Navigating Speed Bumps on the Innovation Highway in Hemophilia Therapeutics

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Abstract

The unprecedented emergence of novel therapeutics for both hemophilia A and B during the last half decade has been accompanied by the promise of even more extraordinary progress in ameliorative and curative strategies for both disorders. Paradoxically, the speed of innovation has created new dilemmas for persons with hemophilia and their physicians with respect to optimizing individual choices from the expanding menu of standard and novel therapies and approaches to symptom or risk reduction, and ultimately, to normalizing the hemophilia phenotype. Among the most disruptive new approaches, challenges remain in the form of the adverse reactions that have been observed with nonfactor therapies, as well as in the uncertain long-term safety profile of potentially curative gene therapy. Together, these challenges have generated uncertainty as to how to adopt novel therapies and treatment strategies across a diverse patient population, creating speed bumps on the hemophilia innovation highway. It is from this perspective that this article discusses the current state of gene therapy and bleeding prophylaxis for hemophilia A and B, as well as prevention and treatment of the factor VIII inhibitor phenotype in hemophilia A. It further posits that these speed bumps may provide important clues to the mechanistic understanding of both symptom manifestation and resilience within the hemophilia phenotype, as well as opportunities to reconsider and reconfigure the current paradigms for symptom prediction and individualized therapeutic decision making.

Introduction

The unprecedented emergence of novel therapeutics for both hemophilia A (factor VIII [FVIII] deficiency) and B (factor IX [FIX] deficiency) during the last half decade has been accompanied by the promise of even more extraordinary progress in ameliorative and curative strategies for both disorders. Paradoxically, the speed of innovation has created new dilemmas for persons with hemophilia and their physicians with respect to optimizing individual choices from the expanding menu of standard and novel therapies and approaches to symptom or risk reduction, and ultimately, to normalizing the hemophilia phenotype. Among the most disruptive new approaches,

challenges remain in the form of the adverse reactions that have been observed with nonfactor therapies, as well as in the uncertain long-term safety profile of potentially curative gene therapy. Together, these challenges have generated uncertainty as to how to adopt novel therapies and treatment strategies across a diverse patient population, creating speed bumps on the hemophilia innovation highway.

It is from this perspective that this article discusses the current state of gene therapy and bleeding prophylaxis for hemophilia A and B, as well as prevention and treatment of the FVIII inhibitor phenotype in hemophilia A. It further posits that these speed bumps may provide important clues to the mechanistic understanding of both symptom manifestation and resilience within the hemophilia phenotype, as well as opportunities to reconsider and reconfigure the current paradigms for symptom prediction and individualized therapeutic decision making.

The mission of extramural Division of Blood Diseases and Resources within the National Heart, Lung and Blood Institute (NHLBI) at the National Institutes of Health (NIH) is to plan and direct the Institute's research, resource and training programs related to the cause, prevention, diagnosis, and treatment of blood diseases; the improved use and safety of blood, bone marrow, and blood products; and the management of blood resources for transfusion and stem cell transplantation. As part of a mission that encompasses disorders of thrombosis and hemostasis, NHLBI acknowledges the ground-breaking progress in the field of hemophilia and the contrasting longstanding barriers to a fundamental understanding of the mechanisms of disease and therapeutic optimization and recognizes the inherent challenge of realizing a precision medicine approach to a rare disorder. This article includes several examples of how the

NHLBI has therefore engaged the hemophilia community to an effort to optimize partnerships and explore opportunities to facilitate critical gap research highlighted by the speed bumps on the hemophilia innovation highway.

Gene therapy at the forefront of therapeutic progress

There has been an explosion of hemophilia gene therapy trials within the last decade (Table 1).[1] Better understanding of the outcome-modifying hepatic immune response to the vector capsid stimulated innovation in the adeno-associated viral (rAAV) vector delivery systems[2] and experimentation with a high specific activity FIX gene product (FIX Padua),[3,4] both of which have facilitated 6 active Phase 1/2 trials for individuals with severe and moderate (≤ 2 U dL^{-1} FIX) hemophilia B, with another poised to begin recruitment within the current year (Table 1).[1] Capitalizing on both gene and vector efficiency, 1 trial has reported unprecedented steady-state plasma FIX activity levels (median: 29.6 U dL^{-1}) over 14 to 52 weeks of follow up, with documented amelioration of the severe hemophilia B phenotype.[5] These outcomes have been achieved with a 1 to 2 log reduction in maximum vector dose (5×10^{11} vg kg^{-1}) and fewer episodes of vector-associated hepatitis than previously reported with AAV gene therapy for hemophilia B.[6,7] The sponsors of this trial have initiated a long-term follow-up study of this cohort. The application of gene editing to hemophilia has so far been infrequent, but the editing of the albumin gene locus to accept and express FIX using proprietary zinc finger nuclease technology in a rAAV6 delivery system[8,9] is being studied in 1 ongoing trial, the results of which are not yet in the public domain (Table 1).

