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Gene therapy for hemophilia: Recent developments and challenges (Review)

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Abstract. Hemophilia is an X-linked disease marked by the absence or impaired function of specific coagulation factors. Hemophilia A is characterized by a deficiency in factor VIII, and hemophilia B is characterized by a deficiency in factor IX. Factor XI deficiency, referred to as hemophilia C, is a very uncommon bleeding illness characterized by moderate symptoms; although the condition is not widespread, standard management poses significant challenges linked to socioeconomic factors. The introduction of FDA-approved gene therapy for hemophilia B in 2022 holds transformative potential; however, certain issues demand careful consideration. The present review provides insight into the existing hemophilia landscape, discusses the latest strides in gene therapy for hemophilia, and examines the hurdles that need to be addressed to make this innovative treatment more accessible to individuals afflicted by this condition.

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1. Introduction

Hemophilia is a relatively rare hereditary bleeding disease characterized by a lack or decreased functionality of particular coagulation components. The prevailing manifestations of hemophilia encompass hemophilia A (MIM No 306700), characterized by a deficiency in factor VIII, and hemophilia B (MIM No 306900), characterized by a deficiency in factor IX. Factor XI deficiency sometimes referred to as hemophilia C (MIM No. 612416), is a very uncommon bleeding ailment characterized by moderate symptoms. This condition is most frequent among individuals of Ashkenazi Jewish descent (1).

The global prevalence of hemophilia A is considered to be ~17.1/100,000 males at birth, whereas the prevalence of hemophilia B is estimated to be ~3.8/100,000 males at birth. Based on current estimations, the global population of individuals affected by hemophilia is ~1,125,000, out of which ~418,000 individuals are diagnosed with severe hemophilia (2).

Clinical manifestations in patients with hemophilia are solely attributable to the absence of a single protein present in minute quantities in the circulation; hence, gene therapy has long been an attractive method for the treatment of hemophilia, as it provides the possibility of a cure by facilitating the natural production of the deficient coagulation factor by transferring a healthy copy of the respective gene (4). It has been demonstrated that a 5% increase in the levels of the deficient coagulation factor can substantially alleviate the symptoms of hemorrhage (3). The present review aimed to shed light on the current status of hemophilia in Iraq. The present review summarizes the recent advances in gene therapy for hemophilia and discusses the remaining obstacles to enhancing the availability of the novel therapy for patients with this disorder.

2. Literature search methods

A literature search was conducted using the PubMed and Web of Science and Google Scholar platforms for multiple combinations of different hemophilia types, epidemiology, management and gene therapy. The present review encompasses articles produced in the English language, encompassing both preclinical and clinical studies, up until June, 2023. The search results were initially screened based on the relevancy of their titles and abstracts, after which the whole contents of the relevant articles were examined. In addition, pertinent

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research of the reference lists of the relevant documents was performed. Publications written in languages other than English were eliminated.

3. Hemophilia status in Iraq

Epidemiological studies on hemophilia in Iraq are limited in number. According to the World Bleeding Disorder Registry (WBDR) report in 2021, a total of 2,567 hemophilia cases have been registered in Iraq. Among these cases, 2,024 were hemophilia A and 543 were hemophilia B (5).

A recent study including four hemophilia centers in Baghdad, Iraq indicated that the prevalence of hemophilia has doubled over a 10-year period, from 7.2 per 100,000 individuals in 2007 to 15.9 per 100,000 population in 2016 (6). This rate appears to be greater than the rates estimated in neighboring countries, including Iran (7.1), Turkey (7.0), Egypt (6) Jordan (4.4), Syria (3.5), and Saudi Arabia (1.4) per 100,000 individuals (6). According to the same study, hemophilia A comprised the majority, namely 72.9%, of all documented cases of hemophilia, with a prevalence rate of 5.9 per 100,000 individuals within the population. Conversely, hemophilia B constituted 24.8% of the cases, with a prevalence rate of 2 per 100,000 individuals within the community and the least prevalent is hemophilia C with a rate of 0.1 per 100,000 individuals (6). A smaller cohort study conducted at the National Center of Hematology in Baghdad, Iraq involving 191 children with an average age of 5.3 years revealed that hemophilia A ranked as the third most registered bleeding disorder among children with a prevalence of 9.4% following von Willebrand disease (MIM No 613160) 86.9% and Glanzmann's thrombasthenia (MIM No 273800) 39.8% (7).

The overall survival estimates for patients with hemophilia fluctuate considerably as a result of treatment accessibility and advancements in care (8). The life expectancy of individuals with hemophilia who have access to modern treatment and prophylaxis approaches is often virtually normal, and the survival rate is approaching that of the general population (8). However, survival bias is a potential concern in studies that involve low- and middle-income countries, which may be a consequence of the fact that neonatal mortality rates are highest in these countries. The premature fatalities that disproportionately affect individuals with severe hemophilia in low- and middle-income countries may be the cause of skewed outcome comparisons (bleeding events and age of diagnosis) between these nations (9). Regrettably, the precise survival rate of hemophilic individuals in Iraq is not adequately documented in the published data.

Local studies reported that ~40-63% of the registered hemophilia reported the severe type (6,10); however, less than half of these patients were on prophylactic therapy and or on-demand therapy (6).

Current therapeutic approaches in Iraq include factor concentrate, tranexamic acid and to a lesser extent, desmopressin (6). However, in a recently published open-label trial conducted across multiple Iraqi centers, the efficacy of emicizumab prophylaxis was evaluated compared to episodic recombinant factor VII treatment in 32 individuals with hemophilia A and high inhibitor titers (7). That study included patients ranging from 1-46 years of age. The findings revealed a significant improvement in various outcomes, including bleeding rates, joint bleeding, hospital admissions, blood transfusions and school or work absences, when patients switched to emicizumab prophylaxis from their previous 6 months of episodic recombinant factor VII treatment. The most commonly observed adverse effect was injection site reactions, while there was no observed risk of developing thrombosis (7).

