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STATE OF THE ART REVIEW

Exploring nonreplacement therapies' impact on hemophilia and other rare bleeding disorders

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Abstract

The management of hemophilia, von Willebrand disease (VWD), and rare coagulation disorders traditionally relied on replacement therapies, such as factor concentrates, to address clotting factor deficiencies. However, in recent years, the emergence of non-replacement therapies has shown promise as an adjunctive approach, especially in hemophilia, and also for patients with VWD and rare bleeding disorders.

This review article offers an overview of nonreplacement therapies, such as FVIII-mimicking agents and drugs aimed at rebalancing hemostasis by inhibiting natural anticoagulants, particularly in the management of hemophilia. The utilization of non-replacement therapies in VWD and rare bleeding disorders has recently attracted attention, as evidenced by presentations at the International Society on Thrombosis and Haemostasis 2023 Congress. Nonreplacement therapies provide alternative methods for preventing bleeding episodes and enhancing patients' quality of life, as many of them are administered subcutaneously and allow longer infusion intervals, resulting in improved quality of life and comfort for patients.

KEYWORDS
hemophilia, mimic factor, nonreplacement therapy, rare bleeding disorders, rebalancing therapy, von Willebrand disease

1 | INTRODUCTION

Bleeding disorders constitute a heterogeneous group of rare inherited conditions characterized by defects in hemostasis, leading to an increased susceptibility to bleeding that affects both males and females. Estimates from the World Federation of Hemophilia latest annual global survey for the year 2022 reveal that 427,685 people worldwide suffer from bleeding disorders [1]. Eighty-four percent of these individuals are afflicted by hemophilia A or B or von Willebrand disease (VWD), while 16% have rare bleeding disorders (RBDs), including inherited deficiencies of fibrinogen, factor (F)II, FV, FV + FVIII combined, FVII, FX, FXI, and FXIII.

Clinical signs and symptoms associated with bleeding disorders vary widely, ranging from asymptomatic to severe life-threatening bleeding, broadly depending on the level of residual clotting factor in the blood [2]. Distinctive symptoms in persons with hemophilia include hemarthrosis and hematoma, whereas those in VWD and RBDs primarily manifest as mucocutaneous bleeds and severe post-operative hemorrhages. Central nervous system bleeding, a life-threatening manifestation, is more frequent in patients with severe FX, FVII, and FXIII deficiencies and afibrinogenemia. In patients with severe FX deficiency, central nervous system bleeding is a serious hemorrhagic manifestation that may appear very early in life [3]. Additionally, gastrointestinal bleeding, reported less frequently in

other RBDs, is a recurrent symptom in FX deficiency. Women and girls with bleeding disorders are more prone to develop obstetric and gynecologic problems than the general population [4]. In hemophilia, an X-linked disease, females are usually asymptomatic carriers with highly variable bleeding symptoms. However, according to the new nomenclature proposed by the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis (ISTH), some carrier women, now defined as symptomatic, may experience bleeding manifestations [5,6]. The most common bleeding symptom in women and girls with VWD is menorrhagia. Spontaneous abortion is frequent in women with afibrinogenemia and FXIII deficiency; nevertheless, umbilical cord bleeding is the first and most frequent sign in neonates with afibrinogenemia [7]. A recent large study reported a prevalence of 32.4% of hemorrhagic ovarian cysts in patients with RBDs [8].

Over the years, significant progress in drug development has ensured excellent bleeding prevention by optimization of prophylactic treatment in persons with hemophilia A and B: drugs with extended half-life, FVIII-mimicking bispecific antibody (emicizumab), and rebalancing therapies (antitissue factor pathway inhibitor [TFPI] antibodies, fitusiran, and serpinPC), some of them still in clinical trials [9–11]. Moreover, gene therapy now represents a novel therapeutic option for hemophilia A and B after the first 2 gene therapy drugs (valoctocogene roxaparvovec and etranacogene dezaparvovec) obtained marketing approval from both European and American regulatory agencies.

In contrast, the treatment of VWD has seen minimal change over the past 3 decades, except for the introduction of recombinant von Willebrand factor (VWF). Current approaches to managing bleeding episodes in VWD patients primarily involve the use of desmopressin, along with antifibrinolytics and various combined FVIII-VWF or pure plasma-derived VWF concentrates.

Management of RBD patients remains challenging due to the limited availability of specific products. The cornerstone of RBD treatment continues to be replacement therapy with fresh frozen plasma, cryoprecipitate, or prothrombin complex concentrates. Plasma-derived concentrates are presently accessible for fibrinogen, FVII, FX, FXI, and FXIII, while recombinant products have been developed solely for FVII and FXIII. The scarcity of RBD cases likely has not spurred sufficient investment by pharmaceutical companies to truly advance therapies for these rare disorders.

This review comprehensively covers all novel nonreplacement therapies, including 2 broad categories: factor mimics and rebalancing therapies, already approved and currently in use for the management of persons with hemophilia A and B or still in clinical trials (Figure). All nonreplacement drugs under development for the treatment of bleeding disorders other than hemophilia are discussed herewith, including data reported at the ISTH 2023 Congress.

