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Molecular and cytogenetic abnormalities in acute myeloid leukaemia and myelodysplastic syndromes

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Acquired chromosomal changes are a characteristic of all tumour cells. In this chapter we shall briefly review the cytogenetic abnormalities that are more specifically associated with acute myeloid leukaemia (AML) and the myelodysplastic syndromes (MDS). Their clinical usefulness and their significance for the understanding of the mechanism of leukaemogenesis will be discussed.

Mitotic abnormalities had already been discovered in tumour cells by the end of the last century (Arnold, 1879). Boveri (1914) was the first to suggest the existence of a relationship between the abnormal chromosome pattern and the malignant phenotype of the cells. Nowell and Hungerford (1960) reported the constant presence of a small marker chromosome, known as the Philadelphia chromosome, in patients with chronic myeloid leukaemia (CML). With the use of banding techniques (Caspersson et al, 1970; Hagemeijer et al, 1979) recurrent patterns of numerical or structural chromosome abnormalities were found to correlate with distinct haematological malignancies.

At several International Workshops on Chromosomes in Leukaemia, defined correlations were found between cytogenetic abnormalities, morphology and immunophenotype, clinical, epidemiological and aetiological factors, as well as prognostic implications for therapeutic response and survival. The development of molecular genetics in the 1980s led to the characterization of the genes involved in the cytogenetic abnormalities. These studies revealed a new mechanism of oncogenesis by illegitimate gene fusion, as a consequence of translocation. Information was, and still is, obtained on the different molecular mechanisms and changes leading to malignant transformation. In addition, these analyses provide insight into the genetic control of normal haematopoiesis.

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CHROMOSOME AND MOLECULAR CHANGES IN AML AND MDS

Cytogenetic findings and clinical significance

Chromosomal changes can be detected in the majority of cases of acute myeloid leukaemia or myelodysplastic syndromes. These changes in karyotype are clonal and an intrinsic feature of leukaemia. With response to treatment the malignant clone may disappear, but in case of relapse the leukaemic cells generally carry the original clonal abnormalities, sometimes with additional changes.

Chromosomal abnormalities can be divided into different categories:

1. *Recurrent structural abnormalities*, such as balanced reciprocal translocations, inversions and insertions. No gain or loss of chromosome material is found, the conserved breakpoints are specific and have often clinical relevance. For instance, adult patients with AML carrying the translocation t(8;21), t(15;17) or inversion inv(16) are associated with AML FAB M2,M3 and M4eo respectively and known to have a better prognosis (Marosi et al, 1992; Swansbury et al, 1994).
2. *Unbalanced aberrations*, which include gain or loss of chromosome material and, therefore, of genetic material. The breakpoints vary from patient to patient. The changes are primary or secondary; they are relatively easy to detect and have been associated with various clinical entities.

The distribution of karyotypes may vary among different age groups. In AML, in children and young adults, there is a predominance of balanced translocations (Table 1a). In the aged or in secondary leukaemia, or MDS arising in patients with a history of toxic exposure, there is a predominance of numerical and unbalanced abnormalities, in particular a loss or deletion of chromosome number 5 (5q–,–5) or #7 (7q–,–7) (Table 1b). Trisomy 8, in contrast, as a sole or secondary change, is the most frequent abnormality in myeloid disorders. Complex karyotypes (more than three aberrations) are particularly ominous in all age groups. The specific implications of each abnormality for clinical response to therapy will be dealt with below.

Gene re-arrangements in AML and MDS

Molecular analysis of a number of recurrent translocations and the inv(16) showed involvement of the genes mapping at or very close to the chromosomal breakpoints. Both genes, one on each of the involved chromosomes, are interrupted and recombined 'in frame' to give rise to a new hybrid gene. The latter is situated on the major or constant recombinant chromosome and encodes a new fusion protein. This has been shown to be a major step in the chain of events leading to malignancy. Expression of a reciprocal product from the second derivative chromosome is sometimes observed. These newly formed oncoproteins are expressed in leukaemic cells, and for some, their oncogenic potential has been demonstrated in animal studies. A

Table 1a. Structural abnormalities found in AML and MDS.

