Early 1970s
First plasma-derived factor VIII (FVIII) and factor IX (FIX) products available [1]

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From 2017
Late-stage trials for gene therapy in haemophilia underway [4]

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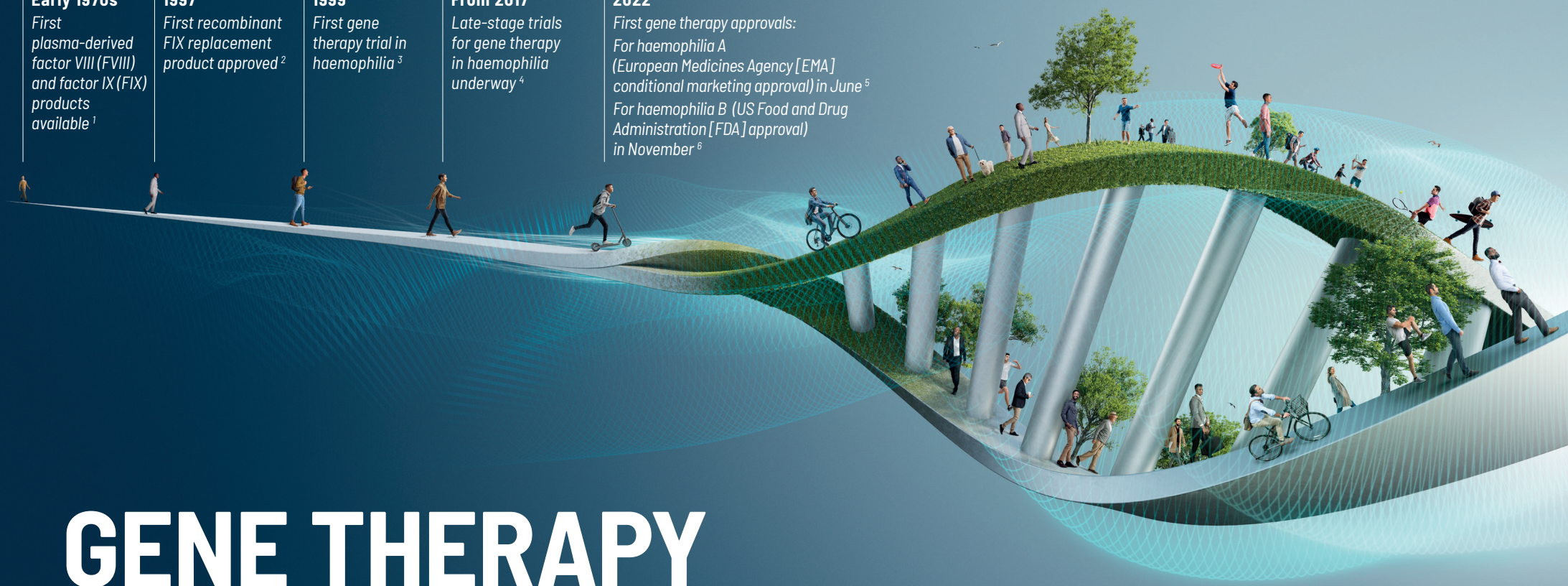


GENE THERAPY FOR HAEMOPHILIA

Date of Preparation: June 2023 GBR-HGX-0011

CSL Behring

Contents

Introduction to haemophilia

What is haemophilia?

Haemophilia is a rare, X-linked, congenital bleeding disorder. Patients with haemophilia lack either functional coagulation factor VIII (FVIII; haemophilia A) or factor IX (FIX; haemophilia B).[7]

FVIII and FIX are necessary for efficient blood coagulation, so a deficiency in these factors can lead to limb- or life-threatening bleeding events–both spontaneous events and events following surgery or trauma.[7,8]

Cause and prevalence of haemophilia A and B[7]

Haemophilia A		Haemophilia B	
FVIII deficiency		**FIX deficiency**	
Caused by inherited or spontaneous mutations in the *F8* clotting factor gene		Caused by inherited or spontaneous mutations in the *F9* clotting factor gene	
80-85%	**24.6**	**15-20%**	**5.0**
of all haemophilia cases	cases per 100,000 males	of all haemophilia cases	cases per 100,000 males

An estimated 1,125,000 males have haemophilia worldwide; of these, 418,000 have the severe form of the disease.[9]

Defining haemophilia severity

The severity of bleeding episodes in haemophilia is related to the degree of clotting factor deficiency in the patient.[7] Patients with mild haemophilia may not display abnormal or prolonged bleeding without major trauma or surgery, while patients with severe haemophilia frequently experience spontaneous bleeding episodes.