Gene therapy for hemophilia A has historically faced even greater challenges than hemophilia B due to the size of the FVIII gene and initially poor protein expression. Consequently, the similarly busy landscape of registered trials is astounding, given that there are 5 active trials with 2 more soon to be initiated (Table 2).[1] Almost all gene therapy strategies employ liver-directed rAAV vectors of various serotypes to deliver the FVIII-SQ B domain-deleted FVIII (BDD-FVIII) gene product.[10] The single exception is the University College London trial, which uses a modified BDD-FVIII transgene, identified in Table 2 as BDD-V3-FVIII.[10] Most trials are in the early stages of recruitment (Table 2). However, the published results of 1 trial indicate that 7 severe (<1 U dL^{-1} FVIII) hemophilia A subjects treated with the highest vector dose of 6×10^{13} vg kg^{-1} had achieved and sustained plasma FVIII levels at 52 weeks of between 19 and 164 U dL^{-1} with significant reduction in their annualized bleeding

rate (ABR), when evaluated at 1 year following therapy.[11] The sponsors of this study have planned 2 single-arm Phase 3 trials, one of which is currently studying the safety and efficacy of a similarly high vector dose in 40 additional participants. The other will experiment with a lower dose of 4×10^{13} vg kg^{-1} in a distinct 40 subject cohort (Table 2).

Undoubtedly, curative gene therapy is moving closer than ever to becoming a viable treatment option for persons with both hemophilia A and B and, extrapolating from long-term studies in hemophilia dogs, eventually for individuals with hemophilia and inhibitors.[12] However, even as we await long-term outcomes that promise to fully elucidate the optimal boundaries of therapeutic safety and efficacy with respect to target FVIII and FIX plasma levels, the persistent problem of acute hepatic immune responses to the highest dose vector-gene infusions, noted in all trials and prompting nearly universal early intervention to abrogate the inflammatory reaction and mitigate the loss of hepatic gene expression, still compromise the dosing of gene product required to achieve sustained and fully prophylactic plasma levels of FVIII and FIX.[13] Furthermore, pre-existing or post-therapeutic anti-AAV antibodies currently exclude up to two-thirds of otherwise eligible patients from entering clinical trials or prevent vector re-administration in the event of waning gene expression over time.[14] Such limitations should serve as speed bumps that slow progress enough to drive further public and private funding of mechanistic research in vector optimization and, more broadly, in gene-based curative strategies.

Moreover, we are reminded that the current array of single arm, primarily Phase 1/2 gene therapy trials serve as first in man proof of principle studies that place us squarely at the beginning of the evidenced-based clinical research agenda in gene therapy. As a recent Cochrane Systematic Review concluded: "Gene therapy for haemophilia is still in its nascent stages and there is need for well-designed clinical trials to assess the long-term feasibility, success and risks ... for people with haemophilia."[15] To that end, efforts are underway at the NHLBI within the NIH in the United States to assist the hemophilia community in configuring the mechanistic research requirements and clinical scientific priorities that would inform the future landscape of the most challenging gene therapy trials for individuals with hemophilia A and FVIII inhibitors.

Innovation and dilemma in bleeding prophylaxis

In the absence of curative gene therapy for the most severe hemophilia phenotypes, the primary goal of treatment is the

Table 1

Gene Therapy Trials for Hemophilia B[1]

Sponsor (Trial Start)	Intervention	Factor IX (FIX) Gene Product	Adeno-Associated Viral (AAV) Capsid	Intended Enrollment	Highest Vector Dose, vg kg^{-1}	Mean % Sustained FIX Activity	Clinical Trials.Gov Identifier	Status
U College, London/St Jude (2010)	scAAV2/8-LP1-hFIXco	FIX	rAAV8	14	2×10^{12}	5.1	NCT00979238	Active, not recruiting
Shire (2012)	AskBio009	FIX Padua	rAAV8	30	3×10^{12}	0	NCT01687608	Active, not recruiting
Spark Therapeutics Pfizer (2015)	SPK-9001	FIX Padua	rAAV-Spark100	15	5×10^{11}	33.7	NCT02484092	Recruiting
UniQure (2015)	AMT-060	FIX	rAAV5	10	2×10^{13}	6.9	NCT02396342	Active, not recruiting
Sangamo Biosciences (2016)	SB-FIX	Zinc finger-FIX	rAAV6	12	NA	NA	NCT02695160	Recruiting
Dimension Therapeutics (2017)	DTX101	FIX	rAAVrh-10	12	5×10^{12}	6.7	NCT02971969	Active, not recruiting
UCL/Freeline Therapeutics	FLT-180	FIX-Padua	rAAV engineered	18	NA	NA	NCT03369444	Not yet recruiting

Table 2

Gene Therapy Trials for Hemophilia A[1]

