overall, had shorter PFS but varied substantially by individual chromosome, with 13q− favorable, whereas deletions of chromosomes 4, 15, and 18 were highly unfavorable, and often occurred with other aberrations and CKT. There are many similarities and some differences between CIT and venetoclax regimens. In both, unmutated IGHV, CKT (vs none), and β2M confer worse outcome, whereas 13q− is favorable. Other factors have interesting differences. Age has no impact on venetoclax regimens, whereas with CIT, it is more adverse at age >65 years as the CIT regimen changes from FCR to BR. The 11q− is well recognized to be adverse with CIT but had no significance with venetoclax. Many aberrations had numbers too small in the CIT arm for statistical analysis. This divergence of outcome with IGHV status with both CIT and venetoclax is consistent with the CLL14 study,[8] whereas with ibrutinib, M-CLL and U-CLL have identical outcomes.[9] It will be interesting to see the longer-term follow-up from the GIV arm.

CLL has a risk of clonal evolution with the development of additional and adverse karyotypes potentially producing more refractory disease that may be therapy driven.[10] Thus far, only small numbers have progressed and fewer still have required next line therapy. Nevertheless, more patients with CIT acquired a higher CKT level and 17p−. This study excluded 17p− and TP53-mutated patients, and so in the real-world environment, this situation will be more complex.

This article nicely demonstrates the importance of karyotype in CLL, both for CIT and now also with venetoclax-based regimens. Readers of *Blood* with an interest in CLL will be rewarded by close study of the main article and its supplementary data. However, just under 900 patients is not enough to evaluate and understand less frequent karyotypes. Karyotypic evaluation must be incorporated into CLL clinical trials, especially those with larger numbers. Furthermore, karyotype can clearly help refine the prognosis and expectations for individual patients and hence should be included in the standard diagnostic workup for all patients with CLL.

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MYELOID NEOPLASIA

Comment on Feng et al, page 460

Flipping the switch in the stem cell niche

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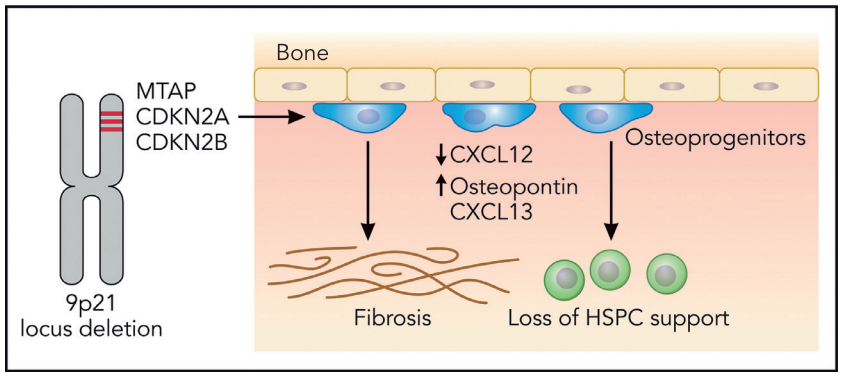
In this issue of *Blood*, Feng et al[1] describe a novel mouse model by which deletion of a single copy of the 9p21 tumor suppressor locus induces an aberrant bone marrow (BM) microenvironment, leading to loss of support for hematopoiesis, increased marrow fibrosis, and development of a fatal myelodysplastic/myeloproliferative (MDS/MPN) disorder.

The BM microenvironment, or niche, is essential for support and regulation of normal hematopoiesis and, likewise, is involved in sustaining and promoting leukemia.[2] Composed of diverse cell types with distinct functions, the BM microenvironment engages in intimate bidirectional cross talk with hematopoietic stem cells (HSCs) and their progeny. The array of cell types composing the niche include multipotent mesenchymal stem cells (MSCs) capable of trilineage differentiation into chondrocytes, osteoblasts, and adipocytes, as well as vascular endothelial cells and immune cells.

