The Most Exciting Time in Hemophilia

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The doctors told my mother when I was diagnosed in 1956, "This is a good time to be born with hemophilia. We'll have a cure in five years." That statement was pretty far from reality, but a cure may actually happen in 5–10 years. And, as new therapies with increasingly longer half-lives are appearing in clinical trials and the marketplace, a different concept of "functional cures" with repeated injections at long intervals is taking hold. Some of these therapies, if given once every two or four weeks, may temporarily suffice until gene therapy does become a reality.

This is the most exciting time in hemophilia research… ever! Today, research is occurring in three main areas.

Research Area 1: Prolonged half-life factor products

What exactly is half-life? Half-life is the amount of time it takes for factor to decrease its circulating concentration by half, or 50%. It's calculated by taking a series of blood samples over a specified time span after infusing clotting factor, and then measuring how much factor remains in each sample. When graphed, these measurements are called pharmacokinetic (PK) curves, and they show how rapidly your body eliminates factor. Half-life of factor may vary from product to product, and from person to person. So it's important to know how long a particular brand of factor lasts in your body, which may be significantly different from the average half-life of that brand. By knowing how quickly you eliminate factor from your blood, your HTC team can tailor a prophylactic dosing schedule specific to your needs.

For instance, immediately after 50 IU/kg of standard factor VIII is infused, the level of circulating factor VIII in the body is at 100%. If the product has a 12-hour half-life, then about 12 hours later, the factor VIII level will be at
50% (half has been eliminated). And 24 hours after the original injection, 25% is left. Two days after the initial injection, the factor VIII level is at 6.25%.

How does this work for a prolonged half-life product? If half-life is on average 50% longer than 12 hours, say 18 hours, then 36 hours after the original injection, factor VIII levels are reduced by two half-lives, so 25% is left. Three days after the initial dose, factor VIII is at 6.25%.

In other words, when using a prolonged half-life product with an 18-hour half-life, you may be able to go an extra day between infusions, as compared to a standard product with a 12-hour half-life.

Prolonged half-life factor VIII

Two prolonged half-life factor VIII products on the US market are Eloctate (from Biogen) and Adynovate (from Baxalta). Eloctate has a half-life of 19 hours, while Adynovate has a half-life of 14.3 hours.

Why are the two half-lives so different? Each product is manufactured using a different technology to prolong the half-life. The different technologies—described next—create different half-lives.

One approach to prolonging the half-life of clotting factor involves harnessing one of the body's natural mechanisms for prolonging the half-life of certain proteins. Clotting factors are proteins, and each protein that circulates in the blood has a different half-life; some proteins last for a few hours, and others last for several weeks. Two proteins in particular, albumin and an immune antibody called IgG, both last for a long time—more than 21 days.[1] The question is, Why do these proteins last a long time and others not so long?

The answer to this question helps us understand how the longer half-lives of some proteins may be exploited to increase the half-lives of clotting factors. Many proteins in the blood are absorbed and broken down by endothelial cells—the cells that line the blood vessels. IgG

usually manages to escape this process. Why? There's an area on the protein, called Fc, which allows the protein to bypass the breakdown process and causes the endothelial cell to eject the protein back into circulation. Scientists at Biogen took advantage of this fact and developed a recombinant form of factor VIII fused to an Fc molecule. With the Fc molecule attached to the factor, the endothelial cells treat the factor as if it were IgG, and eject the factor back into the bloodstream, extending its half-life. Eloctate is the brand name of Biogen's factor VIII Fc-fusion product, and it has a half-life about 50% longer than standard factor VIII products.

