

Fc-fusion technology and recombinant FVIII and FIX in the management of the hemophilias

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Abstract: Prophylaxis with regular infusions of factor VIII (FVIII)- or factor IX (FIX)- containing products is the mainstay of modern hemophilia care. However, this therapeutic regimen is inconvenient, requiring repeated intravenous injections from childhood. Approaches meant to prolong the half-life of FVIII and FIX in plasma have been developed in order to improve the feasibility and acceptability of replacement therapy, extending protection from bleeding, reducing infusion frequency and hence the need for venous access devices in young children. Several strategies have been implemented to enhance the pharmacokinetics of clotting factors, including conjugation with polyethylene glycol and the production by genetic engineering of fusion proteins containing the coagulation factors linked to a long-lived plasma protein such as albumin or the Fc fragment of immunoglobulin (Ig)G. The latter technology is one of the most promising, since the prolongation of FVIII and FIX half-life is obtained by exploiting the physiological binding of the Fc domain to the neonatal Fc receptor. Fc fusion monomers have been obtained with both recombinant FVIII (rFVIII-Fc) and FIX (rFIX-Fc), and data from preclinical and clinical studies showed improved pharmacokinetics for both factors, which are produced in human embryonic kidney (HEK) 293 cells, thus ensuring full human post-translational modifications. In Phase I/IIa studies, rFVIII-Fc and rFIX-Fc showed 1.5–1.7 fold and 3.0–4.0 fold longer elimination half-life, respectively. Similar data have been obtained in the Phase III clinical studies with rFVIII-Fc and rFIX-Fc published recently. Both drugs were satisfactorily safe, particularly with respect to immunogenicity, and no serious adverse event was observed.

Keywords: factor VIII, factor IX, long-acting molecules

Introduction

Hemophilia A and B are congenital bleeding disorders caused by mutations in the genes coding for coagulation factor VIII (FVIII) and factor IX (FIX).¹ Severe hemophilia, characterized by the complete plasma deficiency of these coagulation factors (less than 1%), is epitomized by limb- or life-threatening clinical manifestations such as hemarthrosis, soft-tissue hematomas, retroperitoneal, intracerebral, and excessive post-surgical hemorrhages. Recurrent joint bleeding and soft-tissue hematomas may cause severe arthropathy, muscle contractures, and pseudotumors, leading to chronic pain and disability that often warrant major orthopedic surgery.¹ Prophylaxis of bleeds, involving the regular infusion of plasma-derived or recombinant pharmaceutical products containing the deficient coagulation factor, is the mainstay of hemophilia care, made evidence-based by the results of two randomized clinical trials that showed the capacity of this therapeutic approach to avoid arthropathy.^{2,3} However, the practical adoption and implementation of prophylaxis is inconvenient, because the most widely used

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therapeutic regimens involve repeated intravenous infusions of the deficient coagulation factors FVIII or FIX in order to maintain plasma trough levels at or above 1%.⁴ Prophylaxis is usually delivered according to regimens based upon 2–3 injections per week, or even more frequently, owing to the short half-life (10–14 hours) of FVIII products currently available (slightly longer for FIX products, 15–20 hours).⁵ This need creates problems of venous access, mainly but not only in young children, who often require an alternative access such as central venous lines or arteriovenous fistulae.⁶ In addition, the inconvenience of frequent venipunctures is perhaps one of the reasons why many patients quit continuous prophylaxis at the time of adolescence or adulthood.

With this background, approaches meant to prolong the half-life of coagulation factors in plasma have been developed, in order to improve the feasibility and acceptability of replacement therapy. The potential benefits of the therapeutic enhancement brought about by longer-acting coagulation factors would include extended protection from bleeding and reduced infusion frequency, hence less need for venous access devices. Besides conjugation with polyethylene glycol, genetic fusion to plasma proteins is one of the most promising strategies developed in order to produce long-acting FVIII and FIX. It involves engineering of fusion constructs of these coagulation proteins with either albumin or immunoglobulin (Ig)G. The Fc domain of IgG is engineered to form continuous polypeptides with clotting factors that remain longer in plasma because they are cleared more slowly than the native factor and recycled back into the circulation. So far, this approach has been pursued for both FVIII and FIX, and data from studies in patients with hemophilia are already available,^{7–10} even though at the time of writing no product is yet licensed by regulatory agencies in Europe and in the USA. The purpose of this paper is to review the available knowledge on Fc fusion technology in the treatment of hemophilia A and B.

