associated with complete response (CR) and progression-free survival (PFS) after anti-CD19 CAR T-cell therapy in patients with aggressive B-cell non-Hodgkin lymphoma who received either low- or high-intensity Cy/Flu lymphodepletion. In multivariable analysis, they observed that lower prelymphodepletion serum lactate dehydrogenase reflecting less aggressive disease and greater increase in serum monocyte chemoattract protein-1 (MCP-1) concentration in response to lymphodepletion were associated with better probability of achieving a CR and better PFS. In addition, higher serum IL-7 peak after CAR T-cell infusion was also associated with better PFS. Patients receiving high-intensity lymphodepletion had a higher probability of achieving a favorable cytokine profile, defined as day 0 MCP-1 and peak IL-7 concentrations above their respective medians, compared with those receiving low-intensity lymphodepletion. Within the subgroup of patients who received high-intensity lymphodepletion, the PFS benefit was primarily observed in those achieving a favorable cytokine profile, and multivariable modeling showed that a favorable cytokine profile but not lymphodepletion intensity was associated with better PFS. These observations provide novel insights into the mechanisms by which lymphodepleting conditioning might enhance the efficacy of adoptive T-cell therapies.

The results by Hirayama et al suggest that the biological effects of the lymphodepleting regimen are likely more important than the intensity of lymphodepletion to improve CAR T-cell efficacy. Although this study identified novel biomarkers associated with clinical outcome after CAR T-cell therapy, it also raises a number of questions. For example, it is unclear why only a subset of the patients who received high-intensity lymphodepletion achieved a favorable cytokine profile. Whether it is because of differences in the pharmacokinetics of Cy/Flu, baseline immune profiles, or tumor characteristics in these patients needs to be studied. It is unknown how higher MCP-1 and IL-7 concentrations lead to better PFS. It is possible that IL-7 acts by promoting CAR T-cell proliferation, survival, and persistence,[7] but the mechanism for MCP-1 is less obvious. It is also unknown whether the increase in these markers is solely due to lymphodepletion or to other biological effects of the lymphodepletion regimen. Additional studies to elucidate these mechanisms and confirmation in larger studies are needed before considering systemic administration of IL-7 and/or MCP-1 as a strategy to improve CAR-T efficacy. As the kinetics of CAR T-cell expansion in vivo may be dependent on the costimulatory domain used (eg, CD28 vs CD137) as well as the maturation phenotype of the CAR T cells, it remains to be determined whether the biomarkers identified here will have prognostic significance with other CAR T-cell products that may have different CAR design and/or different manufacturing protocol. Studies to investigate whether the effects of an unfavorable cytokine profile can be overcome by additional engineering of the CAR T cells are also warranted. Finally, as outlined above, because accumulating evidence suggests that Cy/Flu and other regimens administered prior to adoptive T-cell therapies likely work by multiple mechanisms besides lymphodepletion, a more appropriate term for these approaches might be "conditioning regimen" rather than "lymphodepleting regimen," and future research should explore strategies to optimize conditioning regimens to improve clinical outcomes after CAR T-cell therapy.

Conflict-of-interest disclosure: S.S.N. has received research support from Kite/Gilead, Celgene, Cellectis, Poseida, Merck, Acerta, Karus, BMS, and Unum Therapeutics and served as consultant and advisory board member for Kite/Gilead, Celgene, Novartis, Unum Therapeutics, Pfizer, Merck, Precision Biosciences, CellMedica, and Incyte. ■

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DOI 10.1182/blood-2019-03-900928

THROMBOSIS AND HEMOSTASIS

Comment on Muia et al, page 1899, and Zhu et al, page 1909

Hairpin and allosteric regulation in ADAMTS13

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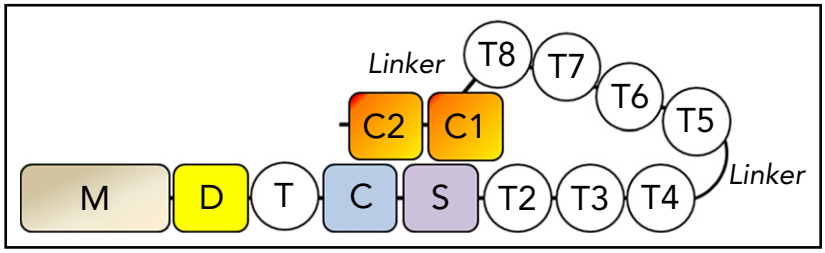
In this issue of *Blood*, Muia et al[1] and Zhu et al,[2] using complementary approaches, provide important insights into the structure and function of ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin-1 repeats, member 13), identifying critical structural features and interactions that allosterically regulate its proteolytic activity on von Willebrand factor (VWF).