Cytogenetic	FAB	Frequency[a]	Prognostic implications	Therapy-related[b]	Other
t(8;21)(q22;q22)	M2(eo)	9%	Good	No	Often –x/–y
inv(16)(p13q22)	M4(eo)	10%	Good	No	
t(16;16)(p13;q22)					
t(15;17)(q22;q21)	M3 M3V	8%	Good	No	Coagulation disorders, ATRA responsive (only M3)
t(11;17)(q23;q21)	M3	<1%	Undetermined	No	
t(11q23), mainly:	M5/M4	10%	Poor	Often (1)	Secondary leukaemia after use of Topo II inhibitors
t(9;11)(p22;q23)	rarely M1/M2				
t(6;11)(p25;q23)					
t(10;11)(p12;q23)					
t(11;19)(q23;p13)					
inv(3)(q21q26)	M1, M4, M6	3–5%	Undetermined	No	AML with dys-megakaryopoiesis or thrombocytosis
t(3;3)(q21;q26)	MDS				
t(1;3)(p36;q21)					
t(9;22)(q34;q11)	M1, M2, M4	1–2%	Poor	No	
t(6;9)(q23;q34)	M2, M4, MDS	1%	Poor	No	Basophils
t(8;16)(p11;p13)	M5b	1%	Poor	No	Phagocytosis
t(16;21)(p11;q22)	M1-M7	<1%	Undetermined	No	
t(1;7)(q10;p10)	M4, MDS	<1%	Poor	Yes (2)	
t(1;22)(p13;q13)	M7	<1%	Poor	No	Hepatosplenomegaly, childhood
t(7;11)(p15;p15)	M2	<1%	Undetermined	No	Orientals
t(3;21)(q26;q22)	M1-M7, MDS, CMLBC	<1%	Poor	Yes (1)	
t(5;12)(q33;p13)	M1-M4, MDS CMLBC	<1%	Undetermined	Yes (2)	
t(3;12)(q26;p13)	AML/MDS	<1%		No	
t(3;5)(q21-25;q31-35)	MDS	<1%	Poor	Yes (2)	

[a] Frequency is given as average overall value for adults with AML. The relative frequency is higher in childhood AML.

[b] 'Therapy-related' signifies that the abnormality is found in the novo as well as in secondary AML or MDS after therapy with (1) inhibitors of DNA Topoisomerase II, or (2) alkylating agents.

Table 1b. Unbalanced abnormalities found in AML and MDS.

Cytogenetic	FAB	Frequency[a]	Prognostic implications	Therapy-related[b]	Other
+4; +11; +21	M4, M1-M7	1–3%	Undetermined	No	
+8; –7; –5	M1-M7, MDS	15–20%	Poor–very poor	Yes (2)	Often in association with other abnormality
5q–; 7q–; 20q-	M1-M7,	10–30%	Very poor	Yes (2)	
12p–; 17p–; i(17q)	MDS, CMLBC	1–5%			
Complex karyotype	M1-M7, MDS	5–15%	Very poor	Yes (2)	≥ 3 Abnormalities

[a] Frequency is given as average overall value for adults with AML. The relative frequency is higher in childhood AML.

[b] 'Therapy-related' signifies that the abnormality is found in the novo as well as in secondary AML or MDS after therapy with (1) inhibitors of DNA Topoisomerase II, or (2) alkylating agents.

number of translocations and their fusion transcripts are now defined, and several mechanisms by which they might play a role in leukaemic transformation are suggested (Table 2).

Table 2. Molecular genetic re-arrangements caused by chromosomal translocations.

Cytogenetic	Involved genes	Fusion gene product	Putative transforming mechanism
t(8;21)(q22;q22)	*ETO* (*CDR, MTG8*); *AML1*	AML1-ETO (CDR, MTG8)	Suppressor myeloid differentiation
inv(16)(p13;q13)	*MYH11*; *CBFB*	CBFB-MYH11	Effects differentiation?
t(16;16)	*MYH11*; *CBFB*		
t(15;17)(q22;q21)	*PML*; *RARA*	PML-RARA (100%) RARA-PML (70%)	Dominant inhibition of promyelocytic differentiation
t(11;17)(q23;q21)	*PLZF*; *RARA*	PLZF-RARA	
t(9;11)(p22;q23)	*AF9*; *MLL*	MLL-AF9	Regulator of transcription effect on early differentiation
t(6;11)(p25;q23)	*AF6*; *MLL*	MLL-AF6	
t(10;11)(p12;p23)	*AF10*; *MLL*	MLL-AF10	
t(11;19)(q23;p13)	*MLL*; *ELL*	MLL-ELL	
t(11q23)	*MLL*; other gene	MLL- other	
t(9;22)(q34;q11)	*ABL*; *BCR*	BCR-ABL	Tyrosine kinase activation
t(6;9)(q23;q34)	*DEK*; *CAN*	DEK-CAN	
t(16;21)(p11;q22)	*FUS(TLS)*; *ERG*	FUS-ERG	
t(3;21)(q26;q22)	*EVI-1*; *AML1*	AML1-EVI-1	Lack of transcriptional activity
	EAP; *AML1*	AML1-EAP	Repressor gene?
	MDS1; *AML1*	AML1-MDSI	
t(5;12)(q33;p13)	*PDGFRB*; *TEL*	TEL-PDGFRB	
t(3;12)(q26;p13)	unknown; *TEL*		

