Haemophilia A - Update 2025

Haemophilia A is a genetic disorder which leads to excessive bleeding after even minor injuries due to a production deficiency of clotting Factor VIII (FVIII). It is a X-chromosomal disease and affects males primarily, most females carry the malfunction silently. The prevalence of haemophilia A is 1 in 5000 males with increasing numbers worldwide. The most common clinical manifestation of severe haemophilia A are frequent joint haemorrhages leading to the development of arthropathies associated with chronic joint pain, disability and a reduced quality of life.

With the biotechnological progress in the 1990's, safe and effective recombinant replacement products became available allowing physicians to not only treat bleedings, but also to prevent them (prophylaxis). The burden of frequent (up to 4 times per week) factor administration via intravenous infusion including the risks of infections (due to catheter infection) and thrombotic complications is however significant. To improve the quality of life, new biotechnological products were developed. Fusing of coagulation factor proteins with proteins that have long circulating half-lives such as immunoglobulin or chemical pegylation, enabled to extend the circulating half-life of FVIII by 1.5 to 3.5-fold. With these extended half-life (EHL) products the burden of treatment could be reduced in most cases to just one or two infusions a week. However, the most problematic issue in the use of plasma-derived and recombinant FVIII products is the development of immunogenicity, i.e. the formation of FVIII allo-antibodies (also called "FVIII inhibitors") that neutralize FVIII function occurring in up to 30% of patients with severe haemophilia A. Bypassing agents such as recombinant activated FVII (rFVIIa) and plasma-derived activated prothrombin complex concentrate (aPCC) are used in such cases. The fastest and most efficient way to eliminate persistent inhibitors is however to induce tolerance to FVIII products by using immune tolerance induction which is effective in 60-80% of treated individuals with haemophilia A.

Recent advancements in haemophilia management have focused on the development of novel non-factor replacement therapies as an alternative to conventional replacement treatment or bypassing agents in patients with haemophilia A with or without inhibitors. Non-factor replacement therapies that have been approved so far include Emicizumab (Hemlibra®) and the anti-TFPI (Tissue Factor Pathway Inhibitor) monoclonal antibody Marstacimab (Hympavzi™). Several novel non-factor replacement drugs are currently in late phase of clinical development such as Mim8 (FVIIIa mimetic), Concizumab (anti-TFPI monoclonal antibody) and Fitusiran (RNAi therapy targeting antithrombin). Non-factor replacement products have a very long half-life permitting sustained activity levels even with weekly or monthly subcutaneous dosing.

Newest drug developments for haemophilia seek to combine a highly potent bleed protection with the lowest possible treatment burden to permit an almost normal life. With the introduction of gene therapy, this goal is getting closer, although there are still some hurdles to take. Valoctocogene roxaparvovec (using AAV5 gene transfer) is the *first* gene therapy for the treatment of haemophilia A, available since August 2022 in the European Union. Future innovative approaches for haemophilia include CRISP/Cas9 gene editing and tRNA-based protein editing, which could potentially cure this hereditary disease.

The **Charité Research Organisation GmbH** has profound experience in the conduction of early phase projects for new medicines to treat haemophilia A. We want to share our expertise in this Whitepaper.

Introduction

Genetic Background of Haemophilia

Haemophilia is a genetic disorder which leads to excessive bleeding after even minor injuries. This is due to a production deficiency of clotting factors either Factor VIII (FVIII, Haemophilia A) or Factor IX (FIX, Haemophilia B). Haemophilia A is more common and occurs in about 70-80% of haemophilia cases. It is a X-chromosomal disease and affects males primarily whereas females carry the malfunction silently [**Figure 1**].

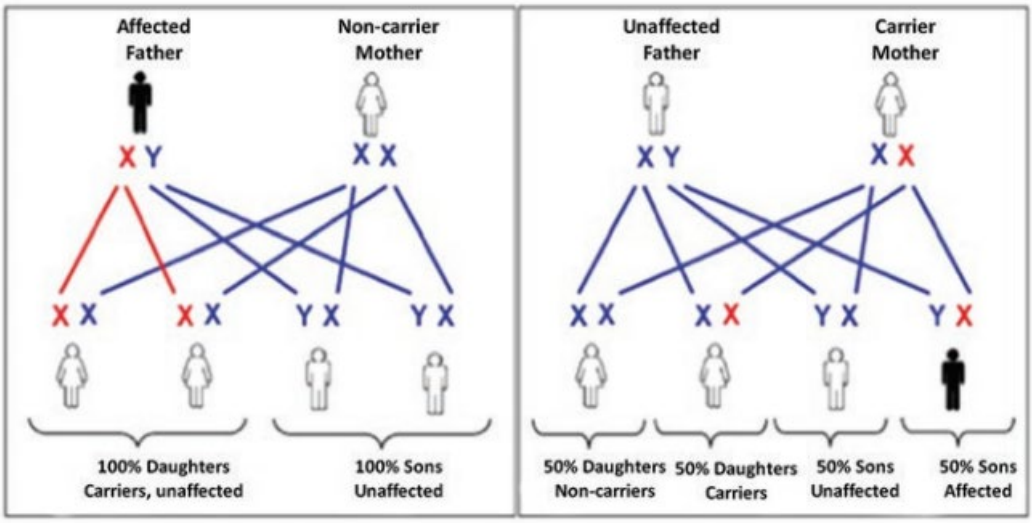

Figure 1. Haemophilia inheritance pattern.
In red affected X chromosome and unaffected chromosomes in blue.

Source: Figure adopted from [López-Arroyo et al., 2021].

But recent analyses of the ATHN dataset (the largest data set of haemophilia carriers in the world) showed that up to half of affected females either meet criteria for having haemophilia or report abnormal bleeding [Puetz & Cheng, 2021]. Importantly, the terms "carriers" or "symptomatic carriers" do not correctly reflect the genetic mutation status of the women and girls with clinically manifested haemophilia. The majority of female haemophilia patients are heterozygous but only those who have factor levels below the haemostatic range also meet the definitions used for haemophilia treatment. Therefore, it is appropriate to apply a classification based on factor level rather than genetic status for treatment purposes [Miller & Bean, 2021]. Homozygous, compound heterozygous or hemizygous females are rare and clearly have haemophilia as they do not have a normal allele.

First Descriptions of a Bleeding Disease

The first description occurred in the Talmud back in the 2nd century in a collection of Jewish laws, which allowed the exemption of boys from circumcision if two previous brothers had died from the procedure due to excessive bleeding. There was just an assumption of a family disease. In 1803, the American physician Dr. John Conrad Otto described an inheritable bleeding disorder in several families in which only males were affected, and in which transmission occurred via unaffected females [Otto, 1803]. Some years later, the German physician Johann Lukas Schönlein described similar observations also as bleeding disorder and in the dissertation of his student Friedrich Hopff the term "haemophilia" was cointed from the Greek heme 'blood' and filia 'affection' [Hopff, 1828].

In the 19th century, haemophilia gained the dubious reputation as „the disease of kings" due to a connection to the British royal family. Queen Victoria (1837–1901) was carrier of the hereditary

disease and her son Prince Leopold, Duke of Albany, and her daughters spread the disease across various European royal families. In 1896, her granddaughter “Princess Alix of Hesse and by Rhine” married Tsar Nicholas II of the Romanovs in Russia. They had five children, and their sole son, Tsarevich Alexei Nikolaevich, suffered from haemophilia. Today we know that the “royal disease” was in fact haemophilia B [Rogaev et al., 2009].

Evolution of Haemophilia Treatment Approaches

People with haemophilia are diagnosed at a very young age. According to a report from the Centers of Disease Control, the median age at diagnosis is 36 months for mild haemophilia, 8 months for moderate haemophilia, and 1 month for severe haemophilia [Kulkarni et al., 2009] and appropriate prophylaxis needs to start upon diagnosis to prevent bleedings and complications. The life expectancy of untreated haemophiliacs was still less than 20 years on average in the middle of the last century. The evolution of treatment approaches targeting haemophilia led to an increased life expectancy reaching nearly the same as that of normal people (**Figure 2**).

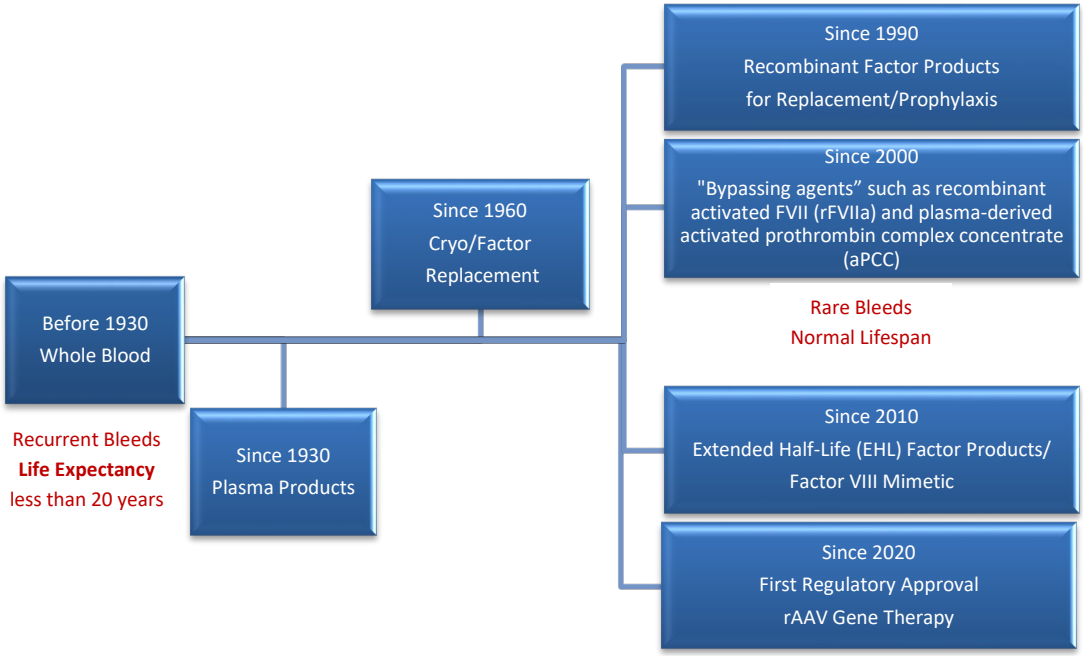

Figure 2. Evolution of treatment approaches targeting haemophilia

It was not until 1936, when Patek and Taylor identified a substance that could reduce the clotting time of haemophilic blood. About 10 years later, the same substance was identified as “antihaemophilic globulin” [Lewis et al., 1946]. In the 1950’s, the production of the first human FVIII preparations was started in Europe resulting in the introduction of a prophylaxis regimen (Malmö protocol) in Sweden. However, until the mid-1960’s haemophilia bleedings were still minimized by direct blood transfusion. In 1965, the discovery of a so called “cryoprecipitate” with FVIII activity in a slowly thawing frozen plasma revolutionized the treatment of haemophilia.

In 1968, the first commercial FVIII concentrates were developed that permitted direct substitution of clotting factors. In the 1980’s the first pasteurized FVIII concentrate Haemate® P was commercially available in Germany followed by the first highly purified pasteurized FVIII concentrate Beriate® P marketed in 1990. Shortly thereafter (1992), the first recombinant FVIII products were introduced. The

introduction of virus-inactivation technologies for blood derived products such as pasteurization (sufficient to inactivate both enveloped and non-enveloped viruses) was crucial to prevent a contamination by blood-borne viruses like the human immunodeficiency virus (HIV) or hepatitis B and C viruses [Schramm et al., 2014]. However, the most problematic issue in the use of plasma-derived and recombinant FVIII products is the development of immunogenicity, i.e. the formation of FVIII allo-antibodies (also called "FVIII inhibitors") that neutralize FVIII function occurring in up to 30% of patients with severe haemophilia A [Ho et al., 2000; Peyvandi et al., 2021]. To reduce the substantial morbidity and mortality associated with immunogenicity, immune tolerance induction (ITI) is also used to eliminate high-responding (anamnestic) "FVIII inhibitors" of recent onset and restore normal factor pharmacokinetics [Dimichele et al., 2007]. If "FVIII inhibitors" are present, the use of so called "bypassing agents" such as recombinant activated FVII (rFVIIa) and plasma-derived activated prothrombin complex concentrate (aPCC) was introduced as first-line treatment in these times [Chai-Adisaksopha et al., 2017].