In myeloid neoplasms, cells from the malignant hematopoietic clone can remodel the microenvironment to form a self-reinforcing leukemic niche that maintains and promotes expansion of the malignant clone at the expense of normal hematopoiesis.[3,4] This is accomplished in part by secretion of inflammatory mediators that instruct MSCs in turn to produce factors promoting leukemic growth. Intriguingly, evidence that an aberrant BM microenvironment itself can initiate myeloid disease comes from studies employing genetic mouse models.[2] Activation of β-catenin specifically in osteoblasts[5] and



Deletion of the 9p21 locus encompassing tumor suppressor genes *MTAP*, *CDKN2A*, and *CDKN2B* leads to development of an MDS/MPN disorder with extensive marrow fibrosis, mediated by reprogramming of MSCs to osteoprogenitors and consecutive imbalance of cytokines in the BM milieu. Professional illustration by Patrick Lane, ScEYEnce Studios.

deletion of Dicer1 only in osteolineage cells[6] both cause myeloid disease with transformation to acute myeloid leukemia (AML), highlighting the importance of osteoblasts as regulators of hematopoiesis.

Feng et al now add compelling new data on the role of the BM microenvironment. The p21 locus on chromosome 9 encompasses several tumor suppressor genes, among them *MTAP*, *CDKN2A*, and *CDKN2B*, which have been implicated in numerous solid cancers. Among hematopoietic neoplasms, monoallelic loss of 9p21 is found in pediatric acute lymphoblastic leukemia and is linked to reduced survival. To examine the effects of 9p21 loss on hematopoiesis in vivo, the authors generated a mouse strain with conditional deletion of the 9p21-syntenic locus. Perhaps surprisingly, $9p21^{+/-}$ haplodeficient mice did not develop acute leukemia but rather extensive marrow fibrosis resulting in a hypoplastic BM and extramedullary hematopoiesis in the spleen and liver. Hematopoiesis was profoundly disturbed with severe anemia, thrombocytopenia, and changes characteristic of myelodysplasia in megakaryocyte-erythroid as well as granulopoietic lineages. Haplodeficient mice also showed abnormal cortical bone formation, pointing to an altered BM microenvironment.

Because the mice developed extensive fibrosis and a hypoplastic BM, serial transplantation of BM cells proved difficult. The authors instead used mononuclear cells from the spleen, the site of extensive extramedullary hematopoiesis, to show that the MDS/MPN disease was fully transplantable. Most strikingly, reciprocal transplantation of donor wild-type (WT) hematopoietic cells into lethally irradiated haplodeficient $9p21^{+/-}$ recipients again resulted in the same MDS/MPN disorder with 100% mortality of the recipients, but transplantation of BM cells from young $9p21^{+/-}$ mice before disease onset into irradiated WT recipients showed no perturbation of hematopoiesis. To further pinpoint the cell of origin, the authors extended their study to specifically induce deletion of 9p21 in osteoblasts, which again was able to fully recapitulate the MDS/MPN phenotype.

Using single-cell RNA sequencing to dissect alterations of the stromal cell compartment, the authors show that deletion of the 9p21 locus leads to expansion of chondrocyte and osteogenic precursors as well as cells with a fibroblast phenotype, with a concomitant reduction in adipogenesis, thus shifting the balance of HSC supportive cells in the niche. Mechanistically, the cytokine milieu of the BM was altered, with increased secretion of CXCL13 and osteopontin and severely reduced levels of CXCL12. Although CXCL12 is a well-recognized cytokine necessary for support of normal hematopoiesis, CXCL13 has been linked to fibrosis in other organs but not previously described as a major player in hematopoiesis. Osteopontin, produced by osteoblasts, is involved in bone remodeling, inflammation, and fibrosis. Osteopontin is also a negative regulator of HSCs. Thus, loss of 9p21 induces reprogramming of MSCs to fibrosis-driving osteoprogenitors and a subsequent switch in the cytokine balance of the BM niche (see figure). This points to osteoblasts as culprits in myeloid disease initiation.

So, do these results translate to the human setting? To answer this question, the authors examined bone samples from patients with MDS. They found elevated levels of CXCL13 in some MDS samples and a correlation between decreased *CDKNB2* gene expression and CXCL12 levels, suggesting there may indeed be a role for loss of tumor suppressors within the 9p21 locus in the MDS niche, but confirmation in a larger patient cohort is needed. As MSCs from patients with MDS are not clonally mutated,[7] indirect mechanisms such as epigenetic changes may play a more important role in shaping an altered niche in human disease. Along this line, decades-old work identified *CDKNB2* as a frequently epigenetically silenced gene in MDS.[8] Treatment of MSCs from patients with MDS with hypomethylating agents restores normal methylation[9] and reverts MDS MSCs to their normal function[9,10] but has not been linked to *CDKNB2* expression.

