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REVIEW

Novel treatments for hemophilia through rebalancing of the coagulation cascade

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Abstract

Hemophilia A and B are inherited hemorrhagic disorders that result from alterations in the coagulation cascade. Aside from spontaneous bleeding, the main complication of hemophilia is hemarthrosis. Progress over the last three decades, specifically prophylaxis using recombinant factor, has prevented hemarthrosis and lengthened patient life expectancies. However, many treatments require frequent dosing up to three times a week, and alloantibodies (inhibitors) against replacement factor continues to be an issue. These problems call for novel treatments for patients with hemophilia. Although there has been progress in extended half-life factors and mimetics of factor VIII, an alternative treatment methodology is to rebalance the activities of pro- and anticoagulant factors through inhibition of the natural anticoagulants: antithrombin, tissue factor pathway inhibitor, protein C, and protein S. This review will explore the efficacy of targeting these inhibitory pathways from preclinical development through clinical trials, and delve into concerns of thrombotic risk.

KEYWORDS
activated protein C, anticoagulant, antithrombin, hemophilia A, hemophilia B, protein S, tissue factor pathway inhibitor

1 | INTRODUCTION

Hemostasis is critical for survival, which is why vertebrate evolution developed a system for the cessation of bleeding, the coagulation cascade. Individuals who have dysfunction in procoagulants are at increased risk of death due to hemorrhage, and the most common deficiencies in secondary hemostasis are in factors VIII (FVIII) and IX (FIX), resulting in hemophilia A and B, respectively.[1] Hemophilia A affects about one in every 5000 males,[2] and hemophilia B affects about one in 25,000 males.[3] Both can be categorized into severe, moderate, or mild disease, based on the baseline coagulant protein activity of less than 1%, 1–5%, and 6–40%, respectively. Spontaneous bleeding and hemarthrosis may occur frequently for patients with severe hemophilia, whereas symptoms may occur only with trauma in patients with mild and moderate disease. Patients with severe hemophilia often show symptoms as early as the neonatal period, such as circumcision bleeding or intracranial hemorrhage.[4] As they become mobile, infants and children with severe hemophilia bruise easily and start to develop joint hemorrhages (hemarthrosis).[5] Repeated hemarthrosis into the same joint can result in a "target joint" that becomes increasingly susceptible to repeated bleeds. Eventually, patients with chronic hemarthroses develop self-fulfilling cycles of reduced mobility and secondary muscular atrophy with even more risk of rebleeding.[6,7]

1.1 | Current treatments for hemophilia

Following the diagnosis, most hemophilia treatment centers in developed countries recommend prophylactic treatment for patients with severe disease.[8] The efficacy of these prophylactic treatments is

Abbreviations: APC, activated protein C; AT, antithrombin; FIX, factor IX; FVa, activated factor V; FVII, factor VII; FVIII, factor VIII; FX, factor X; K1, Kunitz-1; K2, Kunitz-2; PCI, protein C inhibitor; PS, protein S; rFVIIa, recombinant FVIIa; RNAi, RNA interference; TFPI, tissue factor pathway inhibitor; α1AT, α1-antitrypsin

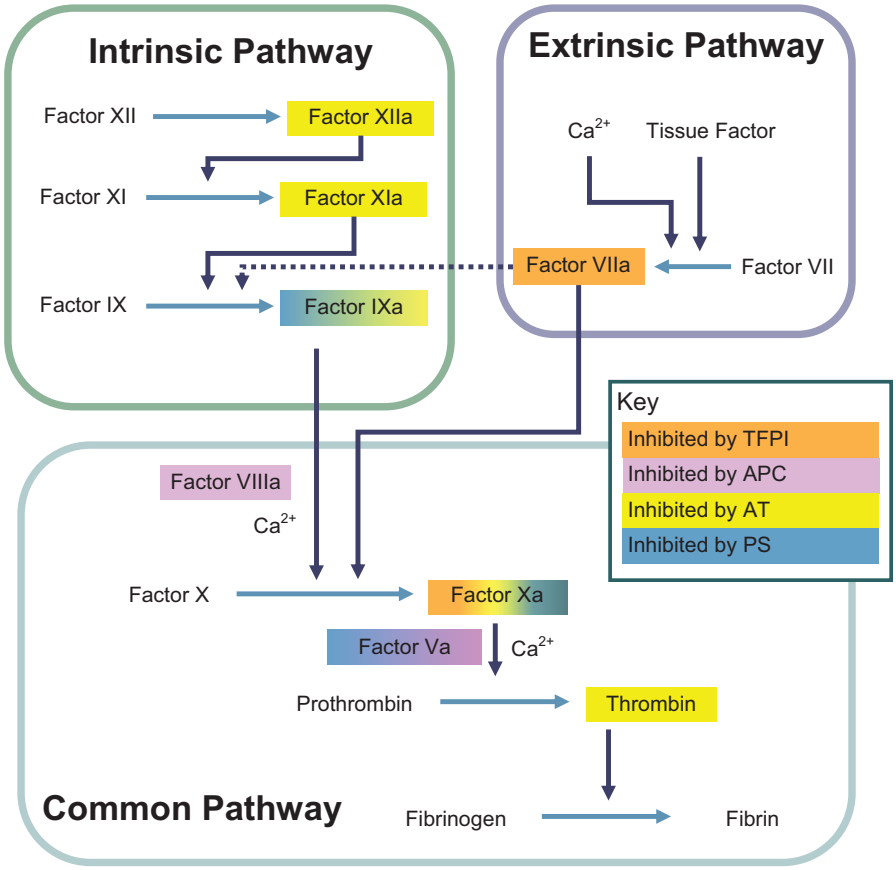

FIGURE 1 Anticoagulants selected for rebalancing and their respective procoagulant targets. APC, activated protein C; AT, antithrombin; PS, protein S; TFPI, tissue factor pathway inhibitor