Fc fusion technology

The Fc domain of immunoglobulins has been previously employed to form fusions with such molecules as cytokines, growth factors, or other proteins used as research tools or therapeutic agents. Fc fusion is an established technology previously used to prolong the half-life of several drugs licensed for the long-term treatment of a number of chronic diseases.^{11–13} Fusion of the Fc domain of IgG to a therapeutic protein prolongs its half-life through binding to the neonatal Fc receptor (FcRn), which is expressed in the epithelial cells lining the intestine, lung, and kidney, with a degree of expres-

sion that is relatively constant throughout life in humans.^{14–16} FcRn is also expressed in the endothelial cells lining the vasculature, the site at which IgG protection most likely occurs.¹⁷ Fc domain binding to FcRn delays lysosomal degradation and favors recycling back into the circulation.^{18,19} Binding with the FcRn is a pH-dependent process, because it occurs intracellularly in acidic endosomes. IgG or Fc fusion proteins are taken up from circulation by nonspecific endocytosis; when the endosomes fuse back to the cell membrane, the neutral pH causes the dissociation of the Fc domain from FcRn, thereby releasing the IgG or Fc fusion protein into the bloodstream and thus escaping endosomal/lysosomal degradation.²⁰ Ultimately, the Fc fragment degrades and does not accumulate in the body.²¹

The Fc domain is a dimer able to bind two molecules of a given therapeutic protein, and first-generation dimeric Fc fusion molecules were successfully used and demonstrated biological activity. However, because there is an inverse relationship between molecular weight and transport efficacy, which is more evident at molecular weights greater than 30 kDa,²² continuing efforts to improve transport efficiency led to the development of second-generation Fc fusion molecules that are dimeric with respect to the Fc domain but monomeric with respect to the therapeutic protein. Referred to as Fc fusion monomers, these molecules have improved properties compared with their dimeric counterparts when the effector molecule is naturally a monomer, because the monomeric configuration improves transport efficiency and provides a greater extension of the circulating half-life. Moreover, many Fc fusion monomers showed enhanced biologic activity if compared with the dimeric configuration.^{23,24} In particular, pertaining to the FIX Fc fusion protein, the FIXFc monomer has enhanced pharmacokinetic properties compared with the FIXFc dimer, especially with respect to maximum concentration (C_{max}) and area under the curve (AUC),²⁴ eventually resulting in a prolonged half-life.

rFVIII₁₋₂Fc and rFIX₁₋₂Fc development

High gene expression, efficient secretion into the cell culture medium, and protein-A affinity purification of Fc fusion proteins render simple their downstream manufacturing processes, by allowing the application of the procedures established for antibody drug manufacturing.²⁵ Using this technology, rFVIII₁₋₂Fc and rFIX₁₋₂Fc were developed by fusing a single molecule of B domain-deleted (BDD) recombinant FVIII (rFVIII) or FIX (rFIX) to the dimeric Fc domain of IgG1 with no linker sequence.^{24,26,27} Both drugs are produced by recombinant DNA technology in human embryonic kidney

(HEK) 293 cells that, at variance with nonhuman mammalian cells, ensure full post-translational modifications, including glycosylation.²⁸ The latter is the most critical post-translational modification of therapeutic proteins because it does influence yield, solubility, stability, clearance rate, and immunogenicity.²⁹ In particular, post-translational modifications such as glycosylation, phosphorylation, and tyrosine sulfation are thought to be responsible for the lower in vivo recovery for rFIX in comparison with plasma-derived FIX (pdFIX).^{30,31}

