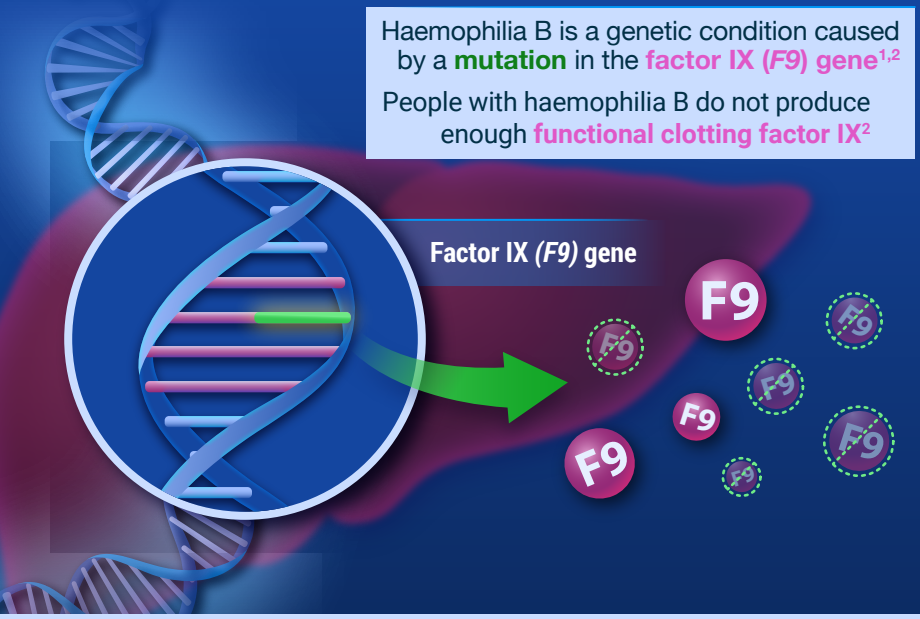
Investigational CRISPR-Based Targeted Gene Insertion for Haemophilia B

For Study Investigator Use Only

CRISPR-based targeted gene insertion for haemophilia B is investigational and the efficacy and safety has not been evaluated by any regulatory authority

Haemophilia B is a genetic condition caused by a **mutation** in the **factor IX (*F9*) gene**[1,2]

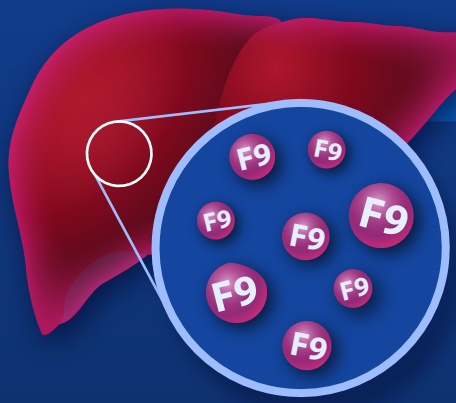
People with haemophilia B do not produce enough **functional clotting factor IX**[2]



Gene insertion aims to permanently restore the body's ability to independently produce factor IX without the need for routine factor replacement therapy[3]

What is Gene Insertion?

Gene insertion is a type of gene editing where a therapeutic gene can be precisely added to specific sections of DNA to restore the body's ability to work as it should[3]

