study using data from the patients' samples. The activity of APY0201 was highest in patient-derived samples with hyperdiploidy (trisomies with one or more odd-numbered chromosomes) and lowest in patients' samples with a t(11;14) translocation. In addition, *ex-vivo* samples with high TFEB levels were sensitive to APY0201. High TFEB levels have been associated with increased autophagic flux suggesting that autophagic flux may be directly related to PIKfyve inhibition. These preliminary results may suggest patient populations that could be enriched for in a future clinical trial.

In conclusion, Bonolo de Campos *et al.* provide exciting data to support the ongoing investigation of therapeutically manipulating targets specific to plasma cell function, particularly protein handling in myeloma.[2] Although the finer details of the actual mechanisms may differ somewhat between multiple myeloma and non-Hodgkin lymphoma, data from this study and those performed in non-Hodgkin lymphoma provide compelling evidence for the role of PIKfyve inhibition in inducing cell death, with changes seen in the autophagy and lysosomal pathways. Notably, this study demonstrates the importance of the inherent genetic differences in myeloma biology and the potential role of PIKfyve inhibitors in targeting a distinct group of genetically defined myeloma to continue this era of personalized medicine.

References

1. Gay F, Engelhardt M, Terpos E, et al. From transplant to novel cellular therapies in multiple myeloma: European Myeloma Network guidelines and future perspectives. Haematologica. 2018;103(2):197-211.
2. Aronson LI, Davies FE. DangER: protein ovERload. Targeting protein degradation to treat myeloma. Haematologica. 2012;97(8):1119-1130.
3. Bonolo De Campos C, Zhu YX, Sepetov N, et al. Identification of PIKfyve kinase as a target in multiple myeloma. Haematologica. 2020;105(6):1641-1649.
4. Hoang B, Benavides A, Shi Y, Frost P, Lichtenstein A. Effect of autophagy on multiple myeloma cell viability. Mol Cancer Ther. 2009;8(7):1974-1984.
5. Aronson LI, Davenport EL, Mirabella F, Morgan GJ, Davies FE. Understanding the interplay between the proteasome pathway and autophagy in response to dual PI3K/mTOR inhibition in myeloma cells is essential for their effective clinical application. Leukemia. 2013;27(12):2397-2403.
6. Hessvik NP, Øverbye A, Brech A, et al. PIKfyve inhibition increases exosome release and induces secretory autophagy. Cell Mol Life Sci. 2016;73(24):4717-4737.
7. Gayle S, Landrette S, Beeharry N, et al. Identification of apilimod as a first-in-class PIKfyve kinase inhibitor for treatment of B-cell non-Hodgkin lymphoma. Blood. 2017;129(13):1768-1778.
8. Kumar S, Kaufman JL, Gasparetto C, et al. Efficacy of venetoclax as targeted therapy for relapsed/refractory t(11;14) multiple myeloma. Blood. 2017;130(22):2401-2409.
9. Pawlyn C, Davies FE. Toward personalized treatment in multiple myeloma based on molecular characteristics. Blood. 2019 14;133(7):660-675.
10. Qiang YW, Ye S, Chen Y, et al. MAF protein mediates innate resistance to proteasome inhibition therapy in multiple myeloma. Blood. 2016;128(25):2919-2930.

The increasing complexity of the management of core-binding factor acute myeloid leukemia

Mark R. Litzow

Division of Hematology and Transplant Center, Mayo Clinic Rochester, Rochester, MN, USA

E-mail: MARK R. LITZOW - litzow.mark@mayo.edu

doi:10.3324/haematol.2020.249110

The core binding factor (CBF) acute myeloid leukemias characterized by the t(8;21) and inv(16)(p13q22)/t(16;16)(p13;q22) cytogenetic abnormalities have long been known to prognostically represent more favorable subcategories of acute myeloid leukemia (AML). These translocations are characterized by the presence of the *RUNX1-RUNX1T1* (*AML1-ETO*) and *CBFB-MYH11* fusion transcripts, respectively. In fact, the t(8;21) was the first cytogenetic abnormality identified in AML in 1973.[1] These CBF-AML subtypes have continued to remain in the favorable risk category in multiple classification systems up to the current time based on their high rate of achievement of complete remission with induction chemotherapy and their relatively low relapse rate.[2] Clinical trials over the years have demonstrated that these two CBF-AML subtypes are particularly responsive to high doses of cytarabine utilized in consolidation regimens. Addition of the immunoconjugate drug, gemtuzumab ozogamicin, to induction chemotherapy further reduces the risk of relapse and improves overall survival in patients with CBF-AML.[3] The favorable results of chemotherapy in patients with CBF-AML have led to the widely accepted practice not to perform allogeneic blood or marrow transplant (alloBMT) in these patients who achieve first remission. This is in contrast to patients with AML with intermediate risk or unfavorable risk features where allogeneic blood or marrow transplant in first remission is a widely accepted practice.