impressive, with earlier studies showing a 40% reduction in mortality.[9] In addition, prophylaxis has been proven to prevent hemarthrosis.[10] However, these treatments are limited by cost,[11] frequency,[12] as well as the development of alloantibodies, otherwise known as inhibitors.[13] Frequent intravenous infusions (two to three times per week) often put a strain on patients and families, especially in young children for whom intravenous access is difficult.[12] Surgically implanted venous access devices ease the burden of infusions, but carry increased rates of infection and even thrombosis.

Besides the cost and frequency of treatment, the main complication of factor replacement is the development of inhibitors that interfere with therapy through direct inhibition or accelerated clearance of infused factor.[8] Approximately 20–30% of patients with severe hemophilia A develop inhibitors, although this rate is lower for patients with severe hemophilia B. The standard treatment for patients with inhibitors had been bypassing agents, including recombinant factor VIIa (rFVIIa) and activated prothrombin complex concentrates (aPCCs), which are so named because treatment circumvents the need for both FVIII and FIX. Unfortunately, these treatments are not as effective as factor replacement. Furthermore, aPCCs are often contraindicated in hemophilia B patients with inhibitors because of development of anaphylaxis against FIX-containing products. In 2017, the FDA approved emicizumab, a bispecific antibody that substitutes for FVIIIa by enhancing the interaction of FIXa and FX together.[14] This new drug has led to emicizumab becoming the de facto standard of care for hemophilia A patients with inhibitors; unfortunately, there is no counterpart for hemophilia B patients with inhibitors.

Coagulation factor inhibitors are a major problem for patients with hemophilia and necessitate innovative therapies. Since hemophilia patients have a deficiency in procoagulant factors, tipping the scale toward bleeding, inhibition of the anticoagulant factors, protein C (PC), protein S (PS), tissue factor pathway inhibitor (TFPI), and antithrombin (AT),[15–17] has been exploited to rebalance coagulation.

1.2 | The anticoagulants

Upon activation by thrombin, PC is a serine protease that along with its cofactor PS inhibits FVIIIa and activated factor V (FVa) (Figure 1). TFPI is a serine protease inhibitor that primarily inhibits the tissue factor: VIIa complex and activated factor X (FXa).[18] There are two human isoforms of TFPI, TFPIα and β, with the former being more potent. Both isoforms contain Kunitz-1 (K1) and Kunitz-2 (K2) domains, inhibiting FVIIa and FXa, respectively. TFPIα additionally has a Kunitz-3 (K3) domain, which facilitates interaction with PS to further enhance FXa inhibition.[19] By itself, PS can directly inhibit FXa, FVa, and FIXa.[20] AT,

the most abundant anticoagulant circulating in plasma,[21] is the primary inhibitor of thrombin; however, it can also inhibit FIXa, FXa, factor XIa (FXIa), and factor XIIa (FXIIa).[22]

The focus of this review is to summarize the novel nonfactor therapies in the preclinical and clinical pipelines that rebalance the coagulation cascade through inhibition of the natural anticoagulants. We will not discuss other nonfactor therapies that mimic FVIII, such as emicizumab, which has been reviewed elsewhere.[23]

2 | INHIBITION OF ANTITHROMBIN

AT is the primary inhibitor of thrombin and other procoagulants, including FIXa and FXa (Figure 1).[17] Inhibition of AT as a treatment strategy was inspired by two observations. AT deficiency in FVIII-deficient mice decreases bleeding compared to those without AT deficiency.[24] Additionally, hemophilia patients who coinherit AT deficiency have been found to have less severe bleeding phenotypes than similar patients with normal levels of AT.[25,26] Fitusiran, also known as ALN-AT3SC, employs RNA interference (RNAi) technology to specifically target AT mRNA in hepatocytes, the site of synthesis and release into the bloodstream (Table 1).[27] This specificity of delivering RNAi to the liver is facilitated by *N*-acetylgalactosamine-conjugated small interfering RNAs.[28]

Initial preclinical studies in hemophilic *FVIII* knockout mice found that those treated with fitusiran displayed significantly improved hemostasis, and were indistinguishable from mice treated with 25 IU/kg recombinant hFVIII treatment.[29] High doses of fitusiran were able to normalize the activated partial thromboplastin time (aPTT) in hemophilic mice, but at moderate doses wild type mice developed disseminated intravascular coagulation (DIC). These results indicate that very low AT concentrations achieved with fitusiran increase the risk of coagulopathy, highlighting the need for careful control of levels.