Sponsor (Trial Start)	Intervention	B Domain-Deleted Factor VIII (BDDFVIII) Gene Product	Adeno-Associated Viral (AAV) Capsid	Intended Enrollment	Highest Vector Dose, vg kg^{-1}	Mean % (Range) Sustained Factor VIII Activity	Clinical Trials.Gov Identifier	Status
BioMarin Pharmaceutical (2015)	BMN 270	BDD-FVIII	rAAV5	15	6×10^{13}	93 (19–164)	NCT02576795	Active, not recruiting
Spark Therapeutics (2016)	SPK-8011	BDD-FVIII	rAAV-LK03	30	NA	NA	NCT03003533	Recruiting
U College, London (2017)	AAV2/8-HLP-FVIII-V3	BDD-V3-FVIII	rAAV8	18	6×10^{12}	NA	NCT03001830	Recruiting
Sangamo Therapeutics (2017)	SB-525	BDD-FVIII	rAAV2/6	20	NA	NA	NCT03061201	Recruiting
BioMarin Pharmaceutical (Phase 3) (2017)	BMN 270	BDD-FVIII	rAAV5	40	6×10^{13}	NA	NCT03370913	Recruiting
BioMarin Pharmaceutical (Phase 3)	BMN 270	BDD-FVIII	rAAV5	40	4×10^{13}	NA	NCT03392974	Not yet recruiting
Shire	Bax 888	BDD-FVIII	rAAV8	10	NA	NA	NCT03370172	Not yet recruiting

NA = not available, BDD-V3-FVIII = modified B domain-deleted factor VIII transgene (McIntosh J et al, Blood, 2013;121:3335–3344).

prevention of spontaneous (nontraumatic) and traumatic hemorrhage into the musculoskeletal system, vital organs, skin, and mucous membranes through the preventative intravenous administration of exogenous clotting FVIII or FIX replacement. This practice, known as prophylaxis, has become the standard of care in high-income countries for children and some adults with primarily severe hemophilia A,[16,17] and is also widely practiced in caring for individuals with hemophilia B.[17–19] The therapeutic principle underlying the first experiences with prophylaxis was the conversion of a severe to a moderate bleeding phenotype by achieving trough factor levels of 1% to 5% of normal.[20]

Prophylaxis is defined as primary, secondary, or tertiary according to published consensus criteria officially adopted by the Scientific and Standardization Committee (SSC) of the International Society of Thrombosis and Hemostasis (ISTH) (Table 3).[21,22] The first spontaneous or traumatic musculoskeletal (muscle and joint) or intracranial hemorrhage has been further specified as triggering indications for the initiation of primary prophylaxis (Table 3).[22] Importantly, there is Grade 1 evidence-based support for the significant decrease in all bleeding, and musculoskeletal hemorrhage, with adherence to thrice weekly primary prophylaxis.[23,24]

However, there are intervening pragmatic considerations in the implementation of primary prophylaxis, particularly for young children with severe hemophilia A. The risk of early musculoskeletal hemorrhage must still be balanced against problematic regular recurrent venous access in a young child. Consequently, several approaches to achieving full-dose prophylaxis evolved prior to the introduction of extended half-life (EHL) clotting factor. In 1 study, the most aggressive approach, directly initiating a regimen of 20 to 50 IU kg^{-1} on a ≥3 times weekly schedule to maintain an FVIII trough level of ≥1 U dL^{-1} (FULL regimen), resulted in 87% of children achieving full-dose prophylaxis by age 4 years. However, 88% of children so

treated also required a central venous access device (CVAD) for factor administration. (Table 4).[25] Alternatively, step-wise approaches beginning with 1 to 2 times weekly administration, with either a 3- to 6-month intentional ramp-up to full-dose prophylaxis (ASAP regimen) or gradual dose augmentation predicated on breakthrough bleeding (PHENOTYPE regimen), resulted in commensurately fewer children requiring CVADs, but fewer also achieving full-dose prophylaxis at an early age (Table 4).[25] Furthermore, delay in achieving fully effective prophylaxis directly correlated with increased rates of breakthrough musculoskeletal hemorrhage and the greater potential for chronic arthropathy in one or more target joints (Table 4).[25]

Importantly, despite the body of evidence for the benefit of primary prophylaxis in severe hemophilia A, there are few phenotypic predictors or biomarkers within this narrow window for critical intervention that can inform a child's risk-benefit ratio for hemorrhage and thus guide the personalized timeline for implementation of full-dose prophylaxis.

The SSC/ISTH guidelines for primary prophylaxis prior to EHL factor include hemophilia B with the recommendation to treat with 35 to 50 IU kg^{-1} every 3 days to twice weekly to achieve a target FIX plasma level trough of 1 U dL^{-1}.[22] This regimen and the criteria for initiation of primary FIX prophylaxis have been extrapolated from hemophilia A data, given the considerably fewer efficacy data for hemophilia B, and accommodates the longer FIX plasma half-life of approximately 16 hours in the pediatric population. In 1 national retrospective study, age at start of prophylaxis similarly trended downward for hemophilia A and B over a decade to a comparable median age of 1.8 and 1.4 years, respectively, in 2007.[18]

However, the incorporation of prophylaxis into the standard of care for children and adolescents has considerably increased clotting factor consumption, adding significant cost to the care of hemophilia in high-income countries. In 1 published model for

Table 3

Consensus Definitions for Hemophilia Prophylaxis[20]

Primary prophylaxis[a]	Regular continuous[b] replacement therapy started in absence of documented joint disease by examination and/or imaging, and before the 2nd clinical joint bleed and age 3 years
Secondary prophylaxis	Regular continuous[b] replacement therapy started after ≥2 joint bleeds but before the onset of joint disease on examination and/or imaging
Tertiary prophylaxis	Regular continuous[b] replacement therapy started after the onset of joint disease documented by examination and plain radiographs

[a] Started immediately or shortly after 1st recognized joint or clinically significant muscle hemorrhage; started immediately after an intracranial bleed; both spontaneous and traumatic bleeding are triggering indications for prophylaxis.