2 | FACTOR MIMICKING THERAPIES

2.1 | Emicizumab

The introduction of emicizumab, the first nonreplacement therapy approved for all age groups by both the European Medicine Agency and the United States (US) Food and Drug Administration (FDA) for hemophilia A, marked a paradigm shift in the hemophilia treatment

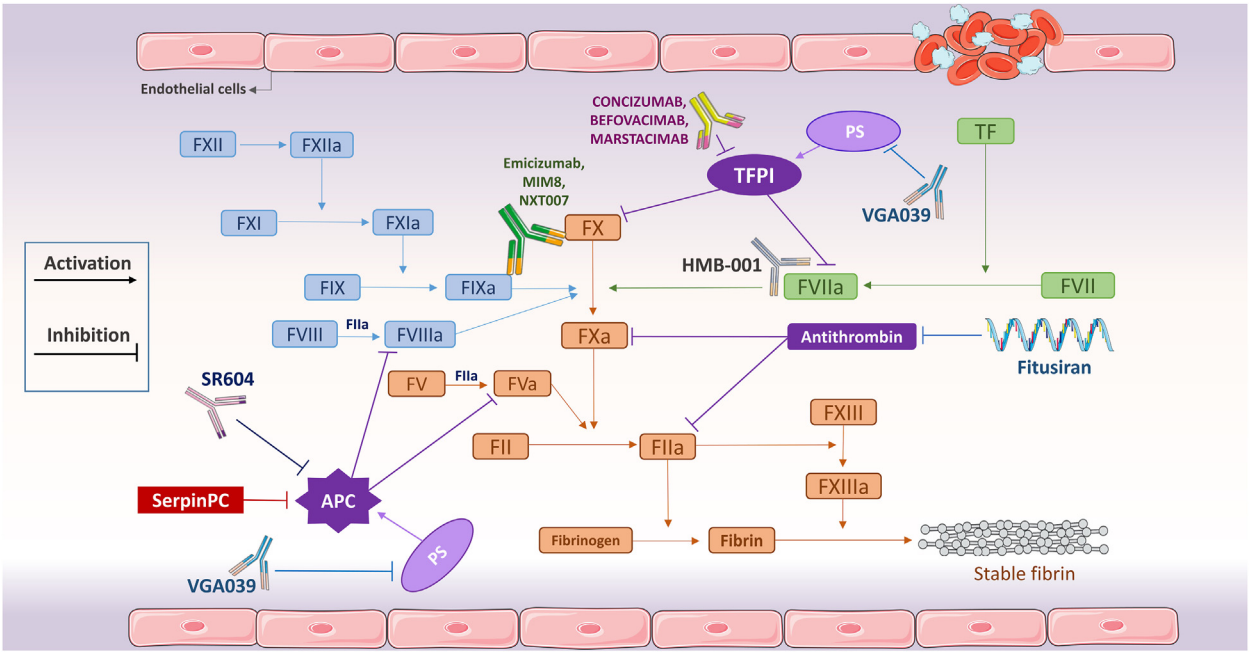

FIGURE Schematic representation of the coagulation cascade with the sites of action of natural anticoagulants and the novel nonreplacement drugs. APC, activated protein C; F, factor; Pro S, protein S; TF, tissue factor, TFPI, tissue factor pathway inhibitor.

landscape. Emicizumab (ACE910), a humanized bispecific monoclonal antibody (mAb) developed by Genentech and Chugai Pharmaceutical, simultaneously binds 2 antigens, FIXa and FX, placing them in spatially appropriate positions to mimic the cofactor function of FVIIIa [12]. The ease of subcutaneous administration and the weekly or monthly infusion frequency facilitate the acceptance of prophylaxis in persons with hemophilia A, in patients without FVIII inhibitors, and especially in those with inhibitors [13,14]. This drug particularly has alleviated venous access problems in children, dramatically changing their therapeutic outlook. Moreover, unlike prophylactic replacement therapies, this drug produces no peak and trough levels but establishes a steady state that ensures a stable and constant level for several weeks, thus effectively preventing most bleeding episodes and offering protection against minor injuries. As of April 2023, the global number of people with hemophilia A treated with emicizumab exceeds 20,000 individuals [15].

The efficacy and safety of emicizumab have been proven in several phase 3 clinical trials of the HAVEN program and in real-life scenarios [16]. Pooled data from HAVEN 1 to 4 clinical studies over a median period of 120.4 weeks showed a decreased annualized bleeding rate (ABR), indicating a significant reduction in bleeding episodes in adults and children with hemophilia A with or without inhibitors following a prophylactic schedule with emicizumab. Analysis of the HAVEN studies also demonstrated adequate control of joint bleeding, indicating that the concentration achieved by emicizumab at steady state (approximately 30-50 μg/mL, corresponding to at least 10-15 IU/dL of FVIII activity) facilitates the control of joint morbidity [17]. However, safety concerns have initially emerged during clinical trials. Five patients experienced serious adverse events: 3 thrombotic microangiopathies and 2 thrombotic events, when persons with hemophilia A with inhibitors concomitantly using bypassing agents like activated prothrombin complex concentrate (aPCC) at doses of >100 U/kg for more than 24 hours and recombinant FVIIa (rFVIIa) [13,18,19]. Preliminary data from *ex vivo* studies suggest that aPCC potentiates the procoagulant effect of FVIII mimetics, likely due to the presence of FIXa that enhances the activity of emicizumab [20]. Thus, even small amounts of FIXa can lead to serious complications, emphasizing the need for careful control of this mimetic factor.

