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Core binding factor AML: New prognostic categories and therapeutic opportunities

Chandrima Sinha, PhD[1], **Lea C. Cunningham, MD**[2], and **Paul P. Liu, MD, PhD**[3]

[1]Postdoctoral Fellow, Bone Marrow Transplant & Cellular Therapy, St Jude Children's Research Hospital, Memphis, TN 38105

[2]Assistant Member, Bone Marrow Transplant & Cellular Therapy, St Jude Children's Research Hospital, Memphis, TN 38105

[3]Deputy Scientific Director, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892

Abstract

Core binding factor (CBF) is a heterodimeric protein complex involved in the transcriptional regulation of normal hematopoiesis. Mutations in CBF-encoding genes result in leukemogenic proliferative advantages and impaired differentiation of the hematopoietic progenitors. CBF molecular aberrations are responsible for approximately 20% of all adult acute myeloid leukemia (AML). Although CBF-AMLs are considered to have relatively good prognosis compared to other leukemia subtypes, they are a heterogeneous group of disorders and modern therapy frequently leads to relapse and the associated morbidity and mortality. Improvements in risk stratification and development of targeted therapies are needed for better outcomes. In this review we provide a brief overview of the molecular basis, prognostic categories and the advanced treatment strategies for CBF leukemias.

Molecular basis of CBF leukemia

Leukemia is a cancer of the developing blood cells caused by mutations leading to either uncontrolled proliferation (class I) or lack of differentiation (class II) or both. The World Health Organization (WHO) classifies AML into several categories based on underlying genetic alterations to facilitate diagnosis and prognosis[1]. Recurrent genetic alterations are frequently observed in AML patients. Among them t(8;21)(q22;q22) and inv(16)(p13q22)/ t(16;16)(p13q22) are the most common and result in generation of corresponding abnormal fusion genes *RUNX1-RUNX1T1* and *CBFB-MYH11*[2], respectively (Figure 1).

Correspondence: P. Paul Liu, 49 Convent Dr., Building 49, Room 3A26, Bethesda, MD 20892; pliu@mail.nih.gov or Lea Cunningham, Lea.Cunningham@STJUDE.ORG.

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Native RUNX1 and CBFβ form a heterodimeric transcription factor complex CBF that regulates normal hematopoietic ontogeny. Core binding factor is comprised of an alpha subunit and a beta subunit. There are 3 alpha subunits (RUNX1-3) and one beta subunit (CBFβ) identified to date. The alpha subunit binds to a consensus DNA sequence TGT/cGGT and the beta subunit stabilizes the interaction between the alpha subunit and DNA but does not interact with DNA independently[3]. Association of CBFβ induces a 40-fold increase in the DNA binding affinity of RUNX1[4]. Therefore both subunits are required for maximum transcriptional efficiency of target genes downstream such as lymphocyte-specific protein tyrosine kinase, granulocyte-macrophage colony-stimulating factor-1 receptor, interlukin-3 and myeloperoxidase[5]. It has been found that RUNX1 also interacts with co-activators p300 and CREB binding protein to mediate transactivation[6]. Fetal mice null for *Runx1* or *Cbfb* die of CNS hemorrhage and lack of fetal liver hematopoiesis on embryonic day 11.5–12.5, demonstrating that CBF is required for definitive hematopoiesis[7–10].

The fusion gene *CBFB-MYH11* was initially identified in 1993[11] and the corresponding fusion protein CBFβ-SMMHC (smooth muscle myosin heavy chain) was identified in inv(16) patient samples in 1996[12]. CBFβ-SMMHC forms large nuclear aggegregates[13], sequesters the alpha subunit RUNX1 in the cytoplasm[14] and arrests differentiation of the inv(16) containing human cell line ME-1[15]. The RUNX1 interacting N-terminal region of CBFβ and the myosin multimerizing C-terminal coiled coil domains of SMMHC direct this sequestration process[16]. CBFβ-SMMHC also prevents the ubiquitin-mediated proteosomal degradation of RUNX1 and generates a stable complex that dominantly inhibits normal CBF function[17]. The t(8;21) was first described in 1973[18] and the *RUNX1-RUNX1T1* fusion gene was identified in 1992[19]. The fusion protein product of t(8;21) is comprised of the DNA binding RUNT homology domain of RUNX1 and most of the RUNX1T1 (ETO) except the first 30 amino acids at the N-terminus[20]. The absence of the C-terminal transactivation domain in the fusion protein RUNX1-RUNX1T1 disrupts normal hematopoiesis in a dominant–negative fashion and therefore specific inactivation of this fusion induces differentiation of the t(8;21) positive Kasumi-1 cell line[21]. RUNX1-RUNX1T1 has also been shown to silence microRNA-193 resulting in increased leukemogenesis by increasing expression of histone deacetylases (HDAC), DNA-methytransferase1 (DNMT) and ultimately decreasing PTEN expression[22]. A common potential mechanism of both of these genetic fusion products is the dominant inhibitory effect on native RUNX1 and finally repression of target genes transcription, as mouse embryos heterozygous for *RUNX1-RUNX1T1* or *CBFB-MYH11* have almost identical phenotypes as the $Runx1^{-/-}$ or $Cbfb^{-/-}$ embryos regarding CNS hemorrhage and hematopoietic defects[7,8,23,24].

