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Review

Advances in biopharmaceutical products for hemophilia

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SUMMARY

Hemophilia is caused by the deficiency of clotting factors due to a single genetic abnormality. Replacement therapies have evolved from plasma-derived to recombinant coagulation factor concentrates but continue to have certain limitations. Monoclonal antibodies are clinical prophylactic treatment options unaffected by inhibitors and have better compliance than coagulation factor concentrates for patients with hemophilia. Gene therapy is a breakthrough in hemophilia treatment, as it drives the hepatic expression of factor VIII or factor IX and requires only a single administration to enable long-term replacement treatment in adult patients. Furthermore, biopharmaceutical products that target new pathways unaffected by inhibitors, including tissue factor pathway inhibitors, activated protein C, and antithrombin, as well as pharmaceutical technology advances to reduce dosing frequency, have demonstrated promising clinical results. This review provides a comprehensive overview of these biopharmaceutical products and explores the future of hemophilia treatment.

INTRODUCTION

Hemophilia A (HA) and B (HB) are X-linked recessive congenital bleeding disorders caused by mutations in the genes responsible for producing coagulation factor VIII (FVIII) or factor IX (FIX), leading to clotting factor deficiencies in the blood plasma. The severity and frequency of bleeding in patients with hemophilia (PwH) depend on the levels of these coagulation factors. Traditional treatment for hemophilia involves intravenous supplementation with virus-inactivated plasma or recombinant coagulation factor concentrates (CFCs), which have established applications in episodic treatment, routine prophylaxis, and perioperative management.[1]

Clotting factor replacement therapies require long-term and frequent intravenous injections to maintain FVIII or FIX at a minimum trough level of 1–3%, which aids in reducing the risk of spontaneous bleeding to an acceptable level and improving the quality of life for PwH.[2] During bleeding or the perioperative period, PwH requires additional CFC injections to achieve the desired peak in factor activity.[3] However, the currently available clotting factor products impose a major treatment burden, with frequent intravenous administration risking damage to the skin and blood vessels, potentially leading to phlebitis.[4]

The use of CFCs may lead to the development of neutralizing antibodies, known as inhibitors, which can hinder the activity of coagulation factors.[5] PwH with persistent inhibitors require prompt immune tolerance induction (ITI) or bypassing agents to treat bleeding.[1,6] However, ITI requires a considerable initial investment and is not always effective. For patients with HB (PwHB) with inhibitors, ITI also carries the risk of allergic reactions and irreversible nephrotic syndrome.[6] Compared with CFCs, bypassing agents are more expensive and associated with a high risk of inducing thrombosis.[2]

Given these limitations, gene therapies, and non-factor drugs, including monoclonal antibodies, small interfering RNAs (siRNAs), and recombinases, have emerged as less burdensome preventive treatment options. With advancements in gene delivery systems, gene therapy products for hemophilia treatment have been approved for marketing and are regulated as biological products.[7] Clinical data indicate that a single intravenous injection can achieve sustained expression of factors in PwH for 5–8 years or longer, with significant clinical benefits.[8,9] Among non-factor drugs, emicizumab is widely used as a prophylactic treatment for patients with HA (PwHA) and is recommended by multiple guidelines and principles.[1,10]

Although currently available products for hemophilia treatment improve the patient's quality of life and health equity, they have drawbacks such as poor clinical compliance or limited use for episodic and perioperative treatment in inhibitor-positive patients. The shortcomings of these products must be carefully considered when exploring strategies for developing new drugs for hemophilia. This review provides an overview of the available biopharmaceutical products for hemophilia, discusses pharmaceutical technology innovations aiding the development of hemophilia therapies, and highlights emerging biopharmaceutical products that are less burdensome with potential for market approval.

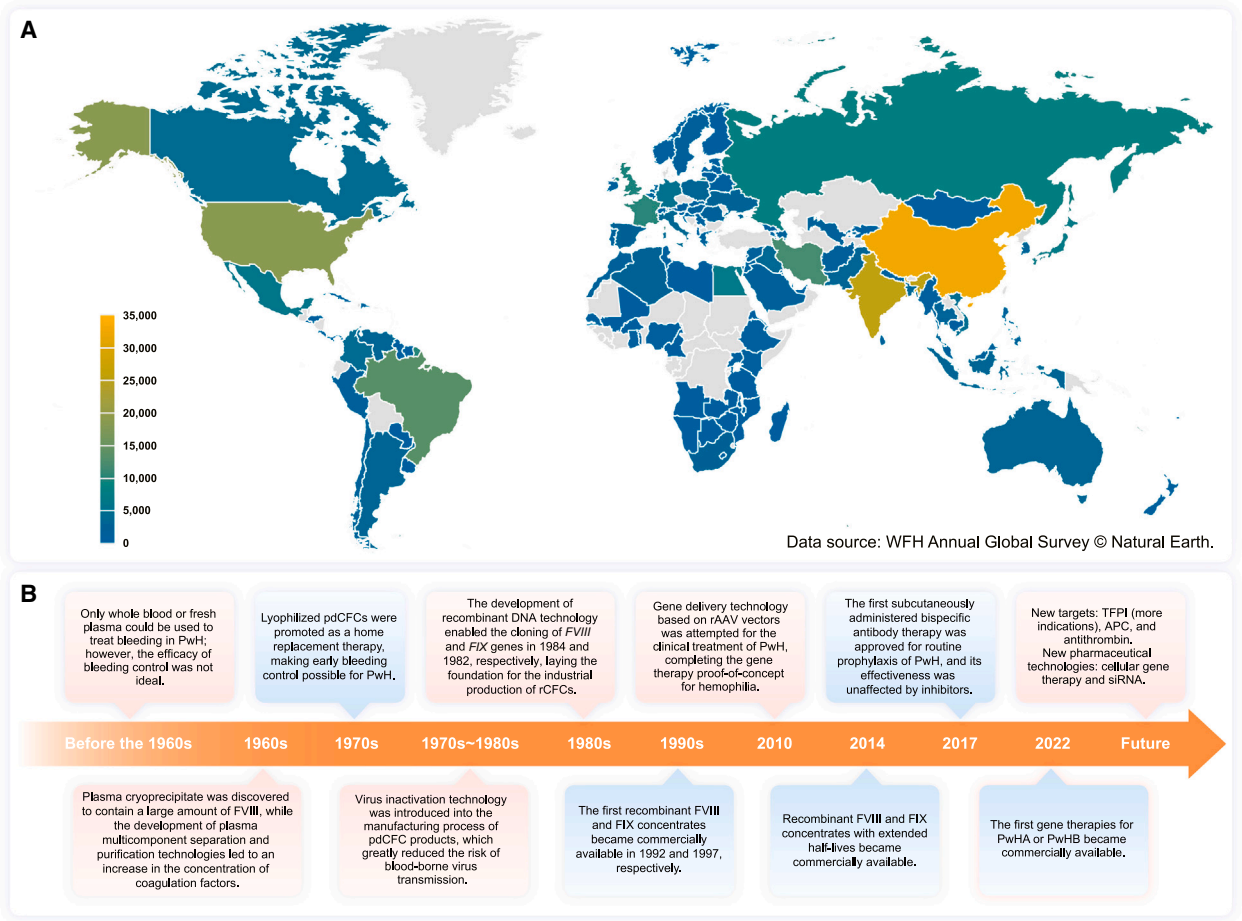

Figure 1. Global distribution of registered PwH in 2022 and the historical timeline of biopharmaceutical products for hemophilia

(A) Total number of PwH in different countries or regions. Countries and regions with no reported data for 2022 are indicated in gray. Data source: WFH Annual Global Survey © Natural Earth. Abbreviations: PwH, patients with hemophilia; WFH, World Federation of Hemophilia.

