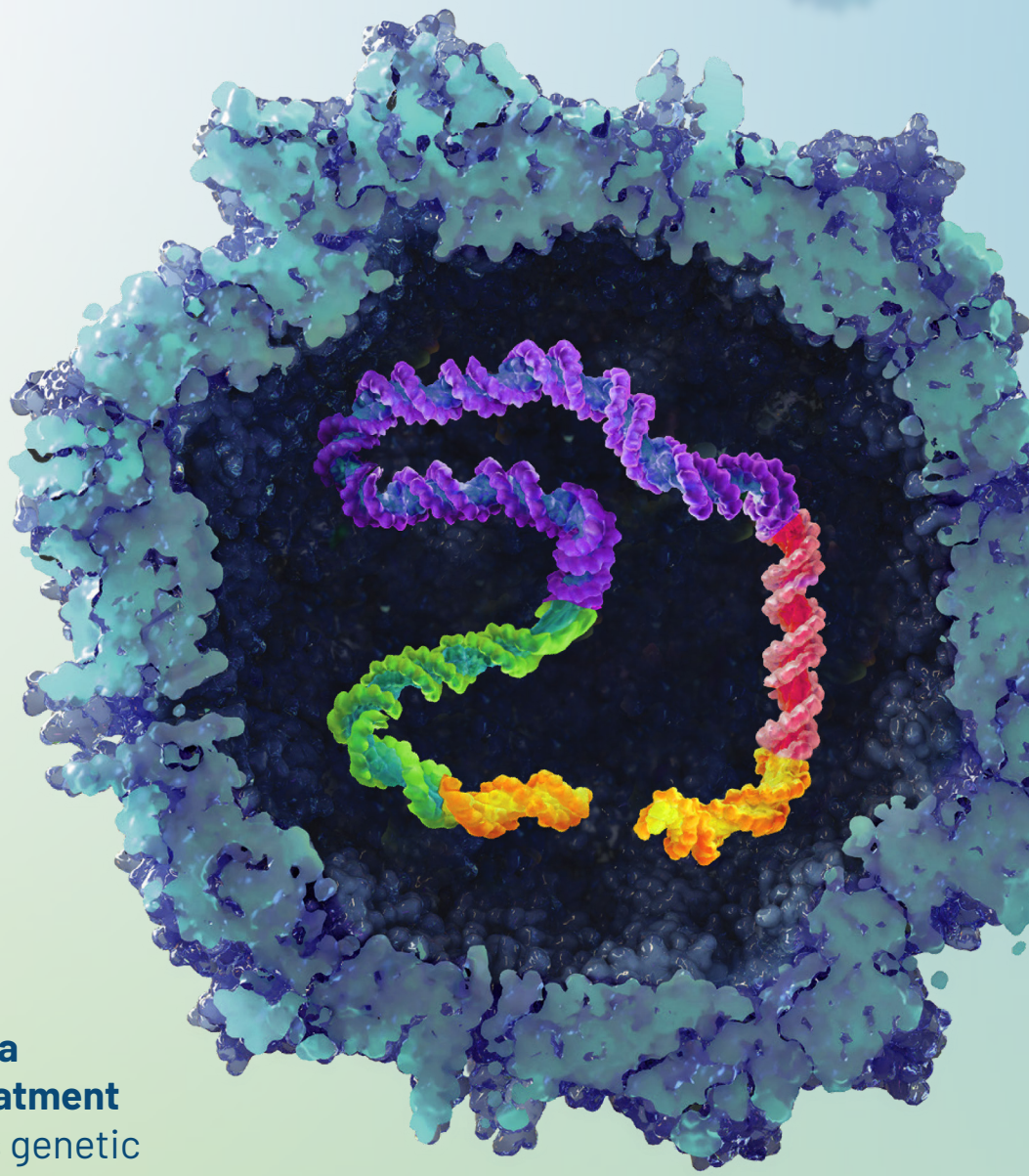
# Gene Therapy
## A Primer



**Gene therapy has the potential to be a transformative treatment approach** that uses genetic material to treat or cure a disease.[1]