Cooperating mutations in CBF leukemia

Murine knock-in models have demonstrated that both CBF fusion genes are necessary but not sufficient to cause leukemia and additional mutations are required for the pathogenesis of CBF leukemias[2,25]. Therefore in preclinical mouse models, mutagenic induction of second mutations are needed for development of AML[2]. In CBF leukemia patients, frequently detected second mutations are NPM1, c-KIT and FLT3. A study with 300 AML patients (16 to 60 years) showed that 48% of the patients have NPM1 mutations[26]. Another study with 481 AML patients indicated that 20% of the CBF-AML cytogenetic group had

FLT3 mutations[27]. On the other hand, KIT mutations have been observed for 6.6–46.1% of CBF-AML patients[28]. NPM1 plays an important role in ribosomal protein assembly, transport, prevents aggregation of nuclear proteins and regulates transcriptional activity of p53[26]. Leukemogenesis occurs when cytoplasmic mutant NPM1 inactivates the tumor suppressor p19Arf in a p53 dependent or independent manner[29]. Inactivation of NF-kappaB renders CBF-AML with NPM1 mutation more sensitive to chemotherapy[29]. Genetic rearrangements that lead to constitutively active hematopoietic receptor tyrosine kinases (RTK) such as FLT3, c-KIT, JAK2 and RAS family members have been identified in CBF-AML patients[2]. These mutations may be particularly amenable for treatment with specific RTK inhibitors[2]. Haploinsufficieny of the tumor suppressors TLE1/4 in t(8;21) and overexpression of MN1 in inv(16) have been observed in addition to the epigenetic and posttranslational silencing of differentiation-inducing transcription factor CEBPA in CBF-AML[30]. There are case reports of rare cooperating mutations such as BCR-ABL[14] and TEL-PDGFRβ[15] fusion proteins in t(8;21) AML[31]. Both are examples of constitutively active tyrosine kinases that provide survival and proliferation advantages to progenitor cells without affecting their differentiation. The synergistic effects of these hyperproliferative phenotypes together with the CBF mutation-associated impaired differentiation lead to the multistep pathogenesis of AML (both class I and class II phenotypes).

Prognosis

Although the CBF genetic rearrangements in AML patients are reported to be associated with relatively favorable prognosis[32,33], only 40–60% of adult CBF-AML patients exhibit long-term survival[28]. Additionally all treatment regimens are associated with significant relapse related morbidity and mortality[34,35].

Molecularly defined genetic abnormalities are important prognostic factors in AML and important for patient management[36]. A study with 201 adults with de novo AML indicated the prognostic significance of karyotype on drug resistance, complete remission (CR) and overall survival (OS) at 5 years[37]. *RUNX1-RUNX1T1* had the best 5 year OS of 50% and for *CBFB-MYH11* the OS was 43%. Normal karyotype was associated with better prognosis in patients older than 55 years. Another study in the Medical Research Council (MRC) with 1612 patients including children and adults up to 55 years of age investigated the effect of pretreatment karyotype on prognosis and subsequent hematopoietic stem cell transplantation (HSCT) in first CR[38]. CBF mutations were found to have favorable outcomes without any differences between de novo and secondary AML in the pediatric group and the prognoses were maintained after HSCT in first CR. An additional MRC study with 1065 older patients (median age 66) indicated that inv(16) and t(8;21) are associated with superior CR, OS and lower drug resistance[39]. Together the findings from these studies suggest that cytogenetically distinct AML subsets are important for risk stratification and prognosis.

