

The hope and reality of long-acting hemophilia products

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Recombinant DNA technology and protein engineering are creating hope that we can address ongoing challenges in hemophilia care such as reducing the costs of therapy, increasing the availability to the developing world, and improving the functional properties of these proteins. Technological advances to improve the half-life of recombinant clotting factors have brought long-acting clotting factors for hemophilia replacement therapy closer to reality. Preclinical and clinical trial results are reviewed as well as the potential benefits and risks of these novel therapies. Am. J. Hematol. 87:S33–S39, 2012. © 2012 Wiley Periodicals, Inc.

Introduction

Over the past several decades, clinical care for hemophilia has improved dramatically [1] (Fig. 1). The development of FVIII and FIX concentrates that can stop or prevent bleeding episodes has reduced the associated morbidity, as well as improved the quality of life and normalized life expectancy [2]. Regular, long-term prophylactic therapy with factor concentrates prevents recurrent hemarthroses and reduces or prevents the development of hemophilic arthropathy [3,4]. This is now the standard of care for the management of children with hemophilia and is increasingly being applied in adult care. Comprehensive hemophilia centers, the adoption of home therapy, and worldwide advocacy efforts are improving health and quality of life throughout the world [5]. Advancements in biotechnology contributed significantly through the development of improved pathogen screening, viral inactivation techniques, and the development of recombinant versions of factors VIII and IX [6]. The broad adoption of recombinant therapy throughout the developed world has significantly increased the supply of clotting factor concentrates and helped advance aggressive therapeutic interventions such as prophylaxis [7,8]. However, this has come at great costs to governments, medical insurers, and patient's families with an estimated annual cost of over \$150,000 per patient in the USA [9,10]. In addition, barriers persist limiting the adoption and adherence of effective prophylactic therapy, particularly in the developing world. Standard prophylaxis regimens (using 25–40 IU/kg of FVIII three times per week or 50–100 IU/kg of FIX twice per week) are difficult for some individuals and suboptimal adherence remains a problem [11]. The development of alloantibodies that inhibit the activity of infused replacement products also remains a significant complication [12]. Despite recent gene therapy strategies that are showing new promise that long-term corrective plasma levels may be achieved [13], a cure for hemophilia has not yet been realized.

Biotechnology has been successful at overcoming similar barriers in other disease states. Recombinant DNA technology and protein engineering remain important platforms to address ongoing challenges in hemophilia care such as reducing the costs of therapy, increasing the availability to the developing world, and improving the functional properties of these proteins. Long-acting biological therapeutics are an incremental advance toward overcoming some of these barriers. Strategies that have been successful for other therapeutic proteins are now being applied to FVIII and FIX and include modifications such as the addition of polyethylene glycol (PEG) polymers and polysialic acids, alternative formulation with polyethylene glycol-modified liposomes and bioengineered fusion proteins. In addition, insights into factor VIII structure and function have allowed

targeted modifications of the protein to increase the duration of its cofactor activity and reduce its clearance in vivo. Similar bioengineering efforts have led to promising modifications to recombinant VIIa, used to treat individuals with hemophilia and inhibitors. Some of these therapies may open up alternative delivery routes such as subcutaneous administration. This manuscript will explore the hope that these advances in therapeutics for hemophilia may lead to improved clinical outcomes, and the reality of the preclinical and clinical results observed, and the potential risks of adverse effects that will need to be explored in the decades ahead.

Themes from the Hemophilia Therapeutic Pipeline

Four major themes have appeared in the research and development pipeline toward new therapeutic agents for hemophilia: (1) lower cost factor concentrates, (2) non-protein therapies, (2) novel bypassing agents for hemostatic control of inhibitors, and (4) half-life extension through bioengineering.

