

# BLOOD

# T Y P E



key updates for medical care providers

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## The New landscape of Therapeutics for the Treatment of Hemophilia

### Introduction

There have been many changes to the treatment landscape of Hemophilia over the past few decades. Initial therapy with blood products was replaced with plasma-derived lyophilized factor concentrates, which allowed for home infusion and improved quality of life. Further purification and viral inactivation processes increased the safety profile of plasma-derived concentrates. Genetic cloning of factors VIII and IX in the 1980's and 1990's has led to the production of recombinant products.

High cost of therapy, the frequency of infusions often requiring venous access devices, and inhibitor formation have been issues that have impacted effective prophylaxis. Further advances in product half-life extension through different technologies including albumin or neonatal Fc fusion, PEGylation, and single chain technology have led to the recent FDA approval of four extended-half-life recombinant products, with several other products in late-stage clinical trials. The manufacturing of several recombinant products (Nuwiq, Eloctate, Alprolix) in Human Embryonic Kidney (HEK) cell lines may result in a product that is more like the human native protein in regards to post-translational modifications. Several nonfactor therapeutic approaches with improved ease of administration are being explored. The hope for gene therapy is on the horizon; however, issues impacting achieving long-term stable factor expression, potential risk of insertional mutagenicity, and hepatic toxicity remain.

### The Generations of Recombinant Factor Products

Initial recombinant FVIII products contained added human albumin as a stabilizer. Third generation products eliminated

human and animal proteins from the culture media and final preparation, and utilized additional viral inactivation steps to further increase safety. FDA approval of Alprolix in March 2014, Eloctate in June 2014, and Nuwiq in September 2015 ushered in the fourth generation of products which are produced in Human Embryonic Kidney (HEK) cell lines, without human or animal protein exposure. It is hoped that these products more closely resemble the native clotting factor and therefore may impact the recognition of the protein by the immune system and potentially translate into a decreased rate of inhibitor development, especially in FVIII deficiency – time will tell.

Table 1: Generations of Recombinant Factor Products

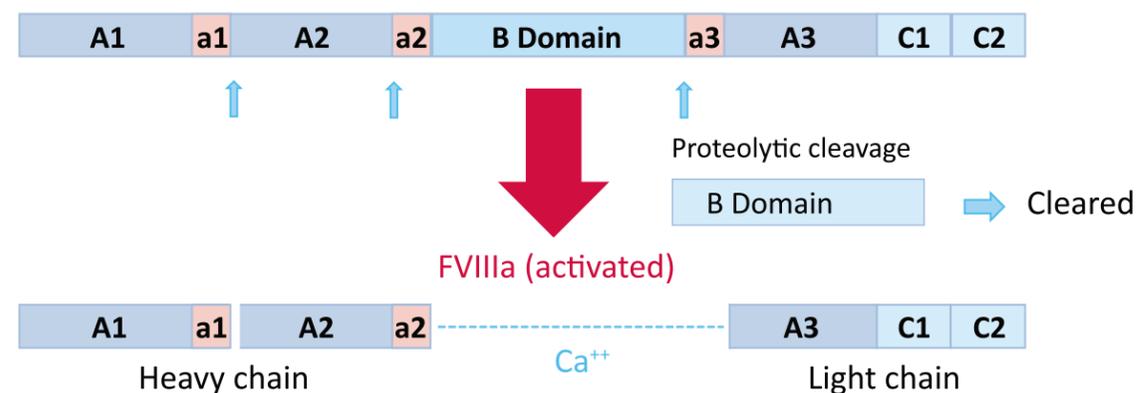
| Generation | Definition   |
|------------|--|
| 1st        | Contains albumin in final preparation, exposed to human or animal proteins during production |
| 2nd        | No albumin in final preparation, exposed to human or animal proteins during production       |
| 3rd        | No human or animal protein exposure  |
| 4th        | Produced in a human cell line, no human or animal protein exposure                           |

### B Domain Modification in FVIII Recombinant Products

The FVIII gene is a large molecule. B-domain modification through deletion or truncation improves production efficiency. Once activated, the B domain deleted or truncated protein is the same as the activated native protein. Currently licensed products that have undergone B-domain modification include Xyntha (Pfizer), Novoeight (Novo Nordisk), Nuwiq (Octapharma), Eloctate (Biogen), and OBIZUR (Baxalta). (See Figure 1, next page.)

Figure 1:

### FVIII



FVIII requires proteolytic activation catalyzed by thrombin or factor Xa. The proteases cleave FVIII at three sites, yielding the active cofactor FVIIIa. The cleaved FVIIIa contains heavy and light chains, which are held together by calcium ions.<sup>56</sup>

### New Recombinant Factor Product Concentrates

The following sections will discuss recombinant products that have been recently licensed or are in late-stage clinical trials. Other recombinant products that were licensed and launched in the United States prior to 2013, or are in earlier stages of development or clinical study will not be reviewed.

