JAK2^{V617F} patients with essential thrombocythemia and analyze MPL cell surface levels. Decreased MPL surface expression in the context of JAK2^{V617F} would clarify that enhanced MPL degradation is a universal feature of JAK2^{V617F}-positive disease. Conversely, lack of a difference between JAK2^{V617F} and JAK2^{WT} would suggest that the mechanism of excessive platelet production in essential throbmocythemia is distinct from that in polycythemia vera.

There are a variety of implications and future directions that arise from these findings. From a clinical standpoint, it is worth considering whether proteosome inhibitors might yield clinical benefit in the setting of JAK2^{V617F}-positive disease, because inhibition of the proteosome could restore normal levels of MPL on the surface of neoplastic cells and, therefore, restore the quiescent/apoptotic effects of high-concentration TPO signaling. Unfortunately, bortezomib has not been found to demonstrate clinical activity in myelofibrosis as a single agent in a phase 2 clinical trial.[8] As such, it is also worth considering whether combinations of proteosome and JAK2 inhibitors would show synergistic effects on cells. Analysis of surface levels of MPL after kinase/proteosome inhibitor therapy may also serve as a useful biomarker for evaluation of drug efficacy.

Although this study helps explain a longstanding observation in myeloproliferative neoplasms, there are still a variety of mechanistic questions to address. These include the precise nature by which proteosomal degradation of MPL is induced in the context of JAK2^{V617F}. It is possible that this is a passive process whereby JAK2^{WT} innately has the capacity to protect MPL from proteosomal degradation and JAK2^{V617F} has lost this ability. However, the fact that JAK kinase inhibition reverses this phenomenon in the context of JAK2^{V617F} suggests this is an active process initiated and/or coordinated by signaling from the JAK2^{V617F} mutant protein. Identifying the precise molecular interactions that allow JAK2^{WT} to protect against and JAK2^{V617F} to promote proteosomal degradation will be critical to enhance our understanding of this process. One useful starting point would be identification of the operationally important ubiquitin ligase. It will also be interesting to study the effect of JAK2$^{WT/V617F}$ heterozygosity versus homozygosity on these signaling events and molecular interactions.

Finally, the observation that TPO can induce either growth or quiescence/apoptosis in a concentration-dependent manner requires further mechanistic exploration. One possibility would be that high concentrations of ligand result in TPO dimers, causing oligomerization of MPL receptors. These aggregated MPL complexes may then form a signalosome with additional receptors and different intracellular components than are normally associated with individual MPL homodimers. Similar mechanisms have been proposed for other ligand/receptor systems.[9] Further interrogation of the precise molecular switches that control this concentration-dependent signaling process will be of great interest and use. In sum, the present study by Pecquet and colleagues has shed light on a long-established feature of myeloproliferative neoplasms. In doing so, their work has suggested novel clinical possibilities as well as a variety of follow-up mechanistic studies to further our understanding of this important process.

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REFERENCES

1. Moliterno AR, Siebel KE, Sun AY, Hankins WD, Spivak JL. A novel thrombopoietin signaling defect in polycythemia vera platelets. *Stem Cells.* 1998;16(Suppl 2):185-192.

2. Moliterno AR, Hankins WD, Spivak JL. Impaired expression of the thrombopoietin receptor by platelets from patients with polycythemia vera. *N Engl J Med.* 1998; 338(9):572-580.

3. Pecquet C, Diaconu CC, Staerk J, et al. Thrombopoietin receptor down-modulation by JAK2 V617F: restoration of receptor levels by inhibitors of pathologic JAK2 signaling and of proteasomes. *Blood.* 2012;119(20):4625-4635.

4. Moliterno AR, Spivak JL. Posttranslational processing of the thrombopoietin receptor is impaired in polycythemia vera. *Blood.* 1999;94(8):2555-2561.

5. Royer Y, Staerk J, Costuleanu M, Courtoy PJ, Constantinescu SN. Janus kinases affect thrombopoietin receptor cell surface localization and stability. *J Biol Chem.* 2005; 280(29):27251-27261.

6. Besancenot R, Chaligne R, Tonetti C, et al. A senescence-like cell-cycle arrest occurs during megakaryocytic maturation: implications for physiological and pathological megakaryocytic proliferation. *PLoS Biol.* 2010;8(9). pii: e1000476.