Lower cost factor concentrates could be realized simply through increasing the competitive landscape of therapeutic options. The Biologics Price Competition and Innovation Act, which was part of the Patient Protection and Affordable Care Act signed into law in 2010 enables the Food and Drug Administration to approve the marketing of biosimilar biological products based on a single reference biological product [14]. Such biosimilars could include minor differences in clinically inactive components provided they exhibit no “clinically meaningful differences” in terms of safety, purity, or potency. Biosimilarity would still need to be demonstrated through analytical studies, animal studies, and a clinical study. To this end, the pipelines of all of the current hemophilia therapeutic manufacturers have expanded such that there may soon be multiple biosimilar options for recombinant FVIII, FIX, and VIIa. However, it remains likely that manufacturers will still seek a standard Biologic License Application for these “follow-on biologics” limiting

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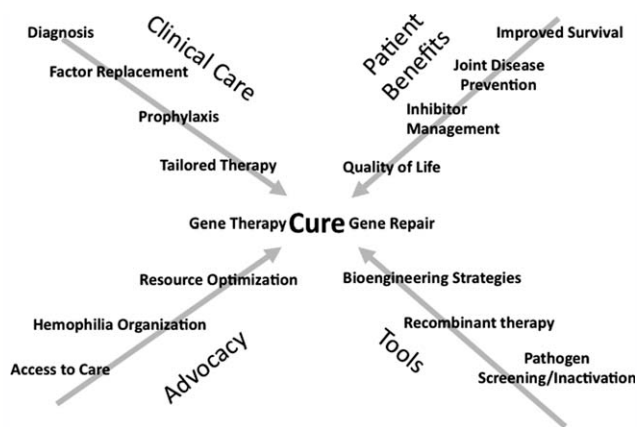


Figure 1. Summary of advances in care for hemophilia.

the aggressiveness of pricing [15]. Enhanced expression systems may also offer a mechanism to produce recombinant proteins at much higher yields than current cell culture systems [16]. Transgenic animals have also shown promise as an efficient source of producing abundant recombinant proteins taking advantage of the high cell density of the mammary gland of livestock [17]. Proof of principle for transgenic expression has now been reported for rFIX [18] and rFVIII [19] and is being investigated to produce rVIIa.

Another novel area of investigation is targeting inhibitors of coagulation. The primary targets have been tissue factor pathway inhibitor (TFPI) and activated protein C (APC). The defective factor Xa generation from the intrinsic tenase complex, characteristic of hemophilia, can be overcome by increasing extrinsic factor X (FX) activation as has been achieved through the use of prothrombin complex concentrates and rVIIa in hemophilia with inhibitors. Alternatively, the extrinsic pathway can also be stimulated by inhibiting TFPI, the key regulator of the extrinsic pathway. TFPI has been effectively targeted by antibodies (anti-TFPI) [20] as well as naturally occurring (fucoidan) [21] and synthetic inhibitors (aptamers and peptides) of TFPI [22]. Inhibition of APC could also enhance thrombin generation and has been targeted by aptamers and RNAi silencing [23].

Novel bypassing agents for managing inhibitors are under development including bioengineered variants of rVIIa to be discussed below. Whereas infusion of factor Xa should be able to bypass the intrinsic pathway (and the blockade from inhibitors) and promote thrombin generation, it is rapidly inactivated in plasma by antithrombin and TFPI resulting in a short plasma half-life. FX circulates as a zymogen and is converted to an active state by proteolysis at a highly conserved site (R¹⁵-I¹⁶), followed by a conformational change. A novel FX variant (FX^{I16L}) is a functional protease with “zymogen-like” properties such that it is ineffectively targeted for inactivation yet retains prothrombinase activity when assembled with factors Va and prothrombin on a phospholipid surface. This results in a novel agent with a prolonged half-life and bypassing activity and has demonstrated efficacy in murine models [24].