Widespread availability of safe and effective recombinant replacement products allowed physicians to not only treat bleedings, but also prevent them (prophylaxis) and became soon standard of care. As coagulation factor products must be given via intravenous infusions, this is problematic for young children. In addition, the burden of frequent (up to 2-4 times per week) factor administration including the risks of infections (due to venous catheter) and thrombotic complications was significant. To improve the quality of life, new biotechnological products were developed [Mannucci, 2023]: Fusing of coagulation factor proteins with proteins that have long circulating half-lives such as immunoglobulin or chemical pegylation, enabled to extend the circulating half-life of FVIII by 1.5-fold and FIX by 4- to 6-fold. With these extended half-life (EHL) products the burden of treatment could be reduced in most cases to just one infusion a week [**Figure 2**]. The most revolutionary development in haemophilia care was the development of a Factor VIII mimetic, the bispecific antibody Emicizumab that binds to both Factor IX and Factor X. Emicizumab can be given subcutaneously as infrequently as once monthly. In addition, Emicizumab significantly reduced the occurrence of breakthrough bleeds. Since recently, a novel FVIII mimetic (called INNO8) designed for *oral* administration entered in clinical development for bleed prevention and long-term prophylaxis in patients with haemophilia A, irrespective of inhibitor status or severity [Lund et al., 2024].

The ultimate goal in haemophilia therapy is to normalize factor levels and never take medication again. Today this can be approached with gene therapy [**Figure 2**]. Gene therapy targets the genetic malfunction of haemophilia directly. In fact, recent gene therapeutics using recombinant adeno-associated virus (rAAV) vectors may achieve normalization of coagulation factor levels for a long period in a patient's life by one-time administration. However, newest data show a decline in the efficiency of rAAV-gene therapeutics starting already after 2 years of administration [Puetz, 2024]. In addition, some safety concerns such as drug induced liver toxicity or development of hepatocellular carcinoma were seen in preclinical animal models and need still to be evaluated in humans in the long-term [Dawaldi et al., 2021; Nguyen et al., 2021; Kapelanski-Lamoureux et al., 2022].

Haemophilia at Advanced Age

Reaching an advanced age with haemophilia means to face problems as their non-haemophilic peers in the general population. One recent study assessed the prevalence of comorbid conditions in more than 2,200 older males with haemophilia receiving care in haemophilia treatment centres in the United States from 2013 to 2021 [Soucie et al., 2022]. They reported that compared with the normal male population, men with haemophilia had a lower prevalence of coronary artery disease, stroke, heart attack, and leukaemia, but a higher prevalence of anxiety, depression, and obesity. Additionally, nearly 3 in 4 men with haemophilia had a history of prior hepatitis C virus (HCV) infection, and about 1 in 4 had a history of human immunodeficiency virus (HIV) infection. Men with haemophilia who had a history of HIV or HCV infection had a higher prevalence of chronic kidney disease, liver cancer, anxiety, and depression than those without infections. Importantly, a higher thrombotic risk seems to be associated with high factor levels or some non-factor therapies and/or co-morbidities such as cardiovascular diseases. The management of bleedings, bleeding-related pain and thrombosis in older haemophiliacs with multiple comorbidities requires an individual, multi-disciplinary approach to support and optimise long-term physical functioning and overall quality of life [Makris et al., 2024].

Haemophilia Patient Numbers

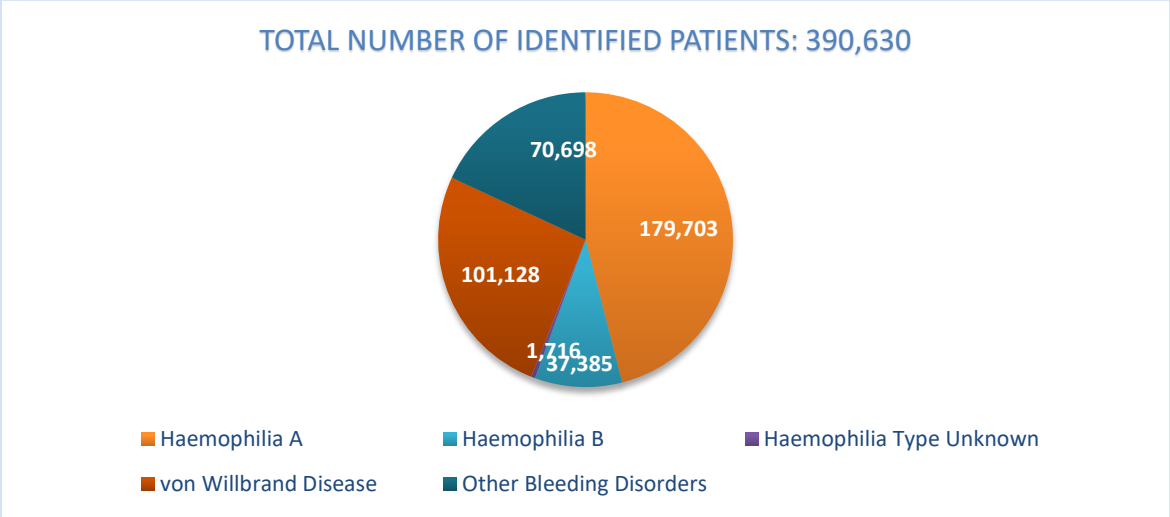

Figure 3. Global Numbers of Patients with Bleeding Disorders (2023)
Source: Data based on [The World Federation of Haemophilia Report on the Annual Global Survey 2023]

Worldwide, the prevalence of haemophilia is 1 in 5000 males for haemophilia A and 1 in 30,000 males for haemophilia B with increasing numbers worldwide (**Figure 3**). An accurate and early diagnosis of haemophilia enables access to treatment and care. The probability of identification of this rare bleeding disorder is however still dependent on the economic standard of the corresponding country. The prevalence of haemophilia varies worldwide, with close to 100% of patients diagnosed in high-income countries and as low as 12% diagnosed in lower-income countries [Coffin et al., 2023]. With the 2nd revision of the 'Guideline on the clinical investigation of recombinant and human plasma-derived FVIII products' [EMA, 2019], it is requested to include all patients with haemophilia in specific haemophilia registries, which may also support post-authorisation studies. In addition, a harmonization of existing national and European haemophilia registries by using a compatible core dataset was required. Germany has a national haemophilia registry supervised by the Paul-Ehrlich-Institut (PEI). In 2023, around 4477 patients with haemophilia A and 858 cases with haemophilia B have been recorded in the German Haemophilia Registry [DHR-Deutsches Hämophilieregister, 2023].

Haemophilia A Severity Grades

According to the WFH Guidelines for the management of haemophilia, the severity of bleeding manifestations in haemophilia generally correlates with the degree of the clotting factor deficiency [Scrivastava et al., 2020] (**Table 1**). The most common clinical manifestation of severe haemophilia A are frequent joint haemorrhages leading to the development of arthropathies associated with chronic joint pain, disability and a reduced quality of life.

Severity	Clotting Factor Level	Bleeding Episodes
Severe	<1 IU/dL (<0.01 IU/mL) or <1% of normal	Spontaneous bleeding into joints or muscles, predominantly in the absence of identifiable haemostatic challenge
Moderate	1- 5 IU/dL (0.01- 0.05 IU/mL) or 1- 5% of normal	Occasional spontaneous bleeding; prolonged bleeding with minor trauma or surgery
Mild	5- 40 IU/dL (0.05- 0.40 IU/mL) or 5- <40% of normal	Severe bleeding with major trauma or surgery; rare spontaneous bleeding

Table 1. Relationship of bleeding severity to plasma clotting factor level
Source: [Srivastava et al., WFH Guidelines for the management of haemophilia 2020]

Current Haemophilia A Management

The primary goal of haemophilia A therapy is the prevention of bleeding (prophylactic care) by replacing the missing blood clotting FVIII via intravenous infusion of commercially available clotting factor concentrates or other haemostasis products [Scrivastava et al., 2020]. The prophylaxis therapy in severe haemophilia needs to be started early in life (before age 3) to prevent musculoskeletal complications from recurrent joint and muscle bleeds. Episodic clotting factor replacement therapy should no longer be considered to be a long-term treatment option, but is still required as an "on demand" treatment option. Usually, infusions of clotting factor concentrates are performed by people with haemophilia themselves or their caregivers in a home treatment setting. As a rule, treatment of haemophilia patients should be managed by specially qualified centers such as the Haemophilia Comprehensive Care Centre (HCCC) and by a Haemophilia Treatment Centre (HTC), which, in collaboration with an HCCC, provides treatment close to home. Adherence to these treatment regimens reduces complications such as intracranial bleedings or joint bleeds and, may increase the life expectancy to almost the same level as that of healthy people and to integration of haemophilia patients into a normal social life.

Traditional FVIII Replacement Products

The main types of coagulation factor concentrates are industrially manufactured human plasma-derived FVIII concentrates (pd-FVIII) and standard half-life recombinant FVIII (SHL-rFVIII) concentrates which must fulfill the requirements for pharmaceutical Good Manufacturing Practice (GMP). The WFH regularly publishes an update of registered clotting factor concentrates including the latest one [WFH Online Registry of Clotting Factor Concentrates (2020)], which lists all currently available products and their manufacturing details. A short overview of these replacement therapies which require frequent infusions up to three times a week to warrant plasma trough FVIII plasma levels of at least 1% for bleeding prophylaxis is given in **Table 2**.

Products should be selected based on the key requirements including product safety (including quality, purity, and viral inactivation) and efficacy. For the plasma-derived factor concentrates, plasma is collected from healthy people, and is separated into components, such as clotting factors. The commercially available products are usually freeze-dried and treated to kill any potential viruses before it is packaged for use. In contrast, the recombinant FVIII concentrates are genetically engineered using DNA technology. Therefore, recombinant FVIII concentrates do not contain any plasma or albumin, and therefore, cannot spread any bloodborne viruses.

Traditional FVIII replacement products (Marketed Products)	FVIII status	Plasma half-life in adults
Human Plasma Derived FVIII (Pd-FVIII)		
Antihaemophilic factor (Hemofil M®, Koate® DVI)	Human full-length	14.5–17.4 h
Antihemophilic factor/ von Willebrand factor (VWF) complex (Alphanate®, Wilate®)	Human full-length FVIII and VWF complex	12.2–17.9 h
Standard Half-Life Recombinant FVIII (SHL-rFVIII)		
Octocog alfa (Advate®, Kogenate® FS, Kovaltry®)	Full-length recombinant	12.16 – 14.2 h
Moroctocog alfa AF-CC (ReFacto® AF)	B-domain deleted–FVIII	13.8 h
Turoctocog alfa (NovoEight®)	B-domain truncated-FVIII	10.3 h
Simoctocog alfa (Nuwiq®)	B-domain deleted	14.7 h
Lonoctocog alfa (Afstyla®)	B-domain truncated single chain FVIII	14.2 h

Table 2. Overview of available Traditional FVIII replacement products

Source: Based on data reported in [Abdelgawad et al., 2024]

In 2024, the International Society on Thrombosis and Haemostasis (ISTH) Haemophilia Guideline Panel issued a conditional recommendation suggesting prophylaxis with plasma-derived FVIII over standard half-life recombinant FVIII for previously untreated individuals with severe haemophilia A who will start prophylaxis with a standard half-life recombinant FVIII concentrate. A shared decision making should consider concentrate availability, costs, and patient preference [Rezende et al., 2024].

