

Diagnosis and Treatment of Hemophilia

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Abstract: Hemophilia A and B are inherited bleeding disorders characterized by deficiency or dysfunction of coagulation protein factors VIII and IX, respectively. Recurrent joint and muscle bleeds are the major clinical manifestations. Replacement therapy with clotting factors, either at the time of bleeding or as part of a prophylactic regimen, is adapted to individual patient needs. The major complication of therapy is the development of neutralizing antibodies. In response, researchers have developed novel agents to both reduce the treatment burden and prevent bleeding regardless of the presence of inhibitors. Another new development, gene therapy, has the potential for a definitive cure. This review summarizes the pathophysiology, clinical presentation, diagnosis, and treatment of hemophilia, as well as information regarding neutralizing antibodies, immune tolerance induction, novel agents, and gene therapy.

Introduction

Hemophilia is an inherited bleeding disorder caused by deficiency or dysfunction of the coagulation proteins factor VIII (FVIII), leading to hemophilia A, or factor IX (FIX), leading to hemophilia B. Together, hemophilia A and B affect approximately 1 in 5000 males. Females who inherit one affected X chromosome can have bleeding symptoms. Over the past 50 years, remarkable success in the treatment of hemophilia has been achieved through modifications of therapy, especially the development of more tailored therapy. In the recent past, the main focus for research in hemophilia became safer and more efficacious factor replacement therapies. Now, however, the focus is shifting toward the development of novel nonreplacement therapeutics and to curative approaches such as gene therapy. As a result, patients with hemophilia—even those with the most severe form—can expect to lead a nearly normal life free of arthropathy if they live in a developed country. In contrast, the majority of patients in developing nations receive little to no treatment and experience early mortality and significant morbidity.¹⁻⁵

Keywords

Factor replacement, hemophilia, hemophilic arthropathy, novel agents

Factor VIII/IX and the Coagulation Cascade

Nature has evolved a highly complex system that balances the procoagulant, anticoagulant, and fibrinolytic systems that function together to maintain blood within the vasculature in a fluid state, while also providing the rapid assembly of a thrombus upon vascular damage. This process is characterized by the sequential activation of 3 vitamin K–dependent serine proteases (FVII, FIX, and FX) and their cofactor complexes (tissue factor, FVIII, and FV). FVIII acts as an essential cofactor for FIX in the intrinsic coagulation cascade, amplifying FIX activity. FVIII is a heterodimer composed of a heavy chain and a light chain that is stabilized by noncovalent interactions between the light chain and von Willebrand factor (VWF). This VWF interaction stabilizes FVIII and inhibits FVIII binding to phospholipids, thereby increasing the half-life of FVIII.^{6,7} Mutations in *F8/F9* genes lead to either deficiency of factors or impaired factor functions, resulting in either hemophilia A or B, respectively.

Clinical Presentation and Diagnosis

The clinical presentations of hemophilia A and B are largely similar, given that the main target site for bleeding is the joints.⁸ The literature contains conflicting data; some studies show that patients with hemophilia B have a lower bleeding frequency than those with hemophilia A,^{9–11} whereas others show similar phenotypic severity.¹² Spontaneous bleeding, which is a hallmark of the disease, occurs in the joints in 70% to 80% of the episodes.^{13,14} The most frequent site of nontraumatic (spontaneous) intra-articular bleeding in both children and adults is the ankle, followed by the elbow and the knee.¹⁴ Approximately half of children with severe hemophilia have a muscle bleed or hematoma by 6 to 8 months of age, when physical activity increases.¹⁵ In severe hemophilia, even spontaneous muscle hemorrhage may occur in the lower legs, buttocks, iliopsoas muscle, and forearms.¹⁶ Recurrent joint bleeds induce a cascade of inflammatory and degenerative processes injuring the synovium, cartilage, and bone. Such joints are referred to as target joints, and they are formally defined by the International Society on Thrombosis and Haemostasis as a joint that has 3 or more spontaneous bleeds within a consecutive 6-month period. Blood in the joint space induces inflammatory changes of the synovium and degenerative changes of the cartilage, which occur simultaneously. The key stimulant for these changes is iron released into the synovial fluid, which is both pro-inflammatory and pro-angiogenic.^{17–19} Neovascularization leads to the formation of new friable vessels, which are more prone to bleeding. This leads to a vicious circle of bleeding, iron accumulation, synovial hypertrophy, and