A critical consideration is how to best combine nonreplacement and replacement therapies in the clinical management of patients during acute bleeding events or surgeries. Clinicians now know how to handle these circumstances effectively, thereby reducing the incidence of complications. Recently, a study analyzed data on the hemorrhagic and thrombotic adverse events associated with emicizumab and extended half-life FVIII products as reported in the public pharmacovigilance EudraVigilance database during the postmarketing phase of these products in 2021 [21]. Despite the limitations of pharmacovigilance data, the report identified an increase in the reporting rate of thrombotic adverse events with emicizumab compared with extended half-life FVIII products accompanied by a lower reporting rate of hemorrhagic adverse reactions [19]. Another recent study utilized data from the FDA Adverse Event Reporting Systems of the US in order to assess the rate of thrombotic adverse events in patients treated with emicizumab. The results showed, in agreement with the European study, that the reported thrombotic adverse events associated with emicizumab in the FDA Adverse Event Reporting Systems data occurred more frequently than those associated with FVIII products [22]. Consequently, enhanced postregistration surveillance is necessary to monitor the long-term safety and efficacy data of this novel agent.

Emicizumab treatment is associated with a low incidence of antidrug antibodies (ADA), specifically at 5.1%, most of which are transient and/or low titer. Furthermore, there is a reported 2.7% incidence of ADA exhibiting neutralizing activity [23]. Progressive loss of emicizumab efficacy and diminished chromogenic FVIII-like activity could be indicators of clinically important ADAs. It must be reiterated that emicizumab is not suitable for treating acute bleeding nor for surgeries that warrant additional replacement therapies.

In recent years, numerous research studies have also explored the potential role of emicizumab in the treatment of VWD and RBDs.

VWF is a large multimeric plasma glycoprotein that plays a central role in hemostasis by stabilizing circulating FVIII and mediating platelet adhesion to damaged vascular subendothelium. VWD is characterized by broad clinical heterogeneity. Type 1 and type 3 VWD refer to a partial or total quantitative defect of VWF, whereas type 2 VWD refers to qualitative VWF defects, including type 2A, 2B, 2M, and 2N [24].

Emicizumab has been used off-label for the management of 10 patients with VWD type 3, including 4 with evidence of a VWF inhibitor requiring treatment with bypassing agents [25–30]. These patients had a severe bleeding tendency, combining mucocutaneous bleeding typical of VWD with hemarthrosis typical of persons with moderate/severe hemophilia A. Treatment of these patients had originally been done with rFVIII concentrates as well as bypassing agents such as rFVIIa and aPCC. However, due to the subsequent reappearance of life-threatening bleeding symptoms and resistance to authorized treatments caused by the emergence of VWF inhibitors, prophylaxis with the off-label emicizumab was proven to be effective. Emicizumab prophylaxis made patients free from bleeding, enhanced their quality of life, and reduced hospital admissions, joint bleeds, and also spontaneous bleeds.

In addition, *in vitro* studies conducted through a perfusion chamber on whole blood samples from patients with VWD showed that emicizumab improved thrombus formation also for blood samples from patients with types 3, 2N, and 2A VWD [31–33]. More recently, Casari et al. [34] tested the effect of emicizumab on VWD type 2A and 3 murine models, verifying its efficacy using the thrombin generation assay, the perfusion chamber, and tail-vein transection. Improved hemostasis was observed only in VWD type 3 murine models but not in VWD type 2A. Since VWF deficiency impairs both primary and secondary hemostasis, type 3 VWD patients with undetectable VWF levels also exhibit FVIII deficiency. Addressing FVIII levels through mimetic factors or alternative nonreplacement therapies could potentially ameliorate the onset of bleeding episodes in patients with VWD type 3. The FVIII mimetic has no effect on the animal model of type 2A VWD associated with increased VWF degradation, which may

interfere with emicizumab or clot formation, but this hypothesis requires further investigation. The efficacy of emicizumab in patients with VWD type 3 should be further tested in appropriate clinical trials; data were presented at the 2023 European Association for Hemophilia and Allied Disorders (EAHAD) conference [34].

2.2 | New next-generation FVIII-mimicking drugs

2.2.1 | Mim8

Mim8, developed by Novo Nordisk, is a novel next-generation FVIII-mimicking bispecific antibody in clinical development. Manufactured from a collection of monoclonal antibodies directed against FIXa and FX, extensive mutational optimization has improved the catalytic activity of FIXa for the activation of FX on the surface of activated platelets, demonstrating higher hemostatic efficacy than emicizumab [35,36].

The pharmacodynamic profile in nonhuman primates is favorable, showing excellent bioavailability with subcutaneous infusion [36]. Phase 1 studies indicate that a single subcutaneous dose of Mim8 is well tolerated with a terminal half-life of about 30 days, supporting weekly to monthly dosage [37]. Currently, Mim8 is in phase 3 clinical trial to investigate its efficacy and safety in adults and adolescents with hemophilia A with or without inhibitors (NCT05053139).

2.2.2 | NXT007

A new emerging technology, FAST-Ig (Four-chain assembly by electrostatic steering technology-immunoglobulin), has been applied to develop NXT007, a new anti-FIXa/FX bispecific humanized IgG4 antibody developed by Chugai [38]. Preliminary data from a phase 1/2 clinical trial in healthy Japanese volunteers (NXTAGE; JapicCTI-194919), presented at ISTH Congress 2023, showed a mean elimination half-life of approximately 10 weeks and single subcutaneous doses were well tolerated without thromboembolic events [39]. A phase 1/2 study to evaluate the safety and efficacy of NXT007 in persons with severe or moderate hemophilia A is ongoing (NCT05987449).

