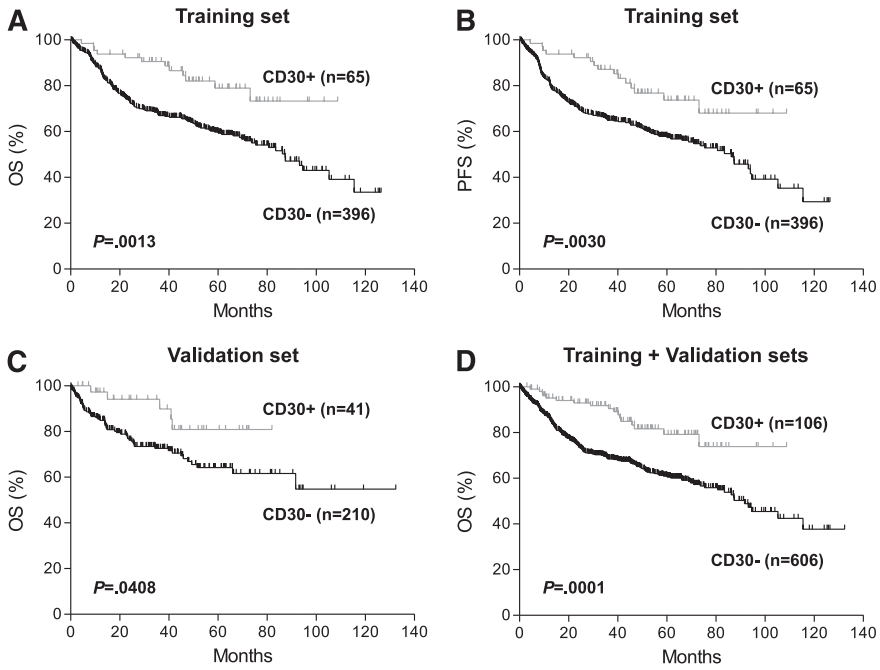

Prognostic impact of CD30 expression in de novo DLBCL. (A-B) Overall survival (OS) (A) and progression-free survival (PFS) (B) of patients with $CD30^+$ vs $CD30^-$ DLBCL in the training set. (C) OS of patients with $CD30^+$ vs $CD30^-$ DLBCL in the validation set. These patients were part of an independent cohort of 442 patients with available survival information (supplemental Table 1). (D) OS of patients with $CD30^+$ vs $CD30^-$ DLBCL in combined training and validation sets. See Figure 2 in the article by Hu et al that begins on page 2715.

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 extramediastinal 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

1. Hu S, Xu-Monette ZY, Balasubramanyam A, et al. CD30 expression defines a novel subset of diffuse large B-cell lymphoma with favorable prognosis and distinct gene expression signature: a report from The International DLBCL Rituximab-CHOP Consortium Program Study. *Blood.* 2013;121(14):2715-2724.
2. Sehn LH, Berry B, Chhanabhai M, et al. The revised International Prognostic Index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP. *Blood.* 2007; 109(5):1857-1861.
3. Perry AM, Mitrovic Z, Chan WC. Biological prognostic markers in diffuse large B-cell lymphoma. *Cancer Contr.* 2012;19(3):214-226.
4. Iqbal J, Meyer PN, Smith LM, et al. BCL2 predicts survival in germinal center B-cell-like diffuse large B-cell lymphoma treated with CHOP-like therapy and rituximab. *Clin Cancer Res.* 2011;17(24):7785-7795.
5. Johnson NA, Slack GW, Savage KJ, et al. Concurrent expression of MYC and BCL2 in diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. *J Clin Oncol.* 2012;30(28):3452-3459.
6. Dang CV, O'Donnell KA, Juopperi T. The great MYC escape in tumorigenesis. *Cancer Cell.* 2005;8(3):177-178.
7. Lenz G, Wright G, Dave SS, et al. Lymphoma/Leukemia Molecular Profiling Project. Stromal gene signatures in large-B-cell lymphomas. *N Engl J Med.* 2008; 359(22):2313-2323.
8. Rimsza LM, Roberts RA, Miller TP, et al. Loss of MHC class II gene and protein expression in diffuse large B-cell lymphoma is related to decreased tumor immunosurveillance and poor patient survival regardless of other prognostic factors: a follow-up study from the Leukemia and Lymphoma Molecular Profiling Project. *Blood.* 2004;103(11):4251-4258.
9. Alizadeh AA, Gentles AJ, Alencar AJ, et al. Prediction of survival in diffuse large B-cell lymphoma based on the expression of 2 genes reflecting tumor and microenvironment. *Blood.* 2011;118(5):1350-1358.
10. Perry AM, Cardesa-Salzmann TM, Meyer PN, et al. A new biologic prognostic model based on immunohistochemistry predicts survival in patients with diffuse large B-cell lymphoma. *Blood.* 2012;120(11):2290-2296.

● ● ● THROMBOSIS & HEMOSTASIS

Comment on Starke et al, page 2773, and on Wang et al, page 2762

von Willebrand factor in its native environment

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In this issue of *Blood*, Starke et al[1] and Wang et al[2] study endothelial cells cultured directly from the blood of patients with von Willebrand disease (VWD), and thereby overcome some limitation of studying VWD mutations in

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,[3-5] but this approach is rarely feasible.

Alternatively, cells can be isolated from peripheral blood that have many properties of vascular endothelial cells.[6] 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.[7] 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.[8]

Wang et al[2] and Starke et al[1] 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.

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REFERENCES

1. Starke RD, Paschalaki KE, Dyer CE, et al. Cellular and molecular basis of von Willebrand disease: studies on blood outgrowth endothelial cells. *Blood.* 2013;121(14):2773-2784.

2. Wang J-W, Bouwens EAM, Pintao MC, et al. Analysis of storage and secretion of von Willebrand factor in blood outgrowth endothelial cells derived from von Willebrand disease patients. *Blood.* 2013;121(14):2762-2772.

3. Booyse FM, Quarfoot AJ, Chediak J, Stemerman MB, Maciag T. Characterization and properties of cultured human von Willebrand umbilical vein endothelial cells. *Blood.* 1981;58(4):788-796.

4. Levene RB, Booyse FM, Chediak J, Zimmerman TS, Livingston DM, Lynch DC. Expression of abnormal von Willebrand factor by endothelial cells from a patient with type IIA von Willebrand disease. *Proc Natl Acad Sci USA.* 1987;84(18):6550-6554.

5. de Groot PG, Federici AB, de Boer HC, d'Alessio P, Mannucci PM, Sixma JJ. von Willebrand factor synthesized by endothelial cells from a patient with type IIB von Willebrand disease supports platelet adhesion normally but has an increased affinity for platelets. *Proc Natl Acad Sci USA.* 1989;86(10):3793-3797.

6. Lin Y, Weisdorf DJ, Solovey A, Hebbel RP. Origins of circulating endothelial cells and endothelial outgrowth from blood. *J Clin Invest.* 2000;105(1):71-77.

7. Hirschi KK, Ingram DA, Yoder MC. Assessing identity, phenotype, and fate of endothelial progenitor cells. *Arterioscler Thromb Vasc Biol.* 2008;28(9):1584-1595.

8. Berber E, James PD, Hough C, Lillicrap D. An assessment of the pathogenic significance of the R924Q von Willebrand factor substitution. *J Thromb Haemost.* 2009;7(10):1672-1679.