### New Standard Half-Life Recombinant Factor Concentrates:

#### Factor VIII Deficiency (Hemophilia A)

##### Novoeight (Turoctogog Alfa):

Novoeight is a third generation B-domain deleted recombinant product manufactured by Novo Nordisk in the Chinese hamster ovary cell line (CHO). It was launched in the United States on March 26, 2015. Median annualized bleeding rate (ABR) was reported as 3.1 in 213 subjects receiving Novoeight for routine prophylaxis. It is approved for prophylaxis and on-demand treatment of bleeding episodes in children, adolescents, and adults with Hemophilia A.<sup>2,3,4,5,6,7</sup>

##### Nuwiq (Simoctogog Alfa, human-cl rhFVIII):

Nuwiq is a fourth generation B-domain deleted recombinant FVIII product produced in a HEK cell line. It is manufactured by Octapharma, and was FDA approved on September 15, 2015. In contrast to currently available commercial rFVIII produced in animal cell lines, Nuwiq is fully sulphated at Tyr1680 and therefore displays higher binding affinity for von Willebrand factor (VWF). A higher VWF binding affinity minimizes circulation of unbound FVIII and reduces FVIII clearance, which may translate into an improved half-life in some patients. Producing a protein that is more similar to the native human FVIII through production in a human cell line has been suggested as a

mechanism to potentially decrease inhibitor development or development of allergic reactions. Nuwiq is approved for prophylaxis and on-demand treatment of bleeding episodes in children, adolescents, and adults with Hemophilia A.<sup>8,9,10,11,12</sup>

##### Kovaltry (Octocog Alfa):

Kovaltry is a third generation full length recombinant FVIII product produced in a Baby Hamster Kidney (BHK) cell line. The FDA approved Kovaltry in March 2016 for use in adults, adolescents, and children with Factor VIII deficiency. Several improvements have been reported with Kovaltry in comparison to its predecessor, Kogenate (rFVIII-FS), including improvements in consistency of glycosylation and expression. Furthermore, coexpression of human heat-shock protein 70 (HSP70), a molecular chaperone involved in the proper folding of proteins and prevention of protein aggregation, enhances the viability of the expression cell line by inhibiting apoptosis and possibly by increasing proper folding of the FVIII protein. LEOPOLD (Long-Term Efficacy Open-Label Program in Severe Hemophilia A Disease) clinical trials enrolled 204 adult and pediatric patients with hemophilia A and a factor VIII level  $\leq 1\%$  as part of either routine prophylaxis, on-demand treatment of bleeding episodes, perioperative management, or pharmacokinetic evaluation of Kovaltry. In LEOPOLD II, patients were randomized to receive on demand therapy, low dose prophylaxis twice weekly (20-30 IU/kg), or higher dose prophylaxis (30-40 IU/kg) three times weekly. As expected, prophylaxis was found to be superior to on demand therapy. Compared to the thrice weekly group, patients in the twice weekly group had higher ABR during the first 6-month period of treatment, but the ABR was comparable between the two groups in the second 6-month period.<sup>13,14,15</sup>

### Factor IX Deficiency (Hemophilia B)

#### Ixinity (Trenonacog Alfa)

Ixinity is a third generation recombinant antihemophilic Factor IX produced in a Chinese hamster ovary cell line (CHO). It is manufactured by Emergent Biosolution, and was FDA approved on April 29th, 2015. Ixinity is licensed for prophylaxis and on-demand treatment of bleeding episodes in adults and adolescents with Hemophilia B.<sup>16,17</sup>

### Von Willebrand Factor Deficiency

#### Vonvendi

Vonvendi (Vonvendi Alfa) is a recombinant Von Willebrand Factor (VWF) that was FDA approved in December 2015 for on-demand treatment of bleeding episodes in adults > 18 years of age with all subtypes of Von Willebrand Disease (VWD). It is a third generation product manufactured in a CHO cell line. A total of 193 bleeding episodes were reported in 22/37 patients with VWD exposed to VONVENDI. Out of these patients, 4 had type 2A, 1 had type 2N, and 17 had type 3 VWD.<sup>18,19</sup>

Unlike currently available plasma-derived concentrates, Vonvendi does not contain a significant amount of FVIII. Therefore, the use of this product requires initial co-administration of recombinant FVIII in patients with low factor VIII levels. Use of this product may be advantageous in VWD patients who require repeated infusions such as those undergoing procedures, as elevated FVIII levels that result from continued use of plasma derived products containing both FVIII and VWF may contribute to development of adverse events such as thrombosis. Vonvendi may prove particularly useful in the perioperative setting for patients with VWD type 3. Unlike plasma-derived VWF concentrates, Vonvendi has no exposure to the human protease ADAMTS13 during the production process and maintains intact high-molecular-weight multimers and ultralarge multimers, which are essential for effective platelet plug formation.<sup>20</sup>

### Extended Half-Life (EHL) Factor Concentrates

Despite the advent of recombinant factor concentrates, hemophilia patient outcomes have remained suboptimal; the frequency of required infusions impacts quality of life, many patients require central venous access devices for continued venous access, and most patients with hemophilia spend the majority of time with factor trough levels below the mild deficiency range.

