a number of oncogenic proteins and receptors (eg, p53, c-Myc, Akt, cyclin D, Cdk4, ALK) that are deregulated in lymphoma, preventing their degradation (see figure). Overexpression of HSPs has been documented in different lymphoma subtypes and it contributes to the oncogenic process. Inhibition of HSPs that specifically bind to proteins involved in lymphomagenesis may be of great value for the treatment of these diseases.

The authors show that HSPH1, a subgroup of the HSP70 family of proteins, is expressed in B-cell lymphomas, preferentially in those with a high proliferation rate. HSPH1 protein expression directly correlates with BCL-6 and c-Myc protein expression in aggressive lymphoma cell lines. In fact, the authors convincingly show that HSPH1 directly binds to BCL-6 and c-Myc, thus acting as a chaperone for these proteins. Most important, in a series of in vitro studies, the authors show that HSPH1 inhibition directly downregulates BCL-6 and c-Myc, which leads to a significant decrease in cell proliferation. To further evaluate this concept, the group tested the growth of an aggressive B-cell lymphoma line in an in vivo model. Mice transplanted with HSPH1–knocked-down tumors had significantly longer survival, and the protein levels of BCL-6 and c-Myc in the tumor cells were decreased, in agreement with the reported in vitro studies. In addition, tumor angiogenesis was also reduced, consistent with the inhibition of the provascularization properties of c-Myc, which may additionally contribute to the antitumoral effect.

Finally, to enhance the clinical relevance of this finding, the investigators analyzed the expression of HSPH1 in primary B-cell lymphomas. In a previous study, they showed that HSPH1 protein expression was correlated with the tumor proliferation rate. Thus, low-grade lymphomas showed a significantly lower HSPH1 tumor expression than high-grade lymphomas. In particular, diffuse large B-cell lymphoma (DLBCL) with a high Ki-67 index and Burkitt lymphoma appear to have higher HSPH1 expression. In line with these data, HSPH1 protein expression in a transformed DLBCL was significantly increased compared with the expression seen in the indolent lymphoma. Remarkably, in c-Myc+ lymphomas, HSPH1 protein expression correlated with that of c-Myc.

Overall, these data suggest that HSPH1 inhibition could potentially be useful in those lymphomas with c-Myc alterations (ie, Burkitt and the so-called “double-hit” lymphomas—mature B-cell lymphomas with a translocation affecting c-Myc in combination with another translocation usually affecting bcl-2). This is of paramount relevance, in particular for the latter, amounting to a poor-prognosis lymphoma for which no effective therapies have been developed so far. A few studies have addressed the inhibition of other HSPs as a treatment of B-cell lymphoma. In particular, HSP90, an important chaperone involved in cancer, is frequently expressed in DLBCL and, importantly, its expression highly correlates with BCL-6, a transcription factor that is frequently deregulated in DLBCL.

Experimental in vitro and in vivo studies showed that a purine-derived HSP90 inhibitor, PUH71, selectively killed primary DLBCL—coexpressing BCL-6 and HSP90, and this approach is being translated into the clinical scenario in patients with lymphoma with the new available HSP90 inhibitors. Although early trials with HSP inhibitors in lymphoma patients have not shown impressive clinical activity, it may be envisioned that a combination of different HSP inhibitors targeting distinct oncogenic proteins may improve their efficacy and may add value to conventional immunochemotherapy.

Pharmacologic inhibitors of HSPH1 have yet to be developed, but the discovery of HSPH1 as a chaperone of c-Myc in B-cell lymphoma presents an important opportunity to design rational, molecularly based treatments for c-Myc–dependent B-cell lymphomas such as Burkitt lymphoma and the “double-hit” DLBCL.

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Comment on Khorashad et al, page 1772

A “RANning” leap with “XPOrt” into TKI resistance

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In this issue of Blood, Khorashad et al1 show that genetic (eg, short hairpin RNA [shRNA]-mediated) or pharmacologic (eg, KPT-330 [selinexor]) inhibition of nucleocytoplasmic protein trafficking restored sensitivity to tyrosine kinase inhibitors (TKIs) and impaired clonogenic potential of chronic myeloid leukemia (CML) cell lines with BCR-ABL1 kinase-independent TKI resistance (see figure). It is increasingly the case that a deeper knowledge of the crosstalk between leukemic stem and progenitor cells and their bone marrow microenvironment is needed to fully understand how the latter influences leukemia development, progression, and resistance to targeted therapies. This also appears to be true for those cases of CML.
Mechanistically, Khorashad et al. have identified signaling pathways associated with BCR-ABL1 kinase-independent TKI resistance by performing a lentiviral shRNA library screen on K562 cells (K562S, imatinib sensitive) and an imatinib-resistant derivative line (K562R) that maintains viability despite suppression of BCR-ABL1 kinase activity.

Genes with a potential role in resistance were selected based on criteria designed to minimize false-positive results. The RAS-related nuclear protein RAN and the karyopherin β family member XPO1 (exportin-1, also called chromosome maintenance protein 1 (CRM1)), two interacting proteins with key functions in nucleocytoplasmic transport, were among the top 5 candidates, suggesting a role for these factors and corresponding signal transduction pathways in BCR-ABL1–independent TKI resistance (see figure).