3 | RATIONALE OF TARGETING NATURAL ANTICOAGULANTS

Rebalancing therapy, as implied by its name, seeks to restore equilibrium to the hemostatic system by selectively inhibiting such natural anticoagulants as TFPI, antithrombin (AT), activated protein C (APC), and protein S (PS).

The maintenance of the hemostatic balance relies on a delicate interplay between procoagulant and anticoagulant agents. Any imbalance in these proteins can result in uncontrolled hemostasis, posing a risk of thrombosis or excessive bleeding. In cases of coagulation factor deficiency, inhibiting an anticoagulant protein may facilitate the rebalancing of hemostasis. Notably, deficiencies in PC and PS, AT III, and TFPI have been observed to ameliorate clinical severity in persons with hemophilia [40]. These observations led to the concept of downregulating natural anticoagulants to correct the hemorrhagic phenotype [41,42].

The rationale for inhibiting natural anticoagulants is also supported by data from animal models, demonstrating that reducing the effects of natural anticoagulants can compensate for the lack of procoagulant factors and promote hemostasis. Thus, inhibiting the activity of natural anticoagulants becomes a potential strategy to alleviate the bleeding phenotype observed in patients with inherited coagulation deficiencies by favoring a procoagulant state and altering the balance of hemostasis [42].

3.1 | TFPI

TFPI, a natural anticoagulant, negatively regulates thrombin generation by inactivating FXa and the FVIIa-tissue factor complex in the extrinsic pathway [43]. TFPI consists of 3 tandem Kunitz-type protease inhibitory domains. Under physiological conditions, the Kunitz-2 domain binds FXa, resulting in its inhibition with the formation of a binary TFPI/FXa complex. Subsequently, the Kunitz-1 domain binds to FVIIa in the tissue factor/FVIIa binary complex, forming a quaternary complex that further inhibits FX. By blocking the action of TFPI, clot formation can occur. Hence, the idea of inhibiting TFPI to promote blood coagulation in persons with hemophilia, irrespective of inhibitor status [44].

The first therapeutic attempt to inhibit TFPI function involved an aptamer, ARC19499 (BAX499), which effectively blocked TFPI, improving clotting time in *in vitro* studies and reducing bleeding in a nonhuman primate model [45]. Unfortunately, its development was discontinued when phase 1 study revealed excessive and unexpected bleeding in the enrolled persons with hemophilia [46]. These findings underscore the importance of comprehending TFPI's role and evaluating potential effects of different therapeutic approaches to inhibit it.

Antibodies targeting TFPI were tested in animal models as early as the 1990s, but clinical-grade antibodies against TFPI only emerged in the 21st century. Concizumab (mAb2021) and marstacimab (PF-06741086) are monoclonal antibodies against TFPI, offering a disruptive therapeutic opportunity for treating persons with hemophilia, with or without inhibitors.

3.2 | Anti-TFPI antibodies

3.2.1 | Concizumab

Concizumab (mAb 2021) is a high-affinity humanized IgG4 mAb directed against the Kunitz-2 domain of TFPI designed to selectively target and block FXa [47]. In animal models, mAb 2021, administered both intravenously and subcutaneously, demonstrated effectiveness

in reducing bleeding. Subcutaneous administration also exhibited adequate bioavailability for hemostasis [48].

Pharmacokinetic studies in cynomolgus monkeys further supported the bioavailability of the subcutaneous administration, indicating that concizumab (mAb 2021) is a potentially valid alternative for the treatment of persons suffering from hemophilia A and B, regardless of inhibitor status [49]. Phase 1 and 2 studies conducted in these patients confirmed concentration-dependent procoagulant effects and a positive safety profile after daily intravenous and subcutaneous administration [50,51]. However, clinical development was briefly halted in 2020 due to 3 nonfatal thrombotic events in the phase 3 trials. Following a risk mitigation plan with a new reduced dosing regimen of drug to mitigate the thromboembolic risk, phase 3 studies of the Explorer program were resumed [52]. In addition, updated indications for the management of mild and moderate breakthrough bleeding episodes have been proposed. Results from phase 3 studies (Explorer 7 and Explorer 8) confirmed the efficacy of daily subcutaneous concizumab prophylaxis compared with on-demand treatment in significantly reducing bleeding episodes [53,54]. In addition, concizumab prophylaxis showed a positive long-term effect (56 weeks) on the target joints, their resolution, and joint bleeding in participants in the clinical trial [55]. This drug, being subcutaneously administered daily, will change the treatment perspective in persons with hemophilia B, especially those with inhibitors. The development of inhibitors is a rare event, affecting only 1.5% to 3% of all patients. However, this complication is associated with considerable morbidity, particularly due to the occurrence of allergic/anaphylactic reactions and nephrotic syndrome [56]. Induction of immune tolerance is frequently unsuccessful and may pose complications, especially in patients with a history of allergy or anaphylaxis. Thus, the introduction of concizumab holds the potential to notably revolutionize the treatment approach for persons with hemophilia B, particularly those who developed inhibitors. Recently, concizumab has been approved by Health Canada in March 2023 for prophylactic treatment of patients aged 12 years and older with hemophilia B and inhibitors [57].

