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Advances in molecular genetics and treatment of core-binding factor acute myeloid leukemia

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Abstract

Purpose of review Core-binding factor (CBF) acute myeloid leukemia (AML) is among the most common cytogenetic subtypes of AML, being detected in approximately 13% of adults with primary disease. Although CBF-AML is associated with a relatively favorable prognosis, only one-half of the patients are cured. Herein we review recent discoveries of genetic and epigenetic alterations in CBF-AML that may represent novel prognostic markers and therapeutic targets and lead to improvement of the still disappointing clinical outcome of these patients.

Recent findings Several acquired gene mutations and gene-expression and microRNA-expression changes that occur in addition to t(8;21)(q22;q22) and inv(16)(p13q22)/t(16;16)(p13;q22), the cytogenetic hallmarks of CBF-AML, have been recently reported. Alterations that may represent cooperative events in CBF-AML leukemogenesis include mutations in the *KIT*, *FLT3*, *JAK2* and *RAS* genes, haploinsufficiency of the putative tumor suppressor genes *TLE1* and *TLE4* in t(8;21)-positive patients with del(9q), *MNI* overexpression in inv(16) patients, and epigenetic and posttranscriptional silencing of *CEBPA*. Genome-wide gene-expression and microRNA-expression profiling identifying subgroups of CBF-AML patients with distinct molecular signatures, different clinical outcomes, or both, have also been reported.

Summary Progress has been made in delineating the genetic basis of CBF-AML that will likely result in improved prognostication and development of novel, risk-adapted therapeutic approaches.

Keywords

acute myeloid leukemia; core-binding factor; gene-expression profiling; *JAK2*; *KIT*; *MNI*

Introduction

Core-binding factor (CBF) acute myeloid leukemia (AML) is characterized by the presence of t(8;21)(q22;q22) or inv(16)(p13q22)/t(16;16)(p13;q22). These chromosome aberrations

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create, respectively, *RUNX1-RUNX1T1* and *CBFB-MYH11* fusion genes, that disrupt subunits α and β of CBF, a heterodimeric transcription factor involved in hematopoietic differentiation [1]. *RUNX1-RUNX1T1* can be generated not only by t(8;21) but also by its infrequent variants, including complex translocations involving one or two additional chromosomes [2–5], and insertions [e.g., ins(21;8)(q22q22;q22) and ins(8;21)(q22;q22q22)] [2,5,6]. Likewise, variants of inv(16)/t(16;16) and cryptic rearrangements leading to *CBFB-MYH11* have been reported [7*,8,9].

CBF-AML is fairly common, with t(8;21) detected in 6% and inv(16)/t(16;16) in 7% of adult *de novo* AML patients [10]. CBF-AML patients have a relatively favorable prognosis, especially if intensive postremission treatment with multiple cycles of high-dose cytarabine is administered [11–13]. However, only 50–60% of CBF-AML patients are cured using contemporary treatment [14–16]. Thus, genetic, clinical, or both, markers are needed that identify CBF-AML patients unlikely to respond to current treatment who could potentially benefit from more intensive, novel, or both, therapies.

Genetic alterations cooperating with core binding factor-acute myeloid leukemia-associated chimeric proteins

Expression of the *RUNX1* and *CBFB* genes appears essential for the development of normal hematopoiesis. In murine knockout models, the homozygous loss of *RUNX1* or *CBFB* caused lack of definitive hematopoiesis and embryonic death [17]. Both mouse and in-vitro models have shown that the *RUNX1-RUNX1T1* and *CBFB-MYH11* fusion proteins are critical for CBF-AML development. However, neither fusion protein alone is sufficient for leukemogenesis [18*,19*]. Thus, for overt leukemia to occur additional genetic, epigenetic alterations, or both are necessary. This is consistent with a multistep model of leukemogenesis, in which two classes of mutations cooperate: one affecting genes encoding transcription factors capable of impairing hematopoietic differentiation, such as *RUNX1-RUNX1T1* and *CBFB-MYH11* (class II mutations), and another involving genes such as *KIT*, *FLT3*, *NRAS* or *KRAS*, whose mutations lead to increased cell proliferation, survival (class I mutations), or both [20].

Secondary chromosome aberrations

Secondary chromosome aberrations are detected at diagnosis in approximately 40% of patients with inv(16)/t(16;16), and 70% of t(8;21) patients [14–16]. The patterns of secondary aberrations are nonrandom, and differ between inv(16)/t(16;16) and t(8;21) patients (Table 1 Table 1). One recent study suggested that secondary aberrations other than +8, +21 and +22 are more common in inv(16)-positive patients with rare, nontype A *CBFB-MYH11* transcripts [21*]. Secondary aberrations, such as +22 in inv(16)/t(16;16) patients, and -Y, -X and del(9q) in t(8;21) patients are less frequent in non-CBF-AML signifying their importance for CBF-AML leukemogenesis [22]. However, molecular alterations associated with +22, -Y, and -X have not yet been elucidated.

In contrast, the *TLE1* and *TLE4* genes have recently been suggested as candidate tumor suppressors residing within the 2.4Mb region at 9q21.32–9q21.33 invariably lost in t(8;21) patients with del(9q) [23**]. The authors knocked down expression of several genes mapped to 9q21.32–9q21.33, and demonstrated that decreased expression of only *TLE1* and *TLE4* affected myeloid cell survival, proliferation and apoptosis. Additionally, *TLE1* and *TLE4* expression was lower in t(8;21) patients with del(9q) compared with normal CD34+ cells or cells from t(8;21) patients without del(9q). Moreover, lower expression of *TLE1* and *TLE4* increased the cell division rate of the t(8;21)-positive Kasumi-1 cell line without del(9q), whereas forced *TLE1* and *TLE4* expression led to apoptosis and death of the Kasumi-1 cells.

These data suggest that haploinsufficiency of *TLE1* and *TLE4* may cooperate with RUNX1-RUNX1T1 in the leukemogenesis of t(8;21) patients with del(9q) [23**].

Gene mutations

Genetic events cooperating with CBF-AML-associated gene fusions may also include activating mutations in genes encoding receptor tyrosine kinases (RTKs) or small GTPases.