Originally, gene fusion in AML was thought to be a highly specific property of exclusive partners. More recently, genes have been identified that may recombine with a variety of other genes; this gives rise to different translocations and to leukaemias which can be of different phenotype or lineage. For example, the *MLL* gene on 11q23 and the *TEL* gene on 12p13 may be involved in AML, MDS as well as acute lymphoblastic leukaemia (ALL).

Numerical changes and deletions result in gene unbalance. The loss of genetic material is suggestive for another mechanism of leukaemogenesis by loss of tumour suppression. Such tumour suppressor genes have been shown to play a role in familial solid tumours. Many studies currently concentrate on the definition of such genes on 5q, 7q, 20q and other chromosomal regions that are frequently lost in AML and MDS.

Methods of detection

Genetic abnormalities of leukaemic cells can be investigated using a number of different techniques: karyotype analysis with banding techniques, fluorescence in situ hybridization (FISH), Southern blotting, reverse transcriptase polymerase chain reaction (RT-PCR) and immunological

detection of a specific oncoprotein. The latter two methods assay for the RNA and protein product of the oncogene.

To obtain cells at the metaphase stage, short-term cultures with or without the use of growth factors or mitogens are applied. *Banding techniques* induce differential staining along the chromosomes, allowing identification of each chromosome by microscopy or on photographs. This approach reveals a complete picture of the genetic changes at the chromosomal level, i.e. both numerical and structural. It also provides information on the complexity of the clonal abnormalities. Sometimes it may also disclose the presence of more than one neoplastic cell population. One of the major disadvantages is the limited resolution of banding. On average, a chromosome band contains from 3 to 5×10^6 DNA base pairs of DNA and may contain up to 100 genes. A deletion of part of a band may be submicroscopic and therefore not detectable.

FISH is based on the property of single-stranded DNA to hybridize specifically with complementary sequences. DNA probes, specific for a chromosome or a given gene, are labelled with non-isotopic haptens such as biotin or digoxigenin (Pinkel et al, 1988). FISH bridges the gap in resolution between conventional cytogenetics and molecular DNA techniques and is becoming more and more sensitive. Larger probes can be used on interphase cells, overcoming the necessity to obtain metaphases and obviating the possible selection that may occur in culture. Interphase molecular cytogenetics allows for the screening of large numbers of cells and hence is ideal for follow-up studies of patients and for the detection of minimal disease.

The most interesting feature of FISH is the possibility of combining it with other identification techniques like cytomorphology or immunophenotyping (van Lom et al, 1993). Using these approaches, specific genetic defects can be assigned to well defined subpopulations of cells and reciprocally selective subgroups of cells can be screened for genetic changes.

The chromosomal breakpoints of specific translocations are generally clustered in one or a few of the introns of the involved genes. These breakpoint clusters can be probed, and *Southern blotting* is a major tool for diagnosing re-arrangement of those genes—such as the *MLL* gene—which have multiple possible targets.

The RT-PCR technique amplifies the specific 'fusion transcript' that is characteristic of a translocation. RT-PCR, in theory, has the power to detect one leukaemic cell among 10^6 cells, but is also extremely sensitive to laboratory contamination.

The advantage of the molecular analysis is its high sensitivity and resolution. The limitation is that only one question at a time can be answered. Molecular techniques are used now to evaluate response to therapy and measure residual disease in remission samples or forecast relapse, or to determine residual leukaemia in autologous haematopoietic stem-cell transplants. The same methods are employed to assess donor chimerism after allogeneic bone marrow transplantation. They are also very convenient to screen particular categories of patient for frequent

aberrations with significant prognostic implications (e.g. screening of leukaemia for the 11q23 translocation).