[b] Continuous defined as intent to treat for 52 weeks y^{-1} but with a minimum a priori defined infusion frequency for ≥45 weeks y^{-1}.

Table 4

Outcome Relative to Primary Prophylactic Strategy[23]

Prophylactic Strategy	Full	ASAP	PHENOTYPE
Age at evaluation		4 years	
Number of patients	66	130	167
Full prophylaxis, %	87	65	38
CVAD required, %	88	34	22
Cumulative no. joint bleeds, median (IQR)*	1 (0–2)	1 (1–3)	3 (1–5)
% without joint bleeds*	32	27	8

CVAD = central venous access device, IQR = interquartile ratio.
* P value < 0.01.

severe hemophilia A, initiation of full primary prophylaxis according to the current recommendations and continued through age 20 at maximally joint-protective doses of 4000 IU kg^{-1} y^{-1} would already result in a mean cumulative factor consumption of 3.2×10^6 IU compared with 0.5×10^6 IU with on demand therapy.[26] Extended throughout a lifetime, mean cumulative use is calculated to reach 19×10^6 IU (vs 3.9×10^6 IU for on demand treatment), with diminishing cost effectiveness.[26] The authors suggest that this model may provide some rationale for severe hemophilia A adults evolving from primary prophylaxis to a treatment plan that switches between non-primary prophylaxis and on demand regimens based on rates of breakthrough hemorrhage and acceptable target levels of musculoskeletal bleeding protection, as well as both availability and cost of clotting factor.[26]

And yet, the trends point to greater adoption of primary prophylaxis beyond childhood. The United Kingdom Haemophilia Centre Directors Organization (UKHCDO) national guidelines recommend continuing primary prevention into adulthood for all except the mildest bleeding phenotypes.[19] The United States, historically a later adopter of national prophylaxis among high-income countries, reported an overall doubling in the use of continuous prophylaxis from 31% to 59% of persons with severe hemophilia A between 1999 and 2010 that included a near doubling of primary prophylaxis to 75% of children and adolescents < 0 years old, as well as an increase from 11% to 51% use in adults between 20 and 30 years of age.[27]

Furthermore, considerable complexity is emerging in any analysis of outcomes in the adult landscape of nonprimary prophylaxis. The UKHCDO guidelines encourage the de novo adoption of secondary and tertiary prophylaxes in adults with ongoing hemorrhage that impacts quality of life.[19] That strategy would be supported by the US national study that reported a similar decrease in joint hemorrhage of approximately 20% among individuals who did and did not adopt secondary and tertiary prophylaxis, suggesting regimen self-selection based on

bleeding phenotype in the adult population.[27] However, the US study also confirmed that while prophylaxis predicted decreased bleeding at any age (P < 0.001), only prophylaxis initiated at age <4 years (P < 0.001) and the absence of obesity (P < 0.001) predicted preserved joint range of motion.[27] Furthermore, in a US health economics study, savings in healthcare utilization fully offset the incremental pharmacy costs associated with prophylaxis among children aged 6 to 18 years, while no offset was observed in older adults (45–64 years) on prophylaxis.[28]

Finally, the evidence base for prophylaxis has been established from outcomes generated from the infusion of plasma-derived and biosimilar recombinant factor concentrates while the clotting factor landscape is rapidly evolving for hemophilia A and B with the registration of multiple EHL FVIII (Table 5) and FIX (Table 6) concentrates.[29] Although many of the products are already marketed in the United States, guidelines for how they might be incorporated into prophylaxis strategy there have not yet been issued. Moreover, published UKHCDO deliberations suggest some substantial quandary about which individuals would benefit and the extent to which the EHLs would necessarily modify the existing regimens.[30] This is particularly so in children with severe hemophilia A, given the small incremental increase in half-life that EHL FVIII products have been shown in preregistration clinical trials.[31–34]

And herein lies the speed bump on the prophylaxis innovation highway—the promise of excellent outcomes in children and adolescents has created the imperative to establish a viable health economic strategy for the adoption of bleeding prevention across the lifespan, and into the low- and middle-income countries where 80% of persons with hemophilia reside,[35,36] that also incorporates the evolutionary experience with the next generation of EHL therapeutics.

As outlined in Figure 1, such a strategy would necessarily require a migration from national guideline-informed standards of care toward algorithm-based person-specific event prediction interventional decision making, driven by standardized and harmonized health and economic outcome data collected as part of clinical care. If accomplished in a timely way, clinical and economic outcome data on current products and treatment strategies would become the baseline against which to evaluate the incorporation of novel and/or curative technologies into the standard of hemophilia care. The goal for data collection and analysis would be the generation of accurate predictive models for individual disease severity and propensity for hemorrhage is derived from standardized deep phenotype data, genomic and transcriptomic information, and a more complete understanding of the environmental determinants of risk. These models would in turn inform person-specific therapeutic decision making in hemophilia prophylaxis, as well as in the overall approach to hemophilia care.

