attractive therapeutic target. The novel and selective CDK9 inhibitor BAY 1143572 is currently being tested in phase 1 studies in patients with advanced solid tumors and acute leukemia.9

Narita et al investigated the efficacy of BAY 1143572 in ATL. They used multiple in vitro models that included ATL-derived or HTLV-1–transformed cell lines, primary leukemic cells obtained from ATL patients, and in vivo xenografts of patient-derived ATL cells in immunocompromised NOG mice. They demonstrate that BAY 1143572 specifically inhibited CDK9-mediated phosphorylation at serine 2 of RNA polymerase II, resulting in decreased levels of c-Myc and Mci-1, thus leading to growth arrest and apoptosis of ATL cells (see figure). These effects were observed both in vitro and in vivo. Notably, a single oral administration of BAY 1143572 on a daily basis resulted in a striking decrease in ATL tumor infiltration in the liver and bone marrow of treated mice. This effect was also associated with decreased human soluble interleukin-2 receptor levels in the serum (indicative of the reduced levels of ATL tumor bulk) and resulted in a significant prolongation of mouse survival (no death in animals treated with BAY 1143572 vs 100% death in the untreated controls).

These findings reveal the importance of the CDK9 signaling in ATL pathogenesis and make CDK9 signaling an attractive and promising molecular and therapeutic target in ATL. The current report suggests that BAY 1143572 and other CDK9 inhibitors warrant testing in ATL patients, either alone or in combination with other effective therapies such as the combination of zidovudine, interferon-α, and arsenic trioxide.

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

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Comment on Newberry et al, page 1125

Traffic lights for ruxolitinib

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In this issue of Blood, Newberry et al provide evidence that clonal evolution in patients with myelofibrosis (MF) who are receiving therapy with ruxolitinib correlates with discontinuation of treatment, mainly because of loss of response or progression, and also predicts shorter survival thereafter.5

The JAK1 and JAK2 inhibitor ruxolitinib has become the standard of care for most patients with MF; it provides rapid and sustained reduction of splenomegaly and improvement in symptoms and quality of life, possibly resulting in prolongation of survival.2 Long-term follow-up showed that about half the patients enrolled in the COMFORT-I and COMFORT-II studies discontinued treatment by 3 years, largely because of loss of response and/or disease progression. The mechanisms of resistance and/or loss of response to ruxolitinib have not yet been deciphered. In cell lines, acquisition of mutations in the predicted ruxolitinib-binding region conferred resistance to JAK inhibitors,3 but such events have not been documented in patients. The phenomenon of persistence to a JAK2 inhibitor (ie, the fact that JAK2V617F-mutated cells survive despite chronic JAK inhibition) was ascribed to heterodimerization between activated JAK2 and other members of the JAK family and was shown to occur in cell lines, murine models, and patients treated with JAK2 inhibitors; however, the clinical relevance of such observations remains unsettled.4

Mutations in JAK2, MPL, and CALR are all involved in the abnormal activation of JAK/STAT signaling. This is not surprising (and it is good for patients) that ruxolitinib is efficacious irrespective of the underlying driver mutation. A JAK2V617F allele burden >50% predicts for better response to ruxolitinib,5 although this observation requires confirmation. In the COMFORT-II study,6 selected nondriver mutations included in the high-molecular-risk (HMR) category (ie, mutations in ASXL1, EZH2, SRSF2, IDH1, and IDH2)7 had no impact on the likelihood of obtaining early spleen response and symptomatic improvement. However, in the same patient cohort as in the Newberry et al report, it was shown by using an enlarged mutation panel that those patients with 3 or more mutations at study entry were more likely to discontinue treatment early and had lower odds of achieving spleen response.8 These latter findings suggest that patients with higher mutation complexity are less likely to obtain sustained responses to ruxolitinib, in line with findings that the number of mutations is independently associated with shorter survival and increased risk of leukemic transformation.9,10 When deciding on initial treatment using ruxolitinib, the above molecular information represents green and yellow lights for starting the treatment (see figure).
The study by Newberry et al has identified variables that are associated with and potentially predict for shorter duration of response to ruxolitinib and provides data on prognosis in patients who discontinued treatment. The core study population consisted of 56 patients with MF enrolled in a phase 1/2 study who discontinued ruxolitinib; in 42 patients, paired samples were available for molecular analysis and were used for target resequencing with a panel of 28 myeloid neoplasm–associated mutated genes (mutations in \( SRSF2 \) that are relatively frequent and harbor negative prognostic significance\(^7 \) were not included in the panel). At the time of treatment discontinuation, hemoglobin and platelet count were reduced compared with baseline, whereas the proportion of patients with transfusion dependency increased from 29% to 43%. These findings are compatible with disease progression and with hematologic toxicity resulting from ruxolitinib. Clonal evolution, defined by the acquisition of 1 or more mutations at discontinuation, was reported in 35% of patients with available paired samples (n = 62); mutations mostly occurred in \( ASXL1 \) (68%), followed by \( TET2, EZH2, \) and \( TP53 \).

Of importance, survival after discontinuation was remarkably shorter for the patients with clonal evolution (6 months) compared with all others (16 months). A platelet count of \(<260 \times 10^9/L \) at baseline or \(<100 \times 10^9/L \) at discontinuation also predicted for shortened survival, whereas acquiring transfusion dependency during ruxolitinib therapy turned out to be the only clinical variable correlated with clonal evolution. The proportion of patients with a complex karyotype at discontinuation rose from 13% at baseline to 25%, but this was independent of clonal evolution. Therefore, acquisition of new mutations during therapy with ruxolitinib would turn the traffic light to red, indicating the need to switch directions (see figure).