Bleeding severity is related to clotting factor level[7]

Severe

- **Clotting factor level <1% of normal**
- Spontaneous bleeding into joints and muscles
- Predominantly with no apparent haemostatic challenge

Moderate

- **Clotting factor level 1 to <5% of normal**
- Occasional spontaneous bleeding
- Prolonged bleeding with minor surgery or trauma

Mild

- **Clotting factor level 5 to <40% of normal**
- Spontaneous bleeding is rare
- Prolonged bleeding with major surgery or trauma

Bleeding in haemophilia

Bleeding can occur in multiple sites in haemophilia patients. Bleeds can cause pain, loss of mobility, or even have fatal consequences, depending on the severity, frequency, and organ system(s) affected.[7] Joint bleeds are a serious complication of haemophilia, and even a single joint bleed can cause irreversible damage.[10]

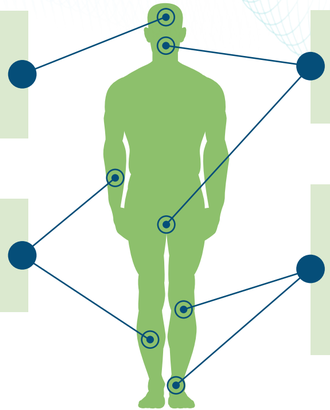
Frequency and characteristics of bleeds at different sites in haemophilia[7,11]

Central nervous system bleeds (<5%)

- Least common but life-threatening
- Include intracranial haemorrhage

Muscle bleeds (10-20%)

- May occur in muscles (in calf, forearm, etc.)



Other major bleeds (5-10%)

- Serious bleeds that occur in other sites (e.g., mouth, nose, genitourinary tract)

Joint bleeds (70-80%)

- Occur most commonly in ankles, knees, and elbows
- May lead to disability and negatively impacts quality of life

Scientific advances in haemophilia

The current standard of care in haemophilia

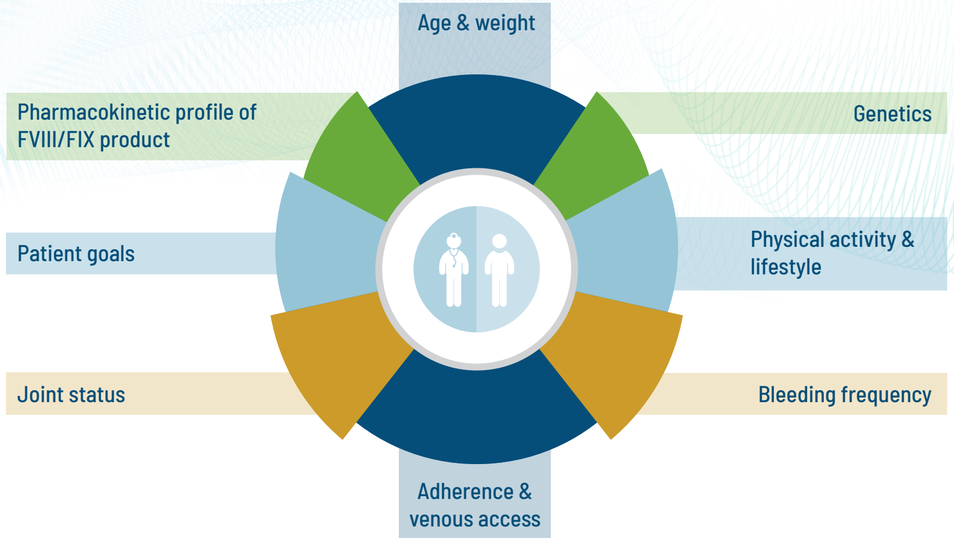
Prophylaxis with factor replacement products is the current standard of care for patients with severe haemophilia. The aim of prophylaxis is to maintain high FVIII/FIX trough levels in order to avoid breakthrough bleeds.[7]

Many patients with severe haemophilia are satisfactorily managed with prophylactic treatment, and have better clinical outcomes than patients who are not on prophylaxis.[12]

Benefits of regular prophylaxis compared to intermittent episodic treatment[13,14]

Bleeding	Joint damage	Disability	Need for orthopaedic surgery	Quality of life
↓	↓	↓	↓	↑

Treatment for patients with severe haemophilia should be individualised based on:[7,15–19]



Research into optimising prophylactic treatment is continuing.