Haemophilia A therapy has become a *highly individualised treatment*. It is important to consider the pharmacokinetics of the coagulation factor concentrates administered to each individual patient, e.g. clearance, volume of distribution and half-life (**Figure 4**).

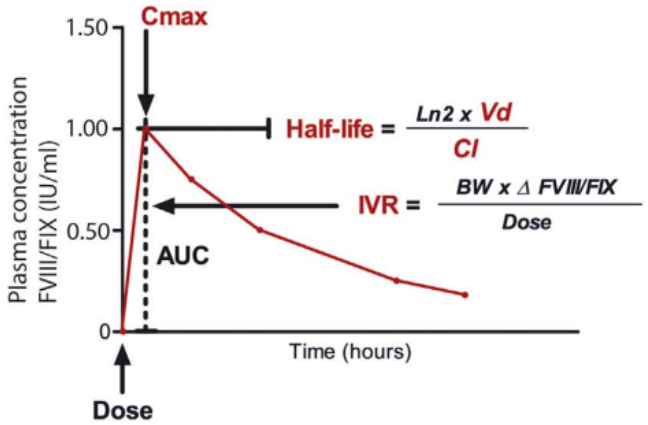

Figure 4. Description of pharmacokinetic (PK) parameters using non-compartimental model

After bolus infusion of clotting factor concentrate, FVIII (or FIX in case of haemophilia B) plasma levels increase until the maximum concentration is reached (Cmax). The in vivo recovery (IVR) is calculated by body weight (BW) (kilograms) x observed increase in FVIII/FIX plasma levels divided by the dose. Half-life is derived from the clearance (Cl) and volume of distribution (Vd) and is defined as the time required for the concentration to halve. Finally, area under de curve (AUC) is the integral of the concentration-time curve.

Source: Figure adopted from Hazendonk et al., 2018

Application of these PK parameters results in a precise estimate of peak FVIII and enables calculation of FVIII concentrations and the formulation of recommendations on frequency and timing of dosing of FVIII concentrates. While prophylaxis with SHL-rFVIII products is considered the gold standard for severe haemophilia A, treatment for the moderate form is often given as an 'on-demand' therapy. In addition, prophylactic therapy with FVIII concentrates can be optimised by individualised pharmacokinetic monitoring consisting of a close peak factor level assessment (within 15-30 minutes after infusion) to verify the calculated dose and, complete PK (10-11 blood samples over a period of 32-96 hours), or with limited sampling in combination with population PK estimates [Scrivastava et al., 2020].

Extended Half-Life-rFVIII-Products in Europe

Traditional standard-half-life (SHL) rFVIII products require frequent infusions up to three times a week to sustain plasma trough FVIII levels (≥ 1%) required for bleeding prophylaxis. But many patients still remain susceptible to spontaneous bleeding at this threshold. In the last decade, a new generation of recombinant FVIII (rFVIII) concentrates with extended half-life (EHL) were developed for bleeding prophylaxis to aim for trough plasma FVIII levels of >3 to 5% or higher to further reduce the risk of bleeding [Scrivastava et al., WFH Guidelines for the management of haemophilia 2020]. Fusion technologies and PEGylation have been proven as successful half-life extension strategies to sustain efficient trough plasma FVIII levels and to reduce the frequency of administration and potentially improving patient compliance. The newly developed EHL-rFVIII products have half-lives ranging from 14 up to 43 hours, which is a breakthrough compared to the SHL-rFVIII products with half-lives from 8 to maximal 12 hours (**Table 3**).

EHL-rFVIII product (Marketed Product)	**Technology used for half-life prolongation**	**Route and frequency of administration**	**Plasma half-life in adults**
Damoctocog alfa pegol (Jivi®)	PEGylation (60 kDa) of rFVIII	Intravenous; 2 times per week	19 hours
Efmoroctocog alfa (Elocta®)	Fusion protein of rFVIII and Fc-region of IgG	Intravenous; every 3 to 5 days	19 hours
Rurioctocog alfa pegol (Adynovi®)	PEGylation (20 kDa) of rFVIII	Intravenous; 2 times per week	14-16 hours
Turoctocog alfa pegol (Esperoct®)	Glyco-PEGylation (40 kDa-PEG molecule)	Intravenous; every 3 to 5 days	18-19 hours
Efanesoctocog alfa (Altuviiio®) (Altuvoct®)	Fusion to Fc-VWF-XTEN polypeptides	Intravenous; Once weekly	43 hours

Table 3. Authorised FVIII products with an extended half-life for haemophilia A

EHL= Extended Half-Life, rFVIII= recombinant Factor VIII, VWF= von Willebrand Factor,
Source: Based on data reported in [Andrade et al., 2024]

The most innovative EHL-product is Efanesoctocog alfa, a recombinant [Antihaemophilic Factor, Fc-VWF-XTEN] fusion protein that is a *first-in-class* high-sustained FVIII therapy enabling treatment and prevention of bleeds (including perioperative prophylaxis) with once-weekly prophylactic dosing for adults and children with haemophilia A. Using Fc fusion technology by adding a region of von Willebrand factor and XTEN polypeptides, it has been shown to break through the '*von Willebrand factor ceiling*' (a known half-life limitation on other factor VIII therapies) [Chhabra et al., 2020]. It is marketed as ALTUVIIIO® in the US, Taiwan, and Japan and as Altuvoct® in Europe.

Formation of Factor VIII Inhibitors

Unfortunately, up to 30% of patients with severe haemophilia A who infuse with clotting FVIII concentrates develop inhibitors (FVIII allo-antibodies) that neutralize FVIII function [Van den Berg et al., 2019; Peyvandi et al., 2021]. The immunological mechanism of inhibitor formation is still a field of intensive research. Due to a largely reduced or missing FVIII expression, patients with haemophilia A are not able to eliminate high-affinity FVIII-reactive T-cells. When these patients are treated with FVIII products, these T-Cells will migrate to the spleen where they induce formation of FVIII allo-antibodies by B cells and/or plasma cells [Barg et al., 2018; Delignat et al., 2018].

The erroneous immune responses are initiated when dendritic cells (antigen-presenting cells, APCs) encounter the 'unfamiliar' replacement factor molecule and present it via HLA class II, marking it for elimination. Co-stimulatory signals via cell surface molecules such as Cluster of Differentiation 40 (CD40), or CD80/86 (which are found in dendritic cells and required for T cell survival), or ICOS ("inducible co-stimulator"), and secretion of pro-inflammatory cytokines are required for T cell activation. Activated $CD4^+$ T helper cells migrate to the spleen where they induce B cell activation and differentiation into memory B cells or the formation of antibody producing plasma cells, which neutralize the infused clotting factor. T regulatory cells (Treg) are potent modulators of adaptive immune responses and can suppress activation of $CD4^+$ T helper cells. Conditions that lead to CD4+ T cell anergy (unresponsiveness) or their deletion also contribute to the prevention of the immune response. Furthermore, Treg may directly suppress activated B cells from differentiating into memory B cells and/or directly suppress inhibitor-producing plasma cells [Sherman et al., 2018] (**Figure 5**).

In addition, infections or surgical interventions are supposed to trigger activation of the complement system thereby enhancing adaptive immune responses (proliferation of autologous CD4+ T helper cells) towards plasmatic FVIII products [Ringler et al., 2023].

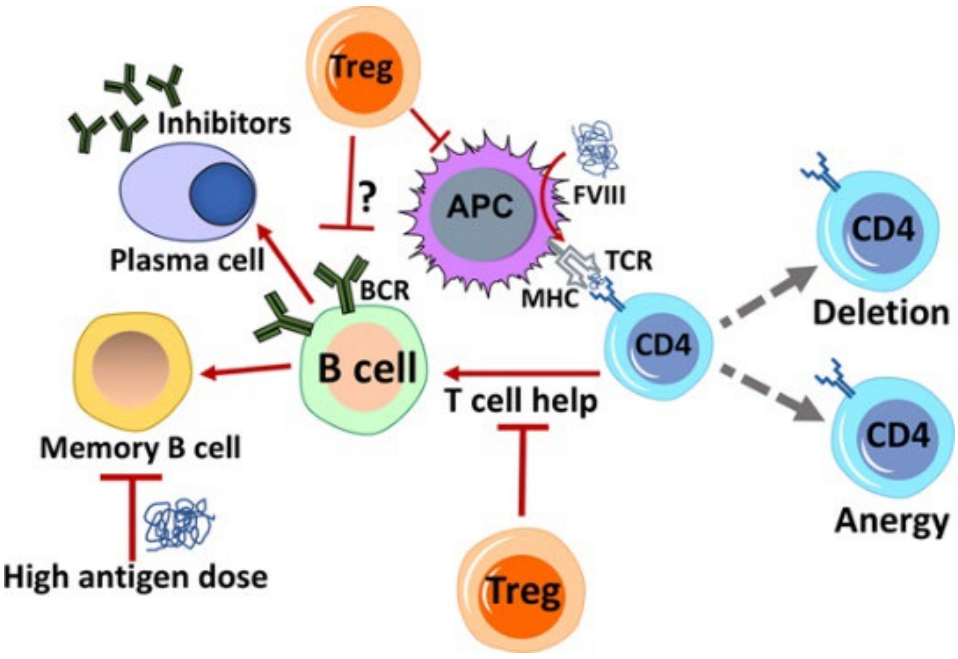

Figure 5. Induction of inhibitor formation through enhanced adaptive immune responses

Source: Figure adopted from [Sherman et al., 2018]

Bypassing Agents

In any patient who fails to respond to adequate FVIII replacement therapy after past responsiveness, the inhibitor titre should be assessed. Inhibitor testing should also be performed before major surgery and if there is suboptimal response to FVIII replacement therapy in the post-operative period. The inhibitor titre is measured by the Bethesda assay or the Nijmegen-modified Bethesda assay [Scrivastava et al., 2020]. The definition of a positive inhibitor for FVIII is a Bethesda titre of >0.6 Bethesda units (BU). For patients with FVIII inhibitors who develop an acute bleed, the treatment should be based on the individual inhibitor titre. In the presence of 'low-titre inhibitors' (inhibitor <5.0 BU), the administration of high dosages of rFVIII products with close monitoring of FVIII activity may be considered. But in case of 'high-titre inhibitors' (inhibitor ≥5.0 BU) these will become ineffective. In this case, so-called bypassing agents (BPA) such as recombinant activated FVII (rFVIIa) and plasma-derived activated prothrombin complex concentrate (aPCC) are recommended to promote clotting. Traditional FDA and EMA licensed BPA are Eptacog alfa® (rFVIIa activates FX to FXa and FIX to FIXa), Eptacog beta® (rFVIIa activates FX to FXa) and FEIBA NF® consisting of multiple coagulation factors (FII, FIX, FX, and FVIIa).

Immune Tolerance Induction

The fastest and most efficient way to eliminate persistent inhibitors is however to induce tolerance to FVIII products by using immune tolerance induction (ITI) which is effective in 60-80% of treated patients with haemophilia A. The frequent administration of high FVIII (antigen) doses directly inhibits memory B cells, leading to down-regulation of inhibitor formation [Jardim et al., 2020; Scrivastava et al., 2020]. Successful ITI is defined as a persistently negative Bethesda titre, accompanied by normal pharmacokinetics, including factor recovery >66% and half-life >6 hours for standard half-life FVIII concentrates. Successful ITI is usually achieved after 2-3 years. Thereafter, FVIII prophylaxis may be initiated or resumed. In case patients experience frequent bleeding during ITI, prophylaxis with BPA products (rFVIIa, aPCC) or non-factor replacement therapy (Emicizumab) may be instituted [Scrivastava et al., 2020].