Table 5

Extended Half Life Factor VIII Products (Adapted From[27])

Product (Company)	Technology	Terminal Half-Life, h	Status[a]
Elocta/Eloctate (Biogen Idec/Sobi)	Fusion protein with IgG1 Fc fragment (rFVIIIFc)	19	Marketed in USA, Canada, and Europe
Adynovate (Baxalta/Shire)	Random PEGylation (BAX 855; 20 kDa)	14–16	Marketed in USA, Japan, and Switzerland
NN7088 (Novo Nordisk)	Site-specific glycoPEGylation (N8-GP; 40 kDa)	19	Biologics License application filed with FDA, February 2018
BAY 94-9027 (Bayer)	Site-specific PEGylation (K1804C PEGylation; 60 kDa)	19	Biologics License application filed with FDA, September 2017
Afstyla (CSL Behring)	Single chain rFVIII (CSL627)	NA	Marketed in USA

NA = not available, rFVIII = recombinant factor VIII.
[a] Status as of July 2018.

Table 6

Extended Half Life Factor IX Products (Adapted From[27])

Product (Company)	Technology	Terminal Half-Life, h	Status[a]
Alprolix (Biogen Idec/Sobi)	Fusion protein with the Fc fragment of IgG1	82	Marketed in USA, Canada, and Europe
Idelvion (CSL-Behring)	Fusion protein with albumin	102	Marketed in USA, Japan, and Europe
N9-GP (Novo Nordisk)	Site-specific glycoPEGylation	93	Marketing in USA, 2018

[a] Status as of July 2018.

The NHLBI is currently supporting research that may ultimately shape the pathway toward a precision medicine approach to prophylaxis and to the holistic care of individuals with hemophilia. The cornerstone of this effort is the NHLBI-wide program entitled Trans-Omics for Precision Medicine (TOPMed), which endeavors to further study cohorts in heart, lung, blood, and sleep science through genomic and transcriptomic characterization, data that will eventually made accessible to researchers worldwide through dbGAP and other data commons.[37] Included in TOPMed is a cohort of 5142 US individuals with hemophilia A who had had baseline FVIII genotyping performed through the national My Life Our Future project. In a separate NHLBI-supported initiative, the PhenX tool, developed by the NIH,[38] is being used to develop standardized measures to characterize hemophilia phenomic data.

Furthermore, NHLBI is working with the hemophilia community to establish a national blueprint for the design of prospective cohorts that leverage national data collection and incorporate standard measures for prioritized outcomes, including patient-reported outcomes. Informatics, biobanking, and ethics expertise from within the NIH and the scientific community is being mobilized to create models for direct data transfer from electronic medical records, as well as policies for centralized biobanking, and streamlined data sharing for individual patient-level data. This project is prioritizing the engagement of persons with hemophilia, exploring private-public funding partnerships, and maximizing training opportunities in epidemiology and data science to ensure the long-term role of strategic national data and biospecimen collection in the future precision medicine approach to risk stratification and therapeutic decision making in hemophilia.

Overcoming inhibitors of healthy outcomes in severe hemophilia A

While the past few decades have ushered in tremendous progress in the prevention of hemorrhagic and transfusion-transmitted complications of hemophilia and its treatment, they have also seen minimal mitigation, if not intensification, of a complication that has the potential to negatively impact the lifelong healthy outcomes that individuals with even severe disease and access to

> National guidelines are replaced by algorithms for person-specific prediction of disease related events and choice & timing of interventions

> Person-specific algorithms for event prediction and interventional decision-making are derived from national and international datasets of individual and population-based health outcomes and economic analyses

> Data collection is as much a part of clinical care as clinical research

> Outcome data on current strategies and products become the baseline for future decision-making when incorporating other novel and/or curative technologies into the standard of hemophilia care

> Accurate predictive models of individual disease severity, as well as propensity for hemorrhage and other bleeding complications, are derived from standardized deep phenotype data informing and informed by transcriptomics

Figure 1. **Moving toward a precision medicine approach to prophylaxis and to hemophilia care in general.**

primary prophylaxis have come to routinely expect. This treatment-related complication is the development of a polyclonal neutralizing anti-FVIII IgG4 antibody that predominantly occurs in 25% to 30% of children with severe hemophilia A after a median number of 14.5 exposures to FVIII replacement therapy at a median age of 15.5 months.[39,40] These anti-drug antibodies (ADAs) bind to FVIII with type 1 kinetics, effectively inhibiting infused FVIII activity and, thus, referred to as inhibitors.[41] The increased morbidity and mortality associated with inhibitors that cannot be eradicated in a timely way, particularly if high titer (≥5 Bethesda Units [BU]),[21] have been well documented.[42,43] Neutralizing antibodies also occur less frequently in nonsevere hemophilia, usually in response to intensive FVIII replacement in the setting of trauma of surgery, and impact the phenotype is a significant but distinctive manner.[44] The natural history of this phenomenon and associated risk factors have been well documented through the study of the European INSIGHT cohort.[45–48] Furthermore, neutralizing polyclonal, predominantly IgG4, anti-FIX antibodies also occur in 2% to 4% of severe hemophilia B patients, and can produce a unique and therapeutically challenging clinical phenotype characterized by anaphylaxis and largely associated with the null genotype.[49–52] Due to their high frequency and disproportionate impact on hemophilia clinical outcomes, this article will focus on the scientific gaps, therapeutic innovation, and ongoing challenges related to FVIII antibodies developing in severe hemophilia A.

