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A BRIEF REVIEW ON CLINICAL ASSESSMENT OF GENE THERAPIES FOR HEMOPHILIA A AND HEMOPHILIA B

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ABSTRACT
Hemophilia A is an X-linked, recessive disorder caused by deficiency of functional plasma clotting factor VIII (FVIII), which may be inherited or arise from spontaneous mutation. The development of inhibitory alloantibodies to FVIII can severely complicate the treatment of genetic cases. Rarely, development of autoantibodies to FVIII results in acquired hemophilia A. In the early-phase hemophilia B trials of the currently licensed products, data has shown durable expression for periods of up to 8 years, with observation ongoing. This gives reasonable confidence that this bar can be met, at least for hemophilia B. To date, the only clinical dataset showing long-term expression (>10 years) is one for hemophilia B, in a trial that started in 2010. However, hemophilia A has not yet been shown to exhibit consistent long-term expression at stable levels above 30%, and predictability remains a problem for all licensed gene treatments for hemophilia (all three are AAV vectors that drive liver-specific expression of the clotting factor). The fact that there are already two authorized gene treatments for hemophilia B makes it unique. Both products are non-inferior and superior to routine prophylaxis in terms of ABR; they both demonstrated mean FIX activity levels well into the mild hemophilia range, with Hemgenix levels slightly higher than Beqvez (34% vs. 26.9% by one stage assay); and nearly two-thirds of phase 3 trial participants experienced no bleeding episodes during the follow-up periods.

KEYWORDS: hemophilia, adeno-associated virus, AAV, factor VIII, factor IX,lentivirus clinical trial.

INTRODUCTION
AAV (adeno-associated virus)–based gene therapy is a new treatment for hemophilia and has recently received approval for the treatment of severe hemophilia A. It does not suffer from the limitations of the current standard treatment (regular prophylactic intravenous injections of the missing clotting factor; subcutaneous injection of a bispecific antibody in hemophilia A) and can, it is hoped, raise the concentration of the missing clotting factor over the long term. AAV-based gene therapy can only be performed once, however, because of the generation of antibodies to AAV.

Signs and Symptoms
The hallmark of hemophilia is hemorrhage into the joints. This bleeding is painful and leads to long-term inflammation and deterioration of the joint (typically the ankles in children, and the ankles, knees, and elbows in adolescents and adults), resulting in permanent deformities, misalignment, loss of mobility, and extremities of unequal lengths. Prolonged increase in intra-articular pressure may eventually lead to osteonecrosis, especially in the femoral head.

With mild hemophilia, hemorrhage is most likely to occur with trauma or surgery. Mild or moderate hemophilia may remain unsuspected until relatively late in life, when an inadequate response to a traumatic challenge suggests the diagnosis.

Signs and symptoms of moderate and severe hemophilia include the following.