KIT—The most common RTK mutations are those in the *KIT* gene, which encodes a member of the type III RTK family. They are found in 12–47% of patients with t(8;21), and in 22–38% of those with inv(16)/t(16;16) [24,25,26*]. In t(8;21) AML, *KIT* mutations occur mostly in exon 17 and confer adverse prognosis, whereas the prognostic significance of *KIT* mutations (both in exon 8 and 17) in inv(16) AML is less well established (Table 2 Table 2) [27–33, 34*]. However, in two recent large studies, the presence of *KIT* mutations (predominantly in exon 17) was associated with a higher relapse risk [27,29]. Although in one study overall survival was not impacted [29], in another, *KIT* mutations affected unfavorably overall survival after adjustment for sex [27]. Notably, *KIT* mutations can be targeted by tyrosine kinase inhibitors, which are selectively active against specific *KIT* mutations. For instance, imatinib is active against various exon 8 mutations and the exon 17 mutation involving codon N822, but not mutations involving codon D816, which can be successfully targeted with dasatinib and midostaurin [35]. Thus, determination of the exact type of *KIT* mutation is necessary for selecting the appropriate tyrosine kinase inhibitor in individual patients.

FLT3—An internal tandem duplication (ITD) within the juxtamembrane domain of the *FLT3* gene, that encodes an RTK protein, is relatively infrequent in CBF-AML, being detected in 2–9% of t(8;21) patients [24,28,36–39] and 0–7% of inv(16) patients [24,28,36–38]. Nevertheless, mouse models have demonstrated that when *FLT3*-ITD coexists with *RUNX1-RUNX1T1* [39] and *CBFB-MYH11* [40*] in leukemic blasts, it collaborates with the gene fusions in inducing acute leukemia.

The second type of *FLT3* mutations, point mutations within the activation loop of the tyrosine kinase domain (TKD), are more frequent in inv(16) patients (6–24%) [24,38,41], but occur in only 2–7% of t(8;21) patients [24,38,39,41]. To our knowledge, the prognostic significance of both *FLT3*-TKD and *FLT3*-ITD in CBF-AML remains to be established. Given their relatively high incidence in inv(16) patients, large studies analyzing prognostic impact of *FLT3* mutations, which constitute potential therapeutic targets for tyrosine kinase inhibitors, are warranted.

JAK2—The most recent addition to the list of potentially leukemogenic mutations in CBF-AML is the activating, gain-of-function *JAK2*V617F mutation, previously reported in most patients with chronic myeloproliferative disorders [42]. *JAK2* mutations are infrequent in CBF-AML, being identified in 4 of 64 (6%) t(8;21) patients and none of 99 inv(16)/t(16;16) patients in one study [43], and in 5 of 138 (3.6%) CBF-AML patients [2 had t(8;21) and 3 inv(16)/t(16;16)] in another [34*]. Additionally, Schnittger *et al.* [44] found *JAK2* mutations in two of three t(8;21)-positive patients with therapy-related AML, but in none of 20 t(8;21)-positive *de novo* AML patients, and suggested *JAK2* mutations might be associated with therapy-related CBF-AML after treatment with anthracyclines and topoisomerase inhibitors. Occasionally, *JAK2*V617F mutation may precede acquisition of t(8;21) during progression from myeloproliferative syndrome to CBF-AML [45*]. However, *JAK2*V617F mutations are also detected in *de novo* CBF-AML [34*,43,46]. Importantly, disease-free survival (DFS) of CBF-AML patients with *JAK2* mutations was significantly worse than DFS of patients

without these mutations in one study [34*]. These results require corroboration. *JAK2* mutations are potential targets for small-molecule inhibitors of JAK2 kinases [42].

RAS—Mutations in the *RAS* genes, *NRAS* and, less often, *KRAS*, both of which encode small GTPases, are common among inv(16)/t(16;16) patients, being detected in over one-third of them; *RAS* mutations are present in 8–11% of t(8;21) patients [24,30]. Thus far, *RAS* mutations have not been correlated with clinical outcome (Table 2). Mutated *NRAS* may become a target for molecularly targeted therapy.

Gene overexpression

Gene overexpression has been recently identified as another type of cooperating genetic event in CBF-AML.

MN1—High expression of the *MN1* gene has been consistently detected in inv(16) patients at both the RNA [47,48,49**] and protein levels [49**]. Carella *et al.* [49**] transplanted bone marrow cells overexpressing MN1 into lethally irradiated mice, which rapidly developed myeloproliferative disease, but not AML. Similarly, mice transplanted with MN1-expressing cells in another study developed a hematopoietic disorder resembling AML and rapidly died [50], suggesting that overexpressed *MN1* is a highly efficient oncogene. As Heuser *et al.* [50] could not detect genetic alterations collaborating with *MN1* overexpression, Carella *et al.* [49**] demonstrated that mice transplanted with cells containing both overexpressed *MN1* and *CBFB-MYH11* quickly developed AML. Thus, *MN1* overexpression seems to constitute a cooperating event in inv(16)-associated leukemogenesis. The mechanism through which *MN1* becomes overexpressed is currently unknown. It is unclear whether *MN1* expression has prognostic significance in inv(16) patients, as it does in cytogenetically normal AML patients [51,52].

Microarray gene-expression studies

Initial microarray gene-expression profiling (GEP) studies identified signatures allowing reliable separation of patients with inv(16)/t(16;16) from those with t(8;21) and from other cytogenetic, molecular genetic AML subgroups [48,53–55], or both. More recent studies have focused on CBF-AML, and investigated whether GEP could improve outcome prediction. Paschka *et al.* [56] analyzed GEP in t(8;21) patients lacking *KIT* mutations and, separately, in inv(16)/t(16;16) patients without secondary aberrations or *KIT* mutations. Gene-expression-based outcome predictors for event-free survival (EFS) dichotomized each cytogenetic group into two subsets with markedly different EFS [56].

Bullinger *et al.* [57**] performed GEP analyses on 93 CBF-AML patients, including both inv(16) and t(8;21) patients. Unsupervised 2-way hierarchical cluster analysis stratified the patients into two groups with different GEPs and significantly different survival. Group I, which had worse overall survival, had more cases with inv(16), higher white blood counts (WBC), and higher incidence of *FLT3*-ITD-positive cases, although the latter were infrequent (4/28 in group I versus 0/52 in group II). Review of the genes expressed differentially between the groups revealed that the prognostically adverse group I was characterized by overexpression of the proto-oncogene *JUN* and constitutive activation of the JNK and MAPK signaling pathways. Conversely, deregulated mTOR signaling and antiapoptotic mechanisms were suggested to play a role in the prognostically more favorable group II. Overexpression of such antiapoptotic genes as *BIRC3* and *BIRC6* is of interest because they encode inhibitors of apoptosis proteins (IAPs), which are potential therapeutic targets for antisense and chemical IAP inhibitors [57**].

