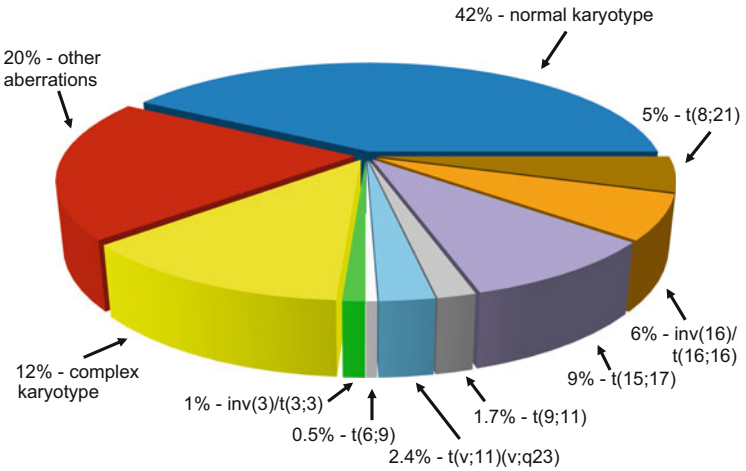

Fig. 13.2 Frequency distribution of patients harbouring chromosome abnormalities among 4,246 adults with AML enrolled onto CALGB companion protocol 8461. "Complex karyotype" denotes the presence of three or more abnormalities other than t(8;21), inv(16) or t(16;16), t(15;17), t(9;11), t(v;11)(v;q23), t(6;9), or inv(3) or t(3;3). "t(v;11)(v;q23)" denotes balanced rearrangements involving band 11q23 other than t(9;11)(p22;q23)

encountered in AML with inv(16), and distinctive pink-colored cytoplasm of neutrophils appear to differentiate t(8;21)-positive patients from other patients with AML with maturation who do not carry this rearrangement [60]. Detection of the t(8;21)/*RUNX1-RUNX1T1* is sufficient to make a diagnosis of AML, even if the overall percentage of marrow blasts is lower than 20 % [10].

Patients with the t(8;21)/*RUNX1-RUNX1T1* have a relatively favourable prognosis [14–17, 52, 53], especially when repetitive cycles of high-dose cytarabine are administered as postremission therapy [61]. While the clinical outcome does not seem to be affected by secondary chromosome aberrations, *KIT* mutations have been repeatedly shown to constitute an adverse prognostic factor [59, 62]. A recent study found that high cumulative incidence of relapse (CIR), but not shorter OS, was associated only with higher relative *KIT* mutant levels, i.e., when a percentage of mutated *KIT* alleles was ≥25 % of total *KIT* alleles [63]. Likewise, shorter OS, but not higher CIR, were found associated with high levels of *FLT3*-ITD [63]. These results await corroboration.

13.3.2 AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/CBFB-MYH11

The second CBF-AML entity is characterized by the presence of inv(16)(p13.1q22), or, less frequently, t(16;16)(p13.1;q22), which together are found in 5–6 % of adult (Fig. 13.2 and data from reference no. [17]) and 6–7 % of paediatric AML patients [17, 25]. At the molecular level, both these chromosome rearrangements fuse *CBFB*

with *MYH11*, a gene mapped to 16p13.1. The chimeric CBFB-MYH11 protein retains the ability to interact with *RUNX1* and block CBF dependent transcription. The genomic breakpoints within *CBFB* and *MYH11* are quite variable and, consequently, more than ten differently sized *CBFB-MYH11* fusion transcript variants have been reported [64]. The most common fusion type A is found in 85 % of patients, followed by type D and type E fusions, each detected in 5–10 % of patients. Although biologic and prognostic implications of different fusions are still unclear, our recent study revealed striking differences in the frequency of secondary chromosome aberrations and *KIT* mutations between patients with type A fusions compared with patients carrying non-type A fusions. Specifically, the latter harboured significantly more often +8 and +21 but less often, +22, which was detected exclusively in patients with type A fusions, as were prognostically unfavourable *KIT* mutations [64].

The presence of the inv(16)/t(16;16)/*CBFB-MYH11* is highly correlated with myelomonocytic marrow morphology and abnormal eosinophils, which constitute a pathognomonic feature of this AML type. The abnormal eosinophils are almost always present, although they may be very rare, comprising no more than as 0.2 % of marrow cells. As in the case of the t(8;21)/*RUNX1-RUNX1T1*, patients with the inv(16)/t(16;16)/*CBFB-MYH11* and marrow blasts percentages <20 % are diagnosed with AML [10].

Patients with the inv(16)/t(16;16)/*CBFB-MYH11* have a relatively favourable prognosis, particularly if their postremission treatment includes three to four cycles of high-dose cytarabine [65]. Among the recurrent secondary chromosome abnormalities, only the presence of +22 has been repeatedly found to reduce the patients' risk of relapse [52, 53, 66], and lengthen their OS duration [22, 66]. On the other hand, mutations in *KIT* [62, 66] and *FLT3* mutations, predominantly *FLT3*-TKD [59, 66, 67], adversely influence the patients' OS. Mutations in *NRAS* and *KRAS* are frequent (acquired by over 50 % of patients), but have not been found to constitute a prognostic factor [63, 66, 67]. However, their presence appears to render AML blasts more sensitive to higher doses of cytarabine given as part of postremission treatment [68].

13.3.3 AML with t(15;17)(q22;q12); PML-RARA

The third category of "AML with recurrent genetic abnormalities" in the WHO classification is acute promyelocytic leukaemia (APL), which constitutes 8–9 % of adult (Fig. 13.2 and data from reference no. [17]) and up to 10 % of childhood AML cases [17, 25]. The underlying molecular event in APL leukaemogenesis is creation of the gene fusion between the *RARA* gene, encoding the retinoic acid receptor α, and one of a number of partner genes, among which *PML* is by far the most frequent (98–99 %). In the majority of patients, the *PML-RARA* fusion gene is generated by a reciprocal translocation t(15;17) or its three- or four-way variants that involve one

or two chromosomes in addition to chromosomes 15 and 17. However, in ~4 % of APL patients, the karyotype may be normal because the *PML-RARA* fusion is formed by an insertion of a tiny segment containing the *RARA* gene into the *PML* locus [69]. Such cryptic rearrangements can be identified only by using reverse transcription-polymerase chain reaction (RT-PCR), fluorescence in situ hybridization (FISH), or next generation mRNA or genome sequencing, but neither marrow morphology nor clinical characteristics and response to treatment differ between patients with the standard t(15;17) and those with hidden alterations identifiable exclusively by molecular techniques [70].

In less than 1 % of APL patients, genes other than *PML* are fused with *RARA* as a result of variant rearrangements, mostly translocations. These variant *RARA* rearrangements and the resulting gene fusions include four reported recurrently, i.e., t(11;17)(q23;q21) and *ZBTB16-RARA*; t(4;17)(q12;q21) and *FIP1L1-RARA*; t(5;17)(q35;q21) and *NPM1-RARA*; and the microscopically undetectable *STAT5B-RARA* fusion [5, 71]. An additional five rearrangements were discovered thus far in single patients, viz, t(11;17)(q13;q21) and *NUMA1-RARA* [72]; t(X;17)(p11;q21) and *BCOR-RARA* [73]; der(2)t(2;17)(q32;q21) and *NABP1-RARA* [74]; t(3;17)(q26;q21) and *TBL1XR1-RARA* [75]; and a complex rearrangement within chromosome 17q involving an insertion of the *RARA* gene into a locus distal to the *PRKAR1A* gene at 17q24.2 and subsequent deletion creating the *PRKAR1A-RARA* fusion [76].