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# Overview of gene therapy

Gene therapy is a type of treatment that has the potential to transform the course of a disease. The goal is to modify a dysfunctional gene or add a functional gene to achieve durable expression of the therapeutic gene to treat a disease.[1,2]

The approach to gene therapy, including gene modification techniques and delivery of the functional gene, varies depending on the type of disease.[2]

## Gene therapy techniques[1,3-6]

**Gene addition or transfer:**
Introduce a functional copy of a missing or dysfunctional gene

**Gene inactivation or silencing:**
Suppress gene expression

**Gene editing:**
Targeted modification of defective genes using editing tools

## Utility of gene therapy

**Monogenic diseases, which are inherited conditions and caused by a single gene mutation, are good candidates for gene therapy.**[6]

In monogenic diseases, gene therapy approaches are predominantly focused on gene addition or gene transfer delivered through a viral vector.[3,6]

## Viral vectors as gene delivery systems

Gene therapy, specifically gene transfer, takes advantage of the ability of viruses to deliver genetic material to host cells. A functional gene is inserted into the viral vector and transferred to target tissue or cells through either *in vivo* or *ex vivo* gene delivery methods.[1,4]

Viral vectors can be engineered to minimize unwanted replication and deliver modified genes to physiologically relevant target tissues and cells.[1,4]

***In vivo*** delivery allows transfer of the viral vector directly to patients.[4,6]

***Ex vivo*** delivery involves extraction and viral transduction of stem cells that are then transplanted back into the body.[4,6]

## Considerations for viral vector design and gene therapy strategies

Gene therapy techniques predominantly utilize a delivery system through a viral vector to aid delivery of the therapeutic gene. Design considerations for gene therapy depend on the primary genetic defect, the size of the modified gene, and the features of the target organ or tissue.[4,6]

- **Size** of the gene cassette
- **Packaging capacity** of the virus
- **Delivery method** (*in vivo, ex vivo*)
- Potential to **integrate** into the host genome
- **Targeting** dividing vs nondividing cells

## Gene therapy by the numbers

**36**

The number of years that gene therapies have been studied in humans.[7]

**>2000**

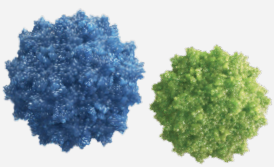
The number of planned or ongoing Phase 1–3 clinical trials of gene therapies as of March 2026.[8]

**41**

The number of gene therapies approved globally for clinical use by Q4 2025.[9]

# Commonly used viral vectors for gene therapy approaches[4]

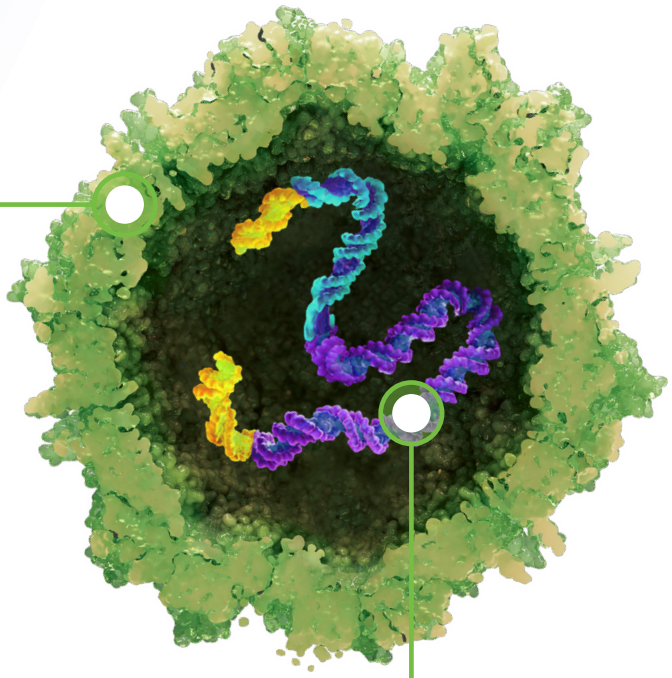
| | *Ex vivo* | | *In vivo* | |
|---|---|---|---|---|
| **Viral vector** | **Retroviral** | **Lentiviral** | **Adenoviral** | **Adeno-associated virus (AAV)** |
| **Host genome integration** | Integrating | Integrating | Poorly integrating | Poorly integrating |
| **Viral genome** | RNA | RNA | DNA | DNA |
| **Cell division required for target cell** | Yes | G1 phase | No | No |
| **Gene packaging capacity** | ~8 kb | ~8 kb | ~8-30 kb | ~5 kb |
| **Immune response to vector** | Low | Low | Extensive | Low |
| **Long-term expression** | Yes | Yes | No | Yes |
| **Main advantages** | Persistent gene transfer in dividing cells | Persistent gene transfer in transduced tissues | Highly effective in transducing various tissues | Elicits few inflammatory responses, nonpathogenic |
| **Main disadvantages** | Risk of insertional mutagenesis[2,10]<br><br>Only transduces dividing cells[7]<br><br>Low transduction efficiency[2] | Risk of insertional mutagenesis[10]<br><br>Scaling up production is often challenging due to labor-intensive methodology[2] | Transient expression[4]<br><br>Strong immune response[4] | Limited packaging capacity[2,4] |