Half-Life Extension Through Bioengineering

The unmet needs in managing hemophilia include reducing the costs of replacement therapy, improving access to the developing world, broadening the adoption of and adherence to prophylactic replacement therapy, reducing or eliminating reliance on central venous access devices, alternative modes of delivery (subcutaneous or oral), and reduced immunogenicity (reviewed elsewhere [25]). Some of these challenges could be overcome through biosimilars and non-protein therapies. Half-life extension of clotting fac-

tors would also be an important step forward as these would require less frequent dosing to maintain effective plasma levels reducing the number of venipunctures per week, perhaps making it easier to adhere to prophylactic regimens. This could enhance the adoption of prophylaxis in young infants when venous access is challenging, perhaps obviating the need for placement of central venous catheters and make prophylaxis in adults less intrusive. A long-acting therapeutic with sufficient bioavailability could make subcutaneous or even oral delivery a reality [26].

Factor VIII

Based on simulations using FVIII half-lives and in vivo recoveries observed during clinical trials in patients receiving prophylaxis, half-lives and infusion regimens (interval between dosing) had the largest effect on predicted FVIII plasma levels whereas in vivo recovery and dose changes exhibited smaller effects [27]. Thus, targeting FVIII half-life extension through bioengineering should have the greatest impact in achieving long-therapeutic windows with protective plasma levels. FVIII is commonly prescribed assuming an average in vivo recovery of 2 IU/dL for each IU/kg dosing and a half-life of approximately 12 hr. Prophylactic regimens have typically been 25–50 IU/kg every other day or three times per week (Monday, Wednesday, and Friday) with a goal to maintain trough FVIII levels above 1 IU/dL [28]. However, large clinical trials in children and adults have shown that FVIII half-life varies from 6 to 25 hr between patients [29,30]. Understanding the mechanisms underlying FVIII clearance and the factors affecting this half-life variability will help advance the strategies to prolong its half-life in vivo.

Following intravenous infusion, the primary determinant of FVIII residence time in plasma is its non-covalent association with von Willebrand factor (VWF), which protects it from proteolysis and cellular uptake, extending its half-life from about 2 hr to 12 hr [31]. Pre-infusion VWF levels correlate to some degree with FVIII half-life with lower levels of VWF observed in blood group O individuals and higher VWF levels with age [32,33]. With a hemostatic challenge, thrombin activation releases FVIII from VWF so that it can exert its procoagulant function. The majority of infused FVIII is otherwise cleared in the liver through interaction with a family of low-density lipoprotein receptor-related proteins (LRP) and heparan sulfate proteoglycan receptors among others [34]. That FVIII clearance may be related to VWF clearance is supported by data correlating FVIII half-life and VWF-antigen/VWF-propeptide ratio. Following synthesis and processing, VWF-propeptide is secreted into plasma at a 1:1 ratio to mature VWF but is cleared independently. The VWF/propeptide ratio has been used as a marker of VWF clearance [35]. In a recent study, FVIII half-life was best predicted by the VWF/propeptide ratio suggesting that FVIII clearance was more dependent on the rate of endogenous clearance of VWF rather than the absolute VWF level [36]. This important aspect of FVIII clearance provides insight into the limitations to the current half-life extension strategies.

The first strategy to be investigated in clinical trials was sustained delivery of rFVIII through association with liposomes as a carrier. Because liposomes are typically cleared very quickly from the circulation, the liposomes were chemically modified with PEG to extend their circulatory half-life. The rFVIII molecules remain unmodified so there is no loss of normal protein-protein interactions (including rFVIII-VWF interaction) and functional activities. Preclinical studies within a hemophilia A mouse model showed that prophylactic infusion of rFVIII reconstituted with PEGylated liposomes (rFVIII-PEG-Lip) prolonged some pharmacokinetic parameters compared with standard

rFVIII and correlated with an enhanced hemostatic efficacy [37]. In a blinded, controlled, crossover, multicenter trial, a single prophylactic infusion of rFVIII-PEG-Lip resulted in a longer bleed-free interval compared with standard rFVIII [38]. Despite these promising results, a subsequent double-blind, randomized, crossover Phase I trial demonstrated that rFVIII-PEG-Lip and standard rFVIII demonstrated similar pharmacokinetic parameters [39]. The potential efficacy of this formulation was most recently investigated in a randomized, active-controlled, double-blind, prophylaxis trial in patients with severe hemophilia A comparing once-a-week dosing of rFVIII-PEG-Lip with three times-per-week dosing with standard rFVIII [40]. This study was terminated midstage when interim analysis indicated that the trial would not meet its efficacy endpoint.

