survival by measuring disease bulk, survival in CLL is now determined by molecular genetic features (p53 or 17p deletion, IgHV unmutated status, and 11q deletion), serum markers of disease proliferation (thymidine kinase and β2-microglobulin), and host factors (age, ECOG performance status, and male sex). In clinical practice and treatment decision making, the most important subgroups are the 25% of patients in the low-risk group, with 5-year survival of 95% and median time to first therapy of >10 years, and the 4% of patients in the very-high-risk group, with a dismal 5-year survival of 19%. Of note, the presence of 17p deletion is required for patients to be classified as very high risk, but even within this p53-deleted subgroup, patients with relatively favorable survival can be identified (eg, those patients without concomitant adverse features and those who do not require immediate therapy). Thus, much of the adverse impact of del(17p) is due to the relative ineffectiveness of current (fludarabine-based) therapy in combating this form of CLL.

What are the caveats against and barriers to the immediate adoption of this prognostic score into clinical practice? First, the score depends on the ability to assay serum thymidine kinase, a prognostic marker that is not routinely measured outside of Germany. Modern methods such as chemiluminescence-based assays may facilitate the adoption of this test by diagnostic and commercial laboratories. Second, the understanding of the genomic landscape of CLL is rapidly evolving, and as data regarding novel mutations such as those in NOTCH1, SF3B1, and BIRC3 accumulate, their roles will need to be re-evaluated in the context of this prognostic model. Additionally, most diagnostic and commercial laboratories test for 17p deletion by FISH, but few currently have the ability to screen for p53 mutations. As the availability of p53 mutation screening increases in clinical practice, the prognostic score will require recalculation to determine whether p53 mutations have an adverse impact as substantial as that of 17p deletion. The authors also acknowledge a degree of patient selection and underrepresentation of older patients from the studies used to generate and validate this index. Finally, the treatment landscape of CLL is changing with the advent of highly active oral small molecules which can be delivered effectively, even in older patients and in those with p53-aberrant CLL, in time, the applicability of the prognostic score in the era beyond chemotherapy will need to be re-evaluated.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

REFERENCES

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Lymphoid Neoplasia

Comment on Mraz et al, page 84

miR in CLL: more than mere markers of prognosis?

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In this issue of Blood, Mraz et al show that microRNA-150 (miR-150) is the most abundantly expressed miR in chronic lymphocytic leukemia (CLL) and affects the threshold for B-cell receptor (BCR) signaling by repressing expression levels of GAB1 and FOXP1. This functional link might explain the described association between expression levels of miR-150 and prognosis.

In 2002, the first link between small noncoding RNAs, known as miRs, and cancer was made by the observation that in CLL, the most common genetic aberration 13q14 deletion, was associated with downregulation of miR-15a and miR-16-1, which reside in the minimally deleted region within 13q14. This seminal observation initiated many studies into the role of miRs in the pathogenesis of cancer in general and especially in CLL. The pioneering work on miR-15a and 16-1, however, also exemplified the complexity of aberrant miR expression and its possible relation with alterations in cancer-specific biological pathways. Although early studies suggested that in CLL miR-15a/16-1 mediated control of BCL2 expression and survival, it took until 2010 to learn that in fact the function of these miRs in B-cell malignancies is exerted mainly by downregulation of genes controlling cell-cycle entry.

Because each miR can affect the expression of hundreds of different genes, which not only differs per cell type but also depends on their developmental stage, and because currently available bio-informatic tools are imperfect in predicting targets via sequence similarities, it has been highly challenging to interpret the pathophysiological relevance of aberrations in miR levels measured ex vivo, or after in vitro manipulation.

Despite these challenges, over the years, several miRs could convincingly be mapped to disease-specific relevant pathways, such as the identification of miR-33a as a component of the chemotherapy resistance network in CLL.
Major advances have been made in understanding the molecular pathogenesis underlying CLL progression and treatment resistance, demonstrating a pivotal role for ligand-independent (“tonic”) and ligand-dependent (“chronic”) BCR signaling in CLL trafficking and activation-induced interaction with the tumor microenvironment. Recent studies in model systems also demonstrated that miRs are involved in BCR signaling. In CLL, a strong hint for a role of specific miRs in the susceptibility to BCR signaling came from observations on differential expression levels of approximately 20 miRs in immunoglobulin heavy-chain variable subgenes (IGHV) unmutated and/or ζ-associated protein of 70-kDa (ZAP-70)–positive CLL cases vs mutated IGHV and/or ZAP-70 negative cases (reviewed in Mraz and Kipps). However, for most of these miRs, their exactly relevant target genes in CLL have not yet been identified and therefore their exact role in the BCR signaling pathway is still largely unclear.

