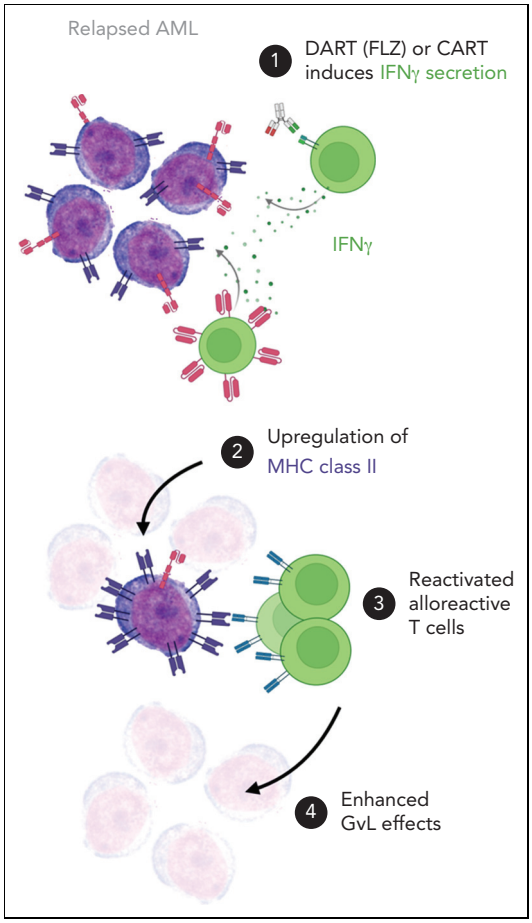

Acute myeloid leukemia (AML) targeted by either the dual-affinity retargeting compound (DART) flotetuzumab (FLZ) or chimeric-antigen-receptor (CAR) T cells upregulate major histocompatibility complex (MHC) class II on their surface, rendering them recognizable by allogeneic effector cells in patients after allogeneic hematopoietic cell transplantation. GvL, graft-versus-leukemia; IFNγ, interferon gamma; TCR, T-cell receptor. Illustration by Helena Jambor.

and NCT04582864). As the response to FLZ has been shown to be related to expression of IFNγ-related genes in the nontransplant setting, a point of interest is whether similar predictive biomarkers can be identified in the post-HCT setting.[9] The current paper by Rimando et al provides a convincing rationale to pursue the clinical testing of T-cell–engaging therapies and CART approaches in patients with relapse of myeloid malignancies after alloHCT.

Conflict-of-interest disclosure: M.B. reports being a scientific advisory board member of and receiving speaker honoraria from Jazz Pharmaceuticals and MSD. ■

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MYELOID NEOPLASIA

Comment on Agrawal-Singh et al, page 1737

Silencing with SAFB: a new role for HOXA9 in AML

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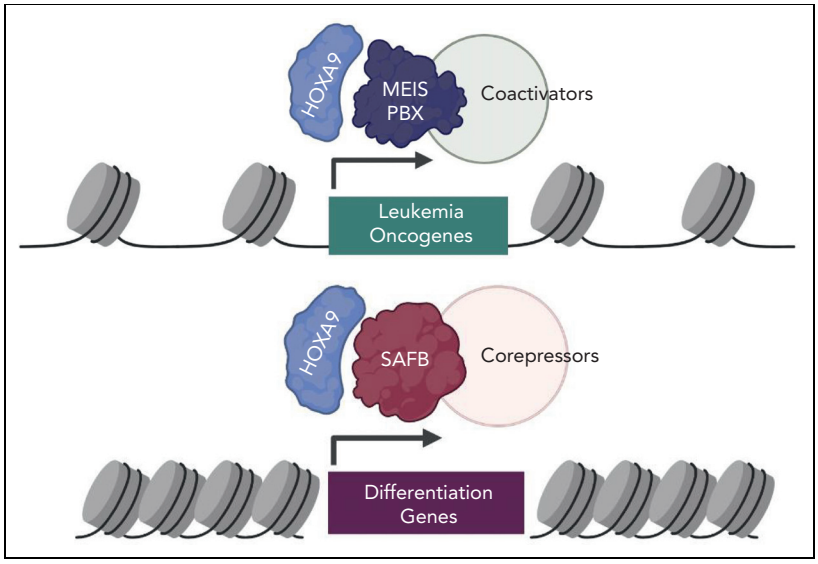
In this issue of *Blood*, Agrawal-Singh et al[1] uncover a novel mechanism by which HOXA9, a transcription factor in acute myeloid leukemia (AML), represses gene expression through interacting with the scaffold attachment factor B (SAFB) protein. These findings bring into focus the transcription repression role of HOXA9 in AML.

