by removing CLL cells from proliferation centers and reducing their overall survival. Morande et al add to this understanding by measuring AID expression in CLL cells that are Ki-67+, and showing that this population of cells is severely reduced after patients begin taking ibrutinib. This means that ibrutinib treatment blocks the means of creating mutation within CLL cells, as well as the means through which clones bearing mutation can be expanded. So why do patients on ibrutinib develop resistance, particularly that associated with mutation of BTK and PLCγ2? The answer to this is not clear but could be related to the amount of treatment received by a given patient prior to ibrutinib therapy. Such patients are particularly prone to developing ibrutinib resistance, likely from clones already containing mutant BTK and PLCγ2 that are reported present at low frequency rather than through de novo mutagenesis.

So how does ibrutinib affect CLL cells in patients? This compound has a high degree of specificity for BTK, and the main downstream targets of this tyrosine kinase in B cells are PLCγ2 and Wiskott-Aldrich syndrome protein (WASp), but this might not be all it does. To more fully understand how ibrutinib therapy impacts CLL cells, Morande et al isolated malignant cells bearing a phenotype associated with having a high proliferative index from CLL patients enrolled in a clinical trial before and during receipt of the drug. They then performed a phospho-protein analysis on these cells and identified that ibrutinib treatment leads to dephosphorylation of JAK1 at Tyr1022, an event that deactivates the function of this kinase. Active JAK1 in cells stimulates the phosphatidylinositol 3-kinase (PI3K) pathway; consistent with its deactivation in ibrutinib-treated CLL cells, the authors observe changes in the phosphorylation of key proteins connected with this pathway that function in controlling proliferation and apoptosis. This could possibly account for the reduction of cells expressing Ki-67 and the increase in CLL cell death that is observed in these patients. The explanation for reduced AID expression is that JAK1 targets STAT6 for phosphorylation, which then translocates to the cell nucleus and is responsible for induction of AID expression. Indeed, Morande et al model this in vitro and show that ibrutinib treatment of CD40L/IL-4-stimulated CLL cells reduces STAT6 phosphorylation and AID expression. Thus, a potentially new role for BTK can be applied that is different to its typical role in the B-cell receptor (BCR)-signaling pathway (see figure panel A). In this new role, BTK is key to modulating JAK1 activation within CLL cells stationed within the microenvironment and exposed to CD40L/IL-4 (see figure panel B). It is known that BTK becomes activated in CD40-stimulated B cells, and, within this context, there may be new substrates, such as protein tyrosine phosphatases, that are able to dephosphorylate JAK1 and STAT6 and downregulate IL-4 signaling. Determining this will require further experiments comparing the effect of ibrutinib on CLL cells incubated with IL-4 alone and with CD40L/IL-4.

The article by Morande et al therefore sets the stage for further work into the mechanism of action of ibrutinib and similar compounds. These compounds can no longer be referred to as just BCR-signaling pathway inhibitors as they seemingly also affect a key pathway responsible for the development and expansion of resistant clones. Thus, ibrutinib is safer than we thought and may be most useful as a frontline therapy.

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REFERENCES


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MYELOID NEOPLASIA

Comment on Pang et al, page 2069

Selective CD117+ HSC exchange therapy

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Imagine a scenario in which defective/malignant hematopoietic stem cells (HSCs) are depleted and replaced by healthy HSCs without hematologic or systemic side effects. In this issue of Blood, Pang et al show that selective CD117+ HSC exchange therapy might become a clinical reality.¹

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) spearheaded somatic stem cell therapy and cellular immunotherapy. It is now a routine procedure, mostly used with the intention of curing myeloid hematopoietic stem and...
progenitor cell (HS(P)C)-derived malignancies. Success of allo-HSCT depends on 2 elements, that is, on lasting engraftment with physiologic function of the substitute HSCs and cotransferred immune cells as well as on eradication of the underlying host malignant hematopoietic disease. Lasting engraftment of donor HSCs requires creation of bone marrow niche space for HSCs and their descendants as well as suppression of host-versus-graft immune rejection. Eradication of the underlying host disease depends on pre-allo-HSCT therapies, but also critically on the donor immune cell–mediated graft-versus-leukemia (GvL) effect, that is, graft-versus-host disease (GvHD) against the host hematopoietic system, including remaining malignant cells. However, although continuously improving, the overall morbidity of current allo-HSCT remains high, in part caused by pretransplant conditioning (chemotherapy, γ-irradiation, immunological immune depletion) and posttransplant immunosuppression for prevention or treatment of GvHD, and associated infectious complications. This largely restricts allo-HSCT to otherwise fit and healthy individuals, which excludes the majority of patients. Moreover, disease relapse after allo-HSCT remains a major cause of death. Thus, improving selectiveness of preconditioning regimens to efficiently deplete host malignant HS(P)Cs while at the same time creating space for incoming donor HSCs, reducing collateral damage to nonhematopoietic tissue, and preserving as well as augmenting donor immune function against remaining malignant cells, are major challenges in the field.