The detailed work of Mraz et al in this issue of Blood takes this next step. They functionally map the most abundantly expressed miR in CLL, miR-150, to the BCR signaling pathway via 2 specific target genes. First, they show that miR-150 levels were significantly lower in cases that used unmutated IGHV or that expressed ZAP-70. To identify miR-150 target genes in CLL, transcriptome analysis was performed and data were verified using 6 database tools. Two genes with evolutionary conserved binding sites for miR-150 were identified, namely GAB1 and FOXP1. The link was functionally validated in cell lines and primary CLL samples by transfection studies. Moreover, roles for both miR-150 and the 2 target genes were shown by measuring calcium fluxes after BCR ligation and RNA interference in cell lines. The BCR-induced calcium fluxes correlated with miR-150 levels in primary CLL samples. Importantly, they also showed that miR-150 levels are significantly lower in cases that use unmutated IGHV or that express ZAP-70. Moreover, patient survival studies indicate an association between expression levels of miR-150, GAB1, FOXP1, and clinical outcome. Although expression levels of miR-150 correlated with ZAP-70 or IGHV status, low-level expression of miR-150 had an independent prognostic value for both overall survival and treatment-free survival.

GAB1 and FOXP1 are involved in essential signaling cascades in both normal and malignant B cells. GAB1 is an adaptor molecule of phosphoinositide 3-kinase (PI3K) (reviewed in Zhang et al). Because the PI3K pathway is activated not only by the BCR, but also by the variable of receptor tyrosine kinases and cytokine receptors, this signaling route is pivotal in microenvironment-induced CLL activation. FOXP1 is a transcription factor associated with the activated B-cell phenotype of diffuse large B-cell lymphoma, and it has been suggested that truncated forms of FOXP1 are functionally associated with subtypes of diffuse large B-cell lymphoma characterized by constitutive nuclear factor-kB activity. It would be of interest to learn the functional consequences of miR-mediated regulation of these genes in the context of microenvironment-induced features of activation such as survival and chemosensitivity, proliferation, and adhesion/migration. In this respect, the observations in model systems that stimulation with anti-CD40 antibodies largely prevented BCR-mediated changes in miR expression levels indeed indicate an intricate interplay with miRs, BCR signaling, and microenvironmental stimuli.

The importance of BCR signaling in the pathobiology of CLL is underscored by the significant clinical activity of inhibitors blocking BCR-associated kinases, specifically Bruton tyrosine kinase and PI3K. Because these need to be administered for prolonged periods, balance of costs vs effectiveness of these novel drugs is highly important. Predictive markers of effectiveness are therefore highly needed. Data provided by Mraz et al suggest that miR-150 expression and its target genes might influence the sensitivity of malignant B cells to these inhibitors.

As always, after a step forward has been taken, we quickly wonder about the implications. One question to consider is: Why and how is miR-150 regulated? If low miR-150...
levels facilitate BCR signaling, which is advantageous to the cells, we might expect selection for this state. Indeed, Mraz et al find that miR-150 levels negatively correlate with disease progression (Rai stage). As for regulation, the authors provide a hint that miR-150 might be controlled epigenetically. Finally, an exciting new field is the development of miR-based therapeutics that either downregulate the function of oncogenic miRs or upregulate the expression of tumor-suppressive miRs, such as the liposome-based miR-34 mimic currently in phase 1 trials (reviewed in Ling et al9).

Because Mraz et al identified miR-150 as a potential key regulator of PI3K and because it has been showed in follicular B cells that miR-185 regulates the expression of Bruton tyrosine kinase,10 it does not take a lot of imagination to speculate on the development of miR mimetics to target key signaling cascades in CLL.

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REFERENCES

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