The development of inhibitors in Iraqi patients with hemophilia was assessed in several small local studies with a prevalence rate ranging between 5-12%. These studies have consistently shown that inhibitors are more commonly observed in individuals with hemophilia A (10-12). Taresh and Hassan (10) conducted an evaluation of inhibitor levels in a group of 118 individuals diagnosed with hemophilia A and 25 individuals diagnosed with hemophilia B, a total of 22 individuals (constituting 18.6% of the sample) were found to have inhibitors. Notably, all these patients were diagnosed with hemophilia A, with a substantial majority (82%) displaying elevated titers. The likelihood of developing inhibitors was significantly elevated in patients with severe hemophilia who had been exposed to factor VIII concentrate at an early age (≤3 months). Furthermore, those who have a familial background of autoimmune disorders and have immune system challenges had a higher propensity for the development of inhibitors (10).

4. Current standard management of hemophilia

The primary purpose of hemophilia therapy is to effectively manage the frequency and severity of bleeding, apart from mitigating the risk of long-term joint degeneration and mortality (13). Depending on the concentration of defective clotting factors, patients are classified as having mild, moderate, or severe hemophilia. Socioeconomic factors greatly affect care standards (4). For severe hemophilia, prophylactic replacement therapy is the recommended treatment to maintain clotting factor levels >1% (13). Financial restrictions prevent 75% of patients with hemophilia in low- and middle-income countries from receiving regular preventive therapy (13). In such circumstances, the guidelines established by the World Federation of Hemophilia (WFH) advocate for the use of low-dose prophylaxis and, in certain cases, on-demand medication as effective strategies for managing bleeding episodes (14). Fresh-frozen plasma or cryoprecipitate, which may cause volume overload and blood-borne pathogen transmission, makes factor leveling harder (4). Thus, hemophilia-related health issues and lower lifespans persist in these countries

Standard recombinant factor VIII and factor IX factors. These factors have a short half-life in the bloodstream; thus, lifelong treatment strategy necessitates the intravenous administration of clotting factor concentrates at least two to three times per week (15).

Extended half-life factor prophylaxis. Pegylation (attaching polyethylene glycol) or fusing clotting factors with proteins such as albumin or Fc have been used to increase factor VII and factor IX stability and half-lives. BIVV001, a novel FVIII fusion protein, has been proposed to boost the half-life of

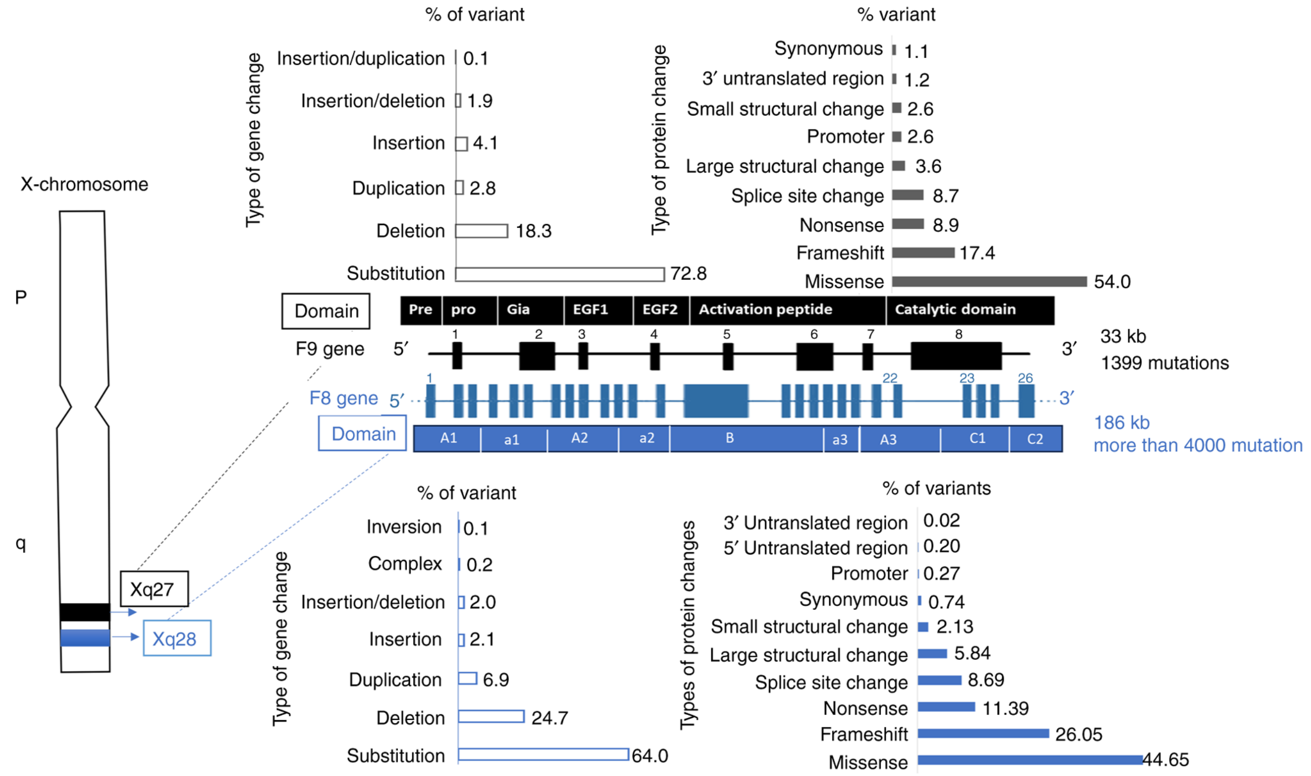
X-chromosome
P
q
Xq27
Xq28
% of variant
Type of gene change
Insertion/duplication 0.1
Insertion/deletion 1.9
Insertion 4.1
Duplication 2.8
Deletion 18.3
Substitution 72.8
% variant
Type of protein change
Synonymous 1.1
3′ untranslated region 1.2
Small structural change 2.6
Promoter 2.6
Large structural change 3.6
Splice site change 8.7
Nonsense 8.9
Frameshift 17.4
Missense 54.0
Domain
Pre
pro
Gia
EGF1
EGF2
Activation peptide
Catalytic domain
F9 gene
5′
1 2 3 4 5 6 7 8
3′
33 kb
1399 mutations
F8 gene
5′
1 22 23 26
3′
Domain
A1 a1 A2 a2 B a3 A3 C1 C2
186 kb
more than 4000 mutation
% of variant
Type of gene change
Inversion 0.1
Complex 0.2
Insertion/deletion 2.0
Insertion 2.1
Duplication 6.9
Deletion 24.7
Substitution 64.0
% of variants
Types of protein changes
3′ Untranslated region 0.02
5′ Untranslated region 0.20
Promoter 0.27
Synonymous 0.74
Small structural change 2.13
Large structural change 5.84
Splice site change 8.69
Nonsense 11.39
Frameshift 26.05
Missense 44.65