That direct PEGylation of rFVIII hasn't come to the clinic sooner may seem surprising, especially since PEGylation has been successfully applied to a number of biologics to extend their circulating half-life. PEG polymers incorporate many water molecules within their hydrophilic structures. The greatest impact of this for a biologic is to increase the effective size of the conjugated protein above the filtration size of the kidney. However, FVIII is already a large glycoprotein and is not cleared by the kidney; therefore this increase in effective size is not an advantage. Any advantage to PEGylation of FVIII is likely through disruption of interaction with cellular clearance receptors. Indiscriminate PEG conjugation could reduce interaction with key activating proteases or assembly within the tenase complex compromising its cofactor function. Alternatively, if PEGylation reduced FVIII-VWF association, the half-life of FVIII could be markedly reduced. Both of these observations have been made since the earliest experimentation with direct PEGylation of FVIII [41,42]. Advances in chemical conjugation and innovative bioengineering have allowed for more precision in selecting the sites for PEG conjugation in order to avoid interfering with these functional protein-protein interactions. Mei et al. generated site-specific PEGylated rFVIII mutants, achieved through linkage to free surface exposed cysteine residues introduced through mutagenesis, that retained full procoagulant function and VWF binding in vitro [42]. These conjugates exhibited improved pharmacokinetics in hemophilic mice and rabbits, and prolonged efficacy in bleeding models in mice consistent with their enhanced half-life in vivo. Another site-specific PEGylation uses targeted conjugation to an O-linked glycan within a truncated B domain linker sequence. This also produced an active molecule which is similar to native FVIIIa. Evaluation in a hemophilia A dog model demonstrated a close to 2-fold prolongation of half-life with an accompanying prolonged hemostatic effect that would support less frequent dosing to maintain minimally effective plasma levels [43]. These and similar strategies have been moved forward to clinical trials (Fig. 2).

An alternative strategy to extend the half-life of biologics has been to fuse them to another protein with a much longer half-life. In one example, rFVIII was fused to the constant region (Fc) of immunoglobulin G (rFVIII-Fc). Fc-containing proteins that are internalized by endothelial cells bind to the neonatal Fc receptor (FcRn) present in the acidified endosome in a pH-dependent manner and are then recycled back to the cell surface, avoiding catabolism in the lysosome, and they are subsequently released back into plasma at physiologic pH. Preclinical studies with rFVIII-Fc show that in circulation it is complexed with VWF and released upon activation to exert cofactor activity that is similar to native FVIII. rFVIII-Fc exhibits ~ 2-fold extension of half-life in hemophilia A mice and dogs [44]. In a first-in-human Phase I study, rFVIII-Fc had comparable re-

| Replacement Therapy | Half-life Extension Strategy | Half-life Impact | Biologic In Development | Clinical Trial Status |
|---------------------|---------------------------------|------------------|---------------------------------------|------------------------------------|
| Factor VIII | Indirect PEGylated liposomes | None | BAY79-4980 (Bayer) | Phase II (terminated mid-stage) |
| | Direct PEGylation | N/A | N8-GP (Novo Nordisk) | Phase III |
| | | | BAX855 (Baxter) BAY94-9027 (Bayer) | Phase I (complete) |
| | Fc Fusion | 1.5- to 1.7-fold | rFVIII-Fc (Biogen Idec) | Phase III |
| Factor IX | PEGylation | 5-fold | N9-GP (Novo Nordisk) | Phase III |
| | Fc Fusion | 3-fold | rFIX-Fc (Biogen Idec) | Phase III |
| | Albumin Fusion | N/A | rIX-FP (CSL Behring) | Phase I |
| Factor VIIa | PEGylation | 4.3-fold | N7-GP (Novo Nordisk) | Phase II (complete) |
| | Albumin Fusion | N/A | rVIIa-FP (CSL Behring) | Preclinical |

Figure 2. Long-acting hemophilia therapeutics in the development pipeline. N/A, not yet available from human clinical trial data.

covery to rFVIII indicating similar affinity for VWF and the observed half-life was 1.5- to 1.7-fold [45].

