used as means to isolate both multipotential progenitors as well as transplantable HSCs in the fetal liver.

In the AGM (aorta-gonad mesonephros region) where HSCs emerge, ESAM$^+$ cells coexpressed c-kit and the endothelial markers Tie2, CD34, and PECAM that are known to be present in fetal HSCs. Interestingly, ESAM$^+$ cells formed a distinct subpopulation that selected all hematopoietic progenitors with lymphoid potential, which is of importance as the establishment of lymphoid potential during the onset of fetal hematopoiesis distinguishes the true multipotential hematopoietic stem and progenitor cells from the earlier, yolk sac–derived progenitors that have limited lifespan and developmental potential. Comparison with the yolk sac further suggested that ESAM^{Hi}ckit$^+$Tie2^{Hi} cells represent emerging HSCs whereas the myeloerythroid progenitor cells are harbored in ESAM^{Lo}ckit^{Hi}Tie2^{Lo} fraction. In contrast to many endothelial markers that become downregulated in HSCs later during development, ESAM expression was not only maintained in the Lin$^-$ckit$^+$Sca1^{Hi} HSC fraction in the adult bone marrow, but its expression level even increased in aging mice.

The finding that ESAM is faithfully expressed in long-term repopulating HSCs and their immediate precursors throughout ontogeny may have many important applications. Purification of hematopoietic cells based on ESAM expression may facilitate investigation of the mechanisms that dictate cell fate toward multipotential HSCs rather than short-lived myeloerythroid progenitors during embryogenesis, and assessment of the development of these distinct hematopoietic programs in vivo as well as in vitro from embryonic stem cells or induced pluripotent cells. Furthermore, the high level and fairly specific expression of ESAM in multipotential hematopoietic cells could potentially be used for tracking HSCs during development and localizing them in distinct cellular niches by various imaging techniques. In addition, unraveling the molecular networks that regulate ESAM expression may give important clues about the cell intrinsic programs that govern the identity and functional properties of multipotential hematopoietic stem and progenitor cells. The importance of the findings in this study is further enhanced by recent findings that ESAM expression in HSCs appears to be conserved between different mouse strains and across species, as human HSCs were also shown to express this marker.[8] Another important question is whether ESAM is functionally required for establishing and maintaining HSC properties, unlike most surface markers associated with HSC potential. Although ESAM$^{-/-}$ mice are viable and fertible and do not exhibit major hematopoietic failure, their hematopoietic lineage distribution is skewed and the number of their HSCs appears to be even slightly higher, implying that ESAM may be functionally involved in HSC-niche interactions in the bone marrow.[8] Nevertheless, as a novel HSC marker with unique characteristics, ESAM is a highly appreciated addition to the "hematopoietic tool box" and will likely benefit both basic and translational studies on hematopoietic stem cells.

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

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● ● ● CLINICAL TRIALS

Comment on Mielcarek et al, page 2888

Where is the start line?

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In this issue of *Blood*, Mielcarek and colleagues report on the results of a retrospective analysis of the outcomes of initial acute GVHD therapy.

Despite advances in the management of complications related to hematopoietic cell transplantation, treatment of acute graft-versus-host disease (GVHD) remains suboptimal. Corticosteroids are the primary front-line therapy for acute GVHD at a standard dose of 2 mg/kg per day prednisone, with response rates of about 50%.[1-3] In this issue of *Blood*, Mielcarek et al report on the outcomes of initial acute GVHD therapy among 773 transplantation patients at the Fred Hutchinson Cancer Research Center from 2000 to 2005.[4] Patients were treated with either the standard dose of 2 mg/kg per day prednisone or low-dose prednisone (1 mg/kg per day) at the discretion of the attending physician. By day 100 after initiating therapy, patients treated in the low-dose group received a mean cumulative dose of 44 mg/kg compared with 87 mg/kg in the standard-dose group. Adjusted outcomes between the 2 groups were not statistically different. In multivariate analysis, treatment with low-dose steroids was associated with a reduction in prolonged hospitalization and a trend toward lower risk for invasive fungal infections. The authors conclude that initial treatment with low-dose prednisone did not compromise acute GVHD control or mortality and was associated with decreased toxicity.

This article addresses an important question regarding the preferred initial steroid dose for patients with acute GVHD. It is clearly desirable to use as low a dose of steroids as possible to reduce the risk of toxicity. However, this goal must be balanced with the need to attain early and durable control of acute GVHD. This single

center large study provides implied uniformity of supportive care as a strength. However, it is important to note that these conclusions must be restricted to patients with grade I-II acute GVHD, as less than 3% of patients treated with low-dose prednisone had severe (grades III-IV) acute GVHD. In addition, this analysis was restricted to adults. Although age has not been a consistent factor in the response to acute GVHD therapy, future trials should include children.

