

# ADVANCES IN LLM

Current Developments in the Management of Leukemia, Lymphoma, and Myeloma

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## Core Binding Factor Acute Myeloid Leukemia

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**H&O** What are the unique characteristics of core binding factor acute myeloid leukemia and how does it differ from other acute leukemias?

**GM** Adult core binding factor (CBF) acute myeloid leukemia (AML) consists of at least two cytogenetic subtypes of AML: t(8;21)(q22;q22) and inv(16)(p13q22) or t(16;16)(p13;q22), commonly abbreviated as t(8;21) and inv(16), respectively, which are currently recognized in specific categories of the World Health Organization classification. These two cytogenetic subtypes of AML have been usually grouped and reported together because they share few molecular and prognostic features. In both subtypes the cytogenetic rearrangements disrupt genes that encode subunits of CBF, a heterodimer protein complex that regulates transcription of several genes involved in normal hematopoiesis. In t(8;21), the *AML1* (also known as *RUNX1*) gene that encodes the subunit alpha of CBF is fused with the *ETO* (also known as *RUNX1T1*, *CBFA2T*, or *MTG8*) gene thereby resulting in the chimeric gene *AML1/ETO*. In inv(16), the *CBFB* gene encoding the subunit beta of the CBF is disrupted and fused with the *MYH11* gene thereby creating the chimeric gene *CBFB/MYH11*. Both fusion genes appear to play an important role in malignant transformation, although, as demonstrated in murine transgenic models, acquisition of additional “hits” is necessary for developing a phenotype with overt leukemia. Additionally, in early reports both cytogenetic subtypes appeared to have a similar outcome, which is generally more favorable than that of other subtypes of AML, with the exception of acute promyelocytic leukemia, in which patients often harbor

t(15;17) and are treated with a combination of all-*trans*-retinoic acid and chemotherapy. However, more recent studies have underscored important biologic and clinical differences that caution us from continuing to treat these two subgroups as a single entity. Recently, for example, it has been reported that distinct differences in microarray gene expression profiles can separate the two subtypes with accuracy near to 100%. This diversity might reflect morphologic and cytogenetic differences between t(8;21) and inv(16) patients evident at diagnosis. Patients with t(8;21) frequently present with the French-American-British (FAB) morphologic subtype M2 and display loss of a sex chromosome (-Y or -X) and/or deletions of the long arm of chromosome 9 [del(9q)] as secondary cytogenetic changes. In contrast, patients with inv(16) more often are diagnosed with the FAB subtype M4Eo and present with this rearrangement as a sole chromosome aberration or with trisomies of chromosomes 22, 8, and 21, if secondary cytogenetic changes are present. Finally, our group has recently shown that after controlling for confounding variables patients with t(8;21) appear to have a significantly worse outcome than those with inv(16), despite similar treatment. Although the basis for such a difference remains to be fully elucidated, a dissimilar response to salvage therapies is likely as patients with inv(16) tend to survive longer after relapse.

**H&O** What is the incidence rate of CBF AML and is it more prevalent in certain subpopulations?

**GM** Altogether these subtypes account for approximately 15–18% of AMLs diagnosed. In the Cancer and Leukemia Group B (CALGB) study, the incidence rates were 7% and 8% for t(8;21) and inv(16), respectively. Both subtypes are generally more prevalent in whites, but when other nonwhite ethnic groups are examined, t(8;21) is more prevalent than inv(16).

**H&O** How are CBF abnormalities detected in a patient?

**GM** As I mentioned, the presence of the FAB M2 or M4Eo morphologies at diagnosis can raise suspicions of t(8;21) or inv(16), respectively. This, however, requires

confirmation with standard cytogenetic analysis. More rapid and perhaps more sensitive diagnostic tests include fluorescence in situ hybridization (FISH) and reverse transcriptase polymerase chain reaction (RT-PCR). The latter may also diagnose those rare cases in which gene fusions occur without cytogenetically detectable chromosome rearrangements.

### **H&O** How does the treatment of CBF AML differ from the treatment of other varieties of AML?

**GM** In 1994, Dr. Mayer and colleagues reported the initial results of CALGB 8525. In this study, it was observed that AML patients receiving high-dose cytarabine have a better outcome than patients who receive intermediate- or low-dose cytarabine (*New England Journal of Medicine*). Subsequently, Dr. Bloomfield and colleagues showed that among patients with AML who receive high-dose cytarabine, those who benefit the most were patients with either t(8;21) or inv(16) (*Cancer Research*, 1998). Finally, Dr. Byrd and colleagues found that not only is the dose of cytarabine important, but the number of courses is also important (*Journal of Clinical Oncology*, 1999 and 2001). Patients who receive three or four courses of high-dose cytarabine have a better outcome than those who receive only one course. Based on this data, CALGB recommends using at least three courses of high-dose cytarabine for the treatment of CBF AML, which is the foundation of current treatment.

However, as we start to better understand the leukemogenic mechanisms leading to CBF AML, I believe that this current approach is destined to change with incorporation of novel molecular targeted compounds in the treatment of these diseases. For example, in t(8;21), the fusion protein AML1/ETO has been shown to recruit histone deacetylases and, by our group, DNA methyltransferase 1. These enzymes, once recruited on the CBF target genes, lead to chromatin deacetylation and promoter hypermethylation, which in turn result in gene transcriptional repression and thereby disruption of normal pathways of hematopoiesis. Thus it can be hypothesized that treatment with compounds such as histone deacetylase inhibitors and DNA methyltransferase inhibitors may be successful in restoring gene transcription and promoting hematopoietic differentiation, as already supported by preclinical and early clinical reports.