7. Tiedt R, Coers J, Ziegler S, et al. Pronounced thrombocytosis in transgenic mice expressing reduced levels of Mpl in platelets and terminally differentiated megakaryocytes. *Blood.* 2009;113(8):1768-1777.

8. Mesa RA, Verstovsek S, Rivera C, et al. Bortezomib therapy in myelofibrosis: a phase II clinical trial. *Leukemia.* 2008;22(8):1636-1638.

9. Chang TL, Gordon CJ, Roscic-Mrkic B, et al. Interaction of the CC-chemokine RANTES with glycosaminoglycans activates a p44/p42 mitogen-activated protein kinase-dependent signaling pathway and enhances human immunodeficiency virus type 1 infectivity. *J Virol.* 2002; 76(5):2245-2254.

● ● ● CLINICAL TRIALS

Comment on Leonard et al, page 4597

Hitting the target in MCL

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One of the major challenges in the evaluation of new therapies for lymphoma and other hematologic malignancies has been the ability to demonstrate changes in important biomarkers and pharmacodynamic end points in the tumor cell population. In this issue of *Blood*, Leonard et al provide an elegant evaluation of the cyclin-dependent kinase (cdk) inhibitor PD0332991 in patients with relapsed mantle cell lymphoma.[1] Their results suggest that the combination of functional imaging using fluorothymidine–positron emission tomography (FLT-PET) and immunohistochemistry can provide important information about target inhibition in tumor cells, and the effects this inhibition has on proliferation and metabolism.

The identification of the signature t(11;14) translocation in mantle cell lymphoma (MCL) has lead to a wealth of information describing the many abnormalities of cell-cycle regulation in this therapeutically challenging subtype of lymphoma, and has accelerated the development and testing of numerous agents that target, at least in vitro, these diverse pathophysiologic processes. Activation and inhibition of signaling proteins, cell-cycle regulatory proteins, and other pathways, however, may be discordant in normal tissues and malignant tumors. Pharmacodynamic assessment that relies on surrogate tissues rather than tumor cells may not be optimal in describing the true effects of targeted therapy. For example, evaluation of the oral BCL-2 inhibitor ABT-263 (navitoclax) in

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.[2] $CD3^+$ T cells and platelets fell rapidly after initiation of therapy, and decreases in platelet number correlated with ABT-263 area under the curve. Unfortunatley, 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.[3] 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.[4] 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.[5] The results of FLT-PET—an emerging functional imaging strategy in MCL and other aggressive lymphomas[6]—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 phospo-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.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Leonard JP, LaCasce AS, Smith MR, et al. Selective CDK4/6 inhibition with tumor responses by PD0332991 in patients with mantle cell lymphoma. *Blood*. 2012; 119(20):4597-4607.

2. Wilson WH, O'Connor OA, Czuczman MS, et al. Navitoclax, a targeted high-affinity inhibitor of BCL-2, in lymphoid malignancies: a phase 1 dose-escalation study of safety, pharmacokinetics, pharmacodynamics, and antitumour activity. *Lancet Oncol*. 2010;11(12):1149-1159.

3. Buckstein R, Kuruvilla J, Chua N, et al. Suntinib in relapsed or refractory diffuse large B-cell lymphoma: a clinical and pharmacodynamic phase II multicenter study of the NCIC Clinical Trials Group. *Leuk Lymphoma*. 2011;52(5):833-841.

4. Pratz KW, Cortes J, Roboz GJ, et al. A pharmacodynamic study of the FLT3 inhibitor KW-2449 yields insight into the basis for clinical response. *Blood*. 2009;113(17):3938-3946.

5. Shapiro GI. Cyclin-dependent kinase pathways as targets for cancer treatment. *J Clin Oncol*. 2006;24(11): 1770-1783.

6. Herrmann K, Wieder HA, Buck AK, et al. Early response assessment using 3′-deoxy-3′-[18F] fluorothymidine-positron emission tomography in high-grade non-Hodgkin's lymphoma. *Clin Cancer Res*. 2007;13(12):3552-3558.

● ● ● PHAGOCYTES & GRANULOCYTES

Comment on Alemdehy 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*, Alemdehy and colleagues examine deletion of *Dicer1* in myeloid progenitor cells using a conditional *Cebpa-Cre* allele.[1] 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.[2] We now understand the mechanism by which small RNA processing occurs.[3] Pri-miRNA species are transcribed by