Scientific advances in gene therapy

50+ years of clinical research[20]

2,500+ active clinical trials in genetic therapy research[21]

Two AAV vector-based gene therapies have been approved by the FDA and EMA[22,23]

Abbreviations: AAV=adeno-associated virus; EMA=European Medicines Agency; FDA=Food and Drug Administration.

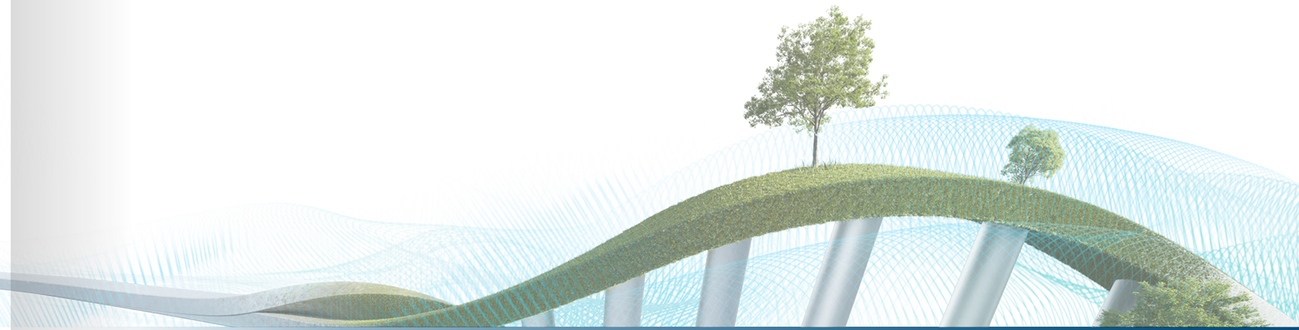
Gene therapy basics

Gene therapy is a technique using genetic material to treat or cure a disease.[24]

There are two methods for gene therapy delivery: *in vivo* gene transfer and *ex vivo* gene transfer.

Functional genes are usually delivered into the cells of the body by inserting them into an inactivated viral shell (the vector), which carries the gene to specific target cells. The gene can be delivered directly to the person (*in vivo* gene therapy) or into cells that have been taken from a patient, then treated and returned to the patient (*ex vivo* gene therapy).[25]

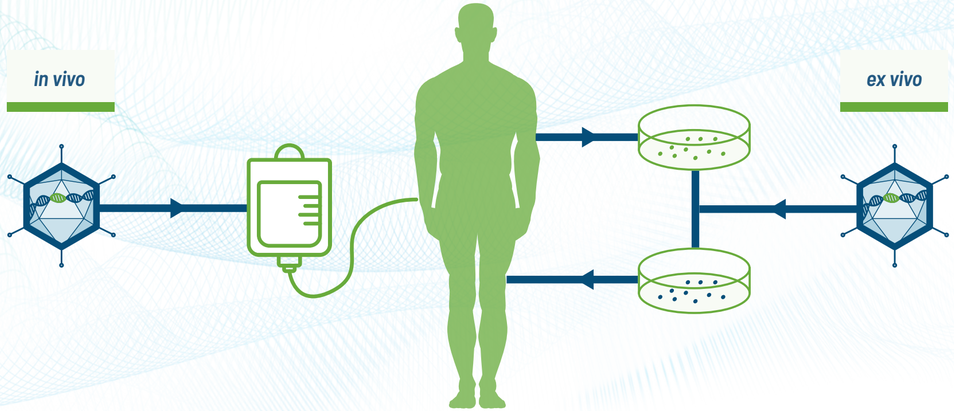
Once inside the target cells, there are different approaches for gene therapy: gene addition and gene editing. Gene addition works by adding the functional gene into the nucleus of target cells. Gene editing refers to genetic engineering in which DNA is inserted, deleted, or modified at a specific location in the genome of a living organism.[24]



Different types of vectors

Many gene therapy technologies use a viral vector as a transfer vehicle for the gene of interest, and multiple types of vectors are available, including adeno-associated virus (AAV) vectors, adenoviruses, lentiviruses, and retroviruses.[21]

