european haemophilia consortium

Novel treatments in haemophilia and other bleeding disorders: a periodic EHC Review

Issue One

May 2018

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A periodic EHC review

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Disclaimer:

EXECUTIVE SUMMARY

Welcome to the first edition of the European Haemophilia Consortium's (EHC) periodic review of novel treatments in haemophilia and other bleeding disorders.

The purpose of this newsletter is primarily to help educate EHC National Member Organisations (NMOs) and help them to provide their members and caregivers with a general overview and understanding of the rapidly evolving landscape of medicinal product development in rare bleeding disorders. The EHC encourages its NMOs to use and adapt this newsletter to their national needs but takes no responsibility for any changes.

The information provided in this newsletter covers recent major developments and is divided by specific type of disorder for which there is an update to report. This newsletter will be updated periodically.

The information provided in this newsletter was compiled from multiple sources, including presentations at recent scientific meetings (e.g. EHC New Technologies workshop, EAHAD Congress), websites (e.g. www.clinicaltrials.gov) and by writing directly to pharmaceutical companies. It was then redrafted and presented in easy-to-understand language. For this we give special thanks and recognition to Declan Noone.

The EHC is also grateful to the New Products Working Group, which has overseen the content and production of this newsletter. Its members include:

- Asst Prof Brian O'Mahony, EHC President
- Dr Radoslaw Kaczmarek, EHC Steering Committee member
- Prof Mike Makris, EHC Medical Advisory Group (MAG) member
- Prof Flora Peyvandi, EHC Medical Advisory Group (MAG) member
- Dr Dan Hart, EHC Medical and Scientific Advisory Group (MASAG) member
- Mariëtte Driessens, EHC volunteer
- Uwe Schlenkrich, EHC volunteer

The EHC greatly welcomes *all* treatment developments that may benefit patients in the future. The EHC takes no position on any product type or class reported in this newsletter.

We hope that the information provided herein is useful and are available for any questions.

Sincere regards,

Brian O'Mahony
EHC President

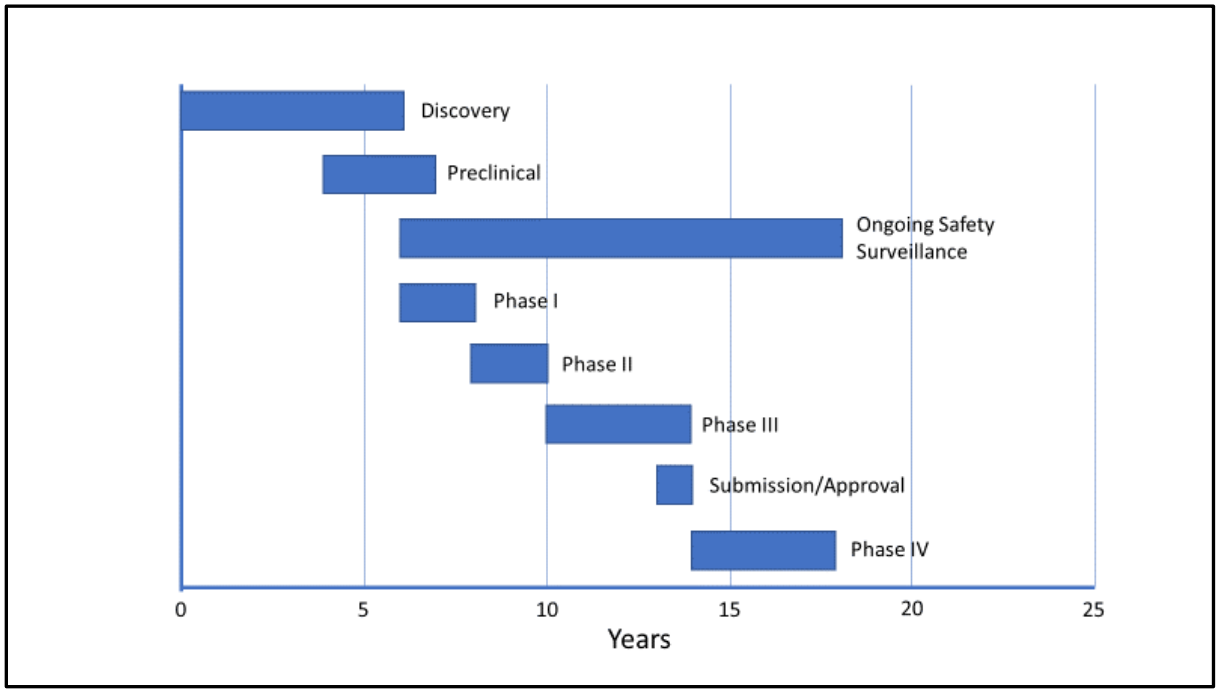
Amanda Bok
EHC CEO

PHASES OF CLINICAL TRIALS

What Are the Phases of Clinical Trials?

Clinical trials are usually conducted in phases that build on one another. Each phase is designed to answer certain questions. Knowing the phase of the clinical trial is important because it can give you some idea about how much is known about the treatment being studied.

Figure 1: Phases and approximate times from inception to clinical trial to approval



Phase I clinical trials: Is the treatment safe?

Phase I studies of a new drug are usually the first that involve people. The main reason for doing phase I studies is to find information about the dose of the new treatment that can be given safely, without serious side effects. Although the treatment has been tested in lab and animal studies, the side effects in people can't always be predicted. These studies also help to decide on the best way to administer the new treatment.

Key points of phase I clinical trials:

- The first few people in the study often get a very low dose of the treatment and are watched very closely. If there are only minor side effects, the next few participants may get a higher dose. This process continues until doctors find a dose that's most likely to work while having an acceptable level of side effects.
- The focus in phase I is looking at what the drug does to the body and what the body does with the drug. In haemophilia, this could be what the addition of a molecule to the factor VIII (FVIII) or factor IX (FIX) does to the protein, or how the drug gets broken down. In terms of gene therapy, this maybe looks at how the body responds to the capsid that the FVIII or FIX gene is delivered in.

- Safety is the main concern at this point. Doctors keep a close eye on participants and watch for any serious side effects. Because of the small number of people in phase I studies, rare side effects may not be seen until later.
- These studies usually include a small number of people. In haemophilia, it this could be between 5 and 20 people.

Phase II clinical trials: Does the treatment work?

If a new treatment is found to be reasonably safe in phase I clinical trials, it can then be tested in a phase II clinical trial. The type of benefit or response the doctors look for in this phase depends on the goal of the treatment and may concern intermediate outcomes (i.e. factor level increase). In extended half-life products (EHLs), they may look for how much factor is needed to stop a bleed with one to two infusions. In gene therapy, they may look at what dose is needed to get a factor level that will provide enough protection and how many bleeds are occurring at these levels.

Key points of phase II clinical trials:

- Usually, a group of 15 to 50 patients with the same type of haemophilia (A, B, vWF, inhibitors) get the new treatment in a phase II study. They're treated using the dose and method found to be the safest and most effective in phase I studies.
- In phase II clinical trials, all patients usually get the same dose. But some phase II studies randomly assign participants to different treatment groups (much like what's done in phase III trials). These groups may get different doses or get the treatment in different ways to see which provides the best balance of safety and effectiveness.
- Phase II studies are often done at major haemophilia centres.

If enough patients benefit from the treatment in this phase, and the side effects are acceptable, the treatment is allowed to go on to a phase III clinical trial. Along with watching for responses, the research team continues to look for any side effects.

Phase I/II

In haemophilia, some trials are Phase I/II. This is often seen in conditions where there are limited numbers of patients available, such as rare diseases. Usually it means that in the first, small (phase I) group of treated patients, doctors not only look at safety but also immediately at effectiveness and if the drug appears safe, a second, larger group is treated and analysed together. Combining phases I and II may allow research questions to be answered more quickly or with fewer patients.

Phase III clinical trials: Is it better than what's already available?

Treatments that have been shown to work in phase II studies must usually succeed in one more phase of testing before they're approved for general use. Phase III clinical trials compare the safety and effectiveness of the new treatment against the current standard treatment or placebo (products without active substance).

This is usually done in randomised studies, i.e. in which it is randomly decided who receives one of the two treatments, to remove possible bias. Randomisation is not often used in haemophilia clinical trials for several reasons. The trial can follow patients on current treatment who are then switching to a new treatment, or record a period of time on the current treatment, followed by patients switching to the new treatment at different levels, e.g. on-demand patients switching to prophylaxis on new treatment, prophylaxis patients switching to the new treatment at new doses, such as from 35IU/kg to 50IU/kg, or pharmacokinetic guided dosing.

Key points of phase III clinical trials:

- These studies are often done in many places around the world at the same time.
- These studies tend to last longer than phase I and II studies.
- Placebos are never used in haemophilia trials. People with haemophilia always get the trial drug.
- As with other studies, patients in phase III clinical trials are watched closely for side effects and treatment is usually stopped if side effects are serious. In haemophilia, the primary side effect that has been examined is the development of inhibitors. For this reason, there are often two Phase III clinical trials, one in previously treated patients (PTPs) and one in previously untreated patients (PUPs).

Submission for approval: New drug application

When phase III clinical trials (or sometimes phase II studies) show a new drug is effective/safe with an acceptable risk of side effects, as with current standard treatment, a new drug application (NDA) is submitted for approval. The authorities (European Medicines Agency [EMA] or the Food and Drug Administration [FDA] in the US) then review the results from the clinical trials and other relevant information.

Based on the review, the authorities decide whether to approve the treatment for use in patients with the type of illness the drug was tested on. If more evidence is needed to show that the new treatment's benefits outweigh its risks, they may ask for more information or even require that more studies be done and they can limit approval until this information is available.

Phase IV clinical trials: What else do we need to know?

Approved drugs are often monitored over a long period of time in phase IV studies. Even after the first three phases of clinical trials, the full effects of the treatment may not be known. Some questions may still need to be answered. For example, a treatment may get approval because it was shown to reduce the number of infusions per week and decrease the number of bleeds per year. However, is the dose high enough to prevent sub-clinical bleeds? Does the new treatment do something that the old treatment does not? Are there rare side-effects? These types of questions may take many more years to answer and are often addressed in phase IV clinical trials.