The current knowledge about the combination of host and environmental risk factors that trigger the innate immune system in the presence of immune "danger signals" have been studied and frequently reviewed, most recently within the last few years[39,40,53,54] (Fig. 2). Predictive modeling of FVIII inhibitor development has been attempted, but has so far been limited by an incomplete understanding of the risk factor landscape.[55]

There has been significant focus on the contribution of FVIII product type (von Willebrand factor-containing plasma-derived vs recombinant)[39,56–64] and recombinant FVIII brand[65,66] to the risk of ADAs, much of which has intensified rather than settled the controversy surrounding this issue. Retrospective studies mostly suggest an increased ADA risk with recombinant FVIII; however, the largest prospective cohort study (RODIN) failed to ascertain a difference in risk.[39,67] Conversely, the international randomized SIPPET trial of 251 mostly previously untreated pediatric patients (PUPs) with severe hemophilia A did demonstrate a significant 87% increased risk of cumulative incidence (CI) of all inhibitors on recombinant FVIII (rFVIII) when compared to plasma-derived (pdFVIII), as well as a similar trend for high titer antibodies.[68] Debate persists about the impact of these results on the complex clinical decision-making process involved in the choice of treatment product type for young hemophilia children, especially given that the CI of all inhibitors was still substantial with the use of pdFVIII (27%), and that the rFVIII product landscape is still rapidly evolving to include novel recombinant biologics that could potentially mitigate inhibitor risk.[69–71]

However, the SIPPET study outcomes provide the strongest rationale to date for post-translational variation between recombinant and plasma-derived FVIII as potential key determinants of the protein's unique immunogenicity among serine proteases in the coagulation cascade.[72–74] Currently, the lack of sufficient actionable knowledge about FVIII immunogenicity constitutes the major remaining speed bump on the innovation highway toward predictably healthy outcomes in severe hemophilia A. Epidemiological and clinical research on FVIII inhibitors has been robust. But future progress may now depend on bringing bedside observations back to the laboratory to inform the mechanistic study of FVIII immunogenicity, the identification of novel druggable targets for inhibitor eradication and tolerance induction, and, ultimately, the rationale design of new biologically active FVIII therapeutics with reduced immunogenicity to minimize or entirely prevent the development of ADAs.[75]

With this goal in mind, NHLBI convened a group of experts in early 2015 to assist in identifying critical scientific gaps in our mechanistic understanding of FVIII immunogenicity. NHLBI issued a funding opportunity announcement https://grants.nih.
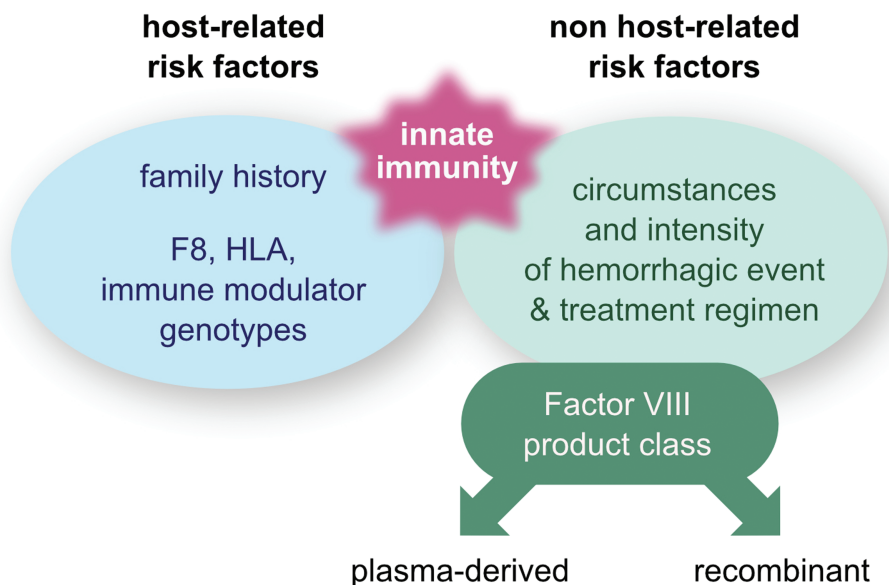

Figure 2. Known risk factors for factor VIII inhibitor development.

gov/grants/guide/rfa-files/RFA-HL-18-014.html in 2017 to stimulate and facilitate the critical science identified by panel of experts from within and outside the field. NHLBI proposed to do this by funding Research Centers of Excellence that would use interdisciplinary teams and bold new approaches to identify FVIII protein-specific triggers and mechanisms underlying the development of anti-FVIII neutralizing antibodies. Applicants in actively engaged disciplines were required to propose basic and translational research that incorporated emerging sciences and technologies that were not yet being exploited in the investigation of FVIII immunogenicity. Collaborations in trans-Omics, glycobiology, the microbiome, and in silico protein design were encouraged. Finally, applicants were required to propose a curriculum to cross train the next generation investigators in interdisciplinary skills development. Teams of interdisciplinary scientists initiated their NHLBI-funded investigation and training activities in 2018.