Transcription factors play pivotal roles in hematopoietic development, and their dysregulation through aberrant expression or translocation is a recurrent feature in AML. HOXA9 is a homeodomain-containing transcription



A model of HOXA9 transcriptional activity in AML. (Top) HOXA9 heterodimerizes with MEIS/PBX proteins to promote activation of oncogenic signaling. Transcriptional activation may involve coactivators such as MLL3/4, proteins that modify chromatin to activate gene expression. (Bottom) A novel mechanism of HOXA9-mediated gene repression described in this study. HOXA9 interacts with the SAFB to repress differentiation-associated genes in leukemia cells. HOXA9 through SAFB recruits corepressors NuRD and HP1γ, proteins that modify chromatin to repress gene expression. Figure created with BioRender.com.

factor of the posterior HOXA gene cluster. HOXA9 plays an important role in hematopoiesis.[2] Despite the mutational heterogeneity of AML, several different genetic mutations and gene fusions lead to aberrant HOXA9 activation in AML.[3] In more than half of all AML cases, HOXA9 is activated through diverse mechanisms, and this activation is associated with a poor prognosis. [3,4] In these AMLs, HOXA9 inactivation impairs leukemogenesis, thus corroborating its role in AML maintenance and making it an attractive therapeutic target.[5]

Most studies on HOXA9 activity in AML have focused on its role as a potent transcriptional activator of downstream targets that promote oncogenesis. These Hoxa9 targets include genes with prominent roles in AML such as *Flt3* and *Myb*.[3] Chromatin occupancy studies have shown that HOXA9 binds to the promoters and enhancer of its target genes, activating their expression and promoting leukemogenesis[6,7] aided by recruitment of coactivators such as MLL3/4 (see figure).[7,8] Interestingly, prior studies have indicated that HOXA9 may also act as a transcriptional repressor on a distinct subset of genes,[6,7] but mechanisms of gene repression by HOXA9 have remained poorly studied.

In the article by Agrawal-Singh et al, the authors purified the HOXA9 protein interactome from an AML cell line using an endogenous HOXA9 antibody. This approach is an improvement over previous studies using HOXA9 that was ectopically overexpressed or using artificial immunoprecipitation tags. The authors identified a number of scaffold/matrix attachment region binding proteins as high-confidence HOXA9 interactors, and they focused on SAFB. The study showed that SAFB loss phenocopies HOXA9 loss in murine models of leukemia in vitro and in vivo. Interestingly, nearly one-third to two-thirds of HOXA9 binding sites were also cobound by SAFB in AML cell line and primary AML cells—demonstrating a striking degree of genomic cooccupancy. There was also a strong overlap in gene expression changes upon loss of the HOXA9 and SAFB. In contrast to need by HOXA9 to recruit coactivators for gene expression, the HOXA9/SAFB complex instead recruited the NuRD corepressor complex and the heterochromatin protein HP1γ to mediate repression of differentiation-associated genes. Targeting the catalytic components of these corepressor complexes using small molecular inhibitors phenocopied the loss of HOXA9/SAFB and showed efficacy in primary human AML patient samples. Arguably, the inhibitors used in this study, panobinostat and chaetocin, may have broader activity beyond suppression of the HOXA9/SAFB complexes; nevertheless, the results presented in this study identify a new target for reversing the repressive activity of HOXA9 in AML. Detailed characterization of HOXA9/SAFB interface will enable precise targeting of this interaction critical for HOXA9 repressive function and leukemia cell survival.

Of note, in characterizing the repressive mechanism of HOXA9 in gene regulation—and in identifying the epigenetic components that mediate this repression—Agrawal-Singh et al shed light on the mechanistic basis by which HOXA9 overexpression maintains leukemia cells in an undifferentiated state. HOXA9 overexpression has at least 2 major effects on hematopoietic cells: enhancement of self-renewal and block of differentiation. The former is relatively well understood, and the current study may help fill in the other half of the puzzle. This study also raises some thought-provoking questions. First, it is well established that HOXA9 forms heterodimers with the 3 amino-acid loop extension (TALE) domain transcription factors, including the MEIS and/or PBX proteins.[3] However, it appears that HOXA9/SAFB predominantly functions independently of HOXA9/MEIS1 and has an opposite effect on transcription. Does HOXA9 form distinct, mutually exclusive complexes with the TALE proteins and with SAFB with activating and repressive functions, respectively? Second, it is not known whether similar mechanisms underly the role of HOXA9 in normal hematopoiesis. To this end, it would be interesting to investigate whether SAFB knockout in hematopoiesis has similar defects in normal hematopoietic reconstitution as *Hoxa9* null hematopoietic stem cells. Finally, the exact role of SAFB in mediating HOXA9 gene repression is still enigmatic. It does not seem to affect the genomic localization of HOXA9. A previous study showed that SAFB has a role in maintaining 3D heterochromatin organization.[9] This raises the interesting question whether the HOXA9/SAFB interaction serves to recruit target genes into a heterochromatic neighborhood for silencing. Future studies may help answer these questions in more detail.