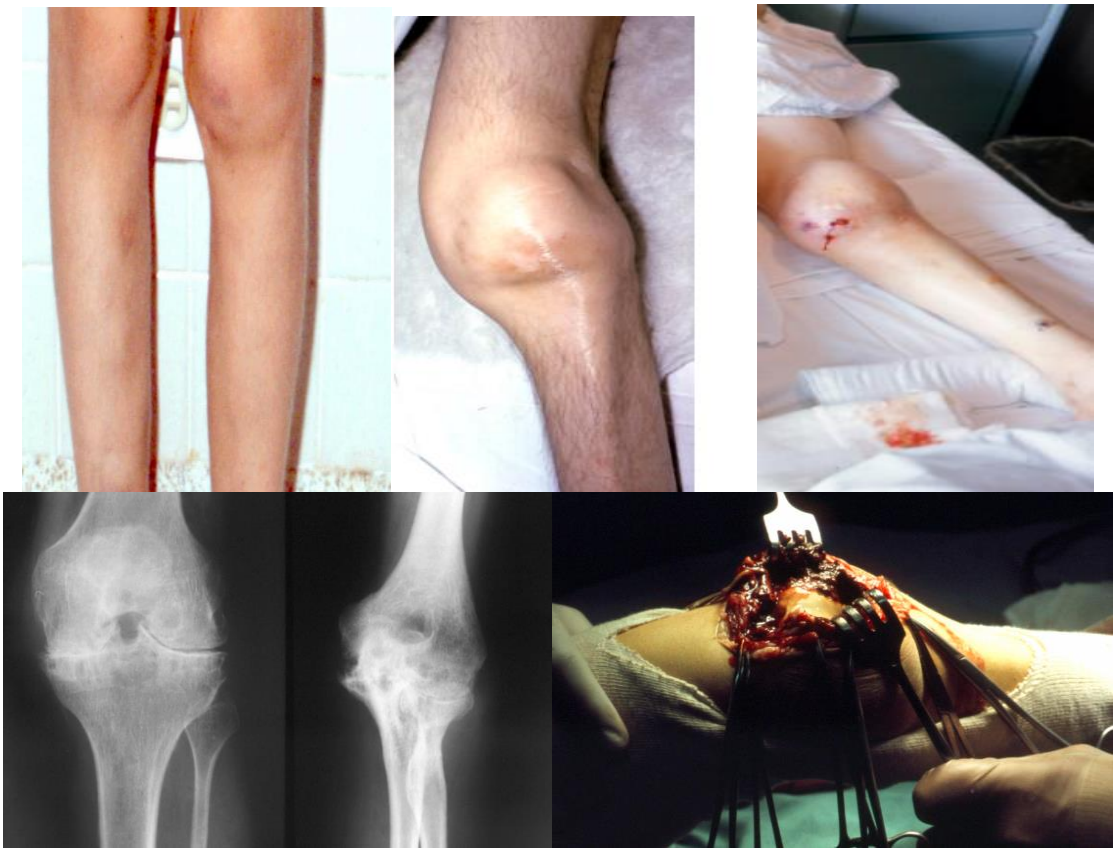
- Neonates: Prolonged bleeding and/or severe hematoma following procedures such as circumcision, phlebotomy, and/or immunizations; intracranial hemorrhage.
- Toddler: Trauma-related soft-tissue hemorrhage; oral bleeding during teething.
- Children: Hemarthrosis and hematomas with increasing physical activity; chronic arthropathy (late complication); traumatic intracranial hemorrhage (life threatening).

Signs and symptoms

Depending on the level of FVIII activity, patients with hemophilia may present with easy bruising; inadequate clotting of traumatic or even mild injury; or, in the case of severe hemophilia, spontaneous hemorrhage.

Signs of hemorrhage include the following.

- General (usuall attributed to anemia secondary to bleeding): Weakness, orthostasis, tachycardia, tachypnea
- Musculoskeletal (joints): Tingling, cracking, warmth, pain, stiffness, and refusal to use joint (children)
- CNS: Headache, stiff neck, vomiting, lethargy, irritability, and spinal cord syndromes
- Gastrointestinal: Hematemesis, melena, frank red blood per rectum, and abdominal pain
- Genitourinary: Hematuria, renal colic, and post-circumcision bleeding
- Other: Epistaxis, oral mucosal hemorrhage, hemoptysis, dyspnea (hematoma leading to airway obstruction), compartment syndrome symptoms, and contusions; excessive/prolonged bleeding with routine dental or other procedures.



Source: Medscape

Diagnosis

Laboratory studies for suspected hemophilia include the following.

- Complete blood cell count
- Screening coagulation studies (prothrombin time [PT], activated partial thromboplastin time [aPTT])
- FVIII assay (clot based or chromogenic)
- FVIII inhibitor assay (Bethesda assay, Nijmegen modified Bethesda assay)

Expected laboratory values are as follows.