(B) Advances in pharmaceutical technology driving the development of biopharmaceutical products for hemophilia. The red background indicates the introduction of biotechnology, whereas the blue background indicates the products. Abbreviations: PwH, patients with hemophilia; pdCFCs, plasma-derived coagulation factor concentrates; rCFCs, recombinant coagulation factor concentrates; rAAV, recombinant adeno-associated virus; TFPI, tissue factor pathway inhibitor; APC, activated protein C; siRNA, small interfering RNA.

SURVIVAL AND MEDICATION STATUS OF PATIENTS WITH HEMOPHILIA REQUIRE MORE ATTENTION

PwH experience major physiological, psychological, emotional, economic, and employment challenges. In low- and middle-income countries, the diagnosis, registration, management, care, and treatment of PwH are not as advanced as in high-income countries. The lack of access to coagulation products makes it difficult to meet the patient's needs.[11] In contrast, PwH in high-income countries predominantly struggle with inhibitors emergence owing to the frequent use of CFCs.[12]

Hemophilia is under-diagnosed globally

According to estimates from the World Federation of Hemophilia (WFH), most PwH worldwide (>75%) have not yet been identified and diagnosed.[13] A meta-analysis using national registries determined the prevalence at birth of PwHA (24.6 per 100,000 males) and PwHB (5.0 per 100,000 males) in high-income countries (Australia, Canada, France, Italy, New Zealand, and the United Kingdom) and estimated the total number of PwH globally to be 1.125 million.[14] However, the number of PwH registered with WFH worldwide in 2022 was only approximately 271,000[15,16] (Figure 1A); accordingly, the number of registered PwH is expected to represent a fraction of the actual total.

More specifically, the total number of PwH registered with WFH in China in 2022 was approximately 32,000,[16] while the Hemophilia Treatment Center Collaborative Network of China estimated 65,000 to 130,000 domestic PwH cases.[17] Similarly, in the United States, although approximately 19,000 PwH have been registered with the WFH,[16] the Centers for Disease Control and Prevention estimates that there are more than 33,000 PwH. These disparities in status may originate from the uneven distribution of medical resources in developed countries, inadequate medical resources in low- and middle-income countries,

challenges in effectively identifying PwH and delays in registering new patients with local regulatory authorities.

Hemophilia is challenging to manage, and PwH is at risk of long-term health complications due to spontaneous, traumatic, and postsurgical bleeding. The associated bleeding patterns and lesions vary widely. Without intervention, multiple organs may experience frequent bleeding, and long-term repeated bleeding in joints can lead to deformity or disability.[18] Various factors influence bleeding patterns. For instance, aging and physical activity can increase the incidence of joint bleeding. Women and girls with heterozygous alleles for hemophilia may also experience heavy or prolonged menstrual and pregnancy bleeding[19]; therefore, the care of female patients should not be ignored.

Intracranial or extracranial bleeding is possible and can be life-threatening in severe cases. Similarly, abdominal bleeding can be challenging to detect and may involve multiple organs, resulting in symptoms, such as pain, distension, vomiting of blood, and blood in the stool. Life-threatening spontaneous or traumatic bleeding can also occur in the chest and throat.[18,20] Given the substantial number of undiagnosed or unregistered PwH worldwide, including female patients who have not yet received care, this population is at a high risk of experiencing potentially life-threatening bleeding episodes without appropriate medical intervention.

Diagnostic and biopharmaceutical products for hemophilia

Personalized treatments based on diagnostic accuracy can minimize the treatment burden on PwH.[21] The one-stage clotting assay (OSA) measures activated partial thromboplastin time to assess plasma coagulation factor activity (FVIII:C, FIX:C). OSA, along with the two-stage (chromogenic) assay (TSA), is widely used worldwide for diagnosing hemophiloia.[22] However, the accuracy is affected by several factors, including age, ABO blood type, and laboratory differences.[23] Meanwhile, individual gene sequencing technologies facilitate the accurate identification of *FVIII* or *FIX* gene mutations, and can therefore be used to screen parental carriers. Sequencing-based prenatal diagnosis can determine whether offspring carry hemophilia-causing genes, supporting early intervention and management before any bleeding occurs.[24]

Prior to the 1960s, researchers attempted to treat hemophilia using direct blood transfusions. The successful separation of CFCs from the plasma in the 1960s and 1970s marked the beginning of modern treatment methods for this illness.[25] Over the next 50 years, plasma-derived CFCs (pdCFCs), recombinant CFCs (rCFCs), monoclonal antibodies, and gene therapies, have become available for PwH (Figure 1B). Since the 1990s, the application of recombinant protein technologies in the biopharmaceutical field has facilitated the use of rCFCs with high safety and monoclonal antibody drugs with improved clinical compliance for hemophilia treatment.[26,27] In 2010, gene therapy utilizing adeno-associated virus (AAV) vectors was first explored for hemophilia treatment, yielding encouraging clinical outcomes.[28] Owing to the advancement of the recombinant AAV (rAAV) vector platform, gene therapy drugs requiring only a single infusion are now available for PwH as long-term replacement treatments.[29]

Imbalances in drug treatment for patients with hemophilia

In 2022, the median global usage of FVIII and FIX per capita was 1.38 and 0.24 IU, respectively.[16] However, disparities in the median per capita usage of traditional replacement therapy CFCs are evident among countries, as a high proportion of affected patients do not receive adequate treatment and care. In 2022, the median FVIII usage per capita was 5.17 IU in high-income countries and 0.03–1.84 IU in low- and middle-income countries (Figure S1).

In high-income countries, most PwH receive prophylactic treatment. The proportion of individuals with severe HA in the United Kingdom who receive prophylactic treatment is 90–93%.[16] Similarly, in the United States, up to 75% of PwHA under 20 years of age receive continuous prophylactic treatment.[30] However, the rate of prophylactic treatment for PwH is extremely low in low- and middle-income countries. In China, only 15% of PwH under 18 years of age receive routine prophylactic treatment,[31] potentially causing arthropathy in 90% of boys with hemophilia.[32]

Prophylactic use of low-to-intermediate doses has led to clinical improvements and is recommended when there is an insufficient supply of CFCs.[33,34] Low-dose prophylaxis for FVIII or FIX concentrates comprises administering 10–15 IU/kg per dose 2–3 times weekly (1,000–1,500 IU/kg per year), while intermediate-dose prophylaxis requires 15–25 IU/kg per dose thrice weekly for FVIII concentrates (1,500–4,000 IU/kg per year) or 20–40 IU/kg per dose twice weekly for FIX concentrates (2,000–4,000 IU/kg per year). High-dose prophylaxis requires 25–40 IU/kg for FVIII concentrates or 40–60 IU/kg for FIX concentrates weekly (>4,000 IU/kg for FVIII or FIX concentrates per year).[1] To improve joint health and promote health equity, it is recommended to maintain FVIII or FIX activity levels in patients with severe hemophilia within the activity level range observed in patients with moderate, preferably mild, hemophilia.[35]

Low-income countries lack resources and access to drugs to treat hemophilia. Hence, local governments should actively work with non-profit organizations to raise awareness about hemophilia among the public and medical staff and improve early diagnosis and treatment. Organizations such as the WFH and hemophilia drug companies should gradually increase humanitarian aid for patients in low- and middle-income countries. Research and development of factor-based biosimilars should be encouraged in middle-income countries with the capacity for the biopharmaceutical production of these drugs. Additionally, the local industrial chains should be employed to reduce the terminal sales price of drugs, economic pressure on patients, and medical insurance pressure on governments.

