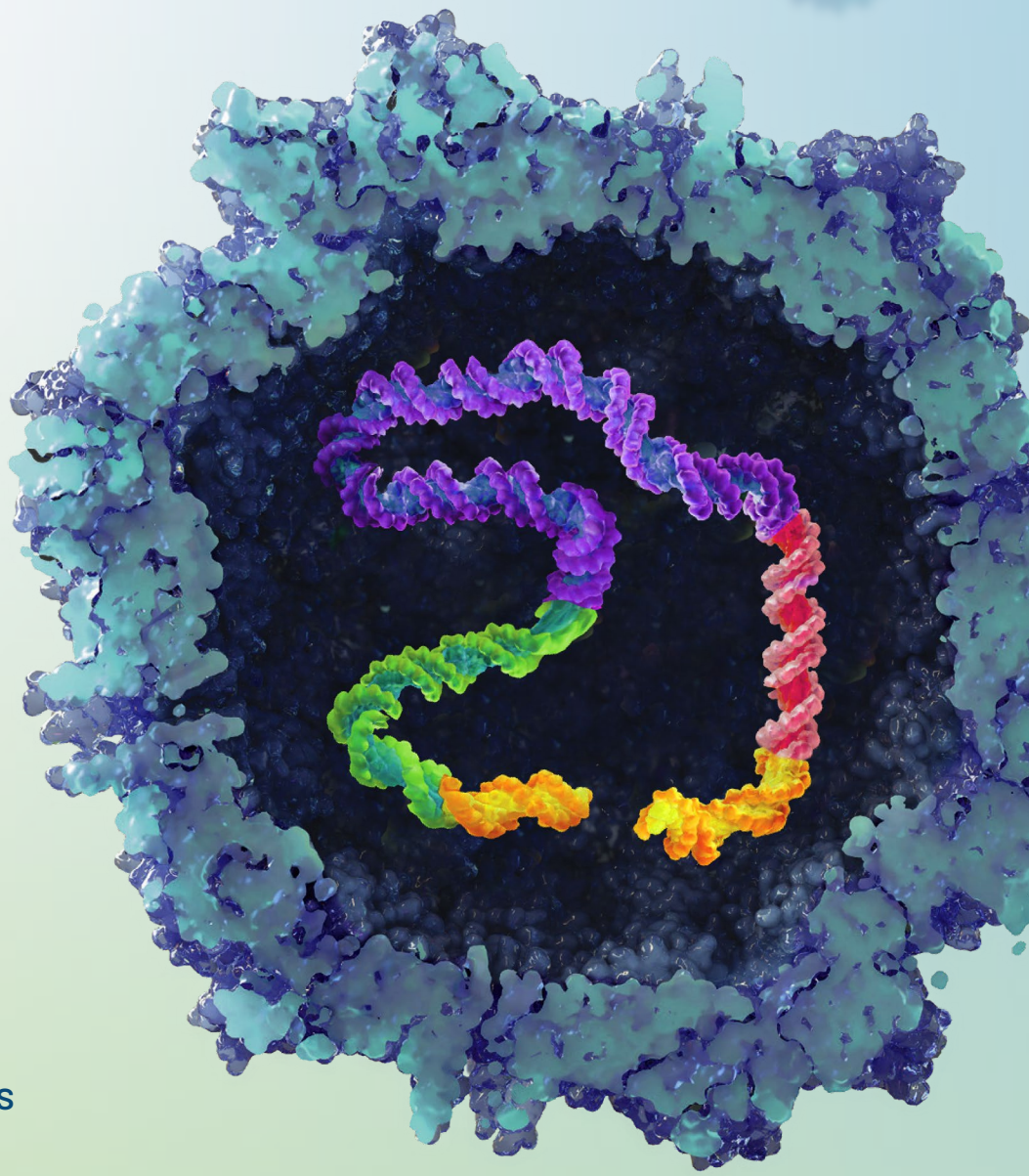
Gene Therapy
A Primer



Gene therapy is a transformative treatment that uses genetic material to treat a disease.[1]

Gene therapy for hemophilia A or B is not approved or for sale in Canada. Before prescribing any product, always refer to the local materials such as the Prescribing Information, Product Monograph, and/or the Summary of Product Characteristics in your country.

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Overview of gene therapy

Gene therapy is a type of treatment that can 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.[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

33

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

>1100

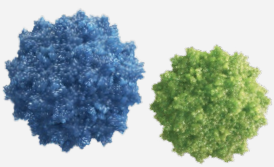
The number of planned or ongoing Phase 1–3 clinical trials of gene therapies as of May 2023.[8]

19

The number of viral vector–based gene therapies approved globally.[7,9-14]

Commonly used viral vectors for gene therapy approaches[4]

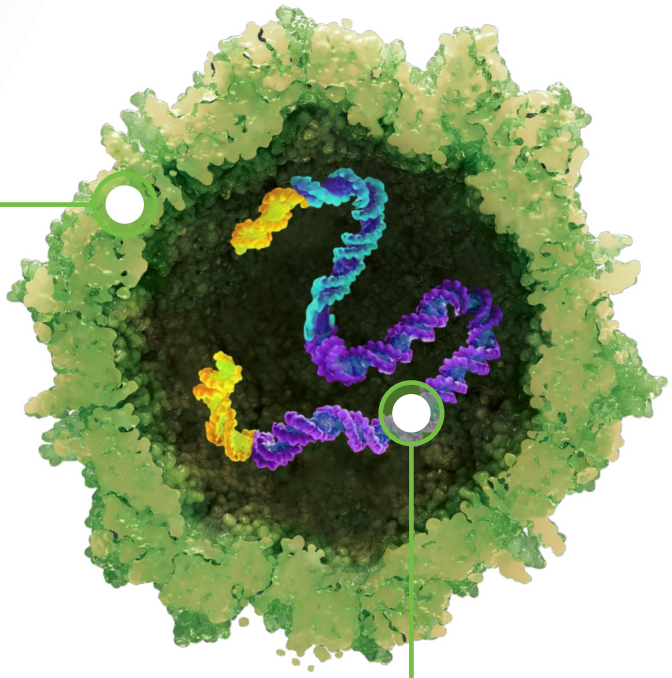
	Ex vivo		In vivo	
Viral vector	**Retroviral**	**Lentiviral**	**Adenoviral**	**Adeno-associated virus (AAV)**
Host genome integration	Integrating	Integrating	Poorly integrating	Poorly integrating
Viral genome	RNA	RNA	DNA	DNA
Cell division required for target cell	Yes	G1 phase	No	No
Gene packaging capacity	~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[2,15] Only transduces dividing cells[7] Low transduction efficiency[2]	Risk of insertional mutagenesis[15] Scaling up production is often challenging due to labor-intensive methodology[2]	Transient expression[4] 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[16]

Can be modified for targeted tissue transduction, minimized host immune response[16,17]

HIV-1 is the most studied lentivirus for gene therapies[16]

GENE CASSETTE (~8 kb)[17,18]

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.[17,19]
- **Promoter**
  Instructs the cell when and where to express the gene that follows.[17,20]

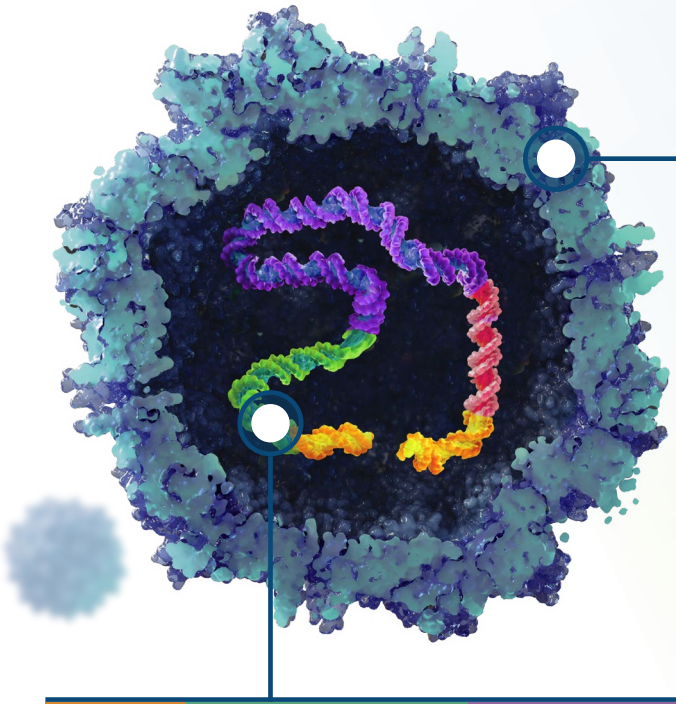
Key features of lentiviral vectors

Integrates into the host genome[4]

Selectively targets specific tissues/organs[16,17]

Durably expresses transgenes after a single administration[4]

Key components of AAV-based gene therapies



CAPSID

Protein shell encoded by the AAV genome[21,22]

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)[23]

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.[22]
- **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.[18,22,23]
- **Transcription Termination Signal**
  DNA regulatory element to end transcription of the transgene.[23]

Key features of AAV vectors

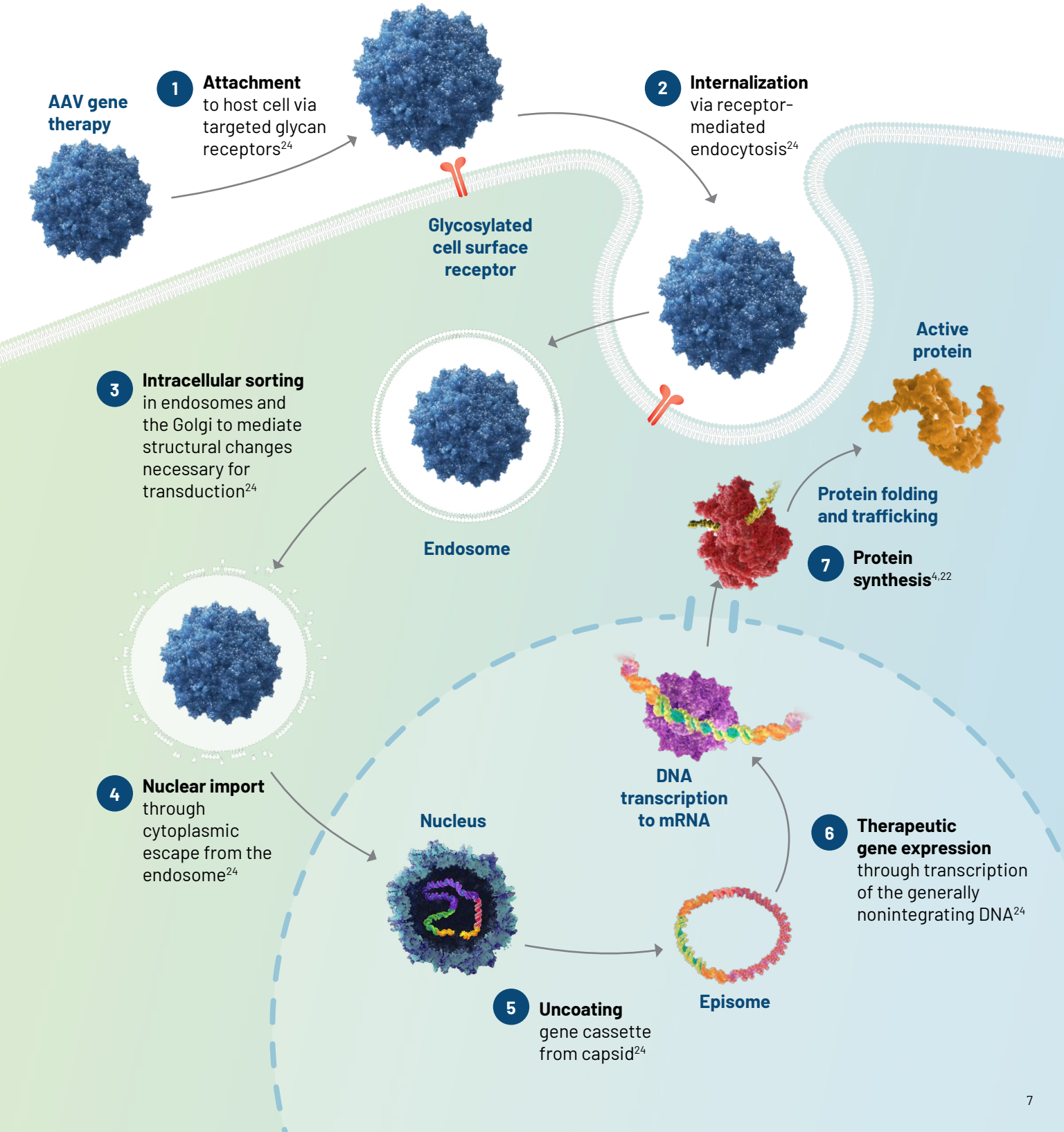
Generally does not integrate into the host genome[4]

Targets specific tissues/organs[4]

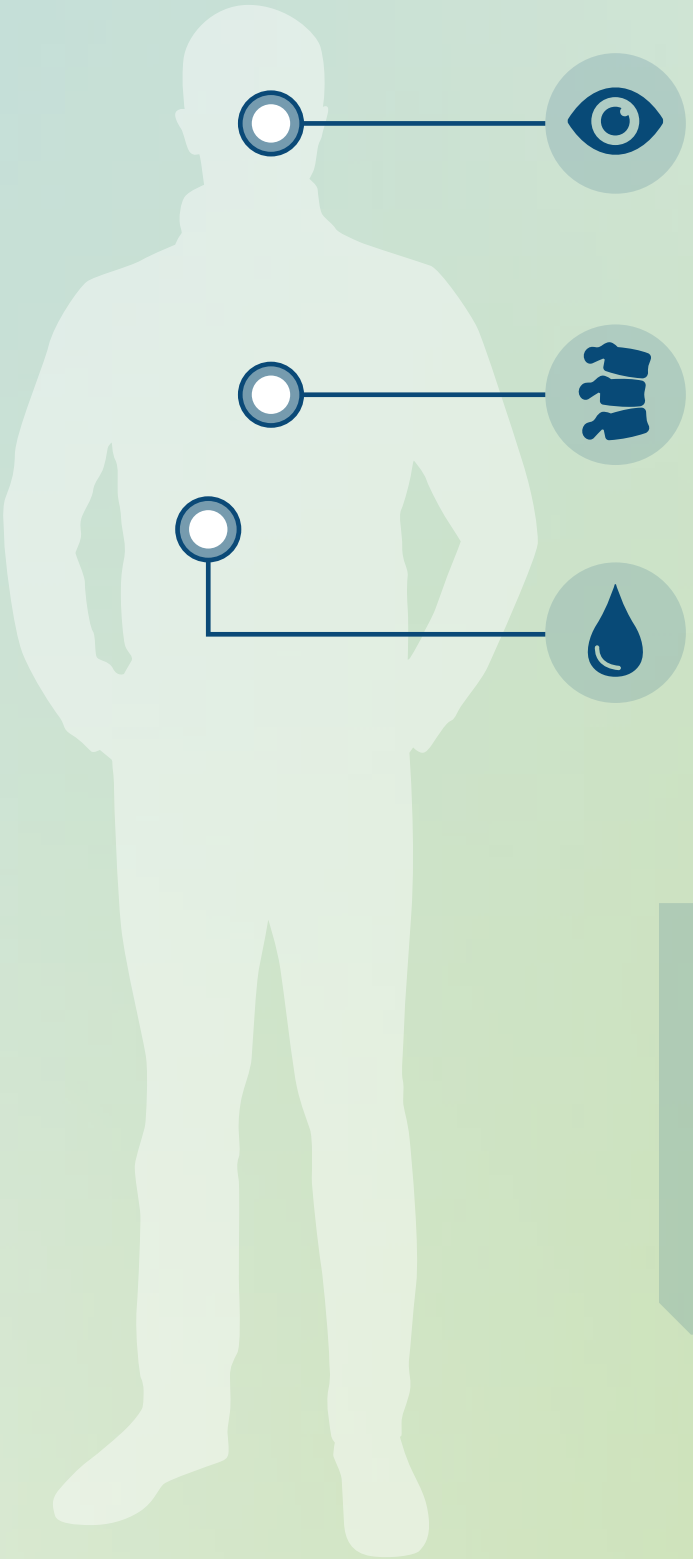
Durably expresses transgenes after a single administration[4]

AAV transduction overview

Transduction of AAV-based gene therapy into targeted host cells is a multistep process.[22,24]



Examples of therapeutic areas utilizing AAV-based gene therapies



Hereditary retinal dystrophy

Restore functional RPE65 in retinal pigment epithelial cells.[2,25]

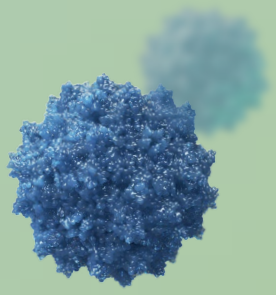
Spinal muscular atrophy

Restore functional SMN1 protein in motor neurons.[2,26]

Hemophilia

Restore functional Factor VIII (FVIII) (hemophilia A) or Factor IX (FIX) (hemophilia B) in hepatocytes.[2,27,28]

AAV-based gene therapies have been approved in some regions to treat a range of diseases.[22,25-28]



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 neutralizing 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.

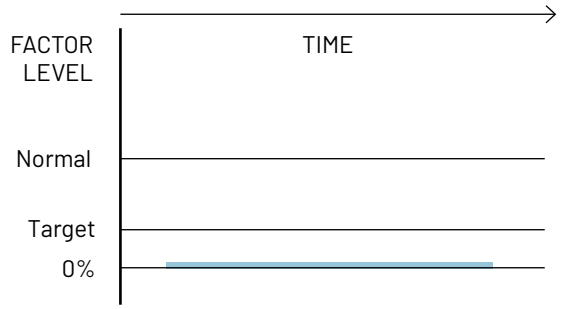
Horizontal transmission
Shedding of infectious vector into the environment.

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]