- Hemoglobin/hematocrit: Normal (or low if associated bleeding)
- Platelet count: Normal
- Prothrombin time (PT): Normal
- APTT: Significantly prolonged in severe hemophilia, but may be normal or minimally prolonged in mild or even moderate hemophilia

Normal values for FVIII assays are 50-150%. Values in hemophilia A are as follows.

- Mild: > 5%
- Moderate: 1-5%
- Severe: < 1%

For treatment of acute bleeds, target levels by hemorrhage severity are as follows.

- Mild hemorrhages (eg, early hemarthrosis, epistaxis, gingival bleeding): Maintain an FVIII level of 30%.
- Major hemorrhages (eg, late hemarthrosis, muscle bleeds): Maintain an FVIII level of at least 50%.

- Life- or limb-threatening bleeding episodes (eg, major trauma or surgery, advanced or recurrent hemarthrosis, major GI bleeding, any head trauma, signs of distal neurovascular compromise of limb or compartment syndrome): Maintain an FVIII level of 80-100%.

Ideally, therapy is individualized to specific patients. However, for general dosing, to find the number of units of factor VIII needed to correct the factor VIII activity level, use the following formula.
Dose in FVIII IU = (weight in kg) x (desired FVIII increase) x (0.5 IU/kg per IU/dL)

FVIII regimens are as follows.
- The second dose should be administered 8-12 hours after the initial dose and is usually one half the initial calculated dose
- Minor hemorrhage requires 1-3 doses of FVIII
- Major hemorrhage requires many doses and continued FVIII activity monitoring with the goal of keeping the trough activity level at least 50%
- Continuous infusions of FVIII may be considered for major hemorrhage or major surgery

The following types of FVIII concentrates are available.
- Plasma-based products: Purified to inactivate viruses

First-generation recombinant products: Produced in mammalian cell lines, contain animal and/or human plasma-derived proteins in cell culture media and in final product.

Second-generation recombinant products: Produced in mammalian cell lines, contain animal and/or human plasma-derived proteins in cell culture media but none in final product.
- Third-generation recombinant products: Produced in mammalian cell lines, contain no animal and/or human plasma-derived proteins in cell culture media or in final product.
- Extended half-life recombinant FVIII products

Desmopressin vasopressin analog, or 1-deamino-8-D-arginine vasopressin (DDAVP), has the following attributes:
- Depending on baseline level and goal factor level, may be considered the treatment of choice for mild and moderate hemophilia A
- Not effective in the treatment of severe hemophilia
- Can be intravenously administered at a dose of 0.3 mcg/kg of body weight in the inpatient setting (Canadian labeling recommends maximum dose of 20 mcg).
- Peak effect is observed in 30-60 minutes
- DDAVP leads to free water retention, which can lead to hyponatremia
- A concentrated DDAVP intranasal spray is available for outpatient use

The following antifibrinolytics are used in addition to FVIII replacement for oral mucosal hemorrhage and prophylaxis.
- Epsilon aminocaproic acid (Amicar)
- Tranexamic acid (Cyklokapron, Lyseda)

Treatments used in patients with inhibitors of FVIII are as follows.
- High doses of FVIII for low-titer inhibitors
- Activated prothrombin complex concentrate (aPCC)
- Activated recombinant FVII (rFVIIa)
- Monoclonal antibodies directed toward restoring FVIII function (eg, emicizumab)
- Porcine FVIII, which has low cross-reactivity with human FVIII antibody
- Desensitization
- Immune tolerance induction (ITI)
- The treatment of patients with inhibitors of FVIII is difficult. Bleeding episodes in patients with low-titer inhibitors (ie, concentrations below 5 Bethesda units [BU]) occasionally can be overcome with high doses of factor VIII. Options in other cases include a variety of agents that bypass FVIII, such as activated FVII and emicizumab; desensitization; and immune tolerance induction.
- In patients who develop synovitis from joint bleeds, injection of radioisotopes into the joint to ablate the synovium (radiosynovectomy) can be used to decrease bleeding, slow progression of cartilage and bone damage, and prevent arthropathy. Unresponsive cases may require arthroscopic synovectomy or arthroplasty.