RAN is involved in the transport of proteins across the nuclear envelope by interacting with karyopherins and changing their ability to bind or release cargo molecules. Cargo proteins containing nuclear localization signals are bound by importins and transported into the nucleus. Inside the nucleus, RAN–guanosine triphosphate (GTP) binds to importin and releases the import cargo. Cargo that needs to exit the nucleus into the cytoplasm binds to exportin in a ternary complex with RAN-GTP.

Upon hydrolysis of RAN-GTP to RAN–guanosine diphosphate (GDP) outside the nucleus, the complex dissociates and the export cargo is released. Khorashad et al. found that RAN and XPO1 synergize to promote nucleocytoplasmic trafficking of cargo proteins through the nuclear pore complex. Although binding of XPO1 to either RAN or cargo protein alone is weak, simultaneous binding of RAN and cargo to XPO1 increases its affinity to both by 1000-fold (see figure). Notably, XPO1-mediated nucleocytoplasmic protein trafficking regulates the function of tumor suppressors and oncogenes (eg, SET, PP2A, p53, p21, p27, NF-κB, Mcl-1, myc, Rb, BRCA1, APC, NMP1, and FoxO3a) that play an important role in survival and proliferation of normal and cancer cells, including different types of lymphoid and myeloid and acute and chronic leukemias (reviewed in Turner et al. and Tan et al.).

Interestingly, Khorashad et al. also identified through the shRNA library screen other pathways whose roles in TKI resistance are yet to be experimentally validated. Among these pathways are genes involved in proteasomal protein degradation, chromatin remodeling, protein biosynthesis, cell-cycle regulation, apoptosis, antioxidation, ubiquitination, and DNA repair. In particular, 5 of the top 50 genes (PSMA1, UBE1, NEDD8, PSMD3, and PSMD1) are associated with proteasome-dependent protein degradation, which has been implicated in TKI resistance of leukemic stem and progenitor cells. Thus, nuclear export and signaling linking the stem/progenitor cell to the microenvironment will further elucidate BCR-ABL1–independent signaling in CML and AML.

XPO1/RAN-mediated export was implicated in many types of solid tumors and hematologic malignancies. Given that XPO1 is a critical regulator of cell proliferation and survival, which is not only overexpressed but also described as a poor prognostic factor in different hematologic malignancies, it is not surprising that different inhibitors of XPO1-mediated export through the nuclear pore complex have been developed. Among these, the selective inhibitors of nuclear export (SINE; Karyopharm Therapeutics) are leptomycin B–based small molecules that irreversibly bind to Cys528 in the cargo-binding groove of XPO1 to prevent
XPO1--cargo interactions (see figure). Previous studies have shown that the closely related SINE compounds KPT-251, KPT-276, and KPT-330 have strong antileukemic activity and minimal and acceptable adverse effects in acute myelogenous leukemia and CML in blast crisis. Notably, the clinically relevant XPO1 inhibitor KPT-330 leads to apoptosis and impairment of leukemic clonogenic potential of leukemic but not normal CD34+ progenitors and significantly increased survival of leukemic mice. Mechanistically, KPT-330 altered the subcellular localization of leukemia-regulated factors, including RNA--binding heterogeneous nuclear ribonucleoprotein A1 and the oncogene SET, thereby inducing reactivation of protein phosphatase 2A tumor suppressor and inhibition of BCR--ABL1 in CML blast crisis cells. Because XPO1 is important for leukemic cell survival, KPT-330 may represent an alternative therapy for TKI-refractory Ph+ leukemias. Thus, the notion that RAN/XPO1 activity controls oncogene kinase-independent drug resistance in both AML and CML further supports the use of the available XPO1 inhibitors in therapeutic protocols for those patients. Notably, the SINE KPT-330 is currently in clinical trials for advanced hematologic malignancies and solid tumors (NCT01607892 and NCT01607895). Furthermore, the work of Khorashad et al opens the gateway to characterize microenvironment--generated signals responsible for altered XPO1 expression/activity and, consequently, to develop strategies to efficiently counteract drug resistance in AML as well as in those cases of CML not responding to TKI monotherapy.

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Comment on Bartels et al, page 1782

The end of the line for neutrophils

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In this issue of Blood, Bartels and colleagues demonstrate that acetylation of the transcription factor CCAAT enhancer binding protein ε (C/EBPε) is essential for terminal neutrophil granulocyte differentiation.1 Neutrophils, the most abundant granulocytes, are essential for host innate immune defense. Neutropenia results from damage to the bone marrow or depletion or destruction of neutrophils by drugs, diseases, or congenital disorders that block neutrophil differentiation. Neutropenic individuals are extremely susceptible to bacterial infection, and febrile neutropenia increases the risk of mortality in cancer patients receiving myelosuppressive chemotherapy.2 Prophylactic use of granulocyte colony-stimulating factor (G-CSF) reduces mortality by increasing neutrophil numbers.2 A better understanding of the mechanisms that regulate granulopoiesis and terminal neutrophil differentiation could spur development of new strategies to overcome neutropenia and improve clinical outcomes.

The C/EBP transcription factor family is essential for granulopoiesis and terminal differentiation of neutrophils.3 C/EBPε is predominantly expressed in immature myeloid cells, and lack of expression leads to a block at the myeloblast stage.3 C/EBPβ is expressed from the metamyelocyte stage and on during maturation.3 C/EBPβ-deficient mice display normal granulocyte differentiation and steady-state levels of neutrophils but are unable to produce neutrophils in response to cytokine

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