However, the two subtypes of CBF-AML are not the same in all respects. Studies going back 15 years or more have pointed out the difference between these two subtypes.[4] Use of next-generation sequencing (NGS) and identification of additional gene mutations in patients with AML have begun to further define differences between the two. One of the first mutational abnormalities found in subsets of patients with CBF-AML were *c-KIT* mutations. The *c-KIT* mutation has been suggested to be associated with a poorer prognosis in CBF-AML patients but, here again, this mutation seems to have less of a prognostic impact in patients with inv(16) compared to those with t(8;21).[5] NGS studies, which are now widely utilized to assess prognosis in many subtypes of AML, have been applied to patients with CBF-AML. Multiple mutations in addition to *c-KIT* have been identified, including genes activating tyrosine kinase signaling, such as *N/KRAS* and *FLT3*. Mutations in genes that regulate

Risk factor	Risk ratio	P
Age	1.031	0.0017
KIT D816V mutation positive (Ref = negative)	4.331	0.0018
KIT D816V mutation non-tested/ missing (Ref = negative)	2.567	0.0036
WBC at diagnosis	1.018	0.0361
Number of chromosomes (Ref = non-pseudodiploidy)	2.552	0.0035

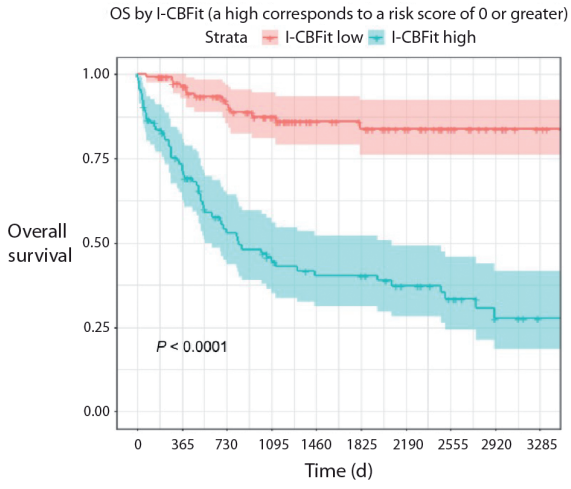

Figure 1. Risk factors and a novel scoring system (I-CBFit) for core-binding acute myeloid leukemia. (A) Risk ratios of risk factors for death or relapse. (B) Overall survival (OS) according to I-CBFit. Ref: reference values; WBC: white blood cell count; d: days.

chromatin conformation or encode members of the cohesin complex have been observed with high frequency in t(8;21) AML, although they are infrequent in inv(16) AML.[6,7] These studies all highlight the heterogeneity of the two subtypes of CBF-AML and have added further complexity to their characterization.

Additionally, monitoring for minimal or measurable residual disease (MRD) by quantitative real-time polymerase chain reaction has been shown to risk stratify patients and predict relapse and, as in other subtypes of AML, has been shown to be a powerful prognostic factor.[8]

Despite the favorable risk categorization of CBF-AML, up to 30-40% of these patients can still relapse after standard intensive induction and consolidation chemotherapy. Once they do relapse, additional re-induction chemotherapy is required to put them into second remission and, generally, these patients are then candidates for alloBMT in second remission in order to prevent subsequent relapse and ensure durable second remissions. Fortunately, these patients can achieve a second remission with chemotherapy in up to 80-90% of the cases. In this setting, addition of gemtuzumab ozogamicin can also help to lessen the risk of subsequent relapse.[9]

Thus, the outcome of patients with CBF-AML who have relapsed and achieve a second remission and subsequently undergo alloBMT is of significant importance in the management of these patients. In this issue of *Haematologica*, Halaburda and colleagues from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation report the results of 631 patients reported to the EBMT registry between 2000 and 2014 with CBF-AML.[10] These patients came from 181 transplant centers, and a little over half of them (n=366) had an inv(16) and 265 of them (42%) had a t(8;21). There were more males with t(8;21) than with inv(16), and time from diagnosis to transplantation, and time from diagnosis to first remission, were also longer in the t(8;21) group. Over half of the patients were transplanted between the years 2010 and 2014, and 21% of patients had additional cytogenetic abnormalities found at diagnosis. Molecular abnormalities were identified, but were reported with low frequency. Leukemia-free survival at 2-5 years was 59% and 54%, respectively, while overall survival probabilities were 65% and 58%, respectively. Relapse risk at two and five years was 20% and 23%, respectively, and non-relapse mortality was 21% and 23% at two and five years, respectively. In multivariate analysis, factors negatively impacting leukemia-free and overall survival were: t(8;21), presence of three or more additional chromosomal abnormalities, and poor Karnofsky performance score of <80%. Furthermore, additional cytogenetic abnormalities and the t(8;21) increased relapse risk. Use of reduced intensity conditioning in the transplant regimen also increased relapse risk. As expected, the presence of MRD assessed by molecular techniques before transplantation was associated with increased relapse risk and inferior leukemia-free survival.