Presence of co-operative NPM1 mutation provides favorable overall survival (OS) after intensive double-induction and consolidation therapy only in the absence of FLT3[26] whereas c-KIT mutations have the worst outcome in CBF-AML patients with 56% relapse rate[28,40]. Though cooperating KIT mutation have not shown any significant effect on OS in inv(16), poorer OS has been observed for patients with t(8;21)[41,42]. Additional FLT3 mutations did

not have any effect on the prognosis in CBF-AML[27]. A study by a Japanese group showed an adverse effect of CEBPA mutation on the OS of the patients with CBF-AML[43]. The prognostic impact of cooperating mutations on inv(16) and t(8;21) is listed in Table 1.

The impact of cytogenetics was also studied in 848 AML patients between 15–83 years of age where patients less than 60 years of age in CR received allo- or auto-SCT[44]. Data indicated that inv(16) and t(8;21) were associated with favorable outcome and should be treated with an intensive regimen (idarubicin, cytosine arabinoside (Ara-C), etoposide (ICE) plus mitoxantrone and intermediate dose Ara-C) for longer disease free survival and allo-SCT should only be considered as salvage treatment for relapsed or refractory patients[28,44].

However, a study with 144 adults with t(8;21) and 168 adults with inv(16) showed better OS and survival after first relapse for the inv(16) group when associated with trisomy 22 and male gender[45]. This study emphasized that specific features of inv(16) and t(8;21) should be consider separately.

According to French-American-British (FAB) classification t(8;21) is usually M2 (80–90%) but sometimes M1 (10%) whereas inv (16) is usually M4. The t(8;21) are more frequently found in younger and non-white patients. On the other hand inv(16) is often associated with secondary cytogenetic abnormalities, higher WBC and blast percentages and mostly found in patients with median age of 41 years[28]. The heterogeneity in clinical manifestation and response to treatments demands their consideration as separate entities.

Diagnosis

Several studies noted that the presence of CBF-AML fusion genes are independent indicators for achievement and duration of complete remission (CR) as well as overall survival rate[46]. Standard cytogenetic analysis can diagnose inv(16) and t(8;21) mutations in metaphase cells for CBF-AML patients. By this method t(8;21) can be easily detected with even suboptimal chromosome preparation whereas inv(16) is hard to detect and frequently misinterpreted as del(16)[47].

To identify subtle rearrangement such as inv(16), an alternative reverse transcriptase-polymerase chain reaction (RT-PCR) based analysis is required. Unlike t(8;21) that produces a single transcript easily detectable by both processes, inv(16) can result in multiple variants of the *CBFB-MYH11* fusion due to the presence of variable breakpoints in both *CBFB* and *MYH11*[48]. In CBF-AML patient samples these fusion genes have been detected without the presence of visible inv(16) and t(8;21). Therefore RT-PCR can detect most of these *CBFB-MYH11* variants and is more sensitive in detecting CBF-AML than cytogenetic analysis[48]. Sometimes even RT-PCR cannot detect the inv(16) as confirmed by the classical southern blot techniques[47]. However false positives have not been documented during cytogenetic analysis in a study with 248 newly diagnosed adult primary AML patients and all but one patient was correctly identified[47]. On the other hand RT-PCR was associated with both false-negative and false-positive results and therefore should not replace cytogenetic analysis for CBF-AML diagnosis.

Cytogenetic analysis can be performed by conventional fluorescent in-situ hybridization (FISH) or by spectral karyotyping (SKY) for multicolor display of different chromosomes[36]. For these cytogenetic analyses, high quality preparation of chromosomes from the patient's bone-marrow is desirable. Flurodeoxyuridine or methotrexate synchronization of bone marrow cells provide optimal chromosome length and increased yield of mitosis after culturing for 6–8 hours[49]. Subsequent cytogenetic analysis is usually performed in at least 20 metaphases according to the International System of Human Cytogenetic Nomenclature[50]. For accurate evaluation of CBF-AML, RT-PCR and FISH should be performed in conjunction with classical banding techniques regardless of phenotype. Recently developed microarray gene expression profiling (GEP) can also separate patients with inv(16) from patients with t(8;21).

A substantial number of AML patients die because of relapse and therefore evaluation of minimal residual disease (MRD) by RT-PCR is beneficial for proving complete eradication of leukemic blasts. Quantitative RT- PCR assays can efficiently identify the fusion transcripts for patients in early or long–term remission after conventional chemotherapy or HSCT and subsequently predict relapse risk based on critical MRD levels, especially for patients with t(8;21)[30]. Several recent publications report the importance of MRD monitoring during and after induction and consolidation therapy, through quantitative RT-PCR to detect the residual fusion transcripts in the bone marrow and peripheral blood, which are useful to predict relapse and OS[51]. Among them a study with 198 CBF-AML patients (age 18–60) indicated that prospective evaluation of MRD is more useful than identification of co-operative mutations for prognosis and treatment stratification to combat relapse[52].

