Review Article

Acute Myeloid Leukemia with the t(8;21) Translocation: Clinical Consequences and Biological Implications

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The t(8;21) abnormality occurs in a minority of acute myeloid leukemia (AML) patients. The translocation results in an in-frame fusion of two genes, resulting in a fusion protein of one N-terminal domain from the AML1 gene and four C-terminal domains from the ETO gene. This protein has multiple effects on the regulation of the proliferation, the differentiation, and the viability of leukemic cells. The translocation can be detected as the only genetic abnormality or as part of more complex abnormalities. If t(8;21) is detected in a patient with bone marrow pathology, the diagnosis AML can be made based on this abnormality alone. t(8;21) is usually associated with a good prognosis. Whether the detection of the fusion gene can be used for evaluation of minimal residual disease and risk of leukemia relapse remains to be clarified. To conclude, detection of t(8;21) is essential for optimal handling of these patients as it has both diagnostic, prognostic, and therapeutic implications.

1. Introduction

Acute myeloid leukemia (AML) is a heterogeneous bone marrow malignancy, and patients with the cytogenetic t(8;21) abnormality represent a subset with specific clinical and biological characteristics [1]. The translocation fuses the AML1 gene (also called RUNX1) on chromosome 21 with the ETO gene (also referred to as the RUNX1T1 gene that encodes the CBFA2T1 protein) on chromosome 8. The criteria for the diagnosis differs from other AML patients; the leukemia cells show biological characteristics that are uncommon in other AML subsets, and the prognosis after intensive chemotherapy is better for these patients than for the majority of AML patients [1]. t(8;21) was the first cytogenetic abnormality discovered in AML [2], and today it offers a unique example of how a cytogenetic abnormality is used to define a distinct subgroup of patients. The AML1 gene has p.d.d. been reported to be involved in 39 different rearrangements and most of them have been detected in myeloid malignancies. In the present paper we describe the biology and clinical characteristics of the most common t(8;21) abnormality.

2. The t(8;21) Abnormality in Human AML

2.1. Frequency and Predisposition. The t(8;21) abnormality is found in approximately 5%–10% of all AML cases and 10%–22% of AML cases with maturation corresponding to the previous FAB class M2 [3–8]. The incidence of AML with favourable cytogenetic abnormalities decreases with age [9, 10]; this is also true for t(8;21) that is most common in children/younger patients [11] and uncommon in patients above 60 years of age [10]. Approximately 10%–20% of children with AML have this translocation [12–15]. The detection of t(8;21) transcripts in Guthrie cards suggests that the initiating events can occur in utero [16]. This is also supported by studies of umbilical cord leukocytes that have described an association between prenatal pesticide exposure (i.e., detection of pesticides in meconium) and the detection of t(8;21) in the leukocytes and the level of

the fusion transcript then correlated with the pesticide level [17].

Tissue-specific genomic organization probably contributes to the formation of this disease-specific translocation. Studies of the nuclear architecture have concluded that chromosomes 8 and 21 tend to colocalize in myeloid cells [18]. Furthermore, the chromatin organization at intron 5 of the RUNX1/AML1 gene, where the sequenced breakpoints have been mapped, are characterized by reduced histone H1 levels and increased levels of hyperacetylated H3 for AML1 expressing cells. Induction of DNA damage can then induce formation of t(8;21) in the HL-60 AML cell line but not in nonhematopoietic HeLa cells [19]. Thus, both the nuclear microarchitecture and epigenetic mechanisms seem to be important for the risk of t(8;21) formation in myeloid cells. The AML1-ETO fusion protein alone is not sufficient for leukemia development (see Section 9), but the protein downregulates the expression of DNA repair enzyme 8-oxoguanine DNA glycosylase (OGG1), which may then lead to additional genetic abnormalities required for development of AML [20].

2.2. AML1-ETO: Variant Rearrangements and Combination with Other Genetic Abnormalities. The t(8;21) generates two fusion genes, AML1-ETO and ETO-AML1, but only the AML1-ETO transcript transcribed from the derivative 8 chromosome is detectable by reverse transcriptase polymerase chain reaction (RT-PCR). Simple reciprocal translocation is by far the most common abnormality for creating AML1-ETO fusions, but the fusion can also occurs through variant rearrangements (Figures 1(a), 2(a)). The translocation can involve several chromosomes and occur together with inversion of the derivative 8 chromosome (e.g., inv(8)(p21;q22)t(8;21), inv(8)(q22q24)) [21, 22]. AML1-ETO fusion can also be a result of insertion, and both ins(21;8) and ins(8;21) have been described [23] (Figure 2(b)). These variant rearrangements can be cryptic and easily overlooked by conventional G-banding, and their frequencies are therefore unknown.

The t(8;21) abnormality is often detected together with additional cytogenetic or molecular genetic abnormalities; a majority of the patients seem to have additional abnormalities (Table 1) [3]. These abnormalities are often numerical, but other translocations or deletions can also be detected.

Chromosome 9 Alternations. Deletion of chromosome 9q, the del(9q) abnormality, is recurrent but uncommon in AML, and it was detected only in 81 out of more than 5000 patients that entered 3 MRC studies [26]. The deletion was then detected in combination with t(8;21) for 29 out of these 81 patients, and the karyotyping indicated that a common area of deletion in region 9q21-22 was present in more than 90% of the cases. Targeted analysis against AML1-ETO was not performed in this study. del(9q) has also been found cooccurring with fusion due to insertion [44]. Based on the results from several clinical studies, the del(9q) abnormality seems to be present in 15%–35% of AML patients with t(8;21) [24, 26]. There was no indication from the MRC data for an adverse prognostic impact of this additional abnormality; patients with t(8;21) together with del(9q) had a 5-year overall survival of 75% in this study [26].

Sweetser et al. investigated the molecular genetics of 43 patients with del(9q) [45]. They described a commonly deleted region of 2.4 Mb containing 11 genes, 7 of these being downregulated in del(9q) AML compared with normal CD34$^+$ hematopoietic cells or AML cells with normal karyotype. Two of these genes, TLE1 and TLE4, are most likely tumor suppressor since, loss of expression in AML1-ETO expressing cells leads to increased proliferation and cell survival.

Numerical Abnormalities. Loss of sex chromosomes is especially common in t(8;21) AML patients (Table 1). Loss of one X chromosome does not seem to have any prognostic impact, whereas a recent study described a weak good prognostic impact for -Y [7].

Other Cytogenetic Abnormalities. Several abnormalities have been described in patients with t(8;21) AML such as trisomy 4 and 8, but these combinations are uncommon, and their possible prognostic impact remains to be investigated. Other abnormalities include tetraploid or near-tetraploid clones [46].

Mutations of KIT. Wang et al. characterized c-kit mutations in a group of 54 AML patients with t(8;21), and they observed mutations for 26 of these patients (Figure 3) [30].

(i) 21 patients had abnormalities in the tyrosine kinase domain, four patients had mutations in the juxtamembrane or extracellular domains, and the last patient had a mutation in the kinase insert sequence between the adenosine triphosphate binding and phosphotransferase regions of the tyrosine kinase domain.

(ii) Seven different point mutations, three internal tandem duplications (ITD), and one amino acid deletion were detected.

(iii) The most common KIT abnormality was the N822K mutation (10/26) followed by three variants of D816 mutations (9/26); this is similar to another study [31].

Thus, many different KIT mutations have been detected in combination with t(8;21), but mutations in the tyrosine kinase domain predominate [30–32]. The incidence of KIT mutations in t(8;21) AML varies between 6%–31% in most clinical studies [30–41], and one exceptional study in children reported an incidence of 43% [47].

The biological impact of different KIT mutations was investigated in a recent experimental study [48]. These authors compared the effects of coexpressing either (i) the more common KITD 814V mutation within the tyrosine kinase domain or (ii) the less common deletion within Exon 8 (E8D419) with AML1-ETO in an experimental animal model. Their observations suggested that the KIT mutations were important for the disease phenotype and the KIT deletion was associated with a less aggressive disease.

