identified or how many ultimately contributed to recommendations. Articles should be rated according to level of evidence, so case report findings and results from advanced phase studies are not given equal weight. Check—though note that meta-analyses garner the same score as randomized control trials. Finally, panelists are locked in a room and forced to listen to Barbra Streisand music until they come to a consensus on recommendations, which are graded according to level of evidence, consistency of results across studies, and applicability to the patient population—in this case, people with MDS. Check. And this took three consensus conferences (I suspect with music being unnecessary).

The ELN authors then took on the broad task of addressing all aspects of MDS, starting with making the diagnosis. They included standard procedures, such as the necessity for a bone marrow biopsy and cytogenetics (although including such obvious procedures in panel recommendations may seem superfluous, neither one is routinely performed, even in the United States)[3] and more sophisticated approaches such as single nucleotide polymorphism arrays to detect cryptic chromosomal defects.[4] They reaffirmed the utility of the World Health Organization classification of myeloid neoplasms[5] and covered the broad collection of risk assessment tools available. Interestingly, while they acknowledged the shortcomings of the International Prognostic Scoring System (IPSS),[6] they recommended its use to stratify risk for all MDS patients, given the large body of data supporting its applicability in therapeutic decisions and the relative paucity of data that have been generated for its revised successor.[7] That will change in due time.

Rather than dividing the therapy section into treatments directed to disease severity (commonly defined as lower- vs higher-risk MDS using the IPSS and based on relative blast percentage, karyotypic abnormalities, and numbers of cytopenias), the ELN focused on each treatment modality itself. They started with watchful waiting, which may have been prescient, given the recent brouhaha over a National Cancer Institute panel's recommendation to stop calling certain premalignant conditions "cancer."[8] Next, without identifying them as such, the authors introduced treatments for higher-risk MDS (stem cell transplantation, cytotoxic therapy, and hypomethylating agents), lower-risk disease (hematopoietic growth factors, immunomodulatory drugs, and immunosuppressive therapy), and ended with supportive care issues, which are germane to both.

In summary, the recommendations are rigorous, and they are comprehensive. But are they useful for those general hematologists and oncologists in practice, flying on their trapezes from patient to patient without a LeukemiaNet? In some ways no, and in some ways yes. In a patient with suspected MDS, the ELN gives equal weight to taking a good history of prior chemotherapy and radiation exposure and to obtaining a family history of Fanconi's anemia and telomere disorders. While 10% of my patients have therapy-related MDS, I have yet to encounter someone with either of the latter conditions. In addition, for the uninitiated, while the therapy section is helpful in assigning levels of evidence to treatments being considered, it is difficult to navigate in answering the question, "for the patient sitting in my clinic with this subtype of MDS, what drug should I use?"

Impressively though, the ELN panel confirms minimum criteria for diagnosing and classifying MDS, which is more challenging than is widely appreciated.[9] They also make clear statements regarding recommendations on controversial topics, such as remission induction therapy preceding stem cell transplantation or the use of iron chelation therapy, along with a recommendation grade. Although I may not agree with all of these recommendations, I can appreciate how they can guide physicians who do not have the time or interest to immerse themselves in the nuances of MDS literature and can keep them from falling to the floor of the Big Top.

Conflict-of-interest disclosure: Dr Sekeres serves on advisory boards for Celgene and Amgen. ■

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● ● ● MYELOID NEOPLASIA

Comment on Walker et al, page 3034

Redirecting traffic using the XPO1 police

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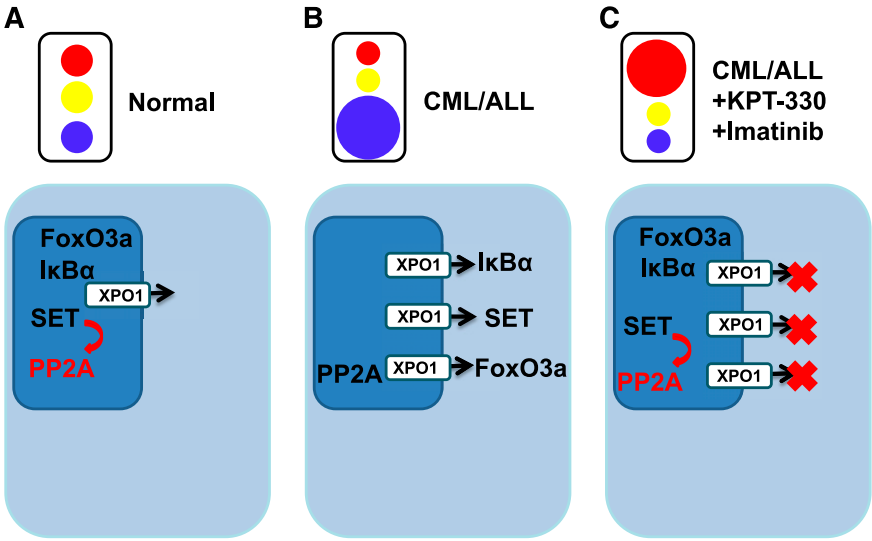
In this issue of *Blood*, Walker et al investigate the preclinical potential of KPT-330, an exportin-1 (XPO1, also known as chromosome maintenance protein 1 [CRM1]) inhibitor, against both accelerated phase (AP) and blast crisis chronic myeloid leukemia (CML-BC) and against Philadelphia chromosome-positive (Ph^+) acute lymphoblastic leukemia (ALL), all of which are diseases of significant unmet clinical need.[1] The authors provide encouraging data from both a leukemic mouse model and a single CML-AP patient, corroborating mechanistic studies suggesting that KPT-330 efficacy relies on targeting abundantly expressed XPO1, followed by the reactivation of protein phosphatase 2A (PP2A).