Non-Factor Replacement Therapies in Haemophilia

Recent advancements in haemophilia management have focused on the development of novel non-factor replacement therapies as an alternative to conventional replacement treatment or bypassing agents in patients with haemophilia A with or without inhibitors with the aim to improve patient outcomes and quality of life [Gualtierotti et al., 2022]. Normal haemostasis depends on a fine-tuned balance between procoagulant and anticoagulant factors. In haemophilia A, the reduction in FVIII leads to a defective thrombin (FIIa) formation resulting in frequent bleedings. The most promising approaches to rebalance the haemostatic system are therefore either to increase thrombin formation by inhibition of physiological anticoagulant factors (such as antithrombin III (ATIII), activated protein C (APC), and tissue factor pathway inhibitor (TFPI)) or by mimicking FVIIIa-activity (FVIIIa Mimetics) (**Figure 6**).

Due to their novel mechanism of action, non-replacement drugs reach stable plasma concentrations at a steady state, facilitating laboratory monitoring to ensure efficacy and safety. Finally, as haemostasis and coagulation are fine-tuned and very complex systems, an excess in thrombin generation may lead to thromboembolic events, which have been reported, although rarely.

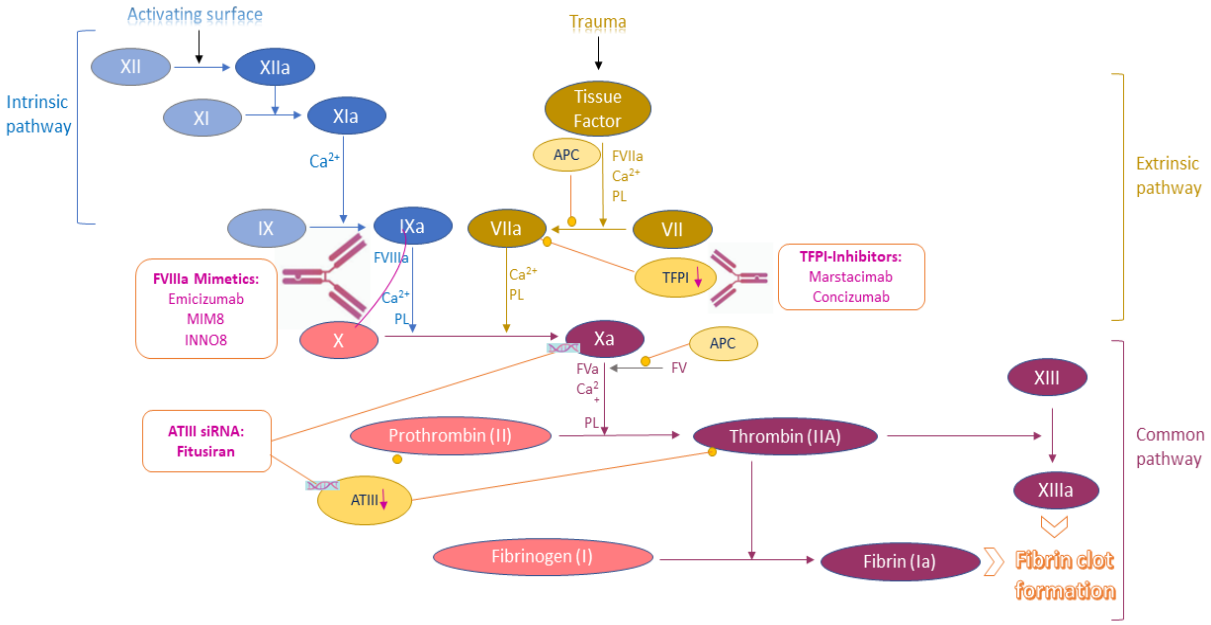

Figure 6. Mechanism of action of approved and novel non-replacement treatments.

APC = activated protein C, TFPI = TF pathway inhibitor

Source: Created with BioRender

Non-factor replacement therapies that have been approved so far in the EU include **Emicizumab** (Hemlibra®, Genentech. Inc./Roche), and the anti-TFPI (Tissue Factor Pathway Inhibitor) monoclonal antibodies **Marstacimab** (Hympavzi™, Pfizer) and **Concizumab** (Alhemo®, Novo Nordisk). Several novel non-factor replacement drugs are currently in late phase of clinical development such as Mim 8 (FVIIIa mimetic) and Fitusiran (RNAi therapy targeting antithrombin). Non-factor replacement products have a very long half-life permitting sustained activity levels even with weekly or monthly subcutaneous dosing (**Table 4**) (**Figure 6**).

Product	Mechanism of action	Route and frequency of administration	Plasma half life
Marketed Products			
Emicizumab (Hemlibra®, Genentech. Inc./Roche)	Bi-specific FVIIIa-mimetic monoclonal antibody; binds FIXa and FX to activate FX (FXa)	Subcutaneous; Every 1, 2 or 4 weeks	30 days
Marstacimab (Hympavzi™, Pfizer)	Anti-TFPI monoclonal antibody (IgG1); increases production of FXa	Subcutaneous; Once weekly	33-65 hours
Concizumab (Alhemo®, Novo Nordisk)	Anti-TFPI monoclonal antibody (IgG4); increases production of FXa	Subcutaneous; Daily	38 hours
Late Phase Investigational Products			
Mim 8 (Novo Nordisk)	Bi-specific FVIIIa-mimetic monoclonal antibody; binds FIXa and FX to activate FX (FXa); supposed to be higher potent in thrombin production than Emicizumab	Subcutaneous; once-weekly or once-monthly prophylaxis	1 month
Fitusiran (Genzyme/Alnylam)	siRNA targeting antithrombin; increases production of thrombin (FIIa)	Subcutaneous; Monthly	3-5 hours

Table 4. Authorised Non-Factor Products

TFPI =Tissue Factor Pathway Inhibitor

Source: Andrade et al., 2024, EPAR Alhelmo (Concizumab)

Emicizumab

Emicizumab (Hemlibra®, Genetech. Inc./Roche) has received FDA (2017) and EMA (2018) approval for the treatment of patients with moderate or severe haemophilia A with and without FVIII inhibitors. Since April 2018 it is available on the German market.

Emicizumab is a recombinant bispecific monoclonal antibody that mimics the function of FVIIIa through a connection of FIX and X. Emicizumab takes over the function of FVIIIa and binds with one arm to FIXa and with the other arm to its substrate FX, thereby initiating the FIXa-mediated activation of FX with formation of the (intrinsic) tenase complex thereby amplifying thrombin generation (thrombin burst). In this way, the blood coagulation cascade can also work without FVIII. The active substance is not affected by inhibitors against FVIII (**Figure 7**).

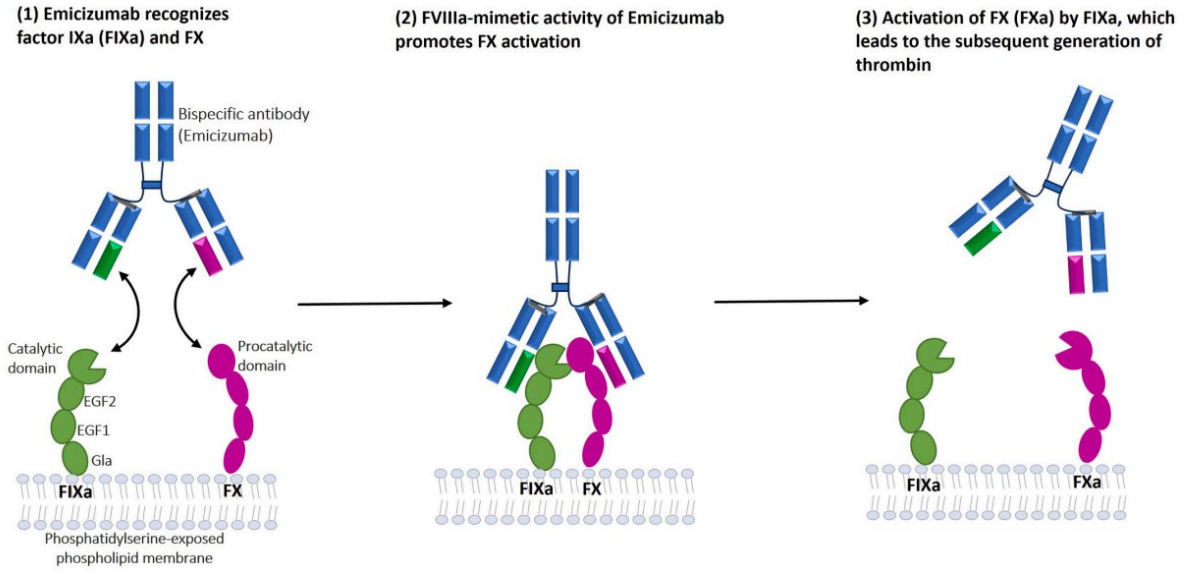

Figure 7. Mechanism of action Emacizumab

The monoclonal antibody Emicizumab bridges activated Factor IXa (FIXa) and Factor X (FX). This bridging action is central to Emicizumab function (FVIIIa-mimetic activity) in facilitating the coagulation cascade and promoting the subsequent generation of thrombin. FXa, in conjunction with other factors, catalyses the transformation of prothrombin to thrombin. This subsequent conversion triggers the creation of a haemostatic plug by converting fibrinogen into fibrin, initiating localized haemostasis. This event can also take place on the activated platelet surfaces.
Source: Figure adopted from [Abdelgawad et al., 2024]

Emicizumab has been approved based on the positive results of the '*HAVEN program*' including 6 clinical phase 3 trials [Andrade et al., 2024]):

- In the HAVEN 1 and 2 trials it has overcome its first challenge in the treatment of haemophilia patients with inhibitors. In the HAVEN 1 trial, Emicizumab prophylaxis compared to no prophylaxis reduced the annual bleeding rate (ABR) by 87 % in male participants aged at least 12 years. In part C of the HAVEN 1 trial, Emicizumab prophylaxis resulted in an ABR that was significantly lower by 79% than the ABR with previous bypassing-agent prophylaxis in a small population of 24 adults and adolescents of at least 12 years [Oldenburg et al., 2017]. In the HAVEN 2 trial Emicizumab showed positive effects in children with less than 12 years of age in various doses and dosing regimens. In a small subgroup of 15 participants previously taking bypassing-agent prophylaxis it reduced the ABR by 99% [Young et al., 2019].
- The HAVEN 3 and HAVEN 4 trials investigated haemophilia A patients without FVIII inhibitors. HAVEN 3 compared patients without prophylaxis against patients with different dosing regimens of Emicizumab which reduced the bleeding rate at least 96% [Mahlangu et al., 2018].

- The HAVEN 5 trial investigated different dose regimens in adult and adolescent patients of the Asia-Pacific region.
- The HAVEN 6 trial showed positive effects in patients with non-severe haemophilia without inhibitors as well.

In a pooled analysis of HAVEN 1-4, the most common adverse event was injection-site reaction (26.8%), and the model-based ABR for treated bleeds across the study period was 1.4 (95% confidence interval [CI], 1.1–1.7). During Weeks 121–144, 82.4% of participants had zero treated bleeds [Callaghan et al., 2021]. No major or minor surgery in the HAVEN 1-4 patient population resulted in death, thrombosis, new FVIII inhibitor development, or unexpected bleed [Santagostino et al., 2023].

However, thrombotic events (TEs) and thrombotic microangiopathies (TMAs) have been identified as risks when taking Emicizumab alongside activated prothrombin complex concentrate (aPCC) at doses of >100U/kg/24 hours for ≥24 hours. Subsequently, TEs and TMAs have been monitored on an ongoing basis in all haemophilia A patients receiving Emicizumab, with or without concomitant use of aPCC, and routine risk minimisation activities have been included in the label.