Ascertainment of the type of the fusion gene is important because it can determine whether the patient will respond to targeted therapy using all-*trans*-retinoic acid (ATRA) and arsenic trioxide (ATO). Therapeutic doses of ATRA have been shown to be effective in patients with the classic *PML-RARA* fusion as well as in those with variant fusions between *RARA* and the *FIP1L1*, *NPM1*, *NUMA1*, *BCOR* and *NABP1* genes [73, 74, 77]. In contrast, APL variants with *ZBTB16-RARA* and *STAT5B-RARA* fusions are resistant to ATRA, and are associated with a worse prognosis. Moreover, only APL with *PML-RARA* has been hitherto responsive to treatment with ATO [77].

Translocation (15;17)/*PML-RARA* and its variants are strongly correlated with distinct marrow morphology, in which abnormal promyelocytes dominate. Two major morphologic subtypes of APL are recognized, namely hypergranular (or typical) present in ~75 % of cases and microgranular (or hypogranular). The latter can sometimes be misdiagnosed morphologically as acute monocytic leukaemia and is associated with very high leukocyte counts with abundant abnormal microgranular promyelocytes, and the presence of the *FLT3*-ITD [10, 78]. Similarly to CBF-AML, patients with the t(15;17)/*PML-RARA* are diagnosed with APL regardless of marrow blast percentage [10].

The prognosis of APL patients with the t(15;17)/*PML-RARA*, which historically had been one of the worst among subtypes of AML, has become the most favourable with the use of treatment regimens containing ATRA and/or ATO, with CR rates of 90–95 % and a cure rate of up to 85 % in recent studies [79]. Secondary abnormalities, which accompany the t(15;17) in roughly one-third of APL patients

at diagnosis and include +8 or trisomy of 8q most frequently, do not seem to affect the patients' prognosis [22, 80]. However, in a recent study patients with a complex karyotype, i.e., two or more aberrations in addition to the t(15;17), had a significantly lower CR rate and shorter OS than patients with non-complex karyotype [81]. Since almost one-half of patients with a complex karyotype had aberrations involving chromosome 17, which mostly led to the loss of 17p and the *TP53* locus, the role of potential loss and/or mutations of *TP53* should be examined in APL with a complex karyotype [82]. *FLT3*-ITD, which is an established adverse prognostic factor in cytogenetically normal AML (CN-AML), has been associated with an increased incidence of induction death among adults [83] and children [84] with APL, but none of the large studies demonstrated significant differences in CR rates [83, 85], risk of relapse [83, 85], CIR [78], DFS [85], event-free survival (EFS) [86], or OS [83, 86] between patients with and without *FLT3*-ITD. However, the presence of both *FLT3*-ITD [78] and secondary chromosome abnormalities [87] was associated with shorter survival among APL patients who experienced a relapse.

13.3.4 AML with t(9;11)(p22;q23); KMT2A(MLL)-MLLT3

Translocation (9;11)(p22;q23) is the most frequent among over 120 chromosome abnormalities involving chromosome band 11q23 and the *KMT2A(MLL)* gene [88]. The *KMT2A(MLL)* gene encodes a DNA-binding protein methylating histone H3 lysine 4 (H3K4), and positively regulating expression of multiple genes including the *HOX* genes [89]. The translocation occurs in ~2 % of adults with AML (Fig. 13.2 and data from reference no. [17]) and results in a fusion of the *KMT2A(MLL)* gene with *MLLT3*, a gene residing at band 9p22 and encoding a nuclear protein containing serine-rich and proline-rich regions, which appear to be important for leukaemogenesis. Almost two-thirds of the cases carry t(9;11) as an isolated chromosome abnormality; +8 is the most frequent secondary aberration, seen in ~20 % of patients; followed by secondary +19 and +21. Morphologically, t(9;11) is strongly associated with acute monocytic and myelomonocytic leukaemias [10].

In most [16, 22, 90–92], but not all [25, 93], studies, patients with the t(9;11) had better clinical outcome that patients harbouring other rearrangements involving 11q23/*KMT2A(MLL)*, referred to in the WHO classification as "variant *MLL* translocations" [10], the recurrent of which are listed in Table 13.1. Consequently, t(9;11) has been classified in the intermediate cytogenetic-risk category [16, 22], whereas the variant *KMT2A(MLL)* translocations, which altogether comprise ~2 % of adults with AML (Fig. 13.2), belong to the unfavourable prognostic group [16, 22]. Interestingly, in a recent large paediatric series [94], +19 occurring as an abnormality secondary to t(9;11) was an independent adverse prognostic factor for incidence of relapse, EFS and OS. In contrast, t(9;11)-positive patients with a secondary +8 had a significantly lower incidence of relapse than children without +8 [94]. These results require corroboration.

13.3.5 AML with t(6;9)(p23;q34); DEK-NUP214

A recurrent t(6;9)(p23;q34) that creates the fusion gene *DEK-NUP214* is relatively rare, being detected in 0.5–0.7 % of adult (Fig. 13.2 and data from reference no. [55]) and 1.4–1.7 % of childhood patients with AML [55, 95]. Secondary cytogenetic abnormalities are uncommon, and ~90 % of the cases harbour t(6;9) or its rare three-way variants as the only chromosome change. On the other hand, the incidence of *FLT3*-ITD in patients with t(6;9) is the highest among AML cytogenetic subtypes, with 67–73 % of t(6;9)-positive patients carrying this mutation [55, 95].

Frequent morphologic features in adults with t(6;9) include increased basophilia (≥2 %), which is otherwise rare in AML, and single or multilineage dysplasia in the marrow [55].

The clinical outcome of patients treated with chemotherapy is very poor both in adults and children [55, 95], and does not seem to be associated with the presence or absence of *FLT3*-ITD [95]. However, the patients' prognosis can be considerably improved by allogeneic stem cell transplantation (SCT), especially in patients who are in CR at the time of transplantation [96]. Moreover, the DEK-NUP214 fusion protein was recently shown to increase cellular proliferation through upregulation of the signal transduction protein mTOR, thus indicating that t(6;9)-positive patients might benefit from treatment with mTOR inhibitors [97].

13.3.6 AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1(MECOM)

Both inv(3)(q21q26.2), and its less frequent variant t(3;3)(q21;q26.2), which in the past was occasionally described as ins(3;3)(q26;q21q26), lead to the fusion of the *RPN1* gene with a gene encoding the zinc finger transcription factor EVI1, currently re-named *MECOM* [31], that is involved in normal haematopoiesis and overexpressed as a result of inv(3) and t(3;3). These chromosome abnormalities are together detected in 1–1.4 % of patients with AML (Fig. 13.2 and data from reference no. [51]), both de novo and secondary to antecedent MDS, and are associated with multilineage dysplasia, abnormal megakaryopoiesis with micromegakaryocytes in the marrow and either normal or increased platelet counts and higher white blood cell counts at diagnosis [51, 98]. The majority of patients carry secondary aberrations in addition to inv(3) and t(3;3), of which monosomy 7 is the most frequent being present in one-half of all patients. In fact, the high incidence of −7 coexisting with inv(3), which in itself is a subtle rearrangement and may be occasionally missed by a cytogenetic laboratory [27], has alerted cytogeneticists to always examine chromosome 3 homologs closely in patients with a seemingly sole −7.

