Received: 17 July 2023 | Revised: 21 November 2023 | Accepted: 28 November 2023

DOI: 10.1111/hae.14907

Haemophilia WFH WILEY

REVIEW ARTICLE

Gene therapy for haemophilia A and B, from basic principles to clinical implementation: An illustrated review

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Abstract

Introduction: With recent approval of the first two gene therapies for haemophilia A and B, educational materials about AAV-based gene therapy are needed by the haemophilia community for a better understanding of this novel therapeutic approach and helping healthcare providers and patients making personalized choices amongst an increasing array of therapeutic options.

Aim: To provide a comprehensive summary of the whole process of AAV-based gene therapy from basic principles to clinical implementation through an illustrated review.

Methods: The authors, with expertise in and knowledge about gene therapy for haemophilia A and B, reviewed relevant articles from PubMed database and translated them into illustrations.

Results: The review is divided into eight illustrated sections providing an overview of gene therapy for haemophilia A and B from haemophilia basics and current treatment landscape, principles of the AAV-based liver-directed gene therapy, through exploring the efficacy and safety results of published phase III clinical trials, current and future challenges, to implementation in clinical practice, including the hub and spoke models and the patient journey.

Conclusion: This illustrated review educates healthcare professionals on AAV-based gene therapy for haemophilia A and B enabling them to further educate their peers and their patients.

KEYWORDS
AAV, bleeds, factor IX, factor VIII, gene therapy, haemophilia

Haemophilia is a hereditary bleeding disorder

Aetiology

Patients without haemophilia

X

F8 gene (~180 kb)[1]
F9 gene (~34 kb)[2]

Clotting factor

FVIII

Functional **FVIII protein**

FIX

Functional **FIX protein**

Patients with haemophilia

Haemophilia A
Haemophilia B

X

Defect in *F8* gene
Defect in *F9* gene

FVIII

Absent or deficient **FVIII protein**

FIX

Absent or deficient **FIX protein**

Clotting factors are produced by the liver.
- FVIII is naturally produced by liver sinusoidal endothelial cells[3].
- FIX is naturally produced by hepatocytes[4].

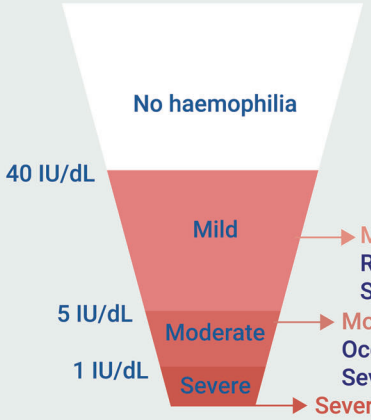
Epidemiology

Patients with haemophilia A — Patients with haemophilia B

/20,000 male births[5]

The prevalence of haemophilia in female patients is lower than in males, accounting for only 3% and 6% of the total patient population in haemophilia A and B, respectively[6]

Haemophilia severity[6]

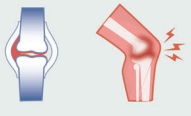


Bleeding symptoms are quite similar for both haemophilia A and B. They depend on the haemophilia severity. Some patients with moderate or mild haemophilia may have severe bleeding phenotype.

Mild:
Rare or no spontaneous bleeding
Severe bleeding with major trauma or surgery

Moderate:
Occasional spontaneous bleeding
Severe bleeding with minor trauma or surgery

Severe:
Spontaneous bleeding mostly into joints or muscles



FVIII, factor VIII; FIX, factor IX

Capsule 1

Approved treatments for patients with haemophilia*

For haemophilia A and B: FVIII and FIX concentrates

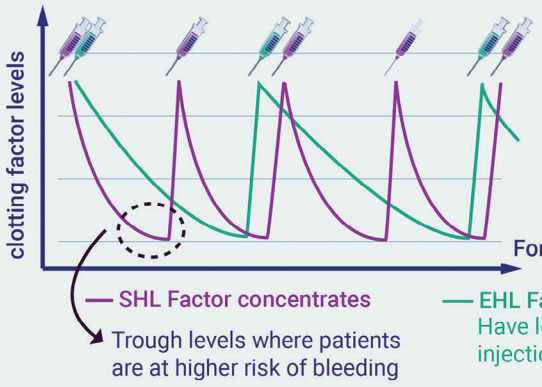
Treatment regimen

intravenous injections with factor concentrate

several times per month or week

for a lifetime

Prophylaxis aimed at preventing bleeds and subsequent arthropathy, is the current standard of care for patients with severe haemophilia[7,8]



