an analogous concept to inhibit both thrombin function and its formation with a bifunctional inhibitor targeting thrombin and factor Xa, the proteinase required for prothrombin activation. Although their approach mirrors the multireactant targeting strategy of endogenous regulation, the twist here is that the bifunctional EP217609 molecule inhibits its 2 targets by different mechanisms.

EP217609 contains a fondaparinux-like component fused through a spacer bearing a biotin moiety to a derivative of N-α-(2-naphthylsulfonylglycyl)-4-amidinophenylalanine piperidide (NAPAP). The fondaparinux-like functionality is intended to bind endogenous antithrombin III with high affinity and enhance the suicide inactivation of factor Xa.[3] On the other hand, the NAPAP analog is intended to reversibly bind to the active site of thrombin and inhibit function.[4] The biotin moiety provides a potential handle for the neutralization of the inhibitory properties of EP217609 by abstraction through high-affinity ligation with avidin. Olson et al's design draws on the established efficacy of heparin pentasaccharide derivatives as anticoagulants without many of the drawbacks of unfractionated heparin but also seeks to resolve the problem of "thrombin-rebound" associated with the cessation of heparin and pentasaccharide therapy.[5]

The cleverest of intentions in designing novel inhibitors can be laid to waste by the complexities of coagulation proteinase enzymology. For example, there is no reason to imagine that specificity, selectivity, or other beneficial features of the individual functional groups will be preserved in the fusion construct. There is also the question of whether the NAPAP-like moiety can effectively inhibit thrombin when EP217609 circulates bound to antithrombin III. Despite these qualifications, the approach seemingly works! A prior version of such a fusion-lacking biotin has shown to be a superior anticoagulant in comparison to its components in animal models of arterial and venous thrombosis and also to prevent "thrombin rebound."[6] A phase 1 study has reported EP217609 to be well tolerated in healthy subjects with neutralization of its anticoagulant effects after the administration of avidin.[7] The drug is now in phase 2 clinical trials for cardiopulmonary bypass.

In a second twist that runs counter to the typical course of anticoagulant development, it is only now that proper expertise has been applied to establish the biochemical basis for the apparent efficacy of EP217609. Olson et al, leading investigators in proteinase, serpin, and heparin enzymology, present a thorough study of EP217609 and related compounds to provide the quantitative basis for its selectivity and the function of the components within the fusion construct. Unexpectedly, they find that incorporation of the NAPAP analog into the fusion increases its affinity for thrombin into the 30pM range, yielding an inhibitor that ranks among the highest affinity inhibitors known for this proteinase. High selectivity for thrombin is evident from its 1000-fold weaker binding constant for the next ranked proteinase of relevance to hemostasis. The fondaparinux-like functionality is also preserved, allowing EP217609 to bind antithrombin with nM affinity and act with similar selectivity to accelerate the inhibition of Xa over other proteinase targets. Importantly, they establish only modest deleterious linkage effects, within a 5-fold range, of ligation at one site in affecting the functionality of the second and vice versa. Finally, they also establish that the basis for inhibitor neutralization by avidin results from an approximately 100-fold decrease in thrombin inhibition and an approximately 30-fold decrease in its binding to antithrombin III. This study highlights the power of enzymology, done expertly, in establishing the basis for the selectivity of this novel class of dual-action anticoagulants and provides the mechanistic foundation to show the way forward for their development as therapeutics.