Treatment

Core binding factor leukemias are among the most frequent cytogenetic subtypes and comprise approximately 15% of all adult acute myeloid leukemias[28]. Although CBF-AML patients have better prognosis, only approximately 40–60% are cured by standard therapy using a backbone of high dose cytarabine treatment in combination with an anthracycline[53,54] which is essentially unchanged for the past 40 years[55]. A study of 285 newly diagnosed patients with AML showed that high dose cytarabine treatment provides the best outcome for CBF-AML patients with 50% demonstrating CR after 5 years[56]. Another study reported a better outcome after intensive cytarabine therapy in t(8;21) but not in inv(16) positive patients[57,58]. A study at MD Anderson reported the potential of fludarabine and granulocyte colony-stimulating factor in augmenting the effectiveness of cytarabine against CBF-AML[59].

Hematopoietic stem cell transplants (HSCT) and intensive chemotherapy are two well-practiced strategies to prevent relapse for AML patients in first remission. A study at MRC with 1063 AML patients (age under 55 years) showed that allogeneic transplantation after intensive chemotherapy reduced the relapse rate in CBF-AML patients[60]. Another study at MRC with 381 patients indicated that addition of auto-HSCT with four courses of intensive chemotherapy reduced the relapse rate and improved the OS for patients with inv(16) and t(8;21)[61]. A study with patients (up to 45 years) in complete remission (CR) showed that though there is no difference in disease free survival rate for patients with inv(16) and

t(8;21), when allo-or auto-SCT were performed after intensive consolidation therapy, the OS was better for younger patients receiving allo-SCT[62]. HSCT is not necessary in first CR for patients with CBF leukemias unless they have relapsed, refractory or otherwise high risk disease[54,63]. However, patients older than 75 years have very poor prognosis and patients over 60 years may be considered for allo-HSCT[28]. The poor-prognostic KIT mutation positive CBF-AML patients are still treated with high dose cytarabine and should also be considered for allo-HCT[28]. For patients who go on to transplant and subsequently present with reduced donor chimerism, reduction of immunosuppression and/or donor lymphocyte infusion (DLI) can sometimes reinduce remission, although there may be risk of graft versus host disease (GVHD) with use of DLI[64].

Susceptibility of leukemic cells to T-cell and natural killer cell-mediated immunosurveillance justifies the use of immunotherapy for preventing relapse. In addition to adoptive transfer of native and genetically modified T cells and NK cells, attempts have been made to sensitize AML cells to cytotoxic immune cells and to upregulate T-cell immunity by vaccination or cytokine treatment[65,66]. However cytokine storm is associated with some types of immunotherapy which may be mitigated by use of cytokine inhibitors or chimeric antigen receptors (CARs) containing engineered NK cells instead[66]. Immune escape is a potential mechanism by which cancer cells can evade immune surveillance after HSCT or other immunotherapies and ways to combat relapse through this mechanism are urgently needed[67].

Approximately 40% of inv(16) and 70% of t(8;21) patients are diagnosed with secondary mutations and therefore treatment specific for those mutations are of particular interest. For example, as constitutively active RTKs are frequently found in patients with *RUNX1-RUNX1T1* and *CBFB-MYH11*fusion genes[2], RTK inhibitors such as dasatinib, imatinib and midostaurin can repress the hyperproliferation of leukemic blasts and warrant investigation as potential therapy for CBF-AML[30,68]. Epigenetic alterations for silencing gene function are often found in CBF-AML and therefore combination therapy with DNMT and HDAC inhibitors are now being used clinically to induce the expression of RUNX1 target genes[69–71]. A list of currently used drugs for CBF-AML treatment has been provided in Table 2.

Mechanistic studies in mice as well as *in vitro* models have demonstrated that the CBF fusion proteins contribute to leukemogenesis through their interactions with their corresponding normal CBF binding partners (CBFβ for RUNX1-RUNX1T1, and RUNX1 for CBFβ-SMMHC)[72,73]. We have conducted a small chemical library screen for inhibitors of these interactions[74]. We identified a benzodiazepine compound, Ro5-3335, which was shown to be effective in suppressing CBF leukemia in animal models[74]. However further modifications to improve pharmacokinetics are necessary before successful clinical implementation.