Recent regulatory approvals of gene therapy products include both *in vivo* AAV-based and *ex vivo* retroviral-based gene therapies for inherited monogenic diseases (those that only affect a single gene).[22,23,26]



The unmet needs in haemophilia

Patients with haemophilia currently have access to a large variety of treatments, including factor replacement products and non-factor replacement therapies.[7,26] Research is continuing to develop additional options.[4]

Despite the advances in haemophilia treatment, the standard of care today requires regular infusions of coagulation factors or non-factor replacement products, which may create a high treatment burden for many patients.[7]

Current unmet needs of patients with haemophilia

Maintaining factor levels

Current treatments with factor replacement products may result in fluctuating factor levels. Falling below minimum levels can increase the possibility of breakthrough bleeds.[7,28]

Patient adherence to treatment

The need for regular infusions can create a high treatment burden for some patients, which ultimately impacts on their quality of life.[29-31]

Joint health

Repeated bleeds into the joints and muscles can cause chronic pain and disability. This can have a negative impact on patients' quality of life.[32,33]

Inhibitor development

The development of inhibitors is a major complication of factor replacement products and is associated with significant morbidity and emotional strain.[34,35]

Psychosocial burden

Patients with haemophilia deal with an array of challenges and emotions related to their condition (e.g., shame, fear, and anxiety). The need for repeated infusions can also place a burden on family and caregivers, and can negatively impact on formal education and employment.[32,36]

Haemophilia as a target for gene therapy

What makes haemophilia a potential target for a gene therapy approach?

Haemophilia is a monogenic disease, meaning it only affects a single gene

- This makes it a good target for gene therapy in order to provide a functional copy of the F8 or F9 gene[37]

Haemophilia is well suited for correction by gene therapy because:

- Bleeding phenotype is responsive to a wide range of factor levels
- Precise regulation of factor levels is not necessary[37]

The efficacy of gene therapy treatment for haemophilia can be readily assessed via measurement of circulating factor levels and quantifiable endpoints[38]

Haemophilia A and haemophilia B are suitable targets for gene therapy

Both haemophilia A and haemophilia B have the aforementioned characteristics, making both diseases suitable targets for gene therapy. There are also some differences in the practical approaches taken for gene therapy in haemophilia A and haemophilia B.

The packaging capacity of the AAV vector, defined by the original size of the AAV vector genome, is approximately 4.7 kilo-bases (kb)–therefore, only small genes can be easily incorporated into the vector. The *F9* gene is only 2.6 kb, which is easier to use compared to the 7-kb *F8* gene (even after the removal of the *F8* B domain [2.6 kb], which is not needed for coagulation function).[39]

In addition, the discovery of a FIX variant (FIX-Padua), which yields an approximately 8-fold increase in activity compared to wild-type FIX, may allow achievement of FIX activity levels with lower vector doses.[39]

The outcome of AAV-mediated gene therapy is the expression of transgenic FVIII or FIX in hepatocytes. The FIX protein is naturally produced by hepatocytes, whereas the FVIII protein is mostly naturally produced by liver sinusoidal endothelial cells.[37]

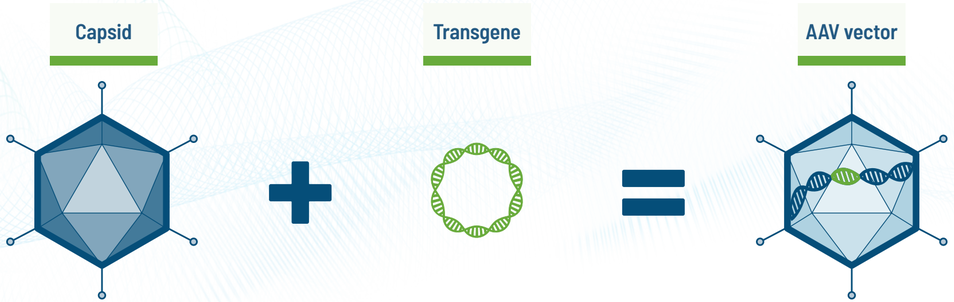
AAV-based gene therapy

What is AAV-based gene therapy?