One caveat to keep in mind in studies assessing the results of alloBMT, particularly in patients with second remission, is that these studies do not take into account the outcome of all patients who have relapsed and they thus select for patients who are able to achieve a second remission and move on to transplant. These studies also do not include an analysis of patients who relapse and fail to achieve second remission, die in the attempt to achieve a second remission, or have significant comorbidities develop which preclude them from proceeding to transplant. This has been described as the "myth of the second remission".[11]

Given the increasing heterogeneity of CBF-AML as outlined earlier, how are we now to determine which patients with CBF-AML should undergo alloBMT in first remission or continue to not be transplanted in first remission and only to proceed to transplant if they relapse and achieve a second remission? There are increasing numbers of prognostic scoring systems that are under development for multiple diseases, and some of those emerging incorporate the availability of identification of additional genetic abnormalities.[12] In particular, a recent publication has reported a novel scoring system for patients with the

t(8;21) subtype of CBF-AML. This study of 247 patients from the United States and Europe identified older age, a *KIT D816V* mutation, a high white blood count, and pseudodiploidy compared with hyper- or hypodiploidy into a scoring system called the I-CBFit. This scoring system demonstrated a disease-free survival rate at two years of 76% in patients with a low-risk I-CBFit score compared with 36% for those with a high-risk I-CBFit score (Figure 1).[13]

Whereas in the past, CBF-AML has been characterized and treated as a monolithic entity in terms of treatment and prognosis, one must now take into account the multiple clinical and laboratory characteristics in order to more expertly characterize the prognosis of these patients so as to design the most appropriate treatment plan and incorporate decision-making toward use of alloBMT in first or second remission. The study by Halaburda and colleagues from the Acute Leukemia Working Party of the EBMT[10] adds a valuable source of information to help understand the pros and cons of these approaches and the outcomes of patients who undergo transplant with CBF-AML in second remission.

References

1. Rowley JD. Identificaton of a translocation with quinacrine fluorescence in a patient with acute leukemia. Ann Genet. 1973;16(2):109-112.
2. Dohner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424-447.
3. Hills RK, Castaigne S, Appelbaum FR, et al. Addition of gemtuzumab ozogamicin to induction chemotherapy in adult patients with acute myeloid leukaemia: a meta-analysis of individual patient data from randomised controlled trials. Lancet Oncol. 2014;15(9):986-996.
4. Marcucci G, Mrozek 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(24):5705-5717.
5. Ayatollahi H, Shajiei A, Sadeghian MH, et al. Prognostic Importance of C-KIT Mutations in Core Binding Factor Acute Myeloid Leukemia: A Systematic Review. Hematol Oncol Stem Cell Ther. 2017;10(1):1-7.
6. Duployez N, Marceau-Renaut A, Boissel N, et al. Comprehensive mutational profiling of core binding factor acute myeloid leukemia. Blood. 2016;127(20):2451-2459.
7. Prieto-Conde MI, Jimenez C, Garcia-Alvarez M, et al. Identification of relapse-associated gene mutations by next-generation sequencing in low-risk acute myeloid leukaemia patients. Br J Haematol. 2020 Mar 2. [Epub ahead of print]
8. Yin JA, O'Brien MA, Hills RK, Daly SB, Wheatley K, Burnett AK. Minimal residual disease monitoring by quantitative RT-PCR in core binding factor AML allows risk stratification and predicts relapse: results of the United Kingdom MRC AML-15 trial. Blood. 2012;120(14):2826-2835.
9. Hospital MA, Prebet T, Bertoli S, et al. Core-binding factor acute myeloid leukemia in first relapse: a retrospective study from the French AML Intergroup. Blood. 2014;124(8):1312-1319.
10. Halaburda K, Labopin M, Mailhol A, et al. Allogeneic stem cell transplantation in second complete remission for core binding factor acute myeloid leukemia: a study from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation. Haematologica 2020;105(6):1723-1730.
11. Forman SJ, Rowe JM. The myth of the second remission of acute leukemia in the adult. Blood. 2013;121(7):1077-1082.
12. Gerstung M, Papaemmanuil E, Martincorena I, et al. Precision oncology for acute myeloid leukemia using a knowledge bank approach. Nat Genet. 2017;49(3):332-340.
13. Ustun C, Morgan E, Moodie EEM, et al. Core-binding factor acute myeloid leukemia with t(8;21): Risk factors and a novel scoring system (I-CBFit). Cancer Med. 2018;7(9):4447-4455.