But even as investigators pursue the science of FVIII immunogenicity, there remains a persistent urgent need to treat individuals with high titer inhibitors with products and strategies that bypass the requirement for FVIII replacement; expediently control acute bleeding in the absence of FVIII; and effectively prevent chronic hemorrhage-associated musculoskeletal morbidity. Bypass therapy with prothrombin complex concentrates (PCCs) was first proven to be effective in the control of acute bleeding in seminal placebo-controlled trials published over 30 years ago by Lusher et al.[76,77] Contemporaneously, Hedner and Kisiel reported on the efficacy of recombinant activated factor VII (rFVIIa) as a therapeutic FVIII bypassing agent in 2 hemophilia patients with high titer inhibitors.[78] By the 1990s, the principle of procoagulant-driven bypass therapy governed the approach to the treatment and prevention of hemorrhage in the presence of inhibitors. An activated PCC (aPCC) and rFVIIa, intravenously administered alone or in combination, became and remained the mainstay of bleeding control for almost 3 decades, with comparable 80% to 90% efficacy for musculoskeletal hemorrhage,[79] and a similar 60% efficacy in bleeding prophylaxis, inferior to the effectiveness of bleeding prophylaxis in the absence of inhibitors.[80,81]

However, during the past 3 years, a plethora of novel strategies to control hemorrhage in the absence of FVIII or FIX replacement have been proposed (Table 7).[82–93] None of these novel agents would be expected to induce or be inhibited by anti-FVIII (or FIX) antibodies. Furthermore, the potential to administer these therapeutics subcutaneously and on a weekly to monthly schedule promises to appreciably improve quality of life in this population in a way that has not been feasible with traditional protein replacement.

The premise underlying most of these approaches is that rebalancing hemostasis would physiologically offset the bleeding diathesis created by a severe deficiency in FVIII or IX, with or without circulating neutralizing antibodies. Two of these novel therapeutics are currently in clinical trials. Phase 3 trials of an antithrombin short interfering RNA (siRNA), Fitusiran (ALN-AT3SC; Alnylam Pharmaceuticals, Sanofi, Cambridge, MA, USA) are ongoing in patients ≥12 years with severe hemophilia A and B, with (ATLAS-INH; NCT03417102) and without (ATLAS A/B; NCT03417245) inhibitors. A patient death from cerebral sinus thrombosis in the preceding Phase 2 trial of this agent required FDA review and replacement dose modification prior to resumption of the Phase 3 trials. Clinical trials of 3 different monoclonal antibodies to tissue factor pathway inhibitor (TFPI) are also ongoing. These include 2 Phase 2 proof of concept studies of concizumab, an IgG4 monoclonal antibody that targets the Kunitz 2 (K2) TFPI domain, in adult hemophilia A and B subjects with inhibitors (explorer 4; NCT03196284, Novo Nordisk A/S, Bagsværd, Denmark) and in adult hemophilia A individuals without inhibitors explorer 5; NCT03196297); a Phase 2 multidose trial of PF-06741086, an IgG1 monoclonal antibody that also targets the K2 TFPI domain, in adult hemophilia A or B participants with or without inhibitors (Pfizer; NCT02974855); and a Phase 1 single escalating and multiple dose study of BAY 1093884, an IgG2 monoclonal antibody that targets both the TFPI Kunitz 1 (K1) and K2 domains, in severe hemophilia A and B with and without inhibitors (Bayer, Berlin, Germany; NCT025571569).

A truly disruptive innovation in effective therapeutics delivery for patients with severe hemophilia A with and without inhibitors has come from the development of emicizumab (ACE910), a bispecific antibody that functions as a FVIIIa-mimetic in the tenase-generating complex with factors IX and X[88,90,91,94,95] (Table 7). Despite some limitations in study design, the initial Phase 1/2 trial in a Japanese cohort reported an impressive short-term decrease in annualized bleed rate, regardless of FVIII inhibitor status, in most subjects with historically severe bleeding phenotypes receiving weekly emicizumab prophylaxis at 1 of 3 escalating doses.[96] These results were replicated in the subsequent Haven 1 Phase 3 trial of 109 hemophilia A participants with inhibitors aged ≥12 years who received 4 weekly subcutaneous 3 mg kg^{-1} doses of emicizumab followed by 1.5 mg kg^{-1} weekly for 24 weeks (NCT02622321).[97] The ABR of 2.9 events (95% confidence interval [CI], 1.7–5.0) among participants who were randomly assigned to emicizumab prophylaxis represented a statistically significant 87% decrease compared with those assigned to no prophylaxis.[97] The prophylactic efficacy demonstrated in this study was pivotal to US licensure of this therapeutic