Preclinical studies of rFVIII-Fc and rFIX-Fc

rFVIII-Fc

The biochemical and functional characterization of rFVIII-Fc in comparison with current rFVIII products confirmed that rFVIII-Fc contains the expected sequence based on the DNA coding sequence and post-translational modifications, such as N-linked glycosylation and tyrosine sulfation.²⁷ rFVIII-Fc has similar in vitro activity to BDD rFVIII, and its specific activity is similar on a molar basis by using a chromogenic assay, proving that the activity of the FVIII moiety is not compromised by Fc fusion.²⁷ Moreover, the in vitro affinity for von Willebrand factor (VWF) was modestly reduced (almost 30% lower than that of BDD rFVIII), a difference that is probably not relevant for the pharmacokinetic profile of the drug.²⁷ rFVIII-Fc has been proven to be as effective as rFVIII in treating tail clip injury in hemophilia A mice and fully corrected the whole blood clotting time in hemophilia A dogs.²⁶ Moreover, owing to the twofold prolonged half-life, rFVIII-Fc showed extended protection from bleeding as well as prolonged correction of the blood clotting time in hemophilic mice and dogs.²⁶ Finally, in FcRn knock-out mice, the half-life of rFVIII-Fc was similar to that of rFVIII, confirming the role of FcRn in half-life improvement of the Fc fusion protein.²⁶

rFIX-Fc

The functional characterization of rFIX-Fc in several animal models (including normal mice, rats, monkeys, and FIX-deficient mice and dogs) showed a 3.0–4.0 fold longer half-life than unmodified rFIX.²⁴ Biochemical characterization by Western blotting confirmed the identity of the FIX protein and peptide mapping showed a gamma-carboxylation pattern similar to that of rFIX, with ten of the 12 sites fully occupied.²⁴ Several other post-translational modifications were evaluated and compared with those of rFIX and pdFIX. In general, rFIX-Fc was comparable with rFIX with respect

to Serine (Ser) 158 phosphorylation and Tyrosine (Tyr) 155 sulfation; however, N-linked glycosylation fingerprints indicated that glycans are not fully sialylated.²⁴ O-linked glycosylated isoforms of the first epidermal growth factor (EGF) domain were similar to those of rFIX but present at different relative ratios, and the degree of beta-hydroxylation of Aspartate (Asp) 64 was greater than that of rFIX or pdFIX.²⁴ The levels of activated FIX were tenfold lower for rFIX-Fc than for either rFIX or pdFIX.²⁴ Finally, in FcRn knockout mice, the half-life of rFIX-Fc was similar to that of rFIX, confirming the role of FcRn in half-life improvement of the Fc fusion protein.²⁴

Pharmacokinetics of rFVIII-Fc and rFIX-Fc

rFVIII-Fc

Preclinical data from animal models showed a twofold longer terminal half-life of rFVIII-Fc than rFVIII.²⁶ The first-in-human Phase I/IIa study (ClinicalTrials.gov identifier: NCT01027377) investigated safety and pharmacokinetic properties of rFVIII-Fc in 16 previously treated adult patients with severe hemophilia A (FVIII <1 IU/dL), who received a single dose of rFVIII (25 or 65 IU/kg), followed by the same dose of rFVIII-Fc.⁷ rFVIII-Fc showed a 1.5–1.7 fold longer elimination half-life (18.8 hours for both rFVIII-Fc doses versus 12.2 and 11.0 hours for 25 and 65 IU/kg of rFVIII), a 1.5–1.6 fold lower clearance and a 1.5–1.6 fold higher total systemic exposure.⁷ Dose-dependent peak plasma concentrations and recoveries were similar, and time to reach 1% FVIII activity was 1.5–1.8 fold longer with rFVIII-Fc than rFVIII across all dosages.⁷ Similar results were subsequently obtained in 28 subjects enrolled in the Phase III clinical study (ClinicalTrials.gov identifier: NCT01181128). rFVIII-Fc terminal half-life was significantly longer for rFVIII-Fc than for rFVIII (19.0 versus 12.4 hours) and, consistently, time to reach 1% FVIII activity was 4.9 days as compared with 3.3 days with rFVIII.⁹ As already reported in the Phase I/IIa study, rFVIII-Fc half-life was positively correlated with the baseline plasma levels of VWF antigen and the incremental in vivo recovery was comparable to that of rFVIII.^{7,9} Comparative pharmacokinetic data obtained by one-stage FVIII coagulation assay were confirmed by chromogenic assay and rFVIII-Fc pharmacokinetic data were highly consistent following repeated doses over 14 weeks of treatment.⁹