In summary, extending the half-life of FVIII has proven to be challenging. Though these strategies appear to be having an impact on the catabolism of FVIII, there has been continued reliance on VWF association similar to the native FVIII molecule. This is likely creating a ceiling for the benefit of these approaches of slightly less than 2-fold half-life extension compared to commercial rFVIII variants.

Factor IX

Though there has been less insight into the mechanisms of FIX clearance and catabolism, the same strategies that have been investigated to extend the half-life of FVIII have had significantly more impact on FIX half-life extension.

Recombinant FIX has also been fused to the Fc of immunoglobulin G (rFIX-Fc) and investigated in hemophilia B mice and dogs [46]. Following intravenous infusion, the half-life of rFIX-Fc was 3- to 4-fold longer than rFIX. When infused into mice deficient in FcRn, the half-life was similar to rFIX suggesting that the enhanced circulatory time is facilitated through FcRn diverting rFIX away from intracellular degradation. Within hemophilia B mice, the whole blood clotting time was corrected through 144 hr for rFIX-Fc compared to 72 hr for rFIX. In addition, when the mice were dosed at 200 IU/kg on days 0, 4, and 8, plasma FIX activity for rFIX-Fc never dropped below 0.1 IU/mL (i.e., 10%), whereas mice receiving rFIX spent the majority of the 4-day interval below 0.1 IU/mL and had trough levels prior to the next dose of <0.01 IU/mL. Within hemophilia B dogs, rFIX-Fc exhibited a terminal half-life of 47.5 hr compared to previous observations of 17-18 hr for rFIX. In addition, following infusion of 140 IU/kg, the whole blood clotting time remained corrected to close to normal levels through 144 hr returning to baseline levels at 168 hr. The safety and pharmacokinetics of rFIX-Fc was subsequently evaluated in previously treated adults with severe hemophilia B [47]. No serious adverse events related to the study drug were observed including no inhibitors. rFIX-Fc had a half-life that was 3-fold longer than that reported for rFIX with a mean terminal half-life for FIX activity of 56.7 hr and a mean residence time of 71.8 hr. Interestingly, the incremental recovery for rFIX-Fc was similar to plasma-derived FIX whereas recovery for rFIX has consistently been lower in all prior pharmacokinetic studies [48,49].

Targeted PEGylation of rFIX has also proven to be a successful strategy. Given the smaller size of the FIX molecule with multiple key interactive domains to facilitate its biologic

activity, indiscriminate PEGylation would risk compromising its activation from zymogen to protease, interfering with FVIIIa cofactor activity and/or preventing efficient proteolytic action on FX. The activation peptide of zymogen FIX is cleaved off upon activation, liberating it from FIXa. The activation peptide has two asparagine (N)-linked glycans which, through innovations in glycoPEGylation, permit targeted enzymatic transfer of 40k PEG [50]. Analysis of the resultant glycoPEGylated FIX peptides (N9-GP) shows them to be 95% monoPEGylated with the 40k PEG to be conjugated roughly equally either to the N-glycan at Asn157 (55%) or Asn 167 (45%). There is a minor proportion of di-PEGylated FIX. Biochemical characterization of N9-GP has shown that the activation peptide (that includes the PEG moieties) is released by physiologic activators such that the remaining FIXa is unhindered, exhibiting enzyme kinetics for FX activation that was indistinguishable from rFIX. Within a 1-stage aPTT assay, the specific activity was reduced (73%) compared to rFIX. However, thromboelastography parameters from hemophilic plasma reconstituted with N9-GP was similar to rFIX. In hemophilia B dogs, N9-GP has a greatly prolonged half-life of 113 hr compared to 16 hr for rFIX. Subsequently, the safety and pharmacokinetics of N9-GP was investigated in 16 previously treated adults with severe hemophilia B [51]. None of the subjects developed inhibitors following a single exposure to N9-GP, though one patient developed transient hypersensitivity symptoms during the administration and was not included in the pharmacokinetic analysis. In the remaining 15 patients, N9-GP had a mean half-life of 93 hr, 5-fold higher than rFIX in these patients. Similar to the observations with rFIXFc, recovery was also markedly improved with incremental recovery for N9-GP 94% and 20% higher compared to rFIX and plasma-derived FIX, respectively. In addition, the volume of distribution of N9-GP was approximately half of that observed for rFIX, suggesting that N9-GP primarily circulates in the blood without any significant tissue distribution.