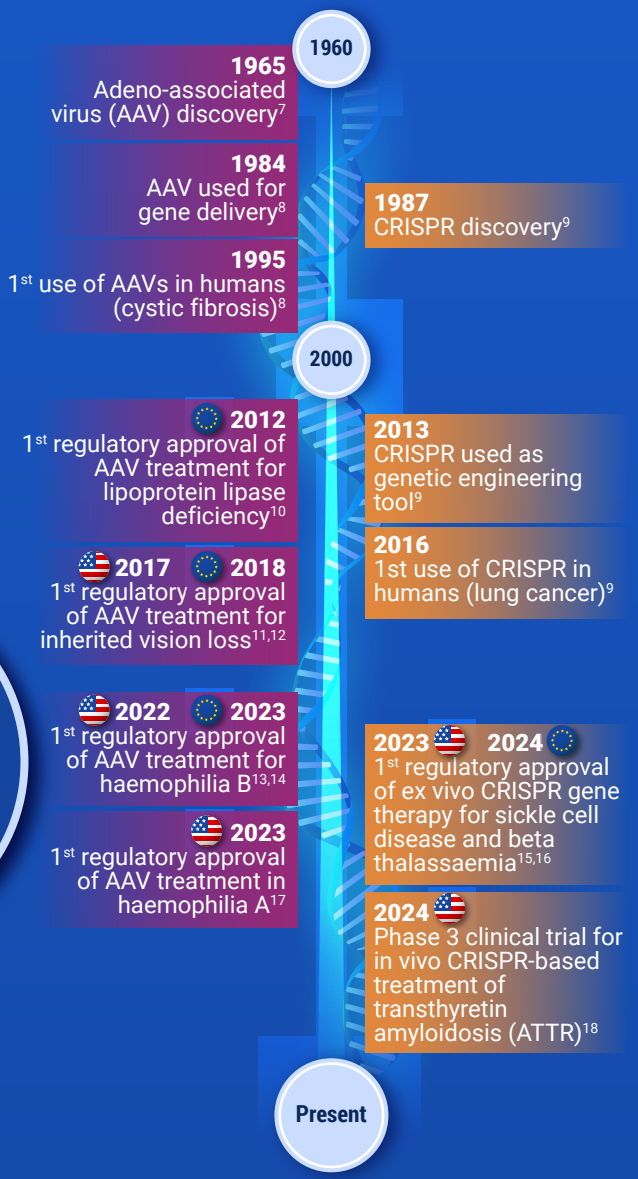


In vivo **CRISPR-based targeted gene insertion** is a type of gene editing being investigated in Haemophilia B. By adding a **therapeutic F9 gene**, the body can make **functional clotting factor IX** on its own[3-5]

In other words...

Genes are like the body's instruction manual. Sometimes, typos in the manual ("mutations") cause the body to miss one of the instructions it needs to produce functional product, such as factor IX in patients with haemophilia B. The goal of gene editing is to carefully correct those typos or insert the right instructions into the manual, so the body can function as it should, and factor IX can be produced[6]

Technological advances leading up to in vivo CRISPR-based targeted gene insertion for haemophilia B investigational studies



