LTα and LTβ are expressed on activated lymphocytes, natural killer cells, and lymphoid tissue inducer cells involved in lymphoid organogenesis. All 3 members are indispensable for the formation of B-cell follicles, germinal centers, and follicular dendritic cells. LTα can either be secreted as a homotrimer or it can be retained on the cell surface in heterotrimeric complexes with LTβ (LTαLTβ2). LTα shares its receptors with TNF, the TNFR1 and TNFR2 receptors, whereas LTαLTβ2 signals through a distinct receptor, the LTβR. LTα, but not LTβ, plays a crucial role in lymphatic vessel function and lymphangiogenesis.5,6

The relevance of LTα-mediated activation of lymphoid microenvironmental endothelium goes beyond the recruitment of T cells in cHL as described in the work of Fhu et al. Various studies have provided compelling evidence that LT signaling is involved in inflammation-induced carcinogenesis, in primary tumor development, and in the host’s adaptive antitumor immune response.3 In lymphoid neoplasia, it was recently shown that a reciprocal cross-talk between stromal and lymphoma cells was facilitated by cell-presented LTαLTβ2, which signaled via LTβR-bearing stromal cells. In a preclinical therapeutic approach, inhibition of this cross-talk with LTβR-immunoglobulin successfully impaired aggressive Myc-driven lymphoma development in mice.8

The study from Fhu et al expands the importance of LTα as a mediator of endothelial cell activation to enhance cellular infiltration in cHL. An unforeseen result of this study was that high concentrations of LTα in HRS cell-derived supernatant and in cHL lymph node tissues enhance hyaluronan expression on endothelial cells. Hyaluronan is a major component of the extracellular matrix and supports hematopoietic but also nonhematopoietic cell migration and proliferation by binding to its ligand CD44. An impact of a hyaluronan-rich tumor microenvironment on the recruitment of inflammatory tumor-associated immune cells and on tumor angiogenesis has been described.9

The data of Fhu et al provide an intriguing link between HRS cell-secreted LTα as an important potent stimulant of endothelial activation and upregulated endothelial expression of adhesion molecules, and hyaluronan, which refines endothelial adhesiveness to naive T cells.

In this regard, it will be exciting to validate the mechanistic concept arising from the work by Fhu et al using primary endothelial cell culture systems and/or suitable transgenic mouse models for cHL. Overall, this work substantially improves our understanding of how HRS cells interact with the microenvironmental endothelium to support recruitment of inflammatory infiltrates in cHL and may lead to future therapeutic approaches.

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

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cells following treatment with AZD1480 was not clearly enhanced in JAK2V617F-positive cells over JAK2-unmutated cells from the same patient. Importantly, the authors found no difference in the NSG-repopulating capacity of CD34+ cells from MF patients pretreated with AZD1480 as compared with cells pretreated with cytokines alone. In aggregate, this work provides further evidence in an MPN patient-derived xenograft model that JAK2V617F-positive MPN disease-propagating stem cells are not effectively targeted by JAK inhibition.

Supporting these findings, we have previously reported that a JAK2 inhibitor was effective at reducing splenomegaly but did not eradicate MPN disease-propagating stem cells in a Jak2V617F genetic murine model. Perhaps most compelling are the recently reported results of the COMFORT II study (ruxolitinib vs best available therapy) where, despite an ~50% reduction in the risk of death in post hoc analysis at 3 years, the median change in the Jak2V617F allele burden from baseline was just ~8.0% at 72 weeks, indicating that any survival benefit for ruxolitinib in MF is not due to preferentially targeting the malignant hematopoietic clone.

The work of Wang et al described in this issue of Blood suggests that splenic-derived MF hematopoietic progenitor cells are more sensitive to JAK inhibition, as compared with MF long-term hematopoietic stem cells (HSCs). Their model therefore proposes that the spleen shrinks because MF progenitor cells undergo apoptosis; however, theJak2V617F allele burden does not fall because MF HSCs survive (see figure). In clinical trials in patients with MF, Jak2V617F allele burden is typically measured from PB DNA, which represents DNA derived mainly from granulocytes and lymphocytes, therefore reflecting downstream progeny of HSCs. Because myeloid progenitor cells are intermediaries in the differentiation of HSCs to PB granulocytes, one might expect that if the expanded JAK2-mutant myeloid progenitor cell population in MF was preferentially sensitive to JAK2 inhibition (as compared with the JAK2-unmutated population), the Jak2V617F allele burden would fall to some degree, but this does not appear to occur in patients with MF treated with JAK inhibitors. Nevertheless, it is possible that PB Jak2V617F allele burden underestimates the effect of JAK inhibitors on erythroid progenitor cells, as terminally differentiated PB erythrocytes do not contain DNA. Because it is currently impossible to measure splenic JAK2V617F allele burden in patients with MF during treatment, we cannot determine whether JAK2-mutant hematopoietic cells within the spleen are preferentially sensitive to JAK inhibitor therapy in vivo. However, differences in the microenvironment of the bone marrow and the spleen could plausibly impact responses to JAK inhibition, with the presence of supporting stromal cells and/or differences in the levels of local inflammatory cytokines potentially rendering bone marrow JAK2-mutated cell populations less sensitive to JAK inhibition in vivo as compared with the same cellular populations within the spleen.

