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REVIEW ARTICLE

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Ex Vivo and In Vivo Gene Therapy Strategies via Viral Vectors: Paving the Way toward a Cure for Hemophilia

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Abstract

Due to the poor quality of life of patients with severe hemophilia, developing a cure method for hemophilia remains pivotal for mitigating associated global health burdens. In the last few years, an increasing number of original research and clinical studies have revealed that gene therapy is a transformative strategy for hemophilia management. Recent advancements in gene therapies, including ex vivo stem cell modification and in vivo viral vector administration, have broadened the scope of gene therapy via viral vectors to address challenges that modulate the specificity of protein expression. This review presents the latest advancements in gene therapy via viral vectors for hemophilia. The vital focus on targeted gene therapy applications for treating hemophilia is not only on exploring administration methods and preconditioning strategies but also on selecting the treatment window and assessing their efficacy. Cell-based therapy offers high specificity and programmability for treating hemophilia A, and in vivo gene therapy provides a safe and straightforward alternative. **(International Journal o Biomedicine. 2026;16(4):430-441.)**

Keywords: gene therapy • hemophilia • stem cells • clinical translation • viral vector

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Introduction

Hemophilia is a rare genetic bleeding disorder that occurs more frequently in males. Severe patients have a strong tendency for spontaneous bleeding after injury. Currently, there is no complete cure for this disease. Without proper medical care, the expected lifespan of patients with severe hemophilia is less than 10 years.[1] Therefore, it is urgent to discover more effective treatments.

Hemophilia is usually caused by a mutation in a single gene. The mutated gene could be the factor VIII (FVIII, F8) or the factor IX (FIX, F9). Both of these factors are important components in the coagulation process.[2] Hemophilia can be classified into types A and B. Hemophilia A is caused by a mutation in the F8 gene, leading to low levels of clotting factor VIII. Hemophilia B is caused by a mutation in the F9 gene, leading to low levels of clotting factor IX.

Although protein replacement therapy has constantly evolved, which has made its half-life longer, the long-term economic pressure is also destroying the lives of patients. In the last few years, gene therapy has emerged as a transformative strategy for hemophilia management. Recent advancements in gene therapies, including *ex vivo* stem cell modification and *in vivo* vector administration, have constituted the main focus. This review presents the different delivery strategies in gene therapy for hemophilia (Fig. 1).

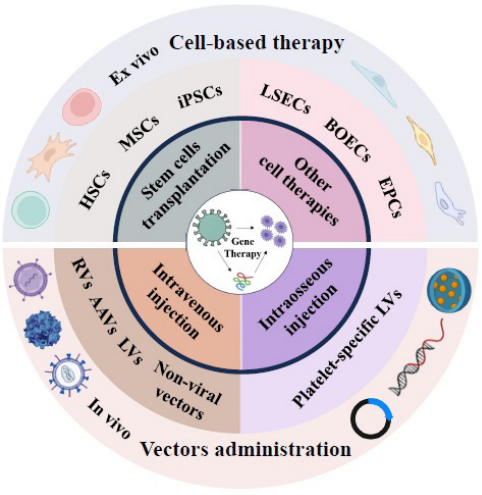


Fig. 1. *Schematic illustration of gene therapies, including stem cell transplantation and in vivo vector administration based on different delivery strategies.*

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Treatments in Gene Therapy for Hemophilia

Recent advancements in gene therapy for hemophilia, including cellular therapy and direct vector injection treatments, have broadened the scope of gene therapy to include challenges that modulate the specificity of protein expression.

Ex vivo approach to gene therapy for hemophilia

The use of cell-based gene therapy represents yet another therapeutic option for severe hemophilia.[3] Several candidate cell types can support F8 production: Hematopoietic stem cells (HSCs) derived from BM, mesenchymal stem cells (MSCs), liver sinusoidal endothelial cells (LSECs), blood-outgrowth endothelial cells (BOECs), and endothelial progenitor cells (EPCs) derived from the differentiation of induced pluripotent stem cells (iPSCs).[4] Fig. 2 shows the schematic illustration of implementation procedures for ex vivo approaches.

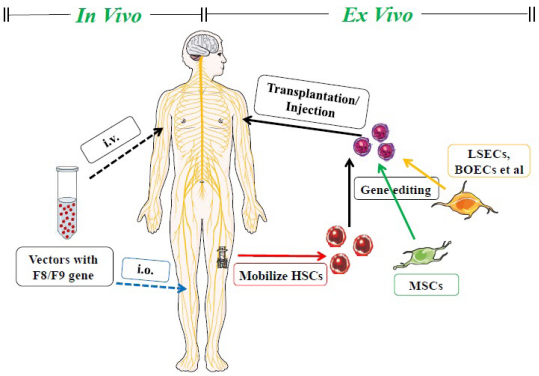


Fig. 2. Schematic illustration of implementation procedures for ex vivo and in vivo gene therapies.

Autologous HSCs transplantation

The most common one is the modified autologous HSC transplantation using vectors carrying the F8 gene. Lentivirus (LV) enables larger gene constructs and, unlike the Adeno-associated Virus (AAV) vector approach, allows the gene of interest to integrate into the host cellular DNA.[5] The latest strategies have leaned towards tissue-specific promoters, especially platelet-specific promoters for HSC transplantation. Most of these strategies were verified in the $F8^{null}$ and $F9^{null}$ mouse model over a decade ago.[6-8] HSCs by LV-F8 led to long-term repopulation of murine recipients with an efficient gene transfer.[9] Shi et al. used a LV-mediated gene transfer system to deliver the human B-domain-deleted F8 (hF8-BDD) expression with platelet-specific αIIb promoter (2bF8) into HSCs, resulting in F8 expression in platelets and sustained therapeutic levels of platelet-F8 with neither inhibitory nor non-inhibitory antibody development.[10]

Recently, a phase 1 trial evaluating the feasibility of HSC gene therapy using platelet F8 in participants with F8 inhibitors, funded by the National Institutes of Health (USA), opened recruitment (NCT03818763). Researchers reported a highly effective case.[11] The participant with a history of F8 inhibitor underwent autologous $CD34^+$ cell transplantation, which was transduced with an LV encoding F8 under the control of the ITGA2B promoter for platelet-specific expression. The level of megakaryocyte FVIII:C was 0.00 mU per 10^6 cells before transduction and 101.32 mU per 10^6 cells after transduction.[12] Also, more clinical studies about HSC transplantation were summarized in Table 1. Other approaches using LVs were under early investigation.[13,14] Merlin et al. used the natural F8 promoter (pF8) to direct gene replacement to F8–secreting cells. This application mediated long-term F8 expression by targeting liver sinusoidal endothelial cells in hemophilic mice, thereby correcting the bleeding phenotype without inhibitor formation.

Other cell therapies

Due to their low immunogenicity, MSCs can be applied without preconditioning before treatment; therefore, numerous immunologic problems have been resolved. Because MSCs can be obtained from the patient's body and reinjected after in vitro modification. Autologous bone-marrow-derived MSCs transduced with an LV expressing coagulation F8 were injected into the left knee joint of cynomolgus monkeys. They confirmed the long-term safety of intra-articular injection of transduced MSCs in non-human primates (NHPs).[15]

Some research on the effectiveness of MSCs for hemophilia was conducted over 10 years ago.[16,17] In animal models, injection of BM-MSC-F8 into HA mice and sheep reduced joint hemorrhage.[18,19] Moreover, Wang et al. generated a transgenic mouse line modified with the hF8-BDD vector, from which hF8-BDD-MSCs were isolated and subsequently transplanted into HA mice. Plasma F8 activities of recipient HA mice increased from 0% to 32%.[20] In a recent study, the protein-coding region of the hF9 gene was inserted to AAVS1. They were transduced into MSCs using the nucleofection method, and high, durable expression of F9 at the protein level was observed both in vivo and in vitro.[21] Also, modified hepatic and pulmonary MSCs capable of secreting coagulation factors could produce endogenous F8.[22,23]

Transplantation of LSECs has been demonstrated in several animal models as a means to treat HA. Kumaran et al. transplanted LSECs into the portal vein and demonstrated liver engraftment and therapeutic correction of HA, with plasma F8 activity exceeding 10% of normal plasma levels.[24]

Blood-derived circulating endothelial cells, referred to as BOECs, represent a clinically promising cell type and exhibit a much higher proliferative potential, which can be expanded in culture for the therapy of HA.[25] BOECs primarily engrafted the spleen and BM after an intravenous (IV) injection. Furthermore, BOECs transfected with F8 can secrete F8 into the plasma, as shown by transplantation into NOD/SCID mice and HA dog.[26,27] F8-transduced BOECs had also been grown into sheets and then transplanted subcutaneously, resulting in partial correction of the disease phenotype in HA mice.[28] It was reported in 2021 that the first preclinical study demonstrated the safety and feasibility of transplantation of GMP-like-produced LV-corrected BOECs within an implantable device for the long-term treatment of HA.[29]

iPSCs are an alternative to embryonic stem cells for HA gene therapy. Firstly, the derivation of iPSCs does not require