Targeted therapy with gemtuzumab ozogamicin (GO), an anti-CD33 antibody conjugated to a calicheamicin derivative, has shown increased OS and reduced relapse rate and can be considered for good risk CBF-AML[75]. In addition, various tumor suppressors (let-7b/7c and microRNA-127) and myelopoietic microRNAs (microRNA-223) are found to be down

regulated in *RUNX1-RUNX1T1* and *CBFB-MYH11* positive leukemic cells and those microRNAs may become potential therapeutic targets for CBF-AML[21,30].

Conclusions

Despite favorable prognosis, modern therapy for CBF-AML is still associated with significant morbidity and mortality due to relapse[34] and infection during intensive chemotherapy[76]. Cytogenetic characterization and discovery of oncogenic molecular events not only enhance our understanding of CBF-AML but also improve risk stratification and development of targeted therapies. Secondary cooperating mutations frequently serve as potential therapeutic targets and occasionally as additional prognostic factors for patients with inv(16) and t(8;21). Further studies are required to develop more effective and targeted therapy to achieve a 100% cure rate for CBF-AML patients.

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Abbreviations

ABL	abelson murine leukemia viral oncogene homolog
AML	acute myeloid leukemia
BCR	breakpoint cluster region protein
CAR	chimeric antigen receptors
CBF	core binding factor
CEBPA	CCAAT/enhancer-binding protein alpha
CR	complete remission
CREB	cAMP response element-binding protein
DLI	donor lymphocyte infusion
DNMT	DNA-methyltransferase
FISH	fluorescent in-situ hybridization
FLT3	Fms-like tyrosine kinase 3
GO	gemtuzumab ozogamicin
GVHD	graft versus host disease
HDAC	histone deacetylases
HSCT	hematopoietic stem cell transplantation
JAK2	janus kinase 2
MN1	meningioma (disrupted in balanced translocation) 1

MRC	Medical Research Council
MRD	minimal residual disease
MYH11	myosin, heavy chain 11
NF-kappaB	nuclear factor kappa-light-chain-enhancer of activated B cells
NK	natural killer
NPM1	nucleophosmin1
OS	overall survival
p53	tumor protein p53
PDGFRβ	beta-type platelet-derived growth factor receptor
PTEN	phosphatase and tensin homolog
RTK	receptor tyrosine kinases
RT-PCR	reverse transcriptase-polymerase chain reaction
RUNX1	runt-related transcription factor 1
RUNX1T1	runt-related transcription factor 1; translocated to, 1 (cyclin D-related)
SKY	spectral karyotyping
SMMHC	smooth muscle myosin heavy chain
TLE1/4	transducin-like enhancer protein 1/4
WHO	World Health Organization

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Summary

- CBF-AML is associated with t(8:21) or inv(16) which results in the abnormal fusion gene *RUNX1-RUNX1T1* or *CBFB-MYH11* respectively. These aberrant fusion genes lead to impaired differentiation of hematopoietic progenitors.
- Advanced FISH based cytogenetic characterization and RT-PCR based detection of CBF leukemia fusion genes are important for correct diagnosis and risk stratification of CBF-AML patients as well as for estimating minimal residual disease (MRD).
- CBF-AMLs are considered to have favorable prognosis but still only approximately 40–60% of patients are cured by standard therapy and relapse remains a major post-treatment complication.
- CBF-AML fusion genes are necessary but not sufficient to cause leukemia. Secondary cooperative mutations promote additional pathogenesis such as hyperproliferation and abrogated differentiation.
- Along with standard cytarabine-doxorubicin therapy, mutation specific targeted therapy, HSCT and immunotherapy hold promise for eradicating relapse and improving cure rates for CBF-AML.