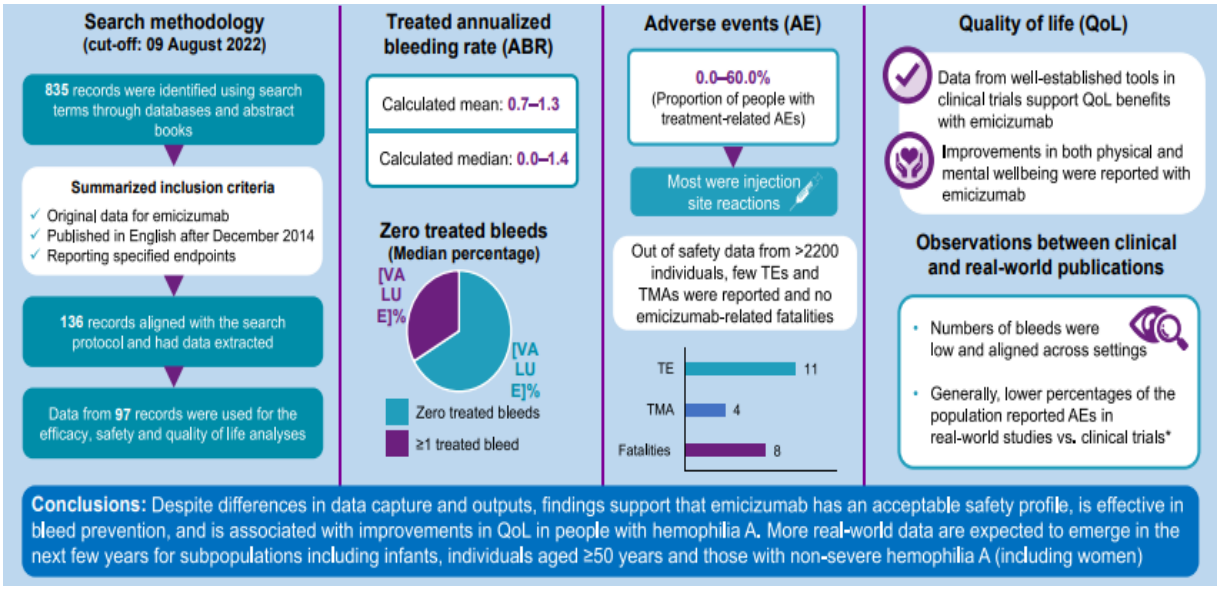

Figure 8. Clinical and real-world data for Emicizumab use in people with congenital haemophilia A regardless of age, disease severity or FVIII inhibitor status.
Source: Figure adopted from [Young et al., 2024]

Long-term safety data for Emicizumab have been analysed by searching the '*Roche Global Safety Database'* for TEs and TMAs from June 2012 to August 2023 [Sarouei et al., 2024]: A total of 97 events were identified in people with haemophilia A, of which 2 TEs and 5 TMAs were associated with concomitant aPCC use, and 90 TEs were not associated with aPCC. Most TEs (67.9%) were associated with pre-existing cardiovascular risk factors and/or risk factors for thrombosis. All 5 TMAs were associated with concomitant use of aPCC at >100U/kg/24 hours for ≥24 hours. This analysis continues to support that TEs and TMAs without concomitant aPCC at doses of >100U/kg/24 hours for ≥24 hours are not an identified risk for people with haemophilia A receiving Emicizumab prophylaxis.

Furthermore, long-term data based on clinical and real-world data published from December 2014 until August 2022 show that Emicizumab prophylaxis is well tolerated and effective in people with congenital haemophilia A regardless of age, severity of disease, or inhibitor status [Young et al., 2024] (**Figure 8**). These data support an acceptable safety profile, bleed prevention, and improved quality of

life with Emicizumab. Therefore, health authorities no longer require special expedited safety reporting for Emicizumab worldwide. However, monitoring and reporting of safety data are still ongoing.

Marstacimab

A further step forward in the management of haemophilia represents the recent FDA approval (October, 2024) of Marstacimab (Hympavzi™, Pfizer) as non-factor replacement, which can be administered as once-weekly (subcutaneous) prophylaxis for patients aged 12 years and older with haemophilia A or B *without* inhibitors. Marstacimab is a human monoclonal IgG1 antibody targeting the Kunitz 2 domain of Tissue Factor Pathway Inhibitor (TFPI), a protein that naturally limits blood clot formation [Malanghu et al., 2023]. By reducing the activity of TFPI, Marstacimab increases thrombin generation and thereby clot formation (**Figure 6**).

Its approval is based on data from the Phase 3 '*BASIS*' trial involving 116 male adult and pediatric patients with severe haemophilia A or B *without* inhibitors. In patients previously treated with on-demand factor replacement therapy, the estimated annualized bleeding rate (ABR) dropped from 38 to 3.2 during Marstacimab treatment. A similar bleeding rate reduction was observed in patients switching from previous prophylactic factor replacement therapy to Marstacimab, with an ABR of 5.08 on Marstacimab compared with 7.85 during factor replacement. The most common side effects observed under Marstacimab treatment were injection site reactions, headache, and pruritus. Marstacimab is associated with a higher risk of blood clots. Safety warnings have been placed regarding the potential for thromboembolic events, hypersensitivity reactions, and embryofetal toxicity [US FDA new approval, October 2024].

Concizumab

With the recent EMA approval (December, 2024) of Concizumab (Alhemo®, Novo Nordisk), a new non-factor replacement therapy entered the EU market. Concizumab is a human monoclonal IgG4 antibody which inhibits TFPI activity through high-affinity binding to the TFPI Kunitz-2 domain, blocking TFPI binding to active Factor X (and thereby preventing its inhibition) and maintaining Factor Xa production by the Tissue Factor-Factor VIIa complex. These activities normalize thrombin generation and result in a reduction in the number of bleeding episodes. (**Figure 6**).

Concizumab can be administered as once-daily (subcutaneous) prophylaxis for patients aged 12 years and older with haemophilia A or B *with* inhibitors. Treatment (using a pre-filled pen) begins with a starting dose based on the patient's body weight for the first day. A lower dose is given from the second day. After 4 weeks of treatment, the doctor can adjust the dose based on blood levels of Concizumab. Patients can inject themselves if they have been trained appropriately.

The pivotal phase 3 study '*Explorer7*' (NCT04083781), conducted in 127 men and boys, aged 12 years or older, with haemophilia A or B with inhibitors, found that Concizumab is effective at reducing the annual bleeding rate (ABR). Among the 33 patients included in the ABR analysis, who received preventive treatment with Concizumab, the ABR decreased to 1.7, compared with 11.8 in patients who did not receive preventive treatment (but instead received on demand treatment with intravenous replacement with clotting factor-containing products). Patients receiving Concizumab also had a slight improvement in pain and physical functioning based on a standardised scoring system (called SF-36v2) [Matsushita et al., 2024; EPAR, Alhemo, December 2024].

Gene Therapy for Haemophilia A

Haemophilia is an ideal entry of researchers into gene therapy. As it is a *monogenetic* X-chromosomal recessive disease, introduction of a functional copy of the F8gene (called F8 transgene) is expected to warrant a stable increase in FVIII plasma level leading directly to the reduction of bleeding events. This may finally reduce or eliminate the need for regular factor replacement therapy.

Adeno-associated viral (AAV) vectors with serotype 5 (AAV5) used for FVIII gene transfer are liver directed where the factor protein synthesis is initiated and the transgene product is finally released into the circulation. There are however some hurdles to overcome such as [Monahan et a., 2021] (**Figure 9**):

(1) *Vector immunogenicity*: The presence of neutralizing antibodies against the AAV capsid can prevent or limit cell transduction, whereas cytotoxic CD8+ T-cell responses can eliminate AAV-transduced cells that present AAV capsid antigens loaded on MHC-I complexes. A careful patient selection is therefore important to assure a successful treatment. Adeno virus infections occur naturally in men and therefore the immune system may have developed neutralizing antibodies. About 3 to 59 % of the population show antibodies to different AAV serotypes, and the lack of a standardized methodology for neutralizing antibodies measurement complicates comparison between studies.
(2) *Potency and efficacy*: The efficiency with which AAV vectors infect and transduce into the desired target cells can impact therapeutic doses and efficacy.
(3) *Genotoxicity*: Although rare, the integration of the AAV vector DNA into the genome of the infected cell may have genotoxic effects.
(4) *Persistence*: The episomal AAV genome in the nucleus of the infected cells can be lost in conditions of cell proliferation (such as liver growth), which may impact therapeutic efficacy.

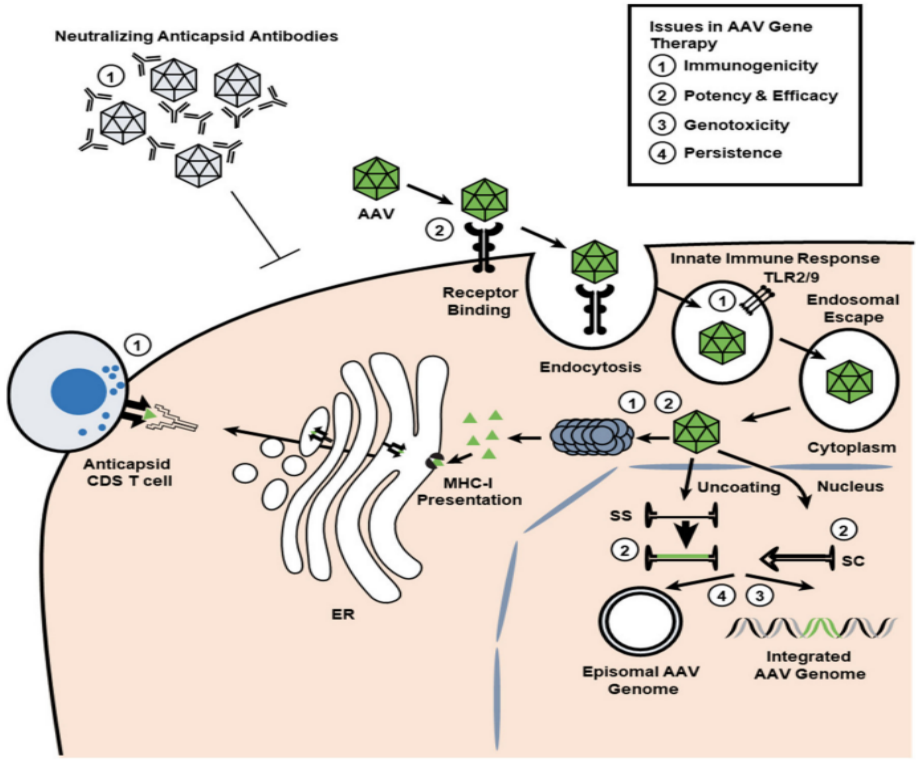

Figure 9. Potential limitations of gene transfer with AAV vectors
Source: Figure adopted from Monahan et al., 2021

First EMA approved gene therapy for haemophilia A

Valoctocogene roxaparvovec (Roctavian®, BioMarin) is the first gene therapy for the treatment of severe haemophilia A, available since August 2022 in the European Union. Valoctocogene roxaparvovec is an adeno-associated virus serotype 5 (AAV5) based gene therapy vector, designed to introduce a functional copy of a transgene encoding the B-domain deleted SQ form of human coagulation factor VIII (hFVIII-SQ). Transcription of this transgene occurs within the liver, using a liver-specific promoter, which results in the expression of hFVIII-SQ. The expressed hFVIII-SQ replaces the missing coagulation factor VIII needed for effective haemostasis.