further bleeding, ultimately leading to permanent joint damage. This does not lead to the same condition in the long-term in all patients, however. Some patients develop chronic synovitis with joint swelling caused by synovial inflammation and hypertrophy but without clear cartilage damage, whereas others develop hemophilic arthropathy with significant osteochondral damage without synovitis.²⁰ Hemophilic arthropathy leads to pain, loss of range of motion, and muscle atrophy, resulting in loss of activities, which further decreases quality of life in patients with hemophilia.^{21,22} Chronic synovitis is more frequent in patients in resource-limited countries, although it can still occur in patients in resource-rich countries despite prophylactic clotting factor replacement therapy. Prophylaxis is the current standard of care, and is generally effective in preventing hemophilic arthropathy.^{21,23}

Another major bleeding complication in hemophilia is intracranial hemorrhage, which is potentially life-threatening. The largest cohort study of surveillance for intracranial hemorrhage in hemophilia is based on data from the Universal Data Collection project in the United States, which included more than 10,000 patients with hemophilia who were followed from ages 1 to 12 years. This study found an incidence of intracranial hemorrhage of 1.9%, a frequency of 390 events per 10⁵ patient-years, and an intracranial hemorrhage mortality rate of 19.6%.²⁴ Prophylactic replacement of FVIII/IX is the optimal treatment regimen for patients with hemophilia, as described above, and is likely to be effective at preventing intracranial hemorrhage as well.^{21,25–27}

The diagnosis of hemophilia is relatively straightforward and when it is suspected, measurement of FVIII or FIX clotting activity will nearly always reveal the diagnosis. It should be noted that all the vitamin K–dependent factors, including FIX, are reduced at birth, whereas FVIII levels are normal (even elevated) at birth. Thus, hemophilia A can be diagnosed immediately after birth even from a sample of cord blood,^{28,29} whereas mild hemophilia B may be difficult to diagnose at birth. In such cases, measurement of the FIX level needs to be repeated after age 6 months.³⁰ Hemophilia is classified into 3 main forms: severe, moderate, and mild, depending on the residual coagulant activity in blood (FVIII:C/FIX:C; Table 1). Patients with a coagulation factor level of less than 1 IU/dL are classified as severe, and constitute about half of diagnosed cases. Moderate hemophilia is defined as factor levels of 1 to less than 5 IU/dL, and mild disease as 5 to less than 40 IU/dL.³¹ Age at presentation, symptoms at presentation, rate of joint bleeds, and inhibitor development may change depending on the patient's factor level.

Identification of the gene mutation is also important because it may help prognosticate the risk of inhibitor formation, and can also identify female relatives

Table 1. Clinical Manifestations of Different Severities of Hemophilia

	Severe	Moderate	Mild
Factor level	<1%	1% to <5%	5% to <40%
Age at presentation	Birth to 3 years	Birth to 10 years	5 to >21 years
Presentation	<ul style="list-style-type: none"> • Family history (prenatal or postnatal screening) • Neonatal bleeding (circumcision, heel sticks) • Bruising (<1 year) • Vaccine-related bleed • Mucosal bleed • Joint bleed 	<ul style="list-style-type: none"> • Family history (prenatal or postnatal screening) • Neonatal bleeding (less likely than in severe cases) • Vaccine-related bleed • Mucosal bleed • Joint bleed 	<ul style="list-style-type: none"> • Post-traumatic bleed • Postsurgical bleed
Risk for inhibitor development	<ul style="list-style-type: none"> • ~25% in FVIII • ~5% in FIX 	~1% to 2%	Very rare
Risk for hemophilic arthropathy	Universal without prophylaxis	Very common without prophylaxis	Rare