While the supply of CFCs in high-income countries has relatively met the needs of PwH,[36] a notable increased risk of inhibitor formation has arisen with repeated use. In patients who develop inhibitors, continued use of CFCs elicits only limited hemostatic effects, while high-titer inhibitors may render hemophilia replacement therapy ineffective.[11,37] Hence, there is an increasing demand for effective non-factor drugs unaffected by inhibitors.

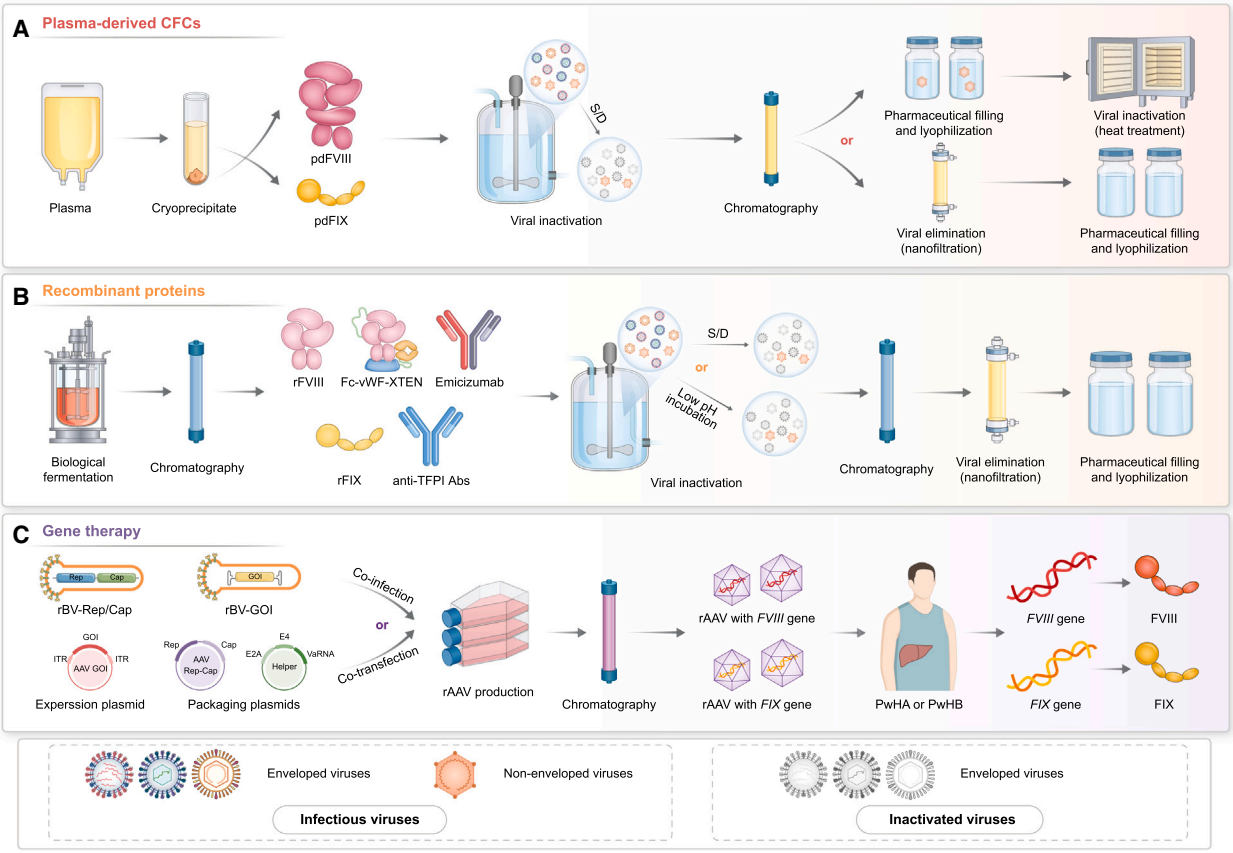

Figure 2. Application of pharmaceutical technologies in drugs approved for hemophilia

(A) Healthy human plasma is the raw material from which blood factors (FVIII and FIX) are separated and purified. Abbreviations: pdFVIII, plasma-derived FVIII; pdFIX, plasma-derived FIX; S/D, solvent/detergent.

(B) Recombinant factors and monoclonal antibodies biosynthesized using recombinant DNA technology. Abbreviations: Abs, antibodies; rFVIII, recombinant FVIII; rFIX, recombinant FIX; S/D, solvent/detergent; TFPI, tissue factor pathway inhibitors.

(C) Viral gene therapies that introduce nucleic acids into patient somatic cells to correct disease-causing mutations. Abbreviations: Cap, capsid genes; GOI, gene of interest; ITR, inverted terminal repeat; PwHA, patients with hemophilia A; PwHB, patients with hemophilia B; rAAV, recombinant adeno-associated virus; rBV, recombinant baculovirus; Rep, replication genes.

BIOPHARMACEUTICALS APPROVED FOR HEMOPHILIA TREATMENT

CFCs remain the most commonly used biopharmaceutical products for treating hemophilia. However, a new recombinant factor drug (efanesoctocog alfa), monoclonal antibody drugs, and gene therapies have demonstrated better clinical compliance than traditional replacement therapies; and the effectiveness of monoclonal antibody drugs is unaffected by inhibitors.[38]

Traditional plasma-derived and recombinant coagulation factor concentrates are the most commonly used therapeutics

Since the 1960s, the development of plasma multi-component separation and purification technologies has improved the concentration of coagulation factors in preparations, resulting in the increased availability of pdCFCs to treat PwH.[39] To generate pdCFCs, mixed plasma is used as raw material, and subjected to a series of purification steps (centrifugation, precipitation, and chromatography); this is followed by viral inactivation and elimination processes, such as solvent/detergent (S/D) treatment, dry heat, or nanofiltration. The resulting solution is then filled and lyophilized to create a pharmaceutical preparation (Figure 2A).