Once the efficacy of fitusiran was well established in animal studies,[29] clinical trials were initiated in hemophilia A and B patients.[30] A phase 1 dose-escalation study was performed on normal subjects without a history of thrombophilia, as well as hemophilia patients without inhibitors using weekly to monthly dosing. Dose-dependent decreases in AT level were observed as low as 89% below baseline, as well as increases in thrombin activity. Hemophiliacs with about 25% of baseline AT had peak thrombin generation within the normal range. This result showed that fitusiran is able to increase thrombin production and potentially restore hemostasis.

Phase 2 trial results have not been published yet, but preliminary data have been shared.[26] The most promising results lie with hemophilic patients with inhibitors. AT levels were decreased by 80% without evidence of thrombosis. Patients previously receiving on-demand factor therapy with an average annualized bleeding rate (ABR) of 12 were reduced to an ABR of 1.7 during fitusiran prophylaxis. Although not a primary outcome measure, 67% of patients did not experience spontaneous bleeds.[26]

These promising results propelled fitusiran into phase 3 clinical trials in order to further evaluate safety and efficacy. Unfortunately, there

TABLE 1 Summary of therapies in clinical trials

	Fitusiran	Concizumab	PF-06741086
Mechanism of action	siRNA inhibition of AT[26,28,29]	Antibody inhibition of TFPI K2 domain[41–43]	Antibody inhibition of TFPI K2 domain[48]
Status	Phase 3 trial[25]	Phase 2 trial[43]	Phase 2 trial[53]
Dosing	Weekly/monthly, subcutaneous[25,29]	Daily, intravenous or subcutaneous[43]	Weekly, subcutaneous[53]
Clinical trial data	Reduced ABR[25] Adverse events: injection-site erythema and pain Serious adverse events: cerebral venous sinus thrombosis, elevated transaminases	Dose-dependent increase in D-dimer, peak thrombin, and ETP[42] Adverse events: headache, flu symptoms, fatigue,[43] nonfatal thrombotic events	Significantly reduced ABR compared to on-demand treatment group[53] Adverse events: injection-site erythema and pain

Abbreviations: ABR, annualized bleeding rate; ETP, endogenous thrombin potential; siRNA, small-interfering RNA; TFPI, tissue factor pathway inhibitor.

have been serious events associated with these studies. A patient suffered from a fatal cerebral venous sinus thrombosis during the open-label extension of the phase 2 study.[31] The patient was incorrectly diagnosed with subarachnoid hemorrhage, which resulted in treatment with excessive FVIII concentrate. The trial was temporarily paused for Alnylam to develop risk mitigation guidelines surrounding breakthrough bleeding, and then restarted a few months later.[32] Other adverse events have included asymptomatic and transient elevation of transaminase levels in 36% of participants.[30] Majority of these participants had a medical history of hepatitis infection and had not received curative treatment. Researchers were unable to identify any dose–response relationships, since these participants were in different dosing groups. Fitusiran was in phase 3 clinical trial; however, due to new adverse events, the nature of which has not yet been revealed, dosing has been suspended until further notice.[33]

Until recently, RNAi was the only method that had been used to inhibit AT activity. However, a recent preclinical study has shown the possibility of using llama-derived single-domain antibodies (also known as nanobodies) that inhibit AT (Table 2).[34] Nanobodies were evaluated through injection of recombinant protein into hemophilia A mice, as well as delivery via adeno-associated viral vectors into hemophilia B mice. Both methods demonstrated that the nanobodies were able to reduce blood loss and did not elicit inhibitory immune responses.

3 | INHIBITING TISSUE FACTOR PATHWAY INHIBITOR

While there is only one clinical therapeutic in development for inhibition of AT, there are several mechanisms that have reached clinical trials for modulation of TFPI. There have been four inhibitors developed that target either the K1 or K2 domain of both isoforms of TFPI: BAX499, concizumab, BAY 2093884, and PF-06741086 (Tables 1 and 2).

3.1 | BAX499

BAX499 is able to inhibit full-length TFPIα through binding to multiple domains.[35] BAX499, formerly known as ARC19499, is an aptamer, which is an oligonucleotide that can fold into a three-dimensional structure.[36] It decreases TFPI inhibition of FXa activity by up to 70%.[37] Ex vivo studies of plasma from hemophilia A and B patients resulted in corrected endogenous thrombin potential and peak thrombin, and BAX499 also decreased bleeding times in primate models of hemophilia A generated through injection of anti-FVIII antibody.[37]

In 2010, BAX499 entered a phase 1 clinical trial based on the success of preclinical studies; however, the trial ended prematurely when patients given BAX499 had an increased number of bleeding events.[38] At the highest dose, plasma studies showed a 25-fold increase of full-length TFPI, likely due to decreased clearance. Due to the increase rate of bleeding events, further development of BAX499 was canceled. Although preclinical studies confirmed that inhibition of TFPI can pro-

TABLE 2 Summary of preclinical targeted therapies

	sdAB	HS02-52G	KRK α1AT	BAY 1896052	GalNac-PS
Mechanism of action	Llama-derived single-domain antibody against AT	DNA aptamer inhibition of APC	Engineered serpin with anti-APC activity	Antibody targeted to exosite of APC	Small interfering RNA targeting the *Pros1* gene in hepatocytes
Ex vivo results	Increased thrombin production[34]	Decreased clotting times[57]	High specificity for APC[64]	Increased ETP and decreased clotting times[66]	Improved ROTEM[69]
In vivo results	Decreased total blood loss in mice[34]	Not performed	Decreased total blood loss in tail clip assays[64]	Decreased total bleeding time in monkeys[66]	Reduced intra-articular bleeding, knee joint swelling[69]

Abbreviations: APC, activated protein C; AT, antithrombin; ETP, endogenous thrombin potential; sdAB, single-domain antibodies.

mote hemostasis, these clinical data are a cautionary note of the risks of anticoagulant factor manipulation.