(a) *Full-length AML1-ETO (A1-E) protein*

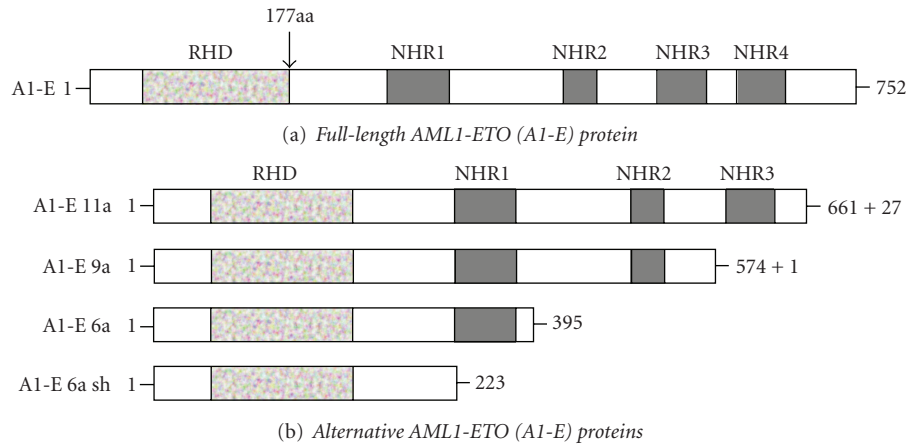(b) *Alternative AML1-ETO (A1-E) proteins*

FIGURE 1: Domain organization of the full-length and alternative AML1-ETO fusion proteins. (a) The full-length AML1-ETO (A1-E) protein is shown, where most of the ETO (RUNX1T1) gene is fused into the N-terminal 177aa of AML1 (RUNX1) gene giving rise to a transcript coding for a protein of 752 amino acids (aa). The AML1 gene encodes the Runt homology domain (RHD) which is a DNA-binding protein, while ETO encodes four highly conserved functional domains called nervy homology domains (NHR1-4). (b) Different fusion transcripts arise due to alternative exon usage and splicing, which give rise to truncated proteins lacking NHR domains. Protein size (i.e., number of aa) is shown on the right with the number of additional aa that were not included in the original sequence. These alternative A1-E transcripts can be coexpressed alongside the full-length transcript and have different leukemogenic capabilities.
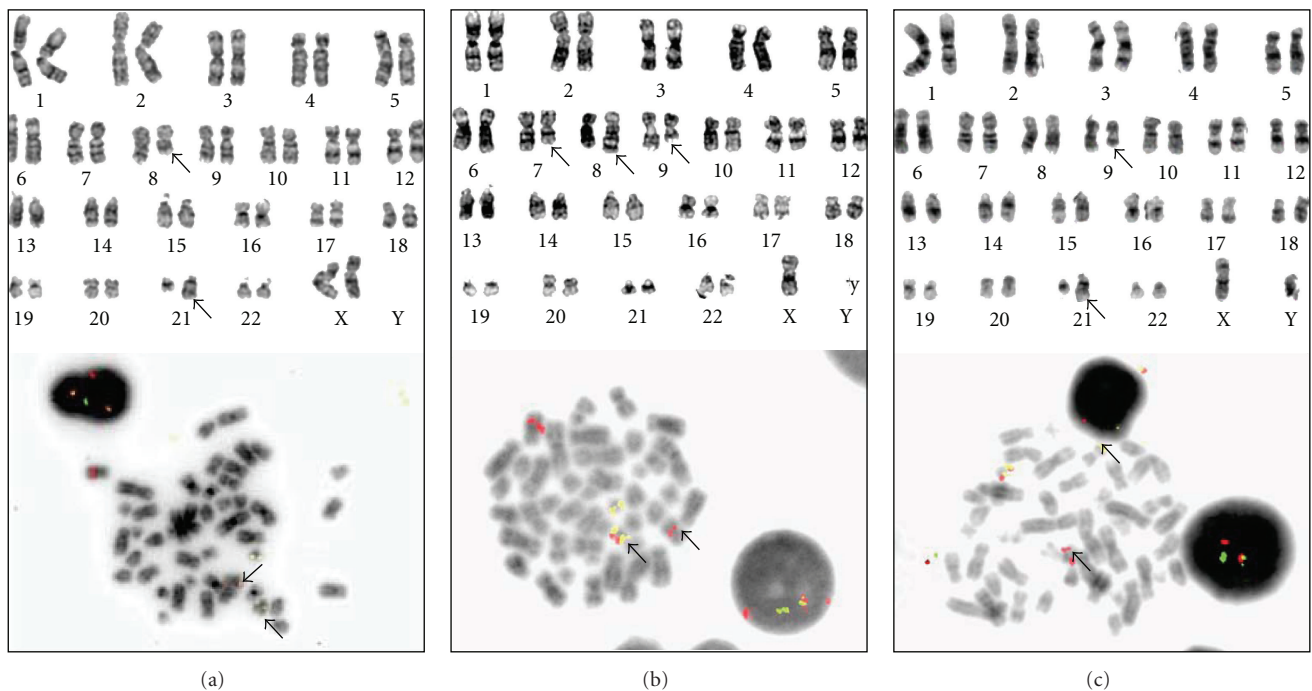

FIGURE 2: Cytogenetic analysis of AML blasts by G-banding and FISH. (a) The derivative chromosomes from a simple reciprocal translocation between 8q22 and 21q22 are detectable by G-banding (upper panel) and the translocation can be verified using FISH probes (lower panel) against ETO and AML1. With this particular probe (Vysis LSI ETV6(TEL)/RUNX1(AML1) ES Dual Color) fusion signals will appear both on derivative 8 and derivative 22. (b) In rare cases, AML1-ETO fusion occurs as a result of insertion. Small insertions can only be detected using FISH probes. In this case, the translocation t(7;8)(q11;q22) between chromosome 7 and 8 and the 9q deletion del(9)(q12q22) were detected. As deletion 9q is rare in AML and can coexist with AML1-ETO fusion, FISH analysis was, therefore, performed. Only one fusion signal on derivative 22 was detected, indicating an ins(21;8)(q22;q22q22) insertion. (c) AML1 can also be involved in translocations with other partners mimicking complex t(8;21). In this case, there is a translocation between 9q22 and 21q22. FISH analysis using in-house split-signal probes against AML1 verified the involvement of these genes, whereas the gene on derivative 9 is unknown.

TABLE 1: Genetic abnormalities commonly detected in combination with t(8;21).

| Abnormality	Frequency in t(8;21) AML	Documented prognostic impact	Reference
Chromosomal abnormalities			
-X in female patients	30%–40%	None	[7, 24, 25]
-Y in male patients	50%–60%	Possible improved	[7, 24, 25]
Del(9q)	15%–35%; most studies state 15%–20%	None	[7, 24–26]
Trisomy 8	8%		[27]
Complex abnormalities	9%–23%	Adverse prognosis	[27–29]
Molecular abnormalities			
KIT mutations	25%–50%	Possible adverse prognosis	[30–41]
JAK2V617F	6%–8%		[36, 42]
Flt3 -ITD	5%	Adverse prognosis	[24, 33, 43]
Flt3 D853	3%–7%		[24, 43]
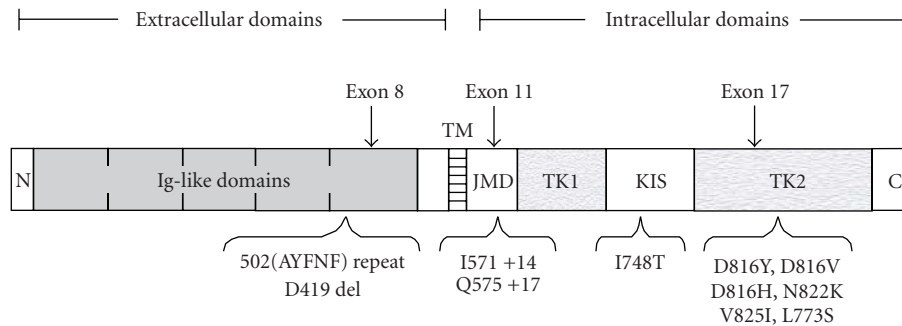
FIGURE 3: General architecture of the c-kit receptor and the mutations described in combination with the t(8;21) abnormality in the study by Wang et al. [30]. The c-kit type III receptor tyrosine kinase consists of an extracellular ligand-binding portion comprising five immunoglobulin-(Ig-) like repeats, a single transmembrane (TM) domain, a juxtamembrane domain (JMD), and a cytoplasmic portion containing and a split tyrosine kinase domain (TK1 and TK2) with a kinase insert sequence (KIS). Locations of c-kit abnormalities found in t(8;21) AML are indicated by the arrows. c-kit mutations are found more frequently within the extracellular fifth immunoglobulin-like domain (exon 8) and the second tyrosine kinase domain which contains the activation loop (exon 17).

The possible prognostic impact of KIT mutations has been investigated in several relatively small studies including 33–54 patients [30, 32, 34, 36] that all have described an adverse impact on relapse rate and/or long-term survival. Relapse rates as high as 70%–80% have been observed [31, 33, 34]. An adverse impact on overall survival was also described in the study reported by Boissel et al. that included 56 patients [33]. However, several recent studies have described no prognostic impact of the KIT mutations in t(8;21) AML both for adult patients [35, 38, 39] and in children AML [40, 47].