Domain organization of ADAMTS13.

Thrombotic thrombocytopenic purpura (TTP) is a devastating and life-threatening disease characterized by the extensive deposition of occlusive platelet and VWF-rich thrombi in the microcirculation. TTP is caused by a deficiency in the VWF-cleaving protease ADAMTS13, which is the only known protein to regulate the adhesive function of VWF. Increasing evidence shows that VWF is involved in many disorders, including arterial and deep vein thrombosis, stroke, atherosclerosis, sickle cell crisis, sepsis, and other thrombotic microangiopathies. There is increasing interest in using ADAMTS13 not only to treat TTP but also to control diverse VWF-related thromboses. Therefore, an improved understanding of its mechanism of action is crucial for its potential usage.

ADAMTS13 is a metalloprotease consisting of the following domains: a metalloprotease (M), disintegrin-like (D), thrombospondin-1 repeat (T), Cys-rich (C), spacer (S), 7 additional thrombospondin-1 repeats (T2-T8), and 2 CUB domains (CUB1-2) (see figure). Although the MDTCS proximal domains are sufficient to cleave a peptide substrate based on the VWF-A2 domain cleavage site sequence, the distal T2-T8 and CUB1-2 domains are required for cleaving VWF multimers under shear stress. Deletion of the distal domains impairs the ability of ADAMTS13 to cleave VWF multimers under shear, but surprisingly enhances its ability to cleave the peptide substrate.[3] This finding led to the idea that the distal domains partially interfere with the proximal domains to maintain the molecule in an autoinhibited state. Consistent with this idea, several monoclonal antibodies specific for the distal domains are also able to relieve autoinhibition.[4] These findings led to the hypothesis that ADAMTS13 assumes a native autoinhibited conformation in which the distal domains interact with the proximal domains, partially inhibiting access to the active center of the protease. Truncation, interaction with antibodies, or change in pH, can allosterically activate the proximal metalloprotease domains. Zanardelli et al showed in surface plasmon resonance studies that the distal domains of ADAMTS13 could bind the D4-CK domain of VWF under low shear stress or static conditions,[5] suggesting that this initial interaction between ADAMTS13 and VWF not only could ctivate ADAMTS13 but also juxtapose ADAMTS13 to further interact with exosites in the A2 domain of VWF that were exposed by high shear stress.[6] If the cleavage site in the VWF-A2 domain is inaccessible, ADAMTS13 would dissociate from VWF and revert to the autoinhibited state.

Crystallographic studies of the DTCS fragment of ADAMTS13 showed that the M and T2 domains that flank the DTCS fragment should be situated at opposite ends of the fragment.[7] To enable CUB1-2 to contact MDTCS, a reversal in the direction of the polypeptide chain must have occurred in the distal domains. In this issue, Muia et al carried out phylogenetic analyses on ADAMTS13 and observed high variability in the number of distal T domains in the ADAMTS13 of 206 species. Pigeon ADAMTS13, which contains only 3 distal T repeats, preserves allosteric regulation. A similar deletion of the T3 to T6 domains in human ADAMTS13, retaining only 3 distal T repeats (T2, T7, and T8), also produces a minimal molecule that preserves allosteric regulation of its peptide cleavage as well as VWF multimer cleavage activities. These studies show that 3 distal T repeats are sufficient to mediate polypeptide chain reversal and preserve interactions that mediate allosteric regulation. In an accompanying paper in this issue, Zhu et al used small-angle X-ray scattering to characterize the molecular envelopes of native and truncated human ADAMTS13 molecules. These studies provided evidence of a hairpin structure in which the 29-residue flexible linker sequence between T4 and T5 apparently formed the apex, and T2-T4 and T5-T8 formed the 2 arms of the hairpin. They also provided supporting evidence for the stable interaction of MDTCS with distal T7, T8, and CUB1. Another 58-residue linker sequence between T8 and CUB1 also facilitated interaction of CUB1 with MDTCS to mediate allosteric regulation. Although not at atomic resolution, these studies provided key evidence of intramolecular interactions essential for allosteric regulation.

Information from the 2 papers provides important insights into the molecular mechanism of allosteric regulation in ADAMTS13. This mechanism includes a substrate-induced conformational change that converts ADAMTS13 into an activated state, primed to engage and cleave the substrate with high specificity. This improved understanding is crucial for the development of ADAMTS13 as a therapeutic agent to treat VWF-related thrombotic disorders.

Conflict-of-interest disclosure: D.W.C. declares no competing financial interests. ■

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DOI 10.1182/blood-2019-02-900563