These therapeutic issues have sparked investigation into development of products with an extended half-life. The technologies that have been applied to recombinant FVIII and FIX products include neonatal Fc receptor (FcRn) or albumin fusion, and PEGylation. In the case of recombinant

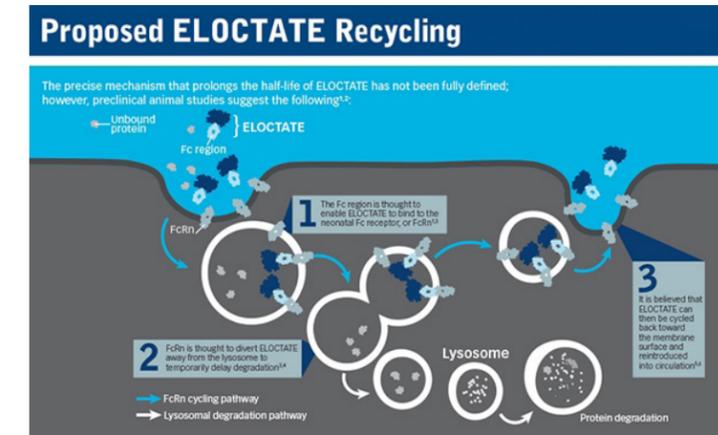
FVIII, Single Chain technology has also been employed.<sup>21</sup>

The normal expected plasma half-life of FVIII is 8-12 hours, and 18-24 hours for FIX. Utilizing current technologies, rFIX plasma half-life can be effectively increased to 4-5 times the standard half-life. The results of rFVIII half-life prolongation have been more modest at 1.4-1.5 times the standard half-life, likely related to the dependence of FVIII half-life on the binding and clearance of endogenous von Willebrand factor.<sup>22</sup> Notably, product half-life is often inversely proportional to patient age, with the pediatric population demonstrating increased clearance rates and volume of distributions.

#### Neonatal Fc receptor fusion/ Albumin fusion

FVIII and FIX can be covalently fused to the Fc domain of human immunoglobulin, IgG1. The Fc domain of the fusion protein then binds to the neonatal Fc receptor, protecting it from intracellular lysosomal degradation and recycling it back into the circulation. This results in prolonged factor plasma half-life through use of a naturally occurring pathway designed to delay lysosomal degradation of immunoglobulins and albumin.<sup>23,24</sup> Covalent fusion between albumin and recombinant factors also utilizes the same neonatal Fc receptor and the endogenous IgG recycling pathway.<sup>25</sup> (See Figure 2, below.)

Figure 2:



Biogen Idec. "Proposed Elocate Recycling." 2016. Accessed June 6, 2016, at: [https://www.eloctatepro.com/en\\_us/home/science/fc-fusion.html](https://www.eloctatepro.com/en_us/home/science/fc-fusion.html). Used with permission.

### FVIII

#### Eloctate (rFVIII-Fc)

Eloctate is a fusion protein composed of B-domain deleted recombinant factor FVIII and the Fc portion of IgG1. It is a fourth generation product produced from a HEK cell line and is manufactured by Biogen. It was the first EHL rFVIII product to be FDA approved in 2014. Pharmacokinetic studies have reported an approximate half-life of 19.7 hours in adults and 12.7-16.4 in children and adolescents.<sup>26</sup> Safety and efficacy has been demonstrated in the adult and

pediatric populations. In a Phase III open-label, partially randomized study, 165 previously treated patients >12 years of age with severe Hemophilia A were randomized to receive: 1) Prophylaxis with 25 IU/kg on Day 1, 50 IU/kg on Day 4 with dose modification for goal FVIII trough level of 1 to 3 IU/dL or higher as needed to maintain good control of breakthrough bleeding; 2) Prophylaxis with 65 IU/kg weekly; and 3) on demand therapy. Median ABR for the three groups were 1.6, 3.6, and 33.6, respectively.<sup>27</sup> A Phase III single-arm pediatric study of 71 patients <12 years of age (Kids A-LONG) administered twice-weekly prophylaxis (25 IU/kg on Day 1, 50 IU/kg on Day 4), with a reported median ABR of 1.96.<sup>28</sup>