Table 1. *Clinical trials about HSCs transplantation on hemophilia.*

Sponsor	Treatment	N participants enrolled	Dose	Approx. Mean activity	Follow-up (Years) / Durability	ABR	Phase	Status	Clinicaltrials.gov Identifier
Shenzhen Geno-Immune Medical Institute	YUVA-GT-F901 (Lentiviral F9 gene-modified autologous hematopoietic and mesenchymal stem cells)	10 (estimated)	NA	NA	NA	NA	I	Unknown	NCT03961243
Shenzhen Geno-Immune Medical Institute	YUVA-GT-F801 (Lentiviral F8 gene modified autologous hematopoietic stem cells and mesenchymal stem cells)	10 (estimated)	NA	NA	NA	NA	I	Unknown	NCT03217032
Medical College of Wisconsin	Auto CD34+PBSC, transduced with a lentiviral vector encoding ITGA2B-F8-BDD	5 (estimated)	NA	NA	NA	NA	I	Recruiting	NCT03818763
Christian Medical College	Auto CD34+PBSC transduced with a CD68-ET3 lentiviral vector encoding a novel coagulation F8 transgene	6	NA	NA	NA	NA	I	Completed	NCT05265767
Expression Therapeutics	CD34+ hematopoietic stem cells transduced with a CD68-ET3 lentiviral vector (encoding the hFVIII gene)	7 (estimated)	NA	NA	NA	NA	I	Not yet recruiting	NCT04418414

human embryos and thus circumvents the ethical debate and supply constraints associated with their use in research or clinical practice.[30] Secondly, the genetic abnormalities causing HA can be corrected using gene-editing technology, followed by the subsequent selection, expansion, and differentiation of iPSCs into endothelial cell progenitors or fully differentiated LSECs. Partial demonstration of such a therapy had been reported during the past 15 years, in which transplantable EPCs were derived from iPSCs and shown to produce F8 in HA mice.[31-33] There has also been an effort to demonstrate the utility of a combined approach that uses gene therapy to direct expression of an hF8 transgene under a megakaryocyte-specific promoter in iPSC-derived cells.[34,35] Other methods of gene editing have been developed for correcting genetic abnormalities in iPSCs, such as the RNA-guided clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 system. Such an approach could be used to correct the F8 gene in patient-derived iPSCs.[36-38] That may offer the possibility of an unlimited source of transplantable EPCs for the treatment of hemophilia.

In vivo approach to gene therapy with IB injection for hemophilia

Compared with autologous HSC transplantation, IV injection has simpler operability and higher safety, manifested as mild immunosuppression before vector IV injection (Fig. 2). The key rate-limiting steps in systemic IV delivery include interactions with serum proteins, blood clearance, vascular escape, attachment, endocytosis, intracellular processing, nuclear entry, and vector genome conversion.[39]

AAV-mediated gene transfer approach

An IV injection is currently the predominant method for administering AAV, with most FDA-approved AAV therapies using this route. Both HA and HB have conducted a considerable number of preclinical animal experiments (including mouse models and large animal models) as well as clinical trials.

Early studies for gene therapy for HB with AAV2 focused on delivery to skeletal muscle, both in animal models (mice and dogs) and humans.[40,41] More recent clinical trials of gene transfer to skeletal muscle have used AAV serotype 1 (AAV1) vectors due to their superior transduction capacity in myocytes.[42,43] Potential complications of gene transfer to skeletal muscle from an immune response against hF9 could occur in some situations; thus, more research on HB, including clinical trials, has shifted to hepatic gene transfer.[44,45] This study used an AAV serotype 8 vector (AAV8), a serotype derived from rhesus macaques that has greater liver specificity than AAV2.[46] Additionally, the prevalence of neutralizing antibodies against AAV8 is lower than that against AAV2 in the human population, allowing more patients to be treated with AAV8 potentially.[47,48] Expression of hF9 in mice and NHPs was elevated with self-complementary AAV (scAAV).[49,50] The clinical trial of hepatic gene therapy for HB used a scAAV8 vector delivered via peripheral vein injection, which allowed F9 activity to persist at around 2-5% of normal.[51] The latest clinical trial in China used a novel, engineered, liver-tropic AAV843 expressing a hyperactive Padua F9 protein, which means FIX:C reached a mean of 36.9 IU/dL, demonstrating fairly therapeutic effects.[52,53] and one of the patients underwent

a right total knee arthroplasty successfully on day 479 after vector infusion without F9 concentrate infusion.[54] Another clinical study showed that the phase 3 BENEGENE-2 trial of Fidanacogene Elaparvovec led to reduced bleeding and stable factor IX expression, particularly as it represented an important alternative FIX-R338L/FIX-Padua AAV5 strategy.[55] Great strides have already been made in clinical trials for AAV-mediated gene therapy for HB. Earlier intramuscular studies provided proof of concept and safety data, while liver-directed gene delivery has shown greater success, though it can still be limited by both NAB and anti-capsid CTL responses.

Given the success AAV has shown as a vector for the treatment of HB, it is logical that several approaches have also attempted to use it for HA.[56] When considering AAV-mediated delivery of F8, the most pressing concerns were packaging capacity and AAV immunogenicity. Early studies over the past 10 years on gene therapy for HA with AAV focused on HA animal models (such as mice). At first, 10^{10-11} AAV/BDD-hF8 particles were injected intravenously into C57BL/6 and NOD/scid mice. All C57BL/6 mice tested developed anti-hF8 antibodies. In contrast, NOD/scid mice expressed hF8, reaching 27% of normal human plasma levels.[57] Then, AAV8-β-actin-canine F8 (cF8), AAV-ApoE-hAAT-hF8-BDD and AAV-ApoE-hAAT-rat F8 (rF8)-BDD were developed to express F8 in HA mice achieving therapeutic F8 levels.[58,59] Due to the success of mouse model research, recent studies have focused on preclinical studies in large animals (such as dogs and NHPs) and phase I/II clinical trials. In the NHP experiments, AAVhu37-F8-BDD was administered intravenously at a dose of 1.2×10^{13} genome copy/kg into five macaques; average hF8 expression was 20.2±5.0% of normal.[60] And the AAV integration profiles were polyclonal, evenly distributed across the genome, and showed a slight bias toward regions of open chromatin and actively transcribed genes, with no enrichment in cancer-associated genes.[61] Similar therapeutic effects and integration characteristics were also observed in the AAV gene therapy for hemophilia dogs.[62] Valoctocogene roxaparvovec (AAV5-hF8-SQ) is an adeno-associated virus 5 (AAV5)–based gene-therapy vector containing a coagulation factor VIII (F8) complementary DNA under the control of a liver-selective promoter. The efficacy and safety of the therapy were previously evaluated in men with severe HA in a phase I and II dose-escalation study.[63-65] There was an open-label, single-group, multicenter, phase III study to evaluate the efficacy and safety of Valoctocogene roxaparvovec in men with severe HA, which showed that the mean F8 activity level at weeks 49 through 52 had increased by 41.9 IU per deciliter, with the longest follow-up period up to 261.1 weeks.[66-69] Other important AAV-F8 programs have also undergone multiyear follow-up, including giroctocogene fitelparvovec[70] and multiyear follow-up data for SPK-8011.[71,72] More clinical studies about AAV-mediated IV delivery gene therapy were summarized in Table 2. Numerous clinical studies of AAV-delivered F8 gene therapy have demonstrated therapeutic F8 production and significant reductions in bleeding. We look forward to learning more about the long-term durability and safety of the treatment as we continue to follow the study participants.

LV-mediated gene transfer approach

LV may represent a complementary strategy for IV-directed gene therapy, for the following reasons: (i) low occurrence or feasible overcoming of immune barriers in humans, since the worldwide prevalence of HIV infection is estimated at 0.8%; (ii) efficient integration of LV in the host cells' genome may be preferred for lifelong maintenance of the therapeutic transgene and potentially allows treatment of pediatric patients without the need for vector re-administration; (iii) larger packaging capacity may make LV better suited for transferring F8 expression cassettes. The absence of prior clinical testing of systemic administration, manufacturing hurdles, and concerns about insertional mutagenesis have, until now, hindered preclinical development of in vivo LV gene therapy directed to the liver.

