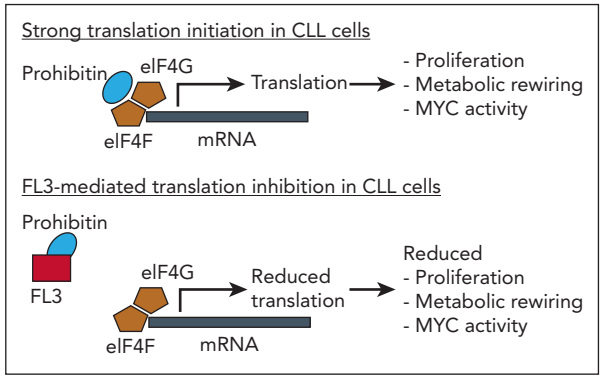

Prohibitins directly binding to the eukaryotic translation initiation complex (shown here are the factors eIF4G and eIF4F) promote strong translation initiation, which results in CLL cell proliferation, metabolic rewiring, MYC activity, and other effects (not shown). FL3 directly binds to prohibitins, thereby replacing them from the translation initiation factors. As a consequence, translation initiation is reduced, causing reduced proliferation, metabolic rewiring, and MYC activity in CLL cells.

has minimal adverse effects) prevent evolution to CLL? Third, there are 2 forms of CLL, 1 with mutated BCR variable region genes and 1 with unmutated BCR. The latter has an unfavorable clinical behavior, and it shows stronger BCR signaling upon activation and also ZAP70-mediated MYC activation.[7,8] Hence, are unmutated CLL cells more vulnerable to translation inhibition than BCR-mutated CLL, so that such a therapy may be useful only for unmutated CLL? Fourth, Richter transformation of CLL, which has a particularly poor prognosis, is often associated with genetic lesions of *MYC* causing increased MYC activity.[9] This points to an even more pronounced translational dysregulation and metabolic rewiring in this transformed form of CLL. Even though translation inhibition with FL3 alone is unlikely to control this aggressive lymphoma, it is certainly worthwhile to study whether Richter transformation is particularly sensitive to inhibition of translation. In that case, this could become a valuable component of a multimodality treatment of Richter transformation.

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MYELOID NEOPLASIA

Comment on Xie et al, page 3184

IL-34: a novel differentiation therapy for AML?

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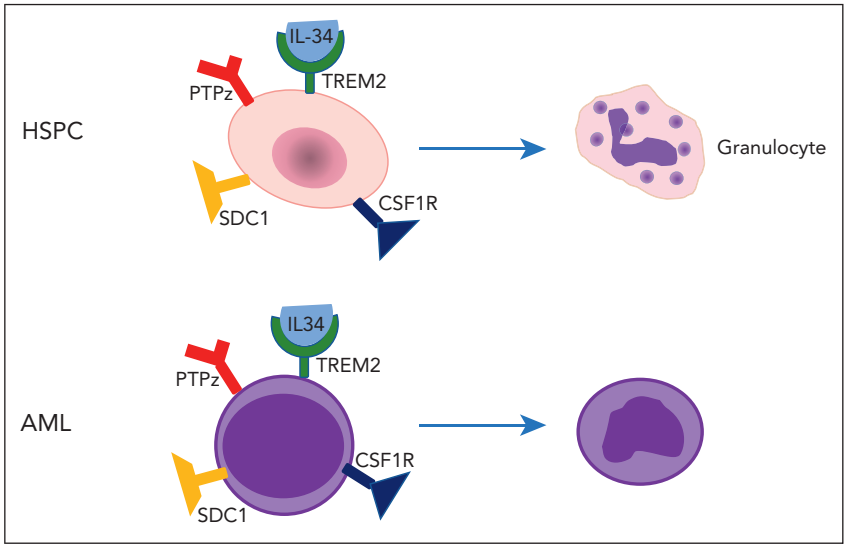
In this issue of *Blood*, Xie et al[1] describe a novel role for interleukin 34 (IL-34) in promoting hematopoietic progenitor and myeloid leukemia differentiation by binding to a previously unrecognized receptor for IL-34, triggering receptor expressed on myeloid cells 2 (TREM2).

Extending their prior observation that osteoclast-specific deletion of tuberous sclerosis 1 (*Tsc1*) results in increased numbers of osteoclasts,[2] the authors evaluated the hematopoietic system and observed increased granulocytic differentiation and enhanced osteoclast production of IL-34, a cytokine previously shown to promote myeloid maturation.[3] The authors investigated whether IL-34 may promote maturation of acute myeloid leukemia (AML) blasts and found that IL-34 induced differentiation of human AML cell lines and primary mouse and human blasts by binding to TREM2 (see figure). In addition, AML cells engrafted into osteoclast-specific *Tsc1*-deleted mice resulted in reduced disease aggressiveness. Impressively, IL-34 treatment of mice engrafted with primary human AML cells or human and mouse AML cell lines resulted in enhanced blast differentiation, reduced disease burden, and improved survival.