rFIX-Fc

The half-life of rFIX-Fc was 3.0–4.0 fold longer than that of rFIX in hemophilic mice and dogs.²⁴ The improved

and prolonged activity was confirmed in the first-in-human Phase I/IIa study (ClinicalTrials.gov identifier: NCT00716716)⁸ conducted in 14 previously treated adult patients with severe and moderately severe (FIX ≤ 2 IU/dL) hemophilia B who received a single dose of rFIXFc (12.5, 25, 50, or 100 IU/kg).⁸ The terminal half-life was threefold longer than that of rFIX (approximately 19 hours), being on average 53.5, 57.6, and 56.5 hours with 25, 50, and 100 IU/kg, respectively.⁸ The incremental recovery of rFIXFc was 0.93 IU/dL per IU/kg and hence similar to that of pdFIX.⁸ Finally, the average time to reach 1% trough FIX activity was 7.3, 10.1, and 12.3 days after 25, 50, and 100 IU/kg, respectively.⁸ Clearance was reduced to 38% of the historical value reported with rFIX, and the maximum concentration was reached immediately after injection of rFIXFc, suggesting a rapid onset of action similar to that of rFIX.⁸ Data from the Phase III study (ClinicalTrials.gov identifier: NCT01027364),¹⁰ recently fully published, confirm the pharmacokinetic profile of rFIXFc on a larger patient sample size (n=123), being the mean half-life of rFIXFc 82.1 versus 33.8 hours for rFIX and the mean time to reach 1% trough FIX activity (after a 50 IU/kg dose) was 11.2 days.¹⁰ The pharmacokinetics of rFIXFc was stable over repeated dosing during several weeks, with a significant increase of AUC and mean residence time and a significant decreased clearance for rFIXFc in comparison with that for rFIX ($P < 0.001$).¹⁰

Clinical development of rFVIII Fc and rFIX Fc rFVIII Fc

The results of an open-label, multicenter Phase III study (A-LONG Study; ClinicalTrials.gov identifier: NCT01181128),⁹ designed to evaluate safety, pharmacokinetics, and efficacy of rFVIII Fc in previously treated patients with severe hemophilia A, has been published.⁹ The study enrolled 165 patients who received rFVIII Fc according to three different treatment regimens: 118 of them received regimens of individualized prophylaxis consisting of 25 IU/kg on day 1 and 50 IU/kg on day 4 to start, followed by 25–65 IU/kg every 3–5 days; 24 received prophylaxis with 65 IU/kg once weekly; and 23 received 10–50 IU/kg (depending on the bleeding severity) for on-demand treatment of bleeding episodes.⁹ The study was finished upon completion of the scheduled pharmacokinetic assessment and achievement of the prespecified number of rFVIII Fc exposure days (EDs), required in order to ensure an acceptable degree of inhibitor detection (ie, a minimum of 104 cases from any treatment arm with at least 50 EDs).⁹ Overall, 153 patients (93%) com-