The intravenous gene therapy approach of LV was first validated in HB experiments. Brown et al. had previously shown that IV administration of LV results in efficient, long-term gene transfer to the liver and achieves phenotypic correction of HB in mice and dogs.[3,73,74] More recently, Milani et al. generated allo-antigen-free and phagocytosis-shielded (CD47hi) LV that allowed supra-normal activity of a human coagulation F9 transgene in NHPs, without evidence of acute toxicity and clonal expansion of transduced cells.[75,76] Meanwhile, IV therapy using LV carrying the F8 gene was evaluated in animal models, showing long-term F8 activity and restoration of hemostasis following IV administration to newborn and adult mice, and normal-range human F8 activity in NHPs.[77] Stein et .al. engineered a feline immunodeficiency virus (FIV)-based LV pseudotyped with the baculovirus GP64 envelope glycoprotein to mediate efficient gene transfer to mouse hepatocytes, and reported that LV IV administration conferred sustained F8 expression in HA mice for several months.[78-80] In addition, Staber et .al. demonstrated the development of a vector system for delivery and therapeutic expression of F8 protein from an optimized cF8 cDNA to achieve safe, long-term expression in hepatocytes. This proof-of-principle study in a relevant small-animal model paves the way for preclinical studies in a canine model of HA.[81]Modified F8 gene constructs were described: LV-F8-N6[82] and LV-F8-N6 replacer with a novel 17-aa peptide (LV-F8-V3),[83] carrying the spleen focus-forming virus (SFFV) promoter (equivalent to 4×10^{7}-1×10^{8} transducing units per mouse), injected into the superficial temporal vein. Shorter F8 constructs that can be more readily accommodated in viral vectors may increase therapeutic efficacy and enable effective gene therapy for HA.

It has been shown that the natural source of most F8 production is endothelial cells,[24] Gene transfer of LV expressing F8 from liver endothelial cells has been proposed, and some encouraging results have been reported in HA mice treated as adults,[84,14] Gong et al. developed an LV-mediated F8-BDD transgene with an endothelial-specific promoter to enable F8 protein expression in endothelial cells, especially liver endothelial cells.[85] However, the stability and turnover of these cells, both during postnatal liver growth and in homeostasis in adulthood,, remain only partially understood.[86]

Table 2. *Clinical trials about AAV-mediated i.v. gene therapy on hemophilia.*

Sponsor	Vector	Manufacturing	N participants enrolled	Dose (vg/kg)	Appox. Mean activity	Follow-up (Years) / Durability	ABR	Phase	Status	Clinicaltrials.gov Identifier
FIX										
St. Jude/ UCL	AAV8-FIX	Mammalian	10	2×10^{11} 6×10^{11} 2×10^{12}	1.9% 2.3% 5.1%	8/stable	2	I/II	Active, not recruiting	NCT00979238
Spark/ Pfizer	SPK-9001 (SPK100-FIX-R338L)	Mammalian	15	5×10^{11}	33.7%	3/stable	0.4	I-III	Completed	NCT02484092
Pfizer	PF-06838435	Mammalian	45	5×10^{11}	26.9%	1.25	1.28	III	Active, not recruiting	NCT03861273
Shire	BAX335 (AAV8-FIX-R338L)	NA	6	2×10^{11} 1×10^{12} 3×10^{12}	3.5% 12% 45% (transient)	1	NA	I/II	Active, not recruiting	NCT01687608
uniQure	AMT-060 (AAV5-FIX)	Insect	10	5×10^{12} 2×10^{13}	4.9% 7.4%	4/stable	0.9	I/II	Completed	NCT02396342
uniQure	AMT-061 (AAV5-FIX-R338L)	Insect	54	2×10^{13}	37%	1.5	0.8	III	Completed	NCT03569891
uniQure	AMT-061 (AAV5-FIX-R338L)	Insect	3	2×10^{13}	31%	6 wk	NA	II	Completed	NCT03489291
Ultragenyx	DTX101 (AAVrh10-FIX)	NA	6	1.6×10^{12} 5×10^{12}	3-8%	1	NA	I/II	Terminated	NCT02618915
Freeline	FLT-180a (AAVS3-FIX-R338L)	Mammalian	6	7.7×10^{11} progressive increased	NA	1	NA	I/II	Terminated	NCT05164471
Freeline	FLT-180a (AAVS3-FIX-R338L)	Mammalian	10	6×10^{11} 1×10^{12} 1.3×10^{12} 2×10^{12}	23-156%	26 wk	0- 2.17 5	I/II	Terminated	NCT03369444 NCT03641703
Sangamo	SB-FIX (AAV6-FIX)	Insect	1	NA	NA	3	NA	I	Terminated	NCT02695160
FVIII										
BioMarin	BMN 270 (AAV5-F8-BDD)	Insect	1 1 6 7	6×10^{12} 2×10^{13} 4×10^{13} 6×10^{13}	<1% 2% 23.2% 52.3%	>1 >1 3/declining expression 4/ declining expression	NA NA 0.5 1.3	I-III	Completed	NCT02576795
BioMarin	BMN 270 (AAV5-F8-BDD)	Insect	144	6×10^{13}	22%	2	NA	III	Completed	NCT03370913
Spark/ Roche	SPK-8016 (AAV-F8-BDD)	Insect	4	5×10^{11}	NA	1	NA	I/II	Completed	NCT03734588
Spark/ Roche	SPK-8011 (AAV-F8-BDD)	Insect	2 3 11 9	5×10^{11} 1×10^{12} 1.5×10^{12} 2×10^{12}	11.5% 20.0% 55.1% 38.7%	1	NA	I/II	Completed	NCT03003533 NCT03876301
Spark/ Roche	SPK-8011 (AAV-F8-BDD)	Insect	12	5×10^{11} 1×10^{12} 2×10^{12}	13% 15% 30%	2.5-3.3/ stable 2-2.5/ stable 1-1.5/ unknown	0.2 1.7 1.7	I/II	Completed	NA
UCL	AAV2/8-HLP-FVIII-V3	Mammalian	3	6×10^{11} 2×10^{12} 6×10^{12}	8-64%	about 1	5	I/II	Active, not recruiting	NCT03001830
Sangamo/ Pfizer	SB-525 (AAV2/6-F8-BDD)	Insect	13	9×10^{11} 2×10^{12} 1×10^{13} 3×10^{13}	NA NA NA 30-250%/ 20-160%	0.6-1.2/un-known	NA	II	Completed	NCT03061201
Sangamo/ Pfizer	SB-525 (AAV2/6-F8-BDD)	Insect	76	NA	NA	>1	NA	III	Active, not recruiting	NCT04370054
Shire	SHP654/BAX88 8 (AAV8-F8-BDD)	Mammalian	21	2×10^{12} 6×10^{12} 1.2×10^{13}	NA	5	NA	I/II	Completed	NCT03370172
Ultragenyx/ Bayer	BAY 2599023/ DTX201 (AAVhu37-F8-BDD)	Mammalian	11	5×10^{12} 1×10^{13} 2×10^{13}	5-20% 8-40% NA	about 1	NA	I/II	Active, not recruiting	NCT03588299

In vivo approach to gene therapy with intraosseous injection for hemophilia

The feasibility of in vivo gene transfer via direct intraosseous (IO) injection of RV and LV into mice was demonstrated ten years ago.[87,88] In addition, in vitro manipulation of stem cells and preconditioning of the subject is not required to achieve a therapeutic benefit. Therefore, this approach may provide a novel treatment for HA. The IO delivery of E-F8-LV using a ubiquitous EF1α promoter initially generated therapeutic levels of F8; however, robust anti-F8 antibody responses ensued, neutralizing functional F8 activity in circulation.[89] Platelets are released from BM megakaryocyte precursors into the circulation, where they play a crucial role in maintaining hemostasis.[90] Platelets may be an ideal vehicle for ectopic F8 expression for several reasons. Miao et al. demonstrated that a single IO injection of LVs can produce long-term stable expression of hF8 in platelets and correct the hemophilia phenotype for at least 5 months after treatment.[91]

Preconditioning before Gene Therapy for Hemophilia

A potential limitation of *in vivo* gene therapy protocols is the induction of virus vector-specific immune responses, which may decrease the vector transduction efficiency and eliminate transduced cells.[92,39,93,94] To reduce immune clearance of viral vectors in the host immune system, researchers have employed various doses and intensities of inhibitors for preconditioning before treatment.

HSC transplantation typically employs relatively intensive immunosuppressive regimens. Cytoreductive therapy can suppress the patient's immune system, reducing the attack on transplanted stem cells and the risk of graft-versus-host disease, and facilitating the implantation of donor cells. The pretreatment protocols for HSC transplantation were classified by the Center for International Blood and Marrow Transplant Research (CIBMTR) as myeloablative conditioning (MAC), nonmyeloablative conditioning (NMAC), and reduced-intensity conditioning (RIC).[95,96] MAC was Alkylating agents (cyclophosphamide, cytarabine, etoposide, and melphalan, et al.) and/or total body irradiation (TBI). The commonly used NMAC regimen consisted of 2 Gy TBI combined with fludarabine or cyclophosphamide, which produced the lowest reduction in blood cell counts. Unlike the MAC regimen, the RIC regimen uses lower doses of the alkylating agents (busulfan, cytarabine, and melphalan) or TBI (usually reduced to less than 70% of the former). The RIC regimen can cause reversible BM suppression (usually within 28 days after HSCT); however, the patient's own hematopoietic function cannot be restored after MAC.