The clinical outcome of patients with inv(3) and t(3;3) has been repeatedly found to be very poor irrespective of the presence of −7 [16, 22], although in one study [51], patients with −7 fared even worse than those who did not harbour this monosomy. At present, allogeneic SCT appears to be the only therapeutic option capable of improving the dismal prognosis of inv(3) or t(3;3)-positive patients [99].

13.3.7 AML with t(1;22)(p13;q13); RBM15-MKL1

In contrast to the aforementioned subtypes of AML, AML with the t(1;22) occurs exclusively in children, 80 % of whom are younger than 1 year, and is very rare, with less than 50 cases reported worldwide to date [5]. The majority of patients have the t(1;22) as the sole chromosome alteration, but complex, hyperdiploid karyotypes are detected in ~40 % of the patients [39]. Strikingly, most infants below the age of 6 months carried the t(1;22) alone, whereas in over 80 % of older children the t(1;22) was part of a complex karyotype [39]. Essentially all patients present with acute megakaryoblastic leukaemia with extramedullary involvement, most often hepatosplenomegaly. While the prognosis was described initially as poor, albeit with a few long term survivors [39], Duchayne et al. [100] reported that those t(1;22)-positive children who did not die early responded well to intensive AML chemotherapy and/or SCT and had prolonged DFS and OS.

13.4 AML with Myelodysplasia-Related Changes

For patients without any of the recurrent genetic abnormalities described above, the WHO classification uses the presence of further specific cytogenetic findings to place some of them in another AML category called “AML with myelodysplasia-related changes”. To be assigned to this AML subtype, the patient with ≥20 % of bone marrow or blood blasts should fulfil one of three criteria: (i) disease progression from an antecedent MDS or myelodysplastic/myeloproliferative neoplasm; (ii) morphologic evidence of multilineage dysplasia in the marrow; or (iii) the presence of one of nine specific reciprocal translocations or eight unbalanced abnormalities and/or a complex karyotype with ≥3 unrelated chromosome changes (Table 13.2) [10]. Notably, fulfilment of the cytogenetic criterion is sufficient for diagnosis even in the absence of the other two criteria. With the exception of the t(3;5)(q25;q35)/*NPM1-MLF1*, which in the past was also described as t(3;5)(q21;q31), and is detected in ~0.4 % of AML patients and associated with an intermediate prognosis [22], the remaining reciprocal translocations are very rare, as is an unbalanced idic(X)(q13), which makes assessment of their clinical significance difficult [101]. Other unbalanced aberrations, save for del(11q) and del(9q), occur more often than not as part of a complex karyotype and are associated with poor outcome. However, the prognostic significance of −5 outside of a complex

karyotype is essentially unknown because it is very rare in patients with non-complex karyotypes. Additionally, patients with a complex karyotype with monosomy 5 ascertained by standard cytogenetic analysis also do not harbour true −5, since almost invariably parts from an apparently missing chromosome 5 are discovered using spectral karyotyping (SKY) [102] or FISH [103] in marker chromosomes and/or unbalanced structural aberrations that are partially recognized in G-banded preparations. Preliminary data indicate that patients with del(9q) differ clinically from patients with other unbalanced abnormalities, which indicates that their inclusion in the WHO "AML with myelodysplasia-related changes" should be reconsidered [101].

13.5 Correlations Between Cytogenetic Findings and Clinical Outcome of AML Patients

Pretreatment cytogenetic findings have been repeatedly proven to constitute one of the most important, independent prognostic factors in AML. This was demonstrated for the first time in the early 1980s by the results of a large, prospective, multi-centre study conducted by the Fourth International Workshop on Chromosomes in Leukaemia [104]. Both subsequent follow-up Workshop studies [11, 105, 106] and several single-institution [12, 107, 108] and large, collaborative multi-institutional studies [13–16, 18–22, 24–26, 109] have confirmed that karyotype at diagnosis is an independent prognostic determinant for achievement of CR, DFS, relapse risk and OS. Consequently, both the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology [110] and the European LeukemiaNet (ELN) [111] stipulate that cytogenetic analysis is an obligatory component of the diagnostic work-up of patients with AML.

A number of large collaborative studies proposed prognostic prioritization systems classifying AML patients into favourable, intermediate or adverse risk groups based on pretreatment cytogenetic findings (Table 13.3) [15, 16, 22]. These cytogenetic risk systems have many common aspects, but also differ with respect to certain features (Table 13.3). In the MRC classification, first proposed in 1998 [14] and revised in 2010 [22], all abnormalities that are not categorized as favourable or adverse, and do not occur together with additional chromosome alterations belonging to either the favourable or adverse group, are classified in the intermediate-risk group. In contrast, both the Cancer and Leukaemia Group B (CALGB; currently known as the Alliance for Clinical Trials in Oncology) [16] and Southwest Oncology Group/Eastern Cooperative Oncology Group (SWOG/ECOG) [15] classifications explicitly categorize particular abnormalities into risk groups, which means that aberrations not frequent enough for analysis remain outside of these prognostic schemata. In addition, SWOG/ECOG and MRC classify patients with a given abnormality into a risk group once [15, 22], whereas CALGB provides risk-group assignment separately for probability of attaining a CR, CIR and OS [16]. As a result, in the latter schema, patients with the same abnormality [e.g., t(6;11)

Table 13.3 Cytogenetic-risk categorization of adult patients with the more frequent chromosome abnormalities in the three major collaborative studies of AML

Abnormality	Cytogenetic risk group				
	CALGB[a]			SWOG/ECOG[b]	MRC[c]
	Probability of CR attainment	Cumulative incidence of relapse	Overall survival		
t(8;21)(q22;q22)	Favourable	Favourable	Favourable	Favourable[d]	Favourable
inv(16)(p13.1q22) or t(16;16)(p13.1;q22)	Favourable	Favourable	Favourable	Favourable	Favourable
t(15;17)(q22-24;q12-21)	NA	NA	NA	Favourable	Favourable
t(9;11)(p22;q23)	Intermediate	Intermediate	Intermediate	Adverse[e]	Intermediate
t(6;11)(q27;q23)	Intermediate	Not classified	Adverse	Adverse[e]	Adverse
t(11;19)(q23;p13.1)	Intermediate	Not classified	Adverse	Adverse[e]	Intermediate[f]
abn(11q23)	Not classified	Not classified	Not classified	Adverse[e]	Adverse
t(6;9)(p23;q34)	Intermediate	Not classified	Adverse	Adverse[e]	Intermediate
inv(3)(q21q26.2) or t(3;3)(q21;q26.2)	Adverse	Not classified	Adverse	Adverse[g]	Adverse
-7	Intermediate	Adverse	Adverse	Adverse	Adverse
del(7q)	Not classified	Not classified	Not classified	Adverse	Adverse
loss of 7q	Intermediate	Not classified	Intermediate	Unknown	Adverse
-5	Not classified	Not classified	Not classified	Adverse	Adverse
del(5q)	Intermediate	Not classified	Intermediate	Adverse	Adverse
loss of 5q	Not classified	Not classified	Not classified	Unknown	Adverse
abn(17p)	Not classified	Not classified	Not classified	Adverse	Adverse
del(11q)	Intermediate	Not classified	Intermediate	Adverse[e]	Intermediate
abn(12p)	Adverse	Not classified	Intermediate	Intermediate/ Unknown[h]	Intermediate
del(9q)	Intermediate	Intermediate	Favourable/ Intermediate	Adverse	Intermediate
complex karyotype ≥3 abnormalities	Adverse	Adverse	Adverse	Adverse	NA

