

Investigational CRISPR-Based Targeted Gene Insertion for Hemophilia B

For Study Investigator Use Only

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

Hemophilia B is a genetic condition caused by a **mutation** in the **factor IX (F9) gene**^{1,2}

People with hemophilia B do not produce enough **functional clotting factor IX**²

Factor IX (F9) gene

F9

Gene insertion aims to permanently restore the body's ability to independently produce factor IX without the need for routine factor replacement therapy³

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 should³

Liver

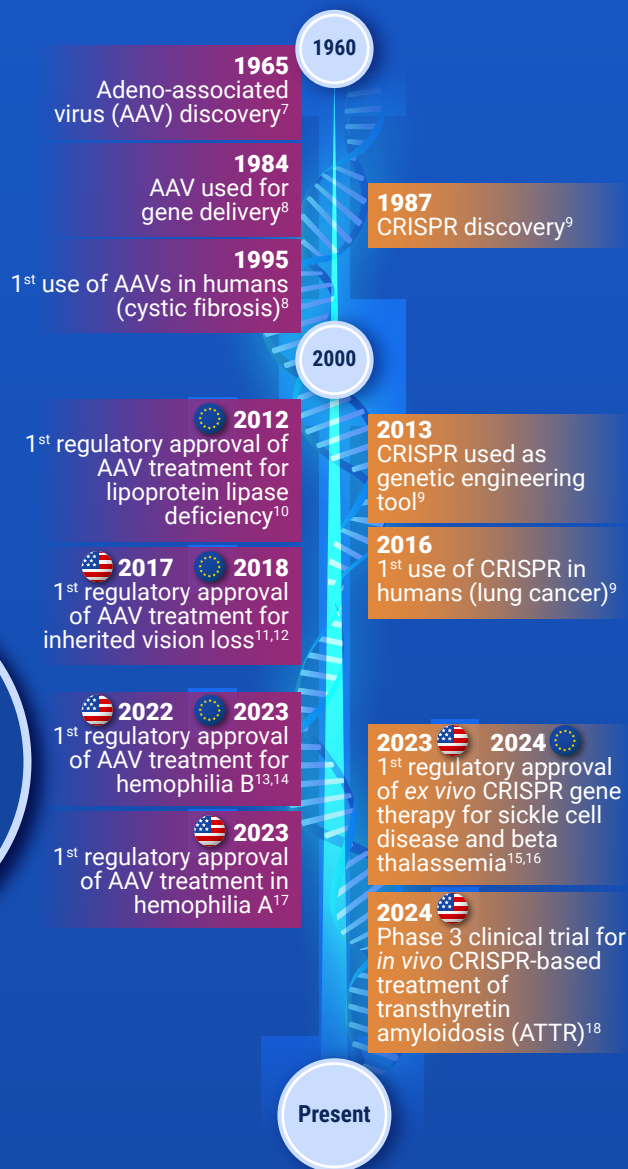
In vivo CRISPR-based targeted gene insertion is a type of gene editing being investigated in hemophilia B. By adding a **therapeutic F9 gene**, the body can make **functional clotting factor IX** on its own³⁻⁵



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 hemophilia 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 produced⁶