HSC transplantation was mostly used with MAC and high-dose TBI (more than 800 cGy),[7,8,10,97-99,35] which was called the myeloablative regimen, and with NMAC and low-dose TBI (220, 440, 660 cGy),[7,10,99] which was called the nonmyeloablative regimen. There was also the RIC applied with (i) 120 mg/m^2 fludarabine and 120 mg/m^2 melphalan,[11,100] (ii) 30 or 50 mg/kg busulfan via intraperitoneal injection on day -1,[13,14] (iii) 25 or 12.5 mg/kg busulfan per day on days -2 and -1 and 10 mg/kg Anti-(murine)-thymocyte globulin (ATG) on day -2(HSC transplantation occurred at day 0).[98] There was no need for pretreatment in MSC therapy. iPSCs therapy preferred to use cyclosporine A (210 mg/L, in drinking water) starting 3 days before transplantation.[33,38]

In the early years, the AAV IV injection for gene therapy mostly involved the use of low-dose immunosuppressants, like RIC (100 mg/body/day cyclophosphamide and 12.5 mg/body/day tacrolimus after vector injection)[58] and glucocorticoids, such as prophylactic prednisone treatment (1 mg/kg per day sustaining 1 week)[52] or (40 mg per day, tapering from week 3 to week 17 or longer)[63] or rituximab and methylprednisolone without exact dosage.[61] In recent years, both animal experiments and clinical trials have increasingly advocated against pre-treatment with immunosuppressive methods.[64] Per-protocol prophylactic glucocorticoids were administered following ALT elevation.[65] Treatment with oral prednisone or prednisolone was started at a dose of 60 mg per day and was tapered over a period of at least 8 weeks.[66]

The immunosuppression of LV IV injection for gene therapy had various strategies. Firstly, participants were received rituximab and cyclophosphamide with different doses (i) 250 mg/m^2, 2 doses at 3 weekly intervals;[82,101,102] (ii) 250 mg/m^2, intravenously at 4 weekly intervals, total of 4 bolus infusions and 300 mg/m^2 slow intravenous infusions every 15 days, total of 8 doses over 4 months.[83] Secondly, participants were pretreated with a corticosteroid (Prednisone, 1 mg/kg oral, or Dexamethasone, 0.2/0.3 mg/kg IV) and an antihistamine (Benadryl, 1 mg/kg i.m., Famotidine, 0.5 mg/kg i.m., or dexchlorpheniramine, 4 mg/kg IV) before vector infusion.[74,76] Only corticosteroid (methylprednisolone, 10 mg/kg/day) was administered intramuscularly as an immunosuppression regimen from day -1 to day 7 or day -3 to day 9 for NHPs (LV administration occurred at day 0).[77]

Immunosuppression significantly affected the efficacy of stem cell transplantation and direct IV gene therapy. An excessively high dose of TBI increased the risk of severe complications for the participants. The low dose of immunosuppression resulted in poor therapeutic effects of transplanted cells and vector administration. Therefore, the appropriate pretreatment solution remains one of the challenges that need to be addressed in the future.

Selection of the Treatment Window Period on Gene Therapy Applications

Ex vivo manipulation and patient conditioning before transplantation are major hurdles that could be overcome by an in vivo approach. In vivo gene delivery to hematopoietic stem and progenitor cells (HSPCs) after systemic LV administration is enabled by the substantial trafficking of these cells from the liver to the BM in newborn mice. So, it is worth discussing and exploring the treatment window period for in vivo gene therapy.

Milani et al. identified a unique window of opportunity in early postnatal life, when high numbers of circulating HSPCs (cHSPCs) were trafficking from residual hematopoietic niches to the BM. The corresponding results were published in Nature in May 2025.[103] To assess the feasibility of in vivo LV gene transfer into HSPCs, they examined the spatiotemporal evolution of the hematopoietic system in C57BL/6 mice. The findings demonstrated that phagocytosis shielding enhanced LV gene transfer into HSPCs. Given the unique window of opportunity for the genetic engineering of HSCs in newborns, in vivo HSPC gene transfer holds strong translational potential across multiple diseases. However, treating HA at or before birth with cells or viruses lacks a clinical safety record to support this approach.

Conclusions

Collectively, cell-based therapy has attracted considerable attention in HA treatment due to its high specificity and programmability, while *in vivo* gene therapy has attracted attention due to its safety and simple clinical procedures. However, the clinical application of cell-based therapy is limited by several challenges, including rapid degradation due to poor cellular uptake, damage to the body caused by strong immunosuppression, and the persistence of therapeutic effects. The problems with *in vivo* gene therapy that limit its clinical application include potential immunogenicity, off-target toxicity, germline alteration, and persistence of therapeutic effects.

There are several critical obstacles to be overcome to realize their clinical potential fully: (i) Under the condition that ethics permits and the procedures are safe, would the treatments be carried out during the perinatal period or not? Only with sufficient and corresponding data from animal experiments and clinical trials can this question be answered. (ii) Whether cell-based therapy or *in vivo* gene therapy, to probe a 'one-and-done' treatment for hemophilia has always been the vital direction we need to strive for.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Author Contributions

Jie Gong conceptualized and designed the review and acquired the funding.
Jie Gong and QiuYi Li wrote the original draft.
Hong Du and Rui Yang revised the manuscript.
All authors have approved the final article.

References

1. Bhardwaj R, Rath G, Goyal AK. Advancement in the treatment of haemophilia. Int J Biol Macromol. 2018; 118:289-295. doi:10.1016/j.ijbiomac.2018.06.084
2. Marchesini E, Morfini M, Valentino L. Recent Advances in the Treatment of Hemophilia: A Review. Biol: Targets Ther. 2021; 15:221-235. doi:10.2147/btt.S252580
3. Brown BD, Cantore A, Annoni A, Sergi LS, Lombardo A, Della Valle P, D'Angelo A, Naldini L. A microRNA-regulated lentiviral vector mediates stable correction of hemophilia B mice. Blood. 2007;110(13):4144-4152. doi:10.1182/blood-2007-03-078493
4. Fomin ME, Togarrati PP, Muench MO. Progress and challenges in the development of a cell-based therapy for hemophilia A. J Thromb Haemost. 2014;12(12):1954-1965. doi:10.1111/jth.12750
5. Gong J, Wang H-L, Chang L-J. Molecular therapeutics of Hemophilia A and B. Expert Rev Hematol. 2022;431-441. doi :10.1080/17474086.2022.2075339
6. Shi Q. Platelet-Targeted Gene Therapy for Hemophilia. Mol Ther Methods Clin Dev. 2018;9:100-108. doi:10.1016/j.omtm.2018.01.011
7. Zhang G, Shi Q, Fahs SA, Kuether EL, Walsh CE, Montgomery RR. Factor IX ectopically expressed in platelets can be stored in α-granules and corrects the phenotype of hemophilia B mice. Blood. 2010;116 (8):1235-1243. doi:10.1182/blood-2009-11-255612
8. Chen Y, Schroeder JA, Kuether EL, Zhang G, Shi Q. Platelet Gene Therapy by Lentiviral Gene Delivery to Hematopoietic Stem Cells Restores Hemostasis and Induces Humoral Immune Tolerance in FIXnull Mice. Mol Ther. 2014; 22 (1):169-177. doi:10.1038/mt.2013.197
9. Kurre P, Anandakumar P, Kiem HP. Rapid 1-hour transduction of whole bone marrow leads to long-term repopulation of murine recipients with lentivirus-modified hematopoietic stem cells. Gene Therapy. 2006;13(4):369-373. doi:10.1038/sj.gt.3302659
10. Shi Q, Wilcox DA, Fahs SA, Fang J, Johnson BD, Du LM, Desai D, Montgomery RR. Lentivirus-mediated platelet-derived factor VIII gene therapy in murine haemophilia A. J Thromb Haemost. 2007;5(2):352-361. doi:10.1111/j.1538-7836.2007.02346.x
11. Eapen M, Malec LM, Armant MA, Johnson BD, Shi Q, Xu H, Du LM, Jerkins JH, Duffy LJ, Bushman FD, Lee C, Petrichenko A, Hematti P, Brazauskas R, Jobe SM, Hari PN, Wilcox DA. Platelet-Targeted Gene Therapy for Hemophilia A with Inhibitor History. N Engl J Med. 2025;392(4):412-414. doi:10.1056/NEJMc2415164
12. Srivastava A, Abraham A, Aboobacker F, Singh G, Geevar T, Kulkarni U, Selvarajan S, Korula A, Dave RG, Shankar M, Singh AS, Jeba A, Kumaar N, Benjamin C, Lakshmi KM,