(continued)

Table 13.3 (continued)

complex karyotype ≥4 abnormalities	NA	NA	NA	NA	Adverse[i]
complex karyotype ≥5 abnormalities	Adverse	Adverse	Adverse	Adverse	NA
None (i.e., a normal karyotype)	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate
-Y	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate
+6	Not classified	Not classified	Not classified	Intermediate	Intermediat
+8 sole	Intermediate	Intermediate	Adverse	Intermediate	Intermediate
+8 with 1 other abnormality	Intermediate[j]	Intermediate	Adverse	Intermediate	Intermediate[k]
+11	Intermediate	Intermediate	Intermediate	Unknown	Intermediate
+13	Intermediate	Intermediate	Intermediate	Unknown	Intermediate
+21	Intermediate	Adverse	Intermediate	Unknown	Intermediate
t(9;22)(q34;q11.2)	NA	NA	NA	Adverse	Adverse
abn(3q)	Not classified	Not classified	Not classified	Adverse	Adverse[l]
del(16q)	Not classified	Not classified	Not classified	Favourable	Intermediate
del(20q)	Intermediate	Not classified	Intermediate	Adverse[m]	Intermediate
abn(20q)	Not classified	Not classified	Not classified	Adverse	Intermediate
abn(21q)	Not classified	Not classified	Not classified	Adverse	Intermediate

abn abnormality, *CALGB* Cancer and Leukaemia Group B, *CR* complete remission, *MRC* United Kingdom Medical Research Council; *NA* not available, *SWOG/ECOG* Southwest Oncology Group/Eastern Cooperative Oncology Group

[a]Data from Byrd et al. [16] who analyzed 1,213 adults (age range, 15–86 years, median 52 years) with de novo AML, excluding patients with t(15;17) and t(9;22)

[b]Data from Slovak et al. [15] who analyzed 609 adults (age range, 16–55 years, median 39 years) with AML

[c]Data from Grimwade et al. [22] who analyzed 5,876 adults (age range, 16–59 years, median 44 years) with de novo or secondary AML

[d]Patients with t(8;21) are classified in the favourable category only if the karytoype is not complex (i.e., comprises one or two abnormalities) and does not contain del(9q). Both del(9q) and complex karyotype were classified in the adverse category

[e]Would be included in "abn 11q" category

[f]t(11;19)(q23;p13.1) was not distinguished from t(11;19)(q23;p13.3) and both were included in the "t(11;19)(q23;p13)" category in this study

[g]Would be included in "abn 3q" category

[h]del(12p) classified in the intermediate category, other abnormalities involving 12p seemingly classified as unknown

[i]After exclusion of patients with abnormalities conferring favourable or adverse prognosis

[j]Any abnormality other than t(8;21), inv(16), t(16;16) or t(9;11)

[k]The abnormality occurring together with +8 may not by classified in the favourable or adverse category

[l]Excluding t(3;5)(q21~25;q31~35)

[m]Would be included in "abn 20q" category

(q27;q23)] may be categorized in the intermediate-risk group concerning probability of induction success, in the adverse-risk group with regard to OS and not classified for CIR, because the number of patients who achieved a CR was too low for CIR risk-group assignment [16].

Nevertheless, in spite of the differences among classifications, many chromosome aberrations are uniformly assigned to either a favourable risk, e.g., t(15;17) and CBF-AML-related inv(16)/t(16;16) and t(8;21), intermediate risk, e.g., −Y, or adverse risk, e.g., inv(3) or t(3;3), −7 and a complex karyotype, categories. Notably, in contrast to CALGB and MRC [16, 22], the SWOG/ECOG classification included patients with del(16q) in the favourable risk-group [15]. However, we believe that unless such patients are shown to carry a misinterpreted inv(16) or t(16;16) and the resultant *CBFB-MYH11* gene fusion, they should not be included in the favourable risk group because del(16q) is typically detected in AML with morphology other than that of acute myelomonocytic leukaemia with abnormal eosinophils, and is not associated with a favourable outcome [112–114].

All classifications agree that complex karyotype is associated with unfavourable prognosis, but the definition of a complex karyotype differs among studies. While SWOG/ECOG [15], CALGB [16], and the German AML Study Group [115] considered as complex karyotypes those containing three or more abnormalities, a multi-centre Italian study defined complex karyotype as "the presence of a clone with more than three cytogenetic abnormalities" [109], and the initial MRC definition specified a complex category as "the presence of a clone with at least five unrelated cytogenetic abnormalities" [14]. In most instances, the definition of complex karyotype did not include patients with t(8;21), inv(16)/t(16;16), or t(15;17) following data showing that patients with these abnormalities constitute separate biological and clinical entities, in which increased karyotype complexity does not affect adversely clinical outcome in a manner comparable to other patients with ≥3 abnormalities [14, 16, 52, 53, 80]. In some studies, the complex karyotype category also excludes patients harbouring t(9;11)(p22;q23) [16, 19] or any balanced rearrangement involving band 11q23 [20]. The revised MRC classification defined complex karyotype as one with four or more abnormalities, which excluded all chromosome changes that themselves bestowed either a favourable prognosis [i.e., t(8;21), inv(16)/t(16;16), t(15;17)] or an unfavourable prognosis [i.e., abn(3q) other than t(3;5); inv(3))/t(3;3); add(5q), del(5q), −5; −7, add(7q)/del(7q); t(6;11), t(10;11)(p12;q23), other t(11q23) excluding t(9;11) and t(11;19)(q23;p13) (19p13.1 or 19p13.3 breakpoint was not specified); t(9;22)(q34;q11.2); −17/abn(17p)] [22]. Finally, the ELN specified complex karyotype as having ≥3 abnormalities in the absence of any balanced rearrangements used by the WHO Classification to denote "AML with recurrent genetic abnormalities" [111]. Because the latter two definitions of complex karyotype have been introduced recently, it is currently unknown whether one of them is better than the other.

Depending on the definition, AML patients with a complex karyotype comprise 10–12 % of all AML patients, if complex karyotype is defined as ≥3 aberrations [15, 16, 115], or 8–9 %, if ≥5 aberrations are necessary for complex karyotype recognition [14–16, 18]. In individual patients, complex karyotypes can comprise

variable numbers of chromosome aberrations that in rare cases can reach as many as 30, but the occurrence of particular structural and numerical abnormalities is not random [116]. Balanced rearrangements (translocations, insertions, or inversions) are relatively rare, and unbalanced aberrations leading to loss of chromosome material (monosomies, deletions, and unbalanced translocations) predominate. Most frequently lost is chromosome material from, in decreasing order, chromosome arms 5q, 17p, 7q, 18q, 16q, 17q, 12p, 20q, 18p, and 3p. Recurrent gains of chromosomal segments are less frequent and are often hidden in marker chromosomes and unbalanced translocations; they most often involve 8q, 11q, 21q, 22q, 1p, 9p, and 13q [116]. In ~80 % of patients with complex karyotype loss of 5q material is detected, whereas in ~50 % of the patients, parts of 7q and 17p are lost. Furthermore, abnormalities of 5q, 7q and 17p often occur together, in the same patient, which means that ~85 % of all patients with a complex karyotype harbour loss of at least one of these chromosome arms [116]. On the other hand, ~5 % of complex karyotype patients have only numerical abnormalities, mostly gains of chromosomes (most often +8, +13, +21, +14, +10, and +19) [117]. Chilton et al. [117] reported recently that OS of AML patients with a complex hyperdiploid karyotype (i.e., 49–65 chromosomes) that included only numerical abnormalities was significantly better than OS of patients with a hyperdiploid complex karyotype that had at least one abnormality associated with an adverse outcome [−5/del(5q), −7/del(7q), t(9;22), *KMT2A(MLL)* translocations except t(11;19)(q23;p13), or abnormalities of 3q or 17p]. Indeed, OS of the former was comparable to OS of patients classified in the MRC intermediate cytogenetic-risk category suggesting that patients with hyperdiploid complex karyotype with numerical abnormalities only should not be considered to have an adverse prognosis [117].