Further questions remain. How do these data relate to driver mutations in HSCs, which are considered initiating events in clonal stem cell disorders such as MDS and AML? Crossing $9p21^{+/-}$ mice with mice carrying a heterozygous knockin allele of *FLT3-ITD* did not result in full-blown AML. *FLT3-ITD* is a late event in leukemogenesis and in itself only causes MPN in murine models. It would be interesting to see how HSCs harboring founding mutations such as *DNMT3A*, *SF3B1*, or others contribute to or alter the disease phenotype in this model.

In the clinic, BM fibrosis is notoriously difficult to treat. Although hypomethylating agents show clinical efficacy in MDS and MDS/MPN disorders, they do not alleviate fibrosis. Looking toward the future, more specific therapies directly targeting the disrupted stem cell niche and imbalances in its milieu may emerge as the BM landscape in myeloid disease becomes dissectible at a single cell level. The model from Feng et al offers the possibility of testing novel niche-based therapies to target fibrosis in vivo.

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TRANSPLANTATION

Comment on Senjo et al, page 477

No tolerance with immune suppression!

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In this issue of *Blood*, Senjo et al[1] show that calcineurin inhibitors (CNIs) suppress donor T-cell progression to terminal exhaustion, thus limiting tolerance induction after hematopoietic stem cell transplantation (HSCT) in a mouse model.

Graft-versus-host disease (GVHD) is still a major cause of transplant-related morbidity and mortality. Prophylactic pharmacologic immunosuppression is required to reduce the incidence and lethality of GVHD after unmanipulated HSCT. CNIs are widely used and are considered the mainstay of posttransplant immune suppression, but, despite their use, acute and chronic GVHD occurs in a large proportion of patients.

Why donor T cells fail to reach a tolerogenic state and induce GVHD in some patients is not well understood. Many studies have focused on identifying the mechanisms of tolerance induction after transplant. Thymic function and the involvement of different donor regulatory cell subtypes (eg, regulatory T cells) have been previously demonstrated to be critically important in the establishment of central and peripheral tolerance.[2,3] More recently, the emergence of exhausted donor T cells has been shown to promote tolerance after transplant.[4,5] Donor T-cell exhaustion is a multistep process, occurs over time after transplant, and leads to impaired T-cell effector function. It has been described in patients who receive posttransplant pharmacologic immune suppression. Donor T cells that reach exhaustion lose their ability to induce alloreactive responses and have limited ability to induce GVHD but also very little capacity to maintain the desired antileukemic effect.[4] Exhausted T cells are still not completely characterized, but they express a combination of inhibitory molecules on their surface (eg, PD-1), upregulate key transcription factors (eg, TOX), and have distinct epigenetic programs.[6]

How T-cell exhaustion occurs and why this phenomenon differs among patients are still open questions. A better understanding of the mechanisms that support donor T-cell exhaustion might provide relevant insights to improve GVHD prevention and to better balance immune reconstitution after allo-HSCT.

To investigate these issues, Senjo et al explored the reasons why CNIs fail to induce tolerance in a mouse model. The authors found cyclosporine suppresses expression of genes promoting progression to T-cell exhaustion and allows for maintenance of donor T-cell effector function. Expression of genes such as *TOX*, a master regulator that promotes T-cell progression to "terminally" exhausted T cells, is reduced by treatment with cyclosporine, and T cells are retained in what the authors called a "transitory" exhaustion status. Senjo et al show with elegant experimental models transitory exhausted donor T cells maintain functional alloreactivity. In fact, these cells contributed to the development of chronic GVHD, and their antileukemic activity could be simply restored by PD-1 blockade. Thus, cyclosporine was found to act as a "double-edged" treatment. In fact, it reduces T-cell proliferation and helps protect from acute GVHD occurrence, but, at the same time, it inhibits tolerance induction and plays a key role in the maintenance of an alloreactive state after transplant (see figure).

Novel insights on how donor T cells differentiate over time is a major contribution by Senjo et al. The authors provide biological evidence of why strategies of posttransplant immune suppression using CNIs fail to induce tolerance. They did show that this failure may actually make it possible to restore antileukemic activity. Such insights are of particular importance taking into consideration the recent availability of different posttransplant cell immunotherapies, from the old-style unmanipulated donor lymphocyte infusions to the new approaches with chimeric