pleted the study, and 111 of them had more than 50 EDs.⁹ The median annualized bleeding rate was 1.6, 3.6, and 33.6 in each of the three treatment arms, respectively, with a clear advantage for patients treated on prophylaxis versus those treated on demand.⁹ On average, patients treated with individualized prophylaxis received a median weekly dose of 77.7 IU/kg at a median dosing interval of 3.5 days; with this regimen, 45% of patients had no bleeds versus the 17% bleed-free rate observed in the weekly prophylaxis arm.⁹ Across all arms, 757 bleeding episodes were treated with rFVIII Fc at a median dose of 27 IU/kg. The efficacy rate was 87% with one injection and 98% with two injections.⁹ Efficacy was also assessed in the surgical setting, and was found to be excellent in eight of nine major surgical procedures performed in nine patients.⁹ With respect to safety, no neutralizing inhibitors were detected in any subject, but five subjects tested positive for low-titer non-neutralizing antibodies directed against FVIII, but not against Fc.⁹ Adverse events were reported in 108 subjects, but only in ten of them were they related to the investigational drug; arthralgia and malaise were the most common.⁹ No thrombotic events, hypersensitivity reactions, and/or anaphylaxis were reported.⁹

The Phase III pediatric study (Kids A-LONG Study, ClinicalTrials.gov identifier: NCT01458106)³² is currently recruiting children with severe hemophilia A aged less than 12 years already exposed to FVIII for a minimum of 50 EDs. This study will assess the safety, efficacy, and pharmacokinetics of rFVIII Fc in approximately 50 children with severe hemophilia A with a prophylactic treatment regimen. Patients who complete the aforementioned Phase III studies have the opportunity to continue treatment with rFVIII Fc in the frame of the ASPIRE extension study (ClinicalTrials.gov identifier: NCT01454739)³³ that will assess long-term safety and efficacy of rFVIII Fc.

rFIX Fc

An open-label, multicenter Phase III study (B-LONG Study; ClinicalTrials.gov identifier: NCT01027364),¹⁰ aimed at evaluating safety, pharmacokinetics, and efficacy of rFIX Fc in previously treated patients with severe and moderately severe hemophilia B was recently completed.¹⁰ The study enrolled 123 patients who received rFIX Fc according to three different treatment regimens: 61 of them received weekly prophylaxis starting at 50 IU/kg, with subsequent pharmacokinetic-driven dose adjustments; 29 received prophylaxis at individualized intervals, with 100 IU/kg starting at every 10 day interval and subsequent adjustment of dosing intervals driven by pharmacokinetic data; and 27 received 20–100 IU/kg

(depending on the severity of bleeding) for on-demand treatment of bleeds.¹⁰ Twelve patients (including eight from the three aforementioned treatment groups) received rFIXFc to cover major surgical procedures.¹⁰ Overall, 115 patients (93%) completed the study.¹⁰ The median annualized bleeding rate was 2.95, 1.4, and 17.7 in each of the three treatment arms, respectively, with a clear advantage for patients treated on prophylaxis versus those treated on demand.¹⁰ The dosing interval for patients treated with individualized prophylaxis increased from the initial 10 days to an average interval ≥ 14 days in 54% of cases.¹⁰ Across all arms, 636 bleeding episodes were treated in 89 patients with rFIXFc at a median dose of 46 IU/kg.¹⁰ Across all regimens, the efficacy rate was 90% with one injection and $>97\%$ with two injections.¹⁰ Efficacy was also assessed in the surgical setting, being excellent in 13 of 14 major surgical procedures performed in 12 patients.¹⁰ A single injection of rFIXFc at a median dose of 91 IU/kg was required in 86% of surgeries to maintain hemostasis during the procedure.¹⁰ Most procedures required up to two injections on the day of surgery, and the majority of patients required no more than three injections during postoperative days 1–3.¹⁰ With respect to safety, no inhibitors were detected in any subject, and only one serious adverse event (obstructive uropathy) was assessed as possibly related to treatment.¹⁰

The Phase III pediatric study (Kids B-LONG Study, ClinicalTrials.gov identifier: NCT01440946)³⁴ is currently recruiting children with severe hemophilia B aged less than 12 years who have already been exposed to FIX for a minimum of 50 EDs. This study will assess the safety, efficacy, and pharmacokinetics of rFIXFc used as prophylactic treatment in approximately 26 children with severe hemophilia B. Patients who completed or will complete the aforementioned Phase III studies have the opportunity to continue treatment with rFIXFc in the frame of the B-YOND extension study (ClinicalTrials.gov identifier: NCT01425723)³⁵ that will assess long-term safety and efficacy of rFIXFC.