Micrnas in core binding factor-acute myeloid leukemia

MicroRNAs are small (19-25 nucleotides), noncoding RNA molecules that regulate expression of genes at the posttranscriptional level by hybridizing to complementary mRNA targets and inhibiting their translation or causing degradation [58]. As aberrant expression of many microRNAs has been demonstrated in several types of cancer [59], including AML [60,61,62**], the role of microRNAs in CBF-AML has only begun to be investigated. Fazi *et al.* [63*] reported that RUNX1-RUNX1T1 chimeric protein caused epigenetic silencing of the microRNA-223, which is involved in regulation of myelopoiesis. Demethylating treatment with 5-azacytidine *in vitro* or forced microRNA-223 expression enhanced microRNA-223 levels and restored cell differentiation, suggesting that microRNA-223 might become a therapeutic target.

Another study assessed expression of 260 microRNAs (using quantitative RT-PCR) in 215 AML patients belonging to several cytogenetic and molecular subsets [62**]. Unsupervised analysis identified distinctive microRNA signatures for most patients with t(8;21) and for most with inv(16), and these distinguished CBF-AML from other subtypes of AML. However, some inv(16) patients clustered among t(8;21) cases and vice versa. Most (83%) t(8;21) cases were grouped in a cluster characterized by mainly downregulated microRNAs, which included members of a known tumor suppressor microRNA family, let-7b and let-7c. Both these microRNAs and microRNA-127, reported to function as a tumor suppressor, were also downregulated in inv(16) AML. The authors speculated that downregulation of microRNA-let-7, which regulates the expression of *RAS* and *HMGA2* oncogenes, and microRNA-127, that regulates *BCL6* expression, might contribute to CBF-AML leukemogenesis [62**].

Epigenetics in core binding factor-acute myeloid leukemia

Epigenetic alterations silencing gene function without changing the DNA coding sequence are intrinsic to CBF-AML leukemogenesis. Under physiologic conditions, the N-terminus of RUNX1 interacts with the CBFB subunit and binds the consensus sequence TGT/cGGT at the promoter regions of its target genes [64]. This allows the RUNX1 C-terminus to interact with a p300-containing co-activator complex resulting in transcriptional activation of RUNX1 target genes via histone acetylation. In t(8;21)-AML, the p300-binding site is replaced by the fusion partner RUNX1T1, which recruits histone deacetylases (HDACs) and induces transcriptional repression of the target genes. Their expression can be restored by treatment with HDAC inhibitors [65,66]. In addition to recruiting HDACs, RUNX1-RUNX1T1 also recruits DNA-methyltransferase 1 (DNMT) [66,67]. This leads to the silencing of RUNX1-target genes by the RUNX1-RUNX1T1 fusion protein through both histone deacetylation and promoter hypermethylation. Combinations of DNMT and HDAC inhibitors induce re-expression of the silenced genes in RUNX1-RUNX1T1-positive leukemic cells [68]. Therapeutic targeting of epigenetic alterations in CBF-AML is now being pursued in the clinic.

Epigenetic alterations may also represent secondary 'hits' contributing to CBF-AML leukemogenesis [69,70]. They include methylation of the promoter region of the *CEBPA* gene, a transcription factor involved in myeloid lineage differentiation, reported recently in inv(16)-AML [71*]. Downregulation of *CEBPA* in inv(16)-AML may also be achieved through posttranscriptional interaction of *CEBPA* mRNA with the RNA-binding protein calreticulin [72] or by microRNA-124a, which is also regulated by promoter methylation [71*]. In t(8;21)-AML, *CEBPA* downregulation may result from inhibition of positive autoregulation of the *CEBPA* promoter by RUNX1/RUNX1T1 protein [73,74*].

Hematopoietic stem-cell transplantation in core binding factor-acute myeloid leukemia

Two recent retrospective studies have addressed the role of hematopoietic stem-cell transplantation (HSCT) in treatment of CBF-AML patients. The first, restricted to adults less than 60 years harboring t(8;21), compared outcomes of 118 patients who received human leukocyte antigen (HLA)-matched sibling HSCT with 132 patients receiving cytarabine-based chemotherapy postremission [75^{**}]. The transplant and chemotherapy groups had similar relapse-free survival (RFS, 5-year rates, 55 versus 64%). Although the transplanted patients had lower relapse rates, this did not translate into improved overall survival because HSCT was associated with a higher treatment-related mortality. With regard to overall survival, patients without secondary –Y or –X had a higher risk of death after HSCT than those receiving chemotherapy, with no difference between treatment groups among patients harboring –Y or –X. In both treatment groups, WBC more than $25 \times 10^9/l$ conferred a higher relapse risk and worse RFS and overall survival. Schlenk *et al.* [75^{**}] concluded that cytarabine-based chemotherapy offers results similar or better than HLA-matched sibling HSCT in first remission.

Another study compared results of autologous and allogeneic HSCT in CBF-AML. It found that both transplantation types resulted in similar outcomes; treatment-related mortality was lower and relapse incidence higher in patients receiving autologous HSCT [76^{*}]. Lower WBC was associated with lower treatment-related mortality and better DFS in t(8;21), but not inv(16)/t(16;16), patients. Among inv(16) patients, women had a lower relapse incidence and a better DFS than men.

Monitoring of minimal residual disease in core binding factor-acute myeloid leukemia

Both CBF-associated fusion transcripts constitute molecular markers useful for assessing MRD. Qualitative PCR assays are of limited value because patients in early or long-term remission may continue to test positive for the fusion transcripts [77]. Assessment of *RUNX1-RUNX1T1* by quantitative RT-PCR (Q-RT-PCR) seems to be more promising. The reduction of initial *RUNX1-RUNX1T1* levels measured by Q-RT-PCR after induction and consolidation therapies was shown recently to influence prognosis. Patients with *RUNX1-RUNX1T1* expression levels below the median after induction and consolidation therapies had a lower cumulative incidence of relapse, and longer EFS and overall survival than patients with transcript levels above the median [78^{*}]. Q-RT-PCR and flow cytometry at the end of treatment have been successfully used to identify t(8;21) patients with higher relapse risk in another study [79]. However, other studies using Q-RT-PCR have not corroborated these results [80^{*},81].

As in t(8;21)-AML, Perea *et al.* [79] reported that higher MRD levels at the end of treatment measured by flow cytometry or RT-PCR were associated with a higher relapse risk in inv(16) AML. In another study, more than 1-log increase in the *CBFB-MYH11* or *RUNX1-RUNX1T1* levels in bone marrow during remission caused more than eight times increase in the relapse risk [80^{*}]. Although promising, the use of MRD analysis for clinical decision making in CBF-AML still requires validation in larger trials.