One caveat of the study by Wang et al is that mutational testing was limited to Jak2V617F and CALR mutations (cytogenetic analysis was also performed). Although Guglielmelli et al did not find any molecular predictors of response to ruxolitinib when they genotyped 14 MF-associated prognostically significant mutations in a subset of the COMFORT II cohort (166 of the 219 total patients were evaluated), earlier studies have indicated that Jak2V617F is not a strong driver of clonal expansion in the CD34+ compartment in MF. This suggests that the presence of coexisting genetic alterations drives clonal evolution in JAK2-mutant MF and that genetic heterogeneity could influence the sensitivity of CD34+ cells from patients with MF to JAK inhibitor therapy. Comprehensive genomic characterization of patient-derived xenografts in MPN would provide valuable information when assessing responses to JAK inhibition and has the potential to identify novel therapeutic targets if combined with in vivo functional genomic and/or drug screening in xenografts.

Wang et al are to be commended for taking a fascinating clinical observation from the JAK inhibitor clinical trials and dissecting it in the laboratory in a true “beside-to-bench” style investigation. Although in the JAK inhibitor era the indication for splenectomy in MF and thus the supply of splenic-derived CD34+ cells for research is likely to be reduced, optimized MPN patient-derived xenografts provide an excellent model for additional studies to further dissect MF biology and therapeutic response. Through
innovative thinking, and inspired by Sutton’s “wisdom,” the authors have opened the vault on a new avenue of MF research.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Comment on Kagoya et al, page 2996

Leukemia cells make ruthless competitors

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In this issue of Blood, Kagoya et al provide evidence for an important role for factors secreted by leukemia cells in damaging and suppressing normal hematopoiesis.1

Recently, much attention has been paid to the role of leukemia-induced changes in the bone marrow microenvironment in selectively promoting growth of leukemia while suppressing the growth of normal stem cell growth. Suppression of normal hematopoiesis is a commonly observed feature of leukemia, but the underlying mechanisms are not well understood.

Establishment and progression of leukemia in vivo require a competitive advantage of leukemic over coexisting normal stem cells in the bone marrow. Although this could be attributed to intrinsic proliferative and survival advantages of leukemic cells resulting from a variety of genetic and epigenetic alterations, differential microenvironmental regulation of leukemic and normal stem cells appears to be an important factor contributing to the establishment of leukemia, especially at early stages of development where normal hematopoiesis may be dominant.2

Studies in chronic myelogenous leukemia (CML), myeloproliferative neoplasm (MPN), and acute myeloid leukemia (AML) have shown the importance of leukemia-induced changes in the microenvironment in promoting disease progression. In CML, it has been shown that in addition to intrinsic BCR-ABL–induced alterations in stem cell growth, leukemia cells induce alterations in chemokine and cytokine expression in the bone marrow microenvironment that contribute to enhanced growth of CML stem cells and suppression of normal stem cells, and provide a selective growth advantage to CML stem cells.3 Interestingly, abnormalities in microenvironmental regulation are improved but not completely corrected by tyrosine kinase inhibitor treatment. BCR-ABL–expressing cells have been shown to induce a remodeling of the niche to their advantage, accompanied by a secondary compromise of their normal cell competitors.4 Recent studies have identified leukemia-induced disruption of sympathetic nerve fibers within the bone marrow of MPN patients and mice expressing the human JAK2V617F mutation, and in mixed–lineage leukemia gene–induced AML models.5,6

Sympathetic nerve damage results in alterations in mesenchymal cells that promote growth of leukemic stem cells at the expense of normal stem cells.

The studies of Kagoya et al extend our understanding of leukemia-induced alterations in normal and leukemic hematopoiesis and its contribution to leukemogenesis. Using a murine model of JAK2V617F MPN, they show that malignant cells not only demonstrated increased reactive oxygen species (ROS) themselves, but also induced elevated ROS and paracrine DNA damage in neighboring normal cells. A screen of candidate factors based on analysis of gene expression and short hairpin RNA–mediated knockdown identified overexpression of lipocalin-2 (Lcn2) in JAK2V617F+ cells as being a significant contributor to paracrine DNA damage. Lcn2 expression in MPN was Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway dependent. In normal hematopoietic cells, Lcn2 treatment resulted in elevated ROS levels, leading to p53 pathway activation, increased apoptosis, and decreased cellular proliferation (see figure). In contrast, JAK2V617F+ cells were not susceptible to Lcn2–induced growth suppression despite elevation of ROS and induction of DNA damage, possibly related to attenuated p53 activation in these cells. These findings suggest that Lcn2, in addition to providing JAK2V617F+ cells with a relative growth advantage by suppressing growth of coexisting normal hematopoietic cells, could also accelerate leukemogenesis through induction of DNA damage.

Previous studies have also identified Lcn2 as being essential to BCR-ABL–induced leukemogenesis.7 Reduction of Lcn2...
Hit the spleen, JAK!

Steven W. Lane and Ann Mullally