Conclusion

The evidence summarized above indicates that in the frame of pharmacokinetic studies coagulation FVIII and FIX produced by Fc fusion technology have a longer plasma half-life than standard factor products, although to a much lower extent for FVIII than for FIX.^{7–10} Consistent with the pharmacokinetic data obtained in Phase I–II studies, published clinical data indicate that it is possible to space factor infusions of at least 3 days in the frame of prophylaxis. Hence, there is some degree of sparing venipunctures compared with standard FVIII, but

the difference is not dramatic. On the other hand, pharmacokinetic data for rFIXFc should allow a higher degree of infusion spacing. For instance, in patients with hemophilia A it can be predicted that replacement therapy with FVIII can be given every third day instead of every other day, totaling 121 intravenous injections annually instead of 182. By the same token, in hemophilia B FIX replacement would be given on average every 10 days instead of every third day, totaling 36 annual infusions instead of 121. The available pharmacokinetic and clinical studies indicate that there were no significant adverse events related to the clinical use of these products. It is not surprising that there were very few adverse reactions of the allergic type, because both the components of the fusion polypeptides are plasma proteins with no foreign chemical agent added. Of course, the main theoretical concern before their use in humans was that these highly engineered protein constructs might be seen as foreign by the immune system and thereby cause the new occurrence of inhibitory antibodies even in previously treated patients; ie, those included in Phase I/IIa and Phase III studies. The currently available data allow to exclude that these products carry a strong immunogenicity signal.^{7–10} On the other hand, these favorable results should be regarded as preliminary and long-term surveillance on inhibitor onset is warranted when these products are licensed, as they may become widely available and more patients may switch to their use from the currently available standard products. It will also be important to collect postmarketing data in previously untreated patients with severe hemophilia; ie, the population at higher risk of inhibitor development.

What other problems require more clinical data to be solved? As mentioned above, Fc fusion technology did not prolong the half-life of FVIII and FIX to the same extent. A possible explanation for that is the tight association of rFVIIIc with VWF, because unlike FIX, FVIII circulates in complex with VWF which has a relatively short terminal half-life of approximately 18 hours. We suspect that the physiological need for FVIII to form complexes with VWF, is a major limitation for the degree to which the half-life of rFVIII products can be extended. In addition, it is not yet clear whether or not there will be problems in the laboratory assay of the fusion protein both in vitro and ex vivo, perhaps due to its variable interaction with phospholipids in the activated partial thromboplastin time (aPTT) reagents used to measure FVIII in patient plasma.

A big issue will of course be the price of these novel products, which is not yet known at the time of writing. Perhaps simplistically, we would suggest that the price should

be that of the standard products added with that of actual factor doses that are spared owing to the longer half-life, plus a bonus for the research that led to the development of these truly innovative coagulation factors. It is necessary to develop a model for assessing cost-effectiveness, with quality of life measurement being an essential component of this. It is difficult, lacking head-to-head comparisons, to establish how the Fc fusion factors compare with other long-acting products that are in the pipeline. The albumin fusion FIX product seems to be appealing,³⁶ because albumin is a physiological component of plasma, like IgG. In principle, these two products should be considered more attractive than those based upon the use of polyethylene glycol, because the latter chemical may theoretically accumulate in the body in lifelong-treated patients, like those with severe hemophilia.

All in all, we believe that Fc-fusion products are an exciting innovation in the field of hemophilia treatment, even though only more widespread field use will answer the many still unresolved questions.

Disclosure

The authors report no conflicts of interest in this work.

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