Investigational *In vivo* CRISPR-based targeted gene insertion for haemophilia B

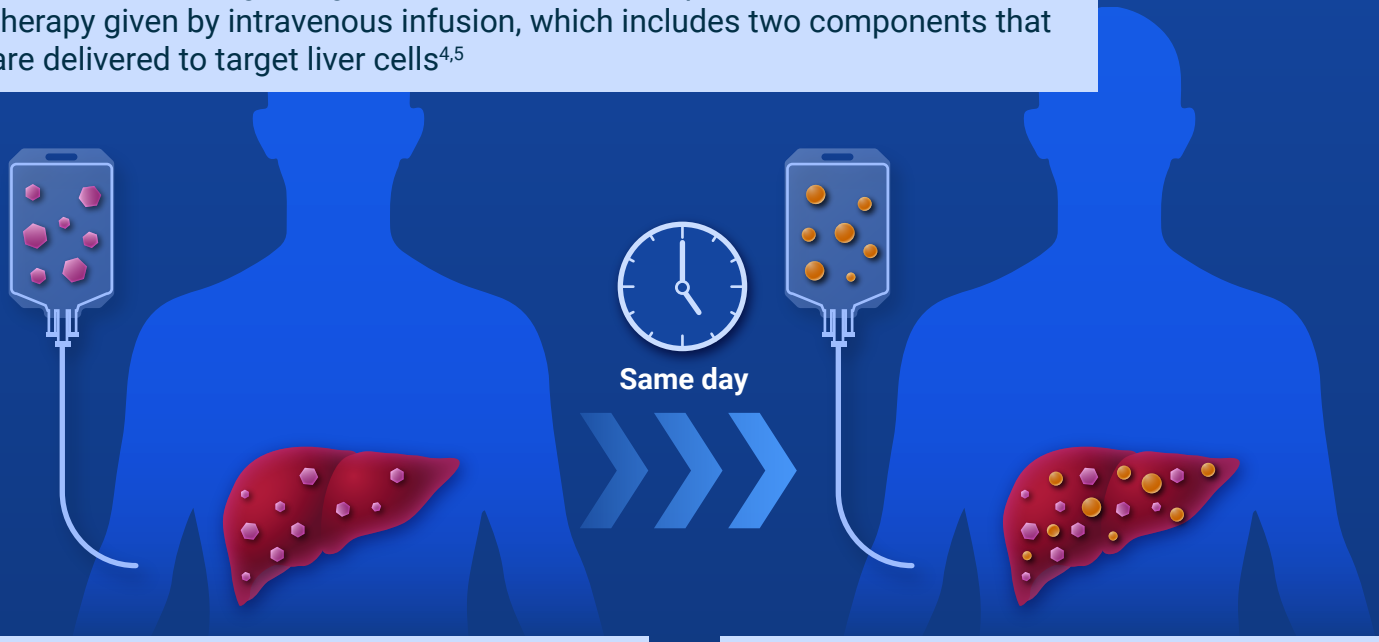
Deeper dive

CRISPR is a versatile tool. CRISPR-based targeted gene insertion for haemophilia B is an investigational in vivo therapy, which means gene insertion takes place in liver cells inside the body. With ex vivo gene therapies, patients' cells are removed and modified outside in a lab before they are reintroduced back to the body[4,5,19,20]

Investigational CRISPR-Based Targeted Gene Insertion is a Two-Step Process:[4,5]

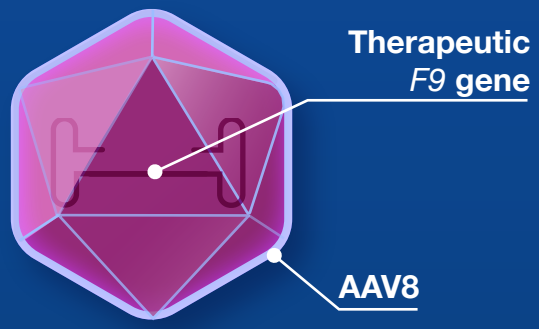
Step 1: Delivery of Therapeutic *F9* Gene and CRISPR

CRISPR-based targeted gene insertion for haemophilia B is an in vivo therapy given by intravenous infusion, which includes two components that are delivered to target liver cells[4,5]



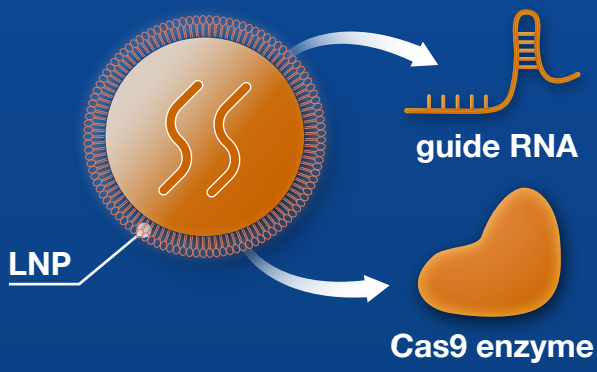
First, the investigational **therapeutic *F9* gene** is delivered to liver cells to provide instructions for making **clotting factor IX**[4,5,8,22]

Then, **a lipid nanoparticle (LNP)** delivers **CRISPR**, a precise tool that is designed to enable targeted gene insertion at a specific location in the DNA[4,5,9,25]



The **therapeutic *F9* gene** is delivered to target liver cells as a transgene using a viral vector known as **AAV serotype 8 (AAV8)**[4,5,8,21,23]

AAVs have been modified, removing and replacing their original genetic material with the **therapeutic *F9* gene**[8,22,23]



CRISPR technology has two parts which work together for gene insertion: a **guide RNA** strand and a **Cas9 enzyme**[3,9,26]

Specially designed **LNPs** are delivery vehicles that carry CRISPR technology and are preferentially taken up by liver cells[24]

Deeper dive

A transgene is a DNA sequence or combination of sequences that can produce functional protein[24]