Figure 1. Diagram illustrating the structure of the factor VIII (F8) and XI (F9) genes and their corresponding coding sequences. The factor VIII gene, is situated on the long arm of the X chromosome at the Xq28 locus. This gene comprises a total of 26 exons that span 187,000 base pairs. The intron located at position 22 (32 kilobases) inside the F8 gene has the greatest size. The IX gene (F9) is characterized by its comparatively compact size and lower level of complexity since it spans a length of 33.5 kilobases located at Xq27 locus with a total of eight exons. Of note, >4,000 variants in the F8 and ~1,399 variants in F9 genes have been recorded. The prevalence of genetic and protein alterations of F8 and F9 are presented in the respective bar charts. Data were accessed form the CDC Hemophilia Mutation Projects (CHAMP and CHBMP): https://www.cdc.gov/hemophilia/mutation-project/?CDC_AAref_Val=https://www.cdc.gov/ncbddd/hemophilia/champs.html.

FVIII to 38 h, protecting patients with hemophilia for longer periods of time (15).

Antifibrinolytic agents. Antifibrinolytic agents are effective in the management of moderate and mild cases of hemophilia. Aminocaproic acid and tranexamic acid inhibit the proteolytic activity of plasmin (14). Desmopressin, on the other hand, stabilizes the already present factor VIII in the plasma by releasing the von Willebrand factor from its storage sites (13).

Targeted therapy. The utilization of immunotherapy, such as emicizumab, represents a notable advancement in the treatment and control of hemophilia. Emicizumab, is a recombinant humanized bispecific IgG antibody that replicates the cofactor activity of the deficient FVIII in individuals with hemophilia A (16). Emicizumab has a prolonged half-life of around 4-5 weeks (15).

5. Factor VIII and IX genotypes

Factor VIII and XI gene characteristics and common mutations are illustrated in Fig. 1. The most prevalent mutation observed in severe hemophilia A is the inversion of introns 22 and 1, which accounts for ~55% of cases and is linked to inhibitor development (17).

In a previous Iraqi study involving 18 patients with hemophilia A, the most frequent mutations in F8 gene were point mutations then inversion mutations followed by frameshift mutations. The severe phenotype was strongly associated with exon 24 mutations with P-value 0.009 and intron 22 with a P-value of 0.036 (18). A larger study included 80 Iraqi Kurdish patients with hemophilia A analyzed the inversion in intron 22 and intron 1 using inverse shifting-polymerase chain reaction reported that among patients with severe hemophilia A, 6.7% had Inv22 and 3.3% (2/60) had Inv1 (19).

Preclinical trials have investigated gene delivery vehicles whether viral or non-viral to establish the safety and efficacy of the gene transfer strategy, ascertain an appropriate starting dose in humans, and evaluate vector biodistribution and sustainability of delivered transduced gene with therapeutic range. A gene transfer strategy for the treatment of any disease requires three components: A therapeutic gene to be transferred, also known as the transgene; a vector or gene delivery vehicle to facilitate the transfer; and a physiologically relevant target tissue in the recipient (20).

In 1989, an early preclinical trial demonstrated that genetically modified *ex vivo* human skin fibroblasts containing human factor IX cDNA could produce factor IX and discharge it into the bloodstream of laboratory animal (21). However, the level was unstable, and it was difficult to determine the duration of transduced cell viability. Subsequent research concentrated on viral vectors, such as adenovirus, adeno-associated virus (AAV) and retrovirus. Adenovirus trials were hindered by immune response induction and hepatotoxicity, whereas

AAV and lentivirus exhibited promise in multiple preclinical scenarios. AVV, a naturally occurring, non-pathogenic virus, was shown to be capable of attaining the long-term expression of the desired gene in various tissues (22).

The sustained repair of the hemophilia phenotype in inhibitor-prone null mutation hemophilia B dogs was achieved with direct liver transduction of an AAV2 vector encoding the factor IX gene in 2009. This approach did not lead to the production of inhibitors (23). Through the use of mouse models, scientists have made the noteworthy observation that hepatic transduction with AAV vectors have the potential to promote the development of immunological tolerance towards factor IX. This process is considered to be mediated by the activation of regulatory T-cells (Tregs) expressing specific markers such as CD4, CD25 (24,25) and FoxP3. In the same manner, studies conducted on dogs and non-human primates have shown sustained transgenic expression over an extended period, with no discernible $CD8^+$ T-cell reaction to the capsid antigens of the vector. This observation holds true regardless of the specific transgene, promoter, or method of delivery (26).

As regards hemophilia A, the capacity of the AAV vector, which is <4.2 kb was restricted in carrying the full length of the F8 gene. The F8 cDNA may be truncated by the removal of the sequence that encodes a nonfunctional domain, known as B-domain deletion (BDD); this strategy was employed in two distinct phase I/II clinical trials for transferring a codon-optimized adeno-associated virus serotype 5 (AAV5) vector in a cohort of 9 patients with severe hemophilia; the results were encouraging with a sustainable increase in F8 activity over a period of 1 year (27).

Due to the absence of innate immunity, retroviral and lentiviral have been found to be attractive vectors for research (28). Retroviral vectors that are capable of transducing non-dividing cells were investigated in pediatric hemophilia, resulting in sustained therapeutic levels of factor VIII in neonatal mice with hemophilia A (26). Efficient retroviral transduction was also achieved in neonatal dogs with hemophilia (29). Immunosuppression was the only condition in which liver-directed LV gene therapy attained therapeutic-range human FVIII activity in non-human primates (30). Lentiviral vectors could be administered intravenously to hemophilic adult mice to achieve therapeutic levels of factor VIII or IX (31,32). However, these vectors were also adept at transducing antigen-presenting cells, and antibodies to the transgene product were detected in the majority of preclinical lentiviral experiments employing ubiquitous promoters, resulting in unstable levels of the factor even when using hepatocyte-specific promoters (26). In non-human primates, liver-directed LV gene therapy achieved therapeutic-range human FVIII activity in non-human primates, albeit only during immunosuppression.