Conclusion

Recent discoveries of gene mutations, changes in gene-expression and microRNA-expression levels and epigenetic events have increased our understanding of CBF-AML leukemogenesis. Many genetic and epigenetic alterations acquired in addition to the t(8;21)/*RUNX1-RUNX1T1* and inv(16)/t(16;16)/*CBFB-MYH11* may serve as prognostic factors, therapeutic targets, or both. It is hoped that ongoing studies will ultimately lead to the design

of more effective, molecularly targeted therapies that will increase the cure rate of patients with CBF-AML.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

1. Speck NA, Gilliland DG. Core-binding factors in haematopoiesis and leukaemia. *Nat Rev Cancer*. 2002; 2:502–513. [PubMed: 12094236]
2. Huang L, Abruzzo LV, Valbuena JR, et al. Acute myeloid leukemia associated with variant t(8;21) detected by conventional cytogenetic and molecular studies. A report of four cases and review of the literature. *Am J Clin Pathol*. 2006; 125:267–272. [PubMed: 16393685]
3. Udayakumar AM, Alkindi S, Pathare AV, Raeburn JA. Complex t(8;13;21)(q22;q14;q22)-a novel variant of t(8;21) in a patient with acute myeloid leukemia (AML-M2). *Arch Med Res*. 2008; 39:252–256. [PubMed: 18164974]
4. Ahmad F, Kokate P, Chheda P. Molecular cytogenetic findings in a three-way novel variant of t(1;8;21)(p35;q22;q22): a unique relocation of the *AML1/ETO* fusion gene 1p35 in AML-M2. *Cancer Genet Cytogenet*. 2008; 180:153–157. [PubMed: 18206543]
5. Mrózek K, Prior TW, Edwards C. Comparison of cytogenetic and molecular genetic detection of t(8;21) and inv(16) in a prospective series of adults with *de novo* acute myeloid leukemia: a Cancer and Leukemia Group B study. *J Clin Oncol*. 2001; 19:2482–2492. [PubMed: 11331327]
6. Gamberdinger U, Teigler-Schlegel A, Pils S, et al. Cryptic chromosomal aberrations leading to an *AML1/ETO* rearrangement are frequently caused by small insertions. *Genes Chromosomes Cancer*. 2003; 36:261–272. [PubMed: 12557226]
7. Monma F, Nishii K, Shiga J, et al. Detection of the *CBFB/MYH11* fusion gene in *de novo* acute myeloid leukemia (AML): a single-institution study of 224 Japanese AML patients. *Leuk Res*. 2007; 31:471–476. [PubMed: 17052753] A relatively large study that using RT-PCR and FISH detected cryptic rearrangements leading to *CBFB-MYH11* among 224 Japanese adults with *de novo* AML.
8. Macedo Silva ML, Raimondi SC, Abdelhay E, et al. Banding and molecular cytogenetic studies detected a *CBFB-MYH11* fusion gene that appeared as abnormal chromosomes 1 and 16 in a baby with acute myeloid leukemia FAB M4-Eo. *Cancer Genet Cytogenet*. 2008; 182:56–60. [PubMed: 18328953]
9. Ohsaka A, Otsubo K, Yokota H, et al. Spectral karyotyping and fluorescence in situ hybridization analyses identified a novel three-way translocation involving inversion 16 in therapy-related acute myeloid leukemia M4eo. *Cancer Genet Cytogenet*. 2008; 184:113–118. [PubMed: 18617061]
10. Byrd JC, Mrózek K, Dodge RK, et al. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with *de novo* acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). *Blood*. 2002; 100:4325–4336. [PubMed: 12393746]
11. Bloomfield CD, Lawrence D, Byrd JC, et al. Frequency of prolonged remission duration after high-dose cytarabine intensification in acute myeloid leukemia varies by cytogenetic subtype. *Cancer Res*. 1998; 58:4173–4179. [PubMed: 9751631]