SPECIFIC ABNORMALITIES IN AML

In this chapter we will deal with the most frequent and clinically most significant chromosomal abnormalities. An overview of all translocations, the involved genes and their fusion products is given in Tables 1 and 2.

t(8;21)(q22;q22)

This is the most frequent translocation apparent in about 10% of adult patients and 20% of children with AML. It is associated with the AML FAB M2, and shows a specific morphology. Typical morphological features of t(8;21) AML include eosinophilia, prominent Auer rods, salmon-coloured granulae, large cytoplasmic globules and vacuoles. The immunological markers CD19 and, less often, CD56, may be present. Loss of a sex chromosome may occur, as well as an interstitial deletion of the long arm of chromosome 9 del(9)(q13q32). Both abnormalities are indicative of clonal progression. Finally, complex variants of t(8;21) have been described. Clinically, AML with t(8;21) is associated with a relatively good prognosis with regard to complete remission (80%) and disease-free survival (67%) probabilities (Marosi et al, 1992; Dastugue et al, 1995). Although relapses do occur in these patients (Fenaux et al, 1989), re-induction of remission is often successful, unless secondary abnormalities occur (Garson et al, 1989).

In t(8;21) part of the long arm of chromosome 8 is reciprocally translocated to the long arm of chromosome 21. On chromosome 21 the *AML1* gene is involved (Myoshi et al, 1991), whereas on chromosome 8 it is the *ETO* gene (also called *CDR, MTG8*). This gene encodes a nuclear protein which normally is not expressed in haematopoietic tissue. The translocation results in the formation of the *AML1-ETO/CDR/MTG8* chimeric fusion gene on the derivative chromosome 8, whose transcript can be detected with the use of an RT-PCR (Nisson et al, 1992; Erickson et al, 1992; Myoshi et al, 1993).

AML1 is a highly conserved gene related to the *Drosophila* pair-rule gene runt encoding a nuclear protein (Erickson et al, 1992; Daga et al, 1992). *AML1* is the human counterpart of the murine gene for the alpha subunit of the nuclear binding protein (PEPB2) (Bae et al, 1993) also known as the core-binding factor (CBF) (Wang and Speck, 1992; Wang et al, 1993). PEBP2/CBF is a protein which is able to bind to the enhancers of T-cell specific genes and to the conserved core site of the mammalian-type retroviral enhancers. Due to alternative splicing, AML-1 comes in various lengths. The normal *AML1* gene (full-length form) acts as a transcriptional activator reporter gene for CBF, GMCSF CSF1R and TCRB sites (reviewed by Nucifora and Rowley, 1995). It is suggested that the shorter form of the *AML1* gene, AML1a, which still contains the runt domain, can act as a suppressor of the reporter gene. Recently, it was found that a larger,

alternative spliced form of the *AML1* gene, called AML1b, is capable of inducing the transcription of a reporter gene. The t(8;21) fusion protein (maintaining the runt homology) appeared to block this transactivation by AML1b (Meyers et al, 1995). This can result in suppression of the normal, myeloid differentiation in cells carrying the t(8;21) (Nuchprayoon et al, 1991). The breakpoints in the *AML1* gene are clustered between exons 5 and 6 (Myoshi et al, 1991), and result in the formation of a constant size fusion transcript regardless of their exact position within the rather large intron (de Greef et al, 1995).

Recently several groups have found that the fusion transcript of AML1-ETO persists in patients in long remission (Nucifora et al, 1993a; Kusec et al, 1994). This finding would suggest that the presence of cells with the t(8;21) in itself does not indicate the presence of residual leukaemic cells or impending relapse, but a quantitative PCR-test should be developed.

t(3;21)(q26.2;q22)

The *AML1* gene is also known to be involved in t(3;21)(q26;q22). This translocation is far less frequent than t(8;21). It is associated not only with (therapy-related) AML but also with MDS and blast crisis of CML. On chromosome 21 the breakpoints in the *AML1* gene are more heterogeneous in comparison to t(8;21) and occur after either exon 5 or exon 6 (Sacchi et al, 1994). On chromosome 3q26 the *EAP* gene (Nucifora et al, 1993b), the *EVI-1* gene (Mitani et al, 1994) and a gene called *MDS1* (Nucifora et al, 1994) have been identified. Chimeric fusions between the *AML1* gene and the *EAP*, *EVI-1* or *MDS1* gene have recently been detected by RT-PCR in patients with t(3;21) (reviewed by Nucifora and Rowley, 1995). The *EAP* gene is recognized now as the ribosomal protein L.22 and belongs to a family of pseudogenes (Toczyski et al, 1993). In a number of patients with t(3;21) the chimeric fusion protein AML-1/EAP did not contain amino acid homology with normal *EAP*. Therefore, the transcript might lack its trans-activational activity and act as a repressor for the *AML1* gene (Nucifora et al, 1993b; Sacchi et al, 1994).