The third-generation, self-inactivating lentiviral vectors further improved safety by splitting the viral genome into separate plasmids, rendering recombinant virus generation even more unlikely (33). In their preclinical study, Wang *et al* (16) injected mice intraosseously with a self-inactivating lentivirus vector encoding FVIII under the control of the Gp1b promoter resulting in partial correction of hemophilia. This led to platelet-producing cells that persistently expressed FVIII inside the animals, resulting in sustained FVIII therapeutic expression levels (16). Additionally, this approach has been proposed to protect FVIII against inhibitor-induced inactivation (34). Non-viral gene transfer includes plasmid and nanoparticles. Dermal fibroblast cells were used for early non-viral gene transfer trials (35). This was achieved by introducing a BDD gene-loaded plasmid into the fibroblasts via electropores made in the cell membranes and then injected into the momentum. After 12-18 months, there were no major side-effects and the FVIII level had increased (35). Lipid-based nanoparticles have been also employed. Prophylaxis with PEGylated liposomes (BAY 79-4980) was found to be safe and effective when compared to rFVIII-FS in equal doses in a phase I, controlled crossover design study (36). The phase II study, on the other hand, was terminated as BAY 79-4980 was unable to demonstrate superior efficacy to rFVIII-FS (37).

6. Clinical trials and FDA approval for gene therapy in hemophilia

Preclinical trials have provided a rough prediction of how humans might respond. Animal models typically demonstrate higher increases in factor VIII and IX levels compared to what has been observed in human trials (3).

Hemophilia B. The very first phase I clinical trial was reported by Lu *et al* (38) in 1993 who used the subcutaneous injection of *ex vivo* skin fibroblasts in 2 patients with severe B hemophilia and achieved increased clotting activity from 2.9 to 6.3% for 6 months in 1 patient only. After ~10 years, recombinant AVV vector was first implicated in factor-IX gene transfer to adult patients diagnosed with severe hemophilia B. Both intramuscular and transhepatic routes failed to sustain plasma transgene expression (39).

The first successful trial was reported in 2011. The administration of codon-optimized Factor IX (co-FIX) contained inside recombinant AAV8 vectors (specifically, scAAV2/8-LP1-hFIXco) was performed intravenously in a total of 6 individuals diagnosed with severe hemophilia B. All individuals expressed 2-11% of normal Factor IX, which improved bleeding (22).

Several subsequent multicentric open-labelled trials were conducted, as summarized in Table I. The preclinical trials used the Padua transgene, which has a naturally occurring single nucleotide mutation (R338 L) that results in a gain-of-function. This mutation, known as FIX-Padua, was applied to enhance the production of FIX by a factor of six to eight (40). The AAV5-hFIXco-Padua vector, known as AMT-061 or etranacogene dezaparvovec®, was used as a replacement for the AMT-060 FIX transgene in order to enhance the level of expression. A single administration of AMT-061 at a dose of 2x1,013 vg/kg successfully halted bleeding for a duration of 26 weeks, without the need for FIX replacement (41). The aforementioned compelling findings initiated the commencement of the worldwide HOPE-B (Health Outcomes With Padua Gene; Evaluation in Hemophilia-B) phase III research (NCT03569891), which aimed to further assess the efficacy of AMT-061 (42). FDA approved AMT-061(CSL Behring's) in 2022 at a cost of US$3.5 million per dose (43) prescribed as $2x10^{13}$ genome copies (gc)/kg IV (2 ml/kg) as a single one-time dose.

Table I. Results of published clinical trials that examined the efficacy of gene therapy in hemophilia B and A.

Sponsor	Authors	Year of publ.	Hemo. type	Type of study	Phase	No. of patients	Baseline F8 IU/dl	Vector	Transgene	Mean/ median[a] follow-up per week	Genome copies/kg	Mean factor activity %	Prophylactic factor replacement status	Trial registration no.	(Refs.)
-	Lu *et al*	1993	B	SC	1	2		rRV N2CMV-IX	FIX	24	-	92%↑	-	-	(38)
-	Manno *et al*	2006	B	MC	1-2	7	<=1	rAAV-2	FIX	111	$2x10^{12}$	Transient ↑for 8 w	-	-	(39)
National Heart, Lung, and Blood Institute	Nathwani *et al*	2011	B	SC	1	10	<=1	scAAV2/8	LP1-hFIXco	165	2c1011, $6x10^{11}$ $2x10^{12}$	1.8 IU/dl, 2.5 IU/dl, 5.1 IU/dl	92%↓ 96%↓	NCT00979238	(22)
Spark Therapeutics and Pfizer	George *et al*	2017	B	MC	1-2a	14	≤2%	AAV-Spark100	FIX-Padua	492	$5x10^{11}$	33.7%	98%↓	NCT02484092	(90)
uniQure B.V	Miesbach *et al*	2018	B	MC	1-2	10	≤2%	AAV5 AMT-060	FIX-Padua	54	$5x10^{12}$, $2x10^{13}$	4.4 IU/dl, 6.9 IU/dl	81%↓ 37%↓	NCT02396342	(91)
uniQure and CSL Behring	Von Drygalski *et al*	2019	B	SC	2b	3	≤2%	AAV5 AMT-061	FIX-Padua	26	$2x10^{13}$	≥40%↑	-	NCT03489291	(41)
Spark Therapeutics	George *et al*	2020	B	MC	1-2	7	<=1	AAV2	hFIX	780	$8x10^{10}$, $4x10^{11}$, $2x10^{12}$	-	84.5↓	NCT00515710	(92)
-	Konkle *et al*	2021	B	MC	1-2	8	≤2%	AAV8 (BAX 335)	FIX-Padua	45.3	$2.x10^{11}$, $1.0x10^{12}$, $3.0x10^{12}$	23-58.5%	N/A	NCT01687608	(93)
Freeline	Chowdary *et al*	2022	B	SC	1-2	17	≤2%	AAVS3 (FLT180a)	FIX-(R338L) Padua	108[a]	$3.84x10^{11}$, $6.40x10^{11}$, $8.32x10^{11}$, $1.28x10^{12}$	51-78%		NCT03369444 NCT03641703	(94)
-	Xue *et al*	2022	B	SC	1	12	<=1	rAAV/BBM-H901	FIX	58[a]	$5x10^{12}$, $2x10^{12}$	36·9%		NCT04135300	(95)
uniQure and CSL Behring	Pipe *et a*.	2023	B	SC	3	54	≤2%	AAV5 AMT-061	hFIX-coPadua	18	$2x10^{13}$	39%	96%↓	NCT03569891	(42)
-	Roth *et al*	2001	A	SC	1	6	<=1	plasmid	factor VIII	52	-	25%↑	-	-	(35)
-	Powell *et al*	2003	A	MC	1	13	<=1	retroviral MoMLV	BDD factor VIII	13-53	2.8, $9.2x10^{7}$, 2.2, 4.4, $8.8x10^{8}$	2.3-19%	-	-	(96)
BioMarin	Rangarajan *et al*	2017	A	MC	1	9	<=1	AAV5 (BMN270)	BDD hFVIII-SQ	52	$6x10^{12}$, $2x10^{13}$, $6x10^{13}$	<1 IU/dl, 1-3 IU/dl, 12-32 IU/dl	35.1%↓ 88%↓ 98%↓	NCT02576795	(27)
BioMarin	Pasi *et al*	2020	A		1-2	15	<=1	AAV5 (BMN270)	BDD hFVIII-SQ	156	$6x10^{12}$ $2x10^{13}$ $6x10^{13}$ $4x10^{13}$	<1 IU/dl <1 IU/dl 20-60 23 IU/dl	100↓ 99.6↓	NCT02576795	(45)