- *In CRISPR-based targeted gene insertion, the transgene, which consists only of the F9 gene and does not carry a promoter, is integrated into the patient's genome*[4,5]

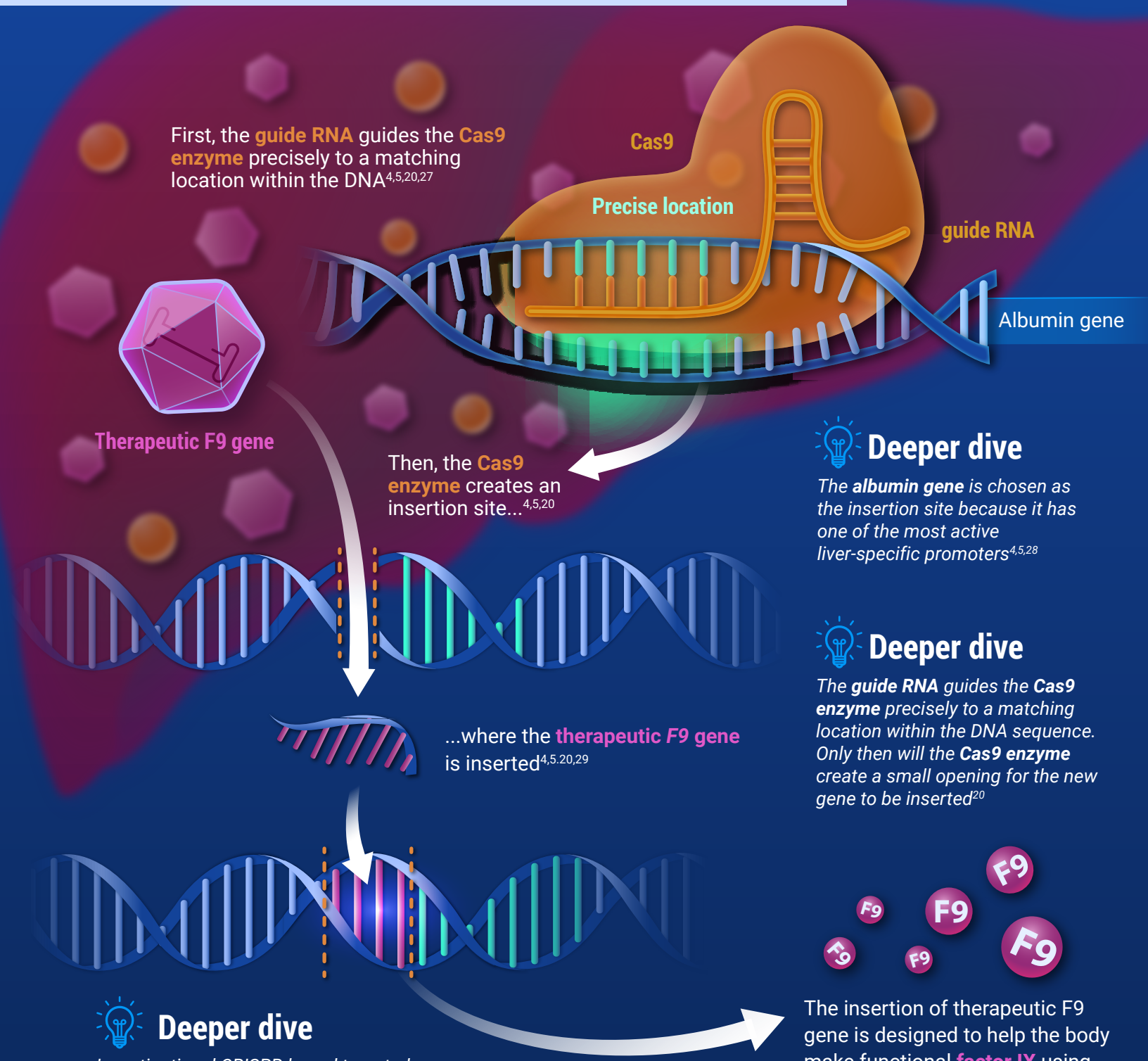
Deeper dive

***LNPs** are used to package the gRNA and the mRNA to encode the Cas9 enzyme, because they are the preferred method for delivering RNA to the liver*[8,25]