The *EVI-1* gene can also be found at 3q26 more downstream of the *EAP* and *MDS1* genes. It encodes a DNA binding protein and contains two domains of zinc finger motifs as well as an acidic domain (Delwel et al, 1993). The *EVI-1* gene is expressed in 30% of patients with AML, MDS or CML-blast crisis (CMLBC), without evidence of 3q26 abnormalities (Russel et al, 1994). Moreover, it is expressed in leukaemias and MDS which carry less frequent 3q26 abnormalities such as t(3;3)(q21;q26) or ins(3;3)(q21q25q26). In AML t(3;3)(q21;q26) as well as inv(3)(q21q26) is associated with disturbances in thrombopoiesis and megakaryocyte development and is often accompanied by other complex cytogenetic abnormalities (Fonatsch et al, 1994). The entire *EVI-1* protein is conserved in the chimeric transcript resulting from the t(3;21) and expressed in the leukaemic cells. Its exact role in the development of leukaemia has not been established yet but it might block stem cell differentiation (Matsugi et al, 1995).

t(15;17)(q22;q21)

The t(15;17) is clinically associated with acute promyelocytic leukaemia (APL), FAB M3, or its microgranular variant M3V, where it is found in 95% of the cases. This leukaemia is characterized by a clonal expansion of haematopoietic progenitor cells, blocked at the promyelocyte stage of differentiation (Rowley et al, 1977). Clinically it is associated with prominent diffuse intravascular coagulation. APL has a relatively good prognosis, and shows the unique property to respond to treatment with high-dose all-*trans* retinoic acid (ATRA), a vitamin A derivative (Huang et al, 1988). The reciprocal translocation results in the fusion of the *PML* gene on chromosome 15q22 to the retinoid acid receptor alpha *RARA* located on chromosome 17q21. The fusion product 5′-PML-RARA-3′, encoded by the der(15), is expressed in all cases. The reciprocal fusion product RARA-PML, encoded by the der(17), is detected in 70% of APL. *PML* is found to be expressed in every human cell line, although in human bone marrow it is restricted to the myeloid lineage (Daniel et al, 1993). Its expression in Hela cells was found to be highest in the G1 phase (Chang et al, 1995). The gene has nine coding exons and a proline-rich N-terminus which resembles the transcription activation domain of some genes. In addition, three clusters of zinc fingers are found followed by an α-helical region. Functionally, it has been suggested that the *PML* gene plays a role as a transcription factor (Kakizuka et al, 1991; de The et al, 1991) and is now known to belong to a new family of zinc-finger transcription factors, i.e. the so-called B-box family (Goddard et al, 1991). Based on the clinical response to treatment with ATRA, it has been suggested that disturbances of the RARA receptor gene which maps to 17q21 play a role in the pathogenesis of APL. A non-functional heterodimer is formed between the *PML* gene and the fusion protein PML-RARA (Perez et al, 1993). Recent data show that disturbance of the normal function and cellular localization of the *PML* gene product in the presence of PML-RARA affects normal cell growth and differentiation (Mu et al, 1994). Also, a positive PML-RARA PCR reaction in patients in clinical remission is associated with impending relapse (Fukutani et al, 1995). Reports have been made of rare cytogenetic variants and masked translocations that involve *PML* and *RARA* as well. Even more rare are two other translocations associated with AML-M3: the t(11;17)(q23;q21) that re-arranges the *PLZF* gene with *RARA* (Chen et al, 1994), and a single report of a t(5;17). So the exact roles of both involved genes and their fusion proteins in the pathogenesis of APL still remain to be established.

inv(16)(p13q22) and t(16;16)(p13;q22)

The inv(16)(p13q22) and t(16;16) are also seen at comparatively high frequency in de novo AML and account for about 16% of cytogenetic abnormalities in these patients. A strong association is apparent between chromosome 16 abnormalities and AML FAB M4Eo, but they have also been found in AML M2, M4 without eosinophilia, M5, MDS and CML

blast crisis (Campbell et al, 1991). Patients usually have a good prognosis (almost 100% of CR), but (leptomeningeal) relapses have been reported (Holmes et al, 1985). For these reasons, detection of this subtle chromosomal re-arrangement is rather important. Probes for FISH analysis are useful to support the diagnosis by conventional cytogenetics.