Inherent in the retrospective design of this study is the presence of potentially confounding variables. The starting steroid dose was chosen at the discretion of the attending physician and as the authors discuss, there must certainly have been a bias toward giving the higher dose steroids to patients with more fulminant GVHD. This is suggested by the fact that only 2.6% of the low-dose steroid patients had severe GVHD compared with 16.3% patients in the standard-dose steroid group.

The 2 treatment groups also differed in a number of important clinical variables. A higher proportion of the low-dose patients had more frequent gut and only limited or no skin GVHD compared with the standard-dose group. The high incidence of gut GVHD in low-dose steroid recipients led to greater use of oral beclomethasone dipropionate, an agent that has been shown to incur a steroid sparing effect and survival advantage in an earlier study.[5]

Notably, despite being associated with less toxicity, the use of low-dose steroids was not associated with less transplantation-related mortality or overall mortality. Although relapse did not differ between all patients in the 2 steroid groups, in a subgroup analysis, risk of relapse was 1.5-fold higher in low-dose steroid recipients than in standard-dose recipients. The groups were balanced with respect to disease risk; however, future prospective trials are needed to determine the impact of steroid dose on this outcome.

In the race to improve outcomes in the treatment of acute GVHD, Mielcarek et al provide compelling evidence that, at least for adult patients with grades I-II acute GVHD, the start line may have been moved back to 1 mg/kg per day. Future prospective studies are warranted to determine the optimal starting dose and to carefully evaluate its consequences for both adults and children and especially for those with severe GVHD. In addition, further identification of patient and graft characteristics predictive of response will lead to a more tailored approach to acute GVHD therapy in the future.

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

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● ● ● THROMBOSIS & HEMOSTASIS

Comment on van den Biggelaar et al, page 3102

VWF and FVIII: the origins of a great friendship

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In this issue of *Blood*, van den Biggelaar and colleagues demonstrate the cotargeting of FVIII and VWF to secretory granules in the absence of a high-affinity interactions between VWF and FVIII, using recombinant VWF type 2N variants.

In addition to mediating the adhesion of platelets to subendothelial tissue at the site of vascular injury, von Willebrand factor (VWF) also serves as the carrier protein for coagulation factor VIII (FVIII) in plasma. The initial point of physical association between these 2 proteins remains unclear. When desmopressin (DDAVP) is administered to mild hemophilia or von Willebrand disease (VWD) patients, both FVIII and VWF are released into plasma. Presumably, VWF is released from endothelial cell Weibel-Palade bodies. While the liver is the major site of FVIII synthesis, the cell within the liver producing FVIII has not been definitively identified. Recent publications suggest that FVIII may be present in selected endothelial populations including human lung microvascular endothelial cells and murine sinusoidal endothelial cells.[1,2]

Several studies have demonstrated that expression of FVIII in VWF-producing cells, such as HUVEC, cultured megakaryocytes, and platelets, results in colocalized storage of VWF and FVIII in endothelial cell Weibel-Palade bodies or in α-granules of platelets and megakaryocytes.[3-6] These studies have suggested that FVIII-regulated storage is secondary to VWF storage and results from a high-affinity VWF-FVIII association early in the secretory pathway. Seemingly in contrast to this conclusion is the observed concomitant release of both VWF and FVIII in type 2N VWD patients after DDAVP administration.[7] Type 2N VWD variants are characterized by a markedly decreased binding affinity for FVIII caused by homozygous or compound heterozygous mutations in VWF that impair FVIII binding. In these patients, the loss of high-affinity VWF-FVIII binding does not promote stabilization of FVIII in plasma and FVIII is degraded fairly rapidly. Given the lack of FVIII binding to 2N variants of VWF, the cellular source of the DDAVP-releasable pool of FVIII is an open question.

The current study by van den Biggelaar et al, which uses several recombinant type 2N VWF variants, provides valuable insight into the relationship between assembly of the VWF/FVIII complex and the cotrafficking of VWF and FVIII to the regulated secretory pathway.[8] The authors employ complementary techniques, Surface Plasmon Resonance (SPR) and a pseudo-equilibrium binding assay to demonstrate a range of mildly to severely reduced FVIII-binding affinity of the 2N variants. Using an HEK293 cell expression system, the authors elegantly demonstrate that, despite the FVIII binding defects, all type 2N variants were able to target coexpressed FVIII and P-selectin to the VWF-containing pseudo–Weibel-Palade bodies. This finally provides a mechanistic basis for the observed DDAVP-induced release of FVIII and VWF in type 2N VWD patients.