PFS between 20 Gy and 30 Gy in patients with a favorable prognosis, the cohort with an unfavorable prognosis (a population similar to that in AHOD0031) had inferior outcomes with 20 Gy. The article by Giulino-Roth et al suggests that lower doses of RT may be associated with different types of cancer; specifically, 6 of the 11 solid tumors were papillary thyroid cancers, a highly curable cancer with a 5-year survival rate of 98.3%.[8] In balancing late effects vs cure, papillary thyroid cancer seems highly preferable to relapsed HL.

In stark contrast to thyroid cancer, all 3 cases of treatment-related AML were fatal and occurred in patients who received ABVE-PC and IFRT. Two patients with available cytogenetics showed an *MLL* locus rearrangement, a finding associated with topoisomerase II inhibitors doxorubicin and etoposide. Despite the low cumulative incidence of 0.2%, the uniform lethality of this complication highlights the continued need to address both the chemotherapy and radiation components when developing new approaches for treating HL.

Continued massaging of traditional therapeutics and technologies is unlikely to have a significant impact on outcomes in HL. Fortunately, a new era in HL treatment may be upon us. Molecular tests, such as that for circulating tumor DNA, may more accurately identify those who need consolidative therapy. Incorporating highly active, novel agents such as brentuximab vedotin, an antibody drug conjugate, or checkpoint inhibitors into earlier lines of therapy is likely to increase the cure rates, hopefully limiting the need for RT and more toxic agents and helping us protect our children.[9,10]

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HEMATOPOIESIS AND STEM CELLS

Comment on Ishii et al, page 1457

FGF-23: a novel actor in stem cell mobilization

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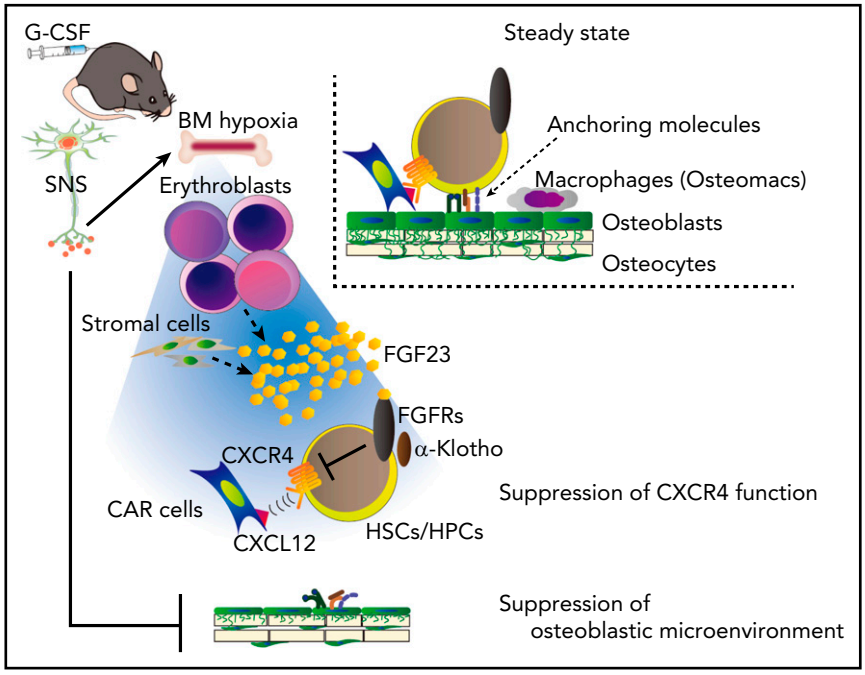
In this issue of *Blood*, Ishii et al[1] identify a novel role for fibroblast growth factor 23 (FGF-23), an osteocyte-derived hormone that regulates phosphate metabolism, in granulocyte colony-stimulating factor (G-CSF)–stimulated mobilization of hematopoietic stem and progenitor cells (HSPCs).

FGF-23 is a hormone secreted by osteoblasts and osteocytes that acts on the kidneys, parathyroid glands, heart, and bone. Now, as reported by Ishii et al, FGF-23 has been shown to play a role in G-CSF–mediated HSPC mobilization. Linkage analysis studies first identified the role of FGF-23 in phosphate wasting disorders.[2] Targeted ablation of FGF-23 demonstrated its role in phosphate and vitamin D metabolism through its actions in the kidney and parathyroid glands.[3] Intact FGF-23 binds with high affinity to a heterodimer of the membrane-bound FGF receptor 1 (FGFR-1) and Klotho proteins, activating canonical FGF-23 signaling.[4]