For haemophilia A with and without inhibitors: emicizumab

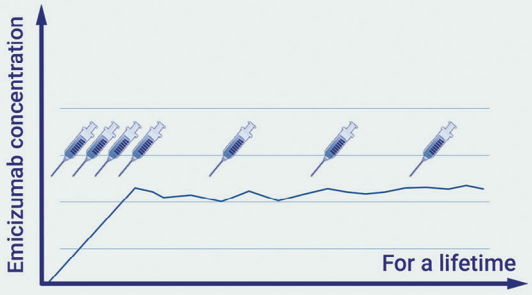
Treatment regimen

subcutaneous injections with emicizumab

up to several times per month

for a lifetime

Emicizumab is a bispecific monoclonal antibody that mimics FVIII[7,9]



The above treatments still need regular administrations and additional on-demand factor concentrates in case of breakthrough bleeds or surgery[10,11].

For haemophilia A and B without inhibitors: AAV-based gene therapy

With only one infusion, gene therapy may have advantages for the treatment of patients with haemophilia by producing stable factor VIII and IX activity levels, providing better bleeding protection and subsequent increased quality of life.

Approved gene therapy products for haemophilia

Haemophilia A: valoctocogene roxaparvovec ROCTAVIAN® (Biomarin) approved in US (Jun/23)[12], EU and UK (Aug/22)[13]

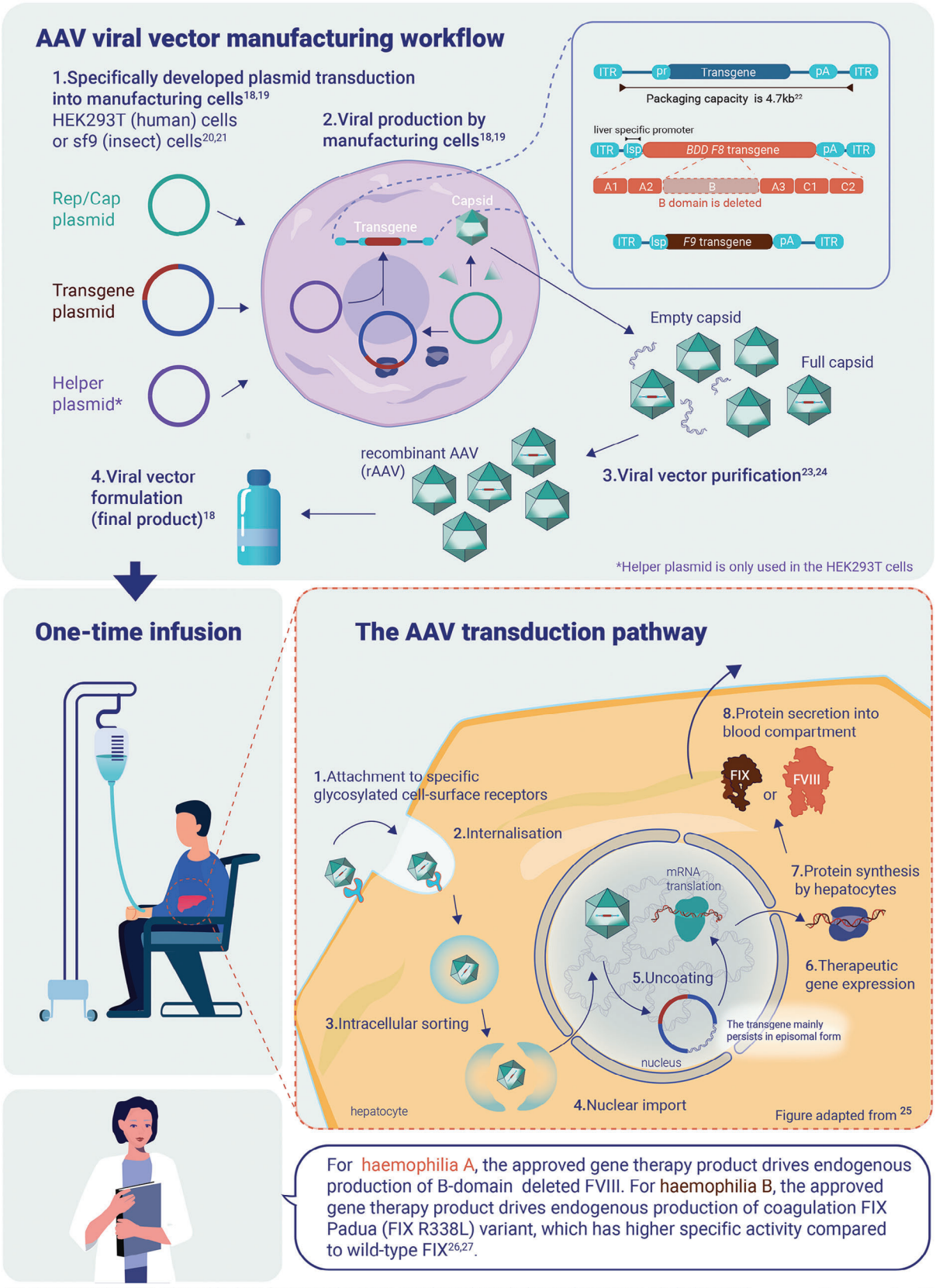
Haemophilia B: etranacogene dezaparvovec HEMGENIX® (CSL Behring) approved in US (Nov/22)[14], EU (Feb/23)[15], UK (Mar/23)[16], and Canada (Oct/23)[17]