Furthermore, we and others have recently shown that in approximately 25–40% of CBF AML patients, mutations of the *KIT* gene encoding a tyrosine kinase receptor involved in signaling pathways of cell proliferation and survival can be found. Most of these mutations result in a gain of function and the encoded protein is constitutively activated in the absence of its natural ligand thereby promoting leukemia cell proliferation and survival. Most

importantly, the presence of these mutations confer on both t(8;21) and inv(16) patients a worse prognosis compared with those carrying the wild type allele. Therefore, it is possible to envision that in the future, once diagnosis of t(8;21) or inv(16) AML is made, patients are screened for such *KIT* mutations and then treated with tyrosine kinase inhibitors, which are currently undergoing clinical trials, in combination with the other therapeutic strategies discussed above. Of course, as distinct *KIT* mutations confer on the leukemia cells different sensitivity to these compounds, it would be also important to fully characterize the exact type of mutations in each patient at diagnosis in order to utilize this approach correctly.

### **H&O** Do these treatments seem to change the genetic signature of the patient?

**GM** These proposed treatments do not change the genomic make-up of the leukemia blasts as they aim to modify aberrant epigenetic changes occurring on CBF target genes and shut off aberrantly activated proteins that sustain leukemogenesis.

### **H&O** What prognostic factors have been identified in CBF AML?

**GM** Thus far, patients with t(8;21) and inv(16) have been grouped as CBF AML and reported together with regard to clinical outcome. However, I believe there is enough clinical, prognostic, and molecular evidence to differentiate the two groups and begin to discuss them as individual entities. In a recent meta-analysis of patients enrolled on different CALGB treatment protocols, we showed that race is an important prognostic factor in t(8;21) AML. It was shown that nonwhite patients are at a higher risk of failing induction chemotherapy compared with the corresponding white population. In addition, nonwhite patients with t(8;21) and secondary cytogenetic abnormalities other than del(9q) had shorter survival compared with nonwhite patients presenting with sole t(8;21) or t(8;21) with del(9q). In contrast, among white patients with t(8;21), these cytogenetic aberration did not impact on clinical outcome. However, the number of nonwhite patients in this study was relatively low, and these results need to be confirmed prospectively. For inv(16), race does not appear to be an important prognostic factor, but older age is associated with shorter survival. Furthermore, patients with inv(16) and one or more secondary abnormality, especially +22, had lower risk of relapse compared to those with inv(16) solely. Finally, more recently, *KIT* mutations, particularly those that occur within the DNA sequences encoding the activation loop domains of the protein, have been showed to predict relapse and/or death in both t(8;21) and inv(16) patients.

## H&O Is the issue of minimal residual disease relevant to the treatment of CBF AML?

**GM** With *inv(16)*, it has been shown that if the presence of the fusion transcript is detected by a sensitive RT-PCR assay during remission, the patient is at a much higher risk of relapse. In general however, it might require several months before patients can achieve a negative RT-PCR status following remission-induction chemotherapy. Patients who do not become negative within the first 9–12 months, however, may be at a higher risk of relapse, although long-term remission has been reported in some cases with persistent detectable minimal residual disease (MRD) beyond this time point. For *t(8;21)(q22;q22)*, the situation is even more complicated because it has been shown that the fusion transcript can be detected in patients who are in complete remission for up to 10 years. Therefore, these results suggest that detection of MRD with a very sensitive RT-PCR assay may not be effective at identifying higher risk for relapse. However, different groups have now shown that it may be possible to identify threshold levels of *AML1/ETO* transcript above which relapse is likely to occur utilizing quantitative RT-PCR assays during remission. In conclusion, there has not yet been a strong prospective study that allows us to use MRD as a prognostic factor for stratification of patients to risk-adapted treatment in both *t(8;21)* and *inv(16)*. As in other types of leukemia, if a patient changes from a negative to a positive MRD status and/or shows persistently high levels of fusion transcripts, he or she is probably at higher risk of relapse. Whether or not this patient would benefit more from a stem cell transplantation at the time of molecular rather than clinical relapse has yet to be demonstrated.

## H&O What is the direction or focus of research currently ongoing into CBF AML?

**GM** Although we have begun to have quite a good understanding of these diseases mechanistically, much more needs to be achieved in order to improve the current clinical results. The *t(8;21)* and *inv(16)* AMLs are generally considered favorable subtypes of this disease, but in reality we should exercise a great deal of caution when we pass this information on to patients. Although with respect to other subtypes, *t(8;21)* and *inv(16)* AMLs may generally have a more favorable outcome, only approximately 50% of the patients achieve long-term survival. Implementation of risk-adapted treatments including stem cell transplantation and participation in clinical tri-

als that incorporate molecular targeted therapies for those patients with a predicted poor outcome are likely to be important initial steps for improving the current clinical results. Furthermore, we hope that important information for more accurate identification of high-risk patients and perhaps discovery of novel therapeutic targets will come from gene expression and microRNA profiling, which are currently under intense investigation.

## Suggested Readings

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