Key points of phase IV clinical trials:

- Phase IV studies look at drugs that have already been approved. The drugs are available for doctors to prescribe for patients, but phase IV studies might still be needed to answer important questions.
- This is typically the safest type of clinical trial to participate in because the treatment has already been studied and might have already been used in many people. Phase IV studies look at safety over time.
- These studies may also look at other aspects of the treatment, such as quality of life or cost effectiveness.

NOVEL TREATMENT IN HAEMOPHILIA A WITHOUT INHIBITORS

Standard half-life (SHL)

The recommended treatment for haemophilia to prevent or treat bleeds is replacement therapy, or replacing the deficient clotting factor. Until recently, people with haemophilia have had access to two types of such treatments: plasma-derived or recombinant clotting factor therapies. Plasma-derived treatment is made from pooled human plasma, while recombinant treatment is produced in live cells grown in a laboratory.

First-generation recombinant FVIII products were generated from the full-length FVIII gene and contained both human albumin (a protein found in blood plasma) and animal proteins in their production. Because of concern about unknown infectious agents, second-generation products removed human albumin, which was previously used as a stabilising agent. Third-generation products no longer use any animal or human components in the production of FVIII products.

Half-life refers to the amount of time the body takes to reduce the amount of clotting factor to half in the bloodstream. Standard half-life of current FVIII products is between 8 to 16 hours with an average of 12 hours.

Up to the end of 2013, there was one 1st generation (Recombinate®, Shire), three 2nd generation (Kogenate FS®, Bayer and the now discontinued Helixate® from CSL Behring and Refacto®, Pfizer,) and two 3rd generation (Advate®, Shire and Refacto AF®/Xyntha®, Pfizer) recombinant FVIII products. By the beginning of 2018, there were an additional three 3rd generation products available in Europe (Kovaltry®, Bayer; NovoEight® Novo Nordisk; Nuwiq®, Octapharma). The increased availability of new products is obviously very good news for patients in terms of access and supply of products, but we must also be aware of the lack of data on new products. In previously treated patients (PTPs) there was no indication of any increased incidence of inhibitors. This is reassuring that there is no unforeseen new problem with each product, particularly with the manufacturing processes, given these patients are well beyond the 50 exposure days (an exposure day – a day on which factor is received), where an inhibitor is most likely to appear. In the previously untreated patients (PUPs), for the three new products, these trials are on-going and due to finish later this year for NovoEight® and Nuwiq® and in 2022 for Kovaltry®.

At the European Association for Haemophilia and Allied Disorders (EAHAD) Congress in February 2018, a question was raised about the importance of PUP data from clinical trials when compared to an intensive post-marketing surveillance. The question focused around the numbers of PUPs in the trials and if there were enough patients enrolled to really identify a strong signal of inhibitor development.

Table 1: Standard half-life clinical trials for previously untreated patients (PUPs) for haemophilia A

Clinical Trial	Name	Company	PUP	Phase			Estimated Time of Completion
				I	II	III	
NCT01493778 (NovoEight®)	Safety and Efficacy of Turoctocog Alfa (NovoEight®) in Prevention and Treatment of Bleeds in Previously Untreated Children with Haemophilia A (Guardian)	Novo Nordisk	Yes				Jun-18
NCT01712438 (Nuwiq®)	Human-cl-rhFVIII (Nuwiq®) in Previously Untreated Patients	Octapharma	Yes				Dec-18
NCT01311648 (Kovaltry®)	BAY81-8973 (Kovaltry®) Paediatric Safety and Efficacy Trial in Previously Untreated Patients (PUPs)	Bayer	Yes				Dec-22

Extended half-life (EHL)

Extended half-life (EHL) products are a new form of FVIII, or FIX for haemophilia B, which stay in the bloodstream longer.

Extended half-life products have been developed to afford patients a treatment regimen that allows for less frequent infusions for reasons such as poor venous access or to facilitate compliance. The other option is that patients stay on their current infusion rate per week and get a higher trough level. This option is often chosen for reasons such as continued bleeds, increased activity levels, severe arthropathy or patients having short half-lives.

The techniques used to increase half-life ($t_{1/2}$) include the following:

1. Fusion with prolonged half-life proteins, such as IgG-Fc
2. Protein modifications
3. Chemistry directed or site specific pegylation

The first available technique, used by Sobi and Bioverativ (a Sanofi company), which can be seen in Elocta® (Eloctate® in the US), is to take a portion of a natural molecule (IgG-Fc) currently in the body and attach this to the FVIII molecule. The half-life extension is 1.5-1.7 increase over current standard half-lives (SHL). This was licensed by the EMA in 2016 with the PUP trial due to be completed in 2019. As this was the first EHL product on the market, there is more information available from real world clinical use. At the EHC New Technologies workshop in November 2017, Prof Johannes Oldenburg presented real world data from the haemophilia centre in Bonn showing a reduction in infusions per year from an average of 175 to 117 in 27 patients; this was primarily driven by 14 patients moving from 3x/week to 2x/week regimens. This corresponded to a 22% reduction in the number of units used per year. A similar experience from the Milan centre was presented by Prof Flora Peyvandi in her plenary talk at EAHAD in February 2018. Additionally, post licensing experience in Canada showed a reduction of 19% in the number of FVIII units used.

The second prolongation of half-life technique that has been used is protein modification, which is applied by CSL Behring to their product Afstyla®. This modifies the FVIII protein to a single chain molecule and in doing so, increases the affinity (attraction) of free FVIII molecules to von Willebrand

Factor (vWF) which carries the FVIII in the blood. This method has resulted in a 1.1 increase in half-life. This product was licenced in Europe in 2017, with PUP data expected by August 2023.

Thirdly is pegylation, which is the most common method used. This method attaches a polyethylene glycol (PEG) molecule to the FVIII molecule. It is used in Adynovi® (Adynovate® in the US) from Shire, currently on the market in the US and Europe, N8-GP from Novo Nordisk and BAY 94-9027 from Bayer, both currently still in clinical trials. The N8-GP trial is expected to be completed by the end of 2018 for both adults and children >6 years. The BAY 94-9027 trials' main trial is completed, and the extension trial aims to be completed by January 2019 in adults and children over 12, and for children <12 in February 2020. PUP trials are expected to be completed by the end of 2023 and 2021 for Adynovi® and N8-GP, respectively. The clinical trial data suggest that the extension of half-life ranges from 1.4- 1.6 times that of SHL products.

Whilst pegylation is a common technology, the size of the peg-molecule attached to the FVIII, as well as where it is attached, varies for each of these products. This has an impact on discussions of long term safety with considerations of potential accumulation of the PEG molecules in different locations in the body and different clearance rates. Whilst a definitive answer is difficult to determine without long-term safety and efficacy data, it has led to a difference in how these products are being licenced between the Food and Drug Administration (FDA) in the US and the European Medicines Agency (EMA), which will impact the patient population that is treated with these products going forward. Adynovate®/Adynovi® is licensed in the US for prophylaxis for children and adults but in Europe the EMA have licenced this only for the use in patients >12 years.

Regardless of strategy, EHL FVIII products generally prolong half-life by approximately 1.5-fold over SHL FVIII. In general, this will allow for a decrease in infusion to twice instead of three times weekly and up to 20% less units needing to be used. It could also be possible to maintain the same infusion rate and obtain higher trough levels.

The increase in half-life for FVIII appears to be limited by the half-life of vWF. Most FVIII circulates with vWF and the clearance of vWF and FVIII occurs mostly together. This makes it difficult to extend the half-life of FVIII beyond that of the half-life of vWF, which results in a ceiling effect on the FVIII half-life increase. When considering the theory that EHL-rFVIII products rely on vWF for circulation, SOBI/Bioverativ (a Sanofi company) are looking into the use of the vWF-XTEN Fusion Protein (rFVIIIFc-VWF-XTEN) (BIVV001) to extend the half-life beyond this ceiling, under a collaboration agreement with Sobi. While currently still in Phase II, clinical preliminary trial data look promising with extension in half-life to 33 hours (2.5-3-fold increase over SHL). Another trial that aims to take a different approach with EHL is from Novo Nordisk, which uses their N8-GP molecule currently in development and aims to use daily subcutaneous infusions with the aim to achieve higher and constant factor levels. Octapharma also have a preclinical programme for a subcutaneous delivery of FVIII.

Although EHL products are promising, the optimal strategy for treatment of bleeds between prophylactic doses and dosing regimens will likely need to be individualized to patient pharmacokinetics accounting for joint damage, physical activities and other factors. EHL products are attractive options for young patients to reduce infusion rates, decrease the need for central lines and may reduce the potential burden of immune tolerance therapy ITI (please see article on inhibitors). However, the immunogenicity (the potential for inhibitor development) of EHLs are unknown and will require extensive longer-term monitoring (including PUPs) to determine whether they are more or less immunogenic than current products.