3.2.2 | Marstacimab

Marstacimab (PF-06741086) is a human IgG1 mAb that binds the Kunitz-2 domain of TFPI, preventing its interaction with FXa [58].

Ex vivo studies in the blood and plasma of persons with hemophilia showed that PF-06741086 induced procoagulant responses, including a reduction of clotting time and restoration of hemostasis [59]; *in vivo* studies in monkeys also demonstrated hemostatic efficacy, supporting further clinical development of this antibody [58]. The first study of PF-06741086 in humans was conducted in healthy volunteers receiving 2 different doses ranging from 30 mg subcutaneously to 440 mg intravenously. Data from this trial demonstrated that single doses of this anti-TFPI antibody were safe and well tolerated. Safety, pharmacokinetic, and pharmacodynamic data supported the transition to the subsequent phases of the clinical study in persons with hemophilia [60]. The study in healthy volunteers was halted prematurely due to thrombotic events, specifically deep vein thrombosis/pulmonary embolism, which manifested in a participant [61].

In phase 2 clinical trial, once-weekly subcutaneous administration of marstacimab in 2 different doses (300 or 150 mg) was assessed in persons with hemophilia A and B with or without inhibitors. Although the study had a limited sample size, the results showed that the administration of marstacimab was well tolerated, associated with good efficacy, and had a favorable safety profile without thrombotic events [62].

Results from phase 3 were recently published in a press release and presented at the ISTH 2023 Congress during a symposium sponsored by a pharmaceutical company [63]. Prophylactic treatment with marstacimab resulted in a clinically relevant reduction in ABRs in persons with hemophilia A and B without inhibitors. Among the 116 patients treated with marstacimab for 1 year, ABRs were 92% lower in the prophylaxis group versus on-demand group. The safety profile was consistent with phase 1/2 results, and the treatment was generally well-tolerated. There have been no thromboembolic events in persons with hemophilia enrolled in clinical trials.

Marstacimab has the potential of becoming an anti-TFPI antibody that offers once-weekly subcutaneous treatment for individuals with hemophilia A or B, regardless of the presence of the inhibitor. European and American regulatory agencies have accepted applications for this drug for the treatment of hemophilia A and B with and without inhibitors, and registration is expected/scheduled for early 2025 and late 2024, respectively.

The rebalancing drugs mentioned earlier, proven effective in persons with hemophilia, may also emerge as therapeutic options for managing individuals with other bleeding disorders. The potential of PF-06741086 to restore thrombin generation in the plasma of RBDs was investigated. Plasma from patients with FV, FVII, and FXI deficiency and VWD type 1, 2A, 2B, and 3 were tested. Inhibition of TFPI in the initiation phase of coagulation resulted in the formation of thrombin that promotes hemostasis in FXI-deficient plasmas and VWF-deficient plasmas. No increase in thrombin generation has been reported in FV- and FVII-deficient plasmas [64].

These studies have highlighted the effectiveness of the therapeutic approach involving the inhibition of the anticoagulant TFPI with antibodies. Thrombotic events have been reported in clinical trials for hemophilia, reiterating the need for caution when TFPI is the target of medical treatment, as the coagulation cascade is a highly balanced system between coagulating and noncoagulating factors. No patient with VWD or RBDs has yet been treated with any of these anti-TFPI antibodies.

3.3 | AT

AT is a heparin cofactor and a member of the serine protease inhibitor family (serpin). AT, a natural anticoagulant, irreversibly inactivates thrombin, FXa, and, to a lesser extent, FIXa, FXIa, FXIIa, kallikrein, and plasmin [65].

Acquired or hereditary AT deficiency results in excessive thrombin production with a significantly increased risk of thromboembolism, mainly in the venous system [66]. In persons with severe hemophilia A or B, the coexistence of AT deficiency is associated with a milder hemorrhagic phenotype, demonstrating that reduced AT activity attenuates their clinical severity [67].

Studies in mice lacking FVIII with heterozygous AT deficiency revealed increased thrombin generation associated with improved hemostasis in a tail-bleeding model [68]. All these observations led to the hypothesis that low levels of AT in persons with hemophilia could be a therapeutic strategy to rebalance hemostasis and promote clot formation.

RNA interference (RNAi) technology was used for targeting AT. RNAi is a biological mechanism to defend against the invasion of exogenous genes derived from infectious viruses. It can theoretically inhibit or silence the expression of any disease-related gene in a sequence-specific manner, making small interfering RNA (siRNA) a promising therapeutic modality [69]. A solution of siRNA delivery system to target hepatocyte cells is the ligand N-acetylgalactosamine (GalNAc) for the asialoglycoprotein receptor [70]. Hepatic asialoglycoprotein receptor, also known as the Ashwell Morell receptor, can recognize and uptake circulating glycoproteins with exposed GalNAc glycans. Conjugation of siRNA with GalNAc surprisingly resulted in targeted delivery in mouse hepatocytes. Furthermore, these conjugates mediated robust and long-lasting silencing of its complementary gene expression in the liver following subcutaneous administration [71].

3.3.1 | Fitusiran (ALN-AT3)

GalNAc–siRNA conjugate (ALN-AT3), specific for a region of SERPINC1 targeting AT, has been considered a viable therapeutic solution to rebalance hemostasis in persons with hemophilia [70]. In preclinical studies conducted in hemophilia A mice and nonhuman primates, fitusiran (ALN-AT3) showed a dose-dependent and long-lasting reduction in AT levels after subcutaneous administration and also induced the generation of thrombin associated with improved hemostasis [72].