Management

FIX is the treatment of choice for acute hemorrhage or presumed acute hemorrhage in patients with hemophilia B. Recombinant FIX is the preferred source for replacement therapy. Ideally, patients with hemophilia should be treated at a comprehensive hemophilia care center.

Management of hemophilia B includes the following.
- Control of hemostasis
- Treatment of bleeding episodes
- Administration of factor replacement products and medications
- Use of factor inhibitors
- Rehabilitation of patients with hemophilia synovitis
- Primary and/or secondary prophylaxis

Treatment may also vary with site-specific locations (eg, joints, mouth, gastrointestinal region, head).

Pharmacotherapy

The following medications are used in the management of hemophilia B.
- Factor IX-containing products (eg, factor IX, recombinant factor IX, factor IX complex)
- Recombinant coagulation factor VIIa
- Recombinant coagulation factor IX

- Antifibrinolytics (eg, epsilon aminocaproic acid, tranexamic acid)
- Antihemophilic agents (eg, desmopressin, anti-inhibitor coagulant complex, human antihemophilic factor, recombinant human antihemophilic factor, plasma-derived prothrombin complex concentrates/factor IX complex concentrates, plasma-derived coagulation factor IX concentrate)
- Monoclonal antibodies (eg, rituximab)
- Tissue factor pathway inhibitor (ie, marstacimab)
- Analgesics (eg, narcotic agents, NSAIDS, acetaminophen with codeine or synthetic codeine analogs)
- Gene therapy (ie, etranacogene dezaparvovec [Hemgenix]; fidanacogene elaparvovec [Beqvez])

Management

The treatment of hemophilia may involve the following.

- Management of hemostasis
- Management of bleeding episodes including hemostatic support and pain management
- Use of factor replacement products and adjuvant medications
- Monoclonal antibodies for prophylaxis (emicizumab, marstacimab)
- Gene therapy
- Treatment of patients with factor inhibitors
- Treatment and rehabilitation of patients with hemophilia synovitis

Antifibrinolytic Agents

Class Summary

These agents are used in addition to factor VIII replacement for oral mucosal hemorrhage and prophylaxis, as the oral mucosa is rich in native fibrinolytic activity. Their use is contraindicated as initial therapies for hemophilia-related hematuria originating from the upper urinary tract because they can cause obstructive uropathy or anuria. They should not be used in combination with prothrombin complex concentrate (PCC).

Aminocaproic acid (Amicar)

This lysine inhibits fibrinolysis by blocking the binding of plasminogen to fibrin and inhibiting plasminogen and conversion to plasmin, resulting in the inhibition of fibrinolysis. The principal drawbacks of this agent are that thrombi formed during treatment are not lysed, and its effectiveness is uncertain. It has been used to prevent recurrence of subarachnoid hemorrhage.

This agent is widely distributed. Its half-life is 1-2 hours. Peak effect occurs within 2 hours. Hepatic metabolism is minimal.

Tranexamic acid injection (Cyklokapron)

This agent is an alternative to aminocaproic acid. It inhibits fibrinolysis by displacing plasminogen from fibrin. It also inhibits the proteolytic activity of plasmin.

Clotting Factors, Gene Therapies

Class Summary

These therapies are designed to introduce a functional copy of a transgene encoding the human coagulation factor VIII.

Valoctocogene roxaparvovec (Roctavian)

Valoctocogene roxaparvovec is an adeno-associated virus serotype 5 (AAV5)–based gene therapy vector, designed to introduce a functional copy of a transgene encoding the B-domain deleted SQ form of human coagulation factor VIII (hFVIII-SQ). The expressed hFVIII-SQ replaces the missing coagulation factor VIII needed for effective hemostasis. It is indicated for severe hemophilia A (congenital factor VIII deficiency with factor VIII activity <1 IU/dL) in adults without pre-existing antibodies to AAV5 detected by an FDA-approved test.