On 16p13 the smooth muscle myosin heavy chain gene is involved (*MYH11*) (Liu et al, 1993a; van der Reijden et al, 1993). On 16q22 the involved gene has been identified as the core binding factor-β gene (*CBFβ* also known as *PEPB2/CBF*) (Liu et al, 1993b). *CBF* is known as a heterodimeric transcription factor which has DNA binding facilities for T-cell and myeloid-specific genes. In addition to the β unit, *CBF* also consists of an α unit. The latter is coded for by the *AML1* gene on chromosome 21 and is responsible for DNA interactions. The β unit acts as stabilizer of this DNA-binding activity. The inv(16) results in an abnormal fusion gene 5-′CBFB/MYH11-3′. A single breakpoint has been identified within *CBFβ*, whereas *MYH11* may show four alternative breakpoints. The fusion product has been identified with the use of RT-PCR (Liu et al, 1993a; Claxton et al, 1994). More recently breakpoint heterogeneity was demonstrated in both genes (Shurtleff et al, 1995). Recent CBFβ-MYH11 fusion transcripts were found in a series of AML M4Eo, but also in about 10% of AML M4 cases without eosinophilic abnormalities (Poirel et al, 1995). Large follow-up series have not yet been reported. In analogy to the alternatively spliced AML1-ETO fusion transcripts, which may lead to truncated CBFα units, alternative splicing in the CBFβ unit has also been identified (van der Reijden et al, 1995). In summary both inv(16) and t(8;21) are found in leukaemias with relatively good prognosis and both are associated with disturbances within the CBF transcription factor. These data may suggest a common mechanism of leukaemic transformation. The fusion proteins in combination with their alternatively spliced products may exert different effects on normal differentiation.

Abnormalities involving 11q23

The *MLL* gene on 11q23, also called *HRX*, *Htrx-1*, *ALL1* (Djabali et al, 1992; Gu et al, 1992; Tkachuk et al, 1992; Domer et al, 1993), is involved in various chromosomal abnormalities and associated with acute lymphoblastic leukaemias (ALL), primary or secondary (therapy-related) AML, mixed lineage leukaemia as well as rare cases of myelodysplasia and malignant lymphomas. The 11q23 abnormalities are found in more than 70% of leukaemias in infants (less than 1 year old) but they are also relatively frequent in children and adults. As the *MLL* gene can fuse to a number of different partner genes, it plays a role in several chromosomal translocations. In ALL, t(4;11)(q21;q23) is the most frequent 11q23 abnormality followed by t(11;19)(q23;p13.3). Patients with ALL and t(4;11) have high WBC, null cell immunophenotype, and are more often of the female sex. In children and adults with de novo AML 11q23 abnormalities are found in 4–6% and most frequently involve t(9;11)(p22;q23),

t(6;11)(q27;q23) and t(11;19)(q23;p13.1). An association of 11q23 abnormalities with FAB M4/M5, high WBC, extramedullary localization, skin infiltration, female sex and poor prognosis, has been established in infants (Sorensen et al, 1994). In adults with AML M4/M5 the presence of 11q23 abnormalities may not predict poor prognosis (Bower et al, 1994). In addition, other 11q23 variants, including t(1;11)(p32;q23), t(X;11)(q13;q23), t(10;11)(p12;q23) and t(11;17)(q23;q25) have been reported in AML. Although translocations are most frequently seen, deletions (del 11q) have also been demonstrated. 11q23 abnormalities are also highly prevalent in secondary leukaemia in patients previously treated with topoisomerase II inhibitors (Gill Super et al, 1993). Many of these translocations exchange chromosomal segments of similar size, and they are therefore difficult to detect in poorly banded metaphases. The prevalence of *MLL* re-arrangement as detected by Southern can be of the order of 10% of the cases.