#### *FIX*

##### *Alprolix (rIXFc)*

Alprolix is composed of rFIX fused with the Fc domain of IgG1. It is a fourth generation product produced in a HEK cell line, and is manufactured by Biogen. The reported half-life was 87 hours in adults, and varying from 6-83.59 hours in the pediatric population.<sup>29</sup> In A Phase III nonrandomized study of patients >12 years of age with hemophilia B (FIX activity < 2 IU/dL), the following treatment groups were evaluated: 1) 50 IU/kg weekly; 2) 100 IU/kg every 10 days; and 3) on-demand therapy. The ABR in the three groups was 3.1, 2.4, and 18.7, respectively.<sup>30,31</sup>

##### *IDELVION (rIX-FP)*

IDELVION is a fusion protein between the C-terminus of recombinant FIX and human recombinant albumin, linked together by a cleavable linker-peptide derived from an endogenous FIX sequence. Upon activation by FXIa or the FVII-TF complex, albumin is cleaved leaving rFIXa. Pharmacokinetic studies from a recent Phase III publication revealed a terminal half-life of 102 hours. Interestingly, the mean troughs for patients on prophylaxis with 40 IU/kg weekly or 75 IU/kg every 2 weeks were 20 IU/dL and 12 IU/dL respectively.<sup>32,33,34,35</sup> This suggests the possibility of achieving higher trough levels than previously possible while minimizing infusion intervals, which may positively impact outcomes or quality of life.

#### *PEGylation*

Half-life extension through site-specific or site-directed PEGylation employs the addition of a large polyethylene glycol moiety to the protein of interest, shielding the protein from proteolysis and reducing hepatic clearance through uptake by lipoprotein-related receptors (LRP1), thereby prolonging circulating drug half-life.<sup>36</sup>

#### *FVIII*

##### *Adynovate (Rurioctacog Alfa Pegol)*

Adynovate is a third generation full length chain extended half-life rFVIII concentrate manufactured by Baxalta. It is produced by covalently binding a branched PEG domain

utilizing a process that ensures 60% of PEG chains are located in the B-domain, which are subsequently cleaved upon activation.<sup>37</sup> Adynovate was U.S. FDA approved on November 13, 2015 for prophylaxis and on-demand treatment of bleeding episodes in adults and adolescents >12 years of age with Hemophilia A. Adynovate's mean half-life is 14.69 hours in adults and 13.43 hours in children, approximately 1.4-1.5 times that of Advate, with the advantage of fewer infusions to achieve targeted factor levels. Clinical trials have not demonstrated evidence of toxicity or altered immunogenicity. The ABR was 1.9 for patients on prophylaxis, with 39.6% of compliant subjects having no bleeding episodes. Median ABR in subjects treated on-demand was 41.5.<sup>38</sup>

#### **Products Not Currently Licensed**

##### *BAY 94-9027*

BAY 94-9027 is a third generation extended half-life rFVIII concentrate that is awaiting licensure by the FDA. It is produced through site-specific conjugation of a PEG molecule to B-domain deleted rFVIII and is manufactured by Bayer. A Phase I clinical trial has reported a half-life of approximately 18.6-19.5 hours in adults.<sup>39</sup> A Phase II/III study in 134 previously treated patients >12 years of age demonstrated preservation of full biologic activity with annual bleeding rate (ABR) ranging from 1.9 to 4.1 in patients receiving prophylaxis and 23 for patients receiving on-demand therapy.<sup>40</sup>

##### *N8 GP (Turoctacog Alfa Pegol)*

N8 GP is a third generation product produced through site-specific glycoPEGylation of B-domain truncated recombinant FVIII. It is manufactured by Novo Nordisk and is awaiting FDA licensure. N8 GP has a reported half-life of approximately 18.4 hours in adults.<sup>41</sup> In a phase III trial of 175 patients receiving prophylaxis and 12 patients receiving on-demand therapy, the ABR was 1.3 and 30.9, respectively.<sup>42</sup>

##### *Single Chain Technology: rVIII-SingleChain (CSL-627)*

rVIII-SingleChain is produced through Single Chain Technology, which covalently links heavy and light chains within a single FVIII chain to increase binding affinity to Von Willebrand Factor. The prolonged VWF half-life is then utilized to provide longer FVIII half-life through decreased clearance and larger area under the curve. In a pivotal phase I/III trial of adults with severe Hemophilia A, 173 patients were followed with 146 receiving prophylaxis with a resultant median ABR of 1.66.<sup>46</sup> Initial data from Phase I trials revealed a half-life of approximately 13.1 hours in adults, not very dissimilar to that reported with standard half-life recombinant factor VIII products. An open-label multicenter phase III study is currently underway (NCT 02172950).