Adeno-associated virus (AAV) vectors are versatile viral vectors that can be engineered to deliver therapeutic genes to specific tissues and cells.[40]

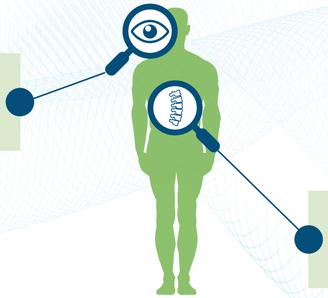
AAV-based gene therapy consists of a capsid (vector) encasing a therapeutic gene (transgene).[40]

Administered in a single dose, AAV-based gene therapy enables the delivery of the transgene inside the target cell, and the cell uses the healthy gene to produce the therapeutic protein to improve or correct the disorder.[40]



Approved indications

AAV-based gene therapy was approved for the treatment of retinal dystrophy in 2017.[41]



AAV-based gene therapy was approved for the treatment of spinal muscular atrophy in 2019.[42]

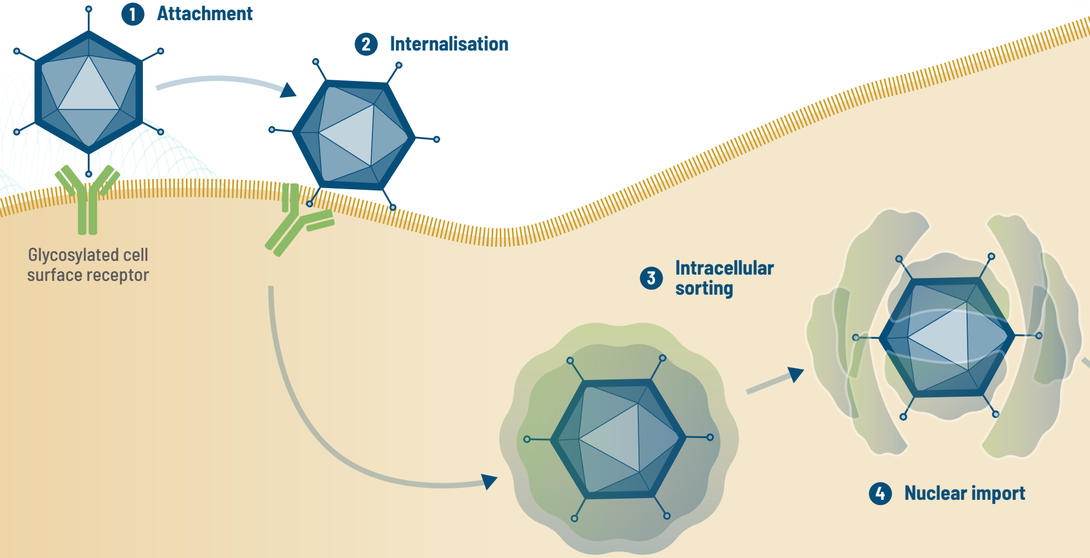
Considerations with AAV vectors

There are many different types of AAV vectors that target specific areas of the body. Some AAV vectors are more precise, while others target a wider, less specific range of cells and tissues.[43]

AAV serotypes can be bioengineered to increase transduction into the target cell.[43]

Pre-existing antibodies to certain naturally occurring AAV serotypes may influence eligibility in clinical trials and the success of certain AAV-based gene therapies.

AAV vector transduction pathway[44,45]



Transduction of the AAV vector is a multistep process:[39,45]

1. **Attachment** to host cell via targeted glycan receptors
2. **Internalisation** via receptor-mediated endocytosis
3. **Intracellular sorting** occurring in endosomes and the Golgi
4. **Nuclear import** through cytoplasmic escape from the endosome and entry into the nucleus
5. **Uncoating** of the vector and release of the transgene
6. **Therapeutic gene expression** through transcription of the non-integrating DNA
7. Protein synthesis
8. Protein secretion

Gene therapy in haemophilia

The goals of gene therapy in haemophilia are to:

Provide long-term benefits with sustained factor activity levels from a single administration of treatment[46]

Reduce or even eliminate spontaneous bleeding and the need for lifelong regular infusions[46]

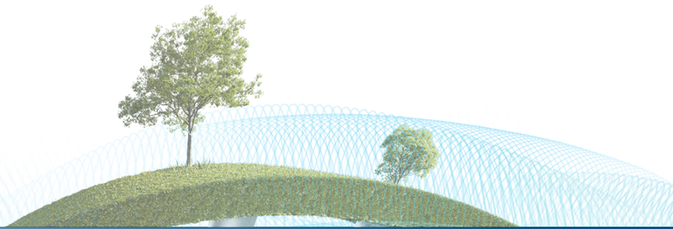
Current status of gene therapy in haemophilia