Other gene therapies are in various stages of clinical development for haemophilia A and B.

*The graphs are for illustrative purposes only. The efficacy and the durability of gene therapy for haemophilia A and B are shown in Capsule 4 and Capsule 6, respectively.
AAV, adeno-associated virus; EC, European Commission; EHL, extended half-life; FDA, Food and Drug Administration; FVIII, factor VIII; FIX, factor IX; SHL, standard half-life

Capsule 2

Principles of gene therapy for haemophilia



For haemophilia A, the approved gene therapy product drives endogenous production of B-domain deleted FVIII. For haemophilia B, the approved gene therapy product drives endogenous production of coagulation FIX Padua (FIX R338L) variant, which has higher specific activity compared to wild-type FIX[26,27].

AAV, adeno-associated virus; BDD, B-domain deleted; FVIII, factor VIII; FIX, factor IX; ITR, inverted terminal repeat; lsp, liver specific promoter; pA, polyadenylation signal; pr, promoter

Capsule 3

Overview of the efficacy of the first approved gene therapies for haemophilia patients

Haemophilia A

Valoctocogene roxaparvovec

Phase III clinical trial: GENEr8-1[28,29]

Study design

134 adult patients with severe haemophilia A (FVIII <1 IU/dL) without FVIII inhibitors.

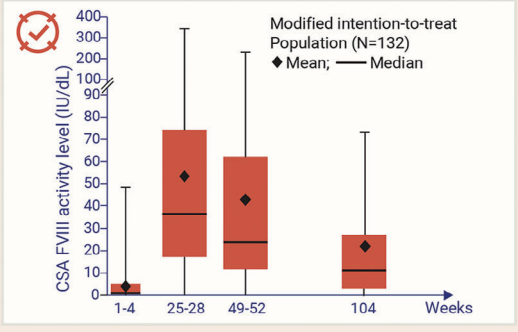
Excluded patients with pre-existing AAV5 NAbs.

Rollover population received FVIII prophylaxis for ≥6 months

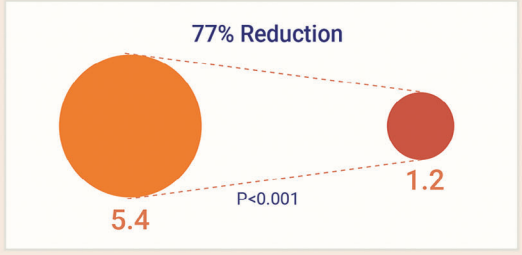
Single infusion AAV5-hFVIII-SQ ($6x10^{13}$ gc/kg).

Primary endpoint: change from baseline in **FVIII activity** after infusion.

FVIII and FIX levels

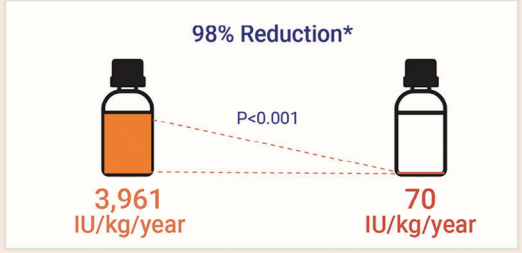


Mean ABR (all bleeds)



From the rollover population from the 270-902 study (n=112)

FVIII and FIX consumption



*From the rollover population from the 270-902 study (n=112)

6 patients (6/134) returned to prophylaxis

Haemophilia B

Etranacogene dezaparvovec

Phase III clinical trial: HOPE-B[30,31]

Study design

54 **adult patients** with **moderate or moderately severe haemophilia B** (FIX ≤2 IU/dL) without FIX inhibitors.