An additional fusion protein strategy has also advanced through the pipeline. Albumin has a half-life of 20 days participating in the same recycling mechanism through interaction with FcRn as described for immunoglobulin. It is also an abundant, naturally occurring plasma protein and has not been shown to be involved in immune defense reactions, increasing enthusiasm for its suitability as a fusion partner. Due to the same challenges of avoiding interfering with the regulation of FIX activation and its protease activity, an innovative fusion strategy was employed. Recombinant human albumin was fused to rFIX through a linker sequence that contains a cleavable sequence identical to the activation site for FIX (rIX-FP) [52]. Thus, following infusion, rIX-FP would potentially benefit from the half-life extending properties of albumin, yet upon activation, albumin and the cleavable linker would be liberated allowing the resultant FIXa to function normally. Preclinical studies with rIX-FP have shown that, depending on the species of animal used, the terminal half-life was up to 5-fold longer than that of rFIX [53]. Clinical studies have recently been initiated.

Factor VIIa

Although rVIIa has proved to be a safe and effective therapeutic for the management of bleeding in hemophilia patients with inhibitors, its short plasma half-life requires a short interval for follow-up dosing and limits its application in prophylaxis. Clinical studies have suggested that higher initial doses may result in a more rapid onset of hemostasis [54,55] and the time from initiation of bleeding to initiation of treatment may have a significant impact on efficacy [56]. In order to overcome these challenges, several bioengin-

ering strategies have been implemented in an attempt to improve rVIIa functionality: (1) increased potency and rate of onset of action of rVIIa through directed molecular evolution and rational design [57,58] and (2) half-life extension through PEGylation, formulation with PEGylated liposomes, and fusion proteins to be discussed below. There are also two N-glycans on the VIIa light chain and protease domains which can be targeted by glycoPEGylation chemistry. GlycoPEGylated rVIIa (N7-GP), is >85% monoPEGylated with 40k PEG and was shown not to change the enzymatic properties of the molecule such that it could interact efficiently with tissue factor (TF) and activate FX [59]. It also retained TF-independent thrombin generation on the surface of activated platelets. Safety and pharmacokinetics of N7-GP were investigated in a Phase I trial in healthy men [60]. N7-GP exhibited a plasma half-life of 15 hr with measurable activity for up to 72 hr after dosing. However, other pharmacokinetic properties were also altered. The maximum FVIIa activity in plasma after administration of 100 ug/kg of N7-GP was 3.8-fold lower than after a 90 ug/kg dose of rVIIa. In addition, the volume of distribution for N7-GP was about half of that observed for rVIIa suggesting N7-GP may circulate primarily in the blood whereas rVIIa is able to be distributed to surrounding tissues. There were no thromboembolic complications in the subjects; however, one blood sample from a single subject was positive for binding (but non-neutralizing) antibodies to N7-GP and FVIIa. In a Phase II trial, three different dose levels of N7-GP were tested in high titer inhibitor patients with hemophilia A and B aged 12–65 years [61]. Since this was designed to also evaluate for efficacy, subjects were eligible with at least two bleeding episodes per month. Following a 3-month observation period, patients were randomized to receive 25, 100, or 200 ug/kg of N7-GP every other day for 3 months (treatment period). N7-GP was safe and well-tolerated and no antibody formation was detected. Pharmacokinetic determinations were similar to those observed in subjects within the Phase I trial. The overall reduction in annualized bleeding frequency across all doses was 44% comparing the observation period to the treatment period. However, a dose-response relationship in the bleed reduction could not be established.