#### *FIX*

##### *N9-GP (Nonacog Beta Pegol)*

N9-GP is a third generation recombinant FIX molecule

covalently attached to a 40-kDA PEG molecule.<sup>43</sup> It is manufactured by Novo Nordisk, and has been submitted for licensure to the FDA. In a phase III study of adults, 15 patients chose on-demand therapy and 59 patients chose prophylactic therapy. The patients who chose prophylaxis were randomized to receive either 10 IU/kg or 40 IU/kg weekly; the half-life was reported as approximately 92 hours. The ABR for on-demand therapy was 16, whereas the ABR for the prophylaxis groups with 10 IU/kg weekly and 40 IU/kg weekly were 3 and 1, respectively.<sup>44</sup> In 25 previously treated pediatric patients, N9-GP has found to have a half-life of 69.6-76.3 hours.<sup>45</sup> A Phase III trial investigating the safety and efficacy of N9-GP in previously untreated patients (ParadigmTM6, NCT 02141074)) is currently underway.

#### **The Future of Extended Half-Life Products**

Recent FDA approval of several extended half-life factor concentrates with others in late stage clinical trials has sparked debate on patient and product selection, with particular emphasis on potential risks and benefits.

Extended half-life products may reduce the number of required infusions in patients with severe hemophilia, with the potential to decrease need for placement of venous access devices and perhaps impact "needle phobia". These products may allow the ability to achieve consistently higher trough levels, with the potential to prevent breakthrough bleeding and subsequent joint disease. Animal models have suggested decreased inhibitor rates in EHL product use; if this data is demonstrated in humans, then these products could improve disease-related morbidity and mortality. This must be proven through clinical studies. Higher endogenous Von Willebrand Factor levels in older adults may translate to longer half-life of EHL rFVIII products. Conversely, the pediatric population may theoretically derive less benefit from rFVIII EHL products in terms of half-life extension. Monitoring continues to be important in both pediatric and adult populations. Given the current plethora of available products, it is important to personalize patient care with consideration of all risks and benefits.

#### **Inhibitor Formation**

The prevalence of inhibitor formation in previously untreated patients is approximately 30% in severe Hemophilia A, and up to 5% in severe Hemophilia B. Complications of inhibitors include failure of standard replacement therapy with difficulty achieving hemostasis, increased morbidity and mortality, and significantly increased surgical risk. Inhibitors remain one of the most severe complications of hemophilia, and are associated with an increased economic burden.<sup>47</sup> Treatment includes instituting a bypassing agent to achieve hemostasis in bleeding episodes, as well as immune tolerance induction therapy to eradicate the inhibitor as appropriate.<sup>48,49,50</sup>

Per the SIPPET trial presented at ASH 2015<sup>51</sup>, rFVIII products were found to be associated with a 1.87-fold higher incidence of inhibitors than the plasma-derived FVIII class that also included VWF. This difference remained even when second generation full length rFVIII concentrates were excluded from the analyses, suggesting that the presence of VWF in the concentrate may have a protective effect. The final publication is awaited for a more detailed analysis of this data. The trial did not include the newest generation of products and these therefore will warrant further study. How this trial will impact care paradigms in pediatric patients has yet to be determined.

Recombinant FVIIa (NovoSeven RT) and plasma-derived activated prothrombin complex concentrate (FEIBA) are FDA approved for the purpose of achieving hemostasis in patients with inhibitors to FVIII or FIX.

OBIZUR is a recombinant porcine sequence FVIII product licensed for treatment of acquired Hemophilia A (autoimmune FVIII inhibitors). The B-domain normally present in naturally occurring porcine factor VIII has been replaced with a twenty-four amino acid linker. Once activated, the resulting rpFVIIa has a comparable activity to the endogenous human FVIIa. Obizur is expressed in a genetically engineered baby hamster kidney (BHK) cell line.<sup>52</sup> The acquired FVIII antibodies often have decreased cross-reactivity to this protein, and therefore it can be used to achieve hemostasis with the added ability to measure FVIII levels through standard assays. Monitoring of levels and for development of anti-porcine factor VIII antibodies is recommended.

#### **Gene Therapy**

Work on development of viable Gene therapy, in particular for Hemophilia B, has continued with the hopes of the potential

for long term expression of levels or even what is termed a “cure”. Several early phase I clinical trials and Adeno-associated virus trials have shown promise.<sup>53,54,55</sup> The two main strategies include using a vector to introduce a transgene into pluripotent cells, which then express the transgene in their progeny and results in long-term gene expression, or using viral vectors to introduce the transgene into long-lived, non-dividing cells.<sup>56</sup> Current challenges include ensuring long-term stable protein expression, vector immunogenicity, hepatic toxicity, and the potential risk of insertional mutagenesis.<sup>57,58,59</sup>

## Conclusions

The hemophilia treatment landscape continues to evolve. New recombinant products with B domain modifications, those produced in human cell lines, and extended half-life factor concentrates present additional treatment options that assist in development of personalized care for the hemophilia patient population. Important considerations include disease severity, targeted levels, patient lifestyle/preferences, prevention of joint disease, and risk of inhibitor development. Treatment with newer products requires continued surveillance for potential long-term outcomes, effects or toxicities. The advent of extended half-life products is exciting, as these may impact improved patient quality of life through fewer infusions and an ability to achieve higher trough levels. The benefits of these technologic advances must be weighed against economic considerations. The impact of new manufacturing technologies on inhibitor development requires further study. Overall, continued advances will increase our ability to impact the morbidity and mortality associated with hemophilia.