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



Investigational *in vivo* CRISPR-based targeted gene insertion for hemophilia B



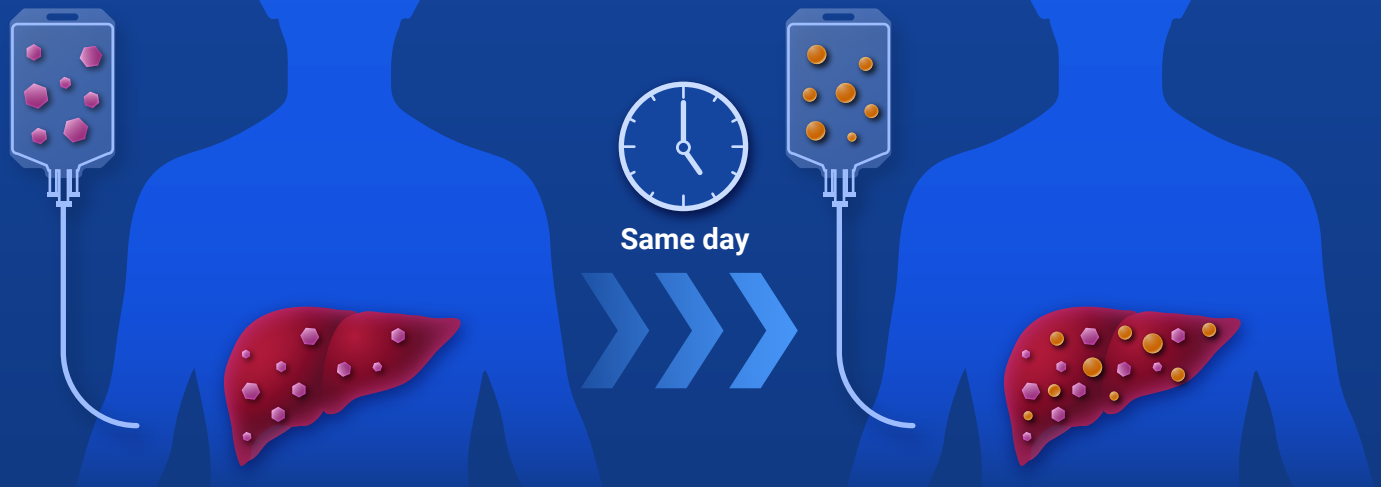
Deeper dive

CRISPR is a versatile tool. CRISPR-based targeted gene insertion for hemophilia 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}

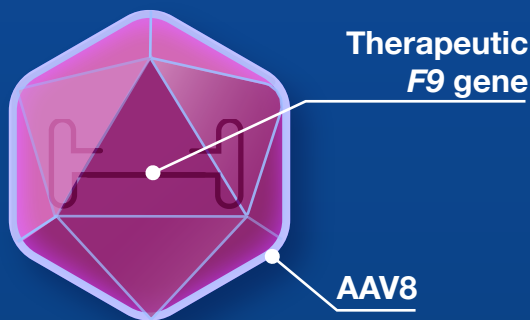
Step 1: Delivery of **Therapeutic F9 Gene** and **CRISPR**

CRISPR-based targeted gene insertion for hemophilia B is an *in vivo* therapy given by intravenous infusion, which includes two components that are delivered to target liver cells^{4,5}



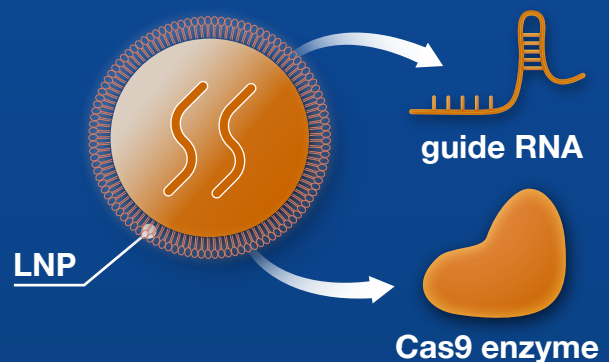
First, the investigational **therapeutic F9 gene** is delivered to liver cells to provide instructions for making **clotting factor IX**^{4,5,8,22}

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 cells²⁵

Deeper dive

A **transgene** is a DNA sequence or combination of sequences that can produce functional protein²⁴

- 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}

First, the **guide RNA** guides the **Cas9 enzyme** precisely to a matching location within the DNA^{4,5,20,27}

Cas9

Precise location

guide RNA

Albumin gene

Therapeutic F9 gene

Then, the **Cas9 enzyme** creates an insertion site...^{4,5,20}

...where the **therapeutic F9 gene** is inserted^{4,5,20,29}



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 inserted²⁰



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 gene³⁻⁵



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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}



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

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