In 2008, Breems et al. [118] proposed recognition of a cytogenetic subset of patients with a particularly adverse outcome named monosomal karyotype (MK). The MK category excludes patients with CBF-AML and APL, and comprises AML patients who harbour two or more autosomal monosomies (i.e., loss of any chromosome but −Y or −X) or have one autosomal monosomy together with at least one structural chromosome abnormality (apparently excluding marker or ring chromosomes) [118]. Thus, MKs are very heterogeneous cytogenetically and frequently include abnormalities that have themselves been independently associated with adverse risk, including inv(3) or t(3;3), rearrangements involving 11q23/*KMT2A(MLL)*, or del(5q). Furthermore, up to 75 % of complex karyotypes are hypodiploid (i.e., contain ≤45 chromosomes, with ≥1 monosomy), and not infrequently ≥1 monosomy can be also found in the remaining complex karyotypes with a pseudodiploid (i.e., with 46 chromosomes) or hyperdiploid (i.e., with ≥47 chromosomes) modal chromosome numbers [116]. Consequently, most patients with a complex karyotype are also deemed to have a monosomal karyotype. While the MK designation has been useful in identifying AML patients with very poor outcomes [118–120], which could be improved to some extent by allogeneic SCT in first CR [121, 122], the marked heterogeneity of MKs makes it unlikely that a single or even a few molecular alterations that could be targeted therapeutically underlie the disease development in all MK patients. Moreover, inclusion of patients

into the MK category depends upon genetic methodology used to detect it. Following application of array-based comparative genomic hybridization and single-nucleotide polymorphism (SNP) genomic profiling, Rücker et al. [123] reduced greatly the number of MK cases from 78 % of all patients with a complex karyotype when MK was identified by standard karyotyping to only 32 %. This happened because many chromosomes deemed lost in G-banded karyotypes (monosomy) were in reality not totally lost, but their parts were found hidden in such structural abnormalities as marker chromosomes, ring chromosomes and unbalanced translocations with partially unidentified chromosome material [123].

Although the molecular consequences of the majority of losses and gains of specific chromosomes and/or chromosome segments in AML patients with complex karyotypes are not yet well characterized, the association between cytogenetic abnormalities of 17p, frequent in complex karyotypes, and mutations in and/or loss of the *TP53* gene causing loss of p53 protein function is well documented [123, 124]. *TP53* loss and/or mutations represent one of the molecular pathways responsible for marked genomic instability of complex karyotypes manifested by the simultaneous presence of multiple related clones and non-clonal cells, creation of complex abnormal chromosomes composed of material from three or more separate chromosomes and/or generation of abnormal "sandwich-like" chromosomes containing several small interchanging segments from two different chromosomes [116]. It has recently been shown that in up to one-half of AML cases with *TP53* mutations, a complex karyotype can arise through a single catastrophic event called chromothripsis, where numerous chromosome rearrangements are acquired simultaneously, instead of through a gradual, stepwise karyotype evolution [125]. Even though AML patients with a complex karyotype and *TP53* alterations have very poor outcome, with CR rates significantly lower and relapse-free survival, EFS and OS significantly shorter than those of patients without *TP53* alterations [123], the presence of chromothripsis has made the dismal prognosis of *TP53*-mutated patients even worse when compared with the outcome of *TP53*-mutated patients without evidence of chromothripsis [125].

The single largest cytogenetic subset of both adult and childhood AML comprises patients with an entirely normal karyotype, although the percentage of adults with CN-AML (40–45 %) is greater than that among paediatric cases (22–24 %). There are data suggesting that the proportion of patients with CN-AML established by standard cytogenetic analysis is overestimated because occasionally such subtle aberrations as t(11;19)(q23;p13.1), inv(3) or inv(16) may escape recognition in preparations of suboptimal quality. To minimize this possibility, CALGB/ALLIANCE pioneered and has been conducting successfully central karyotype review for the last 30 years [27]; central karyotype review is also performed by other cooperative groups [126]. Moreover, some CN-AML patients have been shown to harbour common AML-associated gene fusions, for instance *PML-RARA* or *CBFB-MYH11*, that are a result of microscopically undetectable rearrangements such as cryptic insertions, but these patients are rare and constitute only a fraction of all CN-AML cases [69, 114, 127].

Other cryptic rearrangements that are undetectable on routine cytogenetic investigation seem to be more frequent, but they occur mostly in childhood AML. These include a prognostically adverse t(5;11)(q35.2;p15.4)/*NUP98-NSD1*, detected in 16 % of paediatric and 2 % of adult CN-AML patients [36], and inv(16)(p13.3q24.3) resulting in the *CBFA2T3-GLIS2* gene fusion, which is a frequent rearrangement in childhood acute megakaryoblastic leukaemia and portends an inferior clinical outcome, but to date has not been detected in adults [128]. The latter inversion was initially reported in non-Down syndrome children with acute megakaryoblastic leukaemia, who in all but two cases had an abnormal, often complex, karyotype [128]. A subsequent study analyzed a cohort of 230 children with CN-AML, which did not include any patients with *KMT2A(MLL)*, *CBFB*, *NPM1*, or *FLT3* rearrangements, and detected inv(16)(p13.3q24.3)/*CBFA2T3-GLIS2* in 20 (8.4 %) patients, only one-half of whom was diagnosed with acute megakaryoblastic leukaemia [129]. Finally, a cryptic t(7;12)(q36.3;p13.2)/*MNX1-ETV6* has been occasionally reported in paediatric CN-AML, but to date a vast majority of cases with t(7;12) had an abnormal karyotype, which almost always contained trisomy of chromosome 19 [5]. This suggests that the presence of +19 in a child with AML warrants performing an RT-PCR or FISH analysis to confirm or refute the existence of t(7;12)(q36.3;p13.2)/*MNX1-ETV6*, an abnormality associated with an adverse prognosis [37].