Valoctocogene roxaparvovec has received conditional authorisation by EMA (2022) and FDA (2023) due to the results from a pooled cohort of two multinational phase 3 trials GENEr8-1 (NCT03370913) and GENEr8-2 (NCT03392974) demonstrating endogenous production of factor VIII activity in patients with severe haemophilia A sustained for at least 2 years. In both phase 3 trials, patients with severe haemophilia (Factor VIII activity <1 IU/dL) who were at least 18 years of age and did not have pre-existing anti-AAV5 antibodies or a history of development of factor VIII inhibitors and who had been receiving prophylaxis with factor VIII concentrate received a single infusion of either 6×10^{13} (GENEr8-1) or 4×10^{13} (GENEr8-2) vector genomes of valoctocogene roxaparvovec per kilogram of body weight. In 2022, Ozole and colleagues published the first data of the GENEr8-1 trial, showing that valoctocogene roxaparvovec treatment provided endogenous factor VIII production and significantly reduced bleeding and factor VIII concentrate use relative to factor VIII prophylaxis over a period of 52 weeks post-dose [Ozole et al., 2022] (**Figure 10**). Similar results were obtained in the GENEr8-2 trial.

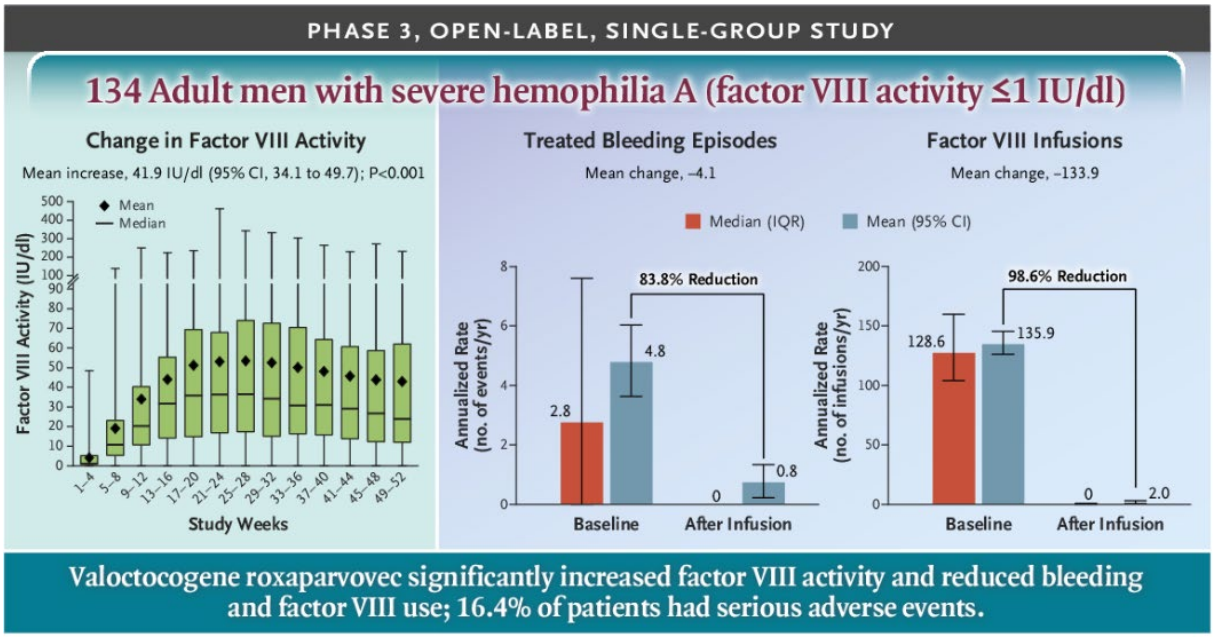

Figure 10. Valoctocogene roxaparvovec gene therapy for severe haemophilia A

Source: Figure adopted from [Ozole et al., 2022]

However, long-term outcomes of both phase 3 clinical trials with valoctocogene roxaparvovec prompt some doubt whether rAAV gene therapy may be a cure or curse for haemophilia patients [Pipe et al., 2023, Mahlangu et al., 2023; Puetz, 2024]: In the long-term, the annualized bleeding rate under rAAV-gene therapy seems not significantly better than the current standard of care. The need for factor replacement was reduced, but not eliminated. The data showed a decline in the efficiency of rAAV-gene therapeutics starting already after 2 years of administration. In addition, the use of the rAAV gene transfer precludes repeated dosing due to auto-antibody formation.

Hepatitis was the principal toxicity reported in both haemophilia A phase 3 trials. At current, it is far from clear, whether complications such as development of hepatocellular carcinoma seen with other recombinant AAV gene therapeutics in preclinical mouse models [Dawaldi et al., 2021; Nguyen et al., 2021; Kapelanski-Lamoureux et al., 2022] or in patients [Ertl et al., 2022] may also occur in haemophilia patients. So far, no tumours have been reported in men after gene therapy in patients with haemophilia [Puetz 2024]. Immunosuppressive treatments are currently employed to stabilise efficacy and to treat drug-induced hepatitis.

Valoctocogene roxaparvovec is contraindicated in patients with neutralizing antibodies to the AAV5 vector, active infections, either acute (such as acute respiratory infections or acute hepatitis) or uncontrolled chronic (such as chronic active hepatitis B), in patients with known significant hepatic fibrosis or cirrhosis, and patients with known hypersensitivity to mannitol. Long term efficacy and safety is currently generated in relevant patient numbers in haemophilia patient registries [Konkle et al., 2020; Symington et al., 2024].

New hopes are emerging with the introduction of Gene editing using the CRISPR/Cas9 technology. It allows the correction of genetic defects with single base-pair precision. It can be performed in cells both outside and inside the body. The first treatment in specific types of beta – thalassemia and sickle cell disease has been approved in the European Union and efforts are undertaken in haemophilia as well, which will be discussed in more detail below in chapter Future Innovative Approaches.

Haemophilia A Drug Pipeline

In the last decade, the big progress in biotechnology facilitated the generation of bispecific antibodies to selected targets which are able to block or mimic physiological effects in humans over a long period of time. For haemophilia patients, the approval of the bispecific antibody Emicizumab (Hemlibra®) marks the beginning of a new era of therapies that are not based on the factor replacement principle. With a subcutaneous injection once a week instead of frequent intravenous infusions (three to four times a week) previously required, patients' quality of life can be significantly improved regardless of inhibitor status. The current drug developments for haemophilia seek to combine a highly potent bleed protection with the lowest possible treatment burden to permit an almost normal life. With the introduction of RNA interference products and gene therapy, this goal is getting closer, although there are still some hurdles to take. Future innovative approaches include CRISP/Cas9 gene editing and tRNA-based protein editing, which could potentially cure this hereditary disease.

In the following, the current drug pipeline for haemophilia A will be presented and discussed according to the development phase of each drug candidate. A tabular overview of the global haemophilia A drug pipeline 2025 available in the public domain at CLINICALTRIALS.GOV is given in the Appendix (**Table 5**).

Phase 3 drug candidates

Mim8 (Denecimig, Novo Nordisk) is a next-generation FVIIIa mimetic (fully human bispecific IgG4 antibody) is currently in development as once-weekly or once-monthly prophylaxis treatment for people living with haemophilia A, with and without inhibitors. Administered subcutaneously, Mim8 bridges Factors IXa/X (FIXa/FX) together upon activation, thereby replacing missing FVIIIa, which effectively restores the body's thrombin generation capacity, helping blood to clot.

The early phase development program included the following trials:

- **FRONTIER1 (Phase 1)** – including a single ascending dose (NCT04204408) and a pharmacokinetic trial (NCT05127473) examined the safety, tolerability, PK and PD of Mim8 in healthy adult males. Mim8 was well tolerated, and there was no severe treatment-emergent adverse event. The PK characteristics of Mim8 were consistent with dose-proportionality. The terminal half-life of Mim8 after a single dose was approximately 1 month, and maximum plasma concentration was reached after 10 days suggesting that Mim8 is suitable as a further long-acting FVIIIa mimetic bispecific antibody for haemophilia A prophylaxis [Persson et al., 2023] (**Figure 11**).
  The **Charité Research Organisation** has mainly contributed to the Frontier1 (Phase 1) trial.

- **FRONTIER1 (Phase 2)** – an open label, multiple ascending dose study (NCT04204408) which evaluated the safety, PK, PD and exploratory efficacy of Mim8 in participants with haemophilia A with or without FVIII inhibitors. Mim8 was well tolerated with no safety concerns and few treated bleeding episodes beyond the lowest dose cohort. The Mim8 PK/PD properties supported weekly to monthly dosing approaches. These data supported the further development of Mim8 [Lenz et al., 2023].

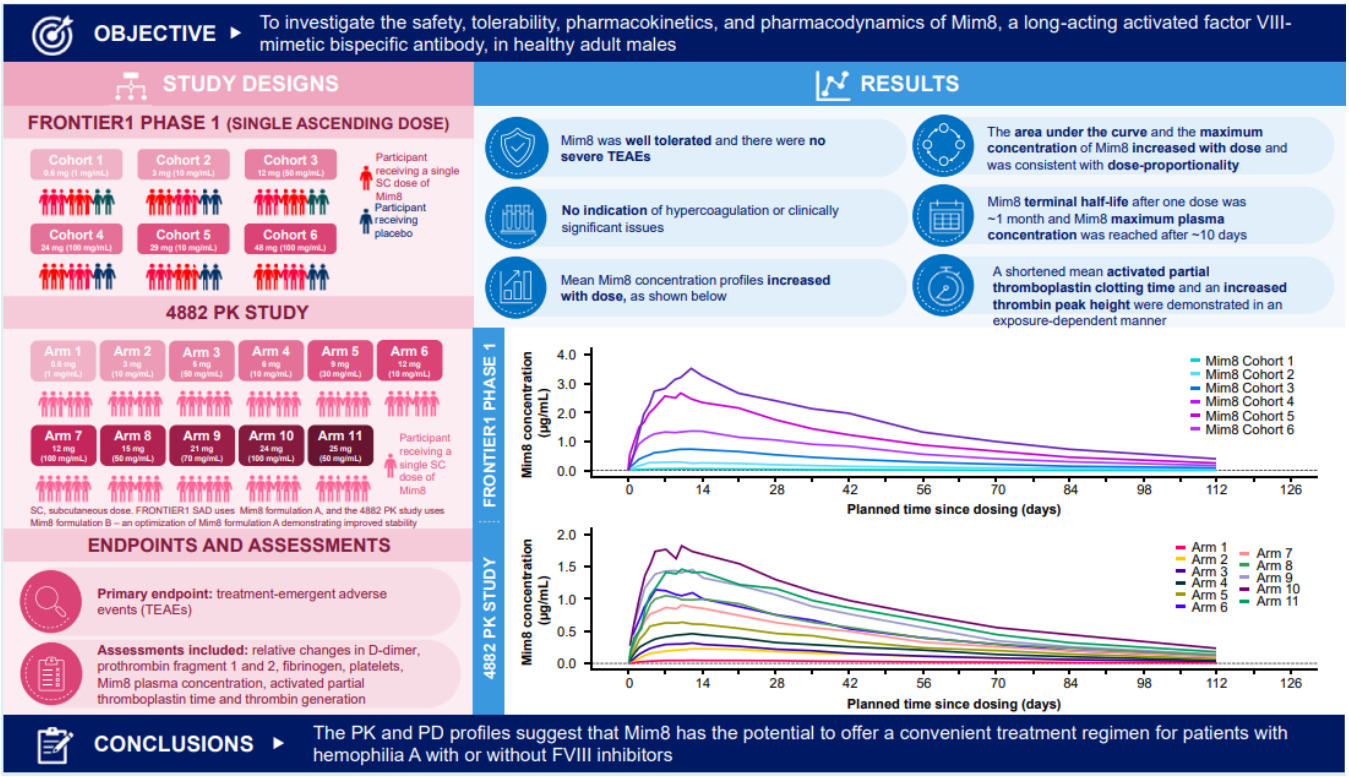

Figure 11. Summary of the Frontier1 (phase1) study results

Source: Figure Adopted from [Persson et al., 2023]

Novo Nordisk plans to submit a marketing authorisation application for Mim8 in 2025 based on the data obtained in the late phase Mim8 programme that includes:

- **FRONTIER2** (NCT05053139) – an open-label, randomized, multinational phase 3 trial for male and female patients (aged ≥12 years) with congenital haemophilia A (of any severity) and with or without inhibitors testing a subcutaneous treatment regimen of once-weekly (QW) or once-monthly (QM) Mim8 prophylaxis. Participants were randomized into 4 treatment arms [Mancuso, et al., 2024] (**Figure 12**).