Various late-stage clinical trials are currently underway, investigating the ongoing efficacy and safety of AAV gene therapies in haemophilia A and haemophilia B.[4]

Potential challenges and remaining issues in haemophilia gene therapy

Despite progress in the development of gene therapy, there are a number of issues that need to be addressed, including:

- **Pre-existing immunity to AAV may limit eligibility for haemophilia gene therapy treatment.[27]** Most clinical trials have excluded patients with pre-existing immunity to the corresponding type of AAV vector used in the trial because the presence of pre-existing AAV antibodies can impair the delivery of AAV vectors.[27] However, in one trial, patients with pre-existing AAV antibodies were not excluded and successful transgene expression was accomplished in the majority of patients with pre-existing anti-AAV antibodies[27,47]
- **AAV vector re-administration is not currently considered possible, and AAV-mediated gene transfer is often viewed as a 'one chance only' therapeutic opportunity.[27]** This is because following administration of AAV vectors, a very robust and long-lived anti-AAV immune response is observed.[27] This immune response is likely to neutralise any AAV vectors that are then re-administered[27]
- **Early transient liver toxicity has been observed in clinical trials, and is marked by mild to moderate increases in transaminase levels.[27,48]** The reasons behind the development of liver toxicity remain unclear; however, some potential mechanisms have been identified and remain targets for ongoing research[27,48]

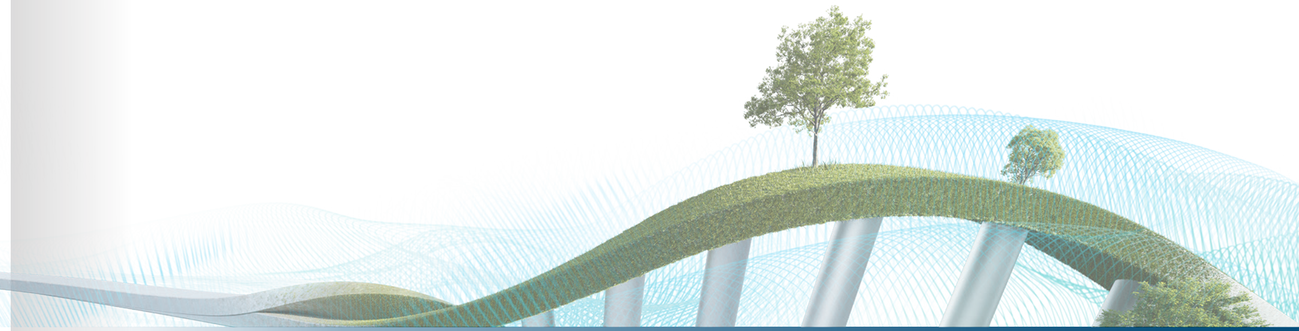


- **Predicting the durability and level of transgene expression in individual patients is very important**[27,49]
  - In terms of **durability of transgene expression**, there are differences seen in clinical studies in haemophilia A and B.[27,49] In haemophilia A, a decline in FVIII levels is seen in some patients over the first 4 years.[27] In haemophilia B, the decline in FIX levels is minimal up to 8 years post administration[49]
  - Regarding the **predictability of clotting factor levels**, currently there is almost no information available to predict individual plasma levels of transgenic protein expression in patients, and significant variability in these levels has been observed in human trials[27]
- **Long-term follow-up of gene therapy patients will be important to determine whether rare, unexpected adverse events will occur.**[27] A theoretical safety advantage of AAV vector delivery is the absence of routine integration of vector sequences into the host genome, thus reducing the risk of long-term insertional oncogenicity.[27] Minimal integration in animal and human studies has been observed, occurring with a frequency of between ~1 per 1,000 and 10,000 cells.[27] However, whether AAV gene transfer is associated with an enhanced genotoxic risk for oncogenicity remains unknown[27]
- **The potential of haemophilia gene therapy in children is unknown.**[27] All haemophilia gene therapy trials to date have only included previously treated adult patients.[27] However, in other diseases, such as spinal muscular atrophy, AAV-mediated gene transfer is being used in very young children[27]
- **The potential of haemophilia gene therapy in patients with inhibitors is also unknown.** To date, patients with current and past histories of FVIII and FIX inhibitors have been excluded from clinical trials[27]

Collaborative patient–physician interaction and a shared decision-making process is key to managing patients' expectations and navigating different treatment options[24,27]

How to get more information

For more information about the advancing science behind gene therapy,
speak to your Regional Business Manager or contact: medinfo@cslbehring.com



References

1. Morfini M. The History of Clotting Factor Concentrates Pharmacokinetics. *J Clin Med*. 2017;6(3):35.

2. BeneFIX Prescribing Information. Accessed April 2023. www.fda.gov/media/73556/download

3. Kay MA *et al*. 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.

4. www.clinicaltrials.gov (NCT03370913, NCT03392974, NCT03587116, NCT03876301, NCT03569891) (Accessed April 2023).

