Until now, 3 long-acting FIX molecules have been studied for weekly prophylaxis, but results from phase 3 trials are available only for 2 of them: nonacog beta pegol and rFIXFc.1,5

In the phase 3 trial reported by Collins et al, weekly prophylaxis with 10 and 40 IU/kg and on-demand treatment with nonacog beta pegol were assessed in patients with severe hemophilia B.1

Many aspects of this new long-acting FIX concentrate deserve consideration:

- The terminal half-life of 96 to 110 hours, which is fivefold longer than that of unmodified FIX, allowed the performance of successful prophylaxis with no more than 1 injection per week. This implies at least a 50% reduction in the number of total injections per year, with significant advantages in terms of quality of life, adherence to prescribed treatment, and less need of central venous lines insertions in the pediatric population.
- The in vivo recovery of nonacog beta pegol was twofold higher compared with standard recombinant FIX, resulting in higher plasmonic FIX levels while using lower doses during weekly prophylaxis.
- As shown in the figure, patients treated with 40 IU/kg/week maintained FIX trough activity well above 25 IU/dL, ensuring good protection from breakthrough bleeds and thus allowing a normal active life. These results support increasing the interval between injections and may allow prophylaxis with 1 injection every 2 to 3 weeks on the basis of individual pharmacokinetics.
- The high efficacy rate by means of a single injection and the successful protection from bleeding into target joints is reassuring. In fact, up to 99% of bleeds were resolved with a single injection, and up to 70% of patients with established target joints at study entry did not bleed in their target joints during the trial.
- A good safety profile was confirmed because no patient developed neutralizing anti-FIX inhibitors.

Finally, at variance with rFIXFc,5 annualized bleeding rates were twofold lower and the success rate of bleeding control with a single injection was slightly higher with nonacog beta pegol, suggesting that this molecule may convey superior efficacy than rFIXFc, although a head-to-head comparison would be needed to confirm this.

In this light, long-acting FIX molecules represent a terrific advance in hemophilia care, and the results presented in the paper by Collins et al confirm this. However, in addition to all the tangible advantages, several aspects still need to be further investigated:

- The long-term safety related to the chronic exposure to the polyethylene glycol moiety;
- The meaning and relevance, if any, of the development of noninhibitory antibodies already reported in the phase 3 trial,1 as well as the immunogenicity of the molecule in previously untreated patients;
- The adequacy and reliability of current clotting factor laboratory assays to predict and monitor treatment efficacy; and
- The cost-effectiveness of the new drug compared with standard products, taking into account the possibility of treatment optimization and individualization.

All in all, with this new molecule, which will hopefully soon be available on the market, the progress of hemophilia replacement therapy has taken an additional step forward to ameliorate treatment feasibility and patients’ quality of life.

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Comment on Xu et al, page 3978

**HMGB1 takes a “Toll” in sickle cell disease**

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In this issue of Blood, Xu et al elegantly demonstrate that high mobility group box 1 (HMGB1) contributes to the endogenous armamentarium to activate Toll-like receptor 4 (TLR4) in sickle cell disease (SCD).1

SCD is the most common monogenic disease, which occurs due to a single amino acid substitution in the hemoglobin, resulting in the expression of sickle hemoglobin (HbS) and sickle-shaped red blood cells (RBC).2 The homozygous disease is characterized by a complex pathophysiology replete with inflammation, oxidative stress, multiorgan damage, pain, and reduced life span. Enormous phenotypic variability contributes to poor disease management resulting in lifelong suffering and poor quality of life. In addition to hemolytic anemia, intermittent and unpredictable episodes of vasoocclusive crises due to adhesion of sickle RBCs in the vasculature impair oxygen and blood supply to the organs, resulting in cumulative organ damage and acute pain.2 It is believed that ischemia/reperfusion injury (I/R) is a major contributor to the complex disease pathobiology,3 but no common targets are defined to ameliorate the clinical manifestations.