Table 6.1 **Factor Brand by Company and Type**

Manufacturer	Product					
	Recombinant			Plasma Derived		
	FVIII	FIX	Inhibitor	FVIII	FIX	Inhibitor
Baxalta	Advate Recombinate Adynovate	Rixubis		Hemofil M	Proplex T Bebulin VH	FEIBA VH
Bayer HealthCare	Kogenate FS Kovaltry					
Biogen	Eloctate	Alprolix				
CSL Behring	Helixate FS Afstyla	Idelvion		Monoclate-P	Mononine	
Emergent Biosolutions	Ixinity					
Grifols				Alphanate	AlphaNine SD Profilnine SD	
Kedrion				Koate DVI		
Novo Nordisk	Novoeight		NovoSeven RT			
Octapharma	Nuwiq					
Pfizer	Xyntha	BeneFix				

Recombinate is a first-generation product.
Kogenate FS and Helixate FS are second-generation. They are the same product.
CSL Behring is licensed to sell Kogenate FS as Helixate FS.
Advate, BeneFix, Ixinity, Kovaltry, Novoeight, Nuwiq, Rixubus, and Xyntha are third-generation products.
Adynovate, Alprolix, Eloctate, Idelvion, and Afstyla are prolonged half-life products.
Baxalta was originally part of Baxter Healthcare International.

Adynovate uses a different technology to prolong its half-life: PEGylation. PEGylation is the process of attaching polyethylene glycol (PEG) to the factor VIII molecule. PEG is a petroleum derivative that is found in a variety of products, from cosmetics to food. Adynovate uses the random addition of PEG, which results in the protein being coated with PEG, protecting it from damage and destruction, and producing a longer half-life. Adynovate's half-life is 14.3 hours, 16% longer than that of standard factor VIII products.

Two other prolonged half-life factor VIII products use a different type of PEGylation, called *site-specific* PEGylation. In contrast to the random PEGylation process used by Adynovate, site-specific PEGylation is highly controlled and results in the attachment of only one PEG molecule on each factor VIII molecule. Bayer has accomplished this by changing one area on the factor VIII molecule to allow it to function as a PEG binding site. Novo Nordisk has taken a similar approach, using a technology called glycoPEGylation. In this process, a single PEG is attached to a sugar that

is attached to a single site on the factor VIII molecule. The precise control in the placement of a single PEG on each factor VIII gives both the Bayer and the Novo Nordisk products an 18- to 19-hour half-life, comparable to Eloctate. Both these prolonged half-life PEG factor VIII products are still in clinical development, but their main clinical trials are completed.

Another approach to prolonging the half-life of factor VIII involves making a slight change in the structure of the factor VIII molecule. Normally, factor VIII is synthesized in the liver as a single long protein, called a *single-chain*. When secreted from the cell, the single-chain factor VIII molecule is broken into two parts, or two chains. Factor VIII travels in the bloodstream as a two-chain molecule. In the approach used by CSL Behring, the two chains of factor VIII are bonded back together to form the more stable single-chain molecule. Data from clinical trials sponsored by CSL Behring indicate the single-chain factor VIII has a half-life of 14.5 hours, similar to Baxalta's Adynovate, and marginally better than standard two-chain factor VIII products with half-lives of 12 hours.

With two prolonged half-life factor VIII products already on the market, and more coming, how will you decide which one to use?

Clinical trial results showed that Eloctate and Adynovate were as effective as standard factor VIII products in stopping bleeding episodes when used on demand. And like standard products, they can prevent nearly all bleeding episodes when administered prophylactically in a variety of dosing regimens. The new products were also shown to be safe—with no unusual adverse events, and no increased risk of inhibitor development. Prophylactic dosing regimens for standard factor VIII products are typically three times a week or every other day to ensure that factor VIII *trough levels* (the factor VIII level just before the next dose) are sufficient to prevent breakthrough bleeding. For prolonged half-life products like Eloctate, dosing once or twice per week is effective in preventing most breakthrough bleeding.

PRODUCTS THAT GIVE US HOPE

Prolonged Half-life Factor Products

	Brand or Clinical Name	**Engineered Protein**	**Company**	**Half-life (hours)**	**Status**
factor VIII	**Eloctate**	rFVIII-Fc	Biogen	19	Available June 2014
	Adynovate	PEG-FVIII	Baxalta	14.3	Available Dec. 2015
	N8-GP	GlycoPEG-FVIII	Novo Nordisk	18.4	Phase 3 (2017–18)
	BAY94-9027	PEG-FVIII	Bayer	19	Phase 3 (2018)
	rVIII-SingleChain	Single-chain FVIII	CSL Behring	14.5	FDA submitted
factor IX	**Alprolix**	rFIX-Fc	Biogen	82.1	Available Mar. 2014
	N9-GP	GlycoPEG-FIX	Novo Nordisk	93	Phase 3
	Idelvion	rIX-FP (albumin)	CSL Behring	101.7	Available Apr. 2016
factor VIIa (for inhibitors)	CSL689	rFVIIa-Albumin	CSL Behring	8.5@1mg/kg	Completed normal human volunteers; now phase 2/3
	MOD-5014	rFVIIa-CTP	Opko	No data yet	Preclinical in dogs; phase 1/2a open

