MLL-AF4+ pro-B acute lymphoblastic leukemia (ALL) in infants represents an aggressive, high-risk type of childhood leukemia arising from prenatally acquired preleukemic t(4;11) chromosomal translocations. However, despite various reported attempts, accurately mimicking MLL-AF4–driven leukemogenesis in mice remains a difficult task. Enforced expression of the MLL-AF4 fusion protein in cord blood–derived human hematopoietic stem cells (HSCs) transplanted into immunodeficient mice either results in the development of malignancies that deviate from the pro-B ALL phenotype as observed in humans, or does not lead to neoplasia at all. Obviously, these discrepancies raise important questions: (I) Does MLL-AF4+ pro-B ALL in infants arise in CD34+ HSCs? (II) Are MLL-AF4 fusion proteins driving leukemogenesis on their own, or are cooperative genetic lesions required? (III) Do MLL-AF4 fusion proteins exert sufficient transforming capacity? For instance, Bur sen et al recently showed that not MLL-AF4, but enforced expression of its reciprocal fusion protein AF4-MLL in murine HSCs or progenitor cells, induced ALL in mice without the requirement of MLL-AF4. In contrast, Tamai et al showed that enforced expression of MLL-AF4 in murine HSCs is sufficient to induce ALL, but demonstrated that the process of transformation is significantly accelerated by cooperative K-Ras mutations. Nonetheless, these experiments remain to be repeated in HSCs of human origin to appreciate the relevance of AF4-MLL and/or RAS activation in the development of MLL-AF4+ pro-B ALL. Moreover, the reciprocal AF4–MLL fusion transcript is present in the majority of, but not all, patients with MLL-AF4+ ALL, and RAS mutations are found in ~25% of the cases. Hence, distinct mechanisms of transformation, as well as the involvement of yet unknown genetic events, cannot be ruled out.

Meanwhile, Dr Pablo Menendez and coworkers have been elegantly addressing the question of the cell of origin from which MLL-AF4+ pro-B ALL may arise. On the basis of their earlier observations that bone marrow–derived mesenchymal stem cells from patients with MLL-AF4+ pro-B ALL harbor and express the MLL-AF4 fusion gene, Menendez et al reasoned that this type of leukemia may well arise in hematopoietic mesodermal or hemangioblastic precursors sprouting from differentiating hESCs. To test this hypothesis, this research group recently created a cellular system to study early hematopoietic development in MLL-AF4–expressing hESCs. Interestingly, introducing MLL-AF4 expression in hESCs enhanced the specification of hemogenic precursors, but impaired further hematopoietic commitment in favor of an endothelial cell fate. Alas, MLL-AF4 expression alone appeared not sufficient to induce leukemia in hESC-derived hematopoietic cells. In the present study, Bueno et al explored the impact of FLT3 activation on the hematopoietic fate of MLL-AF4–expressing hESCs. Patients with MLL-AF4+ pro-B ALL frequently display constitutive FLT3 activation, usually as a result of high-level FLT3 expression, or sporadically from activating mutations within the tyrosine kinase domain. Activated FLT3 positively affects several signal transduction pathways, all of which favor cell survival and proliferation, and supposedly provides (pre-) leukemic cells with a growth advantage and possibly with enhanced transforming capacity. Hence, FLT3 activation may well be an additional genetic event required for MLL-AF4–driven leukemogenesis. Interestingly, Bueno et al show that in MLL-AF4–expressing hESCs, activated FLT3 is capable of abolishing hematopoietic differentiation, indeed suggesting a role for FLT3 activation in the development of MLL-AF4+ pro-B ALL. However, FLT3 activation did not seem sufficient to cooperate with MLL-AF4 in transforming hESC-derived hematopoietic cells. Nonetheless, the presented MLL-AF4–expressing hESC model represents an intriguing experimental system that hopefully soon will also be used to explore the impact of other potential secondary oncogenic lesions. In the meantime, it remains important to keep searching for alternative target cells that may resemble the actual cell of origin, as well as additional (epi)genetic hits that potentiate MLL-AF4–driven leukemogenesis (eg, use of whole-genome sequencing approaches).
CLL is a biologically heterogeneous disease with some patients exhibiting indolent disease for many years and others progressing rapidly and requiring early treatment. The distinction between and even within these groups has become more clear with progressively more sophisticated molecular profiling of this disease. Although common cancer pathways such as p53 play a significant role, more recently B-cell specific abnormalities have been identified and characterized. The most well-defined B-cell specific markers, immunoglobulin heavy chain (IgVH) mutational status, \(^2\), Zap70 expression, \(^4\) and methylation, \(^5\) identify a subgroup of patients with increased responsiveness of the B-cell receptor (BCR) signaling pathway to external stimulation, constitutive activity of this pathway, and more aggressive disease.

In contrast, this article focuses on the subset of patients whose CLL cells display a phenotype associated with molecular anergy, where immunoglobulin M ligation of the BCR does not lead to phosphotyrosine induction or calcium flux. Previously, this group has shown that CLL cells from this subset of patients display constitutive activation of phosphorylated Erk1/2 and activated nuclear factor of activated T cells c1 (NF-ATc1) without Akt phosphorylation. \(^6\) In the current article, this group extends these observations by further characterizing these cells. Similar to previous observations, constitutive activation of phosphorylated extracellular signal-regulated kinase (pErk) was found in 23 of 52 patients. The expression of pErk was correlated with NF-ATc1 nuclear translocation and prolonged survival in culture, as well as markers associated with indolent disease biology, including absence of CD38 expression and low Zap70 expression. Importantly, supporting the idea that these cells are anergic as the result of chronic antigen stimulation, in vitro culture reversed this phenotype and restored sensitivity to BCR engagement. These finding therefore show that CLL cells are anergic in a subset of patients exhibiting indolent disease, that these cells are likely exposed to chronic antigen stimulation in vivo, and that these cells show prolonged in vitro survival, suggesting that anergy can be a mechanism of cell survival.