FIX, factor IX; FVIII, factor VIII.

who might be carriers. Large deletions and nonsense mutations carry the highest risk for inhibitor formation, whereas missense and splicing mutations carry the lowest risk.³² In patients with severe hemophilia A, detection of inversions of intron 22 (reported in 40%-45% of patients with severe disease) and intron 1 (reported in 1%-6% of patients with severe disease) of the *F8* gene are the most common mutations, and thus are usually looked for first, followed by full gene sequencing if they are negative.^{33,34} In moderate and mild hemophilia A, because of the absence of common gene defects, full mutation analysis of the *F8* gene by direct Sanger sequencing is necessary.³⁰ For hemophilia B, sequence analysis is performed on 8 exons, intron-exon boundaries, and the promoter region in *F9*. Unlike in hemophilia A, no common frequent genetic variation occurs.

Factor Replacement Therapy

Prophylaxis refers to treatment with factor concentrate to prevent bleeding and joint destruction, with the aim of maintaining normal musculoskeletal function. Primary prophylaxis refers to the initiation of prophylaxis prior to or shortly after the first joint bleed and requires 2 to 3 infusions per week depending on the factor concentrate, whereas secondary prophylaxis begins after the onset of joint disease.^{35,36} In the past, fixed doses were used for prophylaxis. Now, however, dosing regimens adapted to individual patient needs, known as tailored regimens, are the new direction of treatment. This implies using individual pharmacokinetics with computer-simulated doses and intervals to achieve a predetermined trough activity level.³⁷ On-demand treatment refers to infusion

of the deficient clotting factor at the time of bleeding. It is clear that prophylaxis in patients with severe hemophilia (without inhibitors) is effective at preventing bleeding.^{25,27} In mild forms of hemophilia A, the synthetic vasopressin analogue desmopressin acetate can be used to increase plasma concentrations of FVIII and VWF via intravenous, intranasal, or subcutaneous administration.³⁸

One of the major limitations of factor concentrates is the need to infuse them intravenously and relatively frequently owing to their short half-lives (8-12 hours for FVIII and 18-24 hours for FIX). Bioengineering technology has developed extended half-life recombinant clotting factors with PEGylation or the fusing of factors to another protein with a much longer half-life, such as the fragment crystallizable (Fc) region of immunoglobulin G (IgG) or human albumin, both of which delay lysosomal degradation of the factor and recycle them back into circulation.³⁹⁻⁴² These strategies increase half-life by 3 to 6 times for recombinant FIX and by roughly 1.5 to 1.6 times for recombinant FVIII.

Diagnosis and Management of Neutralizing Antibodies: Inhibitors and Bypassing Agents/Immune Tolerance Therapy

The development of alloantibodies neutralizing factor VIII/IX coagulant activity (inhibitors) represents the main complication of factor treatment of hemophilia, occurring in approximately one-third of previously untreated patients with hemophilia A and in approximately 1% to 5% of those with hemophilia B.^{30,43,44} Development of alloantibodies is associated with increased mortality and