Noncanonical FGF signaling can also be mediated by other FGFRs independent of Klotho, particularly with extremely high FGF-23 levels, as in chronic kidney disease.[4] A potential role of FGF-23 in hematopoiesis was initially suggested by increased red blood cell counts and elevated erythropoietin levels in mice lacking FGF-23. Consistent with an inhibitory role of FGF-23 in erythropoiesis, injection of FGF-23 in wild-type mice decreased erythropoiesis.[5] Patients with chronic kidney failure have elevated levels of FGF-23, which may contribute to anemia by suppressing production of erythropoietin by the kidneys. However, direct effects of FGF-23 on erythropoiesis have been postulated, since the receptors activated by FGF-23, including FGFR-1, FGFR-3, and FGFR-4 and Klotho, are highly expressed in erythroid cells.[4,5] Together these findings suggested that FGF-23 may contribute to suppression of erythropoiesis. On the other hand, data have shown that erythropoietin stimulates murine and human FGF-23 production not only in cells of the osteoblastic lineage but also in



A model for G-CSF–induced mobilization by FGF-23 from erythroblasts. G-CSF or activation of the sympathetic nervous system, inducing hypoxia, increases the expression and release of FGF-23, mainly from erythroblasts (and also partially from stromal cells). Homeostatic mechanisms that contribute to HSPC anchoring to the microenvironment, such as chemoattraction toward CXCL12-abundant reticular (CAR) cells, are suppressed by the high concentration of bone marrow (BM) FGF-23, which counteracts the CXCR4 function via FGFRs. HSC, hematopoietic stem cell; SNS, sympathetic nervous system. See Figure 7 in the article by Ishii et al that begins on page 1457.

hematopoietic cells,[6] specifically in erythropoietic precursor cells.[5,7] Therefore, erythropoietin and FGF-23 may be components of a homeostatic mechanism linking erythropoiesis with skeletal and mineral metabolism.

The authors of the current study found increased levels of FGF-23 in the bone marrow extracellular fluid within the first 24 hours of G-CSF treatment. Since G-CSF induces sympathetic nervous system signals that impact mobilization, the pan β-adrenergic receptor agonist isoproterenol was tested. Isoproterenol also induced FGF-23. Surprisingly, administration of G-CSF or isoproterenol increased FGF-23 expression in erythroblastic cells, identified by flow cytometry as $CD45^{-}Ter119^{+}CD51^{+}$ cells. Hypoxia, which is known to be induced by G-CSF in the bone marrow microenvironment, also increased FGF-23 in an erythroblastic cell line. Next, mice with a global deletion of FGF-23, previously found to have skeletal fragility and abnormal mineral metabolism,[3] were shown to have a decrease in G-CSF–dependent mobilization of HSPCs. Notably, chimeric mice where $FGF\text{-}23^{-/-}$ bone marrow was transplanted in wild-type recipients also had inhibition of G-CSF–dependent stem cell mobilization, suggesting that hematopoietic cells (likely erythroblastic cells) are a critical source of FGF-23 responding to G-CSF mobilization. Mice with targeted deletion of FGF-23 in osteocytes had no reduction in G-CSF–induced stem cell mobilization, although a contribution of osteoblastic FGF-23 could not be excluded using this model.

Next, to investigate the mechanism of this effect of FGF-23 on HSPC mobilization, the authors evaluated CXCL12, the critical cytokine regulating the retention of HSCs in the bone marrow. Mice lacking FGF-23 globally, or in the bone marrow, demonstrated no change in the levels of CXCL12. This suggests that the effect of FGF-23 in stem cell mobilization is not due to modulation of CXCL12 expression. However, FGF-23 did inhibit HSPC migration toward CXCL12, and pharmacologic studies suggested that this may be due to FGF-23–dependent signaling and not the modulation of CXCL12 binding to its receptor.

This novel role of erythroblastic FGF-23 in modulating G-CSF–mediated mobilization may have clinical implications in diseases or iatrogenic conditions in which erythroblasts are decreased. For example, the lack of local FGF-23 due to low erythroblastic numbers in patients with multiple myeloma or patients treated with chemotherapy could contribute to insufficient mobilization after G-CSF. It will also be important to test whether high circulating levels of FGF-23 in chronic kidney disease may impair HSPC retention in the bone marrow. Notably, ongoing phase 2 and 3 studies are evaluating safety and efficacy of burosomab, a fully human monoclonal antibody that inhibits FGF-23, in patients with FGF-23–induced hypophosphatemic rickets/osteomalacia.[8,9] The current work suggests that the hematopoietic consequences of these treatments need to be investigated.

A number of specific questions about the role of FGF-23 in hematopoiesis remain to be addressed. First, additional studies, with targeting of FGF-23 deletion to erythroid populations may further clarify the physiologic role of FGF-23 in erythroblastogenesis and HSPC retention in bone marrow. Moreover, the mechanisms by which FGF-23 regulates mobilization of HSPCs are not clearly elucidated here and should be further explored. Finally, it is unclear if or how this novel action of FGF-23 in regulation of HSPCs contributes to its role in anemia associated with inflammation and chronic kidney disease.

In summary, Ishii and colleagues have delineated the novel role of FGF-23, a mediator of mineral metabolism, in mobilization of hematopoietic stem cells. They have provided persuasive data supporting the importance of local production of FGF-23 by erythroblasts in response to sympathetic signals and G-CSF (see figure). This work adds erythroblasts to the list of hematopoietic cells that may have a regulatory role in the hematopoietic stem cell niche. These novel data also provide additional support to the concept that skeletal and hematopoietic remodeling are linked, demonstrating once more the reciprocal and coordinated regulation of the skeleton and the hematopoietic marrow.

