that TMEM163 is not essential for platelet production or α-granule biogenesis. Although abnormal accumulation of Zn^{2+} was detected in TMEM163-deficient platelets, this did not influence the gene expression of other ZIP/ZnT transporters. Combined deficiency of Rab32/Rab38 small guanosine triphosphatases in mice results in HPS with profound defects in hemostasis.[8] Rab32/Rab38 levels were normal in TMEM163-deficient mice, indicating a unique role for TMEM163-dependent pathways in δ-granule biogenesis.

In addition to TMEM163, other ZIP/ZnT isoforms may be involved in megakaryocyte and platelet Zn^{2+} homeostasis, which regulate distinct Zn^{2+}-dependent signaling pathways.[7] The expression profile and function of ZIP/ZnT isoforms could be dynamic during megakaryopoiesis, changing to optimize Zn^{2+} levels in the cytoplasm and intracellular organelles and regulate Zn^{2+}-responsive genes and transcription factors to support platelet production (see figure). Granular-resident Zn^{2+} store in platelets is also involved in the regulation of the coagulation cascade.[7,9] These complex processes can be further analyzed in mouse models with ZIP/ZnT and HPS/TMEM163 deficiency to determine how defective Zn^{2+} transport and granular Zn^{2+} content are involved in platelet signaling and hemostatic complications in HPS and other storage pool diseases.

The study by Yuan et al has provided new insights into the regulatory mechanism of Zn^{2+} transport in δ-granules and suggests a link between abnormal δ-granule biogenesis and hemostasis. These findings may provide the basis for exploring Zn^{2+}-dependent therapeutic strategies in HPS and other storage pool diseases.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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DOI 10.1182/blood.2021010691

THROMBOSIS AND HEMOSTASIS

Comment on Klamroth et al, page 1818

Prophylaxis in hemophilia: how much is enough?

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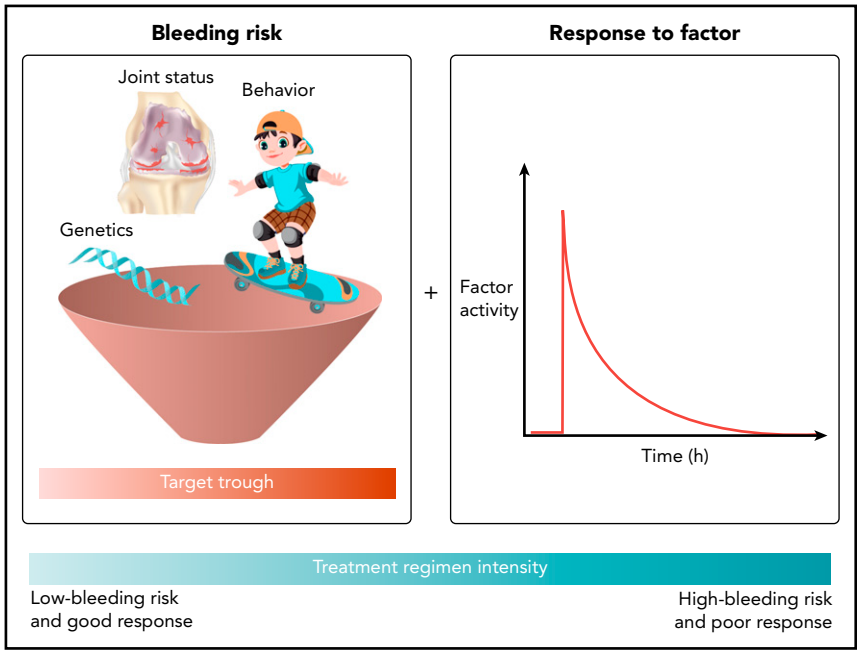
In this issue of *Blood*, Klamroth et al evaluated prophylactic factor replacement therapy targeting one of 2 distinct factor VIII (FVIII) trough levels (1% to 3% vs 8% to 12%) for prevention of bleeding in patients with hemophilia A.[1]

A major consequence of hemophilia is joint bleeding, leading to functional impairment and chronic pain. Continuous prophylaxis is the routine replacement of FVIII/IX via infusion of factor concentrates and was introduced in Sweden in the late 1950s.[2] Its initial use was based on the observation that the frequency of bleeding events was reduced significantly when FVIII/IX levels were kept at ≥1%. Despite



Determining treatment intensity for factor prophylaxis requires estimating bleeding risk, which informs the choice of target trough level, as well as understanding the response to factor replacement therapy, which is determined by the factor product infused and the patient's individual pharmacokinetics.