Table I. Continued.

Sponsor	Authors	Year of publ.	Hemo. type	Type of study	Phase	No. of patients	Baseline F8 IU/dl	Vector	Transgene	Mean/ median[a] follow-up per week	Genome copies/kg	Mean factor activity %	Prophylactic factor replacement status	Trial registration no.	(Refs.)
	George *et al*	2021	A	MC	1-2	18	≤2%	AAV3 SPK-8011	BDD-FVIII	146.4[a]	$5x10^{11}$, $1x0^{12}$, $1.5x0^{12}$, $2x10^{12}$	12%	99%↓	NCT03003533 and NCT03432520.	(50)
Pfizer	Visweshwar *et al*	2021			1-2	11		rAAV 2-6 (PF-07055480)	Modified BDD-FVIII	104	$9x10^{11}$ $2x\ 10^{12}$ $1x10^{13}$ $3x10^{13}$	- - - 30.9%	-	NCT03061201	(48)
BioMarin	Ozelo *et al*	2022	A	MC	3	134	<=1	AAV5 (BMN270)	BDD FVIII-SQ	51	$6x10^{13}$	41.9 IU↑	98.6%↓	NCT03370913	(46)
BioMarin	Mahlangu *et al*	2023	A	MC	3b	132	<1	AAV5 (BMN270)	BDD-FVIII-SQ	104-260	$6x10^{13}$	22.3 U/dl	98.2%↓	NCT03370913	(97)

[a]Indicates that these studies provided the follow-up duration using a statistical average (mean or median). The other studies presented follow-up data in different formats, such as total duration in weeks or in years. SC, single center; MC, multicenteric, AAV, adeno-associated viral; h, human; FIX, factor IX; FVIII, factor VIII; FVIII-BDD, B-domain deleted FVIII; MoMLV, Moloney murine leukemia virus; rRV, recombinant retroviral vector; N2CMV-IX, double-copy retroviral vector driven by human cytomegalovirus enhancer-promote; scAAV2/8-LP1-hFIXco, self-complementary adenovirus-associated virus vector expressing a codon-optimized human factor IX; Publ., publication; Hemo., hemophilia.

Hemophilia A. The large size and complexity of F8 gene has delayed the clinical trials on hemophilia A despite its higher prevalence. Several gene therapies have been developed, and three of them are approaching the final approval (Table I).

In the year 2017, a total of 9 patients were subjected to the administration of a solitary intravenous dosage of BMN 270 (AAV-hFVIII-SQ), also known as valoctocogene roxaparvovec® (27). Over the course of 1 year, the high dosage group demonstrated the maintenance of normalized FVIII activity, as well as the stability of hemostasis and a reduction in the occurrence of bleeding events. A sustained and notable improvement in clinical outcomes was observed throughout the higher dosage groups over the duration of the 5-year follow-up period (44).

In addition to the phase 1/2 dose escalation study and the global phase 3 study GENEr8-1 (45,46), further investigations, referred to as phase 3b research, were conducted to assess the efficacy and safety of valoctocogene roxaparvovec in individuals diagnosed with severe hemophilia A. The firm is now engaged in a phase 1/2 Study using valoctocogene roxaparvovec, which aims to include ~10 individuals who had pre-existing AAV5 antibodies. Additionally, the company is also undertaking a phase 1/2 study targeting individuals with hemophilia A who have either active or past FVIII inhibitors (47). FDA approval for valoctocogene roxaparvovec was deferred until the primary endpoint for ongoing phase III study, 2-year observation, is completed.

SB-525, also known as PF-07055480 (marketed as Giroctocogene fitelparvovec®), is a genetically engineered adeno-associated virus serotype 6 (AAV6) that carries the complementary DNA sequence for B domain deleted human coagulation factor VIII (FVIII). The design of the Giroctocogene fitelparvovec expression cassette aimed at achieving the excellent expression of the FVIII protein specifically in the liver, while also enabling high-yield manufacturing of the vector. The subsequent data obtained from the phase 1 and phase 2 Alta revealed that the rise in FVIII levels was both dose-dependent and sustained. Furthermore, there was a notable reduction in the use of FVIII and an absence of bleeding events seen in the highest dosage group (48). Of note, 4 patients in the highest dose cohort maintained modest to normal mean FVIII activity levels through week 104 (48). The clinical suspension of long-term follow-up research has been implemented by the FDA in response to the need for a review of a proposed protocol revision to the current phase III investigation (NCT04370054, AFFINE) (3).