As a group, patients with CN-AML have been classified in the intermediate prognostic category in all major cytogenetic-risk classifications, because their CR rates, DFS and OS were typically worse than those of adequately treated patients with the t(15;17), t(8;21) or inv(16), but better than the outcome of patients with adverse cytogenetic features [14–16, 22]. However, intensive research efforts undertaken during last the two decades have revealed that CN-AML is very heterogeneous at the molecular level, and the patients belong to molecular subsets with vastly varying prognoses [130]. Those molecular abnormalities that were reported to have an effect on clinical outcome of CN-AML patients are provided in Table 13.4. The presence of mutations in *NPM1* [131–135] and double mutations in *CEBPA* [136, 137] and high expression of *miR-181a* [138] have been associated with a favourable outcome, whereas the patients' prognosis is adversely affected by *FLT3*-ITD [139–142], *KMT2A(MLL)*-PTD [134, 143–145], mutations in *DNMT3A* (both R882 and non–R882 mutations) [146–148], *IDH1* [149, 150], *IDH2* (R172 mutations) [149, 150], *TET2* [151–153], *ASXL1* [154], *RUNX1* [155–157], *WT1* [158–160], and *BCOR* [161], expression of *GAS6* [162], and high expression of *BAALC* [163–166], *ERG* [167, 169], *MN1* [170–172], *SPARC* [173], *DNMT3B* [174], *miR-3151* [175], and *miR-155* [176] (details are provided in Table 13.4). Because leukaemic blasts of some CN-AML patients contain two or more (up to six) prognostic mutations and changes in gene expression, current research efforts concentrate on unravelling how combinations of multiple molecular genetic alterations influence the outcome of CN-AML patients.

Meanwhile, an international expert panel working on behalf of the ELN has recently incorporated three molecular genetic markers whose prognostic significance in CN-AML is best documented, namely *FLT3*-ITD, and *NPM1* and *CEBPA*

Table 13.4 Molecular genetic alterations influencing prognosis of patients with cytogenetically normal acute myeloid leukaemia (CN-AML)

Genetic alteration	Frequency	Associations with clinical outcome
Gene mutations		
Mutations in *NPM1*	46–62 %	Adults younger than 60 years with *NPM1* mutations in the absence of *FLT3*-ITD have significantly better CR rates, EFS, DFS, and OS than patients with wild-type *NPM1* [131–134]
		In patients aged 60 years or older, *NPM1* mutations alone constitute an independent favourable prognostic factor [135]
Double mutations in *CEBPA*	6–8 %	OS of patients with double *CEBPA* mutations was longer than OS of patients with wild-type *CEBPA* and of those with single *CEBPA* mutations. On MVA, double *CEBPA* mutations were an independent favourable factor for OS [136]
		In younger (<60 years) patients, both single and double *CEBPA* mutations conferred longer OS than OS of patients with wild-type *CEBPA*, but only double *CEBPA* mutations retained favourable prognostic significance in MVA for OS, EFS and RFS [137]
FLT3-ITD	28–39 %	Patients with *FLT3*-ITD have significantly shorter CRD, DFS and OS (but not CR rates) than patients who do not harbour *FLT3*-ITD [139, 140]
		Patients with high *FLT3* mutant to *FLT3* wild-type allele ratio have particularly poor prognosis [139, 141, 142]
KMT2A(MLL)-PTD	5–11 %	*KMT2A(MLL)*-PTD had no prognostic significance in intensively treated younger (<60 years) [187], and older (aged ≥60 years) patients [188], but in earlier studies patients with *KMT2A(MLL)*-PTD had shorter CR duration (but not CR rates or OS) [134, 143–145]
Mutations in *DNMT3A*	27–35 % in younger and 33 % in older patients	Overall, *DNMT3A* mutations are associated with shorter DFS (but not OS [147]), with lower CR rates and shorter OS on MVA [146], and shorter EFS and OS, which remained significant on MVA
		In patients aged <60 years, non-R882-*DNMT3A* mutations are associated with shorter DFS and OS [147]. In patients aged ≥60 years, R882-*DNMT3A* mutations confer shorter DFS and OS [147]
Mutations in *IDH1*	13–16 %	Overall, *IDH1* mutations are not associated with outcome [149, 150]
		In patients with *NPM1* mutations and no *FLT3*-ITD, *IDH1* mutations are associated with a higher relapse risk, shorter OS [150] and DFS [149]

(continued)

Table 13.4 (continued)

Mutations in *IDH2*	11–19 %	R172-*IDH2* mutations are associated with lower CR rates [149, 150], increased relapse risk and shorter OS [150]. R140-*IDH2* mutations are not associated with outcome [149]
Mutations in *TET2*	16–30 % overall; 15 % in younger and 29 % in older patients	*TET2* mutations are associated with lower CR rates [151] and shorter DFS [151], OS [151] and EFS [151, 152] and higher RR [152] in CN-AML patients classified in the ELN Favourable Genetic Group. Among patients with *NPM1* mutations but no *FLT3*-ITD, *TET2* mutations confer shorter EFS [152], higher RR [152], and shorter OS [153]
Mutations in *ASXL1*	5–10 % overall; 3 % in younger and 16 % in older patients	In patients aged ≥ 60 years, mostly those in the ELN Favourable Group, *ASXL1* mutations are associated with lower CR attainment probability and shorter DFS, OS and EFS [154]
Mutations in *RUNX1*	13–26 % overall; 6–8 % in younger and 16 % in older patients	*RUNX1*-mutated patients had lower CR rates and worse DFS, EFS and OS than patients with wild-type *RUNX1* [155–157]
Mutations in *WT1*	8–10 % overall; 11–13 % in younger and 7 % in older patients	*WT1* mutations confer worse DFS and OS (but not CR probability) [158]; worse CR achievement probability, CIR, DFS and OS [159, 160]; no impact on RFS or OS [189]
		Patients with *WT1* mutations and *FLT3*-ITD have lower CR rates and worse RFS and OS than patients with *WT1* mutations without *FLT3*-ITD [189]
Mutations in *BCOR*	4 %	*BCOR* mutations are associated with a shorter OS and EFS [161]

Genetic alteration	High vs. low expression	Associations with clinical outcome
Changes in gene expression		
High expression of *BAALC*	Median cut [163–166]	Younger (aged <60 years) high *BAALC* expressers have lower CR rates [164, 165], higher CIR [164] and inferior DFS [163], EFS [163] and OS [163, 165]. *BAALC* expression was confirmed as an independent risk factor on MVA [163–165]
		Older patients (aged ≥60 years) with high *BAALC* expression have lower CR rates, and shorter DFS and OS [166]
High expression of *ERG*	3 quartiles with lower expression vs. the 4th quartile [167, 169]	In younger patients (aged <60 years), high *ERG* expression is associated with lower CR rates [168] and worse CIR [167], EFS [168] and OS [167, 169]
	Median cut [166, 168]	In older patients (aged ≥60 years), high *ERG* expression is associated with shorter DFS [166] and OS [166, 169]

(continued)

Table 13.4 (continued)