Table 2: Extended half-life clinical trials for haemophilia A

Clinical Trial	Name	Company	PUP	Phase			Estimated Time of Completion
				I	II	III	
NCT01731600 (N8-GP)	A Multinational, Open-Label, Non-Controlled Trial on Safety, Efficacy and Pharmacokinetics of NNC 0129-0000-1003(N8-GP) in Previously Treated Paediatric Patients with Severe Haemophilia A (<12 years)	Novo Nordisk					Sep-18
NCT03205163 (rFVIIIFc-VWF-XTEN)	A Safety, Tolerability, and Pharmacokinetics Study of a Single Intravenous Injection of Recombinant Coagulation Factor VIII Fc - Von Willebrand Factor - XTEN Fusion Protein (rFVIIIFc-VWF-XTEN) (BIVV001) in Previously Treated Adults with Severe Haemophilia A (EXTEN-A)	SOBI/Bioverativ (a Sanofi Company)					Sep-18
NCT02994407 (N8-GP)	Safety, Tolerability, and Pharmacokinetics Study of Turoctocog Alfa Pegol Injected Under the Skin in Patients with Haemophilia A	Novo Nordisk					Nov-18
NCT01480180 (N8-GP)	Evaluation of Safety and Efficacy, Including Pharmacokinetics, of NNC 0129-0000-1003 (N8-GP) When Administered for Treatment and Prophylaxis of Bleeding in Subjects with Haemophilia A (>12 years)	Novo Nordisk					Dec-18
NCT0158029 (BAY94-9027)	A Trial Investigating Safety and Efficacy of Treatment with BAY94-9027 in Severe Haemophilia A,>12years (PROTECT -VIII) extension study	Bayer					Jan-19
NCT01775618 (BAY94-9027)	Safety and Efficacy of BAY94-9027 in Previously Treated Male Children with Haemophilia A (<12 years) extension study	Bayer					Feb-20
NCT02615691 (Adynovi®)	Prospective, Multi-center, Open Label Study to Investigate Safety, Immunogenicity, and Hemostatic Efficacy of	Shire	Yes				Jun-23

	PEGylated Factor VIII (BAX 855) in Previously Untreated Patients (PUPs) and Minimally Treated Patients (MTPs) < 6 Years with Severe Hemophilia A (FVIII < 1%)"				
NCT02137850 (N8-GP)	PUPs - Safety and Efficacy of Turoctocog Alfa Pegol (N8-GP) in Previously Untreated Patients with Haemophilia A (PATHFINDER)	Novo Nordisk	Yes		Nov-21
NCT02172950 (Afstyla)	A Phase III Open Label, Multicenter, Extension Study to Assess the Safety and Efficacy of Recombinant Coagulation Factor VIII (rVIII-SingleChain, CSL627) in Subjects with Severe Hemophilia A	CSL Behring	Yes		Aug 23

Non-replacement therapies

There are several strategies that provide a prophylactic effect, but without the use of factor concentrate, hence the name Non-(factor) Replacement Therapies (NRTs). These have two main potential benefits. Firstly, as there is no actual clotting factor infused, the clotting effect is not affected by FVIII inhibitors. Secondly, they use subcutaneous delivery and have longer half-lives, which allow for weekly to monthly dosing. This may also assist with compliance. With current FVIII treatments, including EHLs, if a patient misses a treatment, within hours factor levels drop close to moderate or severe ranges of haemophilia, while NRTs decrease slowly over many days or weeks and their "trough level" provides better protection.

The two main approaches used for NRTs are:

1. FVIII-mimetics
2. Inhibition of physiological anticoagulants

FVIII-mimetics

Emicizumab (Hemlibra®; Hoffman-La Roche Pharmaceutical) is a bispecific antibody that bridges activated factor IX (FIXa) and factor X (FX) in order to restore the function of the missing activated FVIII (FVIIIa) that is needed for effective haemostasis. "Factor VIII activity" of emicizumab in pre-clinical studies is estimated to be equivalent to 10% to 15% of normal FVIII activity levels with weekly subcutaneous injections. Emicizumab has demonstrated efficacy in preventing bleeding in FVIII patients with inhibitors, resulting in the recent approvals from the EMA and FDA for prophylaxis of bleeding episodes in people with haemophilia A with FVIII inhibitors. However, it is not approved for use on demand due to its mode of action and formulation. Additional clotting factor treatments will be required intravenously when breakthrough bleeds occur, as is the case currently with any prophylaxis regimens (bypassing agents or FVIII).

Emicizumab is licensed for prophylaxis treatment of bleeding and not for the treatment of break through bleeding. The adult inhibitor trial (HAVEN 1), demonstrated that patients receiving

emicizumab experienced an 87% reduction in treated bleeding episodes compared with patients not receiving emicizumab. 63% of all patients receiving emicizumab experienced no bleeding episodes that required treatment. Emicizumab prophylaxis demonstrated a 79% reduction in treated bleeding episodes compared to prior bypassing agent prophylaxis, which is the current best standard of care. Long term assessment of treated bleeds in 24-week intervals showed an increase in the percentage of zero treated bleeds from the first 24 weeks to the 48-72-week interval. Overall, the number of bleeds that required treatment reduced over time, possibly because damaged joints have less bleeding, built strength and were protected against further bleeding. This may be the same for FVIII for patients without inhibitors. This will also be a consideration for potential wider use of emicizumab. However, the haemophilia patient population is used to an approach of "if in doubt, treat" and this approach may need to be reconsidered with the use of emicizumab. The next stage is the approval of emicizumab for patients with haemophilia A without inhibitors. This cohort is being studied in the Phase III HAVEN 3 trial with results and potential licensing expected by 2019. Additional trials are looking at extending the time between dosing to once every four weeks in patients with or without inhibitors.

In the Haven 1 trial for patients with inhibitors, three patients that received 100u/kg/24hr of aPCC (FEIBA® from Shire) for ≥ 24 hours (FEIBA® from Shire) developed thrombotic microangiopathy, one of whom continued to have serious bleeding and died of the bleed after the inability to identify the source of the bleed and the patient refusing red cell transfusion due to personal beliefs. In addition, two subjects that received 100u/kg/24hr of aPCC (FEIBA® from Shire) for ≥ 24 hours had thrombotic complications. Studies suggest that aPCC can substantially enhance the thrombin generation of emicizumab and the current hypothesis is that this is a result of presence of FIXa and FX in aPCC. It is currently thought unlikely that this problem will be seen in patients without inhibitors where FVIII concentrate is used, but these adverse events emphasize the potential complications that may arise due to different mechanisms of regulating the clotting process. How best to combine non-factor and factor therapies will likely remain an important issue requiring additional studies and widespread education amongst both clinicians and patients.

There were an additional four deaths reported recently. Three of these four cases were compassionate use requests for patients who had very serious or life-threatening conditions where every other treatment option has been exhausted. There is limited detail on all these cases as the treating clinicians want to ensure that the confidentiality of the patient is respected. However, the statement issued by Roche on March 18th, 2018, clarifies that, in each of the four cases, the assessment of the treating clinician was that the cause of death was unrelated to emicizumab.

In April 2018, a patient with inhibitors, in the Phase III HAVEN 2 clinical trial, developed a neutralising anti-drug antibody to Hemlibra®. As with all therapeutic proteins, there is a potential for the development of anti-drug antibodies with Hemlibra®. The anti-drug antibody resulted in reduced efficacy of Hemlibra® and it was decided to discontinue treatment and the patient resumed his previous treatment. With more than 600 people treated, this is the first confirmed report of a detectable anti-drug antibody that has impacted efficacy. Monitoring for the development of anti-drug antibodies to Hemlibra® is ongoing.

Inhibition of physiological anticoagulants

The other methods that are being investigated under the NRT category use a different approach; however, the overarching concept is broadly similar. In normal clotting, there are factors that promote clotting, such as factor VIII, and also molecules (anticoagulants) that prevent too much clotting (thrombosis). These two types are in a balance, which is disturbed when one type is missing, like FVIII or FIX in haemophilia. The idea of these treatments is to restore the balance at a lower level, by

reducing the levels of the anticoagulants. The NRTs that are being investigated include the inhibition of Anti-Thrombin (AT), a tissue factor pathway inhibitor (TFPI) and activated protein C (aPC).

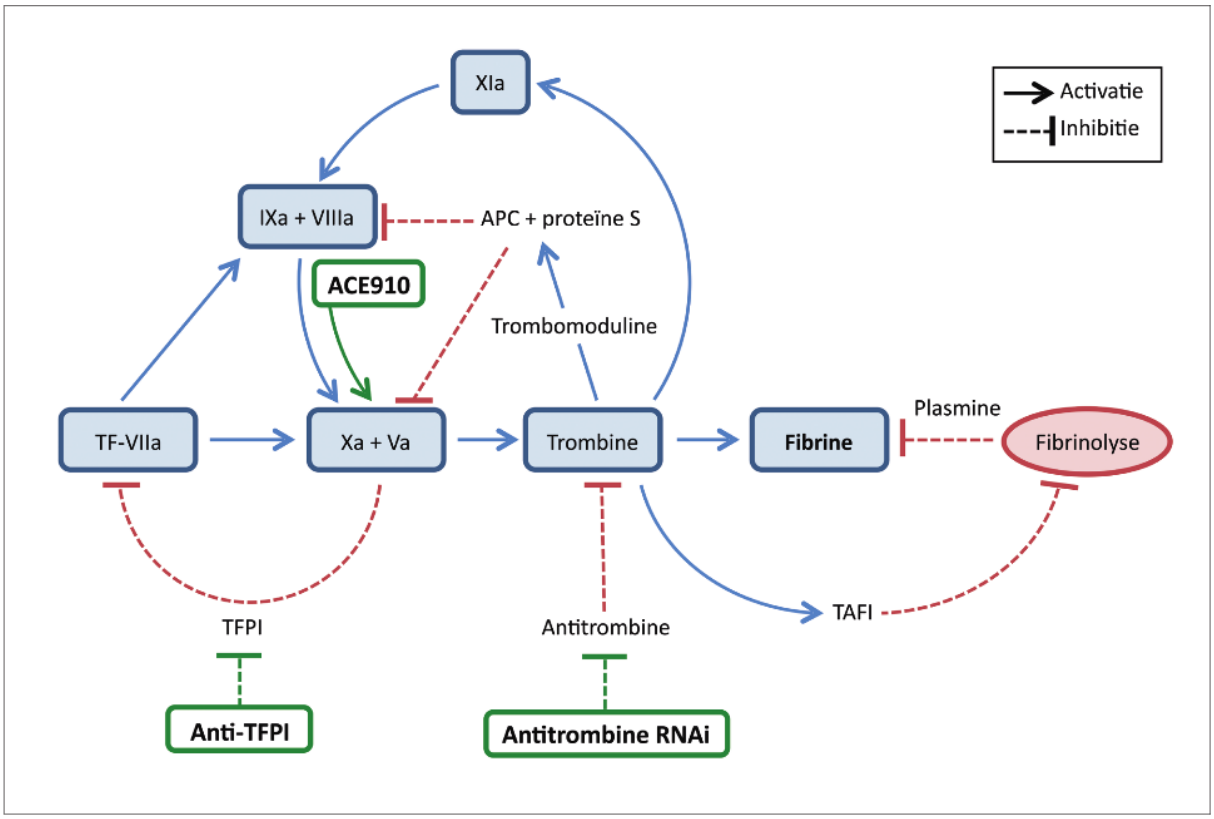


Figure 1. Schematic representation of the coagulation cascade with points of application of the new 'designer drugs' ACE910, anti-TFPI and antithrombin RNAi. APC = activated protein C, TF = tissue factor, TFPI = tissue factor pathway inhibitor, RNAi = RNA interference, TAFI = thrombin activatable fibrinolysis inhibitor. Illustration credit: Dutch Journal for Haematology (NTVH) "New designer drugs in the treatment of haemophilia A."