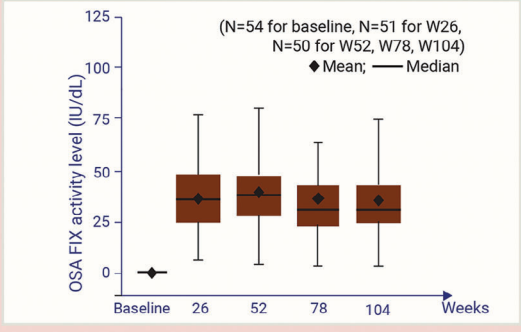
Included patients with pre-existing AAV5 NAbs.

Lead-in: FIX prophylaxis for ≥6 months.

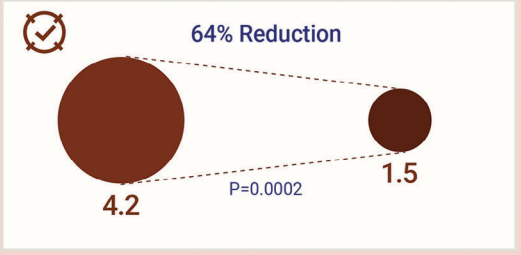
Single infusion AAV5-LP1-hFIXco Padua ($2x10^{13}$ gc/kg).

Primary endpoint: non-inferiority of **ABR** after infusion versus lead-in period.

FVIII and FIX levels

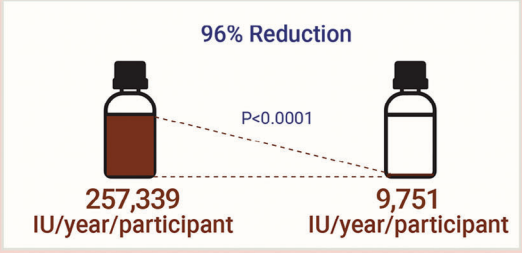


Mean ABR (all bleeds)



From the full analysis population (n=54)

FVIII and FIX consumption



0 patients (0/52) returned to prophylaxis. Two patients did not discontinue prophylactic treatment

AAV5, adeno-associated virus serotype 5; ABR, annualized bleeding rate; CSA, chromogenic substrate assay; gc, genome copies; NAbs, neutralizing antibodies; OSA, one-stage assay

Capsule 4

Short-term safety of the first approved gene therapies for haemophilia

Treatment related* serious AEs

Haemophilia A
Valoctocogene roxaparvovec

Phase III clinical trial: GENEr8-1[28,29]

3.7%

5 patients out of 134

Haemophilia B
Etranacogene dezaparvovec

Phase III clinical trial: HOPE-B[30,31]

0%

0 patients out of 54

Main serious AEs

There were no patients who developed inhibitors to FVIII or FIX.

1 salivary gland cancer
1 acute leukaemia
1 death

1 hepatocellular carcinoma
1 death

All malignancies (studied by molecular analysis) and deaths were considered not related to treatment.

Most common treatment related AEs

	Haemophilia A	Haemophilia B
	AST increase 39 pts — 29.1%	Headache 8 pts — 14.8%
	Nausea 31 pts — 23.1%	Influenza-like illness 7 pts — 13.0%
	ALT increase 108 pts — 80.6%	ALT increase 10 pts — 18.5%

ALT elevation may be associated with partial loss of transgene expression, which can be managed with transient use of immunosuppressive treatments.

Treatment related ALT elevation

79% of all patients received glucocorticoids (106 / 134)

Mean treatment duration of 234.5±116 days
(range 22 to 551)

17% of all patients received glucocorticoids (9 / 54)

Mean treatment duration of 81.4±28.6 days[15]
(range 51 to 130)

In both trials, no new safety signals developed up to 24-months.

Patients should be monitored before and after gene therapy administration[32,33].

*The determination of whether an event was related to the study drug was made by the investigator.
AE, adverse event; ALT, alanine transaminase; AST, aspartate aminotransferase; pts, patients

Capsule 5

Long-term challenges of gene therapy for haemophilia

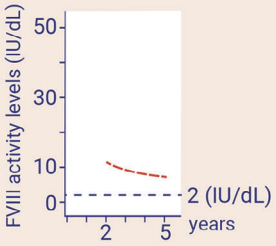
After administration of gene therapy, the patient will develop anti-AAV antibodies, which will preclude redosing[34].