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IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Gandhi et al, page 1468

Sorting biologic subtypes of primary CNS lymphoma

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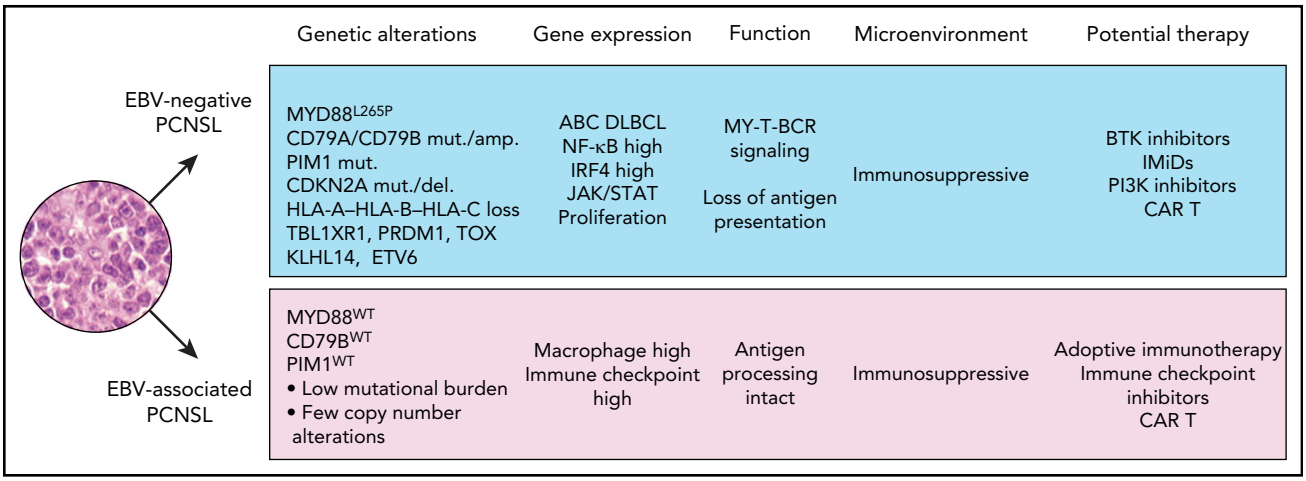
In this issue of *Blood*, Gandhi et al studied 91 cases of primary diffuse large B-cell lymphoma (DLBCL) of the central nervous system (CNS) (PCNSL) and compared the biologic features of tumors associated with Epstein-Barr virus (EBV) to tumors that are EBV$^-$ using digital gene expression signatures and customized hybrid-capture targeted sequencing panels.[1]

The authors identified strikingly distinct genetic and immunologic features based on EBV status, including differences in gene expression, genetic drivers, signatures within the tumor microenvironment, and oncogenic signaling pathway addictions (see figure). Because cases of EBV-associated PCNSL often arise in immunocompromised hosts, a second stratification based on host HIV status was also performed, although biologic differences were less evident. Unsurprisingly, EBV$^-$ PCNSL typically had a gene expression phenotype resembling ABC DLBCL, including mutations of *MYD88*^{L265P}, *CD79B*, and/or *PIM1* in ~80% of cases. Immune evasion was also a prominent genetic feature, as mutations or deletions of *HLA-A*, *HLA-B*, or *HLA-C* were seen in most cases. These genetic features have been reported in PCNSL as well as genetic subtypes of DLBCL with secondary involvement of the central nervous system (SCNSL). These findings underscore the notion that hallmark features of EBV$^-$ PCNSL include constitutive NF-κB activation as well as chronic active B-cell receptor and My-T-BCR signaling that is shared with specific systemic DLBCL subtypes.[2] Conversely, EBV-associated PCNSL was frequently unclassified by gene expression, did not demonstrate cardinal genetic drivers of ABC DLBCL, and exhibited a genomic landscape with few identifiable mutations or copy number abnormalities. Interestingly, EBV-associated PCNSL exhibited a tolerogenic tumor microenvironment, including overexpression of genes associated with T-cell immune checkpoints (PD-L1, PD-L2, LAG-3, TIM-3), macrophages (CD68, CD163), and the antiviral cytokine tumor necrosis factor-α (TNF-α). Paradoxically, the lack of genetic alterations involving HLA class I or II suggests that antigen processing is preserved in these tumors despite their



Primary CNS lymphoma can be subdivided into EBV$^-$ and EBV-associated subtypes based on unique genetic alterations, gene expression signatures, and functional and microenvironmental differences. These biologic differences suggest that potential therapeutic strategies should be tailored within subtypes. ABC, activated B cell; amp, amplifications; BCR, B-cell receptor; BTK, Bruton tyrosine kinase; CAR T, chimeric antigen receptor T cell; del, deletions; IMiD, immunomodulatory imide drug; My-T-BCR, MYD88-TLR9-BCR supercomplex; mut, mutation; PI3K, phosphoinositide 3-kinase.