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Table 7

Novel Strategies for the Prevention of Hemorrhage in Hemophilia ± Inhibitors

Altering the hemostatic balance in hemophilia A and B	
Super Factor Va	Resistant to inactivation by activated protein C (APC)
Zymogen-like Factor Xa Variant Factor Xa^{I116L} (Pfizer)	Resistant to inactivation by serine protease inhibitors
ALN-AT3 SC (Fitusiran; Alnylam)	Short interfering RNA (siRNA) disruption of antithrombin (AT3) translation
Anti-TFPI	Humanized monoclonal antibodies to tissue factor pathway inhibitor (TFPI)
Concizumab (Novo Nordisk)	downregulate TFPI to increase Factor Xa generation
PF-06741086 (Pfizer)	
BAY 1093884 (Bayer)	
Activated Protein C (APC)-specific serpin	α1 antitrypsin Pittsburgh inhibition of APC
Expanding the approach to bypass therapy to hemophilia A	
Emicizumab (ACE910; Hemlibra) (Roche, Genetech)	Factor VIII-mimetic bispecific antibody

in November 2017 (Hemlibra, Roche, Genentech, San Francisco, CA, USA)[98] followed by European registration in February 2018.

Ongoing trials include HAVEN 2 (NCT02795767),[99] examining the HAVEN 1 clinical endpoints in participants ≤12 years old; HAVEN 3 (NCT02847637), studying multiple dosing regimens in hemophilia A noninhibitor participants aged ≥12 years; HAVEN 4 (NCT03020160), studying the safety and efficacy of an every 4-week regimen of 6 mg kg^{-1} emicizumab in hemophilia A inhibitor and noninhibitor participants; and a study of emicizumab prophylaxis to provide bleeding prophylaxis in minor surgery without additional administration (NCT03361137). Based on preliminary data from the HAVEN 3 trial, the US Food and Drug Administration (FDA) has granted Breakthrough Therapy Designation to emicizumab for people with hemophilia A without FVIII inhibitors in April, 2018 (https://www.gene.com/media/press-releases/14713/2018-04-16/fda-grants-breakthrough-therapy-designat/).

Since its licensure in the United States, the demand for emicizumab has been high among hemophilia A patients with and without inhibitors, despite unresolved issues with laboratory monitoring, and the safety signals (5 episodes of thrombosis and/or thrombotic microangiopathy, including 1 fatality from hemorrhage after the TMA had resolved) associated with the concomitant use of high-dose aPCC administration for breakthrough bleeding noted in the HAVEN 1 trial.[97] Although the therapeutic conditions associated with these severe adverse events have led to clinical recommendations for the concomitant use of bypass therapy (https://www.hemophilia.org/sites/default/files/MASAC-Update-on-the-Approval-and-Availability-of-the-New-Treatment.pdf), no pathophysiologic explanation has yet emerged. Furthermore, the development of ADAs to emicizumab, noted in the and original pharmacokinetic studies conducted in healthy volunteers[90,91] but of previously uncertain clinical significance, has now been reported in a single HAVEN 2 trial participant in whom the drug was rendered ineffective (https://www.hemophilia.org/Newsroom/Medical-News/MASAC-Safety-Information-Update-on-Emicizumab-HEMLIBRA).

The unprecedented and simultaneous emergence of multiple-novel therapeutics for the prevention of bleeding in hemophilia with and without inhibitors, and their rapid pathway to licensure and subsequent penetration into the standard of care based on short- and medium-term efficacy data, represent the paradigm of the hemophilia innovation highway. But definitive speed bumps are arising from an incomplete and evolving safety profile and an incomplete understanding of how to optimize their incorporation into an individual's comprehensive care plan. In the case of emicizumab, there are many questions with no immediate answers. If proven effective in the longer term, will its early use in primary prophylaxis alter the epidemiology of FVIII inhibitors? If less effective in preventing major hemorrhage from trauma or during surgery, what will be the immunologic consequences of FVIII rescue in an inflammatory state? Should its use for bleeding prophylaxis in inhibitor patients supplant initial attempts at inhibitor eradication, replace bypassing agents during traditional immune tolerance induction, and/or primarily provide a safe alternative to failed immune tolerance? The answers will come in time, but not without strategic data collection from which to develop precise models for individual inhibitor prediction, and well-designed and executed clinical trials to optimize personalized intervention. Consequently, these questions and more have been prioritized in NHLBI's approach to the development of a

blueprint for future clinical, translational, and basic science research in FVIII immunogenicity and inhibitor prevention and eradication.

Conclusion

These are both exciting and challenging times in the history of hemophilia. In times of tremendous innovation and therapeutic progress, numerous new questions arise as old problems are solved, and with them, the responsibility to take stock and shape new paradigms through emerging science and technology. There has never been a more critical need to bring substantial data to the table to craft the next generation of solutions, nor a more promising toolkit with which to do so.

Acknowledgment

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