Advances in plasma separation technologies have facilitated the conversion of cryoprecipitate and de-cryoprecipitate supernatants into CFCs with high purity and potency. CFCs can be classified into medium, high, and ultra-high.[40] Medium-purity pdFVIII with a specific activity of 1–50 IU/mg is prepared by removing large amounts of impurities and microorganisms via aluminum hydroxide adsorption and polyethylene glycol (PEG) segmented precipitation or PEG glycine precipitation.[41] After precipitation, pdFVIII with a specific activity of 50–200 IU/mg is prepared using ion-exchange chromatography, gel filtration chromatography, or affinity chromatography for high-purity preparation. pdFVIII preparations reaching a specific activity of

1,000–3,000 IU/mg are considered ultra-high purity.[41] Although high- and ultra-high-purity FVIII may be subject to greater losses during production than medium-purity FVIII,[42] they have a higher potency per unit, thereby offering a smaller injection volume and more treatment convenience.

pdFIX is found in the supernatant of plasma following cryoprecipitate removal. Owing to the limitations of separation technologies in the late 1980s, only FIX complex concentrates with relatively low pdFIX purity and other coagulation factors (FII, FVII, and FX) could be prepared.[43] However, after the 1990s, ion exchange, gel filtration, and affinity chromatography enabled the preparation of high-purity pdFIX,[44] reducing the risk of thromboembolic complications in PwHB receiving long-term infusions of FIX complex concentrates.[45]

In addition to the purity of pdCFCs concentrates, viral safety must also be considered.[46] In the 1960s and 1970s, the precipitation and adsorption steps in the preparation process of pdCFCs were insufficient to eliminate all potentially transmissible pathogens posing a major safety risk of causing human infectious diseases. Studies in the 1980s reported that pdCFC use caused human immunodeficiency virus and hepatitis C virus infections in PwH.[47,48] In the 1990s and early 2000s, infectious non-enveloped viruses, such as hepatitis A and parvovirus B19, were also reported in pdCFCs.[49] Viral inactivation and elimination processes are crucial for ensuring the safety of pdCFC products.[50] The European Medicines Agency (EMA) detailed necessary measures in its *Guideline on Plasma-Derived Medicinal Products* to control pathogens, including donor screening, screening of known viral markers in donors and plasma pools, and the introduction of two complementary steps to inactivate and/or remove enveloped and non-enveloped viruses during production.[51] The China National Medical Products Administration also issued the *Technical Methods and Validation Guidelines for Elimination/Inactivation of Viruses in Plasma-derived Products* in 2002, requiring the addition of specific steps to inactivate or eliminate lipid-enveloped and non-lipid-enveloped viruses.[52]

The threat of plasma-derived pathogens and the development of recombinant DNA technology have propelled research and development of recombinant coagulation factor products, which have been approved for marketing since the 1990s (Table S1).

Currently, rCFCs are produced through the large-scale fermentation of mammalian cells, followed by multi-step chromatography purification, S/D treatment or low pH incubation, nanofiltration inactivation and/or virus elimination, and lyophilization with a stable formula (Figure 2B). rCFCs can be divided into three generations. First-generation products are produced using Chinese hamster (CHO) or baby hamster kidney cells, which are cultured in a medium containing animal-derived proteins with human albumin added as an excipient in the final formulation.[53] Second-generation products use human-derived proteins in the cell culture medium, but the final formulation does not contain human albumin.[54] Third-generation products have the same final formulation as the second-generation products, with the addition of human-derived proteins in the culture medium further removed.[55]

In 1992, the first rFVIII, Recombinate, was approved by the United States Food and Drug Administration (FDA), as an alternative to pdCFCs (Table S1). rCFCs avoid the risk of blood-borne pathogen transmission and are equally effective as pdCFCs with similar immunogenicity.[56] Moreover, rCFCs acquisition eliminated the dependence on plasma raw materials and promoted the popularization of prophylactic treatments among PwH. Thus, the proportion of children with severe hemophilia receiving prophylaxis in the United States increased from 33% in 1995 to 52% in 2004,[57] then 86% by 2018.[58] rFVIII products can be classified into structure full-length rFVIII (FL-rFVIII), B-domain-truncated (BDT) rFVIII, and B-domain-deleted (BDD) rFVIII. FVIII synthesized by hepatocytes contains A1-A2-B-A3-C1-C2 domains with a molecular weight of 265 kDa, making the expression of rFVIII considerably more difficult than that of other recombinant proteins.[59] The B domain is not necessary for the coagulation activity of FVIII.[60] Moreover, BDT-rFVIII, BDD-rFVIII, and FL-rFVIII have similar pharmacokinetics, clinical efficacy, and safety characteristics.[61] Removing the B domain increases the levels of FVIII secreted by the expressed cell line by 15- to 25-fold.[62] Therefore, BDT-rFVIII and BDD-rFVIII have been selected as alternatives to FL-rFVIII.

CFCs are used for episodic treatment, routine prophylaxis, and perioperative factor management in PwH (Figure 3). The initial phase of clinical research on hemophilia focused solely on episodic treatment, wherein CFCs were transfused after bleeding to prevent the possible occurrence of severe, life-threatening hemorrhages. However, compared with episodic treatment, routine prophylaxis can prevent severe bleeding in individuals with severe hemophilia, prevent or delay the occurrence and development of joint lesions, and protect the normal function of joints.[63] Unlike patients with severe hemophilia, those with mild-to-moderate hemophilia typically do not receive prophylactic treatment and exhibit a low rate of spontaneous bleeding.[25] Guidelines or recommendations from various medical agencies advise that patients with severe and moderate hemophilia exhibiting severe phenotypes should undergo prophylactic treatment as early as possible,[1,64,65] ideally prior to joint bleeding or before the age of three, to maintain normal joint function.[66] Although long-term prophylactic treatment with CFCs can significantly improve patients' quality of life, the treatment and financial burdens of intravenous injections 1–4 times weekly are significantly heavy. Accordingly, the long-term treatment needs of PwH have directed the pharmaceutical industry to research and promote long half-life products that would improve clinical compliance.

Compared with the standard half-life (SHL)-FVIII, the half-life of extended half-life (EHL)-FVIII is at least 1.3-fold longer.[67] The pharmacokinetic performance of EHL-FIX is superior to that of EHL-FVIII. Compared with SHL-FIX, the half-life of EHL-FIX is 3–5 times longer (Table S1). Between 2014 and 2023, six EHL-FVIII and three EHL-FIX products were approved, increasing the available medication options for PwH. The most commonly used half-life extension technologies include fusion fragment crystallizable (Fc) protein or albumin, PEGylation, and single-chain factor (Figure 3).

After coagulation factors are fused with Fc or albumin, the fusion protein can be recycled into the circulation by binding to the neonatal Fc receptor (FcRn), delaying degradation via the lysosomal pathway and extending the pharmacokinetic half-life of the factor products.[68,69] Factors with site-specific or random

Figure 3. Application scenarios for different biopharmaceutical products in patients with hemophilia

Approved biopharmaceutical products for hemophilia can be classified into three categories: coagulation factor concentrates (CFCs), recombinant monoclonal antibodies, and gene therapies. The products have different characteristics: blue font in the patient type and usage section indicates the common applications, and green indicates the unique advantages of the corresponding products. The half-life of EHL-FVIII and EHL-FIX is at least 1.3-fold and 3-fold greater than that of SHL-FVIII and SHL-FIX, respectively. Small children (SC): 0 to <6 years; older children (OC): 6 to <12 years; adolescents: 12 to <18 years; adults: 18 to <65 years; older adults (OA): ≥65 years. Abbreviations: Abs, antibodies; EHL, extended half-life; FL-rFVIII, full-length recombinant FVIII; gc, genome copies; NFL-rFVIII, non-full-length rFVIII; pdFVIII, plasma-derived FVIII; pdFIX, plasma-derived FIX; PwHA, patients with hemophilia A; PwHB, patients with hemophilia B; rFVIII, recombinant FVIII; SHL, standard half-life; TFPI, tissue factor pathway inhibitor; vg, vector genomes.

