patients with a variety of lymphoma subtypes used early changes in circulating CD3+ T cells and platelets—both require BCL-2 family proteins for their survival—as evidence that this new agent was hitting the desired target. CD3+ T cells and platelets fell rapidly after initiation of therapy, and decreases in platelet number correlated with ABT-263 area under the curve. Unfortunately, these changes did not correlate with tumor response or changes in BCL-2 family protein levels in tumor cells. Similarly, changes in circulating endothelial cells and endothelial cell precursors, a measure of the antiangiogenic effects of a number of targeted therapies, did not correlate with response in patients with diffuse large-cell lymphoma treated with sunitinib.

Treatment with FLT3 inhibitors such as KW-2449 resulted in disappointingly low response rates in acute myeloid leukemia. These low response rates may represent a pharmacokinetic failure; that is, the inability to sustain the inhibition of FLT3 phosphorylation in leukemia cells in vivo compared to the successful sustained inhibition in vitro. The report by Leonard and colleagues describes a novel approach to this recurrent dilemma.

PD0332991 is a pyridopyrimidine with high selectivity for cdk4, producing G1 arrest in preclinical studies and de-phosphorylation of Rb at known cdk4-specific phosphorylation sites. Leonard et al provide important evidence of biomarker modulation after administration of PD0332991 to patients with relapsed MCL. To evaluate changes in cell proliferation, they performed FLT-PET, and for assessment of metabolism, FDG-PET, both before and during the third week of daily administration of study drug. Tissue biopsies were obtained at baseline and on day 21 of cycle one, and assessed using immunohistochemistry for total Rb protein, phospho-Rb, and cell proliferation using Ki-67. The study was powered to detect a 50% reduction in standardized uptake value (SUV) for both FLT-PET and FDG-PET.

Of the 16 evaluable patients, 1 complete response and 2 partial responses by standard imaging criteria were observed (response rate 18.7%); 5 patients including the 3 responders remained on study drug without progression for more than 1 year. Seven patients had a partial metabolic response by FDG-PET by week 3 of cycle one, and 15 had a proliferative response at that point by FLT-PET. Importantly, among informative biopsy pairs (pre- and on-treatment), the reduction in phospho-Rb positive cells was 89%, without changes in total Rb protein (P = .00007, paired t test). Ki-67 staining was also substantially reduced, by 74% (P = .000002). The degree of reduction in phospho-Rb was strongly correlated with reduction in Ki-67, and the phospho-Rb and Ki-67 changes were also correlated with the summed $SUV_{max}$ by FLT-PET. The fact that all 5 patients who stayed on PD0332661 for more than 1 year had a more than 90% reduction is striking.

However, as the authors point out, achieving the protocol-defined threshold biomarker changes did not appear to be sufficient to predict long-term disease control, as substantial reductions in FLT SUV$_{max}$ Ki-67, or phospho-Rb were not correlated with disease stability or response to PD0332991. What is responsible for this discrepancy in the observed data? As in other intracellular pathways in MCL where redundancy likely exists, resistance to pharmacologic inhibition of cdk4/6 may occur via activation of other cell-cycle regulatory proteins such as increased levels of cyclinE-CDK2, or from cdk4-independent activity of cyclin D1. The results of FLT-PET—an emerging functional imaging strategy in MCL and other aggressive lymphomas—were not correlated with FDG-PET response, but both of these tests were performed early, after one cycle of treatment, to try to capture early proliferative effects; correlation of early FLT-PET with FDG-PET performed at a later time point or at treatment completion would add useful information about the utility of the former in evaluating novel agents.

The report by Leonard et al represents an important step forward in the evaluation of targeted agents for the lymphomas. While not all novel therapies can be expected to have as tidy a pharmacodynamic end point as changes in phospho-Rb, and while the correlation between target effect and clinical tumor response was imperfect, this study demonstrates the potential power of combining functional imaging evaluating cell proliferation with tissue biomarker changes in drug development in lymphoma, as well as many other cancers.

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REFERENCES

Comment on Alemdeh et al, page 4723

Chopped and diced: Dicer1 deletion generates myeloid dysplasia

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Gene targeting studies revealed that Dicer1 is required for murine embryogenesis. In this issue of Blood, Alemdeh and colleagues examine deletion of Dicer1 in myeloid progenitor cells using a conditional Cebpa-Cre allele. They show that deletion of Dicer1 is required for viability and that Dicer1 regulates steps of neutrophil maturation.