Although IL-34 has been reported to bind to receptors including colony stimulating factor 1 receptor (CSF1R), protein tyrosine phosphatase receptor type Z1 (PTPz), and syndecan 1 (CD138), deletion of these receptors did not attenuate the differentiation activity of IL-34. Using a combination of approaches, the authors identified TREM2 as a novel receptor required for IL-34's differentiation activity and found that IL-34 binding to TREM2 inhibits



IL-34 induces differentiation. IL-34 induces differentiation of normal hematopoietic/stem progenitor cells (HSPCs) as well as acute myeloid leukemia (AML) blast by binding to its previously unrecognized receptor, TREM2, instead of one of its known receptors: CSF1R, PTPz, or syndecan 1 (SDC1). Although osteoclasts likely represent an important source of IL-34 production, the sources of IL-34 production in patients with AML are unclear, as are the potential consequences of long-term IL-34 administration.

mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) signaling by inducing phosphorylation of RAS protein activator like 1. Notably, IL-34 induced blast differentiation more robustly than small-molecule inhibitors of MAPK/ERK, suggesting that IL-34 regulates additional, as of yet, uncharacterized signaling events. This finding is consistent with prior observations that MEK inhibitors reduce blast proliferation or cell cycle arrest, rather than inducing differentiation.[4,5]

The authors showed that IL-34 serum levels are reduced in patients with AML compared with controls, but given that elevated levels of IL-34 are observed in numerous inflammatory disorders and cancers,[3] and AML and preleukemic states are associated with increased systemic inflammation,[6] it is unclear why serum IL-34 is reduced in patients with AML. We speculate that such differences may be due to AML blast suppression of IL-34 production in the marrow or systemically, or the ability of IL-34 to promote the development of myeloid cells into distinct subtypes that reduce IL-34 production during disease progression.[7] Related to the latter possibility, IL-34 has been reported to have disease-promoting or protective effects depending on cancer type,[3] and thus it would be important to determine its effects during AML pathogenesis. To address these issues, it will be important to determine the source/s of IL-34 production in patients with AML, and such a search should interrogate the various components of the marrow microenvironment as well as tissues outside the marrow because prior studies have shown IL-34 is present in nearly all tissues and is expressed at highest levels in the brain, skin, and lymphoid tissues.[3] In addition, it would be important to determine if IL-34 expression is altered during aging or in preleukemic states such as clonal hematopoiesis and myelodysplastic syndrome, and to determine if IL-34 is required for the outgrowth of mutant clones. Such studies could be complemented by investigations using mouse models of AML that allow systematic evaluation of disease initiation and progression.

Although the IL-34–TREM2 signaling axis appears to be a promising target for the development of novel differentiation therapies in AML, several important questions remain regarding the therapeutic efficacy of targeting this pathway. Although the authors demonstrated that IL-34 can reduce AML clonogenicity, formal experiments testing effects on leukemia stem cells (eg, limiting dilution transplant studies following treatment) were not performed. Concerns also remain regarding whether long-term IL-34 treatment would be possible in patients because although no major toxicities were observed in these studies, the treatment periods were short (14-21 days). In addition, prolonged IL-34 treatment may result in signal attenuation through receptor downregulation or negative signaling feedback mechanisms, which may limit the efficacy of IL-34. Determining which patients may be suitable candidates for IL-34/TREM2-targeted therapies is also an important consideration. Although the authors show that TREM2 is required for the differentiation activity of IL-34, TREM2 expression is heterogeneous among patients with AML, and it remains unclear if differences in TREM2 expression levels impact IL-34 responses. Finally, given the importance of IL-34 in promoting myeloid differentiation and osteoclastogenesis, it would be important to determine if prolonged IL-34 treatment promotes diseases of osteogenesis or inflammation, because recent studies point to both protective effects of low-dose IL-34[8] as well as potential bone toxicities of high levels of IL-34.[9] Although these concerns may ultimately limit the use of IL-34 as a differentiation therapy, direct manipulation of TREM2 signals using other approaches may provide additional potential strategies to induce AML differentiation.