To prove that anergy does indeed lead to cell survival and can potentially be a therapeutic target, the authors treated anergic (pErk1/2\(^+\)) and nonanergic (pErk1/2\(^-\)) CLL cells in culture with inhibitors of mitogen-activated protein kinase kinase 1 and 2 (MEK1/2), extracellular signal-regulated protein kinases 1 and 2 (ERK1/2), or NF-AT. \(^1\) Inhibitors of MEK1/2 and NF-AT were both able to reduce phosphorylation of Erk1/2 and nuclear translocation of NF-AT and restore sensitivity to BCR engagement with short periods of exposure. As well, At 48 hours, anergic cells showed significantly reduced survival compared with nonanergic cells treated with any of these inhibitors (see figure). In addition, an in vivo xenograft experiment in which the authors used the phenotypically anergic Mec-1 cell line showed improved survival in mice treated with an NF-AT inhibitor.

One question raised by these data is whether maintenance of anergy in CLL might be beneficial in some cases, as it is associated with favorable CLL biomarkers as well as potentially an indolent disease course. \(^1\) Although targeting anergy in vitro and in a xenograft model promotes apoptosis, this does not always translate well to the clinic. It will be interesting to discover whether anergy is maintained even in the presence of disease progression and whether the anergic phenotype is maintained after therapeutic intervention. These answers may help determine whether anergy reversal is a viable therapeutic target in a subset of patients.

Inhibition of the anergic signaling pathway selectively reduces survival in anergic CLL cells. CLL cells displaying an anergic phenotype (pErk1/2\(^+\)) or a nonanergic phenotype (pErk1/2\(^-\)) were treated with agents targeting MEK 1/2, ERK 1/2, and NF-ATc1, molecules implicated in the maintenance of anergy. Cells displaying an anergic phenotype showed decreased viability in the presence of these agents compared with nonanergic cells. Adapted from Figure 5 in the article by Apollonio et al that begins on page 3789.
These data also raise the question of how best to target anergy in CLL. One intriguing possibility is combination with other targeted CLL therapies. Currently, much attention is focused on the treatment of CLL with inhibitors of the BCR signaling pathway, especially the phosphatidylinositol3-kinase inhibitor GS-1101 and the Bruton tyrosine kinase inhibitor ibrutinib. Preliminary data with ibrutinib suggest that patients with IgVH-unmutated disease, associated with more active BCR signaling, respond earlier than patients with IgVH-mutated disease. Apollonio et al show that inhibitors of markers of anergy restore BCR sensitivity—perhaps inhibition of anergy before BCR pathway inhibition might improve response. Alternatively, the concept of anergy maintenance through chronic antigen stimulation opens another avenue to target these cells. Biased immunoglobulin gene usage in CLL suggests that in at least a subset of patients’ CLL pathogenesis is antigen driven. If continued antigen stimulation is required for maintenance of anergy, as suggested by these data, inhibition of antigen binding may be another therapeutic strategy to promote apoptosis.

As we move toward more distinct molecular classification of CLL, Apollonio et al identify a subset of CLL patients with a phenotype that could potentially be targeted therapeutically. These data, building on previous work from this group, enhance our knowledge of the biology of this heterogeneous disease and continue to move us forward toward the goal of individualizing targeted therapies for our patients.

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REFERENCES

To shrink or not to shrink

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In this issue of Blood, Andolfo and colleagues show that dehydrated hereditary stomatocytosis (DHSt), an inherited red cell disorder, is associated with a number of distinct germline mutations in PIEZO1, a stretch activated cation channel, in 26 affected individuals from 7 families. The shrinking cell has been an object of curiosity and some bafflement to physiologists and hematologists since it was first seen some 40 years ago. We know that regulation of its volume within narrow limits through a tightly controlled intracellular cation concentration is critical for optimal functioning and survival of the red cell. An autosomal dominant hematolytic anemia characterized by primary red cell dehydration due to decreased cation content was first described by Miller and colleagues in 1971 and is currently designated as hereditary stomatocytosis (HX) or DHSt. These patients typically exhibit mild to moderately compensated hemolytic anemia and the red cells are characterized by increased mean corpuscular hemoglobin concentration and decreased osmotic fragility, both reflecting cellular dehydration. In addition to anemia, a subset of the patients exhibit pre- and/or perinatal edema which recedes spontaneously.

The molecular basis of the disorder, which has been under intense scrutiny for decades, was recently resolved thanks to the identification of mutations in the gene encoding PIEZO1. These came to light in 2 large kindreds studied by Zarychanski and colleagues last year and in 7 additional families described in the present study. PIEZO1 was identified as a protein involved in mechanosensation and stretch-activated cation channel regulation in 2010, and it adds to the impact of that work that, 2 years later, a red cell disorder has been identified as the first human disease stemming from mutations in this gene.

Although the identification of mutations in PIEZO1 leading to red cell dehydration in HX by 2 independent groups is a cause for

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(A) Membrane deformation induced by fluid shear stress acting on normal human discoid red cells and (B) membrane deformation induced by polymerization of hemoglobin upon deoxygenation of human sickle red cells. Do these types of membrane deformation regulate PIEZO1 function?
Survival of the weak (signalers): anergy in CLL

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