5. European Medicines Agency press release 24/06/2022. First gene therapy to treat severe haemophilia A. Accessed May 2023. https://www.ema.europa.eu/en/news/first-gene-therapy-treat-severe-haemophilia

6. US Food and Drug Administration press release 22/11/2022. FDA Approves First Gene Therapy to Treat Adults with Hemophilia B. Accessed May 2023. https://www.fda.gov/news-events/press-announcements/fda-approves-first-gene-therapy-treat-adults-hemophilia-b

7. Srivastava A *et al*. WFH Guidelines for the Management of Hemophilia, 3rd edition. *Haemophilia*. 2020;26(Suppl 6):1–158.

8. Bowen DJ. Haemophilia A and haemophilia B: molecular insights. *Mol Pathol*. 2002;55(2):127–144.

9. Iorio A *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.

10. Oldenburg J. Optimal treatment strategies for hemophilia: achievements and limitations of current prophylactic regimens. *Blood*. 2015;125(13):2038–2044.

11. O'Hara J *et al*. The impact of severe haemophilia and the presence of target joints on health-related quality-of-life. *Health Qual Life Outcomes*. 2018;16(1):84.

12. Manco-Johnson MJ *et al*. Randomized, controlled, parallel-group trial of routine prophylaxis vs. on-demand treatment with sucrose-formulated recombinant factor VIII in adults with severe hemophilia A (SPINART). *J Throm Haem*. 2013;11(6):1119-27.

13. Batty P, Lillicrap D. Advances and challenges for hemophilia gene therapy. *Hum Mol Genet*. 2019;28(R1):R95–R101.

14. Miesbach W *et al*. Long-term analysis of the benefit of prophylaxis for adult patients with severe or moderate haemophilia A. *Haemophilia*. 2020;26:467-477.

15. Thornburg CD, Duncan NA. Treatment adherence in hemophilia. *Patient Prefer Adherence*. 2017;11:1677–1686.

16. Bachelet D *et al*. Risk stratification integrating genetic data for factor VIII inhibitor development in patients with severe hemophilia A. *PLoS ONE*. 2019;14(6): e0218258.

17. Dargaud Y *et al*. Individualized PK-based prophylaxis in severe haemophilia. *J World Fed of Hem*. 2018;24(52):3-17.

18. Poon MC, Lee A. Individualized prophylaxis for optimizing hemophilia care: can we apply this to both developed and developing nations? *Thromb J*. 2016;14(Suppl 1):32.

19. Yu JK et al. Using pharmacokinetics for tailoring prophylaxis in people with hemophilia switching between clotting factor products: A scoping review. *Res Pract Thromb Haemost*. 2019;3(3):528–541.

20. Friedmann T, Roblin R. Gene therapy for human genetic disease? *Science*. 1972;175(4025):949–955.

21. Anguela XM, High KA. Entering the Modern Era of Gene Therapy. *Annu Rev Med*. 2019;70:273–288.

22. Luxturna Summary of Product Characteristics. Accessed April 2023. https://www.ema.europa.eu/en/documents/product-information/luxturna-epar-product-information_en.pdf

23. Zolgensma Summary of Product Characteristics. Accessed April 2023. https://www.ema.europa.eu/en/documents/product-information/zolgensma-epar-product-information_en.pdf

24. Miesbach W *et al*. How to discuss gene therapy for haemophilia? A patient and physician perspective. *Haemophilia*. 2019;25(4):545-557.

25. Prakash V *et al*. Current Progress in Therapeutic Gene Editing for Monogenic Diseases. *Mol Ther*. 2016;24(3):465–474.