High expression of *MN1*	Median cut [170, 172]	In younger patients (aged <60 years), high *MN1* expression (defined using median cut) is associated with shorter RFS and OS and higher RR [170]. When *MN1* expression was used as a continuous variable, higher *MN1* expression was associated with lower CR rates and shorter DFS and OS [171]
	Continuous variable [171]	
		In older patients (aged ≥60 years), high *MN1* expressers had lower CR rates and shorter EFS and OS [172]
High expression of *SPARC*	Median cut [173]	In younger patients (aged <60 years), high *SPARC* expression bestowed lower odds of achieving a CR and shorter OS (which remained significant on MVA) and shorter DFS (not significant on MVA) [173]
Expression of *GAS6*	*GAS6* expression vs. no detectable expression [162]	Patients who expressed *GAS6*, especially those aged ≥60 years, more often failed to achieve a CR and had shorter DFS and OS than patients without *GAS6* expression; all of which remained significant on MVA [162]
High expression of *DNMT3B*	Median cut [174]	Older patients (aged ≥60 years) with high *DNMT3B* expression had fewer CRs and shorter DFS and OS; ($P < 0.001$), which remained significant on MVA [174]
High expression of *miR-181a*	Continuous variable [138]	Younger patients (aged <60 years) with higher *miR-181a* expression have a higher CR rate and longer OS than patients with lower expression [138]
High expression of *miR-3151*	Median cut [175]	Older patients (aged ≥60 years) with high *miR-3151* expression have shorter DFS and OS, than patients with low expression [175]
High expression of *miR-155*	Median cut [176]	Patients with high *miR-155* expression have a lower CR rate and shorter DFS and OS than patients with low expression [176]

CIR cumulative incidence of relapse, *CR* complete remission, *CRD* CR duration, *DFS* disease-free survival, *EFS* event-free survival, *ELN* European LeukemiaNet, *FLT3*-ITD internal tandem duplication of the *FLT3* gene, *KMT2A(MLL)*-PTD partial tandem duplication of the *KMT2A(MLL)* gene, *MVA* multivariable analysis, *OS* overall survival, *RFS* relapse-free survival, *RR* risk of relapse, *vs*. versus

mutations, into a proposed standardized system for reporting cytogenetic and molecular abnormalities in studies correlating genetic findings with treatment outcome in AML [111]. These molecular markers are used to classify CN-AML patients into either the ELN Favourable Genetic Group, which also comprises patients with an abnormal karyotype with either the t(8;21)/*RUNX1-RUNX1T1* or inv(16)/t(16;16)/*CBFB-MYH11*, or into the Intermediate-I Group, which includes exclusively CN-AML patients who belong to one of three Genetic Subsets differing with regard to combinations of *NPM1*, *CEBPA* and *FLT3* mutational status.

The remaining Intermediate-II and Adverse Genetic Groups encompass patients with cytogenetic abnormalities only (for details please see Table 13.5) [111]. The ability of the four ELN Genetic Groups to predict treatment outcome has been recently examined by two large studies, each analyzing over 1,500 AML patients [177, 178]. Both studies yielded similar results showing that application of the ELN reporting system results in a prognostic separation of the favourable and adverse groups from each other and from both Intermediate Genetic Groups for all analyzed outcome endpoints, namely CR rates [178], DFS [178], probability of relapse [177] and OS [177, 178]. By performing multivariable analyses, the CALGB study also demonstrated that the association of ELN Genetic Groups with clinical outcome was independent from other established prognostic factors [178]. Interestingly, both studies revealed a difference between younger and older patients concerning the Intermediate-I and Intermediate-II Groups, with older patients in both Groups having virtually identical outcomes, and younger patients classified in the Intermediate-II Group having a significantly longer OS than the Intermediate-I Group patients (Fig. 13.3). The reasons for superior outcome of younger, but not older, patients in the Intermediate-II as opposed to the Intermediate-I Group are not fully understood. They may be related to striking cytogenetic heterogeneity of the Intermediate-II Group, which, in addition to t(9;11), consists of numerous structural and numerical abnormalities not classified as favourable or adverse whose distribution likely differs between younger and older patients in a manner similar to the age-related differences in the distribution of both the ELN Genetic Groups (Fig. 13.4) and Genetic

Table 13.5 The ELN standardized reporting system for correlation of cytogenetic and molecular genetic data with clinical data in acute myeloid leukaemia [111]

Genetic group	Genetic subset
Favourable	t(8;21)(q22;q22); *RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3*-ITD (normal karyotype)
	Mutated *CEBPA* (normal karyotype)
Intermediate-I	Mutated *NPM1* and *FLT3*-ITD (normal karyotype)
	Wild-type *NPM1* and *FLT3*-ITD (normal karyotype)
	Wild-type *NPM1* without *FLT3*-ITD (normal karyotype)
Intermediate-II	t(9;11)(p22;q23); *MLLT3-MLL*(*KMT2A*)
	Cytogenetic abnormalities not classified as favourable or adverse
Adverse	inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*(*MECOM*)
	t(6;9)(p23;q34); *DEK-NUP214*
	t(v;11)(v;q23); *MLL*(*KMT2A*) rearranged
	−5 or del(5q)
	−7
	abnl(17p)
	Complex karyotype[a]

[a]Complex karyotype is defined as at least three chromosome abnormalities, excluding any of the World Health Organization-designated recurring translocations or inversions, i.e., t(8;21), inv(16) or t(16;16), t(15;17), t(9;11), t(v;11)(v;q23), t(6;9), inv(3) or t(3;3)

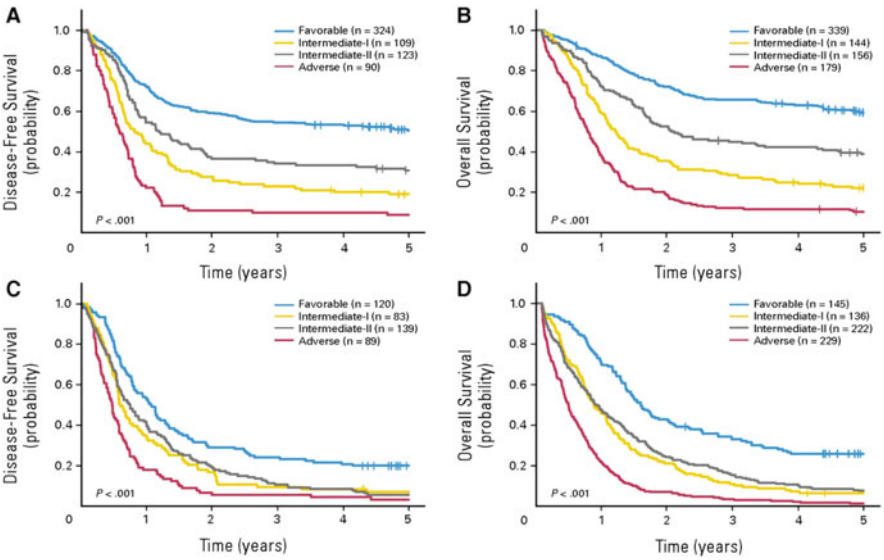

Fig. 13.3 Outcome of patients with de novo AML classified into the four European LeukemiaNet (ELN) Genetic Groups according to the ELN recommendations. (**a**) Disease-free survival and (**b**) overall survival of younger patients aged less than 60 years; (**c**) disease-free survival and (**d**) overall survival of older patients aged 60 years or older (Reprinted with permission from Mrózek et al. [178] © 2012 American Society of Clinical Oncology. All rights reserved)