*By, Magdalena Lewandowska, MD*

## References

- Kumar et al. Changing the Paradigm of Hemophilia Management: Extended Half-Life Factor Concentrates and Gene Therapy. *Semin Thromb Hemost* 2016; 42(01): 018-029. DOI: 10.1055/s-0035-1568877
- Santagostino E., Lentz S.R. et al: Safety and efficacy of turoctocog alfa (NovoEight®) during surgery in patients with haemophilia A: results from the multinational guardianTM clinical trials. *Haemophilia* (2015), 21, 34-40.
- Lentz S.R., Misgav M. et al: Results from a large multinational clinical trial (guardianTM1) using prophylactic treatment with turoctocog alfa in adolescent and adult patients with severe haemophilia A: safety and efficacy. *Haemophilia* (2013), 19, 691-697.
- Kulkarni R., Karim F.A. et al: Results from a large multinational clinical trial (guardianTM3) using prophylactic treatment with turoctocog alfa in pediatric patients with severe haemophilia A: safety, efficacy and pharmacokinetics. *Haemophilia* (2013), 19, 698-705.
- Martinowitz U., Bjerre J. et al: Bioequivalence between two serum-free recombinant factor VIII preparations (N8 and ADVATE)- an open-label, sequential dosing pharmacokinetic study in patients with severe haemophilia A. *Haemophilia* (2011), 17, 854-859.
- Viuff D., Barrowcliffe T.W. et al: International comparative field study of N8 evaluating factor VIII assay performance. *Haemophilia* (2011), 17, 695-702
- Thim L., Vandahl B. et al: Purification and characterization of a new recombinant factor VIII (N8). *Haemophilia* (2010), 16, 349-359.
- Valentino L.A. et al. (2014) 'The first recombinant FVIII produced in human cells - an update on its clinical development programme', *Haemophilia*, vol. 20 (Suppl. 1), pp. 1-9
- ClinicalTrials.gov (2014) Clinical Study to Investigate the Pharmacokinetics, Efficacy, Safety and Immunogenicity of a Recombinant FVIII in Patients With Severe Hemophilia A [Online] <http://clinicaltrials.gov/show/NCT00989196>
- Tiede A. et al. Consumptino of FVIII Concentrate During On-Demand and Prophylactic Treatment With Human-cl rhFVIII In Prospective Clinical Studies in Adult Patients with Severe Hemophilia A. November 15, 2013. *Blood*: 122 (21)
- Tiede A. et al. (2014). Personalized prophylaxis with human-cl rhFVIII in adult patients with severe haemophilia A. Poster presented at the Bari International Conference, Bari, Italy, 3-5 October 2014
- Kannicht C, Ramström M, Kohla G et al. Characterisation of the post-translational modifications of a novel, human cell line derived recombinant human factor VIII. *Thromb Res* 2013; 131: 78–88. Sandberg H, Kannicht C, Stenlund P et al. Functional characteristics of the novel, human-derived recombinant FVIII protein product, human-cl rhFVIII. *Thromb Res* 2012; 130: 80817.
- Sandberg H, Kannicht C, Stenlund P et al. Functional characteristics of the novel, human-derived recombinant FVIII protein product, human-cl rhFVIII. *Thromb Res* 2012; 130: 80817.
- Kavakli K et al. Prophylaxis vs. on-demand treatment with BAY81-8793, a full-length plasma protein-free recombinant factor VIII product: results from a randomized trial (LEOPOLD II). *J Thromb Haemost*. 2015 Mar;13(3):360-9.
- Shah et al. Pharmacokinetic properties of BAY 81-8973, a full-length recombinant factor VIII. *Haemophilia*. 2015 Nov;21(6):766-71. doi: 10.1111/hae.12691. Epub 2015 May 8.
- Ljung R, Kenet G et al. BAY 81-8973 safety and efficacy for prophylaxis and treatment of bleeds in previously treated children with severe haemophilia A: results of the LEOPOLD Kids trial. *Haemophilia*. 2015 Dec 9. doi: 10.1111/hae.12866. [Epub ahead of print]
- Martinowitz U., Shapiro A. et al: Pharmacokinetic properties of IB1001, an investigational recombinant factor IX, in patients with haemophilia B: repeat pharmacokinetic evaluation and sialylation analysis. *Haemophilia* (2012), 1-7.
- Astacio H., Atnikov K. et al: Safety Profile of IB1001, Recombinant Factor IX (trenonacog alfa) in the Treatment of Hemophilia B. Poster presentation at the World Federation of Hemophilia 2-14 World Congress.
- Gill JC, et al. Hemostatic efficacy, safety and pharmacokinetics of a recombinant von Willebrand factor in severe von Willebrand disease. *Blood*. 2015.
- Turecek PL, Schrenk G, Rottensteiner H, et al. Structure and function of a recombinant von Willebrand factor drug candidate. *Semin Thromb Hemost*. 2010;36(5):510-52
- Budde U, Metzner HJ, Müller HG. Comparative analysis and classification of von Willebrand factor/factor VIII concentrates: impact on treatment of patients with von Willebrand disease. *Semin Thromb Hemost*. 2006;32(6):626-635