Investigational CRISPR-Based Targeted Gene Insertion is a Two-Step Process:[4,5]

Step 2: Targeted Insertion of Therapeutic *F9* Gene

Once inside target liver cells, **CRISPR** is designed to precisely target a specific location in the DNA to enable targeted insertion of the **therapeutic *F9* gene**[4,5,20]



Deeper dive

*The **albumin gene** is chosen as the insertion site because it has one of the most active liver-specific promoters*[4,5,28]

Deeper dive

*The **guide RNA** guides the **Cas9 enzyme** precisely to a matching location within the DNA sequence. Only then will the **Cas9 enzyme** create a small opening for the new gene to be inserted*[20]

Deeper dive

*Investigational CRISPR-based targeted gene insertion generates **factor IX** protein that is the same as naturally occurring, functional human factor IX protein*[4,5]

The insertion of therapeutic F9 gene is designed to help the body make functional **factor IX** using the instructions from the therapeutic gene[3-5]

What You Need to Know

CRISPR-based targeted gene insertion for haemophilia B is investigational and the efficacy and safety has not been evaluated by any regulatory authority

Investigational CRISPR-Based Targeted Gene Insertion for Haemophilia B…

…builds on a strong foundation of genetic research that continues to grow and is being investigated in pre-clinical studies and clinical trials[27]

…currently can only be given once. Today, the viral vectors used in treatment can only be introduced to the body once because the body's immune response may prevent them from being delivered again[19]

…targets liver cells (non reproductive cells), so the therapeutic gene and its effects are not aimed to be passed down to offspring[4]

…hoping for paediatric use[4,5]

…aims to be a lifelong treatment after a single dose for haemophilia B, hopefully without the need for routine factor replacement therapy[4,5,19,29]

Deeper dive

If treatment is not successful, patients can discuss with their healthcare practitioners about safely returning to their previous treatment routine or other treatment options that do not use AAVs[4,5,27]

Deeper dive

CRISPR-based targeted gene insertion may be suitable for paediatric use because translational research suggests that the treatment is durable even as the liver continues to grow[4,5]