Normal cells maintain homeostasis by tightly regulating intracellular trafficking of ions, small molecules and proteins. (A) Proteins located predominantly in the nucleus are able to function normally (eg, SET is able to activate its target PP2A, a tumor suppressor). (B) Leukemic transformation abrogates the trafficking of cargo with inappropriate diversions provided by upregulated karyopherins, such as XPO1, providing the "green light" to export specific proteins out of the nucleus. Protein activity is altered (eg, PP2A is no longer appropriately activated). (C) KPT-300 with imatinib treatment introduces a "red light" to halt subverted proteins from being diverted from the nucleus, and nuclear proteins are able to function within their appropriate context.

Normal cellular homeostasis depends on the cell's ability to compartmentalize proteins within specific subcellular compartments. Intracellular transport of proteins to their correct locations is accomplished, in part, by signaling sequences encoded within the proteins themselves. In addition, the nuclear envelope aids cellular organization by forming a barrier restricting passage between the nucleus and cytoplasm. However during carcinogenesis, these normal processes are deregulated, and as a result, nuclear export is subverted. Normal trafficking of critical proteins that maintain regulated cell growth is rerouted, skewing normal flow and creating cellular mayhem (see figure).

At this time, tyrosine kinase inhibitors represent a critical component in first-line therapy for advanced-phase CML and Ph^+ ALL, but unfortunately, the majority of patients experience suboptimal responses and a marginal prolongation of life. Under therapy, minimal residual disease persists and evolves to become fully drug-resistant as the result of clonal heterogeneity, genomic instability, Bcr-abl kinase mutations, and failure to eradicate leukemia-initiating cells, fueling disease relapse. There is therefore an urgent need for improved treatment of these forms of leukemia.

KPT-330 is an oral drug currently undergoing phase 1 studies in patients with advanced, relapsed, and refractory solid tumors (clinicaltrials.gov, no. NCT01607905); hematological malignancies (clinicaltrials.gov, no. NCT01607892); and sarcoma (clinicaltrials.gov, no. NCT01896505). KPT-330 has a novel mechanism of action by inhibiting nuclear-cytoplasmic transport (a key target being XPO1) within cells, triggering significant cellular death and showing promise in multiple cancers.[2-4]

Ions, small molecules, and proteins less than 40 to 65 kDa cross the nuclear membrane in a passive manner; however larger proteins require the assistance of transport molecules called karyopherins. XPO1, a subclass of karyopherins, is able to transport both RNA and proteins mediated by Ran GTPase activating protein. XPO1 possesses the ability to shuttle more than 200 different proteins, including several tumor-suppressor proteins such as retinoblastoma, adenomatous polyposis coli, p53, p21, breast cancer 1, and forkhead box (FoxO), in addition to the oncogene Bcr-abl. In particular cancers, increased XPO1 activity is thought to lead to relocalization of nuclear factors, excluding them from their normal sites of activity and thus favoring cancer initiation, progression, and eventual drug resistance. XPO1 is overexpressed in several cancers, including ovarian, myeloma, pancreatic, osteosarcoma, glioma, and cervical cancer, in which XPO1 expression is negatively correlated with progression-free survival,[5] making KPT-330 a potentially viable therapeutic for these cancers.

Importantly, as shown by Walker et al, XPO1 expression is augmented in CML-AP and CML-BC and in B-ALL ($Ph^{+/-}$). The authors have capitalized on this characteristic to investigate the potential for the clinically relevant karyopherin inhibitor, KPT-330. After complete inhibition of Bcr-abl kinase activity with imatinib, XPO1 was only partially inhibited, suggesting its enhanced expression was both kinase-dependent and kinase-independent in Ph^+ cells. This adds to a growing list of proteins whose aberrant expression is at least partially independent of Bcr-abl kinase, suggesting the need for agents that target alternative mechanisms of aberrant signaling. KPT-330 treatment, at concentrations that have been achieved in clinical trials, induced significant apoptosis (observed to be independent of Bcr-abl inhibition) and impaired the colony-forming ability of CML progenitors, with a concurrent decrease in the activity and transcription of XPO1. The effect of KPT-330 was relatively selective, with a 3-fold difference in EC_{50} for normal $CD34^+$ cells. The authors also demonstrated that induction of XPO1 transcription may be independent of Bcr-abl and, likely, through PI-3K, Akt, or protein kinase C. Mechanistically, treatment with KPT-330 resulted in the nuclear accumulation of SET, CIP2A, IkBα, FoxO3a, p53, and p21, with the redistribution of SET and CIP2A subsequently leading to reactivation of PP2A. Taken together, the data suggest that reactivation of PP2A was responsible for significantly reduced Bcr-abl levels and accounted for at least 50% of the catastrophic apoptosis. KPT-330 showed promise in an allograft model of CML-BC and in a single CML-AP patient in whom KPT-330 therapy led to a reduction in bone pain, splenomegaly, and immature myeloid blasts in the peripheral blood. Unfortunately, the patient refused more