**AAV and lentiviral vectors** are the most common approaches to gene therapy.[4]

Table adapted from Anguela XM, et al. *Annu Rev Med*. 2019;70:273-288.

# Key components of lentivirus-based gene therapies



## ENVELOPE

**Lipids and proteins that encapsulate RNA and enzymes required for genomic integration**[11]

Can be modified for targeted tissue transduction, minimized host immune response[11,12]

HIV-1 is the most studied lentivirus for gene therapies[11]

## GENE CASSETTE (~8 kb)[12,13]


**In addition to the therapeutic gene, or transgene, regulatory elements are necessary for transgene expression.**

- **Long Terminal Repeat (LTR)**
  Nucleotide sequences necessary to signal reverse transcription initiation (5′ LTR), termination (3′ LTR), and packaging of the virus found at each end of the gene cassette. It facilitates integration of the transfer plasmid sequences into the host genome and also contains a signal to render the virus 'self-inactivating' after integration.[12,14]
- **Promoter**
  Instructs the cell when and where to express the gene that follows.[12,15]

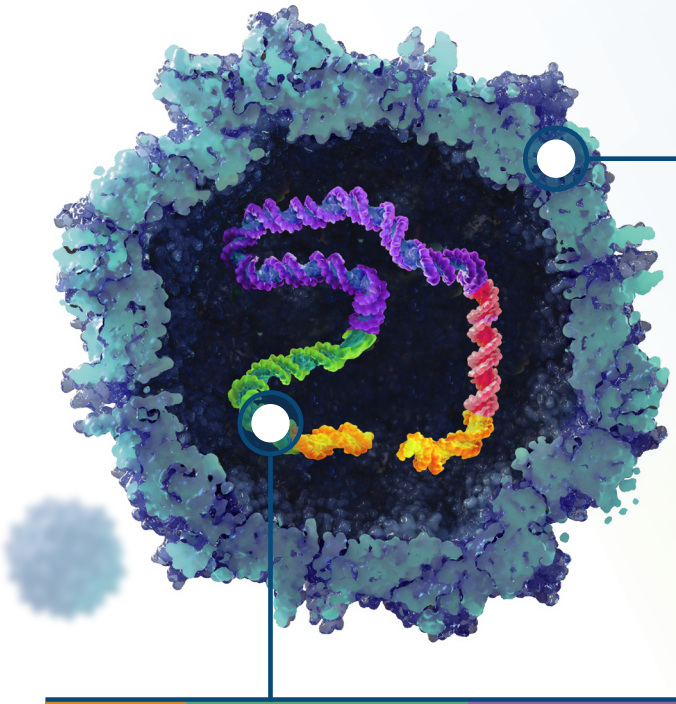
## Key features of lentiviral vectors

Integrates into the host genome[4]

Selectively targets specific tissues/organs[11,12]

Durably expresses transgenes after a single administration[4]