Flt3-Abnormalities. Flt3-ITD occurs at a relatively low frequency in t(8;21) AML and seems to have an adverse prognostic impact also in these patients (Table 1) [33]. Animal models suggest that both AML1-ETO and Flt3-ITD alone are insufficient to cause leukemogenesis, but they may cooperate in inducing AML [49]. Flt3 mutations seem to be associated with an adverse prognosis in patients with t(8;21); a recent preliminary report described a 3-year overall survival of only 26% [35].

JAK2V617F Mutations. Only three studies have investigated these mutations in t(8;21) AML, and they occurred in less than 10% of the patients [35, 36, 42].

Ras Mutations. Mutations in NRas and KRas appear to be more frequent in pediatric than in adult patients, but they do not seem to have any prognostic impact in either group [11, 33, 35, 38]. Even though Ras mutations, thus, do not seem to have any impact on chemosensitivity, experimental studies suggest that these mutations are important in leukemogenesis and promote progression towards transformation in cells expressing the t(8;21) fusion protein [50].

PDGF Receptor Mutations. Results from animal models suggest that PDGF-receptor (PDGFR) mutations may cooperate with AML1-ETO in leukemogenesis (see below), and PDGFRA seems to be frequently expressed in t(8;21) AML cells at least for pediatric patients [51]. However, PDGFRA mutations seem to be very uncommon in these patients although a N870S mutation has been described in the homologous domain of the activating KIT and Flt3 mutations.

Other Molecular-Genetic Abnormalities. Mutations in AML1 and in the hematopoietic transcription factor PU-1 have been described in exceptional patients with t(8;21) AML [25]. The t(8;21) abnormality does not seem to coexist with CEBPA mutations [52], or any of the recently described mutations in IDH 1/2 [53, 54] or DNMT3A [54].

2.3. t(8;21) in Secondary AML. Approximately 10%–20% of all cases of t(8;21) AML are secondary [25, 27] and can be detected after treatment for hematologic malignancies (e.g., T-ALL, Hodgkin's disease, and non-Hodgkin's lymphoma) as well as solid tumors (e.g., breast, lung, prostate, esophageal, and thyroid cancer) [55–57]. Secondary AML seems most common after chemotherapy, but it has also been described after radiotherapy alone [55]. The median latency until diagnosis was 37 months in one study, varying from 11 to 126 months [55].

The secondary form is associated with significantly higher age (median 59 versus 41 years) and higher peripheral blood blast counts than the de novo variant [55]. Secondary forms are also of the M2-subtype according to the FAB classification, Auer rods are detected only for certain patients, and the immunophenotype is similar to de novo forms usually being CD33$^+$CD34$^+$CD117$^+$CD19$^+$CD56$^+$ [55]. A recent study described loss of the Y-chromosome in only 12% of all secondary cases compared with 36% of the de novo t(8;21) subset, whereas the frequencies of other abnormalities were similar for secondary and de novo forms [25]. The overall survival of patients with secondary t(8;21) seems to be significantly inferior to patients with de novo t(8;21) AML [55]. However, even though secondary t(8;21) AML has a reduced frequency of -Y, this decreased frequency of a favorable marker cannot explain the adverse prognosis of secondary t(8;21) AML, because the prognostic impact of -Y in the de novo group is relatively weak [7].

3. Molecular Genetics of t(8;21)

3.1. The AML1-ETO Fusion Gene. As described above (Section 2.2, Figure 2), simple reciprocal translocation is the most common abnormality. The fusion gene can also be formed through complex genetic abnormalities that can be detected by cytogenetic analysis, but these abnormalities may also be cryptic and easily overlooked by conventional G-banding.

The in-frame fusion of AML1 (RUNX1) to ETO (RUNX1T1) generally occurs with break-point in AML1 in intron 5-6 and in ETO in intron 1b-2 [24, 58]. The molecular effects on the fusion protein are given by its structure as illustrated in Figure 1(a) and Table 2. AML1 is a transcription factor that is crucial for hematopoietic differentiation, and binds to enhancers and promoters through its aminoterminal Runt domain; this domain is also present in the AML1-ETO fusion-protein and the protein can, therefore, bind to AML1 target gene promoters. However, even though the fusion protein has binding similarities with AML1, it causes an altered transcriptional activation and has another subnuclear localization than the normal AML1 transcription factor [59]. The ETO/RUNX1T1-encoded protein referred

to as CBFA2T is also a nuclear protein and functions as a transcriptional repressor through its binding to both histone deacetylases and transcription factors [60, 61]. The full-length fusion protein contains all except the 31 N-terminal amino acids. The NHR-2 domain is important for homodimerization and interactions with components of the repressor complexes and seems to be responsible for the reduced intranuclear mobility of the fusion protein [62–64]. Thus, the final result of this combination will often be binding to AML1 target gene promoters resulting in suppression [63]. In addition, the fusion protein is directed to nuclear microenvironments distinct from those where the AML1 molecule resides and for this reason may not bind to all AML1 targets [18, 19]. the fusion protein also colocalizes with core-binding factor β (CBFβ) within the nucleus; this colocalization results in a reduced intranuclear mobility of CBFβ that probably disturbs myeloid differentiation [64].

3.2. AML1-ETO Has Several Splice Variants. Several splice variants of the AML1-ETO gene are present in t(8;21) patient cells, and these are all variants in the ETO part of the molecule [65]. It is generally agreed that the full-length variant alone does not have a leukemogenic effect; this conclusion is based on studies using cell lines as well as in animal models (see Section 6). However, analyses of primary AML cells with t(8;21) have demonstrated that numerous in-frame and out-of-frame transcript variants exist, and these variants seem to result from alternative splicing, internal deletions, or breakpoint region insertions [65]. The full-length molecule encodes a protein of 752 amino acids, whereas the variants are generally shorter (Figure 1(b)).

(i) The AML1-ETO9a variant includes the alternative ETO9a exon and results in a truncated molecule of 575 amino acids that lacks the NHR3 and NHR4 domains [66, 67]. In contrast to the full-length variant, this molecule alone is leukemogenic in a mouse model [66, 67], but this does not seem to be true for an alternative model [68]. A possible explanation for this difference could be that the levels of transcript/fusion protein seem to be lower in the nonleukemic model. The AML1-derived domains together with the ETO-derived NHR-2 domain seem to be critical for this leukemogenic activity. Coexpression of the full-length molecule together with the AML1-ETO9a variant results in earlier onset of leukemia and blockade of myeloid differentiation at an earlier stage. Thus, the various splice variants seem to cooperate in leukemogenesis.

(ii) Another variant contains an alternative exon at the C-terminal end instead of NH4R/Mynd; this exon encodes 27 amino acids in-frame. The variant is expressed in primary human t(8;21) AML cells and the encoded protein seems to reduce repressor activity and tends to form multimeres [69].

(iii) ETO-exon 6a can also be incorporated, potentially giving rise to two different truncated transcripts predicted to encode proteins of 223 and 395 amino acids, respectively [70]. The shorter variant lacks all four

TABLE 2: Molecular structure of the t(8;21) fusion protein, the origin of various domains, and the localization of important molecular interactions.

Origin	Domain (alternative nomenclature)	Molecular interactions
	N-terminal	
AML1	Runt	DNA binding
		Binding to CBFβ with formation of heterodimers
		Binding of other transcriptional regulators
RUNX1T1-derived domains	NHR1 (eTAFH)	Interacts with the nuclear hormone receptor corepressor
		Interaction with the activation domain of E-proteins (E2A and HEB)
	NHR2 (HHR)	Mediates oligomerization with itself or other ETO molecules
		Interacts with the corepressors Sin3, Gfi1, and histone deacetylases 1 and 3
	NHR3 (Nervy)	Interacts with the regulatory subunit of type II cAMP-dependent protein kinase
		This domain together with the NHR4 domain is absent in the leukemogenic AE9a splice variant that naturally occurs in primary human AML cells with t(8;21)
	NHR4 (MYND)	N-CoR and the silencing mediator of retinoid and thyroid hormone receptor (SMRT); these are associated with HDACs
		SON, an RNA/DNA-binding protein
		This domain is absent in the leukemogenic AE9a splice variant
	C-Terminal	

NHR domains whereas the longer variant (referred to as AML1-ETO6a) retains the NHR-1 domain. The AML1-ETO6a variant alone is not leukemogenic in animal models but seems to modulate the activity of the full-length fusion protein.

(iv) The two variants A1bETO and A1cETO were identified in primary human AML cells and have additional sequences upstream to the first AML1 exon. Several in-frame variants of these forms have been identified, and their final effects on the expression of AML1 responsive genes seem to vary from repressive to activating [65].

To summarize, several molecular variants of the AML1-ETO fusion protein exist, and the final effect of this chromosomal translocation thus depends on the balance between these various isoforms.