PEGylation can reduce their proteolysis in the blood and exposure to scavenging receptors, prolonging their survival in circulation.[70] Lonoctocog alfa is a product that covalently links the light chain and BDT heavy chain of FVIII, exhibiting improved stability and high binding affinity to von Willebrand factor (vWF), which may translate into meaningful clinical outcomes.[71] A matching-adjusted indirect comparison confirmed that the annual consumption of lonoctocog alfa was significantly lesser than that of octocog alfa (an FL-FVIII product) and comparable to that of efmoroctocog alfa, a BDD-FVIII product linked to the dimeric

Fc domain of human immunoglobulin G1 (IgG1).[69] Additionally, the annualized bleeding rate (ABR) of lonoctocog alfa was also similar to that of efmoroctocog alfa.[72] Although these three products mentioned above are different, the clinical data have demonstrated the long-acting properties of lonoctocog alfa. FVIII binds to vWF in circulation; hence, the half-life of vWF limits that of EHL-rFVIII and is only 1.3–2.0 times longer than that of SHL-FVIII (Table S1). To further prolong the half-life of rFVIII, efanesoctocog alfa (Fc-vWF-XTEN fusion protein) was constructed by rFVIII fusing to the Fc fragment and adding the D′D3 domain of vWF; it is also covalently combined with two XTEN hydrophilic polypeptides.[73] This design blocks FVIII binding to endogenous vWF, extending the efanesoctocog alfa half-life to 3- to 4-fold that of SHL-rFVIII supported by FcRn.[74,75]

Antibody drugs enrich treatment options for patients with hemophilia and improve quality of life

Recombinant DNA technology is used to express recombinant monoclonal antibodies in CHO cells to prepare emicizumab, concizumab or marstacimab. Subsequently, the fermentation supernatant is collected and prepared through protein A affinity chromatography, ion-exchange chromatography, and viral inactivation and elimination and is finally packaged into finished preparations (Figure 2B).

Emicizumab is a recombinantly expressed bispecific human IgG4 antibody that simulates the function of activated FVIII to bridge activated FIX and FX. Since 2017, emicizumab was approved for marketing in the United States for the prophylaxis of bleeding in PwHA with or without inhibitors.[76] The bleeding rates are significantly lower in individuals treated with emicizumab than in those who previously received FVIII prophylaxis among PwHA with or without inhibitors.[77,78] Emicizumab has also demonstrated excellent pharmacokinetic performance and the frequency of its long-term administration can be once weekly, every 2 weeks, or every 4 weeks[79]

The amino acid sequence of emicizumab has been modified to resemble that of human antibodies, aiming to reduce immunogenicity. However, data from seven phase III clinical trials revealed that 5.1% of PwHA developed anti-drug antibodies (ADAs) after receiving emicizumab; 11.8% of these ADAs reduced emicizumab exposure. Moreover, over 50% of samples containing ADAs showed neutralizing effects.[80] Mim8 is a new bispecific antibody that mimics FVIIIa with an action mechanism similar to that of emicizumab but differs in its amino acid sequence and does not cross-react with anti-emicizumab antibodies.[81] Mim8 has demonstrated positive clinical outcomes in FRONTIER 2 trials and will be submitted for its first regulatory approval by the end of 2024,[82] thus providing patients experiencing emicizumab resistance an alternative.

Concizumab, a humanized IgG4 antibody targeting tissue factor pathway inhibitor (TFPI), was approved in Canada in 2023 for prophylaxis in adolescent and adult PwHB with inhibitors.[83] In phase III clinical trials of concizumab, explorer7 included PwHA and PwHB with inhibitors reported a median ABR of 0, whereas explorer8 included PwHA and PwHB without inhibitors reported a median ABR of 1.7 and 2.8 times, respectively. Both explorer7 and explorer8 reported that the ABRs of PwHA and PwHB with or without inhibitors treated with concizumab were lower than those of PwH without prophylaxis.[84,85] Additionally, patients experienced thromboembolic events in both trials; accordingly, when concizumab was restarted, the initial maintenance dose was reduced from 0.25 to 0.20 mg/kg[84,86]

Marstacimab, a human IgG1 antibody developed by Pfizer that targets the Kunitz 2 domain of TFPI, was approved for marketing in the United States in 2024 for the prophylaxis of adolescent and adult PwHA or PwHB without inhibitors.[87,88] Compared with routine prophylaxis and episodic treatment, in a key phase III clinical trial, marstacimab reduced the ABR in patients with severe HA and moderate-to-severe HB without inhibitors for treating bleeds by 35% and 92% after a 12-month active treatment period, respectively.[87] Marstacimab was administered weekly with non-weight-based flat dosing as a subcutaneous 300 mg loading dose followed by 150 mg once weekly; dose adjustment to 300 mg weekly could be considered. And, no thromboembolic events were recorded in the phase III trial or long-term extension study.[89]

Emicizumab, concizumab, and marstacimab are administered via subcutaneous injection, which improves patient compliance (Figure 3). Although the blood concentration of intravenous CFCs immediately peaks following administration, the time to achieve the maximum plasma concentration of subcutaneous antibodies is as long as several days.[90,91] This accounts for why emicizumab, concizumab, and marstacimab are difficult to use for episodic treatment. To date, consensus on whether emicizumab can be used for the perioperative management of PwHA remains lacking.[92]

As the antibody structure entirely differs from that of FVIII, the hemostatic effects of emicizumab, concizumab and marstacimab are theoretically unaffected by FVIII inhibitors.[93] Clinical trials have demonstrated the effectiveness of emicizumab and concizumab in inhibitor-positive PwH (Table S1). In addition to monoclonal antibody drugs, siRNAs and recombinase therapeutics, such as fitusiran and serpinPC, with no structural homology with FVIII or FIX, are also expected to be adopted for treating inhibitor-positive PwH.[94,95]

Globally, the overall prevalence of inhibitors in PwHA or PwHB is 5–7% and 1.5–3%, respectively.[96,97] The incidence of inhibitors is significantly high at 20–40% in individuals with severe HA and 9–23% in individuals with severe HB.[96,98] For PwH who develop inhibitors, long-term, and frequent supratherapeutic CFC doses may establish peripheral tolerance to coagulation factors and eradicate inhibitors, a therapy known as ITI.[37] Both pdCFCs and rCFCs can be used for ITI in PwH.[6] The success rate of ITI is influenced by various factors.[99] In PwHA, the success rate of ITI can reach 60–70%, while in PwHB, the success rate is relatively low at 13–31%.[100,101] The success of ITI in PwH improves in patients under 7 years old[102]; the inhibitor titer is $\leq$10 BU/mL before initiating ITI, historical and ITI period peak inhibitor titers are $\leq$100 BU/mL[103]; the time between inhibitor diagnosis and ITI initiation is less than 2 years[102] However, the risk of recurrence remains even after successful ITI, a multicenter retrospective cohort study showed a relapse rate of 20–30%.[104] In cases where ITI is ineffective, recurred, or unaffordable for inhibitor-positive patients, novel non-factor replacement therapies with efficacies unaffected by inhibitors can be adopted for effective bleeding control.