12. Byrd JC, Dodge RK, Carroll A, et al. Patients with t(8;21)(q22;q22) and acute myeloid leukemia have superior failure-free and overall survival when repetitive cycles of high-dose cytarabine are administered. *J Clin Oncol.* 1999; 17:3767–3775. [PubMed: 10577848]
13. Byrd JC, Ruppert AS, Mrózek K, et al. Repetitive cycles of high-dose cytarabine benefit patients with acute myeloid leukemia and inv(16)(p13q22) or t(16;16)(p13;q22): results from CALGB 8461. *J Clin Oncol.* 2004; 22:1087–1094. [PubMed: 15020610]
14. Schlenk RF, Benner A, Krauter J, et al. Individual patient data-based meta-analysis of patients aged 16 to 60 years with core binding factor acute myeloid leukemia: a survey of the German Acute Myeloid Leukemia Intergroup. *J Clin Oncol.* 2004; 22:3741–3750. [PubMed: 15289486]
15. Marcucci G, Mrózek K, Ruppert AS, et al. Prognostic factors and outcome of core binding factor acute myeloid leukemia patients with t(8;21) differ from those of patients with inv(16): a Cancer and Leukemia Group B study. *J Clin Oncol.* 2005; 23:5705–5717. [PubMed: 16110030]
16. Appelbaum FR, Kopecky KJ, Tallman MS, et al. The clinical spectrum of adult acute myeloid leukaemia associated with core binding factor translocations. *Br J Haematol.* 2006; 13:165–173. [PubMed: 16939487]
17. Downing JR. The core-binding factor leukemias: lessons learned from murine models. *Curr Opin Genet Dev.* 2003; 13:48–54. [PubMed: 12573435]
18. Peterson LF, Boyapati A, Ahn EY, et al. Acute myeloid leukemia with the 8q22;21q22 translocation: secondary mutational events and alternative t(8;21) transcripts. *Blood.* 2007; 110:799–805. [PubMed: 17412887] This is a comprehensive review of secondary genetic alterations in CBF AML with t(8;21).
19. Müller AMS, Duque J, Shizuru JA, Lübbert M. Complementing mutations in core binding factor leukemias: from mouse models to clinical applications. *Oncogene.* Prepublished on July 7, 2008, as doi: 10.1038/onc.2008.196. This is a comprehensive review of secondary genetic alterations in CBF AML with both t(8;21) and inv(16).
20. Gilliland DG, Jordan CT, Felix CA. The molecular basis of leukemia. *Hematology Am Soc Hematol Educ Program.* 2004:80–97. [PubMed: 15561678]
21. Schnittger S, Bacher U, Haferlach C, et al. Rare *CBFB-MYH11* fusion transcripts in AML with inv(16)/t(16;16) are associated with therapy-related AML M4eo, atypical cytomorphology, atypical immunophenotype, atypical additional chromosomal rearrangements and low white blood cell count: a study on 162 patients. *Leukemia.* 2007; 21:725–731. [PubMed: 17287858] The first study reporting differences in pretreatment characteristics between inv(16)/t(16;16)-positive patients with type A *CBFB-MYH11* fusion transcripts and patients with rare, nontype A *CBFB-MYH11* fusion transcripts.
22. Mitelman, F.; Johansson, B.; Mertens, F., editors. Mitelman database of chromosome aberrations in cancer. 2008. update:<http://cgap.nci.nih.gov/Chromosomes/Mitelman>
23. Dayyani F, Wang J, Yeh J-RJ, et al. Loss of *TLE1* and *TLE4* from the del(9q) commonly deleted region in AML cooperates with AML1-ETO to affect myeloid cell proliferation and survival. *Blood.* 2008; 111:4338–4347. [PubMed: 18258796] A comprehensive study identifying *TLE1* and *TLE4* as potential tumor suppressor genes whose decreased expression as a result of loss of one functional allele due to del(9q) in t(8;21) patients cooperates with RUNX1-RUNX1T1 in CBF AML leukemogenesis.
24. Boissel N, Leroy H, Brethon B, et al. Incidence and prognostic impact of *c-Kit*, *FLT3*, and *Ras* gene mutations in core binding factor acute myeloid leukemia (CBF-AML). *Leukemia.* 2006; 20:965–970. [PubMed: 16598313]
25. Cairoli R, Beghini A, Grillo G, et al. Prognostic impact of *c-KIT* mutations in core binding factor leukemias: an Italian retrospective study. *Blood.* 2006; 107:3463–3468. [PubMed: 16384925]
26. Shih L-Y, Liang D-C, Huang C-F, et al. Cooperating mutations of receptor tyrosine kinases and Ras genes in childhood core-binding factor acute myeloid leukemia and a comparative analysis on paired diagnosis and relapse samples. *Leukemia.* 2008; 22:303–307. [PubMed: 17960171] This study examined *KIT*, *FLT3*, *NRAS* and *KRAS* mutations in a series of pediatric CBF-AML patients both at diagnosis and relapse, and examined prognostic impact of *KIT* mutations.
27. Paschka P, Marcucci G, Ruppert AS, et al. Adverse prognostic significance of *KIT* mutations in adult acute myeloid leukemia with inv(16) and t(8;21): a Cancer and Leukemia Group B study. *J Clin Oncol.* 2006; 24:3904–3911. [PubMed: 16921041]

28. Care RS, Valk PJM, Goodeve AC, et al. Incidence and prognosis of c-KIT and FLT3 mutations in core binding factor (CBF) acute myeloid leukaemias. *Br J Haematol*. 2003; 121:775–777. [PubMed: 12780793]
29. Cairoli R, Beghini A, Ripamonti CB, et al. Prevalence and prognostic impact of *KIT* mutations in acute myeloid leukaemia with inv(16). A retrospective study. *Blood*. 2007; 110:1021a–1022a.
30. Bacher U, Haferlach T, Schoch C, et al. Implications of *NRAS* mutations in AML: a study of 2502 patients. *Blood*. 2006; 107:3847–3853. [PubMed: 16434492]
31. Schnittger S, Kohl TM, Haferlach T, et al. *KIT*-D816 mutations in *AML1-ETO*-positive AML are associated with impaired event-free and overall survival. *Blood*. 2006; 107:1791–1799. [PubMed: 16254134]
32. Shimada A, Taki T, Tabuchi K, et al. *KIT* mutations, and not *FLT3* internal tandem duplication, are strongly associated with a poor prognosis in pediatric acute myeloid leukemia with t(8;21): a study of the Japanese Childhood AML Cooperative Study Group. *Blood*. 2006; 107:1806–1809. [PubMed: 16291592]
33. Goemans BF, Zwaan CM, Miller M, et al. Mutations in *KIT* and *RAS* are frequent events in pediatric core-binding factor acute myeloid leukemia. *Leukemia*. 2005; 19:1536–1542. [PubMed: 16015387]
34. Illmer T, Schaich M, Ehninger G, Thiede C. Tyrosine kinase mutations of JAK2 are rare events in AML but influence prognosis of patients with CBF-leukemias. *Haematologica*. 2007; 92:137–138. [PubMed: 17229652] The first study suggesting that *JAK2* mutations may have prognostic significance.
35. Paschka P. Core binding factor acute myeloid leukemia. *Semin Oncol*. 2008; 35:410–417. [PubMed: 18692691]
36. Kottaridis PD, Gale RE, Frew ME, et al. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. *Blood*. 2001; 98:1752–1759. [PubMed: 11535508]
37. Schnittger S, Schoch C, Dugas M, et al. Analysis of FLT3 length mutations in 1003 patients with acute myeloid leukemia: correlation to cytogenetics, FAB subtype, and prognosis in the AMLCG study and usefulness as a marker for the detection of minimal residual disease. *Blood*. 2002; 100:59–66. [PubMed: 12070009]
38. Thiede C, Steudel C, Mohr B, et al. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. *Blood*. 2002; 99:4326–4335. [PubMed: 12036858]
39. Schessl C, Rawat VPS, Cusan M, et al. The *AML1-ETO* fusion gene and the *FLT3* length mutation collaborate in inducing acute leukemia in mice. *J Clin Invest*. 2005; 115:2159–2168. [PubMed: 16025155]
40. Kim H-G, Kojima K, Swindle CS, et al. FLT3-ITD cooperates with inv(16) to promote progression to acute myeloid leukemia. *Blood*. 2008; 111:1567–1574. [PubMed: 17967943] This study provided evidence of cooperation between *FLT3*-ITD and *CBFB-MYH11* in an animal model of inv(16)-associated AML.
41. Mead AJ, Linch DC, Hills RK, et al. FLT3 tyrosine kinase domain mutations are biologically distinct from and have a significantly more favorable prognosis than FLT3 internal tandem duplications in patients with acute myeloid leukemia. *Blood*. 2007; 110:1262–1270. [PubMed: 17456725]
42. Smith CA, Fan G. The saga of JAK2 mutations and translocations in hematologic disorders: pathogenesis, diagnostic and therapeutic prospects, and revised World Health Organization diagnostic criteria for myeloproliferative neoplasms. *Hum Pathol*. 2008; 39:795–810. [PubMed: 18538168]
43. Döhner K, Du J, Corbacioglu A, et al. JAK2V617F mutations as cooperative genetic lesions in t(8;21)-positive acute myeloid leukemia. *Haematologica*. 2006; 91:1569–1570. [PubMed: 17043013]