Dosage Forms & Strengths

lyophilized powder for reconstitution

- 250 IU/vial
- 500 IU/vial
- 1000 IU/vial
- 2000 IU/vial
- 3500 IU/vial

Hemophilia B

Indicated for children and adults with hemophilia B (congenital Factor IX deficiency) for

- On-demand control and prevention of bleeding episodes
- Perioperative management of bleeding
- Routine prophylaxis to prevent or reduce the frequency of bleeding episodes

Dose calculation

- Dosage and treatment duration depends on the severity of Factor IX deficiency, the location and extent of bleeding, and the patient's clinical condition, age, and recovery of Factor IX
- Calculating the required dose is based on the empirical finding that one IU per kg body weight is expected to increase the circulating level of Factor IX by 1.3 IU/dL in patients aged ≥12 yr, and by 1 IU/dL in patients aged <12 yr
- Required units (IU) = Body weight (kg) x desired Factor IX rise (% of normal or IU/dL) x (reciprocal of recovery [IU/dL per IU/kg]), OR
- Increase in Factor IX IU/dL (or % of normal) = Dose (IU) x recovery (IU/dL per IU/kg)/body weight (kg)
- Adjust the dose based on the individual patient's clinical condition and response

Hemophilia B, Bleeding Episodes
Indicated for on-demand control and prevention of bleeding episodes.