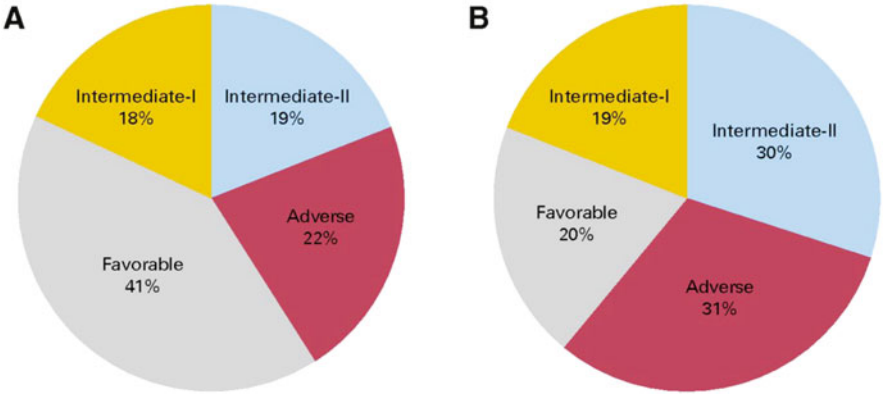

Fig. 13.4 Distribution of the ELN Genetic Groups in younger (**a**) and older (**b**) adults with de novo AML. The Favourable Group is significantly more ($P < .001$) and the Intermediate-II and Adverse Groups are less ($P < 001$) common among younger patients compared with older patients (Reprinted with permission from Mrózek et al. [178]. © 2012 American Society of Clinical Oncology. All rights reserved)

Subsets within these Groups (Fig. 13.5) [178]. These data support the view that the ELN classification should be applied to younger and older patients separately when used for risk stratification of AML patients in prospective clinical trials and in studies correlating genetic findings with clinical outcome [178]. Recent studies

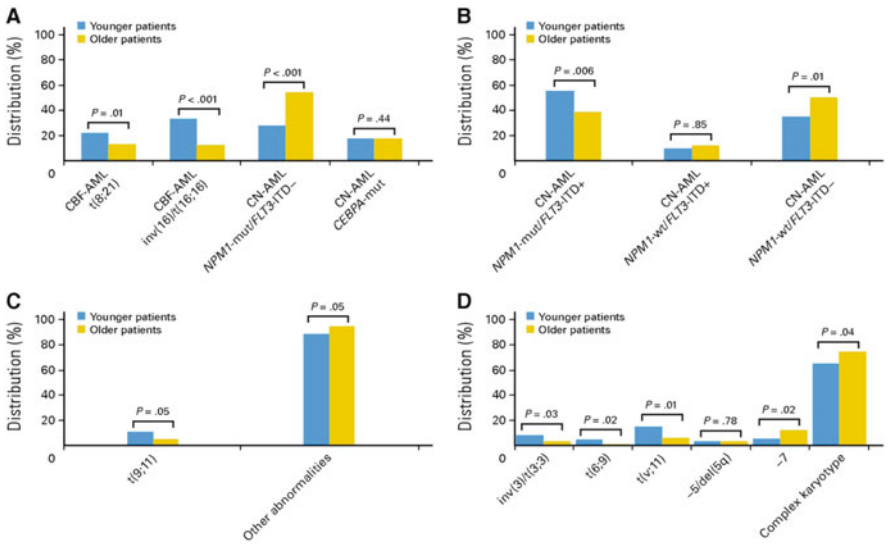

Fig. 13.5 Distribution of the genetic subsets within ELN Genetic Groups in younger and older adults with de novo AML. (**a**) The Favourable Group consists of four genetic subsets. The first two subsets are patients with core-binding factor AML with either t(8;21) or inv(16)/t(16;16). The second two subsets are patients with cytogenetically normal AML (CN-AML) with either *NPM1*-mut/*FLT3*-ITD – (i.e., mutated *NPM1* without *FLT3*-ITD) or *CEBPA*-mut (i.e., mutated *CEBPA*). (**b**) The Intermediate-I Group consists of three genetic subsets of patients with CN-AML and either *NPM1*-mut/*FLT3*-ITD+ (i.e., mutated *NPM1* and *FLT3*-ITD) or *NPM1*-wt/*FLT3*-ITD+ (i.e., wild-type *NPM1* and *FLT3*-ITD) or *NPM1*-wt/*FLT3*-ITD – (i.e., wild-type *NPM1* without *FLT3*-ITD). (**c**) The Intermediate-II Group consists of two genetic subsets of patients with either t(9;11) or other abnormalities (i.e., cytogenetic abnormalities not classified as favourable or adverse). (**d**) The Adverse Group consists of seven genetic subsets: (1) inv(3)/t(3;3), (2) t(6;9), (3) t(v;11) [i.e., various translocations involving 11q23/*KMT2A(MLL)* other than t(9;11)], (4) −5/del(5q) (i.e., monosomy of chromosome 5 or deletion of 5q), (5) −7 (i.e., monosomy of chromosome 7), (6) abnl(17p) (i.e., abnormalities of the short arm of chromosome 17; no patient had this abnormality in our study), or (7) a complex karyotype containing ≥3 cytogenetic abnormalities (Reprinted with permission from Mrózek et al. [178]. © 2012 American Society of Clinical Oncology. All rights reserved)

have provided evidence that testing for such genetic markers as mutations in the *TET2* [151], *ASXL1* [154], and *RUNX1* [156] genes and the expression levels of *MN1* [170], *miR-155* [176] and *miR-3151* [175] may refine the precision of patient risk stratification within the ELN Genetic Groups.

13.6 Concluding Remarks and Future Directions

During the last four decades, cytogenetic studies of AML have identified a large number of recurring chromosomal abnormalities with diagnostic and prognostic significance, and many of them have been dissected molecularly [3, 5, 17]. Cytogenetic analysis of pretreatment marrow has become a mandatory part of the

diagnostic work-up of patients suspected to suffer from AML. Pretreatment cytogenetic findings are being increasingly combined with the results of molecular genetic assays, as exemplified by the ELN reporting system [111], to guide the selection of the most effective treatment approaches. However, the prognostic significance of several recurrent but less frequent chromosomal abnormalities is yet to be established conclusively. They are often categorized in the intermediate-risk category (or the ELN Intermediate-II Genetic Group) by virtue of the absence of evidence that they confer a more favourable or adverse prognosis. Thus, further collaborative studies are needed to collect enough cases with these less common abnormalities to determine how they influence probability of CR achievement, DFS and OS. In addition, prognostic factors depend on the kind of therapy, which means that there is a continuing need for large prospective studies correlating cytogenetic and molecular genetic alterations with clinical outcome of both patients who are treated with contemporary regimens and of patients who are administered novel, experimental therapies often targeting specific genetic rearrangements.

This makes accurate detection of acquired genetic abnormalities of utmost importance. Recently, high-throughput next generation sequencing (NGS) technologies have been used to study AML genomes providing an unprecedented view of intricate interactions of genetic changes contributing to leukaemogenesis in individual patients [8, 179–181]. Although NGS technologies are being used successfully for research purposes they do not seem to be currently ready for routine use in diagnostics and prognostication of AML as stand-alone methods. This is due to the large amount of work and time necessary to corroborate results of these assays, their high, although rapidly declining, cost [179], as well as the need to fine tune bioinformatic algorithms in the programs analyzing sequencing data, which, as a recent study demonstrated [182], may occasionally fail to recognize a pathogenetically essential gene fusion created by a translocation recurrent in AML. It thus remains to be seen whether NGS technologies will be able to entirely supplant classical cytogenetics, FISH and RT-PCR in the future, or whether they will remain a powerful addition to the currently available armamentarium of techniques capable of detecting acquired genetic lesions with clinical significance in AML.

Acknowledgements The authors gratefully acknowledge Jessica Kohlschmidt, Ph.D., for preparing Fig. 13.2. This work was supported in part by the Coleman Leukemia Research Fund.

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