Recognizing the clinical patterns and/or the biomarkers that might predict clinical response in patients with MF receiving ruxolitinib represents a major goal for the appropriate and cost-effective use of this medication. Because survival after discontinuation was significantly shorter among patients who presented evidence of clonal evolution, one might anticipate that the early detection of newly acquired mutations represents a decision node (see figure) in favor of alternative therapies or stem cell transplantation when feasible. Unfortunately, the complexity and cost of genetic tests, uncertainties about when to obtain them during ruxolitinib treatment, and the fact that only one-third of the patients who discontinued ruxolitinib in the study actually had evidence of clonal progression, mark the boundary between theory and clinical practice. At present, such an approach is not feasible in patients treated with ruxolitinib outside clinical studies.

One concerning aspect that could not be dissected in the Newberry et al study is whether the acquisition of additional mutations results from the selective pressure exerted by ruxolitinib on the founding clone, thus facilitating the overgrowth of preexisting mutated clones initially below the detection threshold. Or does it represent true acquisition of novel mutations as a result of clonal genetic instability exacerbated by ruxolitinib? Or is it intrinsically related to the progression of disease and has nothing to do with ruxolitinib? Comparative studies of patients receiving no...
therapy or conventional therapy vs ruxolitinib might provide useful insights in this regard.

One key take-home message for clinicians from the Newberry et al study is that patients who discontinue ruxolitinib experience distally short survival. Novel drugs for the treatment of these patients are urgently needed; unfortunately, there are none on the horizon.

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PLATELETS AND THROMBOPOIESIS

Comment on Machlus et al, page 1132

Balancing the yin and yang of SINE

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In this issue of Blood, Machlus et al report the mechanism and correction of thrombocytopenia in patients treated with selinexor, a member of the growing family of selective inhibitors of nuclear export (SINE) anticancer drugs.1

Acromolecular traffic in and out of the eukaryotic cell nucleus is facilitated by carrier proteins (karyopherins) that interact with cargo, chaperones, nucleoporins, and other components of nuclear pore complexes.2 Achromatin region maintenance 1 (CRM1), exportin 1, or XPO1, is uniquely required for the export of >200 different proteins to the cytoplasm, including nucleophosmin, survivin, p27, APC, BRCA1, and p53.3 The effects of shuttled proteins often depend on their location; for example, in the nucleus, survivin helps attach centromeres to mitotic spindles, whereas in the cytoplasm, it interacts with caspases to inhibit apoptosis.4 These proteins are prominent targets of oncogenic mutations, as are the components of the nuclear transport system that modulate their effects. For example, nucleoporin fusion proteins have been found in leukemias with poor clinical outcomes,4 whereas elevated XPO1 expression is associated with poor prognosis and drug resistance in several cancers.5

XPO1 has long been considered a prominent target for therapeutic inhibition in cancers where its expression is abnormal, and also where it is normal, because in either situation blocking nucleocytoplasmic transport of tumor suppressors and apoptosis inhibitors has the potential to shift the cellular balance away from unregulated survival and proliferation.6 Of the many small-molecule XPO1 inhibitors that have been tested to date, the SINE compounds show a promising combination of anticancer activity, oral bioavailability, and low toxicity. These compounds block XPO1 activity by covalently binding cysteine-528 in the cargo binding pocket. The SINE Selinexor (KPT-330) first entered clinical trials in 2015, and its efficacy is currently being assessed in several phase 1, 2, and 3 clinical trials in patients with advanced, relapsed, or refractory solid and hematological malignancies (https://www.karyopharm.com/pipeline/oral-selinexor-kpt-330/). Although severe treatment-related adverse events have been rare, a major concern has emerged in the form of thrombocytopenia experienced by a majority of patients treated with higher selinexor doses.6

In collaboration with Karyopharm Therapeutics, Machlus et al followed a large cohort of patients with advanced solid tumors in a phase 1 trial of selinexor and observed a general decline in platelet counts that stabilized to ~50% of baseline after 4 weeks of treatment. In laboratory studies, they observed that selinexor had no effect on platelets that would influence their function or clearance, whereas histological examination of mice showing a similar treatment response to patients indicated decreased marrow megakaryocytes (MKs). The effects of selinexor on thrombopoiesis were examined using cultured fetal murine liver cells, where varied timings and doses revealed little effect on late-stage MKs, including those producing proplatelets. The maturation of early-stage MK progenitors, however, was inhibited in a dose-dependent manner, an observation that was replicated in cultured human cord blood–derived CD34+ cells. The mechanism whereby selinexor inhibits early MK development was explored in experiments that first ruled out direct cytotoxicity or induction of apoptosis. In cultured MK, it was observed that selinexor appears to block responsiveness to the developmental regulator thrombopoietin (TPO), which even at 10 times the normal levels had no effect on selinexor-treated cells. Similarly, although TPO-knockout mouse recovered normal platelet counts after TPO injection, this rescue was blocked by selinexor treatment. These results indicated that selinexor interferes with 1 or more developmentally important signaling pathways triggered by the binding of TPO with its receptor c-Mpl. One of these is the JAK/STAT3 pathway, where the signal transducer
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