3.3. The Fusion Protein Has Several Binding Partners. The AML/ETO fusion molecule has several partner molecules [60, 71–78]; nine important partners are listed in the upper part of Table 3. Seven of these partner molecules are involved in the regulation of gene transcription; the molecular mechanisms behind these effects vary between the partners, but involve (i) altered histone deacetylation, for example, regulation of histone acetylation through recruitment of histone deacetylases or the E-protein interaction with the histone acetyltransferase; and (ii) altered DNA methylation

through interaction with DNA methyltransferase. Several of these partner molecules are expected to increase viability, and proliferation and/or decrease differentiation, but their relative contribution to leukemogenesis seems to vary and for example the E-protein interaction seems to be less important [79]. The last two partner molecules mentioned in Table 3 include SON that shows a cytoplasmic localization and is also involved in regulation of proliferation and apoptosis, and protein kinase A (PKA) that based on the initial study does not seem to have any major role in leukemogenesis [78]. Finally, the biological functions and thereby the leukemic activity of the fusion protein can probably be modulated through two mechanisms as described in the lower part of Table 3, either through proteolytic cleavage by calpains or through alternative splicing.

Even though many biological effects of the fusion molecule seem to depend on altered expression of AML1-regulated genes [62–64], other mechanisms involving the ETO portion of the fusion protein may also be important. For example, ETO2(MTG16) has corepressor activity and binds to the transcriptional repressor N-CoR; this binding is inhibited by the fusion protein probably through occupation of the ETO2 binding site by the Mynd/NHR4 domain [80]. This effect seems to be important for the myeloid differentiation block. Another example is the oligomerization domain of ETO that is important for the AML1/ETO-mediated regulation of cell-cycle progression and apoptosis [81].

TABLE 3: Important proteins that directly interact with or modulate the t(8;21) fusion protein.

Molecule	Function of the molecule	Structure: mechanism of interaction	Function/biological effect
Molecular interaction with the fusion molecule as a partner molecules			
GFI1 [71]	Transcription repressor	GF35N and GF36S variant alleles have repressor activity. The fusion protein colocalizes and interacts with the more common GFI136S, and its repressor activity is thereby inhibited; the fusion protein does not colocalize or inhibit the GFI135N repressor activity	GFI1 is a regulator of myeloid differentiation, and the interaction and effect of the fusion protein vary between patients and depend on genetic differences
CBPβ [72]	Transcription repressor	Forms heterodimers with the t(8;21) fusion protein through binding to the Runt domain from AML1/RUNX1	Animal models suggest that CBPβ is important for contribution to the fusion proteins inhibition of neutrophil differentiation, is essential for its growth-enhancing effect and cooperation with receptor-initiated signaling
E-proteins [60]	Transcription factor	DNA-bound E-proteins interact with the histone acetyl transferase p300/CREB binding protein, leading to histone acetylation and initiation of transcription. This effect is silenced by the fusion protein through (i) preventing E-protein/p300 activation and (ii) local recruitment of HDACs	E-proteins are important regulators of growth, differentiation and apoptosis and these functions are probably inhibited through a stable binding to the NHR4 domain of the fusion protein
SMRT/N-CoR complex [73]	Transcriptional regulators	Binding of the complex together with their associated HDACs to AML1 target genes through the fusion protein causes aberrant repression of transcription	Contributes to the differentiation block and attenuates the effect of the fusion protein on cell proliferation
UBF1 [74]	Transcription factor	UBF1 binds ribosomal DNA and regulates RNA polymerase 1 activity (see below); the fusion protein associates with UBF1	Modulation of RNA polymerase 1-mediated ribosomal RNA transcription during interphase
SON [75]	Growth regulation	Binding to the NHR4 domain and is possibly involved in the antiproliferative signaling mediated by this domain	SON shows an abnormal cytoplasmic localization in t(8;21) cells; the functions are largely unknown but it seems to be involved in regulation of proliferation and apoptosis
Histone deacetylases [73]	Acetylation of histones	Direct recruitment of HDACs with silencing of AML1-target hematopoietic genes	There is physical binding between the fusion protein and HDAC1, the final functional effect being regarded as a leukemia-enhancing effect
DNA methyl-transferase 1 [76]	DNA methylation	Silencing of gene expression through methylation, probably functionally linked to HDACs	Contributes to the silencing of gene expression, and is involved in the reduced IL3 expression
Protein kinase A (PKA) [78]	Protein phosphorylation	Binding to the NHR-3 domain of the fusion protein	Even though PKA is important for regulation of cell proliferation, the interaction with AML1-ETO does not seem to have any major impact on proliferation or *in vivo* leukemogenesis
Modulation of the fusion molecule			
Calpains [77]	Proteolytic cleavage	Calpain is required for the induction of blood disorders by the fusion protein in Drosophilia	Calpains cleave a restricted set of protein substrates; one hypothesis is that the enzyme cleaves the fusion protein, and thereby generates a more potent inducer of leukemia similar to the leukemic splice variant. Alternatively calpains may affect leukemic cell migration
Alternative AML1/ETO splicing [62]		One of the splice variants lacks the two carboxyterminal ETO domains	In contrast to the full-length variant this alternatively spliced molecule alone can induce leukemic transformation in experimental models without additional genetic abnormalities

3.4. The Fusion Protein Affects a Wide Range of Biological Functions. The fusion molecule affects a wide range of cellular molecules, and the data summarized in Table 4 illustrates the complexity of the AML1/ETO effects [20, 62, 74, 76, 82–103]. Firstly, gene expression as well as ribosomal function are affected. Secondly, the fusion protein can reduce DNA repair [20]; this is combined with decreased expression of the p53 tumor suppressor, and the final effect is probably an increased risk of new leukemogenic events [104]. A recent study in Drosophila described that the fusion molecule induced increased levels of reactive oxygen species (ROS); these high ROS levels are important for the development

of the AML1-ETO associated phenotype and may also contribute to leukemogenesis through an increased risk of developing additional genetic abnormalities [105]. Thirdly, the responses to hematopoietic growth factors are altered; this is due to altered cytokine release, receptor expression, and probably also downstream intracellular signaling. These events, together with altered cell-cycle regulation, will alter the proliferative capacity of the cells. Finally, the regulation of apoptosis is altered, and the cells show activation of stress responses. Most of these alterations are in favor of increased proliferation and survival and decreased differentiation (Tables 3 and 4), but the fusion protein also has opposite effects, and this may explain why the fusion protein alone cannot induce leukemic transformation (see above).

Two studies have shown that the fusion protein upregulates Connexin 43 (Cx43) (Table 4). Cx43 forms gap junctions and is thereby involved in communication between cells; it has a role both in normal and leukemic hematopoiesis and may also function as an intracellular signaling molecule independent of its role in gap junction formation [106, 107]. Thus, the effect on Cx43 may represent an additional leukemogenic mechanism in t(8;21) AML.

The fusion protein seems to preferentially bind those AML1 target genes with duplicated binding sites in the regulatory elements, and this selectivity may be an important mechanism for dysregulated gene expression and leukemogenesis [108]. Another selective regulatory mechanism is possibly the epigenetic structure at the fusion protein's target sites; a recent study described that the most downregulated genes were characterized by aberrant repressive histone tail changes both at the AML1 consensus as well as the transcription start site [109].

Intracellular signaling is altered in t(8;21) AML. WT1-mediated signaling may contribute to leukemogenesis, and these patients seem to have increased WT1 levels [110]. Proteomic studies suggest that the t(8;21) abnormality also interacts with the p53 network [111]. The survival and growth of t(8;21) AML cells depend on autocrine loops between vascular endothelial growth factor (VEGF) and its receptors (VEGFR) that activate various downstream pathways like PI-3 kinase, Akt, or MEK cascades [112, 113]. Both VEGFR1 and VEGFR2 monoclonal antibodies suppressed the growth of primary AML cells with t(8;21), an effect mainly mediated through reduced phosphorylation of Akt and MEK [114]. In addition, inhibition of VEGFR2 potentiated the growth inhibitory effect of idarubicin for the t(8;21) Kasumi-1 cell line though the mechanisms of this effect are not fully elucidated yet [114]. Another study suggests that VEGF receptor type-2-mediated signaling stimulates proliferation of t(8;21) AML cells, an effect mediated through increased phosphorylation of Akt, and inhibition of this signaling seems to potentiate the effect of cytarabine [115].

The global gene expression profiles of U937 AML cells when expressing different AML-associated fusion proteins were compared in a recent study [116]. The analysis revealed a role of AML1-ETO in downstream pathways known to regulate DNA repair and stem-cell maintenance, including activation of the Notch signaling pathway through Jagged-1 ligand [116, 117]. On the other hand, the effect of AML1-ETO depletion has been investigated by electroporating the t(8;21) carrying Kasumi-1 cell line with specific siRNA [118]. This depletion severely diminished the cell clonogenicity, inhibited G1-S transition, reduced apoptosis and induced senescence. The presence of exogenous G-CSF or GM-CSF could not rescue these cells from senescence but partly counteracted the antiproliferative effect.