Fitusiran (Sanofi Genzyme/Alnylam) is a synthetic small interfering RNA (siRNA) that blocks production of antithrombin, which is currently in Phase III trials (ATLAS 3) for patients with haemophilia (A or B) with and without inhibitors. Monthly subcutaneous injections of fitusiran reduce the levels of antithrombin to approximately 20% of normal and this reduction appears to be efficacious in preventing bleeding, with 33 patients (in Phase II open label extension study) experiencing median annualised bleeding rates (ABRs) of 1.0 and 48% experiencing zero bleeds.

In September 2017, all trials were suspended after a patient died. The patient had treated himself for a musculoskeletal injury with factor concentrate and then developed a headache. He was erroneously diagnosed with a bleed in the brain (subarachnoid haemorrhage) based on CT imaging and was treated with high intensity FVIII concentrate. Sadly, he passed away. After his death, expert review of the CT scans revealed he actually had a clot in the brain (cerebral venous sinus thrombosis), not a bleed. As a result, the clinical trial was suspended. In December 2017, the FDA agreed on the recommencement of the trial with alignment on new clinical risk mitigation measures, including protocol-specified guidelines and additional investigator and patient education concerning reduced doses of replacement factor or bypassing agents to treat any breakthrough bleeds in fitusiran studies.

There are three investigational product trials using the anti-TFPI approaches. PF-06741086 (Pfizer) and concizumab (Novo Nordisk) are in Phase II clinical trials. The third is BAY 1093884 (Bayer), which is in Phase I. Results from Phase I studies demonstrate that the reduction to levels of 20% of normal TFPI

were associated with reduced clotting time and therefore, anti-TFPIs may be used to prophylactically treat patients with haemophilia with subcutaneous (and weekly/monthly) administration.

Another approach is inhibiting activated protein C (aPC). Targeting the anticoagulant effect of aPC has restored haemostasis in haemophilia mouse models in the pre-clinical phase and the company ApcinteX is expected to apply for Phase I trials for haemophilia in the near future.

With the significant potential of improvement in quality of life that NRTs can provide, also come additional unknowns. NRTs do not prevent all bleeding and their impact on the coagulation cascade adds a great deal to the complexity in the management and laboratory monitoring of bleeding events as well as in the condition of haemophilia itself. Treatment with concizumab was associated with elevated D-dimer levels (which are seen in patients with thrombotic disorders) although the clinical relevance of this observation is unknown. The experience with emicizumab and fitusiran should promote a level of caution and the need for education about thrombotic consequences, especially when combining them with other therapies. This can best be achieved by ensuring that NRTs are prescribed and their use monitored by haemophilia comprehensive care centres. The EHC and EAHAD are collaborating on draft principles in relation to the use of NRTs.

There is an additional complexity associated with NRTs in that current assays to measure the clotting factor levels are not appropriate to measure the effect of NRTs. Therefore, additional work will need to be done in order to monitor these therapies in both comprehensive care centres and even more so, outside of specialist centres.

**You can find a table of ongoing clinical trials for non-replacement therapy on the following page.*

Table 3: Non-replacement clinical trials for haemophilia A

Clinical Trial	Name	Company	Product Type	Phase I	Phase II	Phase III	Estimated Time of Completion
NCT02571569 (BAY 1093884)	A Single Escalating Dose and Multiple Dose Study of BAY 1093884 in Subjects with Severe Haemophilia Types A or B, with or without Inhibitors	Bayer	Anti-TFPI				Jul-18
NCT02974855 (PF-06741086)	PF-06741086 Multiple Dose Study in Severe Haemophilia	Pfizer	Anti-TFPI				Nov-18
NCT03363321 (PF-06741086)	PF-06741086 Long-term Treatment in Severe Haemophilia	Pfizer	Anti-TFPI				Nov-18
NCT03196297 (Concizumab)	A Trial Evaluating Efficacy and Safety of Prophylactic Administration of Concizumab in Patients with Severe Haemophilia A Without Inhibitors	Novo Nordisk	Anti-TFPI				Aug-19
NCT02847637 (Hemlibra®)	A Clinical Trial to Evaluate Prophylactic Emicizumab Versus no Prophylaxis in Haemophilia A Participants Without Inhibitors (HAVEN 3)	Roche	Bi-specific Antibody				Sep-19
NCT03020160 (Hemlibra®)	A Study to Evaluate the Efficacy, Safety, Pharmacokinetics, and Pharmacodynamics of Emicizumab Given Every 4 Weeks in Participants with Haemophilia A (HAVEN 4)	Roche	Bi-specific Antibody				Dec-19
NCT03417245 (Fitusiran)	A Study of Fitusiran (ALN-AT3SC) in Severe Haemophilia A and B Patients without Inhibitors	Alnylam/Sanofi	siRNA				Dec-19
NCT03315455 (Hemlibra®)	Efficacy, Safety, and Pharmacokinetic Study of Prophylactic Emicizumab Versus No Prophylaxis in Haemophilia A Participants (HAVEN 5)	Roche	Bi-specific Antibody				Feb-20

Gene therapy

The majority of the trials in gene therapy have been associated with FIX, which has been primarily driven by the limited packing capacity of adeno-associated viral (AAV) vectors. This is because the FIX gene can be easily fit inside an AAV vector, unlike the FVIII gene, which is many times larger and cannot completely fit inside a vector without truncation (making it smaller). However, there are currently five ongoing clinical trials focusing on FVIII. The most advanced of these are the two Phase III trials of Valoctocogene-Roxaparvovec (BMN-270, BioMarin). These are followed by SPK-8011 (Spark), SB-525/PF 07055480 (Sangamo/Pfizer), BAX888 (Shire) and GO-8 (University College London), which are all in initial stages of dosing.

The valoctocogene roxaparvovec gene therapy uses an AAV5 vector containing a FVIII gene of which the B-domain has been deleted (AAV5-FVIII-B-domain deleted), thereby making it smaller. The same genetic construct is used in the manufacture of current recombinant B domain-deleted factor VIII products. A total of 15 patients were enrolled in the Phase II clinical trial. In the two low-dose cohorts, only one patient increased his FVIII activity levels to 2%. In the high-dose cohort (consisting of seven patients), the interquartile FVIII activity range, at 1.5 years, was approximately 50% to 120%, with a median level of 90%; all patients who previously were treated with FVIII prophylaxis stopped prophylaxis, and the median annualised bleeding rate (ABR) and annualised FVIII use after patients achieved FVIII activity levels >5%, were zero for both. In the mid-dose cohort (consisting of six patients), the interquartile FVIII activity range in the three patients with the longest follow-up of one year was approximately 40% to 60%, with a median level of 49%. Similar to the high-dose cohort, all patients stopped prophylaxis, and the median ABR and annualised FVIII use after patients achieved FVIII activity levels >5%, were zero for both. The first Phase III study (GENEr8-1, studying the high dose) was commenced in December 2017, and the second Phase III study (GENEr8-2, studying the mid dose) will commence in the first half of 2018. The SPK-8011 Phase I/II trial uses AAV8-FVIII-B-domain deleted. The first four participants, who have been followed at least 12 weeks post infusion, have reduced their ABR by 82% (100% after week 4) to a mean of 1 (0) annualised bleeds. The first two patients were treated at the low dose, one achieved a sustained mean FVIII activity level of 10% (range 7-11%). The second achieved a mean of 16% (range 6-37%). Two patients were treated at the mid-dose regimen and achieved a sustained mean FVIII activity levels of 9% (range 7-12%) and 13% (range 7-24%). Three more participants have been infused, one at the mid dose level and two at a high dose level.

The other three clinical trials have not presented data on Phase I/II to date, including Shenzhen Geno-Immune Medical Institute, which is using lentivirus, which is an integrating vector (as opposed to AAV, which generally does not integrate into the genome).

AAV vectors

There are several AAV vectors being investigated. For FVIII gene therapy, BioMarin uses AAV5. Spark use bio-engineered AAV8. Sangamo/Pfizer is using AAV2/6 variant and University College London and Shire are using an AAV2/8 variant. All target the liver, which allows the vector to be delivered via a single peripheral IV infusion in the arm. The presence of antibodies (Ab) to the AAVs, which occur in 30% to 40% of the general population depending on the serotype, precludes effective gene transfer and therefore patients with pre-existing Abs to the vectors are excluded from current trials. Shire are carrying out an observational study to assess the seroprevalence of neutralizing antibodies (Ab) to AAV in adults with severe haemophilia A or moderately severe to severe haemophilia B (NCT03185897). Importantly for patients, as these treatments develop, some patients may have antibodies to one AAV vector but not to another, and as a result the pool of patients eligible for these treatments will be increased. Some companies are considering including subjects with low level Abs against their vector.

