been associated with increased risk of venous thromboembolism and atherosclerotic disease.[7,8] Furthermore, complete TFPI deficiency seems to be incompatible with life because no cases have been reported, and animal models completely lacking TFPI result in embryonic lethality. By maintaining extraembryonic TFPI expression and selectively ablating the K1 domain, Castillo et al strategically developed a murine cell line with severe TFPI deficiency. These mice exhibited a prothrombotic phenotype with increased thrombin generation potential in vitro, renal fibrin deposits, and rare cases of brain ischemia.[9] Despite interspecies differences in TFPI, this model will be useful in investigating the pathophysiology of TFPI, a topic of paramount importance because more anti-TFPI therapeutics are being evaluated in clinical trials. Although no thrombotic events or thrombotic microangiopathy were evident in the 2 trials reported by Shapiro et al, the dose-dependent elevation of D-dimer and prothrombin 1+2 fragments raise concerns regarding the potential thrombotic propensity of an iatrogenic TFPI deficiency state, acknowledging the baseline elevation of D-dimer in some cases before treatment with concizumab. One healthy volunteer in the Explorer 1 trial developed superficial thrombophlebitis.[10] In addition, because of concern for less conventional clearance, reduction in fibrinogen, and elevation of D-dimers, more frequent injections of lower concizumab doses were chosen for the phase 2 trials. For the upcoming phase 3 trial, the authors plan to use a loading dose and to start at 0.25 mg/kg once per day. The sequential details in clot formation have not been fully elucidated, and elevated D-dimers may represent transient hypercoagulability with no thrombotic consequence. This particular concern requires careful monitoring.

Ultimately, the results of these trials will provide clinical proof-of-concept regarding anti-TFPI therapy in hemophilia patients and support the further evaluation of concizumab. If further studies confirm the safety and efficacy of anti-TFPI therapies, whether or not these agents can supplant emicizumab in the HA space remains to be determined. Perhaps more importantly, anti-TFPI therapy may have widespread applications in other rare bleeding disorders such as Glanzmann thrombasthenia and Bernard–Soulier syndrome and would provide a viable alternative to recombinant FVIIa prophylaxis for HB patients with inhibitors. As it stands, the care of patients with HA or HB has quickly shifted from conventional therapeutic protein replacement to the mitigation of bleeding via targeted FVIII mimetics, rebalancing natural anticoagulants, and viral vector–mediated gene therapy. It is clear that the next decade of hemophilia care will focus on normalizing the bleeding phenotype because the community standard has shifted from reducing life-threatening bleeding to a more normal life without bleeding events.

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Platelet alloantibody detection: moving ahead

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In this issue of *Blood*, Zhang et al expand on a previous study that described the detection of antibodies against human platelet alloantigens by using gene-edited stem cell–derived target cells.[1]

Antibodies against human platelet alloantigens (HPAs) present on membrane glycoproteins represent a major health problem because they are capable of generating an immune response against platelets that can, in turn, cause a variety of bleeding disorders.[2,3] In addition to genotyping, phenotypic detection of anti-HPA alloantibodies is important for the proper diagnosis, treatment, and/or prevention of diseases such as fetal and neonatal alloimmune thrombocytopenia, posttransfusion purpura, and platelet transfusion refractoriness,

which can have severe clinical outcomes. Currently, platelet alloantigen phenotyping is performed by detecting antibodies against whole platelets as a source of antigens or by monoclonal antibody-based antigen capture assays, each of which have problems with specificity and practicality.[4]

Most HPAs are the result of single nucleotide polymorphisms that have been described on 6 platelet glycoproteins. To date, 6 biallelic HPA isoforms (HPA-1, -2, -3, -4, -5, and -15) and 26 single low-frequency antigens have been identified. Detection of alloantibodies against HPA-3 and HPA-9, which both reside on the C terminus of GPIIb, has proved particularly challenging. Despite being commonly associated with neonatal alloimmune thrombocytopenia, antibodies against these antigens are relatively rare. Furthermore, previous studies have reported difficulties with the specificity of alloantibody detection using standard practices because of epitope complexity.[5-8] For example, the HPA-3 epitope is sensitive to 3-dimensional changes in GPIIb structure, and its recognition depends on glycosylation of nearby residues, which can be lost during platelet storage.

Detection of anti-HPA alloantibodies is further complicated by the fact that antibodies against class I HLA using whole platelet detection can interfere with alloantibody binding to HPA. More specific monoclonal antibody-based antigen capture techniques that can detect antibodies against glyco-modifications on alloantigens are also problematic because they are performed only by select laboratories and can be technically challenging to perform.

Because of these challenges, there is a significant need for alternative, practical methods that can detect evasive alloantibodies with high specificity and sensitivity. A previous article by Zhang et al[9] used CRISPR/Cas9 technology in human induced pluripotent stem cells (iPSCs) to engineer megakaryocyte (MK) progenitors to express low-frequency platelet-specific alloantigens. That proof-of-concept study introduced the possibility of using gene editing of stem cells to express clinically relevant antigens for the detection of specific alloantibodies with 1-step flow cytometric analysis, which uses relatively small amounts of patient sample and is less cumbersome to perform than other types of analyses. However, that study was limited to expressing HPA-1 and did not directly address the issue of class I HLA antibody interference.

In the Zhang et al article in this issue, the authors expand on their original study by engineering iPSC-derived MKs to lack class I HLA antigen expression. These cells were also modified to be type O to avoid blood type incompatibility. HLA-negative, type O founder iPSCs were further edited to express HPA-3 or HPA-9 polymorphisms using the established CRISPR/Cas9 methodology. Importantly, the resulting designer cells were capable of detecting alloantibodies present in patient serum with high specificity and, in some cases, they demonstrated improved sensitivity compared with antigen capture methods. The clinical potential of this method was further demonstrated by showing that anti-HPA-9b alloantibodies in patient sera from unresolved neonatal alloimmune thrombocytopenia cases could be detected.

The authors envision using gene-edited iPSCs for alloantibody detection against additional disease-associated HPAs. Their approach provides clear advantages over existing methods by enabling the expression of particular alloantigens in a whole-cell context without class I HLA interference for highly specific flow cytometric antibody detection. Reports on the utility of this new technology in the clinical setting will surely be eagerly anticipated. Thus, this new study by Zhang et al represents another promising stride toward improving platelet alloantibody detection.

Conflict of interest statement: J.E.I. has financial interest in and is a founder of Platelet BioGenesis, a company that aims to produce donor-independent human platelets from human-induced pluripotent stem cells at scale. He is an inventor on this patent. The interests of J.E.I. were reviewed and are managed by the Brigham and Women's Hospital and Partners HealthCare in accordance with their conflict-of-interest policies. ■

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