Emerging studies are suggestive of the involvement of TLR4 in organ damage and pain at a multicellular level in SCD. We observed that TLR4 transcripts are increased in the spinal cord of mice expressing human HbS compared with control mice expressing normal human hemoglobin.4 Subsequently, Ghosh et al demonstrated that hemin-induced TLR4 activity leads to acute lung injury...
in homozygous sickle mice (HbSS-BERK and Townes), which show phenotypic and hematologic features of SCD. Complementary to these observations, we found increased expression of TLR4 on cutaneous mast cells derived from HbSS-BERK mice compared to those from control mice, and found that activated mast cells lead to neurogenic inflammation and hyperalgesia (pain) in sickle mice, whereas TLR4 deletion in sickle mice results in reduced tonic hyperalgesia and attenuates hyperalgesia induced by hypoxia/reoxygenation ([H/R], which simulates I/R). Concomitantly, Belcher et al demonstrated that heme-induced endothelial TLR4 signaling triggers vasoocclusion in homozygous sickle mice. These basic and translational findings unequivocally suggest that TLR4 may be a common molecular target in multiorgan damage and pain in SCD. Because TLR4 activity is stimulated by HMGB1 released by cell-free heme and in response to inflammation, oxidative stress, and I/R injury, HMGB1 may be instrumental in orchestrating TLR4-induced injury in SCD.

HMGB1 is a nuclear protein involved in chromatin remodeling and regulation of transcription. However, it acts as a damage-associated molecular pattern under a variety of stressors encompassing inflammation, I/R injury, oxidative stress, and cell-free heme, and is released from the nucleus due to cellular activation, autophagy, and cell death, resulting in significantly high cytoplasmic and circulating plasma levels. It also binds to microparticles, further increasing its ability to coactivate multiple receptors. Posttranslational modifications, cellular and extracellular localization, and its ability to bind multiple partners including TLR4 lead to a diverse array of signaling mechanisms, further augmenting inflammation, oxidative stress, autophagy, and apoptosis. Thus, HMGB1 warrants investigation in SCD, which has been initiated by Xu et al.

HMGB1 plasma levels and the ability of HMGB1 to stimulate TLR4 activity have been examined by Xu et al in the HbSS-BERK mouse model of SCD and in human SCD subjects at steady state and in crisis. Their study design, entailing protein expression and functional assays, as well as inclusion of a highly appropriate sickle mouse model and human subjects in parallel with appropriate controls, is commendable. As hypothesized, plasma of sickle mice and human SCD subjects showed significantly higher HMGB1 concentrations compared with control mice and healthy subjects at steady state, and significantly escalated levels of HMGB1 after crisis (H/R injury in mice). Plasma HMGB1 was able to induce TLR4 reporter activity in stably transfected TLR4/ NF-kB/SEAP cells. TLR4 activity mirrored the elevated plasma HMGB1 in sickle mice and human subjects at steady state and after H/R injury and crisis, respectively. Most sickle plasma TLR4 activity was HMGB1 dependent, suggesting that quantity of HMGB1 can be a determinant of TLR4-mediated injury. Treatment of mice with HMGB1 antibody did not influence plasma HMGB1 levels but ameliorated its ability to stimulate TLR4 at steady state and after H/R injury. It is perplexing that even though HMGB1 antibody treatment attenuated TLR4 activity, it did not have any significant influence on liver or lung pathology or on the inflammatory markers serum amyloid protein and soluble vascular cell adhesion molecule 1. It could be that the endogenous TLR4 may be sensitized and that a single dose of antibody may not be adequate to block the ongoing TLR4 activity. A time- and dose-response study would be required to ascertain the role of HMGB1 in organ damage and inflammation in SCD via TLR4-dependent and TLR4-independent mechanisms.

Even though HMGB1 is significantly elevated at steady state and increases further with crisis, it shows a wide distribution and variability within each state in sickle mice and in human subjects with SCD. It is plausible that HMGB1 variability approximates the phenotypic variability and/or the extent of inflammation and tissue injury in SCD. If this scenario is true, HMGB1 could serve as a diagnostic biomarker of disease severity. Because HMGB1 can bind to inflammatory cytokines and microparticles and synergize with cell-free heme, it may have an additive effect on the attendant inflammation and tissue injury in SCD. Therefore, it is critical to recognize the source (or sources) and mechanism of HMGB1 release to attenuate its activity in SCD. Given that TLR4 activation plays a critical role in several pathophysiological conditions, some of which occur in SCD, including organ damage and pain, this study provides a novel unified molecular target—HMGB1—with potential for diagnostic and pharmacotherapeutic development after further investigation in SCD.

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