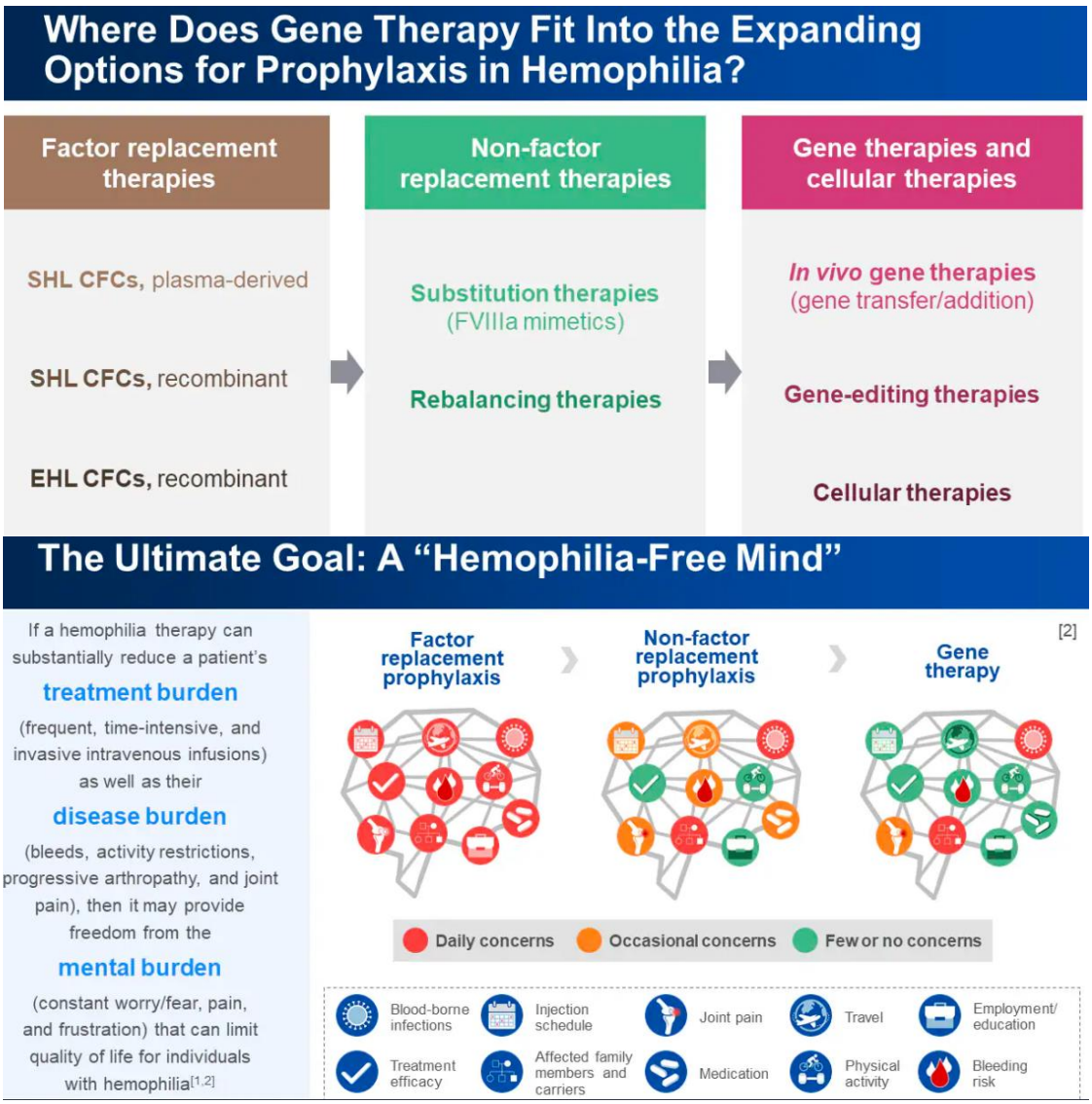
Minor or moderate

- Uncomplicated hemarthrosis, muscle bleeding (except iliopsoas), or oral bleeding
- Maintain circulating FIX at 30-60 % or IU/dL; may repeat q48-72hr; duration of at least 1day, until bleeding stops and healing is achieved; single dose should be sufficient for majority of these bleeds

Major

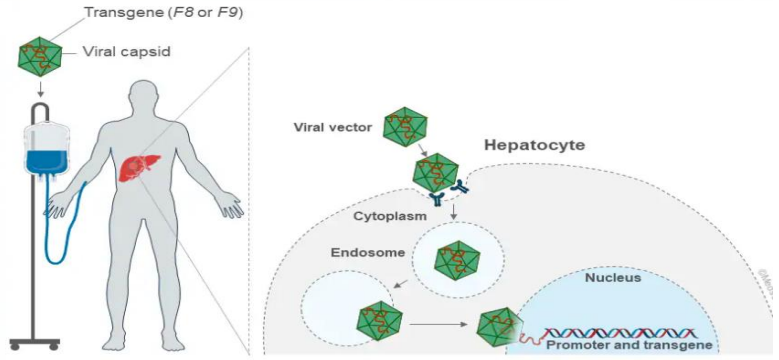
- Life- or limb-threatening hemorrhage; deep muscle bleeding, including iliopsoas, intracranial, and retropharyngeal
- Maintain circulating Factor IX at 60-100 % or IU/dL; may repeat q48-72hr; duration of 7-14 days, until bleeding stops and healing is achieved; consider weekly maintenance dose.

Methods
This review is based on publications retrieved by a selective search in the Medscape, MEDLINE/PubMed database employing the relevant key words, supplemented by expert opinions and the recommendations of the relevant medical societies.



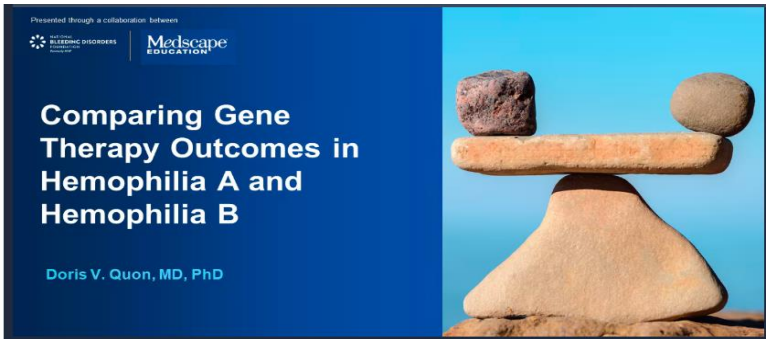
Mechanism of *In Vivo* AAV-Based Hemophilia Gene Therapies