- Dunn A. The long and short of it: using the new factor products. *Hematology* 2015 2015:26-32; doi:10.1182/asheducation-2015.1.26
- Shapiro AD, Ragni MV et al, Kukarni R, et al. Recombinant factor VIII Fc fusion protein: extended-interal dosing maintains low bleeding rates and correlates with von Willbrand factor levels. *J Thromb Haemost* 2014; 12 (11): 178801800
- Dumont JA et al. *Blood*. 2012;119(13):3024-3030.
- Roopenian, D.C. & Akilesh, S. 2007. FcRn: the neonatal Rc receptor comes of age. *Nature Reviews, Immunology*, 7, pp715-725.
- Mahdi AJ, Obaji SG, Collins PW. Role of enhanced half-life factor VIII and IX in the treatment of haemophilia. *Br J Haematol* 2015; 169 (6): 768-776
- Powell JS, Josephson NC et al. Safety and prolonged activity of recombinant factor VIII Fc fusion protein in hemophilia A patients. *Blood* 2012; 119(13):3031-3037.
- Mahlangu J, Powell JS et al: A-LONG Investigators. Phase 3 study of recombinant factor VIII Fc fusion protein in severe hemophilia A. *J Thromb Haemost* 2015 13(6):967-977.
- Young G, Mahlangu J et al. Recombinant factor VIII Fc fusion protein for the prevention and treatment of bleeding in children with severe hemophilia A. *J Thromb Haemost*. 2015 Jun;13(6):967-77. doi: 10.1111/jth.12911. Epub 2015 Apr 23.
- Shapiro AD, Ragni MV et al.. Recombinant factor IX-Fc fusion protein (rFIXFc) demonstrates safety and prolonged activity in a phase 1/2a study in hemophilia B patients. *Blood* 2012; 119 (3): 666-672
- Powell JS, Pasi KJ et al. B-LONG investigators. Phase 3 study of recombinant factor IX Fc fusion protein in hemophilia B. *N Engl J Med* 2013; 369(24): 2313-2323.
- Powell J, Shapiro A et al. Switching to recombinant factor IX Fc fusion protein prophylaxis results in fewer infusions, decreased factor IX consumption and lower bleeding rates. *Br J Haematol* 2015; 168(1):113-123.
- Santagostino E., Martinowitz U. et al: Long acting recombinant coagulation factor IX albumin fusion protein (rIX-FP) in hemophilia B: results of a phase 3 trial. *Blood*. Jan 2016. DOI: <http://dx.doi.org/10.1182/blood-2015-09-669234>
- Lubetsky A., Martinowitz U. et al. 1096 Long-Term Use of Coagulation Factor IX (Recombinant) Albumin Fusion Protein (rIX-FP) in Previously Treated Patients with Hemophilia B in the Prolong-9FP Program. *ASH*, Dec 2015. Session: 322. Disorders of Coagulation or Fibrinolysis: Poster I.
- Santagostino E., Jacobs I. et al. Pharmacokinetic Results of Two Phase III Clinical Studies of Coagulation Factor IX (Recombinant) Albumin Fusion Protein (rIX-FP) in Previously Treated Patients with Hemophilia B (PROLONG-9FP). *ASH*, Dec 2014. Session: 322. Disorders of Coagulation or Fibrinolysis: Poster I
- Santagostino E. PROLONG-9FP clinical development program – phase I results of recombinant fusion protein linking coagulation factor IX with recombinant albumin (rIX-FP). *Thrombosis Research* 131S2 (2013) S7–S
- Dozier JK, Distefano MD. Site-Specific PEGylation of Therapeutic Proteins. *Int J Mol Sci*. 2015 Oct 28;16(10):25831-64. doi: 10.3390/ijms161025831.
- Turecek PL, Bossard MJ et al. BAX 855, a PEGylated rFVIII product with prolonged half-life. Development, functional and structural characterization. *Hamostaseologie* 2012;32 (Suppl 1): S29-S38.
- Konkle BA, Stasyshyn O et al: Pegylated, full=length, recombinant factor VIII for prophylactic and on-demand treatment of severe hemophilia A. *Blood*. 2015 Aug 27; 126(9): 1078–1085
- Coyle TE, Reding MT et al: Phase I study of BAY94-9027, a PEGylated B-domain-deleted recombinant factor VIII with an extended half-life, in subjects with hemophilia A. *J Thromb Haemost*. 2014 Apr;12(4):488-96.
- Boggio LN et al. Bleeding Phenotype with Various Bay 94-9027 Dosing Regimens: Subanalyses from the Protect VIII Study. *Blood* 2013; 124 (21).