SPK-8011 or (samoparvovec®) is a recombinant AAV8 that encodes a B-domain-delete FVIII sequence under the control of a hepatocyte-specific transthyretin promoter (49). Of note, 12 out of 18 participants in a phase 1-2 trial achieved sustained (>12% of the normal value level) 2 years after one-stage factor VIII assay administration with and without glucocorticoids. The annual hemorrhage rate of the participants decreased by 91.5% prior to vector administration; however, 8 participants experienced 33 treatment-related adverse events; 17 were vector-related, including one severe adverse event, and 16 were glucocorticoid-related. Notably, 2 individuals lost all factor VIII expression as a result of an anti-AAV capsid cellular immune response that was insensitive to immune suppression. The phase III trial stopped recruiting in May, 2023 and the results are to be released soon (50).

7. Other potential pathways for gene therapy in hemophilia

Restoring functional hemostasis has been successfully achieved by manipulating natural coagulation inhibitors via the use of antibodies that neutralize tissue factor pathway inhibitors , protease nexin 1,25, activated protein C-specific serpin inhibition and antithrombin inhibition by nanobodies (51).

Post-transcriptional gene silencing RNA interference (RNAi) is an additional approach to restore hemostasis equilibrium in individuals with hemophilia by inhibiting the expression of coagulation-inhibitory serpins (52). Prophylactic therapy, Fitusiran (ALN-AT3, Alnylam/Sanofi), is an RNAi-based therapy developed for patients with hemophilia A and B that targets antithrombin messenger RNA (53). It is synthesized by binding siRNA covalently to a triantennary Ga1NAc ligand which reduces antithrombin production. The safety and tolerability of fitusiran were assessed in a phase I dose escalation study in healthy volunteers and adult participants with moderate-severe non-inhibitor hemophilia A and B. Doses ranging from 0.015 to 1.8 mg/kg resulted in an ~50% reduction in antithrombin levels (54). When antithrombin levels were reduced by >75% from their peak at baseline, the thrombin generation values were comparable to those described in mild hemophilia and at the low end of the range observed in healthy participants (55).

Gene editing is another approach adapted by creating a targeted DNA double-strand break in genomic DNA near the site of desired change. The objective of this approach is to maintain *cis*-regulatory elements that potentially regulate gene function, while mitigating any adverse consequences associated with the mutant gene. This can be achieved using several different nuclease platforms including, but not limited to, meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases, and CRISPR-Cas9. Both AAV and non-viral delivery techniques, including RNPs and lipid nanoparticles have been used to deliver gene-editing components (56).

Pre-clinical studies on hemophilia A have demonstrated that an injection of dual AAV vectors containing *Staphylococcus* pyogenes Cas 9 (SpCas9) and guided RNA with human B-domain deleted FVIII integrated human FVIII into the albumin locus caused the liver in mice to produce FVIII. This technique improved the hemophilia A phenotype for at least 7 months without off-target effects or liver damage, suggesting permanent FVIII substitution (57).

Initial preclinical trials evaluated ZFN, which aimed at locating the normal copy of the clotting factor gene within the albumin intron 1, under the control of the endogenous albumin locus promoter, resulted in high levels of FIX for almost 1 year without any changes in plasma albumin levels, even when only 0.5% of mouse transcripts were modified (58).

Platelet-targeted gene is a promising approach to gene therapy for hemophilia that involves the targeting of FVIII or FIX expression and storage on platelets. This strategy holds promise, not only for enhancing hemostasis, but also for eliciting immune tolerance. The basic notion is to harvest hematopoietic stem cells (HSCs) from the peripheral or cord blood of a patient, insert a platelet-specific promoter into the corrected gene (FVIII or FIX), and then autologously transplant the cells. The study by Shi (59) provides a thorough analysis of this subject.

8. Challenges of gene therapy

Vector related challenges. Viral vectors used in gene therapy, whether they integrate with the host genome (such as retroviruses) or remain episomal (such as adeno-associated viruses or AAVs), carry potential risks (28), including the following:

i) Host immunity. Every vector system used in gene therapy faces its own unique immune response challenges. These challenges can be divided into two categories: Those related to innate immunity and those related to adaptive immunity, which includes memory and preexisting immunity (60).

Immunological evidence of prior exposure to AAV is detectable in a substantial proportion of individuals (30-80%), even in the absence of clinically detectable infection. Even at modest concentrations, anti-AAV antibodies can neutralize the viral vector and limit the viability of gene therapy (3). This poses a significant barrier to the widespread use of AAV-mediated gene transfer, and as a consequence, a number of clinical trials have precluded patients with pre-existing anti-AAV antibodies. In a preclinical study, AAV5 transgene expression was successful even with antibodies targeting AAV5. Primates received rAAV5 with the human secretory embryonic alkaline phosphatase (hSEAP) gene intravenously 6 weeks prior to the first rAAV treatment carrying factor IX had therapeutic levels of factor IX (hFIX) expression, and the researchers determined a threshold of anti-AAV5 antibodies that allowed for the effective re-administration of rAAV5. Anti-AAV5 antibodies did not appear to hinder further AAV5-based therapies (61). The seroprevalence of AAV in patients with hemophilia in developing countries, including Iraq is limited (3). Turkey, for instance, a neighboring country, reported a seroprevalence of pre-existing AAV8 antibodies of (67%), which is relatively high when compared to The Netherlands (27%) and Italy (14%) (62). Hence, epidemiological studies are required to determine the local prevalence.

Further studies involved the use of an endopeptidase that can degrade circulating anti-rAAV-neutralizing antibodies prior to the administration of rAAV gene therapy. Imlifidase, an enzyme derived from *Streptococcus* pyogenes known for its ability to degrade immunoglobulin G (IgG), is currently being studied in transplant recipients (63). In a mouse model of rAAV8-mediated gene therapy, treatment with imlifidase led to a reduction in anti-AAV antibodies and improved the expression of the therapeutic transgene in the liver. This indicates that imlifidase administration shows promise in addressing the challenge of pre-existing antibodies in AAV-based gene therapy (20).

Preclinical trials have found that the ability of host APC transduction varies between retroviruses or lentiviruses vectors (64). Cytotoxic T-lymphocytes and antibodies against transgenic products may result after transduction (26). T-cell activation against the transgene and pseudo-typed lentiviral vectors with diverse glycoproteins has lowered transgene expression over time (64).