Srivastava VM, Shaji RV, Nair SC, Brown HC, Denning G, Lollar P, Doering CB, Spencer T. Lentiviral Gene Therapy with CD34+ Hematopoietic Cells for Hemophilia A. N Engl J Med. 2025;392(5):450-457. doi:doi:10.1056/NEJMoa2410597
13. Doering CB, Denning G, Shields JE, Fine EJ, Parker ET, Srivastava A, Lollar P, Spencer HT. Preclinical development of a hematopoietic stem and progenitor cell bioengineered Factor VIII lentiviral vector gene therapy for Hemophilia A. Hum Gene Ther. 2018;29(10):1183-1201. doi:10.1089/hum.2018.137
14. Merlin S, Fama R, Borroni E, Zanolini D, Bruscaggin V, Zucchelli S, Follenzi A. FVIII expression by its native promoter sustains long-term correction avoiding immune response in hemophilic mice. Blood Adv. 2019;3(5):825-838. doi:10.1182/bloodadvances.2018027979
15. Ohmori T, Mizukami H, Katakai Y, Kawai S, Nakamura H, Inoue M, Shu T, Sugimoto H, Sakata Y. Safety of intra-articular transplantation of lentivirally transduced mesenchymal stromal cells for haemophilic arthropathy in a non-human primate. Int J Hematol. 2018;108(3):239-245. doi:10.1007/s12185-018-2465-8
16. Coutu DL, Cuerquis J, El Ayoubi R, Forner K-A, Roy R, François M, Griffith M, Lillicrap D, Yousefi A-M, Blostein MD, Galipeau J. Hierarchical scaffold design for mesenchymal stem cell-based gene therapy of hemophilia B. Biomaterials. 2011;32(1):295-305. doi:10.1016/j.biomaterials.2010.08.094
17. Dodd M, Marquez-Curtis L, Janowska-Wieczorek A, Hortelano G. Sustained expression of coagulation factor IX by modified cord blood-derived mesenchymal stromal cells. J Gene Med . 2014;16(5-6):131-142. doi:10.1002/jgm.2769
18. Kashiwakura Y, Ohmori T, Mimuro J, Yasumoto A, Ishiwata A, Sakata A, Madoiwa S, Inoue M, Hasegawa M, Ozawa K, Sakata Y. Intra-articular injection of mesenchymal stem cells expressing coagulation factor ameliorates hemophilic arthropathy in factor VIII-deficient mice. J Thromb Haemost. 2012;10(9):1802-1813. doi:10.1111/j.1538-7836.2012.04851.x
19. Porada CD, Sanada C, Kuo C-J, Colletti E, Mandeville W, Hasenau J, Zanjani ED, Moot R, Doering C, Spencer HT, Almeida-Porada G. Phenotypic correction of hemophilia A in sheep by postnatal intraperitoneal transplantation of FVIII-expressing MSC. Exp Hematol. 2011;39(12):1124-1135. doi:10.1016/j.exphem.2011.09.001
20. Wang Q, Gong X, Gong Z, Ren X, Ren Z, Huang S, Zeng Y. The Mesenchymal Stem Cells Derived from Transgenic Mice Carrying Human Coagulation Factor VIII Can Correct Phenotype in Hemophilia A Mice. J Genet Genomics. 2013;40(12):617-628. doi:10.1016/j.jgg.2013.11.002
21. Li S-J, Luo Y, Zhang L-M, Yang W, Zhang G-G. Targeted introduction and effective expression of hFIX at the AAVS1 locus in mesenchymal stem cells. Mol Med Rep. 2017;15(3):1313-1318. doi:10.3892/mmr.2017.6131
22. Sanada C, Kuo CJ, Colletti EJ, Soland M, Mokhtari S, Knovich MA, Owen J, Zanjani ED, Porada CD, Almeida-Porada G. Mesenchymal stem cells contribute to endogenous FVIII:c production. J Cell Physiol. 2013;228 (5):1010-1016. doi:10.1002/jcp.24247
23. Najimi M, Khuu DN, Lysy PA, Jazouli N, Abarca J, Sempoux C, Sokal EM. Adult-Derived Human Liver Mesenchymal-Like Cells as a Potential Progenitor Reservoir of Hepatocytes? Cell Transplant. 2017;16(7):717-728. doi:10.3727/000000007783465154
24. Follenzi A, Benten D, Novikoff P, Faulkner L, Raut S, Gupta S. Transplanted endothelial cells repopulate the liver endothelium and correct the phenotype of hemophilia A mice. J Clin Invest. 2008;8:935-945. doi:10.1172/jci32748
25. Lin Y, Weisdorf DJ, Solovey A, Hebbel RP. Origins of circulating endothelial cells and endothelial outgrowth from blood. J Clin Invest. 2000; 105 (1):71-77. doi:10.1172/jci8071
26. Lin Y, Chang L, Solovey A, Healey JF, Lollar P, Hebbel RP. Use of blood outgrowth endothelial cells for gene therapy for hemophilia A. Blood. 2002;99(2):457-462. doi:10.1182/blood.V99.2.457
27. Ozelo MC, Vidal B, Brown C, Notley C, Hegadorn C, Webster S, Harpell L, Ahlin J, Winterborn A, Handforth J, Arruda VR, Hough C, Lillicrap D. Omental implantation of BOECs in hemophilia dogs results in circulating FVIII antigen and a complex immune response. Blood. 2014;123 (26):4045-4053. doi:10.1182/blood-2013-12-545780
28. Miyata T, Tatsumi K, Sugimoto M, Lillicrap D, Shima M, Ohashi K, Okano T, Matsui H. A novel cell-sheet technology that achieves durable factor VIII delivery in a mouse model of hemophilia A. PLoS ONE. 2013;8(12):e83280. doi:10.1371/journal.pone.0083280
29. Olgasi C, Borsotti C, Merlin S, Bergmann T, Bittorf P, Adewoye AB, Wragg N, Patterson K, Calabria A, Benedicenti F, Cucci A, Borchiellini A, Pollio B, Montini E, Mazzuca DM, Zierau M, Stolzing A, Toleikis PM, Braspenning J, Follenzi A. Efficient and safe correction of hemophilia A by lentiviral vector-transduced BOECs in an implantable device. Mol Ther Methods Clin Dev. 2021;23:551-566. doi:10.1016/j.omtm.2021.10.015
30. Mitani S, Hosoda C, Onodera Y, Takabayashi Y, Sakata A, Shima M, Tatsumi K. Efficient generation of liver sinusoidal endothelial-like cells secreting coagulation factor VIII from human induced pluripotent stem cells. Mol Ther Methods Clin Dev. 2024;32(4):101355. doi:10.1016/j.omtm.2024.101355
31. Xu D, Alipio Z, Fink LM, Adcock DM, Yang J, Ward DC, Ma Y. Phenotypic correction of murine hemophilia A using an iPS cell-based therapy. Proc Natl Acad Sci. 2009; USA 106 (3):808-813. doi:10.1073/pnas.0812090106
32. Gao K, Kumar P, Cortez-Toledo E, Hao D, Reynaga L, Rose M, Wang C, Farmer D, Nolta J, Zhou J, Zhou P, Wang A. Potential long-term treatment of hemophilia A by neonatal co-transplantation of cord blood-derived endothelial colony-forming cells and placental mesenchymal stromal cells. Stem Cell Res Ther. 2019;10(1):34. doi:10.1186/s13287-019-1138-8
33. Rose M, Gao K, Cortez-Toledo E, Agu E, Hyllen AA, Conroy K, Pan G, Nolta JA, Wang A, Zhou P. Endothelial cells derived from patients' induced pluripotent stem cells for sustained factor VIII delivery and the treatment of hemophilia A. Stem Cells Transl Med. 2020;9(6):686-696. doi:10.1002/sctm.19-0261
34. Yakura Y, Ishihara C, Kurosaki H, Kazuki Y, Komatsu N, Okada Y, Doi T, Takeya H, Oshimura M. An induced pluripotent stem cell-mediated and integration-free factor VIII expression system. Biochem Biophys Res Commun. 203; 431(2):336-341. doi:10.1016/j.bbrc.2012.12.096
35. Wang D, Zhang G, Gu J, Shao X, Dai Y, Li J, Pan X, Yao S, Xu A, Jin Y, Huang J, Shi Q, Zhu J, Xi X, Chen Z, Chen S. In vivo generated hematopoietic stem cells from genome