Known and unknowns on the durability of the transgene expression

Haemophilia A
Valoctocogene roxaparvovec

Demonstrated FVIII transgene expression for up to 5 years with a decrease over time[35]

Estimated median FVIII levels extrapolated to 5 years* [28]



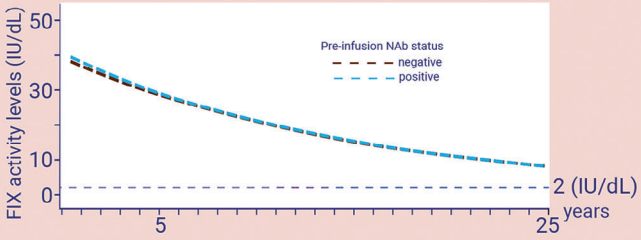
The prediction of durability may depend on the different models and time projections that are used.

*A linear mixed-effects approach was used to obtain estimates of FVIII activity half-life in order to extrapolate median FVIII activity levels beyond the 2-year period. Measurements were obtained with the use of chromogenic substrate assay.

Haemophilia B
Etranacogene dezaparvovec

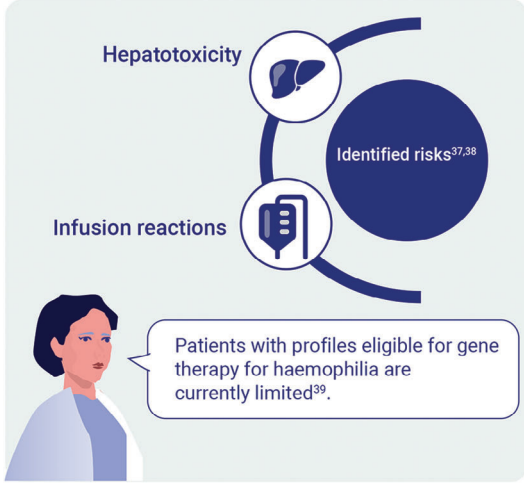
Demonstrated stable FIX transgene expression for 3 years[27]

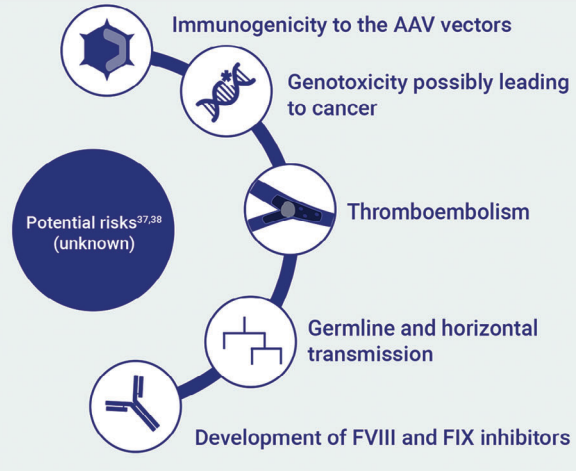
Estimated median FIX activity levels extrapolated to 25 years** [36]



** Post-analysis baseline with 95% prediction intervals. Bayesian approach with pre-infusion AAV5 NAb status as covariate (N=55).

Short-term and long-term follow-up of patients is needed to determine long term efficacy and safety of gene therapy for haemophilia.





AAV, adeno-associated virus; NAb, neutralizing antibody

Capsule 6

Models for implementation of gene therapy for haemophilia in clinical practice

Hub and spoke models[40,41]

The patient journey in gene therapy for haemophilia is complex and multifaceted. Care delivery models may vary between regions and/or countries, and over time.

Hub:
Administration of gene therapy and management centre

HTC and/or HCCC

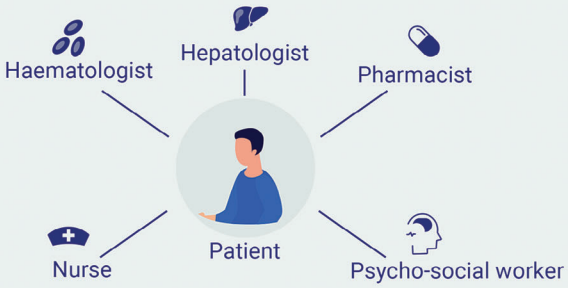
Close collaboration and communication between hub and spoke are required