Gene therapy offers long-term replacement treatment options for patients with hemophilia

If a single gene, *FVIII* or *FIX*, can be delivered into the body to achieve sustained and stable expression, a long-term increase in the activity of plasma factors can be maintained. Consequently, PwH are ideal candidates for gene therapy. Since the 1990s, clinical trials of gene therapy for hemophilia have been attempted, including the *in vitro* transfection of autologous fibroblasts followed by reinfusion, intravenous injection of retroviral vectors, and intravenous injection of adenoviral vectors.[105,106] Although unsuccessful, this represented an important attempt at gene therapy applied for treating hemophilia. In 2010, Nathwani et al. administered the first single intravenous infusion of rAAV serotype 8 vectors containing the *FIX* gene in PwHB. This increased the circulating FIX levels to 1–6% of the normal value, improving the bleeding episodes of patients in the long term, and establishing the concept of gene therapy for hemophilia.[28]

FVIII or *FIX* genes can be efficiently delivered to the liver for specific expression based on rAAV vectors. Three AAV gene therapies are effective for the expression of coagulation factors and approved by the FDA and EMA for hemophilia long-term replacement treatment. The therapies are valoctocogene roxaparvovec for PwHA; etranacogene dezaparvovec, and fidanacogene elaparvovec for PwHB. AAV is a non-enveloped, non-pathogenic parvovirus with a 4.7 kb single-stranded DNA genome.[107] AAV vectors boast a high safety profile, stable long-term expression, and the ability to accommodate relatively large exogenous DNA fragments (5.2 kb).[108] As the size of the FL-FVIII cDNA (7.0 kb) exceeds the loading capacity of AAV vectors,[109] and the B domain of FVIII does not affect its coagulation efficacy, valoctocogene roxaparvovec for PwHA only transfers the BDD-FVIII cDNA (4.97 kb) sequence.[110] Meanwhile, the FIX cDNA (1.5 kb) is relatively small and simple to express. Hence, etranacogene dezaparvovec and fidanacogene elaparvovec carry the complete FIX Padua (R338L) variant gene sequence.[111,112]

The complete rAAV vector preparation process involves three stages: upstream culture, downstream purification, and product packaging (Figure 2C). Production of valoctocogene roxaparvovec and etranacogene dezaparvovec uses the recombinant baculovirus-Sf9 insect cell (Bac-Sf9) system to express rAAV vectors.[113] In contrast, fidanacogene elaparvovec uses the HEK293 three-plasmid transfection system.[114] After the recombinant baculoviruses or plasmids are introduced into the host cells, numerous rAAV vectors are released into the cell culture growth media.[113] The supernatant is harvested for downstream purification, concentration, and filtering of the rAAV vectors to obtain the final product.[113] Once the rAAV vectors carrying the *BDD-FVIII* or *FIX* Padua gene are administered intravenously, hepatocytes consistently express FVIII or FIX for an extended period of time, effectively improving the circulating coagulation factor levels and maintaining the same factor level as that in mild-to-moderate PwH, significantly reducing the risk of bleeding.[115,116]

An open-label, multicenter, single-group phase III clinical trial was conducted with valoctocogene roxaparvovec. The results showed that, after a single infusion of rAAV5 vectors with an integrated FVIII gene, the median FVIII activity level of the participants was maintained at $\geq$ 5 IU/dL in approximately 88% and 75% of the participants at weeks 49–52 and 104, respectively.[117,118] At week 104, the average ABR and the use of FVIII concentrate decreased by approximately 77% and 98%, respectively, compared with the baseline.[118] In a dose-escalating phase I/II clinical study of valoctocogene roxaparvovec, after a single infusion of valoctocogene roxaparvovec, the median FVIII activity level was maintained at > 8 IU/dL for at least 5 years[119] The FVIII level in patients with severe hemophilia is less than 1 IU/dL (1% of normal); after receiving gene therapy, FVIII levels can increase to those of patients with mild hemophilia A (5–40 IU/dL), and remain constant for an extended period. The clinical symptoms of hemophilia are directly determined by the serum factor activity level. For untreated patients with severe and mild hemophilia A, up to 60 and less than one bleeding episode occur per year, respectively.[46]

Single-arm, open-label phase III clinical trials of etranacogene dezaparvovec and fidanacogene elaparvovec showed that compared with the use of FIX for routine prophylaxis, both gene therapies reduced the ABR and FIX usage in PwHB; the activity level of FIX remained stable for a long time.[115] At 12 and 18 months after PwHB received etranacogene dezaparvovec treatment, the average FIX activity level remained at 39% and 34%, respectively, from baseline levels.[115] After PwHB received fidanacogene elaparvovec treatment, changes in the FIX activity level from the baseline were 26% and 25% at 12 and 24 months, respectively.[120] These clinical findings suggest that for PwHB who undergo gene therapy, the activity levels of FIX remain stable over time.

Amid the rapid development of gene therapy for hemophilia, several critical issues require attention. The liver health of patients before and after gene therapy is a crucial factor that cannot be ignored; although hepatocellular carcinoma associated with gene therapy has not been observed, there is still a hypothesized risk of malignancy owing to the integration of the vector into the genome.[121] The three hemophilia gene therapies approved for marketing have shown a self-limiting increase in alanine aminotransferase (ALT) in clinical trials.[8] In a phase III clinical trial of valoctocogene roxaparvovec, approximately 86% of PwHA experienced adverse reactions associated with ALT elevation and required long-term use of corticosteroids for immunosuppressive synergistic treatment.[117] ALT levels may increase due to inflammatory or immune responses induced by AAV gene transfer and may be associated with a decline in transgene expression. Immunosuppressive treatment helps recover ALT levels but does not necessarily restore the initial elevated transgene levels. Additionally, no consensus has been reached on the dose, type, or duration of immunosuppressive treatment.[121]

Patient-specific limitations continue to pose challenges for all AAV gene therapies for hemophilia (Figure 3). These three commercially available gene therapies cannot be administered to children or women.[122–124] Additionally, gene therapy efficacy is affected by pre-existing AAV-neutralizing antibodies in patients. Analysis of 546 serum samples of PwHA worldwide indicated that the positive rate (34.8%) of pre-existing antibodies against AAV5 was the lowest,[125] possibly accounting for why