01. National Bleeding Disorder Foundation. Hemophilia B. https://www.hemophilia.org/bleeding-disorders-a-z/types/hemophilia-b. Accessed March 11, 2024.
02. National Organization for Rare Disorders, Inc. Hemophilia B. https://rarediseases.org/rare-diseases/hemophilia-b/. Accessed March 11, 2024.
03. American Society of Gene & Cell Therapy. Gene editing. https://patienteducation.asgct.org/gene-therapy-101/gene-editing. Accessed March 11, 2024.
04. Data on file. Regeneron Pharmaceuticals, Inc.
05. Sabin L. Novel approaches for gene-based therapies: Targeted gene insertion of Factor 9 as a potential durable treatment for hemophilia B. Lecture presented at: American Society of Hematology; 2023; San Diego, CA.
06. American Society of Gene & Cell Therapy. Hemophilia. https://patienteducation.asgct.org/disease-treatments/hemophilia. Accessed April 11, 2024.
07. Hastie E, et al. *Hum Gene Ther*. 2015;26(5):257–265
08. Wang D, et al. *Nat Rev Drug Discov.* 2019;18(5):358–378.
09. Guo N, et al. *J Adv Res.* 2022;40:135–152.
10. Glybera. Summary of Product Characteristics. UniQure biopharma; 2012.
11. US Food and Drug Administration. FDA approves novel gene therapy to treat patients with a rare form of inherited vision loss. https://www.fda.gov/news-events/press-announcements/fda-approves-novel-gene-therapy-treat-patients-rare-form-inherited-vision-loss. Accessed March 13, 2024.
12. Luxterna. Summary of Product Characteristics. Novartis Europharm Limited; 2018.
13. National Bleeding Disorder Foundation. FDA approves first gene therapy to treat adults with hemophilia B. https://www.hemophilia.org/news/first-hemophilia-b-gene-therapy-approved-by-fda. Accessed March 13, 2024.
14. Hemgenix. Summary of Product Characteristics. CSL Behring GmbH; 2023.
15. US Food and Drug Administration. FDA approves first gene therapy for patients with sickle cell disease. https://www.fda.gov/news-events/press-announcements/fda-approves-first-gene-therapies-treat-patients-sickle-cell-disease. Accessed March 13, 2024.
16. Casgevy. Summary of Product Characteristics. Vertex Pharmaceuticals Limited; 2024.
17. US Food and Drug Administration. FDA approves first gene therapy for adults with severe hemophilia A. https://www.fda.gov/news-events/press-announcements/fda-approves-first-gene-therapy-adults-severe-hemophilia. Accessed March 13, 2024.
18. Intellia Therapeutics. Intellia Therapeutics announces first patient dosed in the phase 3 MAGNITUDE study of NTLA-2001 as a single-dose CRISPR-based treatment for transthyretin amyloidosis with cardiomyopathy. https://ir.intelliatx.com/news-releases/news-release-details/intellia-therapeutics-announces-first-patient-dosed-phase-3. Accessed March 20, 2024.
19. American Society of Gene & Cell Therapy. Vectors 101. https://patienteducation.asgct.org/gene-therapy-101/vectors-101. Accessed March 13, 2024.
20. Asmamaw M, Zawdie B. Mechanism and applications of CRISPR/Cas-9-mediated genome editing. *Biologics.* 2021;15:353–361.
21. American Society of Gene & Cell Therapy. What is gene therapy? Accessed March 13, 2024. https://asgct.org/global/documents/patient-ed-infographics/what-is-gene-therapy.aspx?_ga=2.214286359.1282143957.1713453269-636341200.1705006481&_gl=1*1j3150r*_ga*NjM2MzQxMjAwLjE3MDUwMDY0ODE.*_ga_Q37QKR6TCJ*MTcxMzQ1MzI3OS4xOC4wLjE3MTM0NTMyNzkuMC4wLjA.*_ga_6FB6X4L6XF*MTcxMzQ1MzI4MC4xOC4wLjE3MTM0NTMyODAuNjAuMC4w
22. American Society of Gene & Cell Therapy. Viral vector overview. Accessed March 13, 2024. https://asgct.org/global/documents/patient-ed-infographics/sept-launch-website-material/viral_vector_overview-(7).aspx?_ga=2.218638233.1282143957.1713453269-636341200.1705006481&_gl=1*1vlbvxf*_ga*NjM2MzQxMjAwLjE3MDUwMDY0ODE.*_ga_Q37QKR6TCJ*MTcxMzQ1MzI3OS4xOC4xLjE3MTM0NTM1NTguMC4wLjA.*_ga_6FB6X4L6XF*MTcxMzQ1MzI4MC4xOC4xLjE3MTM0NTM1NTguNDkuMC4w
23. Mietzsch M, et al. *Ann Rev Virol*. 2017;4(1):iii-v.
24. World Federation of Hemophilia. WFH gene therapy registry: Basics of hemophilia, gene therapy, and long-term data collection. Accessed April 11, 2024. https://www1.wfh.org/publications/files/pdf-2212.pdf
25. Kazemian P, et al. *Mol Pharm.* 2022;19(6):1669–1686.
26. Uddin F, et al. *Front Oncol.* 2020;10:1387.
27. American Society of Gene & Cell Therapy. How does gene editing work? Accessed March 13, 2024. https://asgct.org/global/documents/patient-ed-infographics/gene-editing-infographic-(1).aspx?_ga=2.247343236.1282143957.1713453269-636341200.1705006481&_gl=1*pjc2tr*_ga*NjM2MzQxMjAwLjE3MDUwMDY0ODE.*_ga_Q37QKR6TCJ*MTcxMzQ1MzI3OS4xOC4xLjE3MTM0NTM2MTkuMC4wLjA.*_ga_6FB6X4L6XF*MTcxMzQ1MzI4MC4xOC4xLjE3MTM0NTM2MTkuNjAuMC4w
28. Tang J, et al. *Biomed Rep.* 2017;6(6):627–632.
29. American Society of Gene & Cell Therapy. Gene therapy approaches. https://patienteducation.asgct.org/gene-therapy-101/gene-therapy-approaches. Accessed March 11, 2024.

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