- A transgene coding for FVIII or FIX and containing a liver-specific promoter is inserted into a recombinant AAV vector capsid
- High doses of the vector are intravenously infused into the patient's peripheral blood
- **The AAV vectors travel to the liver and enter the hepatocytes via endosomes, where they attach to the nuclear envelope and deliver their genetic payload to the hepatocyte nuclei**



Approved AAV-Based Gene Therapies for Hemophilia

Agent	Hemophilia Type	Approval Status	Vector Serotype	Dose, vg/kg	Summary of Clinical Trials
Valoctocogene roxaparvovec (BMN 270)[1-5]	A	Approved in US and EU *(commercially available in US, Germany, and Italy)*	rAAV5	6×10^{13}	▪ Phase 1/2 (N = 15): safety/efficacy data to 7 years ▪ **Phase 3 GENEr8-1** (N = 144): safety/efficacy data to 4 years ▪ Phase 3b GENEr8-3 (N = 22): prophylactic corticosteroids ▪ Phase 1/2 GENEr8-INH (recruiting, goal N = 20): patients with FVIII inhibitors
Etranacogene dezaparvovec (AMT-061)[6-9]	B	Approved in US, Canada, UK, and EU	rAAV5	2×10^{13}	▪ Phase 1/2 (N = 3): safety/efficacy data to 5 years ▪ **Phase 3 HOPE-B** (N = 54): safety/efficacy data to 3 years ▪ Phase 3b (N = 35): patients with AAV5 neutralizing antibodies
Fidanacogene elaparvovec (SPK-9001/ PF-06838435)[10-13]	B	Approved in US, Canada, and EU	AAVrh74	5×10^{11}	▪ Phase 1/2 (N = 14): safety/efficacy data to 6.5 years ▪ **Phase 3 BENEGENE-2** (N = 51): safety/efficacy data to 2 years



Agent	Approval Status (Marketing Status)	Vector Serotype	N, Efficacy Evaluable (Clinical Trial)	Dose, vg/kg	Summary of Efficacy Data
Valoctocogene roxaparvovec (BMN 270)[1-4]	Approved in US and EU *(commercially available in US, Germany, and Italy)*	rAAV5	134 (GENEr8-1)	6×10^{13}	▪ **ABR (treated)**: 81.3% reduction from baseline at 4 years ▪ **FVIII use**: 92.2% reduction from baseline at 4 years ▪ **Responders returning to prophylaxis**: 18% ▪ **Mean FVIII activity (CSA)**: 16.1 IU/dL at 4 years
Giroctocogene fitelparvovec (SB-525 or PF-07055480)[5-6]	Investigational	rAAV6	50 (AFFINE)	3×10^{13}	▪ **ABR (total)**: 73.8% reduction from baseline at 15 months ▪ **ABR (treated)**: 98.3% reduction from baseline at 15 months ▪ **FVIII use**: 99.8% reduction from baseline at 15 months ▪ **Responders returning to prophylaxis**: 1.3% ▪ **Mean FVIII activity (CSA)**: 40.7% at 3 years
Dirloctocogene samoparvovec (SPK-8011)[7-9]	Investigational	rAAV-LK03	18 (phase 1/2)	5×10^{11} to 2×10^{12}	▪ **ABR (total)**: 91.5% reduction from baseline at 6.5 years ▪ **FVIII use**: 96.4% reduction from baseline at 6.5 years ▪ **Mean FVIII activity (CSA)**: 6.9% (excluding 2 participants)

ABR, annualized bleeding rate; CSA, chromogenic substrate assay; EE, efficacy evaluable.