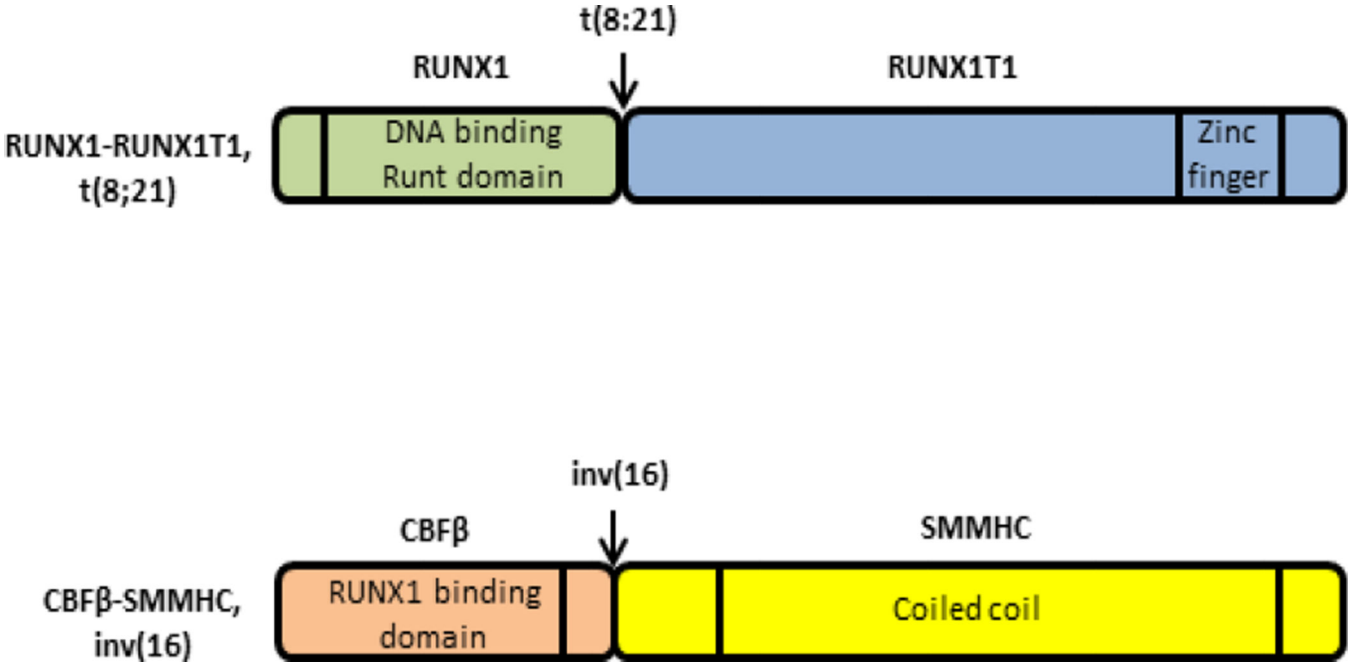

Figure 1. Illustration of fusion protein products from CBF-AML associated mutations and the domains relevant for leukemogenesis

Chromosomal aberrations t(8;21) and inv(16) in CBF-AML produce fusion proteins RUNX1-RUNX1T1 and CBFβ-SMMHC. RUNX1-RUNX1T1 lacks the transactivation domain but retains the DNA binding Runt domain of RUNX1, which is fused to the repressor domain of the RUNX1T1 protein. CBFβ-SMMHC retains the RUNX1 binding domain of CBFβ, which is fused with the coiled coil dimerizing domain of SMMHC.

Table 1

Cooperating mutations and prognosis in CBF AML

Primary mutation	Cooperating mutation	OS	Reference
CBFB-MYH11			
	No cooperating mutation	43%	37
	c-KIT$^+$ CBFB-MYH11	40%	54
	NPM1$^+$ CBFB-MYH11	Better prognosis	26
	FLT3$^+$ CBFB-MYH11	No additional effect	25,27
	N-RAS/K-RAS$^+$ CBFB-MYH11	No additional effect	25
	CEBPA$^+$ CBFB-MYH11	Poorer prognosis	43
RUNX1-RUNX1T1			
	No cooperating mutation	50–60%	37
	c-KIT$^+$ RUNX1-RUNX1T1	14–26%	41,42
	FLT3$^+$ RUNX1-RUNX1T1	No additional effect	25,27
	JAK2$^+$ RUNX1-RUNX1T1	No additional effect	25
	CEBPA$^+$ RUNX1-RUNX1T1	Poorer prognosis	43

Table 2

Currently available treatment for CBF-AML

Classification	Drugs	Mechanism of Action	References
Anti-metabolites	Cytarabine	Inhibit DNA and RNA polymerases	44,53,56
Anthracycline antibiotics	Doxorubicin, Daunorubicin	DNA intercalation	53
Podophyllotoxin	Etoposide	Inhibit topoisomerase II	44
DNA -methyltransferase inhibitor	Azacitidine, Decitabine	Inhibit DNA methylation	71
Histone deacetylase inhibitors	Vorinostat, Valproic acid	Inhibit Histone deacetylation	30,69,70
Kinase inhibitor	Midostaurin, Dasatinib	Inhibit protein kinases nonspecifically	30,68
Antibody-drug conjugate	Gemtuzumab ozogamicin (GO)	CD33 targeted DNA damage	28