Vector design, route and dose affect the gene expression lifespan. Nasal or intratracheal delivery may activate the immune system and induce tolerance (65). Liver-directed gene transfer using a hepatocyte-restricted promoter via hematopoietic lineage-specific microRNA target sequences limits transgene expression in professional antigen-presenting cells and reduces immunological responses (60). The post-transcriptional microRNA-mediated gene suppression has been implicated in confining transgene activity to a particular tissue. This approach involves introducing a specific target sequence into the messenger RNA of the transgene, which corresponds to a microRNA found exclusively in the desired cell type (66). In an alternative methodology, the 2bF9/methylguanine-DNA-methyltransferase (MGMT) LV vector was utilized. This vector incorporates the alpha-2b promoter, FIX and MGMT 140K genes. Following the transduction of HSCs, this vector resulted in a 2.9-fold increase in FIX expression and a 3.7-fold increase in FIX activity within platelets (67,68).

ii) Liver toxicity. The incidence of acute adverse events subsequent to the administration of AAV vectors is relatively infrequent. However, it has been shown that liver toxicity manifested in ~60% of individuals within a timeframe of 4 to 12 weeks subsequent to the therapeutic intervention (27). The observed complication is distinguished by a range of liver enzyme level elevations, as well as a reduction or absence of the transgenic clotting proteins in the circulatory system, primarily caused by the demise of transduced hepatic cells (27). The exact cause of this liver toxicity remains unclear and may vary among patients. Some researchers have related this to viral vector particle load (69). Cytotoxic T-cells targeting AAV capsid peptides have been reported in a number of preclinical trials (70). Others have suggested that a high FVIII transgenes expression increases endoplasmic reticulum stress, which induces hepatocyte apoptosis. While the underlying mechanisms require further investigation, current empirical therapies that aim to manage this issue include oral corticosteroid therapy, commencing at ~1 mg/kg and then gradually reducing the dose over a period of 2-3 months (70). Nevertheless, there are instances where oral steroids prove ineffective in treating liver toxicity, hence requiring the use of intravenous methylprednisolone or other immune regulatory drugs such as tacrolimus or azathioprine (71). In situations where liver toxicity is more prevalent, prophylactic oral steroid schedules have been implemented (71).

iii) Genotoxicity. Long-term mutagenic changes in gene therapy remain a concerning issue. One of the notable benefits associated with AAV vector administration is its tendency to avoid the integration of vector sequences into the host genome. This characteristic significantly reduces the potential danger of long-term oncogenic consequences (71). The thorough genomic analysis of liver biopsies from hemophilic dogs that underwent >10 years of AAV treatment has not revealed any signs of cancerous tumors in the liver (40). Previous studies, however, have demonstrated that hepatocellular carcinoma can develop in neonatal mouse models following recombinant AAV gene transfer (72). Additionally, fragmented wild-type AAV genomes have been detected in liver cancers. In a recently published study, liver biopsy samples from patients who received AAV5-hFVIII-SQ treatment at a dose of $6x10^{12}$ and $4x10^{13}$ vg/kg were collected and analyzed to determine the presence and genomic forms of AAV persistence. The percentage of hepatocytes exhibiting positive staining for vector genomes was 1.3 and 32%, respectively. This staining was observed throughout the liver lobes, resembling findings seen in earlier studies involving non-human primates (73). Additionally, both treatment groups displayed circularized full-length vector genomes and ITR-fusions, appearing both as individual units and linked together in chains, in line with observations from previous research (73).

Insertional mutagenesis is a potential safety concern of lentivirus, which is more likely to occur when dividing cells are transduced. However, newer-generation lentivirus designs have significantly reduced this risk, and there have been no reported cases of leukemic transformation in human gene therapy trials (74). The genotoxicity observed in previous cases involving retroviral vectors could potentially be attributed to the activation of oncogenes by the vector's intrinsic promoter. Consequently, the genetic makeup of the lentivirus has been altered to enable the elimination of the viral promoter during the reverse-transcription mechanism, commonly referred to as self-inactivating lentivirus. The aforementioned alteration has significantly reduced the likelihood of genotoxicity (20).

iv) Germline transmission. Germline viral vector transmission is a serious safety issue. Preclinical studies have not demonstrated AAV in rabbit or dog semen after intramuscular or portal vein rAAV injections (75). In the rAAV2 experiments, semen vector sequences were detected transiently. Subsequent rabbit investigations employing intravascular rAAV vector administration revealed dose-dependent transitory viral detection in semen (76). However, detecting the vector sequences in vasectomy rabbits' semen indicates that viral shedding into the semen did not need germ cells (76). Therefore, regulatory bodies recommend barrier contraception for rAAV-containing semen (77).

Gene related challenges. Therapy durability is the main concern of patients. Uncertainty persists as to whether these levels will stabilize or decline further (71). Investigations on the treatment of the FIX gene in adult patients have demonstrated a minimal decrease in plasma FIX levels for up to 5 years following a single intravenous treatment (78). By contrast, the canine model of hemophilia A maintained therapeutic expression of FVIII for >10 years after AAV vector infusions (79). A human study utilizing an AAV5 vector for FVIII gene transfer revealed a significant decrease in FVIII levels during the first 4 years following vector administration (45). Recently, FVIII levels have reached ~0.20 IU/ml, which continues to provide substantial protection against hemorrhage (45).

It is worth noting that in the general population, natural levels of FVIII and FIX can differ as much as 4-fold among individuals. The intricate balance and complexity involved in the production, secretion and clearance of these proteins is only partially understood at this time (71). Further studies addressing the mechanisms of vector hepatocyte entry, intracellular trafficking, nuclear import efficacy and the behavior of the transgene in terms of remaining episomal or integrating into the host-cell genome are required in order to be able to predict specific coagulation factor levels; these are currently extremely difficult to predict (71).