1. Valoctocogene roxaparvovec-rvox. [PI]. Approved June 2023; 2. Ozelo MC, et al. N Engl J Med. 2022;386:1013-1025; 3. Leavitt AD, et al. Presented at: THSNA 2024; Chicago, IL; April 4-6, 2024. Abstract 345; 4. Symington E, et al. Haemophilia. 2024;30:1138-1147; 5. Leavitt AD, et al. Blood. 2024;143:796-806; 6. Leavitt AD, et al. Blood. 2024;144 (S1): Abstract 1053; 7. George LA, et al. N Engl J Med. 2021;385:1961-1973; 8. George LA, et al. Res Pract Thromb Haemost. 2021;5(2_suppl):67.2, 9. Croteau SE, et al. Presented at: ISTH 2024 Congress; Bangkok, Thailand; June 22-26, 2024. Abstract OC 02.4.

Source: Medscape

DISCUSSION

Gene therapy for hemophilia is based on the transfer of a non-pathogenic and non-replicating recombinant adeno-associated virus (AAV), the viral DNA of which has been replaced by a bioengineered gene cassette, with a tissue-specific promoter and other regulatory elements National Library of Medicine, 2021.

A further advance in gene therapy for hemophilia B was made with the introduction of the Padua variant of the FIX gene, which has five- to 10-fold higher activity and was initially found in familial thrombophilia Simioni *et al.*, 2009. Here, the molecular regulation of activation, inactivation, and cofactor dependence is similar to FIX wild type, but the faster rate of factor X activation leads to hyperactivity and significantly higher factor levels Samelson-Jones *et al.*, 2019.

The first successful trial results on gene therapy for hemophilia were published in 2017 (BMN-270) Chowdary *et al., 2022*, Rangarajan *et al.*, 2017. Six of seven patients in the high-dose group showed sustained normalization of factor VIII activity over a period of 1 year (mean, 93% ± 48), leading to a stabilization of hemostasis as well as to a comparatively sharp reduction in annual factor VIII use from 5286 IU/kg to 65 IU/kg. The primary adverse event was an increase in alanine aminotransferase (ALT) to 1.5 times the upper limit of the normal range or less. Other publications demonstrated the sustained, successively declining expression of FVIII over a period of up to 6 years. None of the patients permanently resumed FVIII prophylaxis, no severe adverse events occurred, and an improvement in quality of life was seen.

At present, quality of life has been investigated in only a small number of patients. It has been demonstrated that the questionnaire commonly used in hemophilia, Haemophilia-Specific Health-Related Quality of Life (Haemo-QoL-A), is also a reliable instrument for the evaluation of quality of life following gene therapy Quinn *et al.*, 2022.

A common side effect of gene therapy is increased liver enzymes, in particular increased ALT, which can lead to a reduction in or loss of therapeutic effect. This occurs more frequently in phase-3 trials on gene therapy for hemophilia A (89%) Ozelo *et al.*, 2022, compared to gene therapy for hemophilia B (17%). Elevated levels of other transaminases, such as aspartate aminotransferase (AST = glutamate oxaloacetate transaminase [GOT]), may also be measured, but not liver synthesis parameters or bilirubin.

Several new approaches to gene therapy for hemophilia have recently entered the clinic and promise to overcome some limitations of the current state of the art. The first clinical trial of gene editing for hemophilia administered three AAV vectors encoding FIX and two zinc finger nucleases (ZFNs) to insert the FIX transgene in the albumin locus, stemming from proof-of-concept data in mice Harmatz *et al.*, 2022, Haurigot *et al.*, 2011. However, the sole participant in that study did not achieve meaningful FIX expression. Nevertheless, encouraging preclinical work with ZFNs prompted further exploration using the newer CRISPR-Cas9 technology, which emerged in the meantime Doudna *et al.*, 2014, Brooks *et al.*, 2024.

CONCLUSION

The promise of gene therapy for hemophilia is the ability to stop bleeding without the requirement for ongoing medication treatment. A hub-and-spoke model, which is a graded and partially overlapping integrated care model, should be used to coordinate the several steps that must be completed in gene therapy. Data transmission and acquisition should be done via electronic platforms.

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