morbidity and decreased quality of life in patients with hemophilia. An inhibitor is a polyclonal high-affinity IgG that is directed against the FVIII protein.⁴⁵ FVIII inhibitors consist of a polyclonal population of antibodies that are targeted to antigenic sites within the A2, A3, and C2 domains of the protein; these epitope targets can change over time.⁴⁶ Two types of inhibitors exist. Type 1 inhibitors completely inactivate FVIII, which is more common in severe hemophilia, and type 2 inhibitors incompletely inactivate FVIII, which is more common in inhibitor patients with mild hemophilia or in patients without hemophilia who develop acquired FVIII inhibitors. In addition to inhibitors, anti-FVIII antibodies are present in healthy individuals and in patients with hemophilia A without exerting coagulant inhibitory activity.⁴⁷ The difference between neutralizing and non-neutralizing antibodies was demonstrated by Hofbauer and colleagues, indicating that anti-FVIII IgG with inhibitory activity has an up to 100-fold higher affinity for FVIII than IgG without inhibitory activity.⁴⁸ Once inhibitors develop, eradication becomes the main goal. Although most inhibitors are either transient or resolve with immune tolerance therapy (further discussed below), 15% of patients with hemophilia A and 3% of those with hemophilia B have persistent inhibitors.⁴⁹ Immune tolerance therapy, which is successful in 70% of patients, requires large amounts of frequently administered factor for many months or even years.^{50,51} Several therapeutic regimens exist for immune tolerance therapy, ranging from low doses of factor given every other day to much larger doses given daily and regimens that incorporate immunosuppressive therapy along with factor infusions (Malmo regimen).⁵¹ Some have advocated using a FVIII product that contains von Willebrand factor because some studies have demonstrated improved outcomes with this approach.⁵²

Patients with high-titer inhibitors frequently develop serious bleeding complications that do not respond to standard factor replacement therapy. Therefore, they must be treated with the bypassing agents recombinant FVIIa (NovoSeven RT; Novo Nordisk) and activated prothrombin complex concentrates (FEIBA, Shire), neither of which is as effective as standard factor replacement in patients without inhibitors.⁵³ Despite these limitations of recombinant FVIIa and FEIBA, these agents have been demonstrated to be effective in the treatment of bleeding and in the prevention of bleeding during surgery. They are less effective, however, at preventing spontaneous and trauma-associated bleeding. Furthermore, they must be infused intravenously over a long period once or twice daily (activated prothrombin complex concentrates) or multiple times a day (recombinant FVIIa), thus imparting a significant treatment burden.^{54,55}

Unmet Needs in Treatment

Several unmet needs exist in the management of hemophilia. Despite the advent of factor replacement therapies and the use of prophylaxis, bleeds still occur, some with serious consequences. In addition, joint disease has not been eradicated, and even in patients with rare bleeding events, the treatment burden of prophylaxis remains a significant problem, even with the extended half-life products. In addition, there is a need for improved therapies to reduce mortality and morbidity in patients with inhibitors, as well as to reduce the treatment burden and improve quality of life.

Between 1999 and 2013, no novel therapies were introduced into the field of hemophilia. However, several new agents (one that is currently available, and others that are in clinical trials) are beginning to address unmet needs. Before factor treatment, patients with hemophilia were spending most of their time in hospitals. Treatment has changed dramatically since the “factor revolution,” enabling patients with hemophilia to treat themselves at home with prophylaxis and lead more normal lives. But now another revolution is taking place, which is the development of products that are not factor replacements. These novel agents have the potential to both reduce the treatment burden and prevent bleeding, regardless of the presence of inhibitors. The next revolution to come is gene therapy, which offers the prospect of curing hemophilia.

Novel Agents and Gene Therapy

Novel therapeutic approaches include bispecific antibodies that mimic the coagulation function of FVIII, inhibition of anticoagulation proteins such as antithrombin with an RNA interference molecule, and tissue factor pathway inhibitor (TFPI) with monoclonal antibodies (Figure). In addition, phase 3 clinical trials of gene therapy have begun for both hemophilia A and B.

Emicizumab-kxwh (Hemlibra, Genentech/Roche) is a novel therapeutic bispecific antibody that mimics the function of FVIII by bringing FIXa and FX together to produce the tenase complex. The tenase complex is essential to the generation of FXa, which in turn is crucial for thrombin generation and hemostasis.^{56,57} It is administered subcutaneously and has been studied in weekly, every-other-week, and every-4-week regimens.