44. Schnittger S, Bacher U, Kern W, et al. *JAK2* seems to be a typical cooperating mutation in therapy-related t(8;21)/*AML1-ETO*-positive AML. *Leukemia*. 2007; 21:183–184. [PubMed: 17096014]
- 45•. Schneider F, Bohlander SK, Schneider S, et al. *AML1-ETO* meets *JAK2*: clinical evidence for the two hit model of leukemogenesis from a myeloproliferative syndrome progressing to acute myeloid leukemia. *Leukemia*. 2007; 21:2199–2201. [PubMed: 17625612] This study reported sequential acquisition of t(8;21)/*RUNX1-RUNX1T1* during evolution from polycythemia vera to AML in a patient harboring the *JAK2V617* mutation.
46. Lee JW, Kim YG, Soung YH, et al. The *JAK2 V617F* mutation in *de novo* acute myelogenous leukemias. *Oncogene*. 2006; 25:1434–1436. [PubMed: 16247455]
47. Ross ME, Mahfouz R, Onciu M, et al. Gene expression profiling of pediatric acute myelogenous leukemia. *Blood*. 2004; 104:3679–3687. [PubMed: 15226186]
48. Valk PJM, Verhaak RGW, Beijen MA, et al. Prognostically useful gene expression profiles in acute myeloid leukemia. *N Engl J Med*. 2004; 350:1617–1628. [PubMed: 15084694]
- 49••. Carella C, Bonten J, Sirma S, et al. *MN1* overexpression is an important step in the development of inv(16) AML. *Leukemia*. 2007; 21:1679–1690. [PubMed: 17525718] An elegant study demonstrating that overexpression of the *MN1* gene occurs recurrently in CBF-AML with inv(16) at both the RNA and protein levels, and that it constitutes an important cooperative event in the pathogenesis of CBF-AML.
50. Heuser M, Argiropoulos B, Kuchenbauer F, et al. *MN1* overexpression induces acute myeloid leukemia in mice and predicts ATRA resistance in patients with AML. *Blood*. 2007; 110:1639–1647. [PubMed: 17494859]
51. Heuser M, Beutel G, Krauter J, et al. High meningioma 1 (*MN1*) expression as a predictor for poor outcome in acute myeloid leukemia with normal cytogenetics. *Blood*. 2006; 108:3898–3905. [PubMed: 16912223]
52. Langer C, Maharry K, Mrózek K, et al. Low *meningioma 1 (MN1)* gene expression to predict outcome in cytogenetically normal acute myeloid leukemia (CN-AML): a Cancer and Leukemia Group B (CALGB) study. *J Clin Oncol*. 2008; 26:374s.
53. Schoch C, Kohlmann A, Schnittger S, et al. Acute myeloid leukemias with reciprocal rearrangements can be distinguished by specific gene expression profiles. *Proc Natl Acad Sci U S A*. 2002; 99:10008–10013. [PubMed: 12105272]
54. Bullinger L, Döhner K, Bair E, et al. Use of gene-expression profiling to identify prognostic subclasses in adult acute myeloid leukemia. *N Engl J Med*. 2004; 350:1605–1616. [PubMed: 15084693]
55. Haferlach T, Kohlmann A, Schnittger S, et al. Global approach to the diagnosis of leukemia using gene expression profiling. *Blood*. 2005; 106:1189–1198. [PubMed: 15878973]
56. Paschka P, Radmacher MD, Marcucci G, et al. Gene expression profiling improves outcome prediction in adult core binding factor (CBF) acute myeloid leukemia (AML): a Cancer and Leukemia Group B (CALGB) study. *J Clin Oncol*. 2007; 25:359s.
- 57•. Bullinger L, Rücker FG, Kurz S, et al. Gene-expression profiling identifies distinct subclasses of core binding factor acute myeloid leukemia. *Blood*. 2007; 110:1291–1300. [PubMed: 17485551] This study used gene-expression profiling to identify two groups of CBF-AML patients with significantly different survival, and involvement of different molecular pathways.
58. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004; 116:281–297. [PubMed: 14744438]
59. Hagan JP, Croce CM. MicroRNAs in carcinogenesis. *Cytogenet Genome Res*. 2007; 118:252–259. [PubMed: 18000378]
60. Garzon R, Volinia S, Liu CG, et al. MicroRNA signatures associated with cytogenetics and prognosis in acute myeloid leukemia. *Blood*. 2008; 111:3183–3189. [PubMed: 18187662]
61. Marcucci G, Radmacher MD, Maharry K, et al. MicroRNA expression in cytogenetically normal acute myeloid leukemia. *N Engl J Med*. 2008; 358:1919–1928. [PubMed: 18450603]
- 62••. Jongen-Lavrencic M, Sun SM, Dijkstra MK, et al. MicroRNA expression profiling in relation to the genetic heterogeneity of acute myeloid leukemia. *Blood*. 2008; 111:5078–5085. [PubMed: 18337557] This study identified distinctive microRNA signatures that distinguished most t(8;21)

and inv(16) patients from other subtypes of AML, and revealed downregulation of microRNA-let-7 and microRNA-127 in CBF-AML.