The safety, tolerability, and pharmacokinetics of fitusiran were evaluated in a phase 1 study. The drug was administered monthly to both healthy volunteers and persons with hemophilia A or B, with and without inhibitors [73,74]. In all clinical trial participants, subcutaneous administration of a 50 or 80 mg dose of this drug resulted in decreased AT levels and increased thrombin production. However, the study had some limitations, such as the absence of a control group, a limited number of participants, and a short duration [73].

In the 2 phase 3 studies (ATLAS-INH and ATLAS-A/B), the efficacy and tolerability of once-a-month prophylaxis with subcutaneous fitusiran 80 mg were compared with on-demand bypassing agents or concentrate products in persons with severe hemophilia A or B, with and without inhibitors [75,76]. Fitusiran prophylaxis demonstrated favorable efficacy and appeared to be well tolerated. However, a transient increase in alanine aminotransferase (ALT) was reported as the most common emerging adverse event in both clinical trials, affecting approximately 23% of participants. This event led to the interruption of fitusiran prophylaxis in around ~7% of patients due to ALT elevations exceeding 5 times the upper limit of the normal value. It is noteworthy that no patients discontinued treatment despite ALT elevations.

The occurrence of dose-dependent, asymptomatic, and transient increases in this liver function test was observed in a small subset of early clinical programs using GalNAc–siRNA conjugates in humans [77]. Among the potential causes for this adverse effect, RNAi-mediated off-target effects have been considered a major driver of hepatotoxicity for GalNAc–siRNA conjugates [78]. Several solutions have been proposed to solve the problem of hepatotoxicity [77].

The fitusiran clinical program was initially suspended and subsequently voluntarily interrupted by the sponsor due to a fatal cerebral thrombosis and additional nonfatal thrombotic events. In total, 5 thrombotic events were reported among 259 treated patients, occurring in conjunction with the use of coagulation factor concentrates or bypassing agents or in association with AT levels less than 10% [79].

To mitigate the risk of thrombotic events, a revised dosing regimen for fitusiran recommended a starting dose of 50 mg every other month, with the option to increase to 80 mg per month based on AT levels targeted between 15% and 35%. This revised protocol facilitated the restart of all paused studies, and ongoing phase 3 ATLAS studies will assess whether the adjusted dosing regimen could enhance the risk–benefit assessment of fitusiran [79]. Given the crucial role of monitoring AT levels in fitusiran prophylaxis and the substantial heterogeneity observed in commercial tests, there is a pressing requirement for a standardized AT measurement method before the approval and subsequent marketing of fitusiran.

Fitusiran is poised to become one of the unconventional options for treating persons with hemophilia B, especially in those who have developed inhibitors, considering the few treatment options available and the related complications. Furthermore, it offers an alternative approach to emicizumab for persons with hemophilia A, both with or without inhibitors. Furthermore, due to its inhibitory action on AT, fitusiran holds potential clinical applicability for the treatment of RBDs such as FV, FVII, and FX deficiencies, which stem from the inability to generate sufficient thrombin to prevent bleeds.

In silico modeling has been employed to simulate the impact of AT reduction on thrombin generation in FV, FVII, and FX deficiencies. This computational model predicted that lowering AT to the 20% level resulted in increased thrombin generation for all these deficiencies [80]. In an *in vitro* study, AT activity was inhibited by an anti-AT antibody in plasma from patients with severe FV, FVII, and FX deficiencies. In these plasma samples, reducing AT to 20% resulted in a negligible improvement in thrombin generation. However, the combination of reducing AT activity and replacing the deficient factor with 1% to 10% of normal significantly increased thrombin generation to near-normal values [81]. These preliminary results support the potential application of fitusiran in some RBDs, although the reduction of

AT may enhance thrombin generation only when a minimal amount of FV, FVII, or FX activity is measured.

3.4 | APC

APC is a powerful anticoagulant, acting through the irreversible inactivation of FVa and FVIIIa, thereby downregulating the generation of thrombin [82]. The physiological significance of PC became apparent after the occurrence of severe thrombotic complications, often fatal, in infants with severe homozygous PC deficiency. Additionally, heterozygous adults with PC deficiency have a higher risk of venous thrombosis [82]. The Leiden mutation is recognized for conferring resistance to APC inactivation to FV. Coinheritance of FV Leiden mutation with hemophilia results in increased thrombin generation that attenuates the frequency and severity of clinical symptoms in patients with low levels of FVIII and FIX [83]. This evidence has been substantiated by *in vitro* and *in vivo* studies, leading to the hypothesis that APC inhibition could fully normalize the hemostatic balance [41,84]. An engineered form of α1-antitrypsin (α1AT), known as α1AT Pittsburg, was developed by Polderdijk et al. [85]. They demonstrated that this serpin mutant strongly inhibits APC and thrombin [85]. To improve targeting specificity for APC, amino acid residues around the cleavage site of the reactive center loop of the serpin were modified, creating a mutated α1AT (SerpinPC) with higher specificity.

3.4.1 | SerpinPC

The inhibitory capacity of SerpinPC on APC was assessed in a hemophilia mouse model, demonstrating that this engineered serpin can restore hemostasis [85,86].