Alternate Treatments for Inhibitors and Hemophilia A and B

Clinical Name	**Engineered Protein**	**Company**	**Dosing Frequency and Delivery**	**Status**
ALN-AT3SC	AT3 RNAi	Alnylam	Twice monthly subcutaneously	Phase 1
ACE910	Anti-FIX/FX (Bispecific MAb)	Genentech/ Roche/ Chugai	1–2 weeks subcutaneously	Phase 3 clinical trials now starting
Concizumab	Anti-TFPI	Novo Nordisk	IV or subcutaneously	Phase 1/2 clinical trials including hemophilia A/B
BAY 1093884	Anti-TFPI	Bayer	No data yet	Phase 1

First-generation: Recombinate. Second-generation: Kogenate FS, Helixate FS. These are the same product; CSL Behring is licensed to sell Kogenate FS as Helixate FS. Third-generation: Advate, BeneFix, Rixubus, Xyntha, Novoeight, Ixinity, Kovaltry, Nuwiq. Prolonged half-life: Eloctate, Alprolix, Adynovate, Idelvion. Kedrion distributes Koate-DVI in the US for Grifols, the manufacturer.

Because all prolonged half-life products were studied in different ways in their clinical trials, it's best to review the product inserts that come with each product (also available online) and then talk to your HTC team about which is best for you.

A safety concern that has been discussed since research into PEGylated factor VIII started more than 10 years ago deserves mention: How does PEG get removed from the body? The body does not metabolize or break down PEG into smaller units, as it does with natural compounds such as proteins and carbohydrates. Small PEGs are more easily excreted than large PEGs. Small PEGs are removed from the blood mainly by the kidneys and then excreted through the urine. Larger PEGs do not easily pass through the kidneys, and it's believed that most are excreted through the liver to the intestine, and then eliminated in the feces. PEGylated factor VIII products use some of the largest PEGs. Because it's not possible to eliminate every molecule of PEG, and because PEG is not broken down, some PEG remains in the body. Research has shown that the impact of PEG remaining in the body seems minimal, but the long-term safety of PEGylated factor VIII products has not been conclusively established: most other PEGylated drugs are used in other diseases for short periods of time and use smaller PEGs. Hemophilia is one of the first instances where PEG will be administered over many years, even decades. So it will be important to understand the safety risks versus benefits when considering a PEGylated factor.

Prolonged half-life factor IX

Three technologies have been used to prolong the half-life of factor IX.

1. Fc fusion is being used in Alprolix, Biogen's Fc fusion factor IX product. Alprolix uses the same Fc technology as Eloctate, but in this case, Fc is fused to factor IX instead of factor VIII. This technology significantly increases the half-life of factor IX over standard factor IX products, from about 19–24 hours for standard products to an average of 86 hours for Alprolix. Most important, this product reduces the number of infusions required to prevent bleeding. Standard half-life factor IX products typically require twice-a-week dosing to maintain good coverage and prevent bleeding. By contrast, prolonged half-life products like Alprolix can be used weekly, and in the clinical trials, about half the patients had good results with every-two-week dosing.
2. Albumin fusion technology is being used in Idelvion, CSL Behring's factor IX albumin fusion product. Idelvion uses the same recycling pathway as Fc; but instead of using Fc, the factor IX molecule is fused to albumin. Because albumin circulates for at least 21 days, it can also be used to extend half-life when fused to other proteins. The latest prolonged half-life product to hit the market, Idelvion was approved by the FDA on March 4, 2016. Idelvion has a very prolonged half-life of 104 hours and can be used once weekly or up to once every two weeks for patients over age 12. In the clinical trials for FDA licensure of Idelvion, breakthrough bleeding was very low for patients treated every one to two weeks.
3. GlycoPEGylation is the same technology Novo Nordisk uses for its prolonged half-life factor VIII product. In this case, Novo Nordisk is using glycoPEGylation to attach PEG to one of two sugars on the factor IX molecule. The clinical trial reports good results and a prolonged half-life of 92 hours. The glycoPEGylated factor IX product, currently named N9-GP, completed a successful phase III clinical trial in 2013 but has not yet been filed for licensure with the FDA.