- 63•. Fazi F, Racanicchi S, Zardo G, et al. Epigenetic silencing of the myelopoiesis regulator microRNA-223 by the AML1/ETO oncoprotein. *Cancer Cell*. 2007; 12:457–466. [PubMed: 17996649] This study demonstrated that RUNX1-RUNX1T1 caused epigenetic silencing of the microRNA-223, and that the increase of microRNA-223 levels by demethylating treatment or ectopic miR-223 expression restored cell differentiation.
64. Yamagata T, Maki K, Mitani K. Runx1/AML1 in normal and abnormal hematopoiesis. *Int J Hematol*. 2005; 82:1–8. [PubMed: 16105753]
65. Liu S, Klisovic RB, Vukosavljevic T, et al. Targeting AML1/ETO-histone deacetylase repressor complex: a novel mechanism for valproic acid-mediated gene expression and cellular differentiation in AML1/ETO-positive acute myeloid leukemia cells. *J Pharmacol Exp Ther*. 2007; 321:953–960. [PubMed: 17389244]
66. Barbetti V, Gozzini A, Rovida E, et al. Selective antileukaemic activity of low-dose histone deacetylase inhibitor ITF2357 on AML1/ETO-positive cells. *Oncogene*. 2008; 27:1767–1778. [PubMed: 17891169]
67. Liu S, Shen T, Huynh L, et al. Interplay of RUNX1/MTG8 and DNA methyltransferase 1 in acute myeloid leukemia. *Cancer Res*. 2005; 65:1277–1284. [PubMed: 15735013]
68. Klisovic MI, Maghraby EA, Parthun MR, et al. Depsipeptide (FR 901228) promotes histone acetylation, gene transcription, apoptosis and its activity is enhanced by DNA methyltransferase inhibitors in AML1/ETO-positive leukemic cells. *Leukemia*. 2003; 17:350–358. [PubMed: 12592335]
69. Jost E, Schmid J, Wilop S, et al. Epigenetic inactivation of secreted Frizzled-related proteins in acute myeloid leukaemia. *Br J Haematol*. Prepublished on June 5, 2008, as doi:10.1111/j.1365-2141.2008.07242.x.
70. Suzuki R, Onizuka M, Kojima M, et al. Preferential hypermethylation of the Dickkopf-1 promoter in core-binding factor leukaemia. *Br J Haematol*. 2007; 138:624–631. [PubMed: 17686056]
- 71•. Hackanson B, Bennett KL, Brena RM, et al. Epigenetic modification of CCAAT/enhancer binding protein α expression in acute myeloid leukemia. *Cancer Res*. 2008; 68:3142–3151. [PubMed: 18451139] This study revealed that methylation of the *CEBPA* promoter is frequent in inv(16) patients and that *CEBPA* mRNA is a target for miRNA-124a.
72. Helbling D, Mueller BU, Timchenko NA, et al. CBFB-SMMHC is correlated with increased calreticulin expression and suppresses the granulocytic differentiation factor CEBPA in AML with inv(16). *Blood*. 2005; 106:1369–1375. [PubMed: 15855281]
73. Pabst T, Mueller BU, Harakawa N, et al. AML1-ETO downregulates the granulocytic differentiation factor C/EBP α in t(8;21) myeloid leukemia. *Nat Med*. 2001; 7:444–451. [PubMed: 11283671]
- 74•. Pabst T, Mueller BU. Transcriptional dysregulation during myeloid transformation in AML. *Oncogene*. 2007; 26:6829–6837. [PubMed: 17934489] This is an excellent review of the mechanisms and consequences of transcriptional dysregulation in AML.
- 75••. Schlenk RF, Pasquini MC, Pérez WS, et al. HLA-identical sibling allogeneic transplants versus chemotherapy in acute myelogenous leukemia with t(8;21) in first complete remission: collaborative study between the German AML Intergroup and CIBMTR. *Biol Blood Marrow Transplant*. 2008; 14:187–196. [PubMed: 18215779] This is a large study demonstrating that outcome of t(8;21) patients receiving cytarabine-based chemotherapy is similar or better than that of patients who underwent HLA-matched sibling HSCT in first CRcomplete remission.
- 76•. Gorin N-C, Labopin M, Frasson F, et al. Identical outcome after autologous or allogeneic genoidentical hematopoietic stem-cell transplantation in first remission of acute myelocytic leukemia carrying inversion 16 or t(8;21): a retrospective study from the European Cooperative Group for Blood and Marrow Transplantation. *J Clin Oncol*. 2008; 26:3183–3188. [PubMed: 18506024] This large study has shown that outcome of CBF-AML patients receiving autologous HSCT in first CRcomplete remission is similar to that of patients receiving allogeneic HSCT.
77. Marcucci G, Caligiuri MA, Bloomfield CD. Core binding factor (CBF) acute myeloid leukemia: is molecular monitoring by RT-PCR useful clinically? *Eur J Haematol*. 2003; 71:143–154. [PubMed: 12930314]

- 78•. Weisser M, Haferlach C, Hiddemann W, Schnittger S. The quality of molecular response to chemotherapy is predictive for the outcome of *AML1-ETO*-positive AML and is independent of pretreatment risk factors. *Leukemia*. 2007; 21:1177–1182. [PubMed: 17377588] This study demonstrated that reduction of *RUNX1-RUNX1T1* levels after induction and consolidation therapies decreased risk of relapse and/or death in t(8;21) patients.
79. Perea G, Lasa A, Aventín A, et al. Prognostic value of minimal residual disease (MRD) in acute myeloid leukemia (AML) with favorable cytogenetics [t(8;21) and inv(16)]. *Leukemia*. 2006; 20:87–94. [PubMed: 16281071]
- 80•. Lane S, Saal R, Mollee P, et al. A 1 log rise in RQ-PCR transcript levels defines molecular relapse in core binding factor acute myeloid leukemia and predicts subsequent morphologic relapse. *Leuk Lymphoma*. 2008; 49:517–523. [PubMed: 18297529] This article reported that 1 log rise in *RUNX1-RUNX1T1* and *CBFB-MYH11* transcript levels allows prediction of impending relapse.
81. Narimatsu H, Iino M, Ichihashi T, et al. Clinical significance of minimal residual disease in patients with t(8;21) acute myeloid leukemia in Japan. *Int J Hematol*. Prepublished on June 16, 2008, as DOI 10.1007/s12185-008-0108-1.

Table 1

Secondary chromosome aberrations in core binding factor-acute myeloid leukemia: frequency and prognostic significance

Secondary chromosome aberration	inv(16)	t(8;21)	Prognostic significance [reference no.]
	% (No. of patients with aberration/total no. of patients studied) ^{a, b}	% (No. of patients with aberration/total no. of patients studied) ^{a, b}	
–Y	2.0% (4/205) ^c	55.3% (157/284) ^c	OS and duration of first CR significantly shorter for male t(8;21) patients with –Y [14]; No prognostic impact [15].
–X	1.3% (2/159) ^d	37.0% (81/219) ^d	No impact on OS of female patients with t(8;21) [14]
del(9q)	0 (0/364)	16.1% (81/503)	Among nonwhite t(8;21) patients, OS of those with del(9q) was better than OS of patients with sole t(8;21) and those with secondary abnormalities other than del(9q) [15]. No impact on OS in white t(8;21) patients [15]. No impact on CR rates, RFS and OS [16].
+22	18.0% (99/551)	0.3% (1/318)	CIR significantly lower for inv(16) patients with +22 compared with CIR of patients with isolated inv(16) [15]. RFS longer for inv(16) patients with +22 than for inv(16) patients without +22 [14].
+8	10.7% (59/551)	6.2% (31/503)	No impact on RFS and OS [14].
+21	4.9% (27/551)	0.6% (2/318)	Not established.
None	60.7% (335/551)	30.2% (152/503)	

CIR, cumulative incidence of relapse; CR, complete remission; OS, overall survival; RFS, relapse-free survival.