# Key components of AAV-based gene therapies



## CAPSID

**Protein shell encoded by the AAV genome**[16,17]

Facilitates binding of the virus to targeted cells or tissues through attachment proteins on its surface

The capsid serotype influences delivery of the functional gene, activation of the human immune system, and the ability to transduce specific cells (tropism). For example, of the 13 AAV serotypes that have been identified, AAV1-3 and 5-9 have a propensity to target liver tissue, as seen in animal studies

## GENE CASSETTE (~5 kb)[18]


**In addition to the therapeutic gene, or transgene, regulatory elements are necessary for transgene expression.**

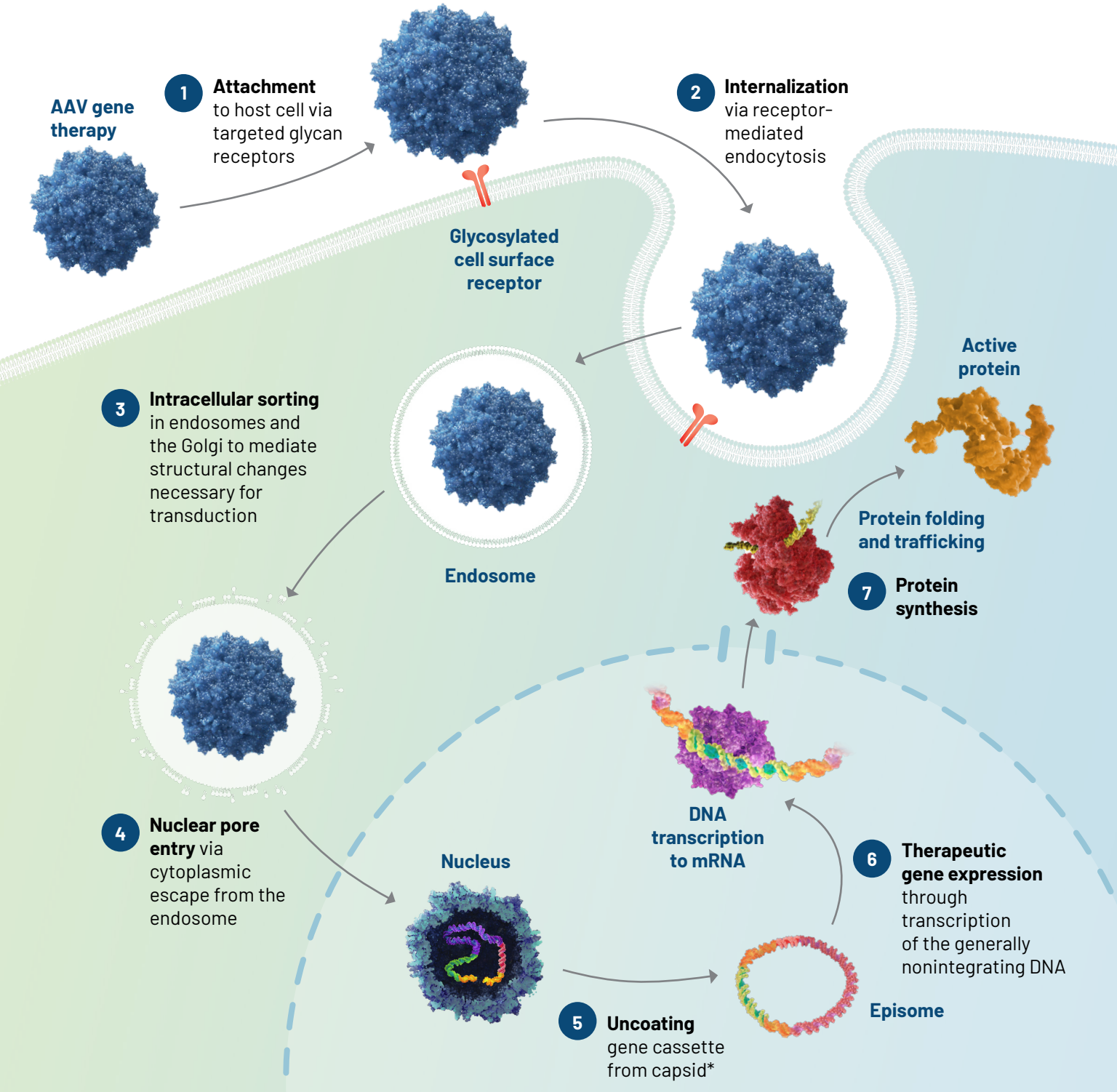
- **Inverted Terminal Repeat (ITR)**
  Sequences of genetic code at each end of the gene expression cassette that drive inter-molecular or intra-molecular recombination to form circularized episomal genomes that can persist in the nucleus.[17]
- **Tissue-specific Promoter**
  DNA that instructs the cell when and where to express the gene that follows. Promoters can contain components that make them more likely to work in specific tissues, eg, liver.[13,17,18]
- **Transcription Termination Signal**
  DNA regulatory element to end transcription of the transgene.[18]