edited induced pluripotent stem cells are functional in platelet-targeted gene therapy of murine hemophilia A. Haematologica. 2020;105 (4):e175-e179. doi:10.3324/haematol.2019.219089
36. Sung JJ, Park CY, Leem JW, Cho MS, Kim DW. Restoration of FVIII expression by targeted gene insertion in the FVIII locus in hemophilia A patient-derived iPSCs. Exp Mol Med. 2019;51(4):1-9. doi:10.1038/s12276-019-0243-1
37. Park CY, Sung JJ, Cho SR, Kim J, Kim DW. Universal Correction of Blood Coagulation Factor VIII in Patient-Derived Induced Pluripotent Stem Cells Using CRISPR/Cas9. Stem Cell Rep. 2019;12(6):1242-1249. doi:10.1016/j.stemcr.2019.04.016
38. Kim DH, Choi SH, Sung JJ, Kim S, Yi H, Park S, Park CW, Oh YW, Lee J, Kim DS, Kim JH, Park CY, Kim DW. Long-term correction of hemophilia A via integration of a functionally enhanced FVIII gene into the AAVS1 locus by nickase in patient-derived iPSCs. Exp Mol Med. 2025;57(1):184-192. doi:10.1038/s12276-024-01375-z
39. Chen Jh, Zhan Lj, Lv Cm, Teng Jb, Cao Cy. Advances and Challenges in Adeno-Associated Virus Gene Therapy Applications of Localized Delivery Strategies. Curr Med Sci. 2025. doi: 10.1007/s11596-025-00084-6
40. Herzog RW, Hagstrom JN, Kung S-H, Tai SJ, Wilson JM, Fisher KJ, High KA. Stable gene transfer and expression of human blood coagulation factor IX after intramuscular injection of recombinant adeno-associated virus. Proc Natl Acad Sci USA. 1997;94(11):5804-5809. doi:10.1073/pnas.94.11.5804
41. Fields PA, Kowalczyk DW, Arruda VR, Armstrong E, McCleland ML, Hagstrom JN, Pasi KJ, Ertl HCJ, Herzog RW, High KA. Role of Vector in Activation of T Cell Subsets in Immune Responses against the Secreted Transgene Product Factor IX. Mol Ther. 2000;1(3):225-235. doi:10.1006/mthe.2000.0032
42. Kay MA, Manno CS, Ragni MV, Larson PJ, Couto LB, McClelland A, Glader B, Chew AJ, J Tai S, Herzog RW, Arruda V, Johnson F, Scallan C, Skarsgard E, Flake AW, High KA. Evidence for gene transfer and expression of factor IX in haemophilia B patients treated with an AAV vector. Nat Genet. 2000;24 (3):257-261. doi:10.1038/73464
43. Flotte TR, Trapnell BC, Humphries M, Carey B, Calcedo R, Rouhani F, Campbell-Thompson M, Yachnis AT, Sandhaus RA, McElvaney NG, Mueller C, Messina LM, Wilson JM, Brantly M, Knop DR, Ye G-j, Chulay JD. Phase 2 Clinical Trial of a Recombinant Adeno-Associated Viral Vector Expressing α1-Antitrypsin: Interim Results. Hum Gene Ther . 2011;22(10):1239-1247. doi:10.1089/hum.2011.053
44. Arruda VR, Stedman HH, Haurigot V, Buchlis G, Baila S, Favaro P, Chen Y, Franck HG, Zhou S, Wright JF, Couto LB, Jiang H, Pierce GF, Bellinger DA, Mingozzi F, Nichols TC, High KA. Peripheral transvenular delivery of adeno-associated viral vectors to skeletal muscle as a novel therapy for hemophilia B. Blood. 2010;115(23):4678-4688. doi:10.1182/blood-2009-12-261156
45. Finn JD, Nichols TC, Svoronos N, Merricks EP, Bellenger DA, Zhou S, Simioni P, High KA, Arruda VR. The efficacy and the risk of immunogenicity of FIX Padua (R338L) in hemophilia B dogs treated by AAV muscle gene therapy. Blood. 2012;120(23):4521-4523. doi:10.1182/blood-2012-06-440123
46. Gao G-P, Alvira MR, Wang L, Calcedo R, Johnston J, Wilson JM. Novel adeno-associated viruses from rhesus monkeys as vectors for human gene therapy. Proc Natl Acad Sci USA. 2002;99(18):11854-11859. doi:10.1073/pnas.182412299
47. Calcedo R, Vandenberghe Luk H, Gao G, Lin J, Wilson James M. Worldwide Epidemiology of Neutralizing Antibodies to Adeno-Associated Viruses. J Infect Dis. 2009;199(3):381-390. doi:10.1086/595830
48. Boutin S, Monteilhet V, Veron P, Leborgne C, Benveniste O, Montus MF, Masurier C. Prevalence of Serum IgG and Neutralizing Factors Against Adeno-Associated Virus (AAV) Types 1, 2, 5, 6, 8, and 9 in the Healthy Population: Implications for Gene Therapy Using AAV Vectors. Hum Gene Ther. 2010;21(6):704-712. doi:10.1089/hum.2009.182
49. Nathwani AC, Rosales C, McIntosh J, Rastegarlari G, Nathwani D, Raj D, Nawathe S, Waddington SN, Bronson R, Jackson S, Donahue RE, High KA, Mingozzi F, Ng CYC, Zhou J, Spence Y, McCarville MB, Valentine M, Allay J, Coleman J, Sleep S, Gray JT, Nienhuis AW, Davidoff AM. Long-term Safety and Efficacy Following Systemic Administration of a Self-complementary AAV Vector Encoding Human FIX Pseudotyped With Serotype 5 and 8 Capsid Proteins. Mol Ther. 2011;19(5):876-885. doi:10.1038/mt.2010.274
50. Chou SC, Huang CP, Su YH, Yu CH, Yang YL, Wang SC, Lin YH, Chen YT, Li JY, Chang YT, Chen SY, Lin SW. Preclinical Evaluation of the Systemic Safety, Efficacy, and Biodistribution of a Recombinant AAV8 Vector Expressing FIX-TripleL in Hemophilia B Mice: Implications for Human Gene Therapy. Int J Mol Sci. 2025;26(13):6073. doi:10.3390/ijms26136073
51. Nathwani AC, Tuddenham EGD, Rangarajan S, Rosales C, McIntosh J, Linch DC, Chowdary P, Riddell A, Pie AJ, Harrington C, O'Beirne J, Smith K, Pasi J, Glader B, Rustagi P, Ng CYC, Kay MA, Zhou J, Spence Y, Morton CL, Allay J, Coleman J, Sleep S, Cunningham JM, Srivastava D, Basner-Tschakarjan E, Mingozzi F, High KA, Gray JT, Reiss UM, Nienhuis AW, Davidoff AM. Adenovirus-Associated Virus Vector–Mediated Gene Transfer in Hemophilia B. N Engl J Med. 2011;365(25):2357-2365. doi:10.1056/NEJMoa1108046
52. Xue F, Li H, Wu X, Liu W, Zhang F, Tang D, Chen Y, Wang W, Chi Y, Zheng J, Du Z, Jiang W, Zhong C, Wei J, Zhu P, Fu R, Liu X, Chen L, Pei X, Sun J, Cheng T, Yang R, Xiao X, Zhang L. Safety and activity of an engineered, liver-tropic adeno-associated virus vector expressing a hyperactive Padua factor IX administered with prophylactic glucocorticoids in patients with haemophilia B: a single-centre, single-arm, phase 1, pilot trial. Lancet Haematol. 2022;9(7):e504-e513. doi:10.1016/s2352-3026(22)00113-2
53. Xue F, Ju M, Zhu T, Zhou Z, Sun J, Yang L, Yan Z, Zhou H, Du X, Zheng C, Zheng J, Wu X, Du Z, Jiang W, Yang C, Xiao X, Liu W, Yang R, Zhang L. Factor IX-Padua AAV gene therapy in hemophilia B: phases 1/2 and 3 trials. Nature Medicine. 2025;32(1):93-102. doi:10.1038/s41591-025-04012-y
54. Xue F, Wang P, Yuan Z, Shi C, Fang Y, Liu W, Wang Y, Xiao X, Yang R, George LA, Zhang L. Total Knee Arthroplasty after Gene Therapy for Hemophilia B. N Engl J Med. 2022;387(17):1622-1624. doi:10.1056/NEJMc2211173
55. Cuker A, Kavakli K, Frenzel L, Wang J-D, Astermark J, Cerqueira MH, Iorio A, Katsarou-Fasouli O, Klamroth R, Shapiro AD, Hermans C, Ishiguro A, Leavitt AD, Oldenburg