Data used to establish proportion of cases with a given secondary chromosome aberration.

^a Adapted with permission from [14,15,16].

^b Percentages for particular secondary aberrations calculated using only those studies that provided relevant data.

^c Percentage of male patients.

^d Percentage of female patients.

Table 2

Prognostic significance of mutations in the *KIT*, *RAS* and *JAK2* genes in core binding factor-acute myeloid leukemia patients

Mutated gene	% mutated (No. of patients with mutation/total no. of patients studied)	Age range, years (median)	Prognostic relevance	Reference
			[0,1-5]inv(16)/t(16;16)	
<i>KIT</i>	30% (18/61)	19–57 (40)	CIR significantly higher for both patients with any <i>KIT</i> mutation and those with exon 17 <i>KIT</i> mutation than for patients with wild-type <i>KIT</i> . OS worse for patients with any <i>KIT</i> mutation in multivariable analysis, after adjustment for sex	Paschka <i>et al.</i> [27]
<i>KIT</i>	32% (20/63)	15–74 (44) ^a	RR significantly higher for patients with <i>KIT</i> mutations in exon 8 compared with patients without exon 8 mutations. No impact on OS.	Care <i>et al.</i> [28]
<i>KIT</i>	31% (13/42) ^b	17–<60 (NR) ^b	RI significantly higher for patients with <i>KIT</i> mutation than for patients with wild-type <i>KIT</i> . No impact on OS.	Cairolì <i>et al.</i> [29]
<i>KIT</i>	22% (10/46)	1–75 (33) ^c	No significant difference in EFS and OS between patients with and those without <i>KIT</i> mutations.	Boissel <i>et al.</i> [24]
<i>KIT</i>	38% (5/13)	<17 (NR)	No significant difference in EFS and OS between patients with and those without <i>KIT</i> mutations.	Shih <i>et al.</i> [26*]
<i>RAS</i> ^d	36% (17/47)	1–75 (33) ^c	No significant difference in EFS and OS between patients with and without <i>RAS</i> mutations.	Boissel <i>et al.</i> [24]
<i>NRAS</i>	36% (41/113)	18–92 (63) ^e	No significant difference in EFS and OS between patients with and without <i>NRAS</i> mutations.	Bacher <i>et al.</i> [30]
			[0,1-5]t(8;21)	
<i>KIT</i>	22% (11/49)	18–71 (37)	CIR significantly higher for patients with <i>KIT</i> mutations compared with those without <i>KIT</i> mutations. OS not significantly different between patients with and without <i>KIT</i> mutations.	Paschka <i>et al.</i> [27]
<i>KIT</i>	13% (8/64)	15–90 (NA)	EFS and OS significantly shorter for patients with exon 17 <i>KIT</i> mutations.	Schnittger <i>et al.</i> [31]
<i>KIT</i>	47% (17/36)	16–76 (40.5)	RI significantly higher and OS shorter for patients with any <i>KIT</i> mutation and for those with exon 17 <i>KIT</i> mutation compared with patients with wild-type <i>KIT</i> .	Cairolì <i>et al.</i> [25]
<i>KIT</i>	12% (6/50)	1–75 (33) ^c	EFS, RFS and OS significantly shorter for patients with <i>KIT</i> mutations.	Boissel <i>et al.</i> [24]
<i>KIT</i>	17% (8/46)	2–15 (7.5)	RR significantly higher, and DFS and OS shorter for patients with <i>KIT</i> mutations compared with those without <i>KIT</i> mutations.	Shimada <i>et al.</i> [32]
<i>KIT</i>	43% (12/28)	<17 (NR)	No significant difference in RR, EFS and OS between patients with and those without <i>KIT</i> mutations.	Shih <i>et al.</i> [26*]
<i>RAS</i> ^f	8% (4/50)	1–75 (33) ^c	No significant difference in EFS and OS between patients with and without <i>RAS</i> mutations	Boissel <i>et al.</i> [24]
<i>NRAS</i>	11% (13/116)	18–92 (63) ^e	No significant difference in EFS and OS between patients with and without <i>NRAS</i> mutations.	Bacher <i>et al.</i> [30]

Mutated gene	% mutated (No. of patients with mutation/total no. of patients studied)	Age range, years (median)	Prognostic relevance	Reference
		[0,1-5]inv(16)/t(16;16) and t(8;21) combined into one CBF AML group		
<i>KIT</i>	41% (11/27) ^g	0–18 (NR) ^h	No significant difference in EFS between patients with and those without <i>KIT</i> mutations.	Goemans <i>et al.</i> [33]
<i>KIT</i>	43% (17/41)	<17 (NR)	No significant difference in EFS and OS between patients with and those without <i>KIT</i> mutations.	Shih <i>et al.</i> [26*]
<i>RAS</i>	41% (8/27) ⁱ	0–18 (NR) ^h	No significant difference in EFS between patients with and those without <i>KIT</i> mutations.	Goemans <i>et al.</i> [33]
<i>JAK2</i>	4% (5/138)	NR	DFS significantly shorter for patients with <i>JAK2</i> mutation compared with those without <i>JAK2</i> mutations. No impact on OS.	Illmer <i>et al.</i> [34*]

CIR, cumulative incidence of relapse; DFS, disease-free survival; EFS, event-free survival; NR, not reported; OS, overall survival; RFS, relapse-free survival; RI, relapse incidence; RR, risk of relapse.

^a Mean age.

^b Numbers and age of patients who received intensive chemotherapy and were analyzed for clinical outcome. The total number of patients studied was 50, including 17 with *KIT* mutations, and their age range was 17–88 years (median 47).

^c Age range and median provided for all patients with CBF AML, including 56 with t(8;21) and 47 with inv(16)/t(16;16).

^d Including 15 patients with *NRAS* and 2 with *KRAS* mutations.

^e Age range and median provided for all 2502 patients included in this study.

^f Including 2 patients with *NRAS* and 2 with *KRAS* mutations.

^g Including 11 patients with inv(16)/t(16;16), 6 of whom harbored *KIT* mutation, and 16 with t(8;21), 5 of whom harbored *KIT* mutation.

^h Age range provided for all 150 patients included in this study.

ⁱ Including 11 patients with inv(16)/t(16;16), 2 of whom harbored *RAS* mutation, and 16 with t(8;21), 6 of whom harbored *RAS* mutation.