## Key features of AAV vectors

| | | |
|---|---|---|
| Poor levels of integration into the host genome[4] | Targets specific tissues/organs[4] | Durably expresses transgenes after a single administration[4] |

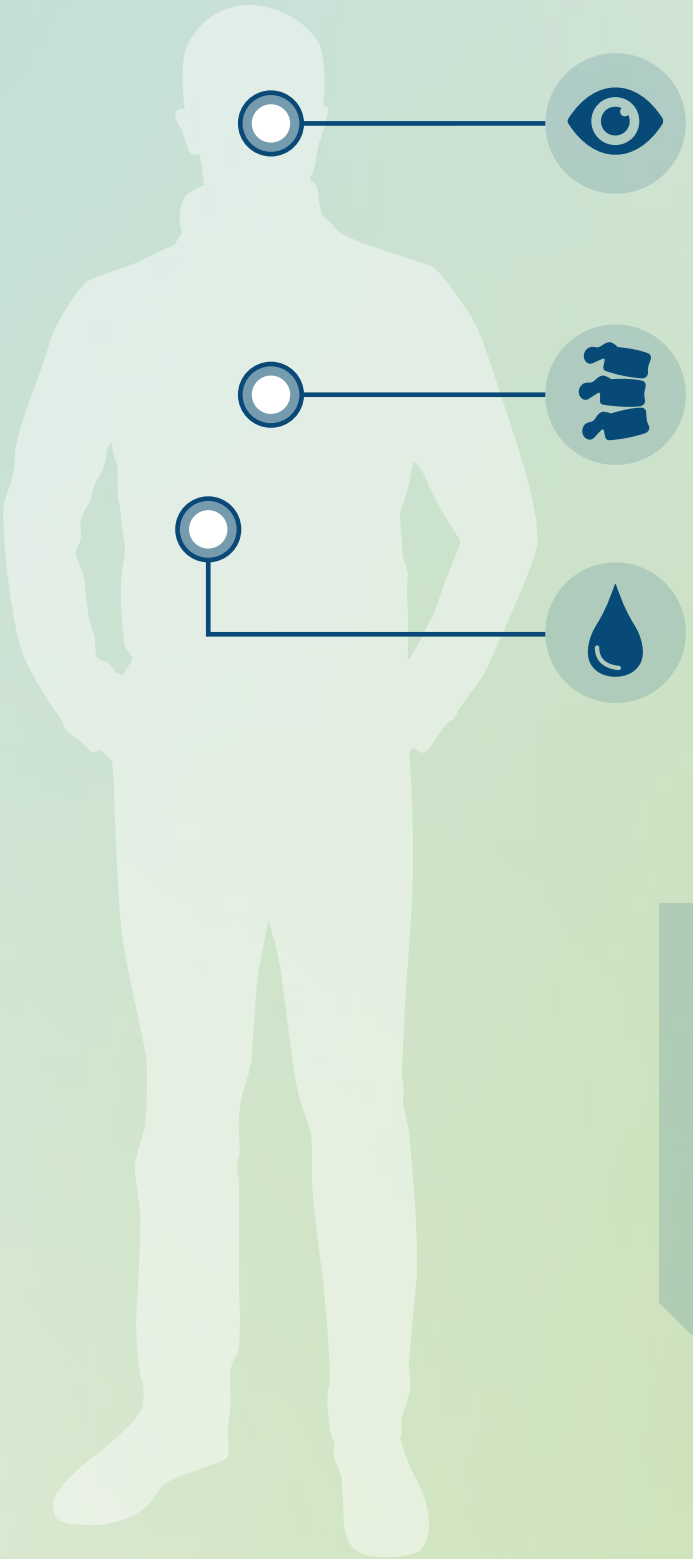
# AAV transduction overview

Transduction of AAV-based gene therapy into targeted host cells is a multistep process.[17,19,20]




*Vector genomes can also undergo integration into the host genome at very low frequencies.

# Examples of therapeutic areas utilizing AAV-based gene therapies



## Hereditary retinal dystrophy

**Restore a functional RPE65 gene in retinal pigment epithelial cells.**[2,21,22]

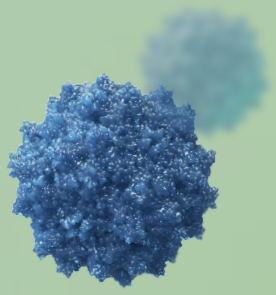
## Spinal muscular atrophy

**Restore functional SMN1 protein in motor neurons.**[2,23,24]

## Hemophilia

**Restore functional Factor VIII (FVIII) (hemophilia A) or Factor IX (FIX) (hemophilia B) in hepatocytes.**[2,25-28]

**AAV-based gene therapies** have been approved in some regions to treat a range of diseases.[17, 21-28]



SMN=survival motor neuron.

# Challenges with AAV-based gene therapy

While AAV-based gene therapy has been shown to be well tolerated and efficacious in some diseases, like any new therapy in development, there are known and unknown risks.[4]