HTC

Spoke:
Referral and management centre

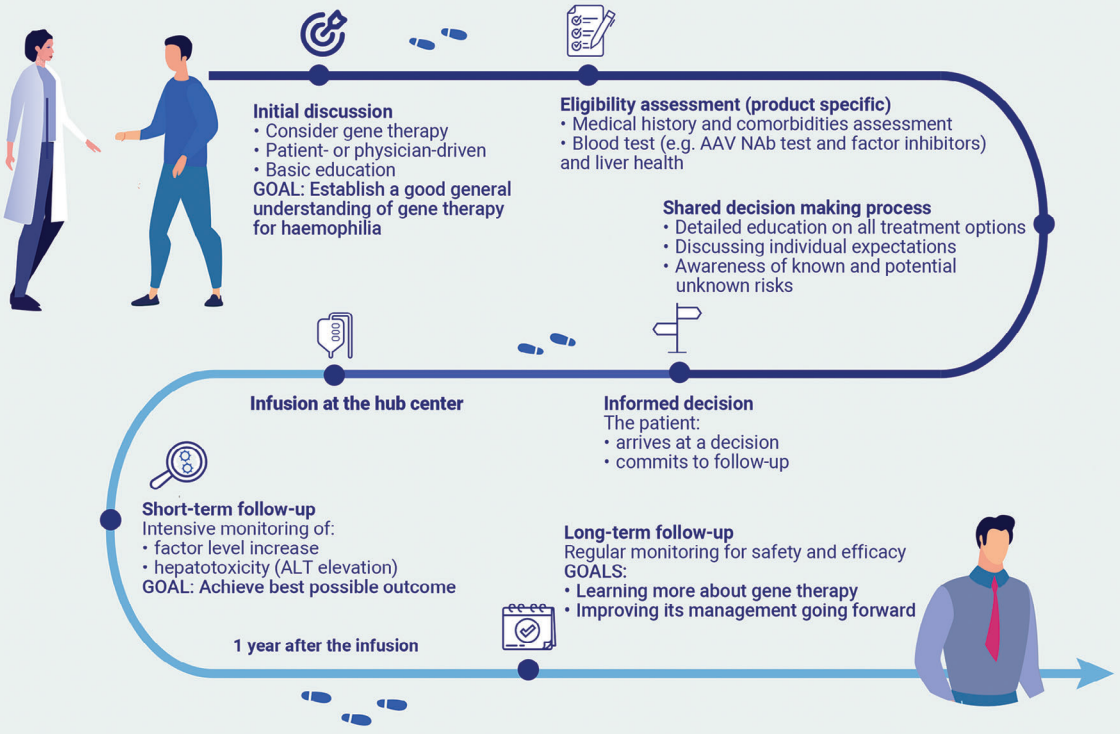
HTC

Multidisciplinary approach



Gaining access to gene therapy for haemophilia patients globally is a key issue, according to the World Federation of Hemophilia[39]. Implementing gene therapy for haemophilia depends on contextual factors and costs.

Patient journey for gene therapy[42,43]



AAV NAbs, adeno-associated virus neutralizing antibodies; ALT, alanine transferase; HCCC, haemophilia comprehensive care centre; HTC, haemophilia treatment centre.

Capsule 7

Summary of gene therapy for haemophilia A and B◊

◊The information displayed in this capsule serves as a general overview and does not provide specific data. Please refer to previous capsules for specifics and all numerical values.
ABR, annualized bleeding rate; ALT, alanine transferase

Capsule 8

AUTHOR CONTRIBUTIONS

All authors were involved in the preparation and the overall conceptualization of the review. Cihan Ay, Laurent Frenzel, and Sandra Le Quellec were also involved in the creation, visualization, and presentation of the published work. All authors validated and approved the published work.

ACKNOWLEDGMENTS

The authors thank Dr.ir. Tonke L. de Jong of COR2ED Medical Affairs, Bottmingen, Switzerland for providing scientific and editorial support, and Dr. Yuva Oz of Art 4 Science, Amsterdam, the Netherlands for providing creative support, which was funded by CSL Behring Europe, Hattersheim am Main, Germany, in accordance with Good Publication Practice (GPP3) guidelines (http://www.ismpp.org/gpp3).

CONFLICT OF INTEREST STATEMENT

Cihan Ay received personal fees for lectures and participation in advisory boards from Bayer, CSL Behring, Novo Nordisk, Pfizer, Roche, LFB, and SOBI. Laurent Frenzel received consultant fees from SOBI, Roche, and Pfizer. Karen Pinachyan and Sandra Le Quellec are full-time employees of CSL Behring.

DATA AVAILABILITY STATEMENT

Not applicable.

ETHICS APPROVAL STATEMENT

Not applicable.

PATIENT CONSENT STATEMENT

Not applicable.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

Not applicable.

CLINICAL TRIAL REGISTRATION

Not applicable.