3.2 | Concizumab

Concizumab is an IgG4 isotype antibody, which binds and inhibits the K2 domain of TFPI with high specificity, blocking the binding of FXa (Table 1).[39] Cynomolgus monkey studies indicate that part of the action is through increased TFPI clearance,[40] but this also leads to the potential that concizumab may need to be administered daily or every other day. One safety concern is coadministration of procoagulant factor products, especially bypassing agents for inhibitor patients. Preclinical animal studies have shown that although use of FVIIIa along with concizumab did lead to increased D-dimer and thrombin–antithrombin complexes (TAT), there were no signs of symptomatic coagulopathy, such as thrombosis.[41]

Explorer 1 was a multicenter, randomized, double-blind, placebo-controlled, and single dose-escalation study that included 28 healthy individuals and 24 hemophilia patients.[42] The results demonstrated that concizumab had a procoagulant effect due to overall dose-dependent increases in D-dimer. In addition to its positive procoagulant effect, there were no serious adverse events reported, and no anticoncizumab antibodies were found. In a phase 1b trial (Explorer 3), concizumab plasma concentrations were measured against peak thrombin generation, and a sigmoidal relationship was found with thrombin generation potential reaching the normal range.[43,44]

These studies were followed by two phase 2 trials, Explorer 4 for inhibitor patients and Explorer 5 for noninhibitor patients.[45] The trials were multicenter and open-label, and patients in both trials received daily subcutaneous injections, with options to increase the dosage for breakthrough bleeding. In the inhibitor trial, the resulting median ABR was 4.5 for patients on concizumab prophylaxis and 20.4 for patients with on-demand use of rFVIIa. In the noninhibitor trial, the average ABR was 7.0, and in both trials, clinical proof of concept was successfully demonstrated.

There are two additional trials in progress, Explorer 7 and Explorer 8, both investigating the safety and efficacy of concizumab as prophylaxis with an injectable pen device for delivery in patients with or without inhibitors. These trials launched in late 2019; however, phase 3 trials were briefly halted for 5 months due to nonfatal thrombotic events in three patients.[46]

3.3 | BAY 1093884 and marstacimab

In addition to concizumab, there are two other inhibitory antibodies against TFPI: BAY 1093884[47] and PF-06741086 (marstacimab).[48] BAY 1093884 binds and inhibits both the K1 and K2 domains of TFPI.[47] There have been two phase 1 clinical trials, the first one assessing pharmacokinetics in patients with severe hemophilia,[49] and the second investigating the safety and tolerability in single escalating and multiple doses.[50] The results of these phase 1 trials have not been published yet. A phase 2 clinical trial was planned to assess the safety and tolerability of weekly subcutaneous dosing.[51] However, this phase 2 trial was terminated due to an unacceptable frequency of thrombosis in the study population.

Marstacimab is an antibody that specifically inhibits the K2 domain of TFPI.[48] A phase 1 clinical trial investigated the safety, tolerability, pharmacokinetics, and pharmacodynamics in healthy volunteers,[52] and a phase 2 trial studied severe hemophilia A and B subjects, with and without inhibitors.[53] Overall, ABRs were significantly lowered with marstacimab prophylaxis.[53] A phase 3 crossover study to compare marstacimab with current methods of treatment for patients with or without inhibitors is currently recruiting.[54] An additional phase 2 trial will investigate the long-term treatment of severe hemophilia patients.

4 | ACTIVATED PROTEIN C (APC) INHIBITION

Compared to the aforementioned treatments, inhibitors of APC are still in the early stages of development. APC is a serine protease that acts as an inhibitor of FVa and FVIIIa (Figure 1).[18] Similar to the rationale to develop inhibitors against other anticoagulants, APC inhibition derived from the observation that the FV Leiden mutation appears to modify bleeding risk in severe hemophilia.[55] This mutation leads to a reduced ability of APC to inactivate FVa, which is associated with a procoagulant phenotype and risk of thrombosis. In addition, impaired APC activity also increases thrombin generation.[56] There are three types of inhibitors in development for APC: an aptamer, an engineered APC-specific inhibitor, and a synthetic peptidomimetic inhibitor. These are all in early development, so little is known about the specificity and toxicity of these inhibitors (Table 2).

4.1 | HS02-52G, a DNA aptamer

HS02-52G is a DNA aptamer that functions by binding the heparin-binding region to inhibit APC anticoagulant function (Table 2).[57] HS02-52G also enhances protein C inhibitor (PCI) activity in inactivating APC. After PCI forms a complex with APC, HS02-52G is quickly released. Because this aptamer only binds to the heparin-binding region of APC, it is able to selectively inhibit APC anticoagulant functions, which can potentially reduce side effects. In ex vivo plasma studies, HS02-52G was able to block APC inactivation of FVIIIa and decrease clotting times in a dose-dependent fashion.[58] One of the benefits of using an aptamer is that an antidote can be easily designed through the use of complementary oligonucleotides. Such an antidote was shown to reverse the effects of HS02-52G on thrombin generation. More studies need to be conducted on the safety and efficacy of this potential treatment in vivo.