All three of these prolonged half-life factor IX products are effective and safe. They each stopped bleeding episodes and prevented almost all breakthrough bleeding when used prophylactically. No increased incidence of inhibitors was detected, and no other unusual adverse events were seen in clinical trials. Dosing regimens are currently every 7–10 days for Alprolix, with half the patients in the clinical trial achieving every-two-week dosing. Idelvion dosing is every 1–2 weeks, and although N9-GP is not yet approved, its clinical trial tested weekly and longer dosing intervals with excellent results.

Research Area 2: Bypassing agents

Developing and managing inhibitors is the greatest unsolved problem in hemophilia today. Bypassing agents like NovoSeven (rFVIIa) and FEIBA offer some control of bleeding in inhibitor patients by skipping the need for factor VIII or factor IX in the clotting cascade. But these agents don't always work when administered prophylactically, and don't control bleeding as well as standard factor VIII and IX in people without inhibitors. Three widely differing scientific approaches are being researched to address the urgent need for better therapies for inhibitor patients.

1. Extending the half-life. One technology involves prolonging the half-life of factor VIIa. Factor VIIa has a very short half-life, about 2.5 hours, and may require several infusions every few hours to bring a bleed under control. CSL Behring is developing a recombinant factor VIIa-albumin fusion product that, in early clinical trials, demonstrated a half-life of 8.5 hours—more than *three times* as long as standard factor VIIa. No data are available yet on factor VIIa-albumin's effectiveness.

These next two novel approaches being researched by several companies don't involve the infusion of any clotting factors or bypassing agents!

2. Using a *bispecific antibody*. You may know that factor VIII works in the clotting cascade by bringing factor IX and factor X together and activating them. These activated factors in turn activate other clotting factors, eventually resulting in the formation of fibrin fibers, the stringy protein necessary for a strong clot. In the absence of factor VIII or IX, little fibrin is formed. This results in weak clots that easily break down, causing prolonged bleeding—hemophilia. Roche and its subsidiary Genentech are developing a new antibody drug (ACE910) to replace factor VIII. Antibodies are Y-shaped proteins produced by the immune system; they have two arms that usually bind or stick to one target, such as an infectious agent like a virus, to eliminate it from the body. Through the process of recombinant DNA technology and genetic engineering, scientists have been able to develop a bispecific antibody that binds to two different molecules. In this case, one arm of the genetically engineered bispecific antibody binds to factor IX, and the other arm binds to factor X. So the antibody latches onto factor IX and factor X in the bloodstream and brings them together—essentially, the bispecific antibody is doing the job of factor VIII.

In early clinical testing, this bispecific antibody was effective in preventing bleeding in factor VIII-deficient patients with and without inhibitors. That's right, this antibody isn't just for inhibitor patients. It may be used by all patients with hemophilia A. And it doesn't require venipuncture—it's administered as a weekly subcutaneous injection. In these trials, patients were protected from most bleeding episodes with ACE910 alone, without the need for prophylactic factor VIII. ACE910 has generated much interest in the hemophilia community and larger-scale clinical trials are under way now.

3. Stopping naturally occurring inhibitors. Another approach that doesn't involve the infusion of factor targets the part of the clotting cascade that shuts down the clotting process. How do we stop bleeding by stopping part of the clotting process? In addition to clotting factors like VIII and IX that participate in forming a blood clot, our bodies also have *naturally occurring inhibitors* that keep the clotting cascade in check by shutting it down. This is necessary to prevent unwanted clotting, possibly resulting in a stroke or heart attack. Think about it: people with hemophilia have enough trouble making clots without their own coagulation inhibitors trying to shut down the process! Perhaps these naturally occurring inhibitors could be neutralized to allow the clotting process to proceed with little or no factor?

