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 dismally 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]

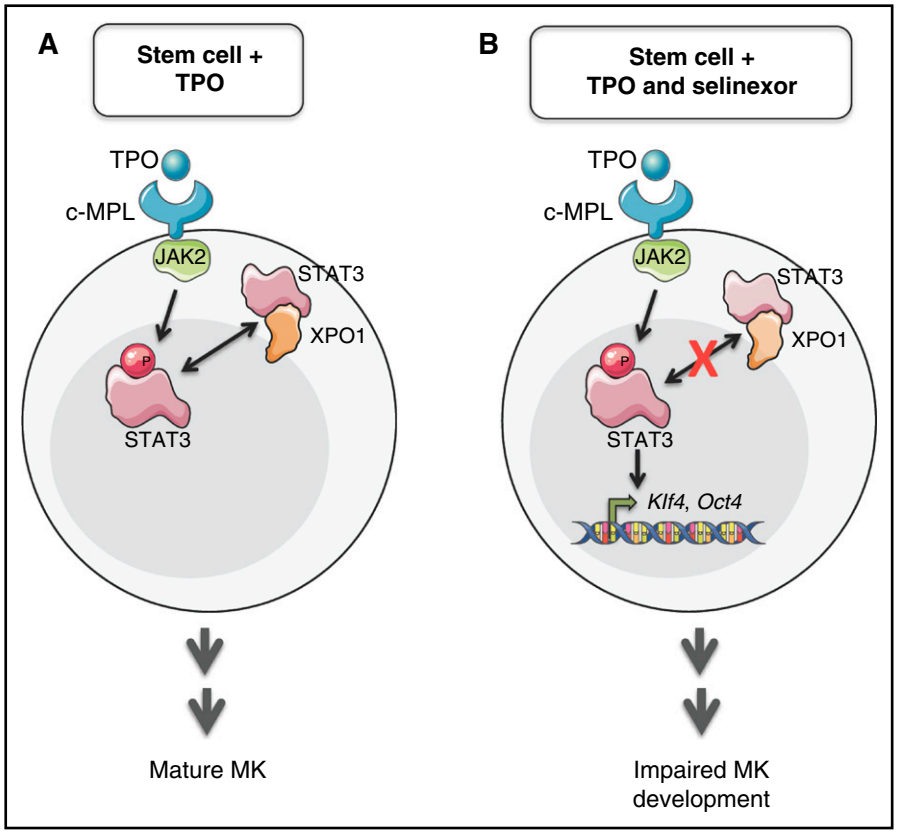
Macromolecular traffic in and out of the eukaryotic cell nucleus is facilitated by carrier proteins (karyopherins) that interact with cargo, chaperones, nucleoporins, and other components of nuclear pore complexes.[2] chromosome region maintenance 1 (CRM1), exportin 1, or XPO1, is uniquely required for 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,[5] whereas elevated XPO1 expression is associated with poor prognosis and drug resistance in several cancers.[6]

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.[7] 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.[8]

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 mice 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



Model of XPO1, STAT3, and selinexor interactions in normal and impaired thrombopoiesis based on the findings of Machlus et al. Binding of TPO by c-Mpl on MK progenitors (A) triggers multiple events, including activation of the JAK2/STAT3 pathway. The accumulation of pSTAT3 in the nucleus is normally limited by XPO1-mediated export of STAT3 to the cytoplasm, but XPO1 inhibition by selinexor (B) allows accumulation of nuclear STAT3, which can be phosphorylated (P) to its active form. These events lead to upregulation of *Klf4* and downstream genes, including *Oct4*, which promotes an undifferentiated state countering the prodifferentiation TPO signal. Thus, selinexor treatment impairs TPO-dependent MK maturation and promotes the onset of thrombocytopenia. See Figure 6G in the article by Machlus et al that begins on page 1132.

and transcription activator STAT3 carries nuclear export signals recognized by XPO1. Using immunofluorescence microscopy, they observed that although total cellular STAT3 remained constant, selinexor treatment led to the accumulation of phosphorylated STAT3 (pSTAT3) in early MK nuclei. pSTAT3 activates Klf4 expression, which via upregulation of Oct3/4 has been shown to maintain stem cells in a pluripotent state.[9] *Klf4* and *Oct4* mRNA expression were both observed to be upregulated in MK progenitors treated with TPO and selinexor, indicating that a major factor contributing to their arrested development is the accumulation of nuclear pSTAT3 due to XPO1 blockade (see figure).

In a final set of investigations, Machlus et al addressed the challenge of alleviating selinexor-induced thrombocytopenia. Clinical trial patients who developed severe (grade 4) thrombocytopenia underwent variable dosing interruptions (8-21 days; 3-6 doses), with or without treatment with the TPO mimetics romiplostim or eltrombopag. A trend toward improved platelet counts was observed in both groups, indicating that an occasional treatment "holiday" with or without supplemental TPO mimetic treatment is effective in moderating severe selinexor-mediated thrombocytopenia.

This study highlights a previously uncharacterized role for XPO1 in early MK development and reveals a likely mechanism for selinexor-induced thrombocytopenia via Oct4-mediated inhibition of progenitor differentiation. Questions remain as to what other processes and molecules (eg, transcription regulators) involved in thrombopoiesis may also be affected by XPO1 blockade, and indeed which ones may not be affected, because even with selinexor treatment a basal level of platelet production was observed. This residual thrombopoiesis may be attributable to the poorly understood TPO-independent mode of platelet production observed in mice and humans lacking expression of TPO or c-Mpl, which may also be relevant to the clinical observation that not all selinexor-treated patients develop thrombocytopenia. For the majority that do, by establishing an effective approach for moderating this treatment-limiting side effect, Machlus et al have made an important contribution to advancing the prospects of developing selinexor and other SINE compounds as anticancer agents. This finding is timely because recent evidence indicates that selinexor may prove uniquely useful against therapeutically challenging KRAS-driven cancers, such as non–small cell lung tumors.[10]

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