4.2 | KRK α1-antitrypsin (serine protease inhibitor)

KRK α1AT was developed through a novel approach by engineering variants into the endogenous serine protease inhibitors (serpins) that inhibit APC,[59] including PCI[60] and α1-antitrypsin (α1AT) (Table 2).[61] APC recognizes a stretch of the exposed reactive center loop of these serpins, forming a complex.[62] However, these endogenous serpins have poor specificity for APC; for example, PCI is also able to inhibit thrombin,[63] and APC-specific reactive center loops were engineered.[64]

To improve the efficacy and specificity of APC inhibition, lysine variants of PCI and α1AT were initially produced and found to be more specific for APC, although functioned at a decreased rate.[64] To alleviate this issue, the α1AT-Pittsburgh mutations were used as the initial template along with the additional lysine variants. α1AT-Pittsburgh itself is a potent inhibitor of APC, but it also has high specificity toward thrombin and FXa. The end product, KRK α1AT, has a high specificity for APC compared to thrombin, FXa, and FXIa. KRK α1AT was able to increase thrombin generation ex vivo and restore hemostasis in hemophilia B mice. Major strengths of KRK α1AT include a half-life of 5–7 days and subcutaneous administration. It likely has a low immunogenic potential, because homologous endogenous serpins are already circulating in plasma. Recruitment has begun for a phase 1a trial in the United Kingdom.[65]

4.3 | BAY 1896052, monoclonal antibody

Recently, scientists at Bayer developed two monoclonal antibodies derived from human antibody Fab fragments (Table 2).[66] The type I antibody specifically binds to the active site, and the type II (BAY 1896052) antibody binds to an exosite on APC. Both were found to have limited interaction with unactivated PC. Because the type II inhibitor was only binding the exosite, it was able to both preserve the cytoprotective function of the APC and improve hemostasis in hemophilia A cynomolgus monkeys.

5 | INHIBITION OF PROTEIN S

PS acts as a cofactor for both APC and TFPI anticoagulant activities,[18] so its inhibition can potentially affect multiple procoagulant pathways. Study of the clinical application of PS inhibition is still relatively new, and thus far there is one proof-of-concept study in a mouse model.[67] Mice with a knockout of the PS gene (*Pros1*) were bred with hemophilia A and B mice. In tail bleeding models, hemophilic mice with complete loss of PS demonstrated significantly less blood loss with mild injury assays. Using an injury-induced acute hemarthrosis model,[68] hemophilia A mice with complete loss of PS were indistinguishable from animals with normal levels of FVIII and PS. This group went on to design a GAlNac siRNA using the same technology as fitusiran, to target *Pros1* gene expression in hepatocytes.[69] Mice treated with siRNA showed reductions in both liver PS mRNA and plasma PS protein, and clotting studies were improved in hemophilia A mice. The injury-induced acute hemarthrosis model showed that siRNA injection was able to reduce intra-articular bleeding and knee joint swelling in the hemophilia model. Unlike fitusiran, PS siRNA-treated animals did not show evidence for DIC. Although not as well developed as other anticoagulants, these data support PS inhibition as a potential treatment for patients with hemophilia.

6 | DISCUSSION/CONCLUSION

Hemophilia is a chronic bleeding disorder that has shown considerable progress in treatment over the last half century. Until the last decade this had primarily consisted of factor replacement, allowing patients to lead nearly normal lives. However, these treatments have downsides, primarily consisting of a burden of intravenous infusions several times per week and development of inhibitors against replacement factors. Emicizumab, a chimeric bispecific humanized antibody that is administered subcutaneously weekly to monthly, is increasingly being adopted by hemophilia A patients with and without inhibitors and addresses both concerns. Hemophilia B care has been revolutionized by long-acting FIX replacement products, enabling infusions on a once weekly basis. And gene therapy for both is in phase 3 trials, with FDA approval expected in the next 2 years. As long as there are no unexpected complications to these options, and given the concerns of thrombosis with some of the rebalancing therapies, it seems likely that the adoption of the latter will be limited.

However, there remains a number of patient groups that still lack highly effective therapies and may be ideal for the rebalancing treatments described above. These include hemophilia B patients with inhibitors and rare coagulation factor disorders for which there are no available concentrates or recombinant factors; for example, deficiencies of prothrombin, factor V, XI, and fibrinogen, and possibly Glanzmann thrombasthenia and severe factor VII deficiency as well. These patients are limited to either bypassing agents or plasma products, approaches that are only partially effective and only slightly better than the state of therapy for noninhibitor hemophilia patients prior to the development of cryoprecipitate and factor concentrates. The rebalancing nonfactor therapies presented in this review could serve as potential treatments for these individuals, and some have already shown efficacy in clinical studies that include hemophilia patients with inhibitors. If these products are successful, this could lead to a more personalized approach to managing these orphan diseases.

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CONFLICT OF INTEREST

Yakun Zhao declares no conflict of interest. Angela C. Weyand has been a consultant for Takeda, Bayer, Sanofi, Genentech, Aptevo, and Kedrion. Jordan A. Shavit has been a consultant for Bayer, Takeda, Sanofi, CSL Behring, Spark Therapeutics, and NovoNordisk.