## Known risks

**Immunogenicity/immunotoxicity** is an immune response to the viral vector and/or the protein encoded by the transgene. While AAV vectors are considered less immunogenic than other vectors, an immune response may pose a challenge to effective gene therapy.[4,29,30]

Antibodies are produced following an immune response to common infections caused by viruses and external pathogens. A subset of antibodies, termed neutralising antibodies (NAbs), persists to protect against future encounters with a virus.[2,29]

NAbs can be produced against the capsid proteins of the viral vector due to the structural similarity of viral vectors and naturally occurring AAVs. About 30%–80% of people have preexisting antibodies to AAVs that are acquired through natural infections. NAb levels may influence eligibility for some gene therapies.[29,31]

The vector may elicit an immune response dependent on the targeted tissue. For example, immune responses can lead to elevated transaminase levels in liver or elevated creatine kinase levels in muscle. Prompt use of immunosuppressants can mitigate elevations in transaminases and creatine kinase.[32]

## Unknown risks[2]

**Potential challenges that have not been observed in the clinic**

**Gene silencing**
Gradual loss of gene expression.

**Phenotoxicity**
Overexpression or ectopic expression of the transgene.

**Vertical transmission**
Germline transmission of therapeutic DNA to offspring.

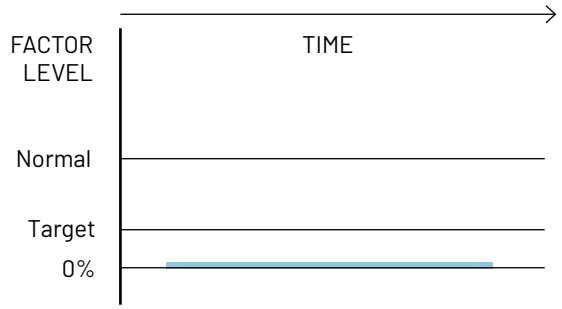
**Horizontal transmission**
Shedding of infectious vector into the environment.