Emicizumab prophylaxis has been studied in a series of trials called the HAVEN trials. HAVEN 1 (A Study to Evaluate the Efficacy, Safety, and Pharmacokinetics of Prophylactic Emicizumab Versus No Prophylaxis in Hemophilia A Participants With Inhibitors) and HAVEN 2 (A Study of Emicizumab Administered Subcutaneously in Pediatric Participants With Hemophilia

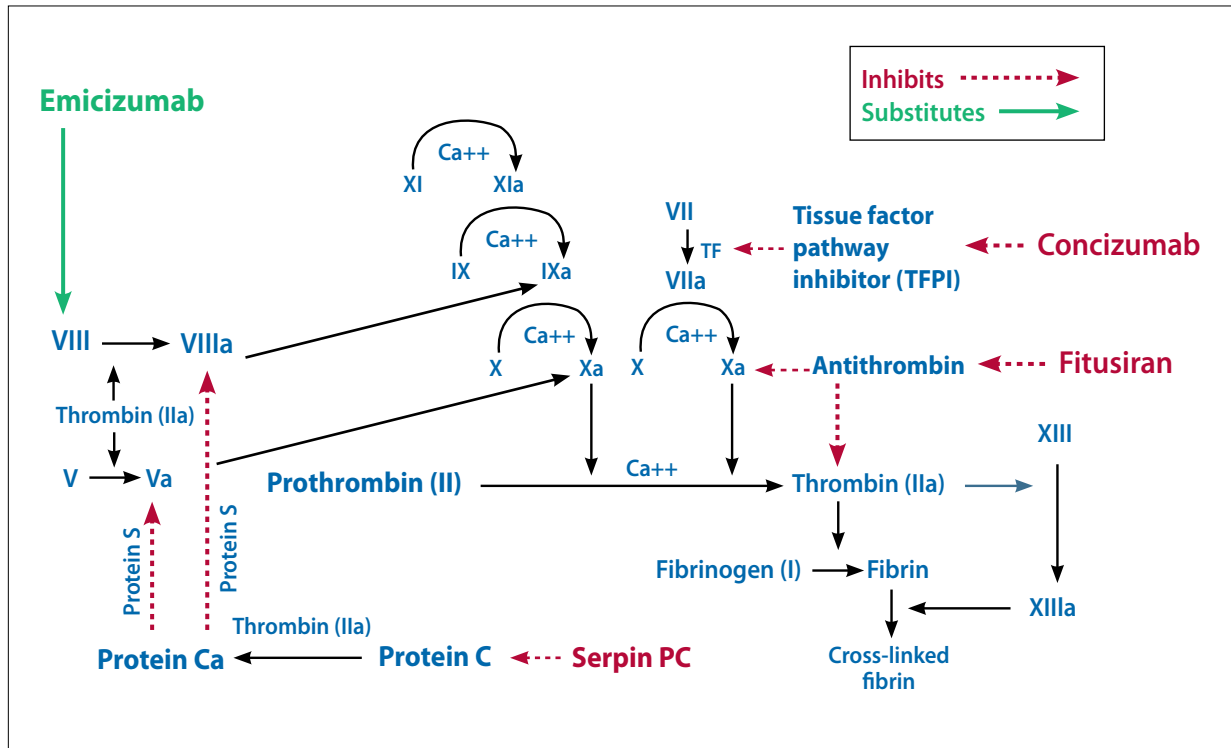


Figure. Novel therapeutics: concizumab, emicizumab, fitusiran, and serpin PC.

A and Factor VIII Inhibitors) were trials in patients with inhibitors who were 12 years and older and younger than 12 years, respectively. Both trials demonstrated significant reductions in annual bleed rates when compared with episodic therapy or prophylaxis with bypassing agents.^{58,59} Serious adverse events were reported in HAVEN 1; a total of 5 participants developed either thrombotic microangiopathy or thrombosis. All of these events occurred when high cumulative doses of activated prothrombin complex concentrates for breakthrough bleeding were given concomitantly with emicizumab. This led to emicizumab having a black box warning regarding the concomitant use of these medications. In addition, it was noted that 3 patients (~1.5%) developed anti-drug antibodies to emicizumab with neutralizing potential, with 1 participant discontinuing emicizumab as a result.^{59,60}

The HAVEN 3 trial (A Clinical Trial to Evaluate Prophylactic Emicizumab Versus No Prophylaxis in Hemophilia A Participants Without Inhibitors) studied emicizumab in patients 12 years and older without inhibitors, and also showed a significant reduction in bleeding compared with episodic therapy and a more modest but important reduction in bleeding compared with FVIII prophylaxis.⁶¹ This study did not report any thrombotic microangiopathy or thrombotic events.