Research is focusing on two powerful, naturally occurring inhibitors: one is tissue factor pathway inhibitor (TFPI) and the other is anti-thrombin 3 (AT3). Both Novo Nordisk and Bayer have made antibodies that can bind to and eliminate TFPI in the bloodstream. These are being tested now in early human clinical trials to see if they can improve clot formation in patients with hemophilia and hemophilia with inhibitors by reducing the negative effect of TFPI on clot formation. No data on effectiveness are available yet.

Alnylam Pharmaceuticals has made a completely different type of molecule to inhibit production of AT3. It's called an RNA interference therapeutic (RNAi). RNA is normally the message that DNA (genes) uses to make proteins in the cell. The RNAi binds to and eliminates AT3 RNA, preventing the liver from making AT3 protein. Clinical trial data on humans suggest that RNAi is effective in preventing bleeding when patients are given a subcutaneous dose sufficient to block the production of most AT3 protein. Bleeding was prevented in patients without the use of any clotting factor. This RNAi—the anti-AT3 molecule (ALN-AT3)—will be tested in larger studies in hemophilia A, hemophilia B, and all inhibitor patients.

The early clinical data for the bispecific antibody (ACE910) and the RNAi (ALN-AT3) are very encouraging when used in both inhibitor and non-inhibitor patients. Larger-scale clinical trials are under way or will start soon to confirm and extend these data. If confirmed, both drugs may offer a meaningful advance for inhibitor patients, and may be used by non-inhibitor patients instead of factor VIII or IX.

Research Area 3: Cell and gene therapy

Our community dreamed of a cure for hemophilia long before my family doctor's 1956 conversation with my mother. Since human gene therapy clinical trials began in earnest in 1990, hemophilia has been touted as an ideal disorder to research and cure. But it's now 2016—are we much further along?

The goal of gene therapy is to somehow put the correct blood-clotting code (DNA) for making factor VIII or factor IX into our cells to enable them to produce factor on their own. This would cure hemophilia.

The problem is, how do we get that code into our cells? In gene therapy, the DNA with the correct code is transported into our cells by viruses. Viruses were chosen to transport the good DNA because they are very good at infecting cells—they have evolved over hundreds of millions of years to become expert at injecting their genetic material into cells. A particular type of virus called *adeno-associated virus*, or AAV, has been the workhorse of gene therapy because when it infects humans, it causes no known disease and typically produces only a mild immune response. These AAV vectors (DNA transporters) have been genetically engineered for gene therapy. To prepare the viral vector for use in gene therapy, the viral genes are removed, and the DNA that makes factor VIII or factor IX is inserted into the viral vector. The virus is then grown to very high quantities and injected into patients. Much of the virus goes to the liver, the normal site of factor VIII and IX production, where it enters the liver cells and delivers the instructions for making factor VIII or IX.

Gene therapy has many challenges. What's the best vector, or transporter, to use without causing an immune response that would destroy it? What vectors could we use that we're not already immune to? What vectors are capable of carrying a large gene such as the factor VIII gene? What vectors can place the gene into a cell's DNA where we want it? The answers to these and many other questions are technically challenging, and that's why it has taken researchers so long to get to this point of some early successes.

Over the past five or six years, a small number of severe hemophilia B patients in a successful clinical trial at University College London (UCL) and the Royal Free Hospital London have been "cured" with gene therapy using a viral vector developed with St. Jude Children's Research Hospital in Memphis. Their factor IX activity is 1% to 6%, and they have few to no bleeding episodes.

Currently, multiple hemophilia B clinical trials are being conducted. They are variations on a theme, testing improvements in all stages of the process, from construction of the vector to the type of factor IX gene to improvements in manufacturing. Results reported to date are cautiously encouraging, with a few more patients making a small amount of normal factor IX. UniQure, a Netherlands-based gene therapy company, has licensed the St. Jude's/UCL technology and has enrolled five people with hemophilia B in a gene therapy clinical trial that started in 2015. Two of

the five patients produced 4.5% and 5.5% levels of factor IX, based on a press release recently published by UniQure.