Ongoing phases 1/2 and phase 2a have indicated a favorable efficacy, safety, and tolerability profile with subcutaneous administration of SerpinPC. The median ABR for overall bleeds in the clinical trials was approximately 90%. Notably, there were no thromboembolic events and, importantly, no nontransient elevations in D-dimer. These data support the potential of SerpinPC to provide clinically efficacious subcutaneous therapy to persons with hemophilia B who have limited treatment options, as well as to those with hemophilia A with and without inhibitors [87].

3.4.2 | SR604 chimeric antibody

Recently, a humanized chimeric antibody, SR604, was designed to selectively block the anticoagulant activity of APC [88]. Preclinical studies in hemophilia A and B mice demonstrated that SR604 can prevent bleeding, and furthermore, this antibody exhibited high bioavailability when injected subcutaneously into cynomolgus monkeys. This novel drug could, therefore, be employed for routine prophylaxis of persons suffering from hemophilia A and B, as well as those with other congenital factor deficiencies.

3.5 | Protein S

PS participates in regulation of coagulation and serves as a cofactor in the APC pathway, improving APC's ability to inactivate FVa and FVIIIa. Additionally, PS plays a crucial role as a cofactor of TFPI in the inhibition of FXa [89]. PS is a key regulator of thrombin generation, considering its dual role in maintaining hemostatic balance. The significance of PS as an anticoagulant is evident in mouse models, where the complete elimination of the PS gene leads to death in utero due to coagulopathy and bleeding [90]. Deficiency of PS in humans elevates the risk of thromboembolic events, with severe cases potentially leading to disseminated intravascular coagulation, underscoring the crucial role of PS as an anticoagulant. Additionally, women with PS deficiency show a heightened risk of late fetal loss [91]. Individuals with hereditary PC/PS deficiency exhibit a 2- to 11-fold increased risk for venous thromboembolism, characterized primarily by deep vein thrombosis and pulmonary embolism.

The inhibition of PS constitutes a potential therapeutic target in hemophilia. The initial strategy employed to inhibit PS involved using siRNA conjugated to the GalNAc ligand to target the expression of the *Pros1* gene, which encodes PS. This siRNA was tested in a mouse model, demonstrating good tolerance and the ability to reduce plasma PS levels, thereby protecting mice from hemophilic arthropathy [92].

3.5.1 | VGA039

Very recently, Vega Therapeutics has developed a mAb named VGA039, specifically targeting PS [93]. VGA039 has the potential to inhibit PS activity across various congenital factor deficiencies. To assess this hypothesis, thrombin generation assays were carried out using several plasma samples from patients with congenital deficiencies of VWF, FV, FVII, FVIII, FIX, FX, FXI, and FXIII. VGA039 demonstrated a dose-dependent promotion of thrombin generation in congenital VWD (type 3, type 2, and type 1), FVII, FVIII, FIX, FXI, and FXIII, but not in FV and FX deficient plasma. The phase 1 clinical trial of VGA039 has commenced, involving both healthy subjects and patients with VWD (NCT05776069).

4 | INNOVATIVE THERAPEUTIC STRATEGIES FOR VWD AND GLANZMANN THROMBASTHENIA

4.1 | KB-V13A12 bispecific nanobody and platelet-inspired hemostatic nanoparticles

The bifunctional molecule KB-V13A12 exhibits binding affinity to both albumin and VWF, thereby extending the endogenous half-life of

VWF. In a mouse model of type 1 VWD, subcutaneous administration of KB-V13A12 resulted in an approximately 2-fold increase in plasma VWF levels lasting up to 10 days, and functional hemostasis was restored, as demonstrated in a tail-clip assay. Synthetic platelet nanoparticles, comprising liposomal nanoparticles containing peptides with the ability to simultaneously bind to collagen, VWF, and activated platelets, have been developed. Results from microfluidic (*in vitro*) and tail-clip (*in vivo*) assays revealed a notable reduction in blood loss in mice with type 2B VWD (35%) and mice deficient in VWF (VWF knockout [KO], 68%). Additionally, an increase in thrombus formation was observed using blood samples from mice with type 2B VWD and VWF KO mice [94].

4.2 | BT200 (rondoraptivon pegol)

BT200 (rondoraptivon pegol) is a pegylated aptamer designed to bind to the VWF A1 domain, effectively increasing VWF and FVIII levels by mitigating their clearance. In the first human trial involving healthy subjects, aptamer BT200 demonstrated the ability to elevate plasma levels of VWF and FVIII. Subcutaneous injections of rondoraptivon pegol were also shown to extend the half-life of substituted FVIII in adult persons affected by severe and nonsevere hemophilia A (NCT04677803) [95]. A prospective phase 2 trial with BT200 focused on patients with type 2B VWD. Results indicated that VWF and FVIII levels increased by more than 2-fold, and platelet counts increased by more than 3-fold [96]. Thrombocytopenic patients exhibited heightened platelet-dependent VWF activity (measured using VWF:GPIbM) and enhanced VWF collagen binding. Additionally, there was a restoration of high-molecular-weight multimers. While these findings are promising, further studies on larger cohorts are necessary for a comprehensive understanding of BT200's therapeutic potential.