Biomarin announced in May 2018 that they have dosed the first patient in a new Phase I/II study evaluating valotocogene roxaparvovec in FVIII patients with pre-existing AAV5 antibodies

Corticosteroids

Liver function is being monitored very carefully in all these studies, firstly to ensure the safety of the patient and secondly to prevent the potential loss of FVIII activity levels. Based on previous studies, corticosteroid use is generally initiated after the increase of alanine aminotransferase (ALT) levels in the liver and to preserve factor activity. There is on-going debate about the prophylactic use of corticosteroids. In the phase III studies of valoctocogene roxaparvovec, prophylactic corticosteroids will not be used in the current trial plans.

Exclusion criteria

The exclusion criteria for patients from these trials include: pre-existing antibodies to the specific AAV vector used, active hepatitis C or B, significant liver dysfunction, liver cirrhosis or late stage fibrosis (stage 3 or 4), liver cancer, and a history of thromboembolic events (e.g. deep vein thrombosis, non-haemorrhagic stroke, pulmonary embolism, myocardial infarction, arterial embolus). What is of most interest is allowance of patients with HIV in the BioMarin study, which will hopefully mean that once on the market, these therapies will have evidence in this cohort of patients. Additionally, the Sangamo trial is not excluding patients who had a transient inhibitor to FVIII in childhood.

Collectively, this emerging data is very encouraging. Long-term follow-up, however, is needed to better assess the impact on constant FVIII activity levels and quality of life. A better understanding of what occurs in the liver at significantly different vector doses for a given AAV serotype is needed. All these studies enrolled adult subjects heavily exposed to FVIII, without any evidence or history of inhibitors. The risk of immune responses both against the vector, particularly if there are differences between vector serotypes (e.g. AAV 5 vs AAV8) and the gene product, will become a prominent discussion topic in future studies that include paediatric patients with less than 50 exposure days. Encouragingly, preclinical studies support the concept that liver gene therapy may provide benefits of immune tolerance induction by continuous expression of the FVIII.

Table 4: Gene therapy clinical trials for haemophilia A

Clinical Trial	Name	Company	Phase I	Phase II	Phase III	Estimated Time of Completion
NCT03001830	Gene Therapy for Haemophilia A. (GO-8)	University College London /Freeline				Apr-19
NCT03003533	A Gene Transfer Study for Haemophilia A (SPK-8011)	Spark				Dec-19
NCT03217032	Gene Modified autoHST for Type A (YUVA-GT-F801)	Shenzhen Geno-Immune Medical Institute				Dec-21
NCT03061201	Dose-Ranging Study of Recombinant AAV2/6 Human Factor 8 Gene Therapy SB-525 in Subjects with Severe Haemophilia A	Sangamo/Pfizer				Jan-22
NCT03370172	Safety and Dose Escalation Study of an Adeno-Associated Viral Vector (BAX888) for Gene Transfer in Haemophilia A Subjects	Shire				Jun-22
NCT03392974	Single-Arm Study to Evaluate the Efficacy and Safety of Valoctocogene Roxaparvovec in Haemophilia A Patients at a Dose of 4E13 vg/kg (GENEr8-2)	BioMarin				Sep-23
NCT03370913	Single-Arm Study to Evaluate The Efficacy and Safety of Valoctocogene Roxaparvovec in Haemophilia A Patients	BioMarin				Sep-23

Standard half-life (SHL)

There was only one recombinant FIX (Benefix®, Pfizer) on the market for almost 20 years. In 2015, Rixubis® from Shire became available in Europe and the US. Ixinity® by Emergent Biosolutions/Cangene also became available in the US but is not being marketed in Europe at this stage. The increased availability in the standard half-life (SHL) market may promote post-marketing trials (Phase IV), which examine a change in dosing regimens aiming at increasing trough levels and reducing weekly infusion rates as well as better understanding the pharmacokinetics of FIX.

Extended half-life (EHL)

The introduction of extended half-life (EHL) products has resulted in a total paradigm shift in the treatment of FIX. Unlike FVIII, where there is a more delicate balance between higher trough levels, fewer infusions and reduction in number of units used per year, the extension gained in half-life using EHLs for FIX is sufficient to achieve all three simultaneously.

The techniques used to increase FIX half-life ($t_{1/2}$) prolongation include the following:

1. fusion with prolonged half-life proteins, such as IgG-Fc and albumin
2. site directed pegylation

There are currently three FIX EHL factor concentrates licenced in Europe. The first EHL available globally was Alprolix®, which is a fusion of the Fc-portion of immunoglobulin G to a single molecule of rFIX (rFIX-Fc) marketed in Europe by Sobi. Alprolix® allows for infusion every 7 to 10 days with some well-controlled patients able to be treated every 14 days. Data from previously untreated patients (PUPs) are expected in June 2019. At the same time as Alprolix® was approved in the EU, Idelvion® from CSL Behring also received approval. Idelvion® is a rFIX protein fused with albumin (rFIX-FP). Refixia® from Novo Nordisk uses a 40 kDa molecule PEG (polyethylene glycol) attached to the FIX (PEGylation). It is approved in the EU for the treatment and prophylaxis of bleeding in patients aged ≥ 12 years with haemophilia B. The estimated completion date for Refixia® PUPs data is October 2020. The half-lives of Refixia® and Idelvion® are increased by almost five-fold.

At the EHC New Technologies workshop in November 2017, Prof Johannes Oldenburg presented data from the haemophilia centre in Bonn on both Alprolix® and Idelvion®. The first thing of note is that there was more rapid uptake in patients switching from SHL FIX product to EHL FIX product compared to FVIII. 18 patients switched to Alprolix® and reduced their infusion rate from 2 or 3.5 times per week (every second day) to once per week, and one patient stayed on his previous regimen of one infusion per week. This led to an overall reduction of 59% infusions over the year. This also corresponded with reduction of 26% in the annual consumption of FIX units. Data from Ireland presented at the EHC New Technologies workshop by Dr Niamh O'Connell showed a switch of 28 adult patients to prophylaxis with Alprolix®, six of whom moved from on demand to prophylaxis. FIX use in units reduced from 47-181 IU/kg/week to 46-63 IU/kg/week and the mean trough level increased from 4% to 8%. Additionally, Canadian experience in post licensing showed a reduction of 50% in the number of IU used.

Prof Oldenburg also presented data on ten patients switching to Idelvion® on an every-seven-day regimen at 35-50 IU/kg. There was a 63% reduction in annualised infusions and 53% reduction in annual factor consumption.

Again, a similar experience was seen at the Milan centre with both Idelvion® and Alprolix® as reported in the other centres, which was presented by Prof Flora Peyvandi in her plenary talk at the European Association of Haemophilia and Allied Disorders (EAHAD) Congress in February 2018.

There are two other trials that are examining the use of subcutaneous injection for the delivery of FIX. CSL Behring have a sub-study Phase IIIb trial to examine the safety and pharmacokinetics of daily subcutaneous (SC) administration of rFIX-FP. At the EAHAD Congress this year, CB 2679d/ISU304 developed by Catalyst Bioscience was presented and it uses a subcutaneous injection of a modified FIX that increases potency to achieve higher trough levels in the mild haemophilia range or potentially normal factor levels, to maintain a steady-state level in the blood. Sobi/Bioverative (a Sanofi company) have announced the development of a subcutaneous therapy with FIX-Fc-XTEN, which is being done under a collaboration agreement with Sobi, however clinical trials have not commenced.

Although EHL products are promising, the optimal strategy for treatment of bleeds between prophylactic doses and dosing regimens will likely need to be individualized to patient pharmacokinetics accounting for existing joint damage, physical activity, and other factors like patient ability for self-management.

These are attractive options for patients to reduce infusion rates, decrease the need for central lines and may reduce the potential burden of Immune Tolerance Induction (ITI) (please see article on inhibitors). As with the FVIII molecule that uses pegylation, concerns about the clearance of the PEG molecule over the long term have led to a difference in how these products are being licenced between the Food and Drug Administration (FDA) in the US and the European Medicines Agency (EMA), e.g. Refixia®/Rebinyn® is licenced by the EMA for those >12 years and the FDA have licensed it for all on-demand but not for prophylaxis. The immunogenicity (the potential for inhibitor development) of EHLs is unknown and will require extensive longer-term monitoring to determine whether they are more or less immunogenic than current products. Trials to administer these products subcutaneously will be watched carefully, amongst others for inhibitor development.

Non-replacement therapies

There are several strategies that provide a prophylactic effect, but without the use of factor concentrate, hence the name Non-(factor) Replacement Therapies (NRTs). These have two main benefits. First, as there is no FIX in these products, their clotting effect is not affected by FIX inhibitors. Second, they use subcutaneous delivery that has very interesting pharmacokinetics and allows for weekly to monthly dosing. This may also assist with adherence.

Two ongoing therapies you may have heard about, emicizumab and concizumab, are not covered in this section. Emicizimab due to its mode of action does not work in patients with FIX deficiency. The second is not applicable due to lack of a clinical trial in this population. For further information on concizumab, please see the article on haemophilia A without inhibitors. The main non-replacement treatments for FIX patients use the inhibition of other anticoagulants.

In normal clotting, there are factors that promote clotting, such as factor VIII or factor IX, and also molecules (anticoagulants) that prevent too much clotting (thrombosis). These two types are in a balance, which is disturbed when one type is missing, like FVIII or FIX in haemophilia. The idea of these treatments is to restore the balance at a lower level, by reducing the levels of the anticoagulants The NRTs that are being investigated are the inhibitor of antithrombin (AT), tissue factor pathway inhibitor (TFPI) and activated protein C (aPC).

Fitusiran (Sanofi Genzyme/Alnylam) is an antithrombin synthetic inhibitor RNA (siRNA), which is currently in Phase III trials for patients with haemophilia with and without inhibitors. Monthly

subcutaneous injections of fitusiran reduces the levels of antithrombin to approximately 20% of normal and this reduction appears to be efficacious in preventing bleeding, with 33 patients (in Phase II open label extension study) experiencing median annualised bleeding rates (ABRs) of 1.0 and 48% experiencing zero bleeds.