The *MLL* gene on chromosome 11 covers about 100 kb and contains at least 21 exons. The MLL protein contains several regions that show homology to that of the *Drosophila* trithorax gene, which is involved in the transcriptional regulation in *Drosophila* embryogenesis. It also contains amino-terminal AT hooks which can bind to AT-rich regions of the minor groove of the DNA double helix (Grosschedl et al, 1994). In this way it may facilitate the action of other DNA-binding factors. The cystein-rich region (CRR), also found on the *MLL* gene product, shows homology with mammalian DNA methyltransferase, which might discriminate between hemimethylated and ummethylated DNA. All these regions lead to the presence of at least two DNA-binding domains (TRX and four zinc-fingers) and suggest a role as a transcriptional regulator. The breakpoints in the *MLL* gene are clustered between exons 5 and 11 of the gene between the two DNA-binding sites. At the present time eight different partner genes have been molecularly characterized. Some of these share sequence homology, such as *AF10* (10p12) with *AF17* (17q25), and *AF9* (9p22) with *ENL* (19p13). However, most do not. Fusion transcripts are found between the *MLL* and its different partner genes, coding for several chimeric proteins (for a review see Bernard and Berger (1995)). Although these chimeric proteins might influence the normal functions of the *MLL* gene, the MLL fusion protein on the derivative chromosome 11 is expressed in all cases and is considered critical in leukaemogenesis (Rowley, 1992; Kobayashi et al, 1993; Downing et al, 1994). Several mechanisms of leukaemogenesis of MLL might be involved—such as competition for targets of normal *MLL* or formation of a new transcription factor by fusion of AT hooks to possible transactivation domains in the product of the partner genes. As a result of the differences between most partner genes, they might contribute in different ways to leukaemogenesis. The fact that leukaemias carrying 11q23 abnormalities can be of myeloid, lymphoid or mixed lineage, might suggest that the critical changes occur in a very early pluripotent progenitor cell, and that the different translocations interfere with the various pathways of differentiation.

Another abnormality, also involving the *MLL* gene, was described in AML; in this abnormality an internal tandem duplication took place within

the gene. The partially duplicated *MLL-1* gene is transcribed into mRNA capable of encoding a partially duplicated protein (Schichman et al, 1994). This self fusion of the gene has been reported in AML with trisomy 11, but also in AML with normal karyotype, and might be considered as another mechanism in leukaemogenesis.

SPECIFIC ABNORMALITIES IN MDS

The myelodysplastic syndromes harbour a variety of disorders which are of clonal origin. They are characterized by ineffective haematopoiesis often in combination with hypercellularity of the bone marrow. A classification can now be made according to the FAB criteria. The morphological classification already made it possible to define patients which need more intensive treatment or even an allogeneic bone marrow transplantation, as they have a poor prognosis (for instance, patients with RAEBt).

In addition to the FAB classification, the chromosomal status can be considered as a major prognostic indicator of survival, leukaemic transformation and response to treatment. Cytogenetic abnormalities are found in 32–73% of primary MDS (Second International Workshop on Chromosomes in Leukemia, 1980; Yunis et al, 1988). They consist of numerical and/or structural re-arrangements. The most frequent abnormalities found are deletion of chromosome 5 (–5/5q–), chromosome 7 (–7/7q–) and chromosome 20 (20q–), –Y, or an additional chromosome 8 (+8). Non-random translocations are far less frequent (1–5%) and involve chromosomes #3, #6, #9, #16 and #21 in the same way as can be found in patients with AML, so they will not be further discussed here. Relations have been found between the different FAB classifications and the presence of specific chromosomal abnormalities (reviewed by Heim and Mitelman, 1995). For instance, del(5q) is found in 50% of MDS type refractory anaemia (RA) and in less than 5% in chronic myelo-monocytic leukaemia (CMML). Monosomy 7 is less frequent in patients with refractory anaemia with ring sideroblasts, (RARS), whereas del(11q) and del(20q) are found more often in these patients, compared to the other MDS subgroups. In the patients with refractory anaemia with excess of blasts (RAEB) or RAEBt, the frequency of chromosomal abnormalities increases, which also may be more complex (Knapp et al, 1985; Heim and Mitelman, 1986). With respect to the prognostic significance of the chromosomal status, various studies show different outcome. The chance for leukaemic transformation has been found to be smaller in patients with an admixture of normal (N) and abnormal (A) metaphases in comparison to patients with all abnormal (AA) metaphases (Pierre et al, 1989; Gonzalez Manzo et al, 1992). Other studies did not confirm this (Yunis et al, 1986; Billström et al, 1988). A recent study of 109 patients showed no relation between FAB and specific chromosomal abnormalities. Patients with three abnormal cell lines or complex abnormalities had the shortest survival. No statistical difference could be found in survival between NN, AN or AA patients (Parlier et al, 1994). Our own analysis of 129 patients with primary MDS also showed that overall

survival is shortest in the presence of multiple chromosomal abnormalities in comparison to single or no chromosomal abnormality (non-published data).