the observed benefit, it was not until completion of the Joint Outcome Study (JOS) that prophylactic factor replacement therapy became widely recognized as standard of care for children with severe hemophilia.[3] However, in adolescents and adults, the routine use of prophylactic replacement therapy lagged until publication of the SPINART study[4] in 2017, which led to more widespread use and a new standard of care. Despite clear benefits, many chose not to use continuous prophylaxis. In US hemophilia treatment centers, only 77.6% of patients with severe hemophilia A aged 11 years or older are on continuous prophylaxis as reported by the Centers for Disease Control and Prevention.[5]

Although achieving a target trough level of 1% reduces bleeding significantly, it does not eliminate bleeding events in all patients. Accordingly, determining the best target trough level for an individual patient is a challenge routinely faced by clinicians. Prior to this study by Klamroth et al, much of our knowledge of the benefit from higher trough levels was derived from modeling[6] rather than from clinical trial data. In the present study, 115 subjects received prophylaxis with rurioctocog alfa pegol (an extended half-life product with a published half-life of 14 to 16 hours)[7] and were randomized to one of 2 target trough level ranges (1% to 3% or 8% to 12%). The dosing regimens used to achieve these target trough levels were derived from individual pharmacokinetic testing. It is not unexpected that higher trough levels resulted in fewer bleeding episodes, and although these data largely confirm predictions based on modeling, it is critical to have made these observations in a randomized clinical trial. Importantly, it was observed that no bleeding events occurred in 42% of those in the 1% to 3% arm vs 62% in the 8% to 12% arm, leaving 38% of subjects continuing to experience bleeding events despite prophylaxis that was significantly more intensive than the current standard of care.

In this study, to achieve a target of 1% to 3%, 39% of subjects required treatment that was more frequent than standard twice-weekly dosing.[8] To achieve the 8% to 12% trough target, 12.1% of subjects required daily infusions, and the majority required every other day infusions: only 1 subject could treat less frequently than every third day and achieve a trough of 8% to 12%. This burden of therapy is significant and may be reflected in the higher number of patients in the higher target arm (25.8%) who were unable to complete the study or had significant protocol deviations compared with the lower target arm (8.7%).

Although useful and commendable for its randomized design, this study is not able to answer what trough level is best. Should the trough level be 1%, 3%, 5%, 10%, or 15%? Many participants (42%) treated with the target trough level of 1% to 3% had no bleeding events during the study period, but some (38%) continued to have bleeding events despite higher target trough levels. Perhaps, the right answer is the trough level at which the patient does not bleed while performing their usual activities of life. Given that individual patient needs vary, it is up to the clinician caring for the patient to weigh the various factors that comprise bleeding risk (see figure) to make a best estimate of the target trough level required to prevent bleeding. In addition to the variables that comprise a patient's bleeding risk, which in turn informs the trough level needed to prevent bleeding, the ability to achieve a trough level is informed by the factor product used and the patient's response to that product (pharmacokinetics). In the past few years, it has become possible to use pharmacokinetics in clinic to individualize treatment regimens and target a specific factor trough factor level. Prior to the availability of pharmacokinetic modeling at the fingertips of clinicians, adjusting prophylactic regimens was often based on trial and error, and applying the results of this study would have been impossible.

This study supports the need to individualize care and makes clear that many patients will need factor replacement regimens that are more intensive than the current standard of care. Unfortunately, challenges remain. It is clear that many patients will not be able to achieve a zero-bleeding state even with current extended half-life products. New products are in development, but often their pricing assumes a use that achieves current standard-of-care results. Accordingly, when used more intensively to achieve better outcomes, treatment becomes even more expensive. In addition, the lack of randomized clinical trial data that compare new products to existing standards of care limits the ability of the community to advance care by failing to elucidate incremental benefit of novel therapies and treatment strategies. We have focused on making treatment easier, which is good, but treatment should also be more effective. These relative evaluations require direct comparisons via randomized trials.

Even if we achieve a zero-bleed state for many patients, it is not likely that joint disease, disability, and chronic pain will be eliminated. The presence of subclinical bleeding is difficult to study and quantify, but its presence is supported by the observation in the JOS that joint damage was seen on magnetic resonance imaging despite a lack of clinically evident bleeding, and by clinical experience of patients with mild hemophilia who develop significant arthropathy in middle age despite never reporting a clinical joint bleed.[3,9] Thus, targeting zero clinical bleeding events does not mean that all joint disease, dysfunction, and pain will be eliminated. This reality underscores the need for better, not just more convenient, therapies.

Conflict-of-interest disclosure: The author reports honoraria from Takeda, Spark Octapharma, and Pfizer and research grants from Novo Nordisk. ■

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DOI 10.1182/blood.2020009603