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● ● ● TRANSPLANTATION

Comment on Kanda et al, page 2409

Related or unrelated donor

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In this issue of *Blood*, Kanda et al have analyzed data from the Japanese Hematopoietic Cell Transplant Registry to address an important question about the risks associated with hematopoietic cell grafts from HLA 1-antigen mismatched related donors contrasted with 8 of 8 HLA allele matched unrelated donors.[1]

The study reported by Kanda and colleagues addressed the question of whether a serologically defined 1-antigen HLA-A, B, or DRB mismatched related donor should be preferred to an "8/8" HLA-A, B, C, DR allele MUD. The study population consisted of 327 1-HLA antigen mismatched related donor transplant recipients and 452 8/8-HLA allele matched unrelated donor transplant recipients accessed through the Japanese Transplant Registry Unified Management Program (TRUMP), which includes data from the Japan Society for Hematopoietic Cell Transplantation (JSHCT) and the Japan Marrow Donor Program (JMDP). Patients underwent transplantation for the treatment of acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia, or myelodysplasia between January 2001 and December 2008. Patients received T cell–replete marrow or blood stem cells and 90% to 97% received a conventional cyclosporine- or Tacrolimus-based regimen for GVHD prophylaxis. The related and unrelated cases differed for certain

pretransplantation risk factors. One-antigen mismatched related donor recipients were more likely to have high-risk disease, to have received peripheral blood rather than marrow stem cells, and be a male receiving a transplant from a female donor than MUD recipients. Results of a multivariate analysis showed that 1-HLA antigen mismatched related cases overall had a lower survival rate compared with 8/8-MUD cases (HR = 1.49; CI = 1.19-1.86). The related donor mismatching effect was most prominent in patients receiving a transplant for standard-risk disease (OR = 1.72; CI = 1.24-2.39). Based on these results, Kanda et al conclude that an 8/8-HLA allele matched unrelated donor should be preferentially selected over a related donor mismatched for one serologically defined HLA-A, B, or DR antigen.[1]

Kanda et al also examined risk of mortality for 1-antigen mismatched related donor transplantations according to mismatched locus, and found that the greatest risk of reduced survival was associated with mismatching for HLA-B[1]. HLA-C locus typing, which was available for a subset of the related patient-donor pairs, revealed that most of the cases mismatched for HLA-B were also mismatched for HLA-C, implying that the remainder of the presumed 1-antigen HLA-B mismatches were probably two antigen mismatches.

The HLA matching data available to Kanda et al for this retrospective analysis were generated with different HLA typing methods.[1] At the time these transplants were performed, the standard clinical practice for HLA typing for the selection of related donor searches was based on serology and matching for the HLA-A, B, and DR antigens, while HLA typing for the selection of an unrelated donor was based on DNA-based genomic typing and matching for HLA-A, B, C, and DRB1 alleles.

Related donor transplant studies from the 1980s found that risk of severe GVHD and mortality was greater with increasing disparity for serologically defined HLA antigens.[2] During the pre-unrelated donor era, mismatched related donor transplantations limited to disparity for 1-HLA antigen were generally deemed acceptable for patients failing conventional therapy when an HLA identical sibling was not available.[2-4]

The utility of DNA-based genomic approaches to HLA genotyping and the importance of allele level HLA matching was initially demonstrated in studies of unrelated donor transplantations,[5,6] and subsequently reinforced by HLA studies facilitated by large transplant registry cohorts.[7-9] Improved statistical power has also made it possible to estimate more accurately the risks of mismatching for antigens and alleles of individual HLA loci, and demonstrated that mismatching for ≥ 2 alleles is associated with progressively increasing risk of severe GVHD and mortality.[6-9]

The analysis presented by Kanda et al leaves open the possibility that a 1-allele HLA-A, B, C, or DR mismatched related donor might be preferred or at least equal to an 8/8-HLA matched unrelated donor. The probability of finding this type of a related alternative donor, however, is very low. Fortunately, recently introduced novel immune suppression strategies have been successful in overcoming the HLA barrier and achieving sustained engraftment without increased risk of severe GVHD, and this has been accomplished with HLA fully mismatched haploidentical donors.[10,11] Future studies are need to confirm these preliminary results, define in more detail the disease-specific indications, and determine the relative advantages of the different alternative donor sources including HLA haploidentical related, matched unrelated, and also umbilical cord blood.

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