posttransplant context is a strategy that has been assessed to decrease GVHD. Although these approaches eradicate alloreactive T cells, they also ablate activated T cells that may mediate desirable T-cell immune activity. In contrast to these earlier data, the authors found that culture conditions in their model enriched ADR T-cell expression on CD8 T cells. Furthermore, ADR T cells preferentially targeted activated CD4 T cells, which likely facilitated the preservation of T-cell antiviral activity.

Of note, relapse is the leading cause of death following allo-HCT. Preclinical and clinical data suggest that adoptive therapy with donor CAR T cells administered following allo-HCT induces less GVHD compared with unmodified donor T cells. However, preclinical models have demonstrated that the potential for GVHD varies depending on aspects of the CAR, including the number of immunoreceptor tyrosine–based activation motifs within the CD3ζ signaling domain and the costimulatory receptor.[8,9] One preclinical model deciphered the biological basis for the attenuation of GVHD with donor CAR T cells, which suggests ways to make CARs from a donor safer and more effective.[8] However, to effectively administer donor CAR T cells following allo-HCT to decrease relapse, studies will need to interrogate how to manage GVHD prophylaxis, as these medications may diminish CAR T-cell activity.

Hence, an approach that diminishes GVHD while maintaining antitumor and antiviral activity is a considerable advance for the allo-HCT field. Further research will need to assess the impact of ADR T cells on chronic GVHD, characterize their effect on the function of regulatory T cells, and evaluate approaches to regulate their persistence. Nonetheless, the authors convincingly demonstrate that CAR-ADR T cells are a novel and innovative strategy to enhance leukemia clearance and diminish acute GVHD. These findings have strong translational implications as to how the administration of engineered donor T cells following allo-HCT may improve clinical outcomes.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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https://doi.org/10.1182/blood.2022018182

THROMBOSIS AND HEMOSTASIS

Comment on Legan et al, page 1221

2B or not 2B: art thou autoinhibitory?

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Just as the line from *Hamlet* "To be, or not to be—that is the question" is perhaps the most famous Shakespearean quote, type 2B von Willebrand disease (VWD) is perhaps the most complex VWD subtype. In this issue of *Blood*, Legan and colleagues present a fascinating study showing how various type 2B causative mutations affect the function of the von Willebrand factor (VWF) autoinhibitory module.[1]

Type 2B mutations are located exclusively in the VWF A1 domain and render the molecule more readily able to interact with glycoprotein Ibα, enhancing platelet binding.[2] In turn, this readiness results in increased platelet consumption that can lead to thrombocytopenia and enhanced cleavage of VWF by ADAMTS13 (VWF-cleaving protease), with a reduction in large VWF multimers.[2,3] An interesting point to note is that although all type 2B mutations are located in the VWF A1 domain, not all type 2B causative mutations are equal in their pathogenetic effects.[4] A recently described structural feature of the A1 domain is what is known as the autoinhibitory module (AIM), which flanks both the N-terminus (N-AIM) and C-terminus (C-AIM) of the Cys1272–Cys1458 disulphide bond.[5] Both of these flanking regions are proline-rich and contain 4 O-linked glycosylation sites; the regions cooperate to stabilize the A1 domain and prevent interaction with platelet glycoprotein Ib alpha chain (GPIbα) until the molecule is activated by shear stress.[6-8] In their study, Legan et al tested the hypothesis that mutations in the A1 domain will differentially affect the autoinhibitory function of the AIM.

They expressed 7 previously reported type 2B variants in an A1 fragment encompassing both AIM modules—P1266L and H1268D—located in the N-AIM; R1306W, V1316M, and R1341Q—located in the A1 domain itself; and L1460V and A1461V—located in the C-AIM. They used a range of biophysical techniques to investigate the impact of

these variants. As perhaps could be expected, all the type 2B variants destabilized the global structure of the A1 domain, with thermal denaturation temperatures at least 5°C lower than wild-type A1. Interesting to note is that the lowest denaturation temperature was observed with the V1316M mutation that is associated with persistent thrombocytopenia. Next, using biolayer interferometry, the authors investigate binding to GPIbα and demonstrate the existence of a low-affinity binding state and a high-affinity binding state. Although these 2 states did not differ in their association rate, the high-affinity interaction dissociated 10-fold more slowly, and approximately 21% of wild-type molecules exhibited the high-affinity state. In contrast, with the exception of the P1266L variant, all the type 2B variants showed a greater proportion of molecules in the high-affinity state. While they are in this high-affinity state, the molecules are therefore more reactive to platelet GPIbα.

Furthering their observations, Legan et al next used the increasingly powerful technique of hydrogen–deuterium exchange (HDX)[9] to probe the conformation of the A1 domain. All the variants demonstrated an increased rate and extent of HDX, compared to wild-type A1, with the greatest effect seen with V1316M. The enhanced HDX of the type 2B variants indicates that these mutations render the molecule more flexible and increase solvent accessibility. Finally, using optical tweezers to mechanically unfold the A1 domain, Legan et al demonstrate that all the mutations caused the domain to unfold at lower forces, consistent with the concept that type 2B mutations lower the threshold to relieve autoinhibition. An interesting observation from this study is the broad link between the extent of the loss of autoinhibition and the severity of the clinical type 2B phenotype. Although all type 2B mutants cause bleeding, significant heterogeneity is present among mutations. This study shows for the first time the detailed underlying mechanisms for this process, and moreover, it highlights the importance of the AIM for effective modulation of VWF function. Further work is now required to illuminate how the VWF A1 domain, and the autoinhibitory module, is involved in activating platelets—*that is* now really *the question*.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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https://doi.org/10.1182/blood.2022019577