26. Tecartus Summary of Product Characteristics. Accessed June 2023. https://www.medicines.org.uk/emc/product/11987/smpc#gref

27. Batty P, Lillicrap D. Hemophilia Gene Therapy: Approaching the First Licensed Product. *Hemasphere*. 2021;5(3):e540.

28. Shapiro A *et al*. Association Of Bleeding Tendency With Time Under Target FIX Activity Levels In Severe Hemophilia B Patients Treated With Recombinant Factor IX Fc Fusion Protein. *Blood*. 2013;122(21):2349.

29. Vasquez-Loarte TC *et al*. Beliefs and Values About Gene Therapy and In-Utero Gene Editing in Patients with Hemophilia and Their Relatives. *Patient*. 2020;13(5):633–642.

30. Witkop M *et al*. Treatment outcomes, quality of life, and impact of hemophilia on young adults (aged 18–30 years) with hemophilia. *Am J Hematol*. 2015;90(Suppl 2):S3–S10.

31. Schrijvers LH *et al*. Adherence to prophylaxis and bleeding outcome in haemophilia: a multicentre study. *Br J Haematol*. 2016;174(3):454–460.

32. Curtis R *et al*. Young adults with hemophilia in the U.S.: demographics, comorbidities, and health status. *Am J Hematol*. 2015;90(Suppl 2):S11–S16.

33. Forsyth AL *et al*. Associations of quality of life, pain, and self-reported arthritis with age, employment, bleed rate, and utilization of hemophilia treatment center and health care provider services: results in adults with hemophilia in the HERO study. *Patient Prefer Adherence*. 2015;9:1549–1560.

34. Castaman G, Matino D. Hemophilia A and B: molecular and clinical similarities and differences. *Haematologica*. 2019;104(9):1702–1709.

35. duTreil S. Physical and psychosocial challenges in adult hemophilia patients with inhibitors. *J Blood Med*. 2014;5:115–122.

36. Palareti L *et al*. Shared topics on the experience of people with haemophilia living in the UK and the USA and the influence of individual and contextual variables: Results from the HERO qualitative study. *Int J Qual Stud Health Well-being*. 2015;10:28915.

37. Perrin GQ *et al*. Update on clinical gene therapy for hemophilia. *Blood*. 2019;133(5):407–414.

38. Arruda VR, Doshi BS. Gene Therapy for Hemophilia: Facts and Quandaries in the 21st Century. *Mediterr J Hematol Infect Dis*. 2020;12(1):e2020069.

39. Doshi BS, Arruda VR. Gene therapy for hemophilia: what does the future hold? *Ther Adv Hematol*. 2018;9(9):273–293.

40. Mitchell AM *et al*. AAV's anatomy: roadmap for optimizing vectors for translational success. *Curr Gene Ther*. 2010;10(5):319–340.

41. Luxturna FDA press release 18/12/2017. FDA approves novel gene therapy to treat patients with a rare form of inherited vision loss. Accessed June 2023. https://www.fda.gov/news-events/press-announcements/fda-approves-novel-gene-therapy-treat-patients-rare-form-inherited-vision-loss

42. High KA, Roncarolo MG. Gene Therapy. *N Engl J Med*. 2019;381(5):455–464.

43. Pipe S *et al*. Clinical Considerations for Capsid Choice in the Development of Liver-Targeted AAV-Based Gene Transfer. *Mol Ther Methods Clin Dev*. 2019;15:170–178.

44. Wang D *et al*. Gao G. Adeno-associated virus vector as a platform for gene therapy delivery. *Nat Rev Drug Discov*. 2019;18(5):358–378.

45. Berry G *et al*. Cellular transduction mechanisms of adeno-associated viral vectors. *Curr Opin Virol*. 2016;21:54-60.

46. Castaman G *et al*. The Arrival of Gene Therapy for Patients with Hemophilia A. *Intl J Mol Sci*. 2022;23(18):10228.

47. Majowicz A *et al*. Therapeutic hFIX Activity Achieved after Single AAV5-hFIX Treatment in Hemophilia B Patients and NHPs with Pre-existing Anti-AAV5 NABs. *Mol Ther Methods Clin Dev*. 2019;14:27–36.

48. Leebeek FWG, Miesbach W. Gene therapy for hemophilia: a review on clinical benefit, limitations, and remaining issues. *Blood*. 2021;138(11):923–931.

49. Nathwani AC *et al*. Adeno-associated mediated gene transfer for hemophilia B: 8 year follow up and impact of removing 'empty viral particles' on safety and efficacy of gene transfer. *Blood*. 2018;132 (Suppl 1):491.

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