A recombinant FVIIa-albumin fusion protein (rVIIa-FP) was developed similar to rIX-FP in which rVIIa and recombinant albumin were joined by a flexible 31-amino-acid glycine serine linker, which was required to optimize the potency of the FVIIa [62]. The incremental recovery of rVIIa-FP was approximately 2-fold higher, and the half-life was approximately 6-fold longer, than rVIIa in a rat model [63]. The molar activity for rVIIa-FP was found to be about 70% of that observed for rVIIa and produced a dose-dependent reduction in clotting factor time by ROTEM analysis in an ex vivo inhibitor blood model. These results have warranted further evaluation in Phase I clinical studies.

Therapeutic Possibilities

Prophylaxis

If there is a benchmark to aim for with respect to clotting factor replacement therapy for an inherited bleeding disorder, factor XIII (FXIII) therapy would be the best achieved to date. FXIII has a long-circulating half-life of 7–12 days with full hemostatic activity even at low concentrations. This allows for routine prophylactic replacement regimens with dosing intervals of between 4 and 6 weeks [64,65]. Such a regimen is minimally intrusive even when initiated in infants and continued through adulthood with high adherence. The half-life extension strategies for FVIII, FIX, and FVIIa discussed above still fall short of this, granted lofty, therapeutic goal. However, if viewed as incremental advances to this

end, we can speculate on their potential impact and on the care of patients with hemophilia A and B or those with inhibitors.

For example, in comparison to a typically prescribed prophylaxis regimen of 25–40 IU/kg of rFIX twice weekly, the authors used the results from the rFIXFc pharmacokinetic data to model that once weekly dosing of rFIXFc at 20 IU/kg, or every 10 days at 40 IU/kg, or even every 2 weeks at 100 IU/kg would be sufficient to maintain a mean trough level above 1% for patients with severe hemophilia B [47]. Considering the additional benefit of improved incremental recovery compared to rFIX, this would seem to be a welcome advance over current therapeutic strategies. Similarly, modeling the pharmacokinetic results from N9-GP or rIX-FP, prophylaxis may be adequate for many patients with once weekly or less frequent dosing. When the same modeling is applied to the FVIII half-life extension strategies, a less than 2-fold prolongation of the half-life would likely mean that patients could move from every other day or three times per week dosing regimens to twice weekly infusions. However, given the wide range of half-lives observed in clinical trials, there may be some patients who can stretch the dosing interval to weekly. The efficacy of these modified prophylactic intervals are being explored in the pivotal trials for these agents. While a prolonged half-life for rVIIa would advance the application of prophylaxis in patients with inhibitors, some of the altered pharmacokinetic properties and lack of a dose-response relationship in bleed reduction may temper enthusiasm to advance the current iteration of glycoPEGylated rVIIa further in clinical trials. The results from the clinical studies with rVIIa-FP are not yet available.

On-demand therapy

Even on-demand therapy could be impacted with these agents. For instance, a longer acting FIX may be able to arrest bleeding with a single infusion due to an extended circulating half-life, whereas ~ 20% of bleeding episodes in hemophilia B require more than a single infusion to achieve bleed control [66]. This could also be realized for hemophilia A management with a longer acting FVIII where not all bleeds are controlled with a single infusion with current rFVIII products. In patients with established target joints, infusion with a product with a more sustained therapeutic window may reduce the risk for early re-bleeding, perhaps altering the typical cycle of bleeding, synovial inflammation, and subsequent re-bleeding. Adults using on-demand therapy may already be infusing once weekly to treat their bleeds. Once they have gained experience using a longer acting agent once per week in this context, it may be easier to transition them to a weekly prophylactic schedule and avoid breakthrough bleeding.