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REFERENCES

1. Shavit J, Ginsburg D. Hemophilias and other disorders of hemostasis. In: *Emery and Rimoins Principles and Practice of Medical Genetics*. Elsevier Science; 2013:1–33.
2. Soucie JM, Evatt B, Jackson D. Occurrence of hemophilia in the United States. *Am J Hematol*. 1998;59(4):288–294.
3. Amy D, Shapiro MD. *Hemophilia B - NORD (National Organization for Rare Disorders)*. Published 2018. https://rarediseases.org/rare-diseases/hemophilia-b/. Accessed June 2, 2019.
4. Carcao M, Moorehead P, Lillicrap D. *Hematology: Basic Principles and Practice*. 7th ed. Elsevier; 2018.
5. Lobet S, Hermans C, Lambert C. Optimal management of hemophilic arthropathy and hematomas. *J Blood Med*. 2014;5:207–218.
6. Pietri MM, Frontera WR, Pratts IS, Súarez EL. Skeletal muscle function in patients with hemophilia A and unilateral hemarthrosis of the knee. *Arch Phys Med Rehabil*. 1992;73(1):22–28.
7. Blanchette VS, Key NS, Ljung LR, Manco-Johnson MJ, Van Den Berg HM, Srivastava A. Definitions in hemophilia: communication from the SSC of the ISTH. *J Thromb Haemost*. 2014;12(11):1935–1939.
8. Franchini M. The modern treatment of haemophilia: a narrative review. *Blood Transfus*. 2013;11(2):178–182.
9. Soucie JM, Nuss R, Evatt B, et al. Mortality among males with hemophilia: relations with source of medical care. The Hemophilia Surveillance System Project Investigators. *Blood*. 2000;96(2):437–442.
10. Manco-Johnson MJ, Abshire TC, Shapiro AD, et al. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. *N Engl J Med*. 2007;357(6):535–544.
11. United States Government Accountability Office. *Medicare: High-Expenditure Part B Drugs*. 2012.
12. Srivastava A, Brewer AK, Mauser-Bunschoten EP, et al. Guidelines for the management of hemophilia. *Haemophilia*. 2013;19(1):e1–e47.
13. Witmer C, Young G. Factor VIII inhibitors in hemophilia A: rationale and latest evidence. *Ther Adv Hematol*. 2013;4(1):59–72.
14. Blair HA. Emicizumab: a review in haemophilia A. *Drugs*. 2019;79(15):1697–1707.
15. Dahlbäck B. Novel insights into the regulation of coagulation by factor V isoforms, tissue factor pathway inhibitorα, and protein S. *J Thromb Haemost*. 2017;15(7):1241–1250.
16. Esmon CT. The protein C pathway. *Chest*. 2003;124(3 Suppl):26S–32S.
17. Jesty J, Lorenz A, Rodriguez J, Wun Tc. Initiation of the tissue factor pathway of coagulation in the presence of heparin: control by antithrombin III and tissue factor pathway inhibitor. *Blood*. 1996;87(6):2301–2307.
18. Smith SA, Travers RJ, Morrissey JH. How it all starts: initiation of the clotting cascade. *Crit Rev Biochem Mol Biol*. 2015;50(4):326–336.
19. Ndonwi M, Tuley EA, Broze GJ. The Kunitz-3 domain of TFPI-α is required for protein S-dependent enhancement of factor Xa inhibition. *Blood*. 2010;116(8):1344–1351.
20. Pilli VS, Plautz W, Majumder R. The journey of protein S from an anticoagulant to a signaling molecule. *JSM Biochem Mol Biol*. 2016;3(1):1014.
21. Opal SM, Kessler CM, Roemisch J, Knaub S. Antithrombin, heparin, and heparan sulfate. *Crit Care Med*. 2002;30(5 Suppl):S325–S331.
22. Palta S, Saroa R, Palta A. Overview of the coagulation system. *Indian J Anaesth*. 2014;58(5):515–523.
23. Weyand AC, Pipe SW. New therapies for hemophilia. *Blood*. 2019;133(5):389–398.