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REFERENCES

1. Lenting PJ, van Mourik JA, Mertens K. The life cycle of coagulation factor VIII in view of its structure and function. *Blood*. 1998;92(11):3983-3996.
2. Yoshitake S, Schach BG, Foster DC, Davie EW, Kurachi K. Complete nucleotide sequences of the gene for human factor IX (antihemophilic factor B). *Biochemistry*. 1985;24(14):3736-3750.
3. Shahani T, Covens K, Lavend'homme R, et al. Human liver sinusoidal endothelial cells but not hepatocytes contain factor VIII. *J Thromb Haemost*. 2014;12(1):36-42.
4. Tatsumi K, Ohashi K, Mukobata S, et al. Hepatocyte is a sole cell type responsible for the production of coagulation factor IX in vivo. *Cell Med*. 2012;3(1-3):25-31.
5. Iorio A, Stonebraker JS, Chambost H, et al. Establishing the prevalence and prevalence at birth of hemophilia in males: a meta-analytic approach using national registries. *Ann Intern Med*. 2019;171(8):540-546.
6. World Federation of Haemophilia. Report on the annual global survey. Accessed August, 2023. Available at: https://www1.wfh.org/publications/files/pdf-2324.pdf
7. Srivastava A, Santagostino E, Dougall A, et al. WFH guidelines for the management of hemophilia, 3rd edition. *Haemophilia*. 2020;26(Suppl 6):1-158.
8. Pipe SW. The hope and reality of long-acting hemophilia products. *Am J Hematol*. 2012;87(Suppl 6):S33-S39.
9. Oldenburg J, Mahlangu JN, Kim B, et al. Emicizumab prophylaxis in hemophilia A with inhibitors. *N Engl J Med*. 2017;377(9):809-818.
10. Leebeek FWG, Miesbach W. Gene therapy for hemophilia: a review on clinical benefit, limitations, and remaining issues. *Blood*. 2021;138(11):923-931.
11. Castaman G, Di Minno G, De Cristofaro R, Peyvandi F. The arrival of gene therapy for patients with hemophilia A. *Int J Mol Sci*. 2022;23(18):10228.
12. FDA. Roctavian—valoctocogene roxaparvovec. 2023. Available from: https://www.fda.gov/news-events/press-announcements/fda-approves-first-gene-therapy-adults-severe-hemophilia
13. EMA. Roctavian—valoctocogene roxaparvovec. 2022. Accessed July, 2023. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/roctavian-0#assessment-history-section
14. FDA. Hemgenix—etranacogene dezaparvovec—Approval Letter. 2022. Accessed July, 2023. Available from: https://www.fda.gov/media/163466/download
15. EMA. Hemgenix—etranacogene dezaparvovec. 2023. Accessed July, 2023. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/hemgenix
16. MHRA. Hemgenix—etranacogene dezaparvovec. 2023. Available from: https://products.mhra.gov.uk/product/?product=HEMGENIX
17. Health Canada. Hemgenix—etranacogene dezaparvovec—Approval Letter. 2023. Available from: https://labeling.cslbehring.ca/PM/CA/hemgenix/EN/Hemgenix-Product-Monograph.pdf
18. Srivastava A, Mallela KMG, Deorkar N, Brophy G. Manufacturing challenges and rational formulation development for AAV viral vectors. *J Pharm Sci*. 2021;110(7):2609-2624.
19. Naso MF, Tomkowicz B, Perry WL 3rd, Strohl WR. Adeno-associated virus (AAV) as a vector for gene therapy. *BioDrugs*. 2017;31(4):317-334.
20. Merten O-W, Gény-Fiamma C, Douar AM. Current issues in adeno-associated viral vector production. *Gene Ther*. 2005;12(Suppl 1):S51-S61.
21. Merten O-W. AAV vector production: state of the art developments and remaining challenges. *Cell Gene Ther Insights*. 2016;2(5):521-551.
22. Batty P, Lillicrap D. Hemophilia gene therapy: approaching the first licensed product. *HemaSphere*. 2021;5(3):e540.
23. Ayuso E, Mingozzi F, Bosch F. Production, purification and characterization of adeno-associated vectors. *Curr Gene Ther*. 2010;10(6):423-436.
24. Adams B, Bak H, Tustian AD. Moving from the bench towards a large scale, industrial platform process for adeno-associated viral vector purification. *Biotechnol Bioeng*. 2020;117(10):3199-3211.
25. Ding W, Zhang L, Yan Z, Engelhardt JF. Intracellular trafficking of adeno-associated viral vectors. *Gene Ther*. 2005;12(11):873-880.
26. Simioni P, Tormene D, Tognin G, et al. X-linked thrombophilia with a mutant factor IX (factor IX Padua). *N Engl J Med*. 2009;361(17):1671-1675.
27. von Drygalski A, Gomez E, Giermasz A, et al. Stable and durable factor IX levels in patients with hemophilia B over 3 years after etranacogene dezaparvovec gene therapy. *Blood Adv*. 2023;10(7):5671-5679.