Evaluating the Risks

Immunogenicity

There have really only been three major therapeutic class introductions for the management of hemophilia over the past 40 years: highly purified plasma-derived factor concentrates, recombinant clotting factors, and the currently explored novel bioengineered recombinant factors. Despite many studies and meta-analyses, debate still rages over whether there is increased immunogenicity for rFVIII [67], which is a remarkable facsimile of the plasma-derived form. More recently, a controversy has been discussed in the literature over whether a minimally modified bioengineered rFVIII, B domain deleted-FVIII, exhibits increased immunogenicity over non-bioengineered rFVIII forms [68–71]. The current slate of novel bioengineered clotting factors include much more significant modifications including fusion to other protein sequences (Fc, albumin), and direct chemical

modification (e.g., PEG) any of which could be new targets for recognition by the immune system. In contrast, some of these strategies, such as PEGylation may actually be associated with a reduced risk for inhibitors as the hydrophilic PEG conjugates could create a “conformational cloud” sterically hindering immune interactions. While the lack of inhibitors is certainly encouraging from the early phase clinical trials to date with these agents, the history in this field suggests this will be an ongoing area of investigation for many years.

PEG

PEGylated products to treat a number of conditions have been approved and safely used for 20 years in both adult and pediatric patients [72]. In addition, PEG can be found in many commonly used consumer products such as tooth-paste, cleansing agents (shampoo, soap), fragrances, and cosmetics. Nevertheless, there have also been recognized unfavorable effects. These can include hypersensitivity reactions, either to the polymer itself or by-products formed during synthesis. These often occur through complement activation and can provoke anaphylactic shock. Pre-existing IgG and IgM anti-PEG antibodies were identified in over 25% of healthy donors and anti-PEG antibodies were induced in some patients within a clinical trial for PEG-asparaginase [73]. Anti-PEG antibodies have also been strongly correlated to rapid clearance of PEG conjugates [74]. Another disadvantage is that PEG is non-biodegradable and excretion relies on urine and stool elimination which occurs very slowly. The fate of PEG at the cellular level, particularly the liver and other reticuloendothelial organs, needs further investigation. There are no systematic long-term studies that clearly show whether PEG is excreted completely, if it is partly retained where it accumulates, and what the potential effects are at those sites of accumulation. For a therapeutic that will potentially be delivered weekly or more often over the life of a person with hemophilia, these are important issues.

Future Directions

Though several of these half-life extension strategies could eventually satisfy regulatory bodies for approval, there is plenty of room for continued innovation. Alternative delivery vehicles for FVIII that would not rely on VWF association for plasma stability could help overcome the limitations in FVIII half-life extension that have been observed. Alternatively, modified forms of rFVIII with enhanced VWF association, such as single chain forms as has been explored recently [75], could provide an incremental increase in plasma half-life. Novel indirect strategies to prolong FVIII half-life could include co-infusion with a bioengineered rVWF with extended half-life. In addition, if the current longer-acting factors were partnered with any of the non-protein therapies such as the TFPI inhibitors, the hemostatic protection could be further enhanced at the tail end of the pharmacokinetic curve when plasma levels have reached minimally hemostatic concentrations. This could conceivably push the dosing intervals for longer-acting FVIII closer to weekly dosing and much longer for longer-acting FIX, perhaps realizing a therapeutic regimen that is much closer to that used for FXIII replacement. Alternatively, these agents may allow for much more aggressive prophylactic regimens targeting trough levels that are much higher to better manage surgery, target joints, or facilitate more active lifestyles. Despite some caution as we enter this new era of hemophilia management, the prospects for the future have never been brighter.

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