Overall the Agrawal-Singh et al study identifies a novel, transcriptional repressive function for HOXA9, a transcription factor dysregulated in a large proportion

of AML, opening up new avenues for therapeutic intervention in AML.

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

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TRANSPLANTATION

Comment on Lohmeyer et al, page 1755

New mechanisms of GVHD suppression by Tregs

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In this issue of *Blood*, Lohmeyer et al uncover how regulatory T cells (Tregs) affect the transcriptomic, metabolic, and functional states of pathogenic effector T cells to blunt graft-versus-host disease (GVHD) in mouse models of allogeneic hematopoietic cell transplantation (allo-HCT).[1]

The infusion of Tregs has emerged as an attractive approach to mitigate GVHD, which is the main life-threatening and life-altering immune complication of allo-HCT. In preclinical mouse models, Tregs prevent severe GVHD without eliminating the potent graft-versus-tumor effects of allo-HCT, a desirable pattern of immunomodulation that has inspired clinical translation.[2-4] Recent reports in patients include promising strategies of early Treg administration ahead of conventional T cells (Tcons) that mediate GVHD, in some cases without any pharmacological immunosuppression.[5] Yet, it remains unknown how Tregs keep pathogenic T cells on a tight leash to achieve beneficial immunomodulation in vivo.

To gain new insights, Lohmeyer et al used a well-established mouse model of major histocompatibility complex (MHC)-mismatched allo-HCT, deploying a combination of transcriptional profiling and T-cell receptor (TCR) clonality index analysis in Tregs vs $CD4^+$ and $CD8^+$ Tcons purified from syngeneic or allo-HCT recipients (see figure).[1] Key comparisons focused on Tcons transplanted in the presence or absence of Tregs, and on Tregs before and after transplantation. Two nonmutually exclusive models of protection were considered: a quantitative impact of Tregs on the activation of alloreactive T-cell clones and/or qualitative effects on their pathogenic functions. The authors observed an increased clonality index both in Tcons and in Tregs after transplantation into allogeneic as compared with syngeneic hosts, consistent with alloantigen-driven selection of a restricted TCR repertoire in both populations. Interestingly, Treg administration did not affect the clonality index of Tcon populations recovered from allo-HCT recipients. Instead, Tregs exerted transcriptional effects on $CD4^+$ and, to a lesser extent, $CD8^+$ Tcons, enhancing the expression of some anti-inflammatory and Th2 signature genes and favoring an oxidative phosphorylation program at the expense of glycolysis, among other changes. Paired transcriptomic analysis showed increased transcripts encoding interleukin-10 (IL)-10 and IL-35 components in Tregs, as well as upregulated IL-10- and IL-35–mediated gene expression signatures in alloreactive Tcons exposed to Tregs in vivo, indicating that these pathways are potential mediators of GVHD suppression. Treg administration also correlated with decreased T-cell accumulation in the gut, a key GVHD target organ. Importantly, Tregs did not prevent alloantigen-driven Tcon activation or upregulated expression of cytotoxic effector genes essential for T-cell–mediated antitumor activity. Altogether, Lohmeyer et al made interesting predictions about Treg activation and functions after allo-HCTs that will need mechanistic testing and should inspire human investigations, particularly when Tregs are administered without interference from calcineurin inhibitors or other pharmacological agents.

In terms of the 2 models of Treg-mediated GVHD protection that the authors intended to test, the data were most consistent with a model implying qualitative effects of Tregs on Tcon pathogenic functions after transplantation rather than a quantitative impact on Tcon activation. Indeed, Treg administration preserved overall T-cell activation/differentiation per transcriptomic criteria and did not interfere with alloantigen-driven Tcon clonal restriction, thus maintaining the full breadth of the alloreactive Tcon repertoire. Together with the preserved induction of cytotoxic effector gene programs, the broad pool of alloreactive Tcon arising in the presence of Tregs may account for the previously reported capacity of Treg administration to spare beneficial graft-versus-tumor effects.[2-4]