In this issue of Blood, Pang et al successfully tackle several of these challenges with 1 approach. Using a monoclonal antibody targeting c-Kit (CD117), a transmembrane receptor tyrosine kinase, they demonstrate HSC niche space generation as well as host malignant HS(P)C eradication, followed by sustained engraftment of donor HSCs. Specifically, they show that (1) CD117 is expressed on normal human HSC and myelodysplastic syndrome (MDS) HS(P)C at similar levels; (2) the mouse anti-human CD117 monoclonal antibody SR-1 inhibits binding of the natural ligand stem cell factor (SCF) to CD117; (3) SR-1 inhibits proliferation of healthy human cord blood and bone marrow HS(P)C in vitro and permanently depletes healthy human HS(P)Cs from previously engrafted NSG mice; (4) SR-1 (and AMG 191, a clinical-stage humanized version of SR-1) depletes...
The work on human HSCs and their malignant counterparts by Pang et al builds on prior studies demonstrating HSC “niche-clearing” by a CD117 blocking anti-mouse antibody (ACK2), which permits engraftment of congenic HSCs in immunodeficient recipients. This also works in fully immunocompetent mice if Fc-mediated antibody functions are enhanced through blockade of the SIRPα-CD47 myeloid immune checkpoint. Alternatively, pretransplant conditioning with ACK2 treatment and low-dose irradiation allows mouse HSC engraftment in immunocompetent mice. Exctingly, in parallel findings to the study presented here, it was shown that in immunocompetent mice, a single dose of drug-conjugated anti-mouse CD117 antibody followed by HSCT results in full donor chimerism without impairment of immune functions or mature blood cell counts.

Taking all this together, it is tempting to speculate about a new area of “selective immune-mediated HSC exchange therapy” in myeloid malignancies as well as in nonmalignant, inherited hematopoietic/immune-system disorders. In this scenario, step 1 would be the exclusive eradication of diseased and healthy host CD117+ HS(P)Cs; step 2 would require termination of the anti-CD117 activity; and step 3 would be transfer of healthy CD117+ HS(P)Cs, which seamlessly produce fully functional hematopoietic cells (see figure). However, several questions still need to be addressed: (1) what is the exact in vivo mode of action of the anti-CD117 targeted therapy and what might be the optimal effector strength of the immune approach in different clinical situations? The current antibodies seem to work both via inhibition of natural ligand (SCF) binding and via FC-mediated toxicity. One can speculate that anti-CD117 efficacy can be engineered and enhanced by Fc optimization, drug-antibody conjugates (as already done4), generation of T-cell engaging and activating antibody constructs, or even by using anti-CD117 chimeric antigen receptor (CAR) T cells with a switch-off mechanism; (2) what will be the on-target toxicities on other hematopoietic and nonhematopoietic cells? CD117 is expressed on HSCs and early myeloid and lymphoid progenitor cells, but also on mast cells, which are relatively abundant in many tissues. CD117 is also expressed in nonhematopoietic tissues as, for example, by certain cells in the central nervous system, interstitial pacemaker cells in the gastrointestinal tract, some kidney cells, and melanocytes. Although no nonhematopoietic toxicities were reported in the in vivo models, there might be species-specific expression and reaction differences. Side effects might depend not only on the variable susceptibility of different cell types toward blockade of SCF signaling but also on the immune-effector strength applied (ranging from nonmodified immunoglobulin G to CAR T cells); (3) can recipient immunosuppression, which is required to prevent incoming HSC rejection and GVHD, both major causes of HSCT-associated morbidity, be relevantly reduced with such an approach in the genetically heterogeneous human population? This may require additional immunological depletion of host cells and possibly transplantation of HSC-enriched grafts with subsequent lymphocyte transfers for protective immunity and GvL.

Although the discussed approaches might still appear visionary, the authors of the study are already testing the clinical translation in patients with severe combined immunodeficiency, which is a situation that resembles the preclinical research (ClinicalTrials.gov identifier: NCT02963064). Indeed, they report encouraging toxicity-free engraftment in the first treated patients. Thus, one can be optimistic that studies like the one discussed here will pave the way to “selective HSCT” in hematopoietic malignancy, correction of genetically inherited hematopoietic diseases, and possibly even for the purpose of tolerance induction in solid organ transplantation.

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REFERENCES


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Selective CD117+ HSC exchange therapy
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