The HAVEN 4 trial (A Study to Evaluate the Efficacy, Safety, Pharmacokinetics, and Pharmacodynamics of Emicizumab Given Every 4 Weeks in Participants With Hemophilia A) evaluated every-4-week dosing in both inhibitor and noninhibitor patients 12 years and older and demonstrated an equivalent efficacy to the weekly or every-other-week dosing schedules studied in the other trials.⁶² Seven deaths have been reported in patients on emicizumab (1 death in the HAVEN trials and 6 deaths with compassionate use of the drug), although none of them were deemed related to the medication.

As a result of the trials above, emicizumab has been approved by the US Food and Drug Administration (FDA) for the prevention of bleeding in patients of all ages with hemophilia A with and without inhibitors. It has also been licensed in numerous other countries for patients with inhibitors, with a noninhibitor indication pending. The approved dosing regimens include weekly, every-other-week, or every-4-week dosing.

Concizumab, which is being developed by Novo Nordisk, is another novel therapeutic. It is a humanized monoclonal antibody that inhibits domain 2 of the TFPI molecule.⁶³ TFPI, one of the natural anticoagulants, is a Kunitz-type serine protease inhibitor consisting of 3 domains. Domain 1 binds to the tissue factor–activated factor VIIa (TF-FVIIa) complex, domain 2 acts on FXa,

and domain 3 interacts with protein S.⁶⁴ In vitro and in vivo studies have demonstrated the high-affinity binding of concizumab to all forms of TFPI (free and cell surface-bound).^{63,65} A phase 1 study has been completed,⁶⁶ and a phase 2 study has been initiated and will evaluate the safety, pharmacodynamics, and pharmacokinetics of concizumab administered at increasing doses subcutaneously in participants with hemophilia A (NCT02490787). No serious adverse events or antibodies to concizumab have been reported.⁶⁶

Another natural anticoagulant is antithrombin, which is the natural inhibitor of thrombin and FXa. One approach to decreasing production of antithrombin is the use of small interfering RNA, which interferes with post-transcriptional expression of the antithrombin protein. Fitusiran, which is being developed by Alnylam and Sanofi Genzyme, utilizes this technology to suppress the hepatic synthesis of antithrombin.⁶⁷ The phase 1 dose-escalation study enrolled 4 healthy volunteers and 25 participants with moderate or severe hemophilia A or B who did not have inhibitory alloantibodies. Healthy volunteers received a single subcutaneous injection of fitusiran or placebo, whereas participants with hemophilia received 3 injections of fitusiran administered either once weekly or once monthly. As a result, once-monthly subcutaneous administration of fitusiran resulted in dose-dependent lowering of the antithrombin level and increased thrombin generation in participants with hemophilia A or hemophilia B who did not have inhibitory alloantibodies.⁶⁸ Rebalancing the hemostasis can also be achieved by suppression of another natural anticoagulant, activated protein C (APC). A serpin (serine protease inhibitor) was developed to specifically inhibit APC. The biggest concerns of this novel drug are blocking APC's anti-inflammatory and cytoprotective role. However, APC-specific serpin is unlikely to affect the anti-inflammatory functions of APC, and long-term follow-up is therefore needed to test this treatment.⁶⁹

