indicate some possible directions for future investigations. However, to consider this group of CD30⁺ DLBCL as a distinct entity is premature at this point. The findings of this study should be validated in future independent studies. It is important that PMBCL be carefully excluded, possibly by using defined molecular signatures from previous GEP studies, as PMBCL can present as extramedullary lesions.

With the limitations of single-marker IHC studies as discussed previously, should we still continue with this type of investigation? There are likely to be a number of biomarkers representing major biological determinants that could influence outcome even in the presence of other confounding factors. Their measurement could have immediate impact on patient management. Preferably, multiple such markers can be identified and assayed as a panel to determine if the panel is more robust and have greater predictive power compared with individual markers. A multimarker approach has been attempted in a number of studies: LIM Domain Only 2 (LMO2)/TNFRSF9,9 MYC/BCL2,5 and cell of origin/microenvironment.10 However, this could well be a transitional stage. We should move toward a more comprehensive, mechanism-based evaluation. The goal of our future studies should not only be finding reliable predictors of outcome, but the predictors should inform us of the mechanisms responsible for adverse outcome and the options available to improve it. The tools for this modern investigation, such as gene and micro RNA expression profiling and next-generation sequencing, are available. It is possible to construct a comprehensive picture for each tumor that will help us understand its strengths and vulnerabilities and its response to therapeutic perturbations. However, this information needs to be coupled with well-designed, large clinical studies, and a partnership of multiple disciplines and institutions is essential. The authors of this paper should be applauded for organizing such a large multi-institutional effort that makes their study feasible.

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

REFERENCES
transfected cell systems that cannot reproduce key aspects of von Willebrand factor (VWF) expression in endothelium.

VWD is a relatively common bleeding disorder that is caused by mutations in the gene encoding VWF. VWF has binding sites for several physiological ligands, and mutations can impair VWF assembly, intracellular storage, secretion, and biological function in a bewildering number of ways to produce complex disease phenotypes. Furthermore, VWF is a multimeric protein assembled from the products of 2 alleles, which makes it possible for a single bad allele to disrupt the entire protein.

We have made a lot of progress understanding VWD by expressing recombinant mutant VWF in transfected cells and seeing what happens. For example, these studies have shown how defects in multimer assembly or stability lead to VWD type 2A, how gain-of-function mutations with exaggerated affinity for platelets produce VWD type 2B, and how defects in binding to factor VIII cause VWD type 2N, an autosomal recessive mimic of hemophilia A. In the last few years, we have also learned a great deal about the pathophysiology of VWD type 1, which is the most common type of VWD but in many ways is also the most perplexing variant.

However, several inherent features of heterologous expression systems limit what can be learned. Most nonendothelial cell types do not target recombinant VWF to Weibel-Palade-like organelles and cannot be used to assess VWF storage or regulated secretion. Patients are usually heterozygous, but modeling the heterozygous state in transfected cells is difficult. Transfected cDNA constructs usually are overexpressed with strong viral promoters, which can introduce artifacts. In addition, focusing exclusively on exons ignores mutations that may reduce transcription, alter splicing, or change mRNA stability. In a few fortuitous cases, these limitations have been overcome by characterizing the behavior of mutant VWF genes in human umbilical vein endothelial cells that were obtained as the patient was born, but this approach is rarely feasible.

Alternatively, cells can be isolated from peripheral blood that have many properties of vascular endothelial cells. These blood outgrowth endothelial cells (BOEC) express endothelial cell surface antigens, store VWF in Weibel-Palade bodies, form capillary-like tubules when cultured in Matrigel, organize into blood vessels in vivo, and proliferate in culture for several passages. In a nice proof-of-principle study, BOECs from a compound heterozygous patient with VWD type 2N were used to identify abnormal VWF mRNA splicing, a premature stop codon, markedly impaired intracellular transport, and abnormal storage of VWF in Weibel-Palade bodies. None of these effects could be discovered by the expression of sequence variants in the exons of the patient’s VWF genes.

Wang et al and Starke et al have built on this foundation, studying BOECs from total of 8 patients with VWD type 1 and 5 patients with VWD type 2 variants. The phenotype of BOECs was stable for at least 8 passages in culture. For a single heterozygous patient, polymerase chain reaction demonstrated reduced transcription of the mutant VWF gene. Although patients with VWD type 1 had decreased levels of VWF mRNA, as might be expected, a striking finding was that many had complex phenotypes with increased intracellular retention in the endoplasmic reticulum, abnormal storage in misshapen Weibel-Palade bodies, and impaired secretion of functional VWF “strings” on the endothelial cell surface. The behavior of VWF in BOECs appears to correlate well with clinical features of VWD, including whether a patient responds satisfactorily to treatment with 1-desamino-8-D-arginine vasopressin (desmopressin).

These reports demonstrate that BOECs can provide unprecedented insight into the pathogenesis of VWD. However, additional experience will be needed to assess the reliability of the approach. The reproducibility of BOEC phenotypes needs to be established more completely, comparing different isolates from the same donor. Success in isolating BOECs seems to be donor-dependent. VWF expression varied significantly between healthy donors, and some donors never yielded BOECs. Nevertheless, BOECs enable studies of VWF phenotypes that are not otherwise accessible. For the first time, it appears possible to analyze the interactions between VWF alleles in a patient’s own endothelial cells to evaluate the interplay between alleles at the level of transcription, splicing, mRNA stability, multimer assembly, Weibel-Palade body morphology, and response to secretagogues. In this respect, the study of BOECs may represent a new standard for the characterization of mutations in VWD, and perhaps for other diseases that affect endothelial cells.

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

REFERENCES


von Willebrand factor in its native environment

J. Evan Sadler