The discovery that double-stranded RNA (dsRNA) specifically suppresses gene expression in Caenorhabditis elegans was a transformative event that has dramatically changed cell and molecular biology research in eukaryotic organisms. We now understand the mechanism by which small RNA processing occurs. Pri-miRNA species are transcribed by

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PHAGOCYTES & GRANULOCYTES

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RNA polymerase II and are processed in the nucleus by the Drosha RNAse into pre-miRNAs (comprising 60 to 70 nucleotides). After transport into the cytoplasm, the premiRNAs are further processed by an RNAse enzyme termed Dicer. miRNAs (and experimentally designed siRNAs) are generated consisting of 22 nucleotide RNA duplexes with two-nucleotide 3’ overhangs. Dicer is part of the RNA-induced silencing complex that functions downstream of miRNA processing.

Early studies demonstrated that constitutive loss of Dicer1 results in embryonic lethality at E8.5 because of loss of brachyury-expressing stem cells. 3 Conditional alleles of Dicer1 have shown the requirement of the enzyme in B cell 4 and T cell development. 5, 6 Interestingly, conditional deletion of Dicer1 in mouse osteoprogenitors resulted in a myelodysplasia phenotype, which progressed into a secondary leukemia with splenomegaly, anemia, an increase in leukemic blasts in the bone and blood, and myeloid sarcomas in a small subset of animals. 8 Inducible deletion of Dicer1 using Mx1-Cre resulted in defective competitive repopulation and reduced reconstitution in secondary transplant recipients. miRNA profiling revealed a locus on chromosome 19 expressing 3 miRNAs. Remarkably, expression of miR-125a increased long-term multilineage reconstitution and one of miR-125a targets was shown to be the proapoptotic protein Bak1. 9 These previous studies help to frame the current analysis. A conditional Dicer1 allele was crossed with Cebpa-Cre to delete the enzyme in granulocyte-macrophage progenitors (GMPs). Cebpa-Cre;Dicer1 mouse died shortly after birth, likely due to defective Cebpa-dependent induction of the lung epithelium in late gestation, 10 similar to mice with conditional deletion of Dicer1 driven by a Sonic Hedgehog transgene. 11

To circumvent the perinatal lethality observed in this model, Alemdehy et al transplanted fetal liver cells from mutant and control embryos into lethally irradiated recipient mice. No quantitative differences in Lin-/Sca-1/Kir2.4, common myeloid progenitors (CMPs), GMPs, or megakaryocyte erythroid progenitors (MEPs) were observed in Dicer1-deficient mice. However, there was a 50% reduction in colony-forming unit–granulocyte macrophages (CFUGMs) from Dicer1 mutants. Culture of Lin−;Dicer1−/− progenitors in GM-CSF resulted in myeloid cell dysplasia with myeloid cells with a hypossegmented nucleus, typical of Pelger–Huet Anomaly. Hypossegmented and bilobed neutrophils were also observed in vivo. This disease has been correlated with mutations in the Lamin B receptor. 12 No changes in Lamin B receptor expression was observed in Dicer1-null GMPS. Whether Dicer1 affects other genes in the Lamin B receptor pathway or whether additional genes play a role in Pelger–Huet Anomaly remains to be resolved. Dicer1-deficient neutrophils also appear to have defect migration as Ly-6GposDicer1−/− mice, 8 no myeloproliferative disease or acute myeloid leukemia was observed.

The effect of Dicer1 deletion on gene expression in GMPs was examined by expression profiling. There were 300 significantly up-regulated transcripts including Bim, K-Ras, HmgA2, Hoxa9, and p21. Gene dosage appeared to also play a role as loss of 1 allele of Dicer1 affected transcript levels. While it is not surprising that Dicer1 plays a critical role in many cellular lineages, there is remarkable specificity in phenotypes observed with the conditional alleles of Dicer1 reported to date. Whether individual lineages have a unique miRNA target as has been shown with inducible Mx1-Cre remains to be explored. 9 Several miRNAs play critical roles in the regulation of hematologic malignancies when gain-of-function or loss-of-function mouse modeling experiments are analyzed. 13 To identify the precise targets involved in lineage-specific determination, miRNA add-back screens should be devised. It will be interesting to determine whether single miRNAs will regulate specific cell progenitors as has been demonstrated for miR-125a. 9 Gaining an understanding of the underlying biology of miRNAs will help validate this interesting group of macromolecules for therapeutic modulation.

REFERENCES


VASCULAR BIOLOGY

Comment on Bhattacharya et al, page 4798

NHERF-2 silences the silencers

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Hyperresponsiveness to growth factors underlies a wide variety of human diseases, including hemangiomas, the most common tumor of childhood. Hemangiomas have been found to be clonal neoplasms of endothelial cells, with somatic mutations in unknown genes likely to be responsible for their development. In this issue of Blood, Bhattacharya et al discover a novel mechanism of endothelial quiescence mediated by the Na+/H+ exchanger regulatory factor-2 (NHERF-2) gene, which might play a role in regulating responses to exogenous growth factors. 1
Chopped and diced: *Dicer1* deletion generates myeloid dysplasia

Dwayne L. Barber