than a single week of therapy, and duration of response was unclear.

Previous studies have attempted to achieve similar nuclear protein entrapment, using leptomycin B. One such study exploited the observation that Bcr-abl translocates to the nucleus once bound by imatinib.[6] The authors performed experiments demonstrating that once Bcr-abl was located in the nucleus under imatinib exposure, treatment with leptomycin B trapped nuclear Bcr-abl. By excluding Bcr-abl from the cytoplasm, it was unable to aberrantly phosphorylate multiple proteins with resultant apoptosis. Although theoretically elegant, these studies were performed in Bcr-abl overexpressing fibroblasts and mouse bone marrow cells. Subsequent studies using normal and CML human $CD34^+$ cells demonstrated that this strategy conferred mild cytostatic effects, but little cytotoxicity, to leukemic progenitors.[7] Other clinical studies with leptomycin B (elactocin) determined that this drug was unsuitable for further development, as it induced severe gastrointestinal toxicities, leading to anorexia and malaise.[5]

The new studies completed by Walker et al provide an encouraging basis for more fundamental preclinical examinations of KPT-330 in conjunction with tyrosine kinase inhibitors. The synergistic potential of these drugs should be assessed in primary samples and in a more disease-relevant mouse model. It would also be important to determine whether this drug combination can affect tumor-initiating populations in advanced-phase CML and ALL. This study fits with the ongoing paradigm shift in thinking away from single-targeted agents to those agents able to abrogate multiple aberrant pathways simultaneously, as represented here by alterations in tumor suppressors PP2A and p53 and oncogenes Bcr-abl, Akt, NF-kB, and c-Myc.[8]

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Comment on Harper et al, page 3045

An open-and-shut case?

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In this issue of *Blood*, Harper and colleagues explain how the mutation responsible for a common hereditary elliptocytosis results in the instability of the membrane and the shape of the cell. The conformational equilibrium of the spectrin dimers in these cells favors the "closed" state, with a consequent inhibition of tetramer formation, loss of membrane skeletal continuity, and shear resistance of the membrane.[1]

The unique shape and mutability of the red cell have perplexed and fascinated hematologists ever since Antonie van Leeuwenhoek observed in 1675 that: "when he was greatly disordered, the globules of his blood appeared hard and rigid, but grew softer and more pliable as his health returned: whence he infers that in a healthy body they should be soft and pliable." Altered red cell shape is routinely used to classify red cell membrane disorders, such as hereditary spherocytosis and hereditary elliptocytosis. The past few decades have seen much progress in our understanding of the mechanistic and structural basis of these disorders.[2] We now know that the elliptocytic phenotype is the result of weakening of the lateral interactions between skeletal proteins, most often between the pairs of spectrin dimers that make the tetramers (see figure).

What is less appreciated is the complex behavior of red cells during flow in vivo, when they experience a large range of fluid shear stresses in the vasculature, and in particular the contribution of the continuous "tank-tread" motion of the membrane during flow through the capillary bed.[3-5] Although the structural perturbation of the red cell skeleton during tank treading is not fully defined, there is evidence of constant dissociation and reassociation of spectrin tetramers during induced membrane deformation.[6] Thus a mutation that alters the propensity of spectrin dimers to associate to tetramers, or for spectrin tetramers to transiently dissociate in response to deformation, will influence the capacity of the membrane to undergo flow-induced dynamic deformations.

A universal feature of hereditary elliptocytosis associated with any of the known mutations in α- and β-spectrin is the reduced ability of the spectrin dimers with mutant subunits to form the tetramers that regulate deformability and mechanical stability of the red cell membrane.[2,7] Although many of the identified mutations reside in domains of spectrin subunits directly involved in dimer-dimer contact interfaces account for the reduced proportion of tetramers in elliptocytes,[8] a puzzling aspect is that some of the identified mutations, such as L260P in α-spectrin, lie far from the dimer contact site. It is in this context that the explanation by Harper et al of the manner in which an α-spectrin mutation, remote from the dimer self-association site, can cause a perturbation of that site is of such interest. This mutation (L260P) occurs in a common form of hereditary elliptocytosis. The results show that the mutation increases the stability of the closed conformation of the