However, in September 2017 all trials were suspended after a patient died. The patient had treated himself for a musculoskeletal injury with factor concentrate and then developed a headache. He was erroneously diagnosed with a bleed in the brain (subarachnoid haemorrhage) based on CT imaging and was treated with high intensity FVIII concentrate. He sadly passed away. After his death, expert review of the scans revealed he actually had a clot (cerebral venous sinus thrombosis), not a bleed. As a result, the clinical trial was suspended. In December 2017, the FDA agreed on the recommencement of the trial with alignment on new clinical risk mitigation measures, including protocol-specified guidelines and additional investigator and patient education concerning reduced doses of replacement factor or bypassing agents to treat any breakthrough bleeds in fitusiran studies.

There are two products using the anti-TFPI approaches, PF-06741086 from Pfizer, which is in Phase II clinical trials and BAY 1093884 from Bayer in Phase I. Results from Phase I studies demonstrate reduction to levels of 20% of normal TFPI, which are associated with reduced clotting time and hence may be used to prophylactically treat patients with haemophilia with subcutaneous (and weekly/monthly) administration.

Another approach is inhibiting activated protein C (aPC). Targeting the anticoagulant effect of aPC has restored haemostasis in haemophilia mouse models in the pre-clinical phase and the company ApcinteX is expected to apply for Phase I trials for haemophilia in the near future.

With the significant potential in improvement in quality of life that NRTs can provide, also come additional unknowns. NRTs do not prevent all bleeding and their impact on the coagulation cascade adds a great deal to the complexity in the management of bleeding events as well as the condition of haemophilia itself.

The experience with emicizumab and fitusiran should promote a level of caution about thrombotic consequences, especially when combining therapies. There is an additional complexity associated with NRTs, in that current laboratory assays to measure the clotting factor levels may not be the most appropriate and additional work will need to be done to monitor these therapies in both comprehensive care centres and even more so outside specialist centres.

Table 5: Non-replacement clinical trials for haemophilia B

Clinical Trial	Title	Company	Product Type	Phase I	Phase II	Phase III	Estimated Completion Date
NCT03417245 (Fitusiran)	A Study of Fitusiran (ALN-AT3SC) in Severe Haemophilia A and B Patients without Inhibitors	Alnylam/Sanofi	siRNA				Dec-19
NCT02974855 (PF-06741086)	PF-06741086 Multiple Dose Study in Severe Haemophilia	Pfizer	Anti-TFPI				Nov-18
NCT02571569 (BAY 1093884)	A Single Escalating Dose and Multiple Dose Study of BAY 1093884 in Subjects with Severe Haemophilia Types A or B, with or without Inhibitors	Bayer	Anti-TFPI				Jul-18

Gene therapy

The FIX gene is a great candidate for gene therapy as its small size fits inside the adeno-associated viral vector (AAV). Due to the limited packing capacity of AAV vectors, most of the early studies were focused on haemophilia B. The original Nathwani gene therapy trial used an AAV8 capsid with a FIX-wild type (i.e. normal FIX). This resulted in FIX levels of between 1.5% to 4% in the low-dose and mid-dose cohort. In the high-dose cohort, FIX levels rose to 5% converting all severe patients to mild haemophilia and achieving 90% reduction in annualised bleed rate. This study also showed the significant impact of increased levels of alanine aminotransferase (ALT) a liver enzyme in the blood in 4/6 (60%) patients. Oral steroids were used to reduce the reaction in the liver, reducing the ALT level and preventing a significant reduction in factor levels. The enrolment of these studies was slow, not because of lack of interest but to gain a better understanding of what was occurring in the liver and to examine the long-term effects as well as to determine the next steps. To date, there has been no evidence of long term adverse events. Additionally, the factor levels have remained stable, which is interesting as, with the generation of new cells in the liver combined with the fact that the AAV vector is non-integrating, one might expect the factor levels to drop over time. So far, this has not been the case, and the improvement has been maintained with very limited use of factor concentrates.

There are two options in achieving higher factor levels and move closer to normal factor level. First, you can increase the dose of the AAV vector. The problem with this is, increasing the dose may cause a greater immune reaction in the liver and hence possibly not achieve any increase in factor levels. As gene therapy with that vector is currently a one-time opportunity and no re-treatment is possible because of immune reaction to the viral capsid, this is not ideal. This option is being studied further to determine what the mechanisms in the liver are that cause the reaction and potentially minimise their effect.

The second option is increasing the specific activity of the FIX protein by introducing a mutation. This means that for the same size of FIX gene, you get much greater activity than expected from the factor amount. This is what the next generation of studies aim to take advantage of as it avoids the requirement for higher doses of AAV. This approach was identified after finding a man who had a FIX level of almost 700%, which is an eight to 10-fold increase in normal FIX activity. This FIX variant is known as the Padua mutation and is being used by several gene therapy trials to increase factor activity level responses.

The phase I/II clinical gene therapy trial of SPK- 9001/PF 07055480 by Spark/Pfizer used the Padua mutation FIX and reported that in 10 patients it achieved a mean FIX activity level of 34% (range of 14-81%) with all patients stopping prophylaxis and no immune response (inhibitors). Two out of 10 patients developed increased ALT levels in the liver and were started on steroids, which prevent loss of the FIX level achieved. The study is due for completion in March 2023 in terms of follow-up (five years) but it is likely that Phase III trials will commence before this. UniQure's Phase I/II trial of AMT-060, carried out with an AAV5 vector and FIX wildtype gene, used higher dose levels than some other trials and achieved levels of 5% to 7%. ALTs increased in three patients (30%) without loss of FIX activity, and follow up will be continued within the trial until May 2021. In the uniQure Phase IIb and III trials beginning in 2018, the FIX-Padua gene will replace the FIX gene used with AMT-060 and will be known as AMT-061, which will aim to increase the levels of Factor IX closer to normal.

University College of London (UCL) is continuing to follow the patients from their landmark academic trial (*N. Engl. J. Med*. 371, 1994-2004, 2014), which has now closed further recruitment. Meanwhile they have launched another phase I/II trial for haemophilia B with a next-generation AAV vector through the London-based gene therapy start-up, Freeline Therapeutics. Active recruitment of

subjects with severe haemophilia B from centres in the UK has begun, with plans to extend trial widely later this year.

Shire are carrying out an observational study to assess the seroprevalence of neutralizing antibodies (NAb) to AAV in adults with severe haemophilia A or moderately severe to severe haemophilia B (NCT03185897).

Shenzhen Geno-Immune Medical Institute is currently the only trial using lentivirus, which is an integrating vector (as opposed to AAV, which generally do not integrate into the genome). Results are expected in December 2021. The Sangamo approach (SB-FIX) is using therapeutic Zinc Finger Nuclease (ZFN) genome editing, which will be delivered by an AAV vector. This is intended to function by placing a corrected copy of the factor IX transgene into the genome of the patients' own hepatocytes.

Corticosteroids

Liver and immune cell function is being monitored very carefully in all these studies, firstly to ensure the safety of the patient and secondly to prevent the loss of FIX levels. Based on previous studies, corticosteroid use is generally initiated after the increase of ALT levels in the blood. The consideration of using steroids prophylactically is part of an on-going debate.

Exclusion criteria

The exclusion criteria for patients from these trials are pre-existing antibodies to the specific AAV vector used, active hepatitis C or B, significant liver dysfunction, liver cirrhosis or late stage fibrosis (stage 3 or 4), liver cancer, and a history of thromboembolic events (e.g. deep vein thrombosis, non-haemorrhagic stroke, pulmonary embolism, myocardial infarction and arterial embolus). UniQure's phase IIb and III study will not exclude patients on the basis of pre-existing antibodies to AAV5.

Collectively, these emerging data are very encouraging. Long-term follow-up, however, is needed to better assess the impact of constant factor levels and quality of life. A better understanding of what occurs in the liver at significantly different vector doses for a given AAV serotype is needed. All these studies enrolled adult subjects heavily exposed to FIX, without any evidence or history of inhibitors.

Table 6: Gene therapy clinical trials for Haemophilia B

Clinical Trial	Title	Company	Phase			Estimated Completion Date
			I	II	III	
NCT02396342	Trial of AAV5-hFIX in Severe or Moderately Severe Haemophilia B	UniQure				May-21
NCT03217032	Gene Modified autoHST for Type A or B Haemophilia	Shenzhen Geno-Immune Medical Institute				Dec-21
NCT01687608	Open-Label Single Ascending Dose of Adeno-associated Virus Serotype 8 Factor IX Gene Therapy in Adults with Haemophilia B	Shire				Nov-30
NCT02484092	A Gene Therapy Study for Haemophilia B	Spark/Pfizer				Jan-19
NCT03369444	A Factor IX Gene Therapy Study (FIX-GT)	UCL/ Freeline				Mar-19
NCT02695160	Ascending Dose Study of Genome Editing by Zinc	Sangamo Therapeutics				Jan-21

	Finger Nuclease Therapeutic SB-FIX in Subjects with Severe Haemophilia B				
NCT03489291	Dose Confirmation Trial of AAV5-hFIXco-Padua	uniQure			Aug-23

NOVEL TREATMENT IN HAEMOPHILIA AND INHIBITORS

If haemophilia had a calendar, 2018 would be the year of the inhibitor. This is highlighted by discussions at international conferences relating to prophylaxis with bypassing agents (BPAs), new products and increasing numbers of clinical trials with new or existing products so patients with inhibitors can begin to move toward a level of treatment that has been achieved in those without inhibitors.

An inhibitor is a high-affinity antibody response that specifically neutralises the procoagulant activity of the relevant clotting factor, causing difficulty in managing bleeds. Inhibitors are characterised in two ways — by the titre and by the immune response. The titre refers to the inhibitory capacity of the patient's plasma to neutralise clotting factor in normal plasma. A high-titre inhibitor is defined as having 5 Bethesda units (BU) or higher, and a low-titre one is defined between a cut off value (usually 0.6 BU) and 5 BU. Patients whose titre is less than 5 BU are divided into those in whom a rapid anamnestic response to factor infusion occurs (i.e. high responders) and those in whom such a response does not occur (i.e. low responders). This characterisation is important because patients with a low titre and low responding inhibitors can be treated with standard replacement therapy, albeit at increased doses to overwhelm the inhibitor. Patients with a high titre or high responding inhibitors can only be treated effectively with BPAs, unless the inhibitor is eradicated.