- Tiede A, Bran B et al: Enhancing the pharmacokinetic properties of recombinant factor VIII: first-in-human trial of glycoPEGylated recombinant factor VIII in patients with Hemophilia A. *J Thromb Haemost* 2013; 11(4): 670-678.
- Giangrande P, Chowdary P et al: Clinical evaluation of novel recombinant glycopegylated FVIII (turoctocog alfa pegol, N8-GP): efficacy and safety in previously treated patients with severe hemophilia A- results of pathfinder (™) 2 international trail. *J Thromb Haemost* 2015 13:176.
- Negrier C, Knobe K et al. Enhanced pharmacokinetic properties of a glycoPEGylated recombinant factor IX: a first human dose trial in patients with hemophilia B. *Blood* 2011; 118 (10): 2695-2701.
- Collins PW, Young G et al. Recombinant long-acting glycoPEGylated factor IX in hemophilia B:a multinational randomized phase 3 trial. *Blood* 2-14;124 (26): 3880-3886.
- Carcao M, Zak kM et al. Safety, efficacy and pharmacokinetics of nonacog beta pegol (N9-GP) in rophylais and treatment of bleeding episodes in previously treated pediatric hemophilia B patients. *Blood* 2014; 124 (21)
- Mahlangu J, Kuliczowski K et al. RVIII-SingleChain, results of the pivotal phase I/III PK, efficacy and safety clinical trial in adults and adolescents with severe hemophilia A. *J Thromb Haemost* 2015; 13:86-86
- Gringeri A., Mantovani L., Scalone L., Mannucci P. (2003) Cost of care and quality of life for patients with hemophilia complicated by inhibitors: the COCIS Study Group. *Blood* 102: 2358–2363
- Calvez T, Chamobst H et al: Recombinant Factor VIII products and inhibitor development in previously untreated boys with severe hemophilia. *Blood*. 2014 Nov 27;124(23):3398-408. doi: 10.1182/blood-2014-07-586347. Epub 2014 Sep 24
- Collins PW, Palmer BP, Chalmers EA, et al; UK Haemophilia Centre Doctors' Organization. Factor VIII brand and the incidence of factor VIII inhibitors in previously untreated UK children with severe hemophilia A, 2000-2011. *Blood* 2014;124(23):3389-3397.
- Gouw SC, van der Bom JG, Ljung R, et al; PedNet and RODIN Study Group. Factor VIII products and inhibitor development in severe hemophilia A. *N Engl J Med* 2013;368(3):231-239.
- Blood Abstracts: 57th Annual Meeting Abstracts*. Vol. 126, Issue 23, 3 Dec 2015.
- Kruse-Jarres R, St-Louis J, Greist A, Shapiro A et al. Efficacy and safety of OBI-1, an antihemophilic factor VIII (recombinant), porcine sequence, in subjects with acquired haemophilia A. *Haemophilia* (2015), 21, 162–170
- McIntosh J, Lenting PJ, Rosales C et al. Therapeutic levels of FVIII following a single peripheral vein administration of rAAV vector encoding novel human factor FVIII variant. *Blood* 2-13; 121 (17): 3335-3344
- Nathwani AC, Nienhuis AW, Davidoff AM. Our journey to successful gene therapy for hemophilia B. *Hu Gene Ther* 2014; 25 (11): 923-926
- Nathwani AC, Reiss UM, Tuddenham EGD, et al. Long—term safety and efficacy of factor IX gene therapy in Hemophilia B. *N Engl J Med* 2013; 3711 (21):1994-2004
- Chuah MK et al. Gene therapy for hemophilia. *J Thromb Haemost* 2013; 11 (Suppl 1):99-110.
- Roth DA, Tawa NE, Proper J et al. Implantation of non-viral ex vivo genetically modified autologous dermal fibroblasts that express B-domain deleted human factor VIII in 12 severe hemophilia A study subjects. *Blood* 2002; 100 (11) 116a-117a
- Powell JS, Ragni MV, White GC II et al. Phase 1 trial of FVIII gene transfer for severe hemophilia A using a retroviral construct administered by peripheral intravenous infusion. *Blood* 2003; 102 (6): 2038-2045.
- Qiu X, Lu D, Zhou J et al. I mplantation of autologous skin fibroblast genetically modified to secrete clotting factor IX partially corrects the hemorrhagic tendencies in two hemophilia B patients. *Chin Med J (Engl)* 1996; 109 (111): 832-839.
- Fay PJ. Activation of factor VIII and mechanisms of cofactor action. *Blood Rev*. 2004 Mar; 18(1): 1-15.



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