The effects of AML1-ETO on leukemic stem-cells have not been investigated in detail. However, the effect of AML1 alone on normal hematopoietic stem-cell homeostasis was investigated in a recent study [119], and an enrichment of quiescent stem-cells was observed in AML1-deficient bone marrow. These results suggest a negative regulatory effect of AML1 on normal stem-cells. Thus, the AML1-ETO fusion molecule may then have an opposite effect of AML1 on leukemic stem-cells and cause an enrichment of quiescent AML stem-cells similar to the AML1 deficiency through its suppression of AML1 target genes (see Section 3.1).

4. Alternative Methods for Detection of t(8;21) in Human AML

Cytogenetic analysis by G-banding is highly recommended at the time of diagnosis of all AML patients [120]. Although this time-consuming method requires the leukemic cells to be captured in mitosis, it is still the best screening method for detection of leukemia-specific chromosomal aberrations. t(8;21) is readily detectable by G-banding (Figure 2), but it can also occur due to variant rearrangements (complex translocations, inversion, and insertion) that may be overlooked if additional fusion-specific analyses like fluorescence in situ hybridization (FISH) or reverse transcriptase polymerase chain reaction (RT-PCR) are not used (Figure 2) [121, 122]. Detection of the fusion protein is less common; the protein can then be detected by Western blotting, but a recently described method using immunobeads for flow cytometric detection seems promising and may represent a future alternative [123].

FISH analysis for AML1-ETO fusion should be performed using locus-specific probes. The advantage with this method is that there is no need for cells in metaphase, and it can, therefore, be performed also in bone marrow biopsies or bone marrow/blood smears. Commercially available dual color probes against AML1 and ETO resulting in fusion-signals are most commonly used (Figure 2). Particular care has to be taken when interpreting the signaling patterns from interphase analyses, and dual-color double-fusion probes should be used in order to reduce the false-positive rate. Although rare, one has to be aware that variant AML1-ETO rearrangements can give only one fusion signal (Figure 2). Probes covering the whole AML1 gene will also detect other AML1 rearrangements, and instead of fusion signals, the AML1 rearrangements will then be seen as two weaker signals that can be misinterpreted as trisomy 21 in interphase analyses. The chromosomal band 21q22 where the AML1 gene is situated, has been reported involved in 55 different rearrangements, and several of these can be overlooked by G-banding, and the majority of these abnormalities have

TABLE 4: Important molecular mechanisms involved in AML1/ETO-induced leukemic transformation.

Molecule	Function	Effect of AML1/ETO on the molecule	Final effect on t(8;21) cells
		Altered gene transcription	
PU.1 [82]	Transcription factor	Decreased expression by AML1/ETO	Inhibition of differentiation
C/EBPα [83, 84]	Transcription factor	Downregulation of C/EBPα; the normal function of this factor is regulation of differentiation and inhibition of proliferation	Increased proliferation and inhibition of differentiation
C/EBP β [85]	Transcription factor	The normal function is transcriptional upregulation of C/EBPα	Altered regulation of differentiation and proliferation through reduced expression of C/EBPα
POU4F1 [86]	Transcription factor	The POU4F1 levels are significantly correlated with the fusion protein levels. One study described differential regulation of 140 genes by this factor, and half of them are also AML1/ETO targets	POU4F1 probably contribute to the gene expression signature associated with t(8;21) AML
PAX5 [87, 88]	Transcription factor	Increased expression at the mRNA and protein level	Aberrant expression of B lymphocyte markers, including CD19, CD79a
		RNA-rependent mechanisms and ribosomal functions	
μRNA [62, 89]	Regulatory RNA molecules	The fusion protein selects a set of μRNAs (miR); it occupies the miR-24-23-27 locus and upregulates their expression	Modulation of proliferation and differentiation through the effects on miR24
		miR24 downregulates the mitogen-activated protein kinase phosphatase 7 and enhances the downstream signaling through phosphorylation of c-jun and p38 kinases	
		Silencing of miR223 through epigenetic mechanisms	Altered regulation of myelopoiesis through effects on mir223
RNA polymerase I [74]	Transcriptional regulators	The fusion protein seems to localize to the nucleolar organizing regions during mitosis, associates with metaphase chromosomes and occupies ribosomal DNA repeats during interphase together with UBF1 (see Table 3)	In contrast to AML1 the fusion protein seems to be a positive regulator of rDNA transcription. Transcription regulated by RNA polymerase 1 seems to increase the proliferation of transformed cells (discussed in [74])
		DNA damage and repair	
OGG1[20]	DNA repair	OGG1 is an important part of the DNA base excision repair pathway, its expression is downregulated by the fusion protein	High OGG1 levels are associated with an adverse prognosis; the downregulation may increase chemosensitivity
DNA damage [90]	Carcinogen-DNA adducts	Increased formation of aromatic hydrocarbon-DNA adducts and upregulation of the cytochrome P450 1A1 enzyme that metabolizes polycyclic aromatic hydrocarbons	This effect may contribute to an increased susceptibility to additional genetic damage
Increased intracellular ROS [105]	Altered signalling. DNA damage?	AML1-ETO causes increased intracellular levels of reactive oxygen species (ROS) in Drosophila	ROS are important for induction of the AML1-ETO associated phenotype and may also increase the risk of additional genetic abnormalities
Increased mutation frequency [91]		Predisposition to leukemia progression	Predisposed for additional genetic effects that are required for leukemogenesis
		Cytokine-mediated growth regulation	
IL3 [76]	Hematopoietic growth factor	Decreased gene expression	Decreased growth factor-dependent proliferation
M-CSF receptor [92]	Growth factor	M-CSF is a growth-enhancing hematopoietic growth factor	Increased cytokine-dependent AML cell proliferation

TABLE 4: Continued.

Molecule	Function	Effect of AML1/ETO on the molecule	Final effect on t(8;21) cells
G-CSF receptor [93]	Growth factor	G-CSF is a growth-enhancing hematopoietic growth factor	Increased cytokine-dependent AML cell proliferation
BCL2 [94]	Antiapoptotic signaling	Upregulation by the AML1-ETO fusion protein	Increased antiapoptotic signaling
C/EBPε [92, 93]	Transcriptional regulator	Induction of G-CSF receptor expression; upregulation of the myeloid-specific promoter for the M-CSF receptor	Increased growth factor receptor expression and thereby increased cytokine-dependent proliferation by t(8;21) cells
NF1 [95]	Tumor suppressor	Decreased expression of the Neurofibromatosis1 (NF1) tumor suppressor	Decreased protein levels are associated with increased response of primary AML cells to GM-CSF
Tyrosine receptor kinase A [96]	A part of the nerve growth factor receptor (NGF)	Upregulation of this growth factor both at the mRNA and protein level	NGF is released by bone marrow stromal cells; in addition, AML1-ETO expressing cells show increased proliferation in response to growth factors
		Cell-cycle regulation	
p21 [97]	Negative cell-cycle regulator	Increased mRNA and protein levels of p21	p21 is a cell-cycle inhibitor, this effect may contribute to the absence of leukemogenesis in the presence of t(8;21) alone
p27^{kip} [98]	Negative cell-cycle regulator	Increased expression caused by either a direct effect of the fusion protein or by Cx43	Cell-cycle inhibition
SSX21P [99]	Cell-cycle regulation?	Low expression of this molecule is associated with low expression of CDC20; possibly causing attenuation of the spindle checkpoint	Altered cell-cycle regulation, increased risk of aneuploidy?
Disrupted spindle checkpoint [100]	Aneuploidy	Disruption of the spindle checkpoint during cell-cycle progression	Increased risk of aneuploidy
		Regulation of apoptosis and stress responses	
Annexin A1 [101]	Proapoptotic, antiproliferative	Downregulated at the gene expression level by the fusion protein	The molecule has proapoptotic and antiproliferative effects; these functions are thus inhibited
Connexin 43 (Cx43) [98, 102]	Gap junction component	Increased expression of Connexin 43 in cells with t(8;21), possibly both a direct and an indirect effect mediated via c-Jun	Cx43 often inhibits cell proliferation both through gap junction dependent and independent mechanisms; this effect may contribute to the lack of leukemogenesis by the full-length fusion protein
p53 [91]	Tumor suppressor	Activation of the p53 pathway	Possibly increased chemosensitivity and thereby contribution to the good prognosis of these patients
TXNIP [103]	Part of stress responses	Involved in reactive oxygen stress responses, AML cells with t(8;21) have increased protein levels of this molecule. The mechanism is not known	High levels inhibit the proliferation of myeloid progenitor cells; this may contribute to the good prognosis of these patients

been found in AML/MDS [124]. If only interphases are available, for analyses, the methodological alternative is to verify AML1 rearrangements by using AML1 dual color split-signal probes (Figure 2(c)). Thus, due to the possibility of other less common AML1 rearrangements, the detection of t(8;21) may require a careful interpretation of additional molecular analyses before a final conclusion can be reached.