Using on-demand treatment to treat bleeding episodes in people with inhibitors can be less effective than in those without inhibitors leading to increased joint damage and a significant impact on quality of life (QoL) as well as increased rate of haemophilia-related deaths. Although prophylaxis with BPAs in those with inhibitors does improve outcomes, especially when started early, and reduce joint and other types of bleeds (45%-72%), it often does not achieve this to the same degree as prophylaxis in non-inhibitor patients. There is also a variable response to prophylaxis, which highlights the need for personalised treatment in people with inhibitors.

With the licensing by the European Medicines Agency (EMA) of Hemlibra® (emicizumab from Roche) in February 2018 for routine prophylaxis of bleeding episodes in people with haemophilia A with factor VIII inhibitors, this is a very promising time. Studies have shown a reduction of >70% in bleeding rates in inhibitor patients compared to their previous prophylaxis.

Based on the present knowledge, prophylaxis should be considered in inhibitor patients who have experienced a life-threatening bleed, frequent musculoskeletal bleeds, and spontaneous bleeds causing significant impairment of QoL and in patients who have failed Immune Tolerance Induction (ITI). The treatment goal for patient suffering from longstanding inhibitors should be the same as for haemophilia patients without inhibitors.

Table 7:On-going bypassing agents, ITI and non-replacement therapy clinical trials for inhibitors

NCT Number	Title	Sponsor	FVIII	FIX	Phase I	Phase II	Phase III	Completion Date
NCT02448680 (Wilfactin®)	A Phase III Study on the Safety, Pharmacokinetics and Efficacy of Coagulation Factor VIIa	LFB (USA)	Yes	Yes				Aug-17
NCT02919800 (MOD-5014)	A Single-dose, Dose-escalation Study of a Long-acting MOD-5014 in Healthy Adult Male	Opko Biologics	Yes	Yes				May-18
NCT02484638 (CSL689)	Study of Recombinant Factor VIIa Fusion Protein (rVIIa-FP, CSL689) for On-demand Treatment of Bleeding Episodes in Patients with Haemophilia A or B with Inhibitors	CSL Behring	Yes	Yes				Jun-18
NCT02571569 (BAY 1093884)	A Single Escalating Dose and Multiple Dose Study of BAY 1093884 in Subjects with Severe Haemophilia Types A or B, With or Without Inhibitors	Bayer	Yes	Yes				Jul-18
NCT03407651 (Marzeptacog Alfa)	Study of Coagulation Factor VIIa Variant Marzeptacog Alfa (Activated) in Adult Subjects with Haemophilia A and B	Catalyst Biosciences	Yes	Yes				Jul-18
NCT03417102 (Fitusiran)	A Study of Fitusiran (ALN-AT3SC) in Severe Haemophilia A and B Patients with Inhibitors	Alnylam/Sanofi	Yes	Yes				Jul-19
NCT03196284 (Concizumab)	A Trial Evaluating the Efficacy and Safety of Prophylactic Administration of Concizumab in Haemophilia A and B Patients with Inhibitors	Novo Nordisk	Yes	Yes				Oct-19
NCT03103542 (Elocta®/Eloctate®)	Study of rFVIIIFc for ITI in Haemophilia A Patients with Inhibitors Who Have Failed Previous ITI Therapies (ReITIrate)	Bioverativ (a Sanofi Company) /Sobi	Yes					Apr-20
NCT03191799 (Hemlibra®)	A Study to Evaluate the Safety and Tolerability of Prophylactic Emicizumab in Haemophilia A Patients With Inhibitors	Hoffmann-La Roche	Yes					Sep-20
NCT03093480 (Elocta®/Eloctate®)	A Study to Evaluate Efficacy of rFVIIIFc for Immune Tolerance Induction (ITI) in Severe Haemophilia A Participants With Inhibitors Undergoing the First ITI Treatment (verITI-8 Study)	Bioverativ (a Sanofi Company) /Sobi	Yes					Dec-20

Bypassing agents

There are several activated recombinant factor VII (rFVIIa) products in clinical trials occurring internationally, with LR769 from LFB USA completing their trial at the end of 2017 and MOD-5014 by Opko Biologics expected to finish trials in May 2018 for the market in Israel. Additionally, CSL Behring are using their fusion technology to extend the half-life of rFVIIa by attaching albumin to the molecule (rFVIIa-FP), which is currently in Phase III. The half-life of rFVIIa-FP at the highest dose investigated in the study was 8.5 hours, which represents a three to four-fold half-life extension compared with rFVIIa. Marzeptacog alfa from Catalyst Bioscience, currently in Phase II trials, is a FVIIa that has a higher clot-generating activity and longer activity at the site of bleeding. It also has the potential to be infused subcutaneously for prophylactic treatment for those with inhibitors. Trial results are expected in July 2018.

Immune Tolerance Induction (ITI)

In terms of treatment for those with an inhibitor, the best option has always been the eradication of the inhibitor, using high doses of FVIII for approximately 12-18 months. With the advent of new products and different treatment approaches, this view might change in terms of the day-to-day treatment of people with an inhibitor. However, when it comes to treating the bleeds that occur with new therapies, surgery or responding to a trauma, the most predictable response to bleeding in these cases will be treatment with FVIII. The coming years may see a significant evolution in the way Immune Tolerance Induction (ITI) is used to eradicate an inhibitor.

In the SIPPET study, patients treated with plasma-derived factor VIII containing von Willebrand Factor had a lower incidence of inhibitors than those treated with recombinant Factor VIII. One of the proposed reasons for this was that as the FVIII molecule is mostly attached to the von Willebrand Factor molecule and, as a result, the von Willebrand Factor molecule may cover the parts of the FVIII molecule that the inhibitor attaches to. This may give time for the body to become accustomed to the foreign FVIII, reduce its immune response and stop producing the inhibitor. There are some international trials further examining the impact of using FVIII products containing von Willebrand Factor to eradicate inhibitors.

With the idea of preventing the inhibitor attaching to specific sites on the FVIII molecule, the question could be theorised, could an extended half-life product be beneficial? Would a different molecule, such as Fc being attached to the FVIII molecule, provide a similar protection by covering the same parts as the von Willebrand Factor? The ReITIerate and verITI-8 trials Sobi/Bioverativ (a Sanofi Company) for patients who have failed ITI and those with first time ITI, respectively, will aim to see if there is any additional benefit of using the Fc bound Factor VIII for ITI. Results for these trials are expected in 2020.

Non-replacement therapies (NRT)

The development of Non-(factor) Replacement Therapies (NRTs) that mimic the FVIII and/or FIX has the potential to have the biggest impact on quality of life in patients with inhibitors, especially those who have failed ITI. Of added benefit is that many of these NRTs are administered via subcutaneous infusions weekly or monthly.

Emicizumab (Hemlibra®; Hoffman-La Roche Pharmaceutical) is a bispecific antibody that bridges activated factor IX (FIXa) and factor X (FX) in order to restore the function of the missing activated FVIII (FVIIIa) that is needed for effective haemostasis. The “Factor VIII activity” of emicizumab in preclinical studies is estimated to be equivalent to 10% to 15% of normal FVIII activity levels with weekly subcutaneous injections. Emicizumab has demonstrated efficacy in preventing bleeding in FVIII patients with inhibitors, resulting in the recent approvals from the EMA and the FDA for prophylaxis of bleeding episodes in people with haemophilia A with FVIII inhibitors. However, it is not approved for

use on demand due to its formulation, so additional clotting factor treatments will be required intravenously when breakthrough bleeds occur as is the case currently with any prophylaxis regimens (BPA or FVIII).

Emicizumab is licensed for prophylaxis treatment of bleeding and not for the treatment of break through bleeding. The adult inhibitor trial (HAVEN 1), demonstrated that patients receiving emicizumab experienced an 87% reduction in treated bleeding episodes compared with patients not receiving emicizumab. 63% of all patients receiving emicizumab experienced no bleeding episodes that required treatment. Emicizumab prophylaxis demonstrated a 79% reduction in treated bleeding episodes compared to prior bypassing agent prophylaxis, which is the current best standard of care. Long term assessment of treated bleeds in 24-week intervals showed an increase in the percentage of zero treated bleeds from the first 24 weeks to the 48-72-week interval. Overall, the number of bleeds that required treatment reduced over time, possibly because damaged joints have less bleeding, built strength and were protected against further bleeding. This may be the same for haemophilia A patients without inhibitors. This will also be a consideration for potential wider spread use of emicizumab. However, the haemophilia patient population is used to an approach of "if in doubt, treat" and this approach may need to be reconsidered with the use of emicizumab. The next stage is the approval of emicizumab for patients with haemophilia A without inhibitors. This cohort is being studied in the Phase III HAVEN 3 trial with results and potential licensing expected by 2019. Additional trials are looking at extending the time between dosing to once every four weeks in patients with or without inhibitors.

In the Haven 1 trial for patients with inhibitors, three patients that received 100u/kg/24hr of aPCC (FEIBA® from Shire) for ≥ 24 hours (FEIBA® from Shire) developed thrombotic microangiopathy, one of whom continued to have serious bleeding and died of the bleed after the inability to identify the source of the bleed and the patient refusing red cell transfusion due to personal beliefs. In addition, two subjects that received 100u/kg/24hr of aPCC (FEIBA® from Shire) for ≥ 24 hours had thrombotic complications. Studies suggest that aPCC can substantially enhance the thrombin generation of emicizumab and the current hypothesis is that this is a result of presence of FIXa and FX in aPCC. It is currently thought unlikely that this problem will be seen in patients without inhibitors where FVIII concentrate is used, but these adverse events emphasize the potential complications that may arise due to different mechanisms of regulating the clotting process. How best to combine non-factor and factor therapies will likely remain an important issue requiring additional studies and widespread education amongst both clinicians and patients.