Overall, these studies are notable for their comprehensive evaluation of IL-34 function in normal and malignant hematopoiesis, identification of a novel receptor for IL-34, and exciting proof-of-concept studies indicating that IL-34–based differentiation strategies are a promising strategy to treat AML. Given the relatively limited success of differentiation therapy as stand-alone therapy in AML,[10] it will be important to determine if IL-34 can be used in combination with frontline or maintenance therapies. Although such studies could be performed using preclinical in vitro and in vivo models, ultimately the answer regarding the efficacy of these strategies likely will require clinical trials. We look forward to future studies that further explore the potential of using IL-34 or other TREM2-targeting strategies to treat patients with AML.

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

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RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Bennett et al, page 3199

Manipulating hepcidin in polycythemia vera

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In this issue of *Blood*, Bennett et al[1] provide elegant data supporting the mounting evidence of the critical role of hepcidin, the master regulator of iron metabolism, in the pathophysiology of polycythemia vera (PV). Another very recent article by Stetka et al, in *Blood*, also explored hepcidin and PV.[2]

By total number, most of our body's cells are small red blood cells (RBCs), estimated to represent ~24.9 trillion of a total of ~29.6 trillion cells (~29.6 × 10^{12}), whereas myocytes and adipocytes make up the majority by mass.[3] Erythropoiesis produces ~200 billion RBCs daily (~2 million per second), requiring ~80% of circulating iron, of which systemic distribution is finely regulated by the liver hormone hepcidin. Hepcidin acts by occluding the sole cellular iron exporter ferroportin, thus reducing absorption of dietary iron and recycling of iron from macrophages. High levels of hepcidin reduces iron availability, whereas hepcidin deficiency can ultimately lead to iron overload.[4] Not surprisingly, the pathological expansion of erythropoiesis in PV is almost invariably associated with iron deficiency (ID) because of exaggerated need, which is further exacerbated by therapeutic phlebotomies. This imbalance results in a complex dysregulation of hepcidin, because of opposing stimuli (see figure). The net effect is a variable degree of hepcidin suppression, predominantly due to ID and the ensuing need to increase iron absorption, partially counteracted by proinflammatory cytokines (reviewed by Ginzburg et al[5]). The work by Bennett et al identifies hepcidin as a key driver of PV phenotype severity, using 2 complementary approaches. Firstly, unbiased genome-wide association studies (GWASs) on 2 large populations (United Kingdom Biobank and FinnGen) found that single-nucleotide polymorphisms (SNPs) in the *HFE* gene were strongly associated with the risk of developing PV. This was particularly evident for the SNP corresponding to the common C282Y mutation, whose homozygosity is associated with hepcidin deficiency and clinically overt hemochromatosis, although with a low penetrance.[4] Of note, individuals who have homozygous C282Y tend to have slightly higher hemoglobin (Hb) levels than noncarriers.[4,6] Common *HFE* SNPs have also been positively associated with Hb and RBC parameters in large meta-analyses of multiple GWASs.[7] Studies on clonal hematopoiesis of indeterminate potential (CHIP) have suggested that the classical acquired somatic *JAK2* V617F is far more frequent than PV in the general population (up to 3.1% vs a prevalence of *JAK2*-associated myeloproliferative neoplasms of 6-8 per 10 000).[8] The reasons for this discrepancy are unclear. It has been hypothesized that the acquisition of *JAK2* V617F is only a part of the story, with the presence of inherited factors possibly being equally important.[9] Until recently, few alleles predisposing to *JAK2* V617 CHIP were identified in the *JAK2* gene itself and in genes controlling cellular aging (*TERT*), epigenetic regulation (*TET2*), and erythroid/megakaryocyte development (*GFI1B*), among others.[9] The work by Bennett et al confirms the strong association of a known germ line *JAK2* SNP (tagging the 46/1 haplotype) with PV and adds the identification of *HFE* as a modifier of PV phenotype severity by predisposing to hepcidin deficiency. Such a genetic background would be likely permissive by favoring iron fueling for exaggerated erythropoiesis when *JAK2* somatic mutations are acquired and reach a certain allele burden.

To corroborate this hypothesis, Bennett et al manipulated hepcidin in a series of elegant experiments in a PV mouse model. Indeed, genetic ablation of liver hepcidin worsened the erythroid phenotype, whereas a remarkable amelioration was obtained by increasing hepcidin (see figure). From a practical standpoint, such experiments provide a further strong rationale for the use of hepcidin mimetics in patients with PV, which are already under active investigation (recently reviewed by Handa et al[10]). In particular, the hepcidin mimetic rusfertide has been demonstrated to virtually eliminate the need for phlebotomy in patients with PV in 2 phase 2 clinical trials (NCT04057040 and NCT04767802). Moreover, the drug improved iron parameters and reduced constitutional symptoms likely due to ID in non-erythroid compartments, with no serious safety signals. Results from an ongoing multicenter placebo-controlled phase 3