# Focus on gene therapy in hemophilia

Hemophilia is an X-linked, hereditary, monogenic bleeding disorder that results in the absence or deficiency of coagulation FVIII (hemophilia A) or FIX (hemophilia B). Currently, there is no cure for hemophilia and the standard of care for moderate or severe disease is lifelong prophylaxis with factor replacement therapies. Episodic treatment is also recommended as needed.[33,34]

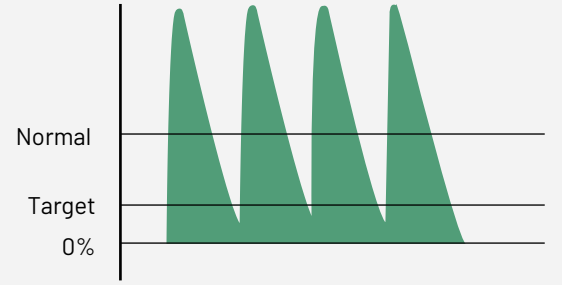
**The goal of gene therapy in hemophilia** is to significantly reduce bleeding episodes and factor replacement usage. Liver-directed gene therapy for hemophilia with AAV vectors has the potential to maintain endogenous factor levels after a single intervention.[4]

## Factor levels by treatment regimen[35]



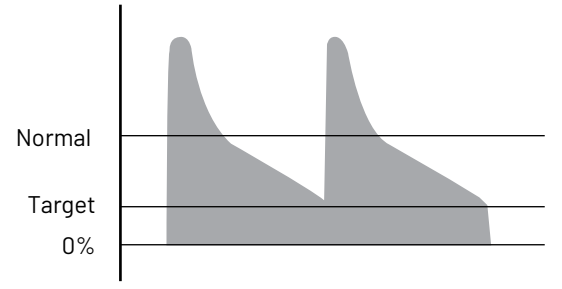

### No treatment

Inadequate factor levels at all times (severe hemophilia).[4,33]



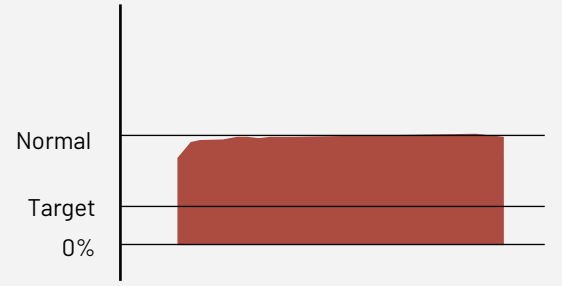

### Standard factor therapy

Factor levels spike with every dose, often dipping below adequate levels.[33,34]




### Long-acting factor therapy

Requires less frequent dosing and maintains levels at or above what is necessary for prophylaxis, but still requires regular administration and results in fluctuating factor levels over time.[34]




### Goal of gene therapy

A single administration holds the potential to sustain above-target factor levels for months or years at a time.[4]

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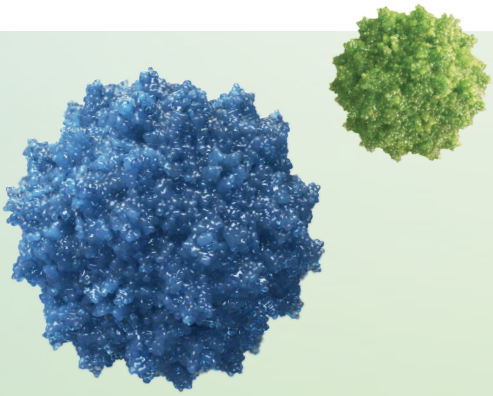
# **Gene therapy:** Key takeaways

**Monogenic diseases** are caused by a single gene mutation and **are good candidates for gene therapy**.[6]

**Viral vectors are used to deliver gene therapy** through *in vivo* or *ex vivo* administration.[4]


**AAV and lentiviral vectors are the most commonly studied in gene therapy** and have unique characteristics for durable expression of a gene. Generally, AAV vectors have a low integration, non-negligible rate and are commonly studied using *in vivo* gene therapies whereas integrating lentiviral vectors are suitable for *ex vivo* applications.[4]



**AAV-based gene therapy has demonstrated tolerability and efficacy in some diseases**.[2,21-28]

Hereditary retinal dystrophy

Spinal muscular atrophy

Hemophilia

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April 2026
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MED-MYS-HGX-00011 | APR2026