Some specific abnormalities found in MDS are now known to involve gene structures which might play a role in the development of the disease. For example, mutations of the *RAS* oncogene and *P53* gene have been reported in MDS but their exact role remains to be established.

ABNORMALITIES INVOLVING CHROMOSOME 5

Deletion 5q

The interstitial deletion of the long arm of chromosome 5 (5q–) can be found in patients with de novo and therapy-related MDS or AML. In the latter groups it can be considered as a bad prognostic sign. Within the 5q– at least four different interstitial deletions can be identified, spread between 5q13 and 5q35. Of these, 5q31 is the segment which was found to be deleted in most patients (Pedersen and Jensen, 1991). Molecular studies have revealed the presence of several genes playing a role in proliferation and/or differentiation located on 5q31. These are the interleukin genes (IL-3; IL-4; IL-5; IL-9), the genes for GM-CSF; for CSF-1 receptor (*v-FMS*), CD14, the zinc finger transcription factor EGR1 and the interferon response factor (IRF1). The use of FISH made it possible to analyse two distinct critical loci on 5q31 in patients with secondary AML and MDS. Both regions called D5S89 and EGR1 were deleted, whereas the interstitial region was conserved (reviewed by Nagarajan, 1995). The loss of the D5S89 locus involves genes that play a role in myeloid growth and differentiation and is found in RAEBt and AML with poor prognosis; it has been suggested that D5S89 is the MDS/AML tumour suppressor locus. The telomeric region near the *CSFR1* gene is considered as regulator of erythroid and megakaryocytic differentiation and therefore as RET locus. Further studies are essential to isolate all critical genes and to establish the consequences of their loss in patients with 5q–.

t(5;12)(q33;p13)

The above translocation can be found in Ph-negative CML, chronic myeloproliferative disorders as well as in MDS-CMMOL, although it seems to be not very frequent in this group. On the molecular level, the platelet-derived growth factor receptor-B (*PDGFRB*) gene is involved at 5q33, with the *TEL* gene at 12p13 (Golub et al, 1994). The latter gene is a member of the ETS family of transcription factors. In MDS it has also been found in a variant t(10;12)(q24;p13) (Wlodarska et al, 1995). Recently it has been found to form fusion transcripts also with other genes such as *ABL* or *AML1*. In these cases often a loss of the other *TEL* allele occurs leading to a complete loss of wild-type *TEL* in the leukaemic cells. Deletion of TEL is found in 15% of children with acute lymphoblastic leukaemia (Stegmaier et al, 1995).

SUMMARY

With the use of molecular techniques it is now possible to define even subtle chromosomal abnormalities and the fusion products resulting from translocations. Defined clinical correlations can now be made and prognostic implications are already found. For instance, patients with AML carrying t(8;21), t(15;17) or inv(16) have a better prognosis for long-term survival. This is also illustrated by Figure 1, which shows data of the Dutch HOVON AML study. The definition of patients with a bad or good prognosis has already resulted in the adjustment of treatment protocols. In the near future, with the use of more defined molecular techniques, we might be able to characterize the chromosomal abnormality of each patient, to individualize his treatment and to recognize very early relapses.

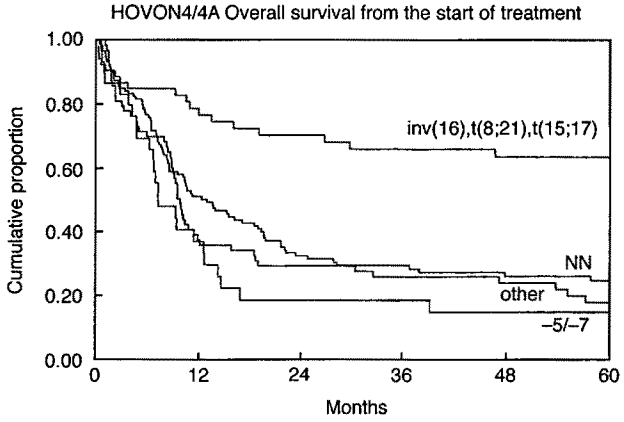

Figure 1. Overall survival in relation to cytogenetic abnormalities in 582 adult patients with AML, treated according to the HOVON 4(A) protocol.

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