24. Bolliger D, Szlam F, Suzuki N, Matsushita T, Tanaka K. Heterozygous antithrombin deficiency improves in vivo haemostasis in factor VIII-deficient mice. *Thromb Haemost*. 2010;103(06):1233–1238.
25. Shetty S, Vora S, Kulkarni B, et al. Contribution of natural anticoagulant and fibrinolytic factors in modulating the clinical severity of haemophilia patients. *Br J Haematol*. 2007;138(4):541–544.
26. Machin N, Ragni MV. An investigational RNAi therapeutic targeting antithrombin for the treatment of hemophilia A and B. *J Blood Med*. 2018;9:135–140.
27. Ragni MV, Georgiev P, Mant T, et al. Fitusiran, an investigational RNAi therapeutic targeting antithrombin for the treatment of hemophilia: updated results from a Phase 1 and Phase 1/2 extension study in patients without inhibitors. *Blood*. 2016;128(22):2572.
28. Nair JK, Willoughby JLS, Chan A, et al. Multivalent N-acetylgalactosamine-conjugated siRNA localizes in hepatocytes and elicits robust RNAi-mediated gene silencing. *J Am Chem Soc*. 2014;136(49):16958–16961.
29. Sehgal A, Barros S, Ivanciu L, et al. An RNAi therapeutic targeting antithrombin to rebalance the coagulation system and promote hemostasis in hemophilia. *Nat Med*. 2015;21(5):492–497.
30. Pasi KJ, Rangarajan S, Georgiev P, et al. Targeting of antithrombin in hemophilia A or B with RNAi therapy. *N Engl J Med*. 2017;377(9):819–828.
31. World Federation of Hemophilia. *Alnylam Suspends Fitusiran Dosing due to Thrombotic Event in Phase 2 Open-Label Extension Study*. https://news.wfh.org/alnylam-suspends-fitusiran-dosing-due-thrombotic-event-phase-2-open-label-extension-study/. Accessed December 12, 2020.
32. World Federation of Hemophilia. *Update: FDA Lifts Suspension of Fitusiran Trial*. https://news.wfh.org/update-fda-lifts-suspension-fitusiran-trial/. Accessed December 12, 2020.
33. World Federation of Hemophilia. *Global Dosing Hold in Fitusiran Trials Initiated by Sanofi Genzyme to Investigate New Adverse Events*. https://news.wfh.org/global-dosing-hold-in-fitusiran-trials-initiated-by-sanofi-genzyme-to-investigate-new-adverse-events/. Accessed December 12, 2020.
34. Barbon E, Ayme G, Mohamadi A, et al. Single-domain antibodies targeting antithrombin reduce bleeding in hemophilic mice with or without inhibitors. *EMBO Mol Med*. 2020;12(4):e11298.
35. Waters EK, Genga RM, Thomson HA, et al. Aptamer BAX499 mediates inhibition of tissue factor pathway inhibitor via interaction with multiple domains of the protein. *J Thromb Haemost*. 2013;11(6):1137–1145.
36. Chang J-Y, Chantrathammachart P, Monroe DM, Key NS. Studies on the mechanism of action of the aptamer BAX499, an inhibitor of tissue factor pathway inhibitor. *Thromb Res*. 2012;130(3):e151–e157.
37. Waters EK, Genga RM, Schwartz MC, et al. Aptamer ARC19499 mediates a procoagulant hemostatic effect by inhibiting tissue factor pathway inhibitor. *Blood*. 2011;117(20):5514–5522.
38. Dockal M, Pachlinger R, Hartmann R, et al. Biological explanation of clinically observed elevation of TFPI plasma levels after treatment with TFPI-antagonistic aptamer BAX 499. *Blood*. 2012;120(21):1104.
39. Hilden I, Lauritzen B, Sørensen BB, et al. Hemostatic effect of a monoclonal antibody mAb 2021 blocking the interaction between FXa and TFPI in a rabbit hemophilia model. *Blood*. 2012;119(24):5871–5878.