RT-PCR is a rapid and sensitive method for detection of the fusion transcript. Although simple to perform, the method is not without challenges. RNA is readily degraded, and tests for RNA quality have to be performed [125].

RT-PCRs are capable to detect one leukemic cell in 10^5-10^6 normal cells [126], and this demands precautions to avoid cross-contamination leading to false positive results. Furthermore, the presence of leukemia-specific fusion transcripts has been detected in tissue from healthy donors when using nested PCR. Single round PCR should, therefore, be sufficient at diagnosis [127]. False negative results can occur if the assay is not designed to detect all different known fusion and transcript variants. As described above (Section 3.2), several AML1-ETO variants have been described due to alternative promoters and splicing, and

different variants can occur in the same patient. However, most variants include exon 3–5 in AML1 and exon 2–4 in ETO [17]. These regions can thus be used for design of the primers; the problem of false-negative results can thereby be reduced, and standardized protocols for RT-PCR have now been published [128–131]. RT-PCR should thus be regarded as a rapid and sensitive methodology, and it can even be used for simultaneous screening for several prognostically important fusion transcripts [132].

5. The Biological and Clinical Characteristics of Human t(8;21) AML

5.1. The Diagnostic Criteria for AML in Patients with t(8;21). The general diagnostic criterium for AML is detection of at least 20% blasts in the bone marrow. However, t(8;21) AML shows morphological signs of neutrophil maturation. Rare cases can, therefore, be seen with bone marrow blast counts below 20%; according to the WHO classification, such cases should also be classified and treated as AML and not as myelodysplastic syndrome [3].

5.2. Morphological and Immunophenotypic Characteristics of t(8;21) AML. The morphology of the leukemic cells has been described by Arber et al. [3], the most common features being relatively large blasts with basophilic cytoplasm, often numerous azurophilic granules and a perinuclear clearing. Auer rods are common and may be detected in blasts or immature neutrophils. The cells show maturing to promyelocytes and myelocytes, and mature neutrophils, possibly with morphological signs of dysplasia, are also present in the marrow. Among the dysplastic signs are abnormal nuclear segmentation (e.g., pseudo-Pelger-Huet anomaly) and cytoplasmic staining abnormalities like pink staining in mature neutrophils. Eosinophilic precursors are often increased; basophils and/or mast cells are also increased sometimes. The erythroid cells and megakaryocytes are morphologically normal. The abnormal differentiation of the leukemic cells may even cause a morphological picture similar to chronic myeloproliferative neoplasias. Lee et al. [133] described a patient with variant t(8;21) as a complex t(8;10;21) (q22;q24;q22) abnormality; this patient presented with morphological findings in blood and bone marrow mimicking atypical chronic myeloid leukemia.

The blasts express myeloperoxidase and are typically $CD13^+CD34^+HLA-DR^+$ [134]. There are also immunophenotypic signs of granulocyte maturation with subpopulations expressing CD15 or CD65, eventually as a part of asynchronous maturation with concomitant expression of CD34 [134, 135]. Aberrant expression of the lymphoid markers CD19, PAX5, and eventually cytoplasmic CD79a is common [87]. CD56 can also be expressed especially for patients with KIT mutations [134, 135], whereas CD19 expression is uncommon for patients with this mutation [136].

5.3. Extramedullary Manifestations of AML. Myeloid sarcoma is a rare condition that can involve almost any site of the body, but especially lymph nodes and skin (for references, see [137]). Other common sites are head and neck soft and subcutaneous tissue and the orbits, whereas intrathoracic manifestation is uncommon [138, 139]. Such tumors can be the first manifestation and precede the primary bone marrow manifestation by several months, or it can represent the first manifestation of a relapse [137–141]. Myeloid sarcoma has been reported in 15% of patients with t(8;21) AML [27]. However, t(8;21) is a relatively rare cytogenetic abnormality in myeloid sarcomas; a recent cytogenetic study of 74 patients with such sarcomas reported that the t(8;21) abnormality was detected only in 2%-3% of these cases [137]. The sarcomas in patients with t(8;21) can be located to uncommon regions, including (i) intracerebral tumors [142] or intraspinal sarcoma with spinal cord compression [143] (ii) abdominal affection either as ovarial infiltration with ascites [144] or compression of nerves or nerve plexuses (e.g., presacral tumors) with neurological symptoms [145]; (iii) skeletal or heart muscle affection [146]; or (iv) pulmonary involvement with initial symptoms resembling nodular or interstitial pneumonia [133, 139]. Even though these lesions may give serious local symptoms at the time of diagnosis, at least in children detection of granulocytic sarcoma in t(8;21) AML does not seem to have a major impact on the long-term survival [147].

5.4. Myelomastocytosis and Myelomastocytic Leukemia. Systemic mastocytosis and AML, including the t(8;21) variant of AML, can show similarities. Firstly, activating KIT mutations are common both in t(8;21) AML and in systemic mastocytosis [1]. Secondly, systemic mastocytosis can occur in combination with other hematological malignancies, usually myeloid malignancies (AML, myelodysplastic syndrome or chronic myeloproliferative neoplasms) and in the WHO classification, this is termed systemic mastocytosis with associated hematological nonmast cell disease [1]. Thirdly, even though the combination of mastocytosis and t(8;21) AML seems rare [148], the t(8;21) AML variant can show phenotypic similarities with mastocytosis and has increased serum tryptase levels (see below). The KIT mutations are detected both in AML and mast cells in those rare cases when mastocytosis is associated with AML; this observation demonstrate that both cell types then are derived from the same clone [149–151].

Due to the low number of published cases, it is not possible to give general guidelines about the treatment of the myelomastocytic leukemia variants. The bone marrow mast cells seem to become more prominent after intensive AML therapy, but this may simply be due to mast cell chemoresistance as evidenced by their persistence after chemotherapy. Some patients have been treated with allogeneic stem-cell transplantation, and the very limited experience with this therapeutic strategy suggests that the type of conditioning therapy may be important for eradication of both AML and mast cells [149, 150].

A recent study investigated serum tryptase levels in AML [152]. Increased levels were detected in nearly half of AML patients and were associated with t(8;21) and KIT mutations. The levels decreased after chemotherapy [150, 152], suggesting that serum tryptase can be used as a marker of treatment

response in these patients. Thus, altered tryptase levels seem relatively common in t(8;21) AML, but morphological myelomastocytosis is rare. One possible explanation for the increased levels could be tryptase release by the AML cells as an ectopic phenotypic characteristic. Alternatively, the levels may reflect an activation/stimulation of tryptase release by normal mast cells. The association with KIT mutations suggests that AML cell release is most likely.

5.5. Differences between Core-Binding Factor AML with t(8;21) and inv(16)/t(16;16). Even though t(8;21) and inv(16)/t(16;16) are both regarded as core-binding factor AMLs with good prognosis, the two forms show several differences in their pretreatment features. This has been reviewed in detail by Mrózek and Bloomfield [43]. Firstly, t(8;21) AML is more frequent in African than white Americans, it has lower white blood cell counts and lower percentages of blasts in the bone marrow. Secondly, extramedullary disease is less frequent in t(8;21), especially lymphadenopathy, splenomegaly, gingival hypertrophy, and skin/mucosa involvement. Thirdly, the frequency and patterns of secondary cytogenetic abnormalities differ. A secondary chromosomal abnormality is detected in 70% of patients with t(8;21) but only in one-third of inv(16) patients. As described above (Section 2.2), the most frequent secondary abnormalities in t(8;21) is loss of sex chromosomes and deletion of 9q, whereas in inv(16), the most common are +22, +8, del7q, and +21. For mutations detected by molecular genetic methods, both KIT and Ras mutations occur, but they are more frequent in inv(16), than in t(8;21) leukemia [33]. These pretreatment differences suggest that the molecular mechanisms in the leukemogenesis differ between these two groups.

Mrózek and Bloomfield [43] also discussed possible prognostic differences between t(8;21) and inv(16)/t(16;16) AML. Observations from several studies suggest that relapse of t(8;21) is less responsive to salvage treatment, and these patients, therefore, have a lower overall survival. This difference may be caused by the additional genetic abnormalities; the KIT mutations associated with t(8;21) AML seem to have adverse prognostic effects whereas +22 in inv(16) has been associated with lower relapse risk in some studies. Finally, there is possibly also an influence of genetic factors/race on the response to chemotherapy in t(8;21) patients.

