interactions with Trp60D and Trp215, respectively. These structural similarities raised concern that aDabi-Fab may interact adversely with some of thrombin's many substrates. Although the aDabi-Fab lacks proteolytic machinery, it might contain some structural elements that could, for example, compete in the exosite reactions of the protease so important for its function.[5] Unwanted anticoagulant or procoagulant activities of aDabi-Fab could compromise its safety in clinical use. It was therefore important to eliminate the possibility of these secondary interactions by demonstrating absence of binding of aDabi-Fab to physiological substrates of thrombin using surface plasmon resonance. Likewise, aDabi-Fab was shown to not directly influence platelet aggregation. Thus, aDabi-Fab would appear to competitively inhibit the binding of thrombin to dabigatran, while avoiding mimicking any of the exosite driven activities of thrombin. Schiele et al[1] further supported this contention by demonstrating with thrombin-dependent in vitro functional clotting assays that aDabi-Fab is not active in the absence of dabigatran. Finally, it was shown that aDabi-Fab was also an effective in vivo antidote of dabigatran anticoagulant activity in rats, in which rapid reversal of anticoagulant effect in clotting assays was demonstrated.

Crucial to the success of this antidote to dabigatran will be additional studies of anticoagulant reversal in animal models of bleeding and, ultimately, in clinical investigation and trials of humans. In the meantime, this study does represent an important step in the development and use of the new oral anticoagulant agents. It shows that provision of a specific antidote is feasible and therefore suggests an effective means of controlling the anticoagulants in unpredictable clinical situations. If ultimately proven safe in human use, this will resolve what has until now been a pivotal limitation of these agents and provide an effective and safe strategy for situations in which immediate reversal of the anticoagulant effect is required.

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

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● ● ● **TRANSPLANTATION** ▬

Comment on Blyth et al, page 3745

Pharmacotherapy versus T lymphocytes for CMV

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In this issue of *Blood*, Blyth et al report a phase 2 study in 50 allogeneic hematopoietic stem cell transplant (HSCT) recipients who received donor-derived cytomegalovirus (CMV)–specific cytotoxic T cells (CTLs) and compare outcomes with a group of concomitant controls who were transplanted at the trial centers but who did not receive CTLs.[1]

They show no significant difference in the incidence of acute or chronic graft-versus-host disease (GVHD) between groups or in the rate of CMV reactivation, but they observed a significant reduction in the percentage of patients who required CMV-directed antiviral therapy and in the total number of treatment days per patient in the cohort receiving CTLs. Notably, administration of virus-specific T cells did not induce acute or chronic GVHD, confirming other reports that virus-specific CTLs are not functionally alloreactive in vivo.[2] The authors therefore conclude that donor-derived CMV-specific T cells reduce the requirement for CMV-directed pharmacotherapy after allogeneic stem cell transplantation. Although their conclusions are limited by their nonrandomized trial design, this is nonetheless the first publication to compare outcome in patients who received donor CMV CTLs with a control group.

It has been 20 years since the first reports showed that donor-derived CMV-specific CTLs reconstituted protective donor immunity to CMV after allogeneic stem cell transplantation.[3] These observations have since been confirmed in a number of studies that include several hundred patients,[4,5] which justifies definitive testing in late-phase randomized trials. The report from Blyth et al, however, suggests that CMV reactivation rate should not be the end point for such studies because they observed no significant difference between CTL recipients and controls. This finding is expected, because even normal seropositive individuals have viral reactivations that are controlled by expansion of memory T cells. The investigators instead observed a difference in the requirement for CMV-directed antiviral therapy, since a higher percentage of patients who did not receive CTLs eventually had to receive such therapy and for a longer median duration than those who received CTLs. One limitation in the Blyth et al study is that decisions on starting and stopping antiviral therapy were made by the individual clinicians on the basis of their standards of practice, illustrating the importance of careful design of future randomized trials to ensure that such decisions follow a standardized procedure. It will also be beneficial to design future randomized studies to include comparative effectiveness analyses so the effect of CTLs on overall cost of antiviral and supportive therapy (including granulocyte colony-stimulating factor for drug-induced neutropenia) can be measured.

Before large-scale pivotal clinical studies of CTLs can be initiated, it will be necessary to simplify the manufacturing process to eliminate the requirement for viruses during production. Blyth et el initially used dendritic cells pulsed with an HLA2-restricted immunodominant peptide NLV from the CMV pp65 antigen to stimulate CTLs but then transitioned their

manufacturing methodology to use dendritic cells genetically modified by an adenoviral vector encoding the full pp65 antigen. Their first approach was restricted to donors who were HLA A0201–positive and produced CTLs with restricted specificity that may have been predisposed to escape mutants. The second process, however, although it produces a broader immune response, is lengthy and requires live virus. Three alternative approaches may overcome these limitations. The first uses clinical-grade overlapping peptide pools derived from viral antigens to expand T cells with multiple antigen specificities.[6,7] Alternatively, peptide-stimulated T cells can be isolated after interferon gamma (IFN-γ) capture. Both have shown encouraging responses as prophylaxis or treatment of CMV infection.[8,9] Finally, investigators have directly selected T cells that are reactive with CMV peptides by using magnetically labeled peptide multimers (streptamers). When CMV-specific T cells from the transplant donors were selected in this way and transferred to HSCT recipients with recurrent CMV reactivations, Schmitt et al observed reconstitution of CMV immunity and clearance of elevated CMV viral load.[10] Although the limitations of this approach include the large volume of

donor blood required for manufacturing, the requirement for donors to express HLA alleles for which viral peptides are available and to have a high frequency of circulating CMV-specific T cells, streptamer selection is being tested in a phase 3 randomized trial for CMV reactivation (NCT01077908).

The availability of several CMV-specific T-cell products manufactured with simpler methodology is finally allowing late-phase testing of CMV-specific CTLs to definitively show that they can prevent and treat CMV disease after HSCT, and Blyth et al have provided a roadmap for designing such studies by showing the importance of judicious choice of end points, the need for standard criteria for instituting and stopping antiviral drugs, and the desirability of incorporating comparative effectiveness analyses.

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