4.3 | HMB-001

The HMB-001 bispecific antibody has demonstrated the capability to bind and accumulate endogenous FVIIa in circulation. This leads to the localized activation of FX and the subsequent production of thrombin upon vessel injury, achieved by placing FVIIa on activated platelets through the TREM-like transcript 1 receptor. A combination of *ex vivo* and animal model studies has provided compelling evidence that HMB-001 can elevate endogenous FVIIa activity to a therapeutically effective level, particularly in Glanzmann thrombasthenia. The initial results from the first-in-human clinical trial phase 1/2 of HMB-001 in patients with Glanzmann thrombasthenia were presented at the EAHAD Congress 2024 [97,98]. This ongoing study tested the antibody at 3 different doses (0.2, 0.5, and 1.25 mg/kg). The pharmacokinetic profile supports dosing every 2 weeks or less frequently, considering its half-life of approximately 10 days. Pharmacodynamic data showed a dose-dependent increase in FVII and FVIIa levels, along with a reduction in prothrombin time. No serious adverse events were observed during this phase of the study, and there were no thrombotic events or apparent changes in D-dimer.

5 | CONCLUSIONS

The hemophilia treatment landscape has evolved from traditional products to innovative therapies featuring FVIII activity mimics and anticoagulant rebalancing drugs. These nonreplacement therapies offer versatility, effectively treating hemophilia A and, in the very near future, hemophilia B, regardless of inhibitor presence. These drugs have revolutionized prophylaxis, easing the burden of this treatment regimen, enhancing adherence for adults and pediatric patients, and ultimately improving their quality of life.

However, the absence of novel treatments for RBDs remains a challenge. Limited advancements have been made in implementing treatment options for these RBDs. Nonreplacement drugs like marstacimab, fitusiran, and VGA039 are under development for their potential to activate hemostasis in diseases such as VWD, FV, FVII, FX, FXI, and FXIII. Despite these efforts, we are still far from having an alternative drug to treat these rare deficiencies.

International organizations (like ISTH, European Haemophilia Consortium, EAHAD, and World Federation of Hemophilia) support the dissemination of new therapies, clinical trial data, guidelines, and recommendations for treating hemophilia and other RBDs. The aim of these organizations is to ensure top-quality clinical care by addressing the challenges faced by individuals with these disorders, educating both medical professionals and the public, and promoting scientific research.

5.1 | Thromboembolic risk with nonreplacement drugs

Nonreplacement drugs represent novel therapeutic approaches and offer numerous benefits by addressing significant shortcomings of traditional replacement therapies. However, despite their advantages, these drugs may also present pitfalls and unforeseen risks, such as thrombotic and thrombotic microangiopathy events. Thrombotic events have primarily been observed in patients concurrently receiving other clotting products for bleeding treatment. The thromboembolic risk may be particularly elevated in persons with hemophilia with inhibitors, as they may require bypassing agents for managing breakthrough bleeds. Additionally, the risk of thrombotic complications arising from the concurrent use of these new drugs with factor replacement therapies (plasma-derived or rFVIII and FIX products) and other hemostasis products must be carefully assessed, especially during and after surgery when patients require coverage to prevent bleeding events.

Patients with cancer or critical conditions face heightened risks of bleeding and thrombotic complications. Adjustments to replacement therapy may be necessary to manage the bleeding risks. Similarly, in persons with hemophilia with sepsis, close monitoring and prompt

administration of clotting factor replacement therapy are essential. However, the suitability of nonreplacement therapies in these scenarios warrants further investigation and evaluation.

It is crucial for healthcare providers to carefully assess the overall risk-to-benefit profile of nonreplacement therapies in individuals with hemophilia, considering factors such as the severity of bleeding disorders, the presence of inhibitors, and individual comorbidities. Close monitoring for signs and symptoms of thrombosis is essential when utilizing these novel drugs, and precautionary measures should be implemented to mitigate the risk of thrombosis.

6 | FUTURE DIRECTIONS

Antibodies currently dominate therapeutic interventions, and recently, researchers from the University College of London have proposed an innovative therapeutic approach using a novel FVIII mimetic antibody, whose sequence is cloned into an adeno associated virus expression cassette (Bi8). In FVIII KO mice, Bi8 demonstrated stable expression, showing the potential use of an adeno associated virus vector to encode information for a bispecific antibody [99].

The potential application of new drugs originally designed for treating persons with hemophilia could be extended to treat VWD and other rare hemorrhagic disorders facilitated by digital artificial intelligence. These tools have the potential to optimize the drug development process for people affected by the rarest bleeding disorders.

Advances in precision medicine approaches may enable personalized treatment strategies not only for hemophilia but also for individuals with VWD and RBDs, considering their distinct biomarker profiles and clinical characteristics. This personalized medicine approach could enhance treatment efficacy and minimize adverse events by tailoring therapy to individual patient needs. Overall, novel nonreplacement therapies hold great promise for the future management of VWD and RBDs. Continued research and innovation in this field are essential to transition these promising therapies from preclinical to clinical stages, thereby improving outcomes for patients worldwide.

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AUTHOR CONTRIBUTIONS

F.P. and I.G. drafted the manuscript and conducted a literature review focused on hemophilia. O.S. and S.M. reviewed von Willebrand disease and rare bleeding disorders. All authors contributed to and approved the final manuscript, with F.P. overseeing the entire process.

RELATIONSHIP DISCLOSURE

F.P.: speaker fees for educational programs/symposia organized by Spark and Takeda; advisory boards/consultant for Sanofi, Sobi, Roche, Biomarin, and CSL Behring.

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