28. Ozelo MC, Mahlangu J, Pasi KJ, et al. Valoctocogene roxaparvovec gene therapy for hemophilia A. *N Engl J Med*. 2022;386(11):1013-1025.
29. Mahlangu J, Kaczmarek R, von Drygalski A, et al. Two-year outcomes of valoctocogene roxaparvovec therapy for hemophilia A. *N Engl J Med*. 2023;388(8):694-705.
30. Pipe SW, Leebeek FWG, Recht M, et al. Gene therapy with etranacogene dezaparvovec for hemophilia B. *N Engl J Med*. 2023;388(8):706-718.
31. Coppens M, Pipe SW, Miesbach W, et al. Adults with haemophilia B receiving etranacogene dezaparvovec in the HOPE-B phase 3 trial experience a stable increase in mean factor IX activity and durable haemostatic protection after 24 months' follow-up. 16th Annual Congress of European Association for Haemophilia and Allied Disorders 2023, 7–10 February 2023, Manchester (PO156).
32. EMA. roctavian, INN-valoctocogene roxaparvovec—Summary of Product Characteristics. Accessed July, 2023. Available at: https://www.ema.europa.eu/documents/product-information/roctavian-epar-product-information_en.pdf
33. EMA. Hemgenix, INN-etranacogene dezaparvovec—Summary of Product Characteristics. Accessed July, 2023. Available at: https://www.ema.europa.eu/documents/product-information/hemgenix-epar-product-information_en.pdf
34. Muhuri M, Levy DI, Schulz M, McCarty D, Gao G. Durability of transgene expression after rAAV gene therapy. *Mol Ther*. 2022;30(4):1364-1380.
35. Pasi KJ, Laffan M, Rangarajan S, et al. Persistence of haemostatic response following gene therapy with valoctocogene roxaparvovec in severe haemophilia A. *Haemophilia*. 2021;27(6):947-956.
36. Shah J, Kim H, Sivamurthy K, Monahan PE, Fries M. Comprehensive analysis and prediction of long-term durability of factor IX activity following etranacogene dezaparvovec gene therapy in the treatment of hemophilia B. *Curr Med Res Opin*. 2023;39(2):227-237.
37. EPAR. Summary of risk management plan for Roctavian (BMN 270; valoctocogene roxaparvovec). Accessed July, 2023. Available at: https://www.ema.europa.eu/en/documents/rmp-summary/roctavian-epar-risk-management-plan-summary_en.pdf
38. EPAR. EU risk management plan for Hemgenix (etranacogene dezaparvovec). Accessed July, 2023. Available at: https://www.ema.europa.eu/en/documents/rmp-summary/hemgenix-epar-risk-management-plan_en.pdf
39. Pierce GF, Coffin D, Members of the WFH Gene Therapy Round Table Program Committee and Organizing Committee. The 1st WFH gene therapy round table: understanding the landscape and challenges of gene therapy for haemophilia around the world. *Haemophilia*. 2019;25(2):189-194.
40. Miesbach W, Chowdary P, Coppens M, et al. Delivery of AAV-based gene therapy through haemophilia centres—A need for re-evaluation of infrastructure and comprehensive care: a Joint publication of EAHAD and EHC. *Haemophilia*. 2021;27(6):967-973.
41. Miesbach W, Baghaei F, Boban A, et al. Gene therapy of hemophilia: hub centres should be haemophilia centres: a joint publication of EAHAD and EHC. *Haemophilia*. 2022;28(3):e86-e88.
42. Noone D, Astermark J, O'Mahony B, et al. The journey of gene therapy in haemophilia—putting the patient at the centre of the hub and spoke model. *J Haem Pract*. 2022;9(1):156-166.
43. Le Quellec S, Breederveld D, Coppens M, Pinachyan K, The paradigm shift of gene therapy for haemophilia: impact on the patient journey. ESGCT 29th Annual Congress In collaboration with BSGCT Edinburgh, UK October 11–14, 2022 (P129).

How to cite this article: Ay C, Frenzel L, Pinachyan K, Le Quellec S. Gene therapy for haemophilia A and B, from basic principles to clinical implementation: An illustrated review. *Haemophilia*. 2023;1-11. https://doi.org/10.1111/hae.14907