6. Animal Models of t(8;21) AML

Several animal models of t(8;21) AML have been developed, including transgenic and knock-in models, conditional knock-in, as well as chimeric models [153]. The models are important for our current understanding of the role of the AML1-ETO fusion protein in leukemogenesis (for detailed discussion and references see [153]). AML1 knock-out causes embryonic death due to bleeding complications, and knock-in models of the AML1-ETO fusion gene resulted in a similar embryonic phenotype, suggesting that repression of AML1-regulated genes are important in the fusion model. Furthermore, one of the transgenic models directed the fusion gene into the stem-cell compartment and its expression

was then significantly lower in lymphoid cells than in myeloid progenitors. This observation underlines the association between AML1-ETO expression and myelopoiesis. Furthermore, transgenic mice that express the AML1-ETO fusion molecule specifically in myeloid cells appeared to be healthy and developed AML only if they in addition were exposed to a mutagen [154]. These observations are consistent with the hypothesis that the AML1-ETO fusion protein alone is not sufficient for development of AML; additional abnormalities have to be present [154]. Similar observations have been made in other conditional knock-in models and models based on retroviral transduction to the stem-cell compartment; the fusion protein alone often results only in increased proliferative capacity and altered regulation of differentiation. In one of these abnormalities an increase in immature eosinophils similar to the human t(8;21) AML was observed (discussed in detail in [153]).

Other chimeric models based on retroviral transduction have also been published (reviewed in [153]). Transfection of the AML1-domain alone from the fusion protein had no effect on hematopoiesis, showing that the ETO-derived part of the molecule is required for development of the hematopoietic abnormalities. Furthermore, the AML1-ETO molecule has also been transfected to mice with other abnormalities, including (i) deficiency of the myelosuppressive Interferon regulatory factor (IRF), (ii) mutations in receptor tyrosine kinases such as TEL/PDGFβR and Flt3; and (iii) downregulation of the p21 cell-cycle inhibitor. All these combinations resulted in development of AML. Thus, the additional abnormalities cooperated with AML1-ETO in leukemogenesis.

7. The Global Gene Expression Profile, microRNA (MIR) Expression, and Epigenomic Profile in Human t(8;21) AML

Global gene expression profiling in AML has revealed that major prognostic subgroups based on genetic markers are recapitulated in large-scale gene expression patterns [4, 155, 156]. These global profiles have identified specific signatures for patients with the t(8;21) abnormality [4, 155, 156], and its prediction can be made with almost 100% specificity and sensitivity [157]. There is an overlap between the expression profiles for the t(8;21) and inv(16) abnormalities [119], but approximately one third of the transcripts are specific for the t(8;21)-associated profile [119]. RUNX1T1/ETO itself has been identified as the most discriminative gene for the t(8;21) cluster [4, 155], but several other genes are also frequently up- or downregulated. Of special interest is probably the transcription factor POU4F1 that is important for embryonic brain development but without any known role in normal or leukemic hematopoiesis. This gene is frequently upregulated in t(8;21) AML [156]; this upregulation is probably not directly caused by the AML1/ETO fusion protein [86] but the unique transcription profile of t(8;21) AML is probably largely attributed to POU4F1 [86]. Finally, AML1-ETO downregulates genes involved in multiple DNA repair pathways, a possible explanation for the increased in vitro DNA damage and p53 activation in these cells

[91, 104]. Based on the results summarized in Table 4 we conclude that the t(8;21) fusion protein alters the expression levels of a wide range of molecules, and thereby affects the regulation of several intracellular processes; this conclusion is also supported by the t(8;21)-associated microarray profile (e.g., altered transcriptional regulation and DNA repair).

Lück and coworkers identified two distinct gene expression signatures among t(8;21) and inv(16) AML based on the presence of KIT mutations [158]. The KIT mutated cases were then characterized by deregulation of genes belonging to the NFκB signaling pathway [159]. On the other hand, Bullinger and coworkers classified t(8;21) and inv(16) AMLs in favorable and unfavorable prognostic subsets based on supervised analysis of gene expression, the two distinct groups being characterized by altered expression of genes involved in the MAP-kinase and the mTOR pathways, respectively [160]. All these pathways are suggested to play a role in leukemogenesis and are considered as potential therapeutic targets [161, 162].

A major part of the published clinical studies do not find any significant prognostic impact of KIT mutations (Section 2.2). The observations from the studies of global gene expression profiles described above and the different effects on intracellular signaling pathways for KIT mutations (the NFκB pathway is affected) compared with chemosensitivity (MAP kinase)/chemoresistance (mTOR) are consistent with the hypothesis that KIT mutations do not have any major prognostic impact. This may be true at least for the chemotherapy regimen used in these German/Austrian studies [158, 160]. A possible explanation for the different prognostic impact between various studies may be that the prognostic impact depends on differences between the chemotherapy regimen.

The global gene expression analyses have identified additional genes than those summarized in Table 4, whose altered expression may be clinically relevant [4, 155, 156]. Firstly, PRAME (preferentially expressed antigen of melanoma) is upregulated, and this AML-associated antigen is now used in vaccination trials and may also be a candidate marker for detection of minimal residual disease (MRD) [163, 164]. Secondly, the structural membrane protein CAV1 is also upregulated, and this molecule is possibly important for chemosensitivity [165]. Among the down-regulated genes are the cystein protease CTSW cathepsin W, the cancer-associated actin-bundling protein LCP1 (lymphocyte cytosolic protein 1), the actin-regulatory protein CAPG (capping protein, gelsolin-like) and the semaphorin receptor PLXNB2 (plexin B2). These genes seem to be involved in cancer cell migration/invasion, cancer-associated angiogenesis or tumor progression [166–169].

It has been demonstrated that patient-derived AML cells express a specific signature of microRNA, a class of small noncoding RNAs involved in regulation of protein coding mRNA [170, 171]. Most microRNAs seem to be downregulated in t(8;21) AML except the miR126/126∗ that is upregulated [170]. miR126/126∗ can inhibit apoptosis, increase cell viability, and enhance colony formation possibly by interacting with AML1-ETO itself [171]. Finally, studies in the t(8;21) positive Kasumi-1 cell line suggest that the microRNA signature seems to be different in the CD34$^+$CD38$^-$ AML cell subset which is believed to harbor a major part of leukemic stem-cells [172].

DNA methylation and histone modifications are important epigenetic mechanisms of gene regulation [173]. These mechanisms seem to play essential roles both independently and cooperatively in malign transformation and progression [173]. This is also true for AML, where different cytogenetic subgroups, including t(8;21), seem to be characterized by distinct epigenetic modifications [174–176]. However, the clustering seems to be less pronounced based on methylation data compared to gene expression data [174]. The t(8;21) DNA methylation cluster harbors patients not having the AML1-ETO fusion gene, even though they seem to have a similar prognosis and also share other features with t(8;21) patients [175]. Furthermore, human hematopoietic stem-cells transduced with the AML1/ETO fusion gene failed to reproduce the epigenetic signature [176], supporting the theory that other mutations are needed to create the fully malign phenotype [154]. In contrast to data from methylation studies, modification of histone H3 Lysine 9 methylation was recently demonstrated to show only minor differences between different cytogenetic groups [177].

8. The Prognostic Impact of t(8;21) in Human AML

8.1. Patients Receiving Conventional Intensive Chemotherapy. t(8;21) is usually associated with a relatively low risk of relapse. A recent MRC report analyzed the survival data for 5876 AML patients (median age 44 years) including 421 patients with t(8;21) [7]. The long-term disease-free survival for this subset was 61%, and a similar high survival has also been observed in other studies [178, 179]. No difference in overall survival was then observed when comparing patients with t(8;21) alone versus patients with additional cytogenetic abnormalities the only possible exception being loss of the Y chromosome that was associated with an improved survival of borderline significance in the MRC study. A French study investigated elderly patients (median age 67 years) with CBF-AML, including 60 patients with t(8;21) [27]. These patients received induction treatment with an anthracycline combined with cytarabine; 80% of the patients achieved complete remission after one induction cycle and 88% after two cycles. However, despite this high remission rate, the median 5-year overall survival for these patients was only 31%. A high white blood cell count (WBC) at diagnosis, poor performance status, and del(9q) were all associated with an adverse prognosis, whereas administration of intensive consolidation treatment was associated with better survival. High WBC is in general considered as an adverse prognostic factor in t(8;21); this is mainly based on clinical studies indicating an unfavorable outcome for patients with high WBC alone [27] or high WBC plus a high percentage of bone marrow blasts [180]. AML t(8;21) with high WBC (usually defined as $>20 \times 10^9$/L) is often considered to have a less favorable prognosis similar to the intermediate prognostic group in clinical studies [181]

(e.g., the HOVON 102 AML/SAKK 30/09 study; EudraCT number 2009-011613-24). Finally, it should also be mentioned that the adverse prognostic impact of KIT mutations that is observed in certain studies may be explained by an association between KIT mutations and high peripheral blood blast counts [37].