The goal of gene therapy is to cure hemophilia such that patients no longer need to be concerned about bleeding, and do not need any factor replacement therapy. Successful gene therapy results in endogenous expression of the clotting factor, leading to steady-state levels and a sustained duration of action. This would liberate individuals from prophylaxis and the need for regular intravenous infusions. In addition, it is postulated that endogenous expression of factor could be less immunogenic because it would have altered interaction with the immune system and could potentially even be a more effective tolerizing therapy in those with inhibitors.⁷⁰ Gene therapy offers an opportunity for one-time intervention and, if it allows for discontinuation of prophylaxis, could result in enormous cost savings over the course of a lifetime (depending on how it is priced), as current costs are estimated to be more

than \$300,000 per year (mostly for the cost of the clotting factor concentrates).⁷¹ In addition, more than 75% of individuals in the developing world have limited or no access to any factor replacement therapy, so gene therapy could be an intervention that could dramatically alter the outcomes for hemophilia patients around the world.⁷² The primary tools for gene transfer have included nonviral vectors, retroviral vectors, adenoviral vectors, lentiviral vectors, and adeno-associated virus vectors, which are currently the vectors of choice for hemophilia A and B.⁷³ Academic proof of concept for hemophilia gene therapy was achieved in a clinical trial for hemophilia B.⁷⁴ The success of this clinical trial has driven an explosion of activity in hemophilia gene therapy programs across the world. The smaller size of the FIX complementary DNA (cDNA) sequence (1.4 kb) compared with the FVIII cDNA sequence (4.4 kb) has allowed researchers to more easily identify suitable vectors to transfer the FIX cDNA into potential target cells, making the overall progress of gene therapy more rapid with hemophilia B compared with hemophilia A (at least in the earlier trials). Techniques such as B domain deletion and codon optimization of the FVIII molecule have been successfully employed to overcome the mismatch in the size of the FVIII cDNA and the capacity of the adeno-associated virus vector. Several clinical trials of gene therapy are ongoing for hemophilia A and B (Table 2).

Conclusion

Over the past 50 years, the diagnosis and treatment of hemophilia have improved considerably. The half-life limitations of factor concentrates have led to the development of new extended half-life factors aimed at reducing the treatment burden while maintaining efficacy and safety. Although the half-lives of recombinant factor IX products have been extended by 3 to 6 times, the prolongation of recombinant factor VIII's half-life remains only partly successful, with an increase of roughly 1.5 to 1.6 times. Furthermore, the recent development and FDA approval of the first nonfactor product could significantly alter the hemophilia treatment landscape and significantly improve the management of patients with inhibitors, and more new nonfactor molecules are in development. Finally, gene therapy offers the potential for a cure. Thus, the future of hemophilia treatment looks bright. We hope to accomplish the current mission of the World Federation of Hemophilia: treatment for all. Perhaps one day, we will see a cure for all.

Disclosures

Dr Kizilocak has no disclosures. Dr Young has received honoraria and consulting fees from Alnylam, Bayer, Bioverativ,

Table 2. Current Clinical Trials of Hemophilia A and B Gene Therapy

Disease	Identifier	Intervention	Phase	Sponsor(s)
HA	NCT03001830	AAV2/8-HLP-FVIII-V3	1	University College London ^a
HA	NCT03003533	SPK-8011 (recombinant AAV)	1/2	Spark Therapeutics
HA	NCT03061201	SB-525 (AAV2/6)	1/2	Sangamo Therapeutics
HA	NCT03370913	Valoctocogene roxaparvovec	3	BioMarin Pharmaceutical
HA	NCT03370172	BAX 888	1/2	Shire
HB	NCT03369444	FLT180A	1	University College London ^a
HB	NCT03307980	SPK-9001	2	Pfizer
HB	NCT02695160	SB-FIX	1	Sangamo Therapeutics
HB	NCT03569891	AAV5-hFIXco-Padua	3	UniQure Biopharma BV

AAV, adeno-associated virus; HA, hemophilia A; HB, hemophilia B.

^a US location: St Jude Children's Research Hospital

CSL Behring, Genentech/Roche, Grifols, Kedrion, Novo Nordisk, Shire, Spark, and UniQure.

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