Ethical concerns

In children. With the continuous advancement of gene therapy, it is anticipated that it will gain even more appeal as a therapeutic alternative for children. However, 'children are not just small adults', and customizing gene therapy to hemophilia younger children will requires special considerations (80). Ethical issues arise when delivering life-altering medications to young children, given the extended lifespan of hemophilia patients, with emerging long-term safety evidence while current standard managing options are available (80). In addition, the strong humoral immune response following first vector delivery hinders successful vector reinfusion. Furthermore, the non-integrating characteristic of AAV vectors renders them difficult to be administered to children, since proliferating cells lose a considerable portion of the vector during liver enlargement (3).

In Muslim-based societies. Islamic ethics are interconnected with science, religion and law. Qur'anic exogenesis, scholastic theology, morality and knowledge acquisition are all sources from which Islamic ethics is derived. They advocate for the prevention of corruption and damage, as well as the acquisition of an advantage or interest. Perhaps the most frequently employed jurisprudential premise for medical treatment, including somatic gene therapy, is the theory of advantage or interest. As long as the concept of fundamental Islamic pillars of public benefit are prioritized in somatic gene therapy, the technique is ethical (81).

Cost-related challenges. The issue of gene therapy expenses is a key obstacle, not only in developing countries, but also in developed nations (71). The authorized gene therapy product AMT-061 for hemophilia B by CSL Behring's put forward a cost of US$3.5 million per dose (82).

A cost analysis conducted prior to the launch of the first gene therapy product found that the total cost per person for gene therapy was $1.0 million, resulting in 8.33 quality-adjusted life years (QALYs), while prophylactic treatment cost $1.7 million and yielded 6.62 QALYs (82). Over a lifetime, the average total cost per patient was estimated to be $16.7 million (with a range of $823,000 to $73.9 million) for those receiving valoctocogene roxaparvovec, with a gain of 18.07 QALYs (ranging from 0.06 to 23.38 QALYs). Patients using prophylactic exogenous FVIII protein had an average total cost of $23.5 million (with a range of $75,000 to $69.6 million) and gained 17.32 QALYs (ranging from 0.05 to 22.54 QALYs). These results confirmed that valoctocogene roxaparvovec was associated with a cost reduction of $6.8 million per patient (4).

The restricted availability of options and financial constraints in lower- and middle-income nations, such as Iraq, are influenced by the high cost of therapy, which is determined by the expenses associated with alternative treatments in high-income countries. The potential for substantial improvement in patient outcomes exists via the use of subsidized, single-dose gene therapy, contingent upon the facilitation of talks and international partnerships that would establish a fair and acceptable price structure (83).

Outcome-based and finance-based payment methods are two different approaches that have been suggested (84). The previous approach links compensation to a mutually agreed-upon clinical outcome. Compensation may be disbursed based on attainable accomplishments, either on an annual basis if the desired outcome endures, or by partial or whole reimbursement of the initial payment if the outcome diminishes after the initial full payment (84). In this strategy, the absence of agreement on the precise definition of a cure and quality of life for hemophilia presents a notable obstacle in addition to the lack of a control group in all gene therapy clinical trials in hemophilia (85). To overcome these difficulties, certain approaches have been suggested. Several strategies may be used to enhance the validity and reliability of clinical research. These strategies include the utilization of data derived from prior clinical trials exploring alternative therapies or real-world evidence to construct synthetic historical cohorts for comparative analysis. Additionally, forecasts about the potential outcomes of clinical trials can be made, and data can be collected from real-world registries after approval (86).

Financial schemes that are based on financial considerations have fewer practical factors to consider (84). These interventions are particularly appropriate in cases when the projected patient outcomes are foreseeable; however, the population of possible patients is either vast or unclear. In a number of instances, a capitation model is used, wherein a predetermined sum is levied irrespective of the number of recipients. This approach may be used in a subscription-based model, whereby a fixed amount of money provides access to an infinite number of patients for a duration of 1 year (84). Alternatively, it is possible to employ a volume-based method in which the cost per patient lowers after a specified threshold is reached. In some cases, the duration of payment is prolonged across multiple years rather than being disbursed as a single amount at the beginning. In order to mitigate the potential risk, one possible approach is to include a number of individuals or corporations that may act as payers or to transfer a part of the responsibility to reinsurers. The users have provided a D to support their statement (4).

Patient- and community-related challenges

Social awareness. The introduction of new healthcare advancements requires sufficient healthcare education and population awareness. A survey conducted by the International Society on Thrombosis and Hemostasis focused on healthcare teams and scientists to assess their understanding and awareness of gene therapy, particularly as regards hemophilia (87). The survey included responses from physicians (66% of respondents), with 59% directly involved in caring for hemophilia patients. The results of that study indicated that ~33% of doctors had challenges when attempting to articulate the core scientific concepts behind AAV gene therapy (87). Furthermore, a significant proportion of doctors, namely 40%, acknowledged their lack of confidence in effectively addressing patient queries pertaining to gene therapy. A survey was undertaken by the WFH, which included the participation of 103 national member organizations and 109 clinicians from 76 countries (88). The findings of that study revealed that a notable proportion of patients (68%) exhibited a main level of comprehension about gene therapy. By contrast, a notable percentage of medical professionals (44%) exhibited only a rudimentary or moderate grasp of the topic (88).

Health status of patients. A considerable number of patients with hemophilia have a history of HIV, hepatitis B, or hepatitis C virus infection. All these patients, in addition to those with other inflammation, cardiovascular and neoplastic diseases ,were excluded from clinical trials (89). A study conducted in Iraq revealed that the prevalence rates of hepatitis C virus, hepatitis B virus and HIV infections among individuals with hemophilia were 22.9, 0.9 and 0.2%, respectively (6). A number of trials excluded patients who had factor inhibitors and AAV-neutralizing antibodies, as well as pediatric or elderly populations (4).

Post-marketing trials have the potential to accommodate these patients and loosen the stringent eligibility criteria commonly employed in premarketing trials, although the cost remains a prohibiting factor.

9. Conclusion and future perspectives

The approval of gene therapy for patients with hemophilia B is expected to be followed shortly by the approval of another agent for hemophilia A. As we enter the era of gene therapy, it is crucial to prioritize education for healthcare providers and the community to ensure they are well-prepared to embrace these advancements. Regional collaboration and the establishment of multinational-sponsored centers can aid in the development of the necessary infrastructure, while ensuring hematologists receive adequate training to effectively deliver this cutting-edge treatment.

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Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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