A recent study described an adverse prognostic impact of high bone marrow cellularity in patients with t(8;21) [182]. The authors found that the bone marrow cellularity was the single most important prognostic parameter in these patients, and they classified their patients in three groups with (i) neither leukocytosis nor increased bone marrow cellularity (ii) only leukocytosis (cutoff 9.1×10^9/L),and (iii) patients with increased marrow cellularity with or without leukocytosis. The survival after chemotherapy was lowest (32% overall 5-years survival) for the last group. These results strongly suggest that bone marrow cellularity should be further evaluated as a possible prognostic parameter in these patients.

t(8;21) is also regarded as a good prognostic marker in pediatric AML. The complete remission rate close to 100%, eventfree survival exceeding 69% and overall survival exceeding 80% have recently been reported for children included in the MRC-AML10, MRC-AML12 and AML-BFM 98 studies and with no effect of additional chromosomal abnormalities on prognosis [183, 184]. Other pediatric protocols have reported eventfree survival below 50% [15, 185, 186], and the reasons for this discrepancy remain to be identified. However, children with relapsed t(8;21) AML seem to have a chance to be cured by salvage therapy [183, 184].

8.2. Experiences with Allogeneic Stem-Cell Transplantation in t(8;21) AML. Two large studies each including more than 300 patients have investigated the outcome after allogeneic stem-cell transplantation for AML patients with inv(16) and t(8;21) [187, 188]. Kuwatsuka et al. described no difference between patients in first remission receiving allogeneic and autologous stem-cell transplantation, and a long-term disease-free survival of 15%–20% was seen even for 85 patients undergoing allotransplantation not in complete remission [187]. As for patients receiving conventional therapy, additional cytogenetic abnormalities did not have any prognostic impact for these patients either. Similarly, Gorin et al. observed a comparable survival for auto- and allotransplanted patients of approximately 60%, and additional cytogenetic abnormalities had no significant prognostic impact, but high white blood cell counts had an adverse impact [188].

The smaller study by Schlenk et al. showed no advantage of allotransplantation compared with conventional intensive chemotherapy [189], thus supporting the two studies described above. In contrast, Shin et al. observed that the survival for allotransplanted patients had improved during the last years and was significantly better than for patients receiving high-dose cytarabine in a retrospective multicenter study based on distribution of questionnaires to each participating center [190]. In our opinion, most of the currently available data suggest that allogeneic stem-cell transplantation usually should not be recommended

for t(8;21) patients in first complete remission. However, the presence of additional KIT mutations, high WBC counts and secondary leukemia have been associated with an adverse prognosis (see Sections 2.2, 2.3, and 8.1), suggesting that allotransplantation should be considered even in first complete remission for the younger subsets of these patients at least when an optimal donor is available.

The possible prognostic impact of KIT mutations in combination with t(8;21) was discussed in detail in Section 2.2. This question has been addressed in several clinical studies, and both adverse prognosis and no prognostic impact have been observed [30–36, 38–40, 47]. It was recently recommended that core-binding factor AML with KIT mutations should be classified as intermediate with regard to prognosis [191]. However, it is in our opinion, difficult to recommend allogeneic transplantation for these patients with its risk of early transplant-related mortality as long as the results are conflicting and several studies show no prognostic impact of the KIT mutations after conventional chemotherapy.

8.3. Disease-Stabilizing Therapy in t(8;21) AML. The combination of all-trans retinoic acid (ATRA) together with valproic acid or another histone deacetylase inhibitor is now tried for disease-stabilization in human AML [192, 193]. Most of the patients included in these studies have not been suitable for intensive chemotherapy; many of them had relapsed disease, and only a small minority had t(8;21). Experiments in the t(8;21) positive Kasumi-1 cell line have demonstrated that the AML1/ETO fusion protein recruits an HDAC-containing repressor complex to the promoters of AML1 target genes [73]. Valproic acid and probably also other HDAC inhibitors cause a dissociation of the fusion molecule from the HDACs. Histone acetylation thereby leads to transcriptional reactivation and increased proapoptotic signaling [73, 101].

Only a few clinical studies have investigated the effect of ATRA in t(8;21) AML, and the results are conflicting. Treatment with ATRA alone for 40 days induced complete hematological remission in one patient [194], whereas others have reported that coexpression of the t(15;17), and the t(8;21) encoded fusion proteins can be associated with ATRA resistance [195].

9. Detection of Minimal Residual Disease in t(8;21) AML

9.1. Methodological Strategies for Detection of MRD. Although detection of the AML1-ETO fusion transcripts represent a favorable prognostic marker, up to 30% of patients will experience a relapse of the disease [7, 178, 179]. MRD means remaining leukemia cells in a patient judged to be in complete hematological remission according to conventional morphological criteria. AML1-ETO-transcripts can persist in patients with t(8;21) even after stem-cell transplantation with GVHD and in long-time CR [196–200], but an increase in the fusion transcript expression seems to be predictive of relapse [201].

RT-PCR is a sensitive approach which identifies leukemic cells in 10^5-10^6 normal cells. By using quantitative RT-PCR it is easy to follow the patient through the course of the disease [202]. Leroy et al. assessed the prognostic value of real-time quantitative PCR in 21 AML1-ETO patients treated by the same protocol and who all achieved CR. Blood and bone marrow were collected at diagnosis, at CR, after intensive consolidation therapy and every 3–6 months thereafter; the median followup time being 15 months. The relapse rate was higher in patients with high pretreatment fusion transcript expression, and the absence of recurrent disease correlated with posttherapeutic absolute transcript levels below 10^{-3} compared to the Kasumi-1 cell line, or more than 3 log decrease of transcript levels compared to the levels at the time of diagnosis [202].

In a study by Ommen et al. various chromosomal aberrations appeared to have different relapse kinetics and therefore optimal sampling intervals might differ; the best MRD sampling interval for AML1-ETO seems to be every fourth month [203]. However, clinical utility of monitoring MRD with such high sensitivity is still under investigation and standardization of sampling procedures, handling and shipment of samples as well as the PCR analyses are required. Standardization of the assays is also necessary to allow comparison of results in different studies and for setting a threshold for AML1-ETO-transcript expression that defines molecular relapse [120, 204].

Multiparameter flow cytometry is less sensitive, but can be applied in the evaluation of MRD in most AML-cases and provides additional information about remaining cells (reviewed in [204]). The method is rapid and detects the presence of 10^{-4} leukemic cells, but it is not as specific as PCR due to the possibility of phenotypic shifts in relapsed disease. Tandem analysis with RT-PCR and flow cytometry can improve MRD detection [205]. Interphase FISH may also have a potential as an adjunct analysis to cytomorphology, cytogenetics, or multiparameter flow cytometry in the identification of MRD, since strong agreement between these methods has been described in large cohorts [206–208].

9.2. Clinical Consequences of MRD Detection. Benefit from preemptive treatment has been shown in PML-RARA acute promyelocytic leukemias [209, 210], but for other acute myeloid leukemia variants, few investigators have taken clinical action on detection of molecular relapse. For clinical utility, it has been shown that kinetics of the AML1-ETO decline correlate to relapse rate and outcome [202, 205, 211]. Monitoring the different alternative splice variants of the fusion transcript has also shown that persistence of the exon9a variant is indicative for later relapse [131].

We previously described that the translocation can be detected on Guthrie cards and may thus occur *in utero* (Section 2.1), the fusion protein alone is not sufficient for leukemogenesis (Section 6) and (iii) the translocation can persist for years after allotransplantation without any signs of leukemia relapse (Section 9.1). Taken together, these observations further support the hypothesis that detection of the fusion transcript is not sufficient for evaluation of the relapse risk. One should rather use

serial determinations; the detection of increasing AML-ETO transcript levels or eventually the kinetics of the increase may be more reliable to evaluate the risk of an imminent relapse.

10. Concluding Remarks

The t(8;21) variant of human AML is a heterogeneous subset characterized by a common disease-specific molecular translocation. This variant is often referred to as core-binding factor AML together with the inv(16)/t(16;16) variants, but these two cytogenetically identified AML subsets show several biological and clinical differences. In contrast to other AML patients the diagnosis of t(8;21) AML can be made even when less than 20% leukemic blasts are present in the bone marrow. The disease is characterized by (i) ectopic expression of B-cell associated molecules; (ii) additional genetic abnormalities are common; (iii) the leukemic cells show specific global gene expression and microRNA profiles; and (iv) usually there is a low risk of leukemia relapse after high-dose cytarabine therapy. Despite the common fundamental cytogenetic characteristics, it should always be remembered that this specific cytogenetic abnormality identifies a heterogeneous subset of patients.

Conflict of Interests

The authors report no potential conflict of interest.

Acknowledgment

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