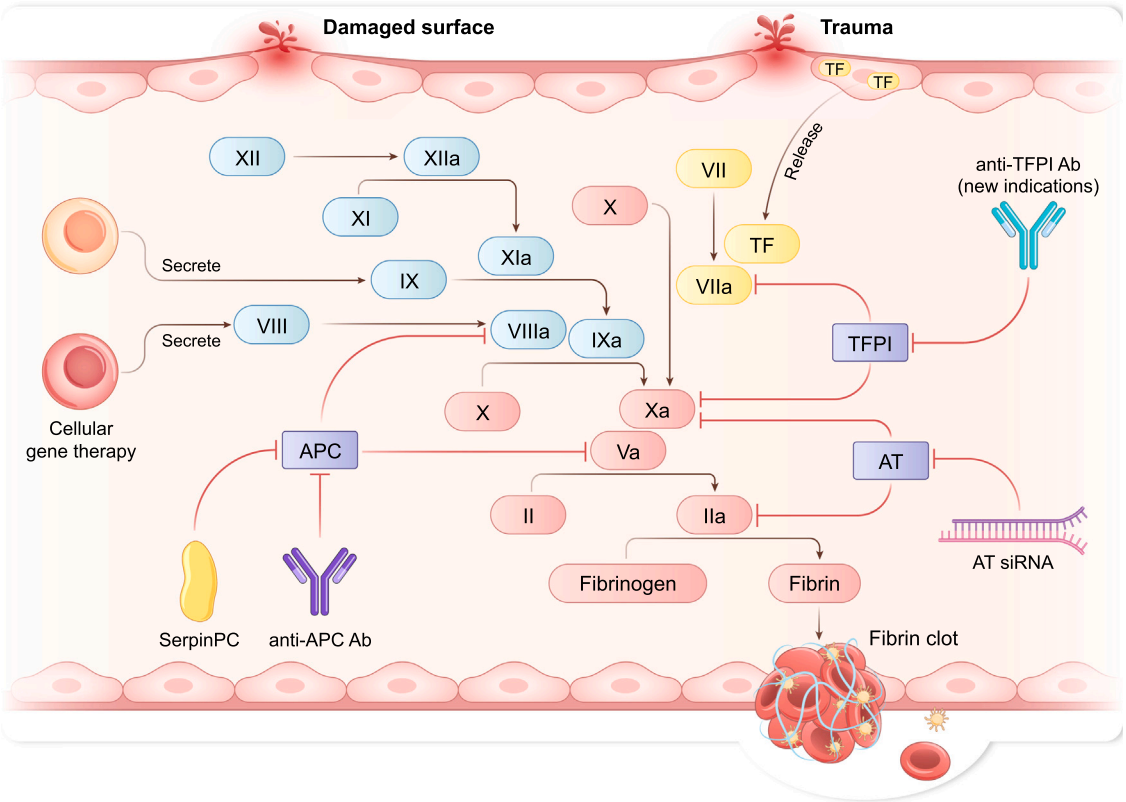

Figure 4. Innovative treatments for hemophilia associated with coagulation pathways

Interventions targeting specific processes in the coagulation cascade have shown promising results in clinical trials. Abbreviations: Ab, antibody; APC, activated protein C; AT, antithrombin; siRNA, small interfering RNA; TF, tissue factor; TFPI, tissue factor pathway inhibitor.

rAAV5 is used as a gene delivery vector for valoctocogene roxaparvovec and etranacogene dezaparvovec.

FUTURE THERAPIES FOR HEMOPHILIA

Emerging targets and advanced pharmaceutical technologies signify the future of hemophilia treatment, and cannot be ignored. In addition to traditional FVIII and FIX targets, clinical trials are assessing agents that regulate the balance between anticoagulant and procoagulant effects, including TFPI (new indications), activated protein C (APC), and antithrombin. Importantly, their efficacy is not affected by FVIII or FIX inhibitors (Figure 4; Table 1). A recently approved antibody drug targeting TFPI has shown encouraging clinical results at a once-weekly dosing frequency and is being tested clinically in inhibitor-positive PwH and specifically children with hemophilia under the age of 12 years. This is expected to expand the clinically applicable populations of anti-TFPI antibody drugs, warranting further attention.

Novel biopharmaceutical products for hemophilia, including siRNAs and cellular gene therapies, have elicited significant improvements in reducing the frequency of dosing in clinical trials (Figure 4 and Table 1). The aforementioned therapeutic drugs under development for hemophilia are designed to improve patient compliance, reduce dosing frequency, and provide potential solutions for inhibitor-positive patients.

Investigating new targets for hemophilia management and potential new indications for anti-TFPI antibody drugs

Concizumab, which targets TFPI, has been approved for marketing in Canada as a prophylactic treatment for PwHB with inhibitors. However, as its half-life is only 38 h; thus, it must be administered subcutaneously once daily.[91] The necessity for frequent dosing may be a significant factor hindering its widespread marketing approval. Although concizumab can effectively treat PwHA and PwHB with inhibitors, Health Canada has not approved concizumab for PwHA with inhibitors.[83]

The development of antibodies against TFPI will hopefully lead to the approval of hemophilia drugs with a broad spectrum of applications. Although marstacimab has been approved for marketing in the United States, the approved indications do not include PwH with inhibitors. The phase II clinical trial of marstacimab included a small number of inhibitor-positive PwH, and was found to be safe and well tolerated; moreover, the overall ABR was quite low.[88] Currently, a phase III clinical trial of marstacimab in inhibitor-positive PwH is underway, and the results may be announced by 2025.[87] Additionally, the ongoing BASIS KIDS clinical trial aims to study the safety and efficacy of marstacimab in children with hemophilia aged 1 to <18 years[87] These clinical trials will have greater significance for expanding the patient population in which marstacimab could be used.

Table 1. Exploration of new targets or pharmaceutical technologies for treating hemophilia

Product type/name	Patient population Target	Trial No.	Administration method Frequency	Latest clinical results	References
Antibody					
Marstacimab	PwHA and PwHB with and without inhibitors TFPI	NCT03363321	Subcutaneous Once-weekly	FDA has approved marstacimab for PwHA or PwHB without inhibitors, and the BASIS clinical trial for marstacimab in inhibitor-positive PwH, as well as the BASIS KIDS clinical trial in children aged 1 to <18 years with or without inhibitors, are both ongoing.	Pfizer[87] and Matino et al.[89]
SR604	PwHA and PwHB with and without inhibitors APC	NCT06349473; CTR20241608 (CDE)	Subcutaneous Once every 2 weeks (tentative)	SR604 exhibited prophylactic and therapeutic efficacy in tail-bleeding and knee-injury mouse models of HA and HB expressing human APC (pre-clinical). CDE has approved SR604 for phase I clinical trials.	Jiang et al.[126] and CDE[127]
Recombinase					
SerpinPC	PwHA and PwHB APC	NCT04073498; NCT05789524; NCT05789537	Subcutaneous Once every 2 or 4 weeks	SerpinPC treatment reduced the median all-bleed ABR to 1.0, representing a 96% reduction from the pre-exposure baseline ABR (phase I/IIa). A phase II clinical trial is underway.	Baglin et al.[95] and Centessa Pharmaceuticals[128]
Cellular gene therapy					
Auto CD34$^+$ cells transduced with LV to produce FVIII	PwHA without inhibitors FVIII	NCT03818763	Intravenous Only once	No reports of spontaneous bleeding or a need for "on-demand" factor VIII post-infusion following the demonstration of whole blood vector copy number (phase I).	Wilcox et al.[129]
BE-101 (autologous primary human B cells medicine engineered to insert the human FIX gene)	PwHB FIX	Pre-clinical	Intravenous Unknown	A single dose of BE-101 could achieve active and sustained FIX levels. The data confirmed the expected biodistribution of FIX-expressing B cells in bone marrow tissue, where they engrafted stably over time (pre-clinical). FDA has approved BE-101 to conduct phase I/II clinical trials.	Be Biopharma [130]
SiRNA					
Fitusiran	PwHA and PwHB with and without inhibitors Antithrombin	NCT03417245; NCT03417102	Subcutaneous Once-monthly	Fitusiran prophylaxis showed hemostatic efficacy and statistically significant reductions in ABR (phase III).	Young et.al.[131] and Srivastava et al.[132]

ABR, annualized bleeding rate; APC, activated protein C; CDE, the Center for Drug Evaluation of the China National Medical Products Administration; CFCs, coagulation factor concentrates; FDA, US Food and Drug Administration; FIX, factor IX; FVIII, coagulation factor VIII; HA, hemophilia A; HB, hemophilia B; LV, lentiviral vector; PwHA, patients with hemophilia A; PwHB, patients with hemophilia B; siRNA, small interfering RNA; TFPI, tissue factor pathway inhibitor.