In other hemophilia B gene therapy studies, researchers are using a super-active form of factor IX, called factor IX Padua, that was first discovered in 1998 and identified in 2009 in a man in Italy who was experiencing excessive clotting. This factor IX variant is being evaluated for gene therapy by several groups because it might solve one of the current problems with gene therapy—low "expression" rates; in other words, low factor levels produced as a result of gene therapy. The factor IX Padua variant might solve this problem because it has about seven times the activity of normal factor IX. So an expression of 2% using a normal factor IX gene would be equivalent to an expression of 14% using factor IX Padua—truly resulting in a cure for hemophilia B, even if not much factor IX protein is made. Recently Baxalta, Spark, and Dimension have taken this factor IX variant into clinical trials. Baxalta has reported some variable but, in some cases, positive data.

And what about factor VIII? Factor VIII is much more difficult to work with than factor IX because of its large size. The viral vector used by almost every gene therapy trial is adeno-associated virus (AAV). Viruses have evolved to carry their own genetic material and not much else. To be used for gene therapy, most of the viral DNA has to be removed and our human DNA "payload" inserted. Some viral vectors can carry larger genetic payloads than others. Unfortunately, AAV can carry only a relatively small payload, and the factor VIII gene overstuffs this virus, making production and manufacturing difficult. Still, BioMarin has an AAV-factor VIII product in clinical studies, and other gene therapy companies are actively pursuing this method too.

Gene therapy for hemophilia may benefit from using a vector other than AAV—one that can carry a larger payload and is more easily developed into a therapeutic product. Research is ongoing on at least one additional viral vector system, lentiviruses, which have been successfully used for gene therapy on bone marrow stem cells. Diseases such as sickle cell disease and some immunodeficiency diseases have been cured by using gene therapy to introduce corrected genes into bone marrow cells removed from the patient. The cells are then grown to large quantities outside the body and reinjected into the patient, curing or partially curing the disease.

Speaking of stem cells, these are the cells in the body that have the ability to produce any type of cell, including those cells that make up our tissues and organs. If cells from a hemophilic patient's liver could be removed via biopsy,
cultured, and turned into stem cells in the laboratory, then the gene for factor VIII or IX could be placed into the stem cells, which could be grown in the lab to large quantities. These stem cells could then be changed into liver cells, and reinjected into the same patient. The new liver cells would make and secrete factor VIII or IX into the blood and cure hemophilia. Is this science fiction? Maybe not. This kind of research worked in mice in two different laboratories, in Korea and the Netherlands.

It's the most exciting time in hemophilia. Many technically cutting-edge research groups have been attracted to hemophilia because it seems an easy target for gene and cell therapy. Hemophilia is an attractive disorder for researchers to work with because the protein involved—factor VIII or factor IX—does not have to be produced within strict limits like, say, insulin: too much insulin could kill you, but almost any level of factor will have a therapeutic effect. Though hitting the target hasn't been easy, given the number of failures over the past 15 or more years, the incremental progress made by scientists brings our community closer to the goal of a permanent cure.

Dr. Glenn Pierce was responsible for the development and approval of Eloctate and Alprolix, the first prolonged half-life products, when he was senior vice president of hematology, cell and gene therapy and chief medical officer for the hemophilia program at Biogen. Before that, Glenn was vice president of US Research at Bayer HealthCare, responsible for the preclinical testing of Bayer's PEGylated FVIII. Glenn retired from Biogen in 2014, and is a

consultant to BioMarin and Genentech/Roche, and an advisor to Alnylam. He lives in California, and travels frequently for hemophilia causes, especially the World Federation of Hemophilia's Expanded Humanitarian Aid program. He is on the board of directors of WFH and serves on NHF's Medical and Scientific Advisory Council (MASAC). Glenn had hemophilia until a liver transplant in 2008.

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1. Albumin (or human serum albumin) is the most common protein found in blood plasma and makes up about 50% of plasma proteins. One of its functions is to transport different compounds throughout the body. IgG, or immunoglobulin G, is a Y-shaped protein used by the immune system to fight infections by inactivating infectious agents such as viruses or marking them for removal or destruction by other immune system cells.