JB, Ozelo MC, Teitel J, Biondo F, Fang A, Fuiman J, McKay J, Sun P, Rasko JEJ, Rupon J. Gene Therapy with Fidanacogene Elaparvovec in Adults with Hemophilia B. New England Journal of Medicine. 2024;391(12):1108-1118. doi:10.1056/NEJMoa2302982
56. Herzog RW. Gene therapy for hemophilia. Front Biosci (Landmark Ed). 2015;20(3):556-603. doi:10.2741/4324
57. Chao H, Mao L, Bruce AT, Walsh CE. Sustained expression of human factor VIII in mice using a parvovirus-based vector. Blood. 2000;95(5):1594-1599. doi:10.1182/blood.V95.5.1594.005k34_1594_1599
58. Ishiwata A, Mimuro J, Kashiwakura Y, Niimura M, Takano K, Ohmori T, Madoiwa S, Mizukami H, Okada T, Naka H, Yoshioka A, Ozawa K, Sakata Y. Phenotype correction of hemophilia A mice with adeno-associated virus vectors carrying the B domain-deleted canine factor VIII gene. Thromb Res. 2006;118(5):627-635. doi:10.1016/j.thromres.2005.11.006
59. Zhang W, Mao J, Shen Y, Zhang G, Shao Y, Ruan Z, Wang Y, Wu W, Wang X, Zhu J, Chen S, Xiao W, Xi X. Evaluation of the activity levels of rat FVIII and human FVIII delivered by adeno-associated viral vectors both in vitro and in vivo. Blood Cells Mol Dis. 2018;73:47-54. doi:10.1016/j.bcmd.2018.09.004
60. Greig JA, Nordin JML, White JW, Wang Q, Bote E, Goode T, Calcedo R, Wadsworth S, Wang L, Wilson JM. Optimized Adeno-Associated Viral-Mediated Human Factor VIII Gene Therapy in Cynomolgus Macaques. Hum Gene Ther. 2018. doi: 10.1089/hum.2018.080
61. Russell CB, Vettermann C, Agarwal S, Witt E, Clark W, Arens J, Fronza R, Obrochta Moss KM, Kasprzyk T, Robinson TM, Tran H, Kenet G, Raheja P, Lester W, Eggan K, Zoog S. Recombinant Adeno-Associated Virus Integration Profiles in Nonhuman Primates and Gene Therapy Participants after Treatment with Valoctocogene Roxaparvovec. Hum Gene Ther. 2025;36(13-14):945-955. doi:10.1089/hum.2024.236
62. Nguyen GN, Everett JK, Kafle S, Roche AM, Raymond HE, Leiby J, Wood C, Assenmacher C-A, Merricks EP, Long CT, Kazazian HH, Nichols TC, Bushman FD, Sabatino DE. A long-term study of AAV gene therapy in dogs with hemophilia A identifies clonal expansions of transduced liver cells. Nat Biotechnol. 2020;39(1):47-55. doi:10.1038/s41587-020-0741-7
63. Rangarajan S, Walsh L, Lester W, Perry D, Madan B, Laffan M, Yu H, Vettermann C, Pierce GF, Wong WY, Pasi KJ. AAV5-Factor VIII Gene Transfer in Severe Hemophilia A. N Engl J Med. 2017;377(26):2519-2530. doi:10.1056/NEJMoa1708483
64. Pasi KJ, Rangarajan S, Mitchell N, Lester W, Symington E, Madan B, Laffan M, Russell CB, Li M, Pierce GF, Wong WY. Multiyear Follow-up of AAV5-hFVIII-SQ Gene Therapy for Hemophilia A. N Engl J Med. 2020;382(1):29-40. doi:10.1056/NEJMoa1908490
65. Pasi KJ, Laffan M, Rangarajan S, Robinson TM, Mitchell N, Lester W, Symington E, Madan B, Yang X, Kim B, Pierce GF, Wong WY. Persistence of haemostatic response following gene therapy with valoctocogene roxaparvovec in severe haemophilia A. Haemophilia. 2021;27(6):947-956. doi:10.1111/hae.14391
66. Ozelo MC, Mahlangu J, Pasi KJ, Giermasz A, Leavitt AD, Laffan M, Symington E, Quon DV, Wang J-D, Peerlinck K, Pipe SW, Madan B, Key NS, Pierce GF, O'Mahony B, Kaczmarek R, Henshaw J, Lawal A, Jayaram K, Huang M, Yang X, Wong WY, Kim B. Valoctocogene Roxaparvovec Gene Therapy for Hemophilia A. N Engl J Med. 2022;386(11):1013-1025. doi:10.1056/NEJMoa2113708
67. O'Mahony B, Dunn AL, Leavitt AD, Peyvandi F, Ozelo MC, Mahlangu J, Peerlinck K, Wang J-D, Lowe GC, Tan CW, Giermasz A, Tran H, Khoo T-L, Cockrell E, Pepperell D, Chambost H, López Fernández MF, Kazmi R, Majerus E, Skinner MW, Klamroth R, Quinn J, Yu H, Wong WY, Robinson TM, Pipe SW. Health-related quality of life following valoctocogene roxaparvovec gene therapy for severe hemophilia A in the phase 3 trial GENEr8-1. Journal of Thrombosis and Haemostasis. 2023;21(12):3450-3462. doi:10.1016/j.jtha.2023.08.032
68. Leavitt AD, Mahlangu J, Raheja P, Symington E, Quon DV, Giermasz A, López Fernández MF, Kenet G, Lowe G, Key NS, Millar CM, Pipe SW, Madan B, Chou S-C, Klamroth R, Mason J, Chambost H, Peyvandi F, Majerus E, Pepperell D, Rivat C, Yu H, Robinson TM, Ozelo MC. Efficacy, safety, and quality of life 4 years after valoctocogene roxaparvovec gene transfer for severe hemophilia A in the phase 3 GENEr8-1 trial. Research and Practice in Thrombosis and Haemostasis. 2024;8(8). doi:10.1016/j.rpth.2024.102615
69. Leavitt AD, Mahlangu J, Raheja P, Symington E, Quon DV, Giermasz A, Kenet G, Lowe G, Key NS, Millar CM, Pipe SW, Chou S-C, Klamroth R, Mason J, Chambost H, Peyvandi F, Majerus E, Pepperell D, Tan CW, Yu H, Chavele K-M, Ozelo MC. Durability of efficacy, safety, and quality of life 5 years after valoctocogene roxaparvovec gene transfer for severe hemophilia A: final phase 3 GENEr8-1 trial results. Research and Practice in Thrombosis and Haemostasis. 2026;10(3). doi:10.1016/j.rpth.2026.103416
70. Leavitt AD, Konkle BA, Stine KC, Visweshwar N, Harrington TJ, Giermasz A, Arkin S, Fang A, Plonski F, Yver A, Ganne F, Agathon D, Resa MdlA, Tseng L-J, Di Russo G, Cockroft BM, Cao L, Rupon J. Giroctocogene fitelparvovec gene therapy for severe hemophilia A: 104-week analysis of the phase 1/2 Alta study. Blood. 2024;143(9):796-806. doi:10.1182/blood.2022018971
71. Elkouby L, Armour SM, Toso R, DiPietro M, Davidson RJ, Nguyen GN, Willet M, Kutza S, Silverberg J, Frick J, Crosariol M, Wang Y, Wang C, High KA, Sabatino DE, Anguela XM. Preclinical assessment of an optimized AAV-FVIII vector in mice and non-human primates for the treatment of hemophilia A. Molecular Therapy - Methods & Clinical Development . 2022;24:20-29. doi:10.1016/j.omtm.2021.11.005
72. George LA, Monahan PE, Eyster ME, Sullivan SK, Ragni MV, Croteau SE, Rasko JEJ, Recht M, Samelson-Jones BJ, MacDougall A, Jaworski K, Noble R, Curran M, Kuranda K, Mingozzi F, Chang T, Reape KZ, Anguela XM, High KA. Multiyear Factor VIII Expression after AAV Gene Transfer for Hemophilia A. New England Journal of Medicine. 2021;385(21):1961-1973. doi:10.1056/NEJMoa2104205
73. Cantore A, Nair N, Della Valle P, Di Matteo M, Màtrai J, Sanvito F, Brombin C, Di Serio C, D'Angelo A, Chuah M, Naldini L, VandenDriessche T. Hyperfunctional coagulation factor IX improves the efficacy of gene therapy in hemophilic mice. Blood. 2021;120(23):4517-4520. doi:10.1182/blood-2012-05-432591
74. Cantore A, Ranzani M, Bartholomae CC, Volpin M, Valle