There were an additional four deaths reported recently. Three of these four cases were compassionate use requests for patients who had very serious or life-threatening conditions where every other treatment option has been exhausted. There is limited detail on all these cases as the treating clinicians want to ensure that the confidentiality of the patient is respected. However, the statement issued by Roche on March 18th, 2018, clarifies that in each of the four cases, the assessment of the treating clinician was that the cause of death was unrelated to Hemlibra®.

In April 2018, a patient with inhibitors, in the Phase III HAVEN 2 clinical trial, developed a neutralising anti-drug antibody to Hemlibra®. As with all therapeutic proteins, there is a potential for the development of anti-drug antibodies with Hemlibra®. The anti-drug antibody resulted in reduced efficacy of Hemlibra® and it was decided to discontinue treatment and the patient resumed his previous treatment. With more than 600 people treated, this is the first confirmed report of a detectable anti-drug antibody that has impacted efficacy. Monitoring for the development of anti-drug antibodies to Hemlibra® is ongoing.

A long-term extension study to evaluate the product is in Phase III trials and is expected in September 2020.

The other methods that are being investigated under the NRT category use a different approach. However, their concept is broadly similar. In normal clotting, there are factors that promote clotting, such as factor VIII or factor IX, and also molecules (anticoagulants) that prevent too much clotting (thrombosis). These two types are in a balance, which is disturbed when one type is missing, like FVIII or FIX in haemophilia. The idea of these treatments is to restore the balance at a lower level, by reducing the levels of the anticoagulants. The NRTs that are being investigated are the inhibitor of anti-thrombin (AT), tissue factor pathway inhibitor (TFPI) and activated protein C (aPC).

Fitusiran by (Sanofi Genzyme/Alnylam) is an anti-thrombin synthetic inhibitor RNA (siRNA), which is currently in Phase III trials for patients with haemophilia with and without inhibitors. Monthly subcutaneous injections of fitusiran reduce the levels of anti-thrombin to approximately 20% of normal levels and this reduction appears to be efficacious in preventing bleeding, with 33 patients (in Phase II open label extension study) experiencing median annualised bleeding rates (ABRs) of 1.0 and 48% experiencing zero bleeds.

However, in September 2017, all trials were suspended after a patient died. The patient had treated himself for a musculoskeletal injury with factor concentrate and then developed a headache. He was erroneously diagnosed with a bleed in the brain (subarachnoid haemorrhage) based on CT imaging and was treated with high intensity FVIII concentrate. He sadly passed away. After his death, expert review of the scans revealed he actually had a clot (cerebral venous sinus thrombosis), not a bleed. As a result, the clinical trial was suspended. In December 2017, the FDA agreed on the recommencement of the trial with alignment on new clinical risk mitigation measures, including protocol-specified guidelines and additional investigator and patient education concerning reduced doses of replacement factor or bypassing agents to treat any breakthrough bleeds in fitusiran studies.

There are three products using the anti-TFPI approaches: PF-06741086 from Pfizer and concizumab from Novo Nordisk, both of which are in Phase II clinical trials, and BAY 1093884 from Bayer in Phase I. Initially anti-TFPIs were considered for use in conjunction with bypassing agents for patients with inhibitors. However, results from Phase I studies demonstrated a reduction to levels of 20% of normal TFPI, which were associated with reduced clotting time and hence may be used to prophylactically treat patients with haemophilia with subcutaneous (and weekly/monthly) administration.

Another approach is inhibiting activated protein C (aPC). Targeting the anticoagulant effect of aPC has restored haemostasis in haemophilia mouse models in the pre-clinical phase and the company ApcinteX is expected to apply for Phase I trials for haemophilia in the near future.

With the significant potential in improvement in quality of life that NRTs can provide, come additional unknowns. NRTs do not prevent all bleeding and their impact on the coagulation cascade adds a great deal to the complexity in the management of bleeding events as well as to the condition of haemophilia itself. Treatment with concizumab was associated with elevated D-dimer levels (which are considered in patients with thrombotic disorders) although the clinical relevance of this observation is unknown. The experience with emicizumab and fitusiran should promote a level of caution about thrombotic consequences, especially when combining therapies.

There is an additional complexity associated with NRTs, in that current laboratory assays to measure the clotting factor levels may not be the most appropriate and additional work will need to be done in order to monitor these therapies in both comprehensive care centres and even more so outside specialist centres.

Gene therapy

For many years the hope for those with haemophilia without inhibitors was gene therapy, which would allow people to live a life free of infusions, bleeds and progressive joint damage. The hope for those with inhibitors was the same. While the non-inhibitor population may see this by 2023, it is unlikely that this will be the case for those with inhibitors as the current clinical trials exclude people with a history of an inhibitor for now.

There are some considerations in terms of the mechanism of action of inhibitors, and how they may be affected, that currently justifies the present exclusions from the trials. In the current trials, the gene therapy is administered and then there is an initial response where the FVIII or FIX levels increase. In those without inhibitors, the calculation of the amount of FVIII and FIX that is being produced is relatively simple: the gene is injected, the body produces factor to a certain level and it is measured. In some cases, the body then produces an immune response to the capsid, the envelope that the gene is delivered in. This response is detected by raised ALT levels in the liver. If this happens, then there is a reduction in the expression of the FVIII or FIX that is being produced, which again can be measured.

The measured response of the production of FVIII or FIX and the time it takes to be detected might be different in patients with an inhibitor. There is reason to hope that gene therapy may be an effective therapeutic option for people with inhibitors in the future. However, currently no clinical trials are ongoing for patients with a current inhibitor.

One of the first steps towards the inclusion of patients with inhibitors in clinical trials is the Sangamo trial, which is not excluding patients who had a transient inhibitor in childhood. The EHC very much look forward to presenting the first gene therapy in patients with inhibitors in the future in this section. If you would like to get more detail on the current trials, please see the other articles in this newsletter.

NOVEL TREATMENT IN VON WILLEBRANDS DISEASE

There have been several changes over the last few years in products containing von Willebrands Factor (vWF). These have been primarily focused on changing the ratio of vWF to FVIII. Products used to have a ratio close to 1 IU of vWF to 2 IU of FVIII (1:2). As patients with von Willebrand Disease (vWD) in most cases have normal FVIII levels, there are a number of considerations that need to be taken into account when treating people who have vWD with products that contain FVIII as well as von Willebrands Factor.

Wilate® from Octapharma is closer to 1 IU of von Willebrand Factor to 1 IU of FVIII. Voncento® from CSL Behring has a ratio of 2.4 IU of von Willebrands Factor to 1 IU of FVIII. LFB's Willfact®/Willfactin® is a highly purified VWF concentrate with a VWF/FVIII.

In December 2015, the first recombinant von Willebrand Factor product became available in the US, called Vonvendi® from Shire. This product has not yet received licencing in Europe, with trials in prophylaxis and paediatrics due to be completed in 2019 and 2020, respectively. A trial on women with heavy menstrual bleeding in Type 1 vWD patients is also on going and is expected to be completed by 2022. In Europe, the product will be submitted for licensing under the name of Veyvondi®.

PHARMACEUTICAL COMPANIES LANDSCAPE

There have been a significant number of changes in the haemophilia landscape over the last five years, not just in the number and variation of new products, but in the spinning off, mergers and acquisitions by companies, which has led to a lot of changes in company names. This is a brief article that summarises some of these changes in the companies mentioned in this newsletter. Whilst this is not very useful on an individual country level, as the company that supplies the products in that country will be based on the name that is registered, it may be useful when trying to search for information on products and trials that may be carried out by one of the companies in a partnership, or one where the names may have changed during development.

In 2015, Baxter spun off a new company called Baxalta, which had a heavy focus on haemophilia products. In June 2016, Baxalta then got purchased by Shire. Shire has received a bidby Takeda.

Swedish Orphan Biovitrum (Sobi) has been involved in the process of development and manufacturing of recombinant protein drugs since the technology was first developed around 30 years ago. In 2004, Biovitrum started to manufacture Wyeth's (now Pfizer's) ReFacto® and ReFacto AF/Xyntha®. Sobi partnered with Syntonix on the development of extended half-life products. Syntonix was subsequently acquired by Biogen Idec and in 2016 Biogen Idec spun off their haemophilia business into a company called Bioverativ. In terms of the partnership, Sobi and Bioverativ (a Sanofi company) collaborate on the development and commercialisation of Alprolix® and Elocta®/Eloctate®. Sobi has final development and commercialisation rights in the Sobi territory (essentially Europe, North Africa, Russia and most Middle Eastern markets). Bioverativ has final development and commercialisation rights in North America and all other regions in the world excluding the Sobi territory and has manufacturing responsibility for Elocta®/Eloctate® and Alprolix®.

In March 2018, Sanofi announced the acquisition of Bioverativ. Through that and two other transactions - the planned acquisition of Ablynx and the above-outlined agreement on fitusiran - they are building a franchise in the field of rare blood disorders. Chugai, the Japanese company that developed ACE910, which became emicizumab and is marketed as Hemlibra®, has a strategic alliance with Roche and Genentech. Roche will market Hemlibra® in Europe, Genentech will market it in the US and Chugai will market it in Japan.

In the gene therapy space, Spark Therapeutics haemophilia B gene therapy is being developed in collaboration with Pfizer. However, Spark Therapeutics retains global commercialisation rights for the haemophilia A gene therapy. Pfizer has partnered with Sangamo in haemophilia A. Sangamo will be responsible for conducting the SB-525 Phase I/II clinical study and certain manufacturing activities. Pfizer will be operationally and financially responsible for subsequent research, development, manufacturing and commercialisation activities for SB-525. Bayer has partnered with Ultragenix for haemophilia A gene therapy, and is scheduled to start Phase I study later this year (2018).

With so many new treatments, including gene therapy, the haemophilia landscape will undoubtedly change again over the coming months and years. Updates will be provided in this newsletter on a periodic basis.