40. Agersø H, Overgaard RV, Petersen MB, et al. Pharmacokinetics of an anti-TFPI monoclonal antibody (concizumab) blocking the TFPI interaction with the active site of FXa in Cynomolgus monkeys after iv and sc administration. *Eur J Pharm Sci*. 2014;56:65–69.
41. Lauritzen B, Olling J, Abel KL, et al. Administration of recombinant FVIIa (rFVIIa) to concizumab-dosed monkeys is safe, and concizumab does not affect the potency of rFVIIa in hemophilic rabbits. *J Thromb Haemost*. 2019;17(3):460–469.
42. Chowdary P, Lethagen S, Friedrich U, et al. Safety and pharmacokinetics of anti-TFPI antibody (concizumab) in healthy volunteers and patients with hemophilia: a randomized first human dose trial. *J Thromb Haemost*. 2015;13(5):743–754.
43. Eichler H, Angchaisuksiri P, Kavakli K, et al. A randomized trial of safety, pharmacokinetics and pharmacodynamics of concizumab in people with hemophilia A. *J Thromb Haemost*. 2018;16(11):2184–2195.
44. Eichler H, Angchaisuksiri P, Kavakli K, et al. Concizumab restores thrombin generation potential in patients with haemophilia: pharmacokinetic/pharmacodynamic modelling results of concizumab phase 1/1b data. *Haemophilia*. 2019;25(1):60–66.
45. Shapiro AD, Angchaisuksiri P, Astermark J, et al. Subcutaneous concizumab prophylaxis in hemophilia A and hemophilia A/B with inhibitors: phase 2 trial results. *Blood*. 2019;134(22):1973–1982.
46. Hemophilia News Today. *Novo Nordisk Pauses 3 Clinical Trials of Concizumab Amid Safety Concerns*. https://hemophilianewstoday.com/2020/03/18/novo-nordisk-pauses-three-clinical-trials-of-concizumab-due-to-safety-concerns/. Accessed April 23, 2020.
47. Gu J-M, Zhao X-Y, Schwarz T, et al. Mechanistic modeling of the pharmacodynamic and pharmacokinetic relationship of tissue factor pathway inhibitor-neutralizing antibody (BAY 1093884) in Cynomolgus monkeys. *AAPS J*. 2017;19(4):1186–1195.
48. Parng C, Singh P, Pittman DD, et al. Translational pharmacokinetic/pharmacodynamic characterization and target-mediated drug disposition modeling of an anti–tissue factor pathway inhibitor antibody, PF-06741086. *J Pharm Sci*. 2018;107(7):1995–2004.
49. Bayer. *A Study to Assess Pharmacokinetics and Pharmacodynamics Following Administration of BAY1093884 in Patients With Severe Hemophilia*. Published 2019. https://clinicaltrials.gov/ct2/show/NCT03481946?term=BAY1093884&rank=1. Accessed August 11, 2020.
50. Bayer. *A Single Escalating Dose and Multiple Dose Study of BAY 1093884 in Subjects With Severe Hemophilia Types A or B, With or Without Inhibitors*. Published 2018. https://clinicaltrials.gov/ct2/show/NCT02571569?term=BAY1093884&rank=3. Accessed August 11, 2020.
51. Bayer. *A Multiple Escalating Dose Study of BAY1093884 in Adults With Hemophilia A or B With or Without Inhibitors*. Published 2019. https://clinicaltrials.gov/ct2/show/NCT03597022. Accessed August 11, 2020.
52. Cardinal M, Kantaridis C, Zhu T, et al. A first-in-human study of the safety, tolerability, pharmacokinetics and pharmacodynamics of PF-06741086, an anti-tissue factor pathway inhibitor mAb, in healthy volunteers. *J Thromb Haemost*. 2018;16(9):1722–1731.
53. Pfizer. *PF-06741086 Multiple Dose Study in Severe Hemophilia*. Published 2018. https://clinicaltrials.gov/ct2/show/NCT02974855?term=PF-06741086&rank=3. Accessed August 11, 2020.
54. Pfizer. *Study of the Efficacy and Safety PF-06741086 in Adult and Teenage Patients With Severe Hemophilia A or B*. Published 2019. https://clinicaltrials.gov/ct2/show/NCT03938792?term=PF-06741086&rank=1. Accessed August 11, 2020.
55. Arbini AA, Mannucci PM, Bauer KA. Low prevalence of the factor V Leiden mutation among "severe" hemophiliacs with a "milder" bleeding diathesis. *Thromb Haemost*. 1995;74(5):1255–1258.
56. Bos MHA, Meijerman DWE, Van Der Zwaan C, Mertens K. Does activated protein C-resistant factor V contribute to thrombin generation in hemophilic plasma? *J Thromb Haemost*. 2005;3(3):522–530.
57. Müller J, Isermann B, Dücker C, et al. An exosite-specific ssDNA aptamer inhibits the anticoagulant functions of activated protein C and enhances inhibition by protein C inhibitor. *Chem Biol*. 2009;16(4):442–451.
58. Hamedani NS, Rühl H, Zimmermann JJ, et al. In vitro evaluation of aptamer-based reversible inhibition of anticoagulant activated protein C as a novel supportive hemostatic approach. *Nucleic Acid Ther*. 2016;26(6):355–362.
59. Polderdijk SGI, Baglin TP, Huntington JA. Targeting activated protein C to treat hemophilia. *Curr Opin Hematol*. 2017;24(5):446–452.
60. Elisen MG, von dem Borne PA, Bouma BN, Meijers JC. Protein C inhibitor acts as a procoagulant by inhibiting the thrombomodulin-induced activation of protein C in human plasma. *Blood*. 1998;91(5):1542–1547.
61. Heeb MJ, Griffin JH. Physiologic inhibition of human activated protein C by alpha 1-antitrypsin. *J Biol Chem*. 1988;263(24):11613–11616.
62. Huntington JA, Read RJ, Carrell RW. Structure of a serpin–protease complex shows inhibition by deformation. *Nature*. 2000;407(6806):923–926.
63. Rezaie AR, Cooper ST, Church FC, Esmon CT. Protein C inhibitor is a potent inhibitor of the thrombin–thrombomodulin complex. *J Biol Chem*. 1995;270(43):25336–25339.
64. Polderdijk SGI, Adams TE, Ivanciu L, Camire RM, Baglin TP, Huntington JA. Design and characterization of an APC-specific serpin for the treatment of hemophilia. *Blood*. 2017;129(1):105–113.
65. *The Safety and Tolerability of SerpinPC in Healthy Men and in Men With Severe Blood Disorders (Haemophilia A and B)*. https://clinicaltrials.gov/ct2/show/NCT04073498. Accessed October 22, 2020.
66. Zhao X-Y, Wilmen A, Wang D, et al. Targeted inhibition of activated protein C by a non-active-site inhibitory antibody to treat hemophilia. *Nat Commun*. 2020;11(1):2992.
67. Prince R, Bologna L, Manetti M, et al. Targeting anticoagulant protein S to improve hemostasis in hemophilia. *Blood*. 2018;131(12):1360–1371.
68. Øvlisen K, Kristensen AT, Valentino LA, Hakobyan N, Ingerslev J, Tranholm M. Hemostatic effect of recombinant factor VIIa, NN1731 and recombinant factor VIII on needle-induced joint bleeding in hemophilia A mice. *J Thromb Haemost*. 2008;6(6):969–975.
69. Prince RE, Schaeper U, Dames S, et al. Targeting protein S using small interfering RNA is well tolerated and protects mice with hemophilia A from acute hemarthrosis. *Blood*. 2020;136(Supplement 1):20–21.

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