PD, Sanvito F, Sergi LS, Gallina P, Benedicenti F, Bellinger D, Raymer R, Merricks E, Bellintani F, Martin S, Doglioni C, D'Angelo A, VandenDriessche T, Chuah MK, Schmidt M, Nichols T, Montini E, Naldini L. Liver-directed lentiviral gene therapy in a dog model of hemophilia B. Sci Transl Med. 2015;7 (277):277ra228. doi:10.1126/scitranslmed.aaa1405
75. Milani M, Annoni A, Bartolaccini S, Biffi M, Russo F, Di Tomaso T, Raimondi A, Lengler J, Holmes MC, Scheiflinger F, Lombardo A, Cantore A, Naldini L. Genome editing for scalable production of alloantigen-free lentiviral vectors for in vivo gene therapy. EMBO Mol Med. 2017;9(11):1558-1573. doi:10.15252/emmm.201708148
76. Milani M, Annoni A, Moalli F, Liu T, Cesana D, Calabria A, Bartolaccini S, Biffi M, Russo F, Visigalli I, Raimondi A, Patarroyo-White S, Drager D, Cristofori P, Ayuso E, Montini E, Peters R, Iannacone M, Cantore A, Naldini L. Phagocytosis-shielded lentiviral vectors improve liver gene therapy in nonhuman primates. Sci Transl Med. 2019;11 (493):eaav7325. doi:10.1126/scitranslmed.aav7325
77. Milani M, Canepari C, Liu T, Biffi M, Russo F, Plati T, Curto R, Patarroyo-White S, Drager D, Visigalli I, Brombin C, Albertini P, Follenzi A, Ayuso E, Mueller C, Annoni A, Naldini L, Cantore A. Liver-directed lentiviral gene therapy corrects hemophilia A mice and achieves normal-range factor VIII activity in non-human primates. Nat Commun. 2022;13(1):2454. doi:10.1038/s41467-022-30102-3
78. Stein CS, Kang Y, Sauter SL, Townsend K, Staber P, Derksen TA, Martins I, Qian J, Davidson BL, McCray PB. In Vivo Treatment of Hemophilia A and Mucopolysaccharidosis Type VII Using Nonprimate Lentiviral Vectors. Mol Ther. 2001;3(6):850-856. doi:10.1006/mthe.2001.0325
79. Kang Y, Xie L, Tran DT, Stein CS, Hickey M, Davidson BL, McCray PB. Persistent expression of factor VIII in vivo following nonprimate lentiviral gene transfer. Blood. 2005;106(5):1552-1558. doi:10.1182/blood-2004-11-4358
80. Sinn PL, Goreham-Voss JD, Arias AC, Hickey MA, Maury W, Chikkanna-Gowda CP, McCray JPB. Enhanced Gene Expression Conferred by Stepwise Modification of a Nonprimate Lentiviral Vector. Hum Gene Ther. 2007;18(12):1244-1252. doi:10.1089/hum.2006.127
81. Staber JM, Pollpeter MJ, Anderson CG, Burrascano M, Cooney AL, Sinn PL, Rutkowski DT, Raschke WC, McCray PB. Long-term correction of hemophilia A mice following lentiviral mediated delivery of an optimized canine factor VIII gene. Gene Ther. 2017;24(11):742-748. doi:10.1038/gt.2017.67
82. Ward NJ, Buckley SM, Waddington SN, Vandendriessche T, Chuah MK, Nathwani AC, McIntosh J, Tuddenham EG, Kinnon C, Thrasher AJ, McVey JH. Codon optimization of human factor VIII cDNAs leads to high-level expression. Blood. 2011;117(3):798-807. doi:10.1182/blood-2010-05-282707
83. McIntosh J, Lenting PJ, Rosales C, Lee D, Rabbanian S, Raj D, Patel N, Tuddenham EG, Christophe OD, McVey JH, Waddington S, Nienhuis AW, Gray JT, Fagone P, Mingozzi F, Zhou SZ, High KA, Cancio M, Ng CY, Zhou J, Morton CL, Davidoff AM, Nathwani AC. Therapeutic levels of FVIII following a single peripheral vein administration of rAAV vector encoding a novel human factor VIII variant. Blood. 2013;121(17):3335-3344. doi:10.1182/blood-2012-10-462200
84. Merlin S, Cannizzo ES, Borroni E, Bruscaggin V, Schinco P, Tulalamba W, Chuah MK, Arruda VR, VandenDriessche T, Prat M, Valente G, Follenzi A. A novel platform for immune tolerance induction in hemophilia A mice. Mol Ther. 2017;25(8):1815-1830. doi:10.1016/j.ymthe.2017.04.029
85. Gong J, Yang R, Zhou M, Chang L-J. Improved intravenous lentiviral gene therapy based on endothelial-specific promoter-driven factor VIII expression for hemophilia A. Mol Med. 2023;29(1):74. doi:10.1186/s10020-023-00680-z
86. Suzuki Y, Kakisaka K, Matsumoto T, Nogami K, Katagiri H, Takahara T, Takikawa Y. Orthotopic liver transplantation for haemophilia A may not always lead to a phenotypic cure of haemophilia A: A case report. Haemophilia. 2018;24(6):e420-e422. doi:10.1111/hae.13604
87. McCauslin CS, Wine J, Cheng L, Klarmann KD, Candotti F, Clausen PA, Spence SE, Keller JR. In vivo retroviral gene transfer by direct intrafemoral injection results in correction of the SCID phenotype in Jak3 knock-out animals. Blood. 2003;102(3):843-848. doi:10.1182/blood-2002-12-3859
88. Worsham DN, Schuesler T, von Kalle C, Pan D. In Vivo Gene Transfer into Adult Stem Cells in Unconditioned Mice by in Situ Delivery of a Lentiviral Vector. Mol Ther. 2006;14 (4):514-524. doi:10.1016/j.ymthe.2006.05.014
89. Miao CH. Hemophilia A gene therapy via intraosseous delivery of factor VIII-lentiviral vectors. Thromb J. 2016;14(S1):41. doi:10.1186/s12959-016-0105-1
90. Michelson AD. How Platelets Work: Platelet Function and Dysfunction. J Thromb Thrombolys. 2003;16(1/2):7-12. doi:10.1023/b:Thro.0000014586.77684.82
91. Wang X, Shin SC, Chiang AFJ, Khan I, Pan D, Rawlings DJ, Miao CH. Intraosseous Delivery of Lentiviral Vectors Targeting Factor VIII Expression in Platelets Corrects Murine Hemophilia A. Mol Ther. 2015;23(4):617-626. doi:10.1038/mt.2015.20
92. Nayak S, Herzog RW. Progress and prospects: immune responses to viral vectors. Gene Ther. 2009;17(3):295-304. doi:10.1038/gt.2009.148
93. Mingozzi F, High KA. Immune responses to AAV vectors: overcoming barriers to successful gene therapy. Blood. 2013;122(1):23-36. doi:10.1182/blood-2013-01-306647
94. Yin L, He H, Zhang H, Shang Y, Fu C, Wu S, Jin T. Revolution of AAV in Drug Discovery: From Delivery System to Clinical Application. J Med Virol. 2025;97(6):e70447. doi:10.1002/jmv.70447
95. Gyurkocza B, Sandmaier BM. Conditioning regimens for hematopoietic cell transplantation: one size does not fit all. Blood. 2014;124(3):344-353. doi:10.1182/blood-2014-02-514778
96. Chaudhry M, Ali N. Reduced-intensity conditioning hematopoietic stem cell transplantation: looking forward to an international consensus. Blood Res. 2015;50(2):69-70. doi:10.5045/br.2015.50.2.69
97. Gong J, Chung TH, Zheng J, Zheng H, Chang LJ. Transduction of modified factor VIII gene improves lentiviral gene therapy efficacy for hemophilia A. J Biol Chem. 2021;297 (6):1-13. doi:10.1016/j.jbc.2021.101397
98. Journal of Clinical InvestigationShi Q, Wilcox DA, Fahs SA, Weiler H, Wells CW, Cooley BC, Desai D, Morateck PA, Gorski J, Montgomery RR. Factor VIII ectopically targeted to platelets is therapeutic in hemophilia A with high-titer inhibitory antibodies. J Clin Invest. 2006;116(7):1974-1982. doi:10.1172/JCI28416

99. Kuether EL, Schroeder JA, Fahs SA, Cooley BC, Chen Y, Montgomery RR, Wilcox DA, Shi Q. Lentivirus-mediated platelet gene therapy of murine hemophilia A with pre-existing anti-factor VIII immunity. J Thromb Haemost. 2012;10 (8):1570-1580. doi:10.1111/j.1538-7836.2012.04791.x
100. Bacigalupo A, Ballen K, Rizzo D, Giralt S, Lazarus H, Ho V, Apperley J, Slavin S, Pasquini M, Sandmaier BM, Barrett J, Blaise D, Lowski R, Horowitz M. Defining the Intensity of Conditioning Regimens: Working Definitions. Biol Blood Marrow Transplant. 2009;15(12):1628-1633. doi:10.1016/j.bbmt.2009.07.004
101. Nathwani AC, Gray JT, McIntosh J, Ng CYC, Zhou J, Spence Y, Cochrane M, Gray E, Tuddenham EGD, Davidoff AM. Safe and efficient transduction of the liver after peripheral vein infusion of self-complementary AAV vector results in stable therapeutic expression of human FIX in nonhuman primates. Blood. 2006;109(4):1414-1421. doi:10.1182/blood-2006-03-010181
102. Nathwani AC, Gray JT, Ng CY, Zhou J, Spence Y, Waddington SN, Tuddenham EG, Kemball-Cook G, McIntosh J, Boon-Spijker M, Mertens K, Davidoff AM. Self-complementary adeno-associated virus vectors containing a novel liver-specific human factor IX expression cassette enable highly efficient transduction of murine and nonhuman primate liver. Blood. 2006;107(7):2653-2661. doi:10.1182/blood-2005-10-4035
103. Milani M, Fabiano A, Perez-Rodriguez M, Hernandez RJ, Zecchillo A, Zonari E, Ottonello S, Basso-Ricci L, Canepari C, Volpin M, Iannello V, Capo V, Quaranta P, Seffin L, Russo F, Biffi M, Ormoli L, Brombin C, Carlucci F, Forlino A, Filibian M, Montini E, Scala S, Villa A, Bueren JA, Rio P, Aiuti A, Cantore A, Naldini L. In vivo haemopoietic stem cell gene therapy enabled by postnatal trafficking. Nature. 2025; 643(8073):1097-1106. doi:10.1038/s41586-025-09070-3

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