Befovacimab, another monoclonal antibody that simultaneously targets the Kunitz 1 and Kunitz 2 domains of TFPI, was discontinued in a non-randomized, open-label phase II clinical trial owing to severe adverse reactions of thrombosis in three cases.[133] These findings suggest that the development of antibodies targeting TFPI should focus on the risk factors of thrombosis. Future studies should focus on a safe and effective window for anti-TFPI treatment to enhance patient outcomes and minimize potential adverse effects.

APC, an anticoagulant inactivating FVa and FVIIIa, can effectively degrade FVIIIa and FVa on phospholipid membranes to inhibit coagulation.[134] SerpinPC is a highly specific recombinant serine protease inhibitor that enhances thrombin activity in sites with tissue damage and shortens the coagulation time

in PwH by reducing the activity of APC in circulation.[135] So far, two phase II clinical trials have evaluated the efficacy, safety, tolerability, and pharmacokinetics of serpinPC in patients with severe HA and moderate-to-severe HB, with or without inhibitors (NCT05789524, NCT05789537). The humanized antibody SR604 targets APC to block its anticoagulant activity, as demonstrated in mouse models with hemophilia.[126] Moreover, SR604 does not induce apparent toxic effects in mice with hemophilia and demonstrates excellent bioavailability in non-human primates via subcutaneous injection.[126] Accordingly, the Center for Drug Evaluation of the China National Medical Products Administration (CDE) has approved a clinical registration trial for SR604 to prevent and treat bleeding episodes in PwHA, PwHB, and patients with congenital FVII deficiency.[127]

Recent advances in pharmaceutical technologies for treating hemophilia

Cellular gene therapy addresses hemophilia by editing cells *in vitro* and reinfusing them into the patient to produce coagulation factors (Figure 4). Currently, cellular gene therapy using lentiviral vectors is available to modify a person's own CD34-positive cells, facilitating the production of FVIII in megakaryocytes through the platelet-specific *ITGA2B* gene promoter. This approach is being evaluated in a phase I clinical trial (NCT03818763).[129]

BE-101, a cell therapy that encodes autologous B cells to express FIX, has passed the Investigational New Drug application of the FDA and is expected to be studied in a phase I/II clinical trial in the second half of 2024 to evaluate the safety and preliminary efficacy of BE-101 in patients with moderate-to-severe hemophilia.[130]

Combining new targets and new pharmaceutical technologies

The rapid development of siRNA technology has provided novel options for treating various rare diseases. siRNA drugs can selectively silence the mRNA expression of disease-related genes, thereby preventing target gene expression. These drug pipelines have high clinical translatability and shorter development timelines than conventional drugs.[136] The potential of siRNAs targeting antithrombin, protein S, and heparin cofactor II for treating hemophilia has been verified in animal models and clinical trials.[137,138]

Among these drugs, fitusiran, jointly developed by Alnylam and Sanofi, is the most advanced siRNA therapeutic for hemophilia (Figure 4). Two phase III clinical trials evaluating its prophylactic treatment efficacy and safety in patients with severe HA and HB, with or without inhibitors, are nearly complete. Clinical data show that a monthly subcutaneous injection of fitusiran significantly reduces the ABR in PwH.[131,132] As of June 2024, the application for the listing of fitusiran was accepted by the FDA and CDE.[139] Additionally, siRNA drugs are generally less expensive than conventional treatment modalities, making them potentially safe, effective, and economical treatment options for PwH.[136]

CONCLUSIONS

PwH requires lifelong treatment to limit the frequency and severity of bleeding; innovative delivery methods and biopharmaceutical products have significantly improved the quality of life and medication choices for PwH. Subcutaneous drug administration and gene therapies have alleviated the inconvenience of frequent intravenous CFC injections, and non-factor drugs allow inhibitor-positive PwH to select treatments without inhibitor restrictions.

However, there remains a risk of not effectively managing thromboembolism or thrombotic microangiopathies if non-factor hemostatic drugs are used during breakthrough bleeding or perioperative management. This risk is indicated by a black box warning on the package insert that comes with emicizumab.[79] Gene therapies aim to provide effective and long-term replacement treatment options for PwH with a single intravenous treatment, eliminating the need for regular prophylaxis. However, strategies are required to reduce associated costs. Challenges with pre-existing immunity to AAV vectors and the applicability of treatment to minors and female patients remain to be addressed. Extensive clinical testing is also warranted to determine treatment longevity and safety.

To improve treatment outcomes and reduce adverse effects, researchers are developing siRNA drugs, recombinases, and cellular gene therapies. Developing innovative biopharmaceutical products and allowing clinical use may positively impact market prices and national bidding behavior, benefiting more PwH.[140] In low- and middle-income countries, there remains an unmet clinical demand for traditional replacement therapies, with CFC usage per capita significantly lower than that in high-income countries.

As clinicians gain more experience with CFCs and new biopharmaceutical products, a growing interest in personalized hemophilia treatments has emerged. The pharmacokinetics of CFCs in PwH can significantly vary, with half-lives differing by 2- to 4-fold between individuals.[141] Existing dosage calculations and intervals are unsuitable for all patients; and therefore, prophylactic treatment should be guided by the actual pharmacokinetics of the individual PwH.[141–143] Non-factor gene therapies and monoclonal antibody drugs have individualized control measures for safety and effectiveness. Gene therapies require consistent long-term monitoring of multiple liver enzymes post-administration, with regimens selected based on individual results.[122–124] Concizumab requires pre-dose plasma concentration measurements 4 weeks following treatment initiation to determine maintenance doses.[91]

The focus on hemophilia treatment has driven progress in biotechnology, leading to breakthroughs in gene therapy and bispecific antibody pharmaceuticals. Research and development of related drugs and personalized prophylactic treatments will offer valuable insights into treating other single-gene diseases.

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AUTHOR CONTRIBUTIONS

D.Y. and J.Z.W. conceptualized the review. J.Z.W. and X.L.L. drafted the article. J.Z.W. X.L.L., H.C.Y., and Y.L.H. organized the data. All authors read and approved the final article.

DECLARATION OF INTERESTS

J.Z.W., X.L.L., and D.Y. are paid employees of Chengdu Rongsheng Pharmaceuticals Co., Ltd. H.C.Y is an employee of China National Biotec Group Company Limited. D.Y. and Y.L.H are employees of Beijing Tiantan Biological Products Co., Ltd. These companies are involved in the development of rFVIII-Fc, rFVIIa, pdFVIII, and pdFIX. The views presented here should not be considered endorsements of any specific products or company.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

No AI was used in the preparation of this review.

SUPPLEMENTAL INFORMATION

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