  Positive results from FRONTIER2 (pivotal trial) were presented as *late breakthrough abstract* at the ISTH Meeting in June 2024, showing that Mim8 was efficacious in reducing the annual bleeding rate (ABR) for patients aged 12 years or above with haemophilia A regardless of inhibitor status [Mancuso, et al., 2024]: Both coprimary-endpoints were reached; Mim8 QW and QM prophylaxis treatment was superior to on-demand treatment and CFC prophylaxis (run-in period) for reducing ABR for treated bleeds. In the trial, Mim8 showed a safe and well-tolerated profile in line with previous trials. A low rate of injection-site reactions was observed. No deaths or thromboembolic events were reported. No neutralizing anti-Mim8 antibodies were detected. In addition, Mim8 prophylaxis improved some patient-reported outcomes such as physical functioning [Hermans et al., 2024]. Following the completion of the 26-week main phase of the trial, a 26-week extension phase is ongoing.

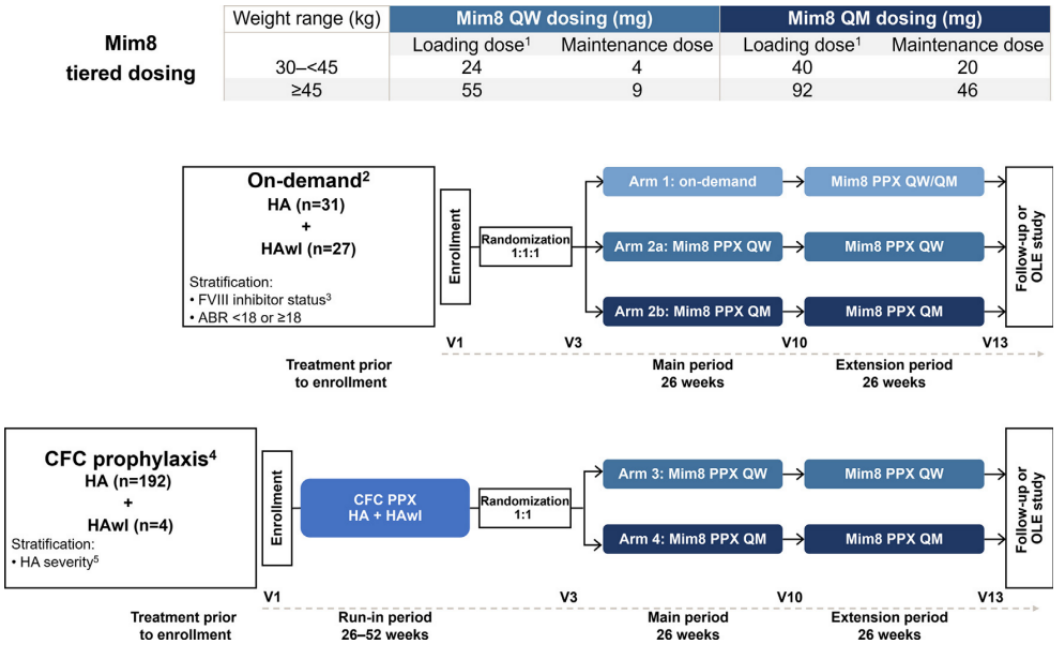

Figure 12. Study design of the phase 3, open-label, randomized, controlled FRONTIER2 study (NCT05053139)

[1] An initial loading dose was administered once in order to rapidly achieve steady-state levels of Mim8, followed by maintenance dose in the next scheduled dosing visit; [2] Patients treated with on-demand/no prophylaxis prior to enrolment were required to have had ≥5 bleeds in the 26 weeks before screening, for which FVIII concentrates or a bypassing agent had been prescribed; [3] The patient's FVIII inhibitor status was defined by use or prescription of bypassing agents in the past 6 months; [4] Patients with FVIII activity ≥1% who were receiving prophylactic treatment were required to have had ≥1 bleed in the 26 weeks before screening, for which FVIII concentrates or a bypassing agent had been prescribed; [5] Severity of haemophilia defined by severe (endogenous FVIII activity < 1%) or non-severe (endogenous FVIII activity ≥ 1%) at diagnosis.
ABR = annual bleeding rate, CFC = clotting factor concentrate, HA = patients with haemophilia A without inhibitors, HAwI = patients with haemophilia A with inhibitors, n = number of exposed patients, OLE = Open-Label Extension study FRONTIER4, PPX = prophylaxis, QW =once-weekly, QM = once-monthly, V = Visit.

Source: Figure adopted from Mancuso et al., 2024

- **FRONTIER3** (NCT05306418; EudraCT:2020-003467-26) – a 52-week safety and efficacy phase 3 trial in 70 paediatric patients with haemophilia A, with or without inhibitors (1-11 years). People will receive once-weekly Mim8 during the first 26 weeks and may subsequently choose to receive once-monthly Mim8. The interim analysis showed that about 74% of children with haemophilia A who were treated weekly with Mim8 did not experience any bleeding requiring treatment. The results suggest that Mim8 is both effective and well tolerated and that the treatment can reduce disease burden through flexible dosing options. After 26 weeks, 31 participants opted to switch to monthly dosing, while 38 participants continued with weekly treatment [Mahlangu et al., 2025].
- **FRONTIER4** (NCT05685238) – an open-label extension study enrolling participants from FRONTIER1 (Phase 2-Arm 1) or several phase 2/3 studies of Mim8 (Arm 2). The results of an interim analysis investigating the safety and efficacy of Mim8 prophylaxis once every two weeks (Q2W) in Arm 1 of FRONTIER4 (26 weeks) were presented at the 66th ASH meeting (2024) showing that Mim8 Q2W prophylaxis was safe and well tolerated with no participants discontinuing treatment. Few participants experienced treated bleeding episodes with Mim8 Q2W dosing and a large proportion had zero treated bleeding episodes. These data are consistent with the results from the FRONTIER2 study [Matsushita et al., 2024].
- **FRONTIER5** (NCT05878938) – a 26-week phase 3 trial investigating PK, PD and safety of switching from previous Emicizumab to Mim8 in adults and adolescents with haemophilia A, with or without inhibitors. The trial was completed in July 2024; publication of study results is expected in 2025.

Fitusiran (Sanofi Genzyme) is a potential '*first-in-class*' small interference RNA therapeutic in development as non-factor replacement therapy for haemophilia A or B with or without inhibitory antibodies to FVIII or FIX. Fitusiran is designed to lower antithrombin, a protein that inhibits blood clotting, with the goal of promoting thrombin generation to rebalance haemostasis and prevent bleeds (**Figure 6**). Fitusiran is generated using ESC-GalNAc conjugate technology (Alnylam Pharmaceutical Inc.), which enables subcutaneous dosing with increased potency and durability. The late phase fitusiran programme includes:

- **ATLAS-AB** (NCT03417245) was a randomized, open-label phase 3 study investigating the efficacy and safety of fitusiran in males ≥12 years with severe haemophilia A or B *without* inhibitors who had previously been treated with on-demand clotting factor concentrates. Study participants (n=120) were randomized 2:1 to receive either once-monthly 80 mg subcutaneous fitusiran prophylaxis or on demand clotting factor concentrates (CFC) [Srivastava et al., 2023]: This study showed that 51% of participants without inhibitors (40 out of 79) who received fitusiran 80 mg monthly prophylaxis experienced zero bleeds compared to 5% (2 out of 40) in the on-demand CFC group. Increased alanine aminotransferase concentration was the most common treatment-emergent adverse event in the fitusiran group and hypertension was the most common in the on-demand CFC group.
- **ATLAS-INH** (NCT03417102) was a randomized, open-label phase 3 study designed to evaluate the safety and efficacy of fitusiran in males ≥12 years with severe haemophilia A or B *with inhibitors* to FVIII or FIX. Study participants (n=57) receiving on-demand treatment with bypassing agents (BPA) were randomized in a 2:1 ratio to receive once-monthly 80 mg subcutaneous fitusiran prophylaxis or continue with on-demand BPA [Young et al., 2023]: This study showed that 66% of participants with inhibitors (25 out of 38) receiving fitusiran 80 mg monthly experienced zero bleeding episodes compared to 5% (1 out of 19) receiving an on-demand BPA after 9 months of treatment. The most frequent treatment-emergent adverse event in the fitusiran prophylaxis

group was increased alanine aminotransferase in 13 (32%) of 41 participants in the safety population. Suspected or confirmed thromboembolic events were reported in two (5%) participants in the fitusiran prophylaxis group. No deaths were reported.

Across both clinical studies, subcutaneous fitusiran prophylaxis resulted in statistically significant reductions in annual bleeding rate by about 90% compared to on-demand in the control arms [Srivastava et al., 2023, Young et al., 2023]. In addition, the convenient route of administration and long durability of fitusiran prophylaxis reduced the overall treatment burden and improved quality of life compared to on-demand treatments. Further phase 3 trials investigated whether a reduction in dose and frequency of fitusiran prophylaxis regimen may improve the benefit risk profile of this innovative approach:

ATLAS-OLE – a phase 3 open-label extension study of fitusiran prophylaxis using lower doses and less frequent dosing (with most on every two months regimen, Q2M) showed that maintaining antithrombin activity levels between 15-35% resulted in clinically meaningful bleed control and a substantially improved safety profile (i.e. reduction in the previously observed incidence of thrombotic events, liver enzyme elevations, gallbladder inflammation or gallstones) in people with haemophilia A or B, with or without inhibitors [Young et al., 2024].

Surgical experience with fitusiran prophylaxis is currently restricted to 60 major surgeries (including 24 in people with haemophilia with inhibitors) occurring during the fitusiran development programme in participants who were on the 80 mg QM or revised antithrombin-based Q2M dose regimen. Major surgeries were safely and effectively conducted with fitusiran prophylaxis following bleeding management guidelines, regardless of inhibitor status. Reversal of lowered antithrombin levels is considered not necessary [Scrivastava et al., 2024].

Regulatory submissions for fitusiran for the treatment of haemophilia A or B in adults and adolescents with or without inhibitors have been completed in China, Brazil and the US with a US Food and Drug Administration (FDA) target action date of March 28, 2025. The FDA also granted fitusiran *Breakthrough Therapy Designation* for haemophilia B with inhibitors in December 2023.

Giroctocogene fitelparvovec (Pfizer/ Sangamo Therapeutics) is a novel, investigational gene therapy using a bio-engineered AAV6 capsid and a modified B-domain deleted human coagulation FVIII gene. After a single infusion of giroctocogene fitelparvovec, people with haemophilia may produce FVIII by themselves for an extended period of time.

The late phase giroctocogene fitelparvovec programme includes:

AFFINE (NCT04370054) – an open-label, multicenter, single-arm phase 3 study evaluated the efficacy and safety of a single infusion of giroctocogene fitelparvovec in adult male participants (n=75 dosed participants) with moderate to severe haemophilia A (FVIII:C≤1%) for the study duration of 5 years. Study participants included in the assessments of the key endpoints of the primary efficacy analysis (n=50) completed a minimum 6 months of routine FVIII replacement prophylaxis therapy during the Run-in study (NCT03587116) providing data to compare with post giroctocogene fitelparvovec treatment.

The primary endpoint was met, measured as the total annualized bleeding rate (ABR) from Week 12 through at least 15 months of follow up post-infusion compared with routine FVIII replacement prophylaxis treatment. Following a single infusion of 3×10^{13} vector genomes of giroctocogene

fitelparvovec per kilogram of body weight, giroctocogene fitelparvovec demonstrated a significant reduction in mean total ABR compared to the pre-infusion period (1.24 vs 4.73; one-sided p-value=0.0040) [Press Release Pfizer, 24-Jul-2024]. Key secondary endpoints were met demonstrating that 84% of participants maintained FVIII activity >5% at 15 months post-infusion (one-sided p-value = 0.0086) with the majority of participants having FVIII activity ≥15%, and the mean treated ABR showed a statistically significant 98.3% reduction from 4.08 in the pre-infusion period to 0.07 post-infusion (from Week 12 up to at least 15 months; one-sided p-value < 0.0001). Throughout the study, among all dosed participants, one participant (1.3%) returned to prophylaxis post-infusion.

Giroctocogene fitelparvovec was generally well tolerated. Transient elevated FVIII plasma levels ≥150% were observed in 49.3% of dosed participants with no impact on efficacy and safety results. Serious adverse events were reported in 15 patients (20%), including 13 events reported by 10 patients (13.3%) assessed as related to treatment. Treatment-related adverse events generally resolved in response to clinical management.

Giroctocogene fitelparvovec has been granted *Fast Track* and *Regenerative Medicine Advanced Therapy designations* from FDA, as well as *Orphan Drug designations* in the U.S. and the European Union. In December 2024, Sangamo Therapeutics announced that it will regain development and commercialization rights to giroctocogene fitelparvovec (previously co-developed with, and licensed to Pfizer Inc.) following a decision by Pfizer to terminate the global collaboration and license agreement between the parties [Press Release, Sangamo Therapeutics, 30-Dec-2024].

Phase 2 drug candidates

ASC618 (ASC Therapeutics) is a transformational in-vivo AAV *second generation* gene therapy for haemophilia A, where FVIII is biosynthesized and secreted in the liver. ASC618 is a recombinant AAV8-vector incorporating a novel liver-specific promoter and a bioengineered, codon-optimized B domain-deleted FVIII variant [Brown et al., 2014]. In preclinical studies, ASC618 exhibited at least a 10-fold increase in the biosynthesis and secretion of FVIII compared with native human FVIII bioengineered gene constructs. ASC618 is supposed to increase durability of clotting factor biosynthesis and secretion by minimizing cellular stress and induction of the unfolded protein response [Pipe et al., 2022].

ASC618 is currently investigated in a phase 1/2 clinical trial (NCT04676048) to evaluate its safety, tolerability, and preliminary efficacy given as a single infusion in adult male patients with severe or moderately severe haemophilia A (FVIII activity ≤ 2 IU/dL). Study completion is expected by end of 2026. The program has received IND clearance and was granted *Fast Track* and *Orphan Drug Designations* by the FDA and *Orphan Medicinal Product Designation* by the European Commission.

Phase 1 drug candidates

Inno8 (Novo Nordisk) is a novel FVIII mimetic designed for *oral* administration for bleed prevention and long-term prophylaxis in patients with haemophilia A, irrespective of inhibitor status or severity [Lund et al., 2024]: Inno8 was made by connecting two heavy-chain only VHH fragments recognizing human FIXa and FX, respectively, followed by extensive protein engineering to enhance the in vitro potency. With a total molecular weight of approximate 30 kDa, Inno8 is five times smaller than an IgG antibody. Inno8 was further engineered to bind human serum albumin in circulation thereby extending the plasma half-life. Using in vitro global haemostatic assays, Inno8 was demonstrated to achieve similar in vitro activity as the sequence-identical analogue of Emicizumab (Hemlibra®) at approximately 90-fold lower concentrations. Moreover, Inno8 was shown to be orally available and exhibited a long systemic half-life of approximately 113 hours in beagle dogs permitting a once-daily oral prophylaxis

treatment of haemophilia A patients. Inno8 is currently investigated in the *first-time-in-human* trial **VOYAGER1** (NCT06649630) to determine the safety, tolerability, PK, PD and immunogenicity of single intravenous and multiple oral doses of Inno8 in healthy male participants. Study completion is expected in September 2025.

NXT007 (Hoffmann-La Roche) is a next-generation bispecific antibody that mimics the role of FVIII in the coagulation cascade. The safety, tolerability, PK, PD, immunogenicity, and efficacy of multiple ascending doses of NXT007 is currently tested in a Phase 1/2 (NCT05987449), open-label, non-randomized, global, multicenter, multiple-ascending dose study in adult and adolescent male participants with severe or moderate haemophilia A with or without FVIII inhibitors. Participants will receive NXT007 administered subcutaneously, 2 loading doses once every two weeks (Q2W) followed by once every 4 weeks (Q4W) maintenance doses up to 7.5 years. Study completion is expected in 2032.

Future Innovative Approaches

CRISPR/Cas9 gene editing: Clustered regularly interspaced short palindromic repeats (CRISPR)/ CRISPR-associated (Cas) protein 9 (CRISPR/Cas9) system belongs to an acquired immune defence system, whereby bacteria employ its DNA cleavage and disruption capabilities to combat viruses and bacteriophages which is increasingly used in medicine for a number of monogenic diseases [Pickar-Oliver & Gersbach, 2019]. The incredible fast development of genome editing techniques using the CRISPR/Cas9 systems is now opening the possibility to rectify mutations in single gene diseases such as haemophilia A. The deficiency in coagulation FVIII is due to a defect in the F8 gene which spans 186 kilobases of the long arm of the X chromosome (Xq28) and includes 26 exons [Bowen, 2002]. Using CRISPR/Cas9 gene editing technology may achieve sustained and stable expression of the F8 gene in haemophiliacs to potentially cure the disease. In a recent research study, a high level and stable FVIII expression was obtained applying a CRISPR-SpCas9 genome editing method to target and integrate a highly activity variant of FVIII (p18T-BDD-F8-V3) into the mouse ROSA26 genomic locus in vitro. The successful exogenous gene knock-in at the cellular level was unaffected by various types of mutations. These findings lay the foundation for future in vivo studies of haemophilia A [Zhao et al.,2024].

tRNA-Based Protein Editing: This technique is a fundamentally novel approach to target genetic diseases through tRNA-based protein editing using anticodon engineered tRNAs to overwrite nonsense mutations that would otherwise result in truncated, non-functional proteins. Thus, *first-in-class* tRNA-based therapeutics may address a broad spectrum of genetically defined diseases and cancer. It could be directed at restoring full-length and functional FVIII protein in people with severe haemophilia A.

HCB-101 (hC Bioscience) is an anticodon-engineered tRNA designed to suppress nonsense mutations. It is delivered as a lipid nanoparticle to target the liver. The preclinical data presented at the World Federation of Haemophilia 2024 World Congress demonstrate successful targeting of the liver in mice as well as successful in vitro production of full-length, functional FVIII despite the presence of a premature termination codon (PTC) that would otherwise result in a truncated non-functional protein. This approach has the potential for application in about 20% of severe haemophilia A cases. Build on these preclinical data, a Phase 1 clinical trial for severe haemophilia A is planned to be started in 2025 [European Haemophilia Consortium, Review August 2024].

Concluding Remark

As the haemophilia treatment landscape is rapidly transforming, it is important to establish systems to constantly monitor developments in emerging and gene therapies for haemophilia and make them available as soon as possible following approval by regulatory authorities [Scrivastava et al., 2020].

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Appendix

Haemophilia A - Current Drug Pipeline

Product (Sponsor)	Mechanism of Action/ Route of administration	Main Outcome Measures/ Endpoints/Results	NCT (clinicaltrials.gov)
Phase 3			
Denecimig (Mim8) (Novo Nordisk)	Next-generation FVIIIa mimetic (fully human bispecific IgG4 antibody/ Subcutaneous administration	**FRONTIER2** (pivotal trial): Mim8 treatment QW or QM up to 52 weeks in HA of any severity with or without inhibitors ○ Primary endpoints achieved: Reduced number of treated bleeds with and without coagulation factor prophylaxis, and with or without inhibitors ○ Safety endpoints achieved: Low number of injection site reactions, no deaths or thromboembolic events ○ Other endpoints: No evidence of neutralizing anti-Mim8 antibodies	NCT05053139: *Completed 2024.* MAA planned in 2025
Fitusiran (Sanofi/Genzyme)	Potential *'first-in-class'* antithrombin siRNA lowering antithrombin levels	ATLAS-AB and ATLAS-INH (pivotal trials): Fitusiran prophylaxis treatment QM up to 9 months in severe HA with or without inhibitors compared to on-demand CFC or on-demand BPA treatments ○ Primary endpoint achieved: Significant reduction in mean annual bleeding rate during efficacy period ○ Safety profile: Occurrence of few thrombotic events and some liver transaminase elevations >3xULN (defined as AESI) were observed ○ Other endpoints: Only few ADAs without effect on antithrombin lowering	NCT03417245: *Completed 2021* NCT03417102: *Completed 2020* New Drug Application for fitusiran accepted for review by the US Food and Drug Administration, with a PDUFA date of March 28, 2025.
Giroctocogene fitelparvovec (Pfizer/ Sangamo Therapeutics)	Gene therapy using a rAAV vector (AAV6 capsid and a modified B-domain deleted human coagulation FVIII gene).	AFFINE (pivotal trial): A single injection of Giroctocogene fitelparvovec in moderate to severe HA without inhibitors who discontinued routine FVIII prophylaxis for a study duration of 5 years ○ Key endpoints achieved: Significant reduction in mean ABR from Week 12 through at least 15 months post-infusion, 84% of participants maintained FVIII activity >5% at 15 months post-infusion ○ Safety profile: Occurrence 13 SAEs (13.3%) judged as related to treatment (generally resolved in response to clinical management)	NCT04370054: *Active, Not Recruiting (accessed 24-Apr-2025).* Study completion expected in 2028. *Fast Track* and *Regenerative Medicine Advanced Therapy designations* from FDA, as well as *Orphan Drug designations* in the U.S. and the EU.

Product (Sponsor)	Mechanism of Action/ Route of administration	Main Outcome Measures/ Endpoints/Results	NCT (clinicaltrials.gov)
Phase 2			
ASC618 (ASC Therapeutics)	AAV8-based gene therapy incorporating a novel liver-specific promoter and a bioengineered, codon-optimized B domain-deleted FVIII variant. ASC618 expresses human FVIII protein in the liver through Intravenous infusion	Phase 1/2 clinical trial: Primary & Secondary Endpoints: ○ Safety, Tolerability & preliminary efficacy of a single infusion of ASC618 in adult male patients with severe or moderately severe haemophilia A (FVIII activity ≤ 2 IU/dL).	NCT04676048: *Recruiting* Study completion expected December 2026
Phase 1			
Inno8 (Novo Nordisk)	Novel, highly potent FVIII mimetic replacing the function of the missing FVIII Oral administration	FTIH trial: Primary & Secondary Endpoints: ○ Safety, tolerability, PK, PD and immunogenicity of Inno8 single intravenous doses and multiple oral doses once daily for 10 days	NCT06649630: *Recruiting* Study completion expected in September 2025.
NXT007 (Hoffmann-La Roche)	Novel FVIII mimetic (next-generation bispecific antibody) Subcutaneous injection	Phase 1/2 trial: Primary & Secondary Endpoints: ○ Safety, tolerability, PK, PD, immunogenicity, and efficacy of NXT007 multiple ascending doses administered subcutaneously every 4 weeks up to 7.5 years in in adult and adolescent male participants with severe or moderate haemophilia A with or without FVIII inhibitors	NCT05987449: *Recruiting* Study completion expected in 2032

Table 5. Haemophilia A - Current Drug Pipeline

BPA = Bypassing Agents, CFC = clotting factor concentrates, EU = European Union, HA = haemophilia A, FVIII = Factor VIII; MAA = marketing authorization application; PK = pharmacokinetics, PD = pharmacodynamics, QM = once monthly, QW = once weekly, rAAV = recombinant adeno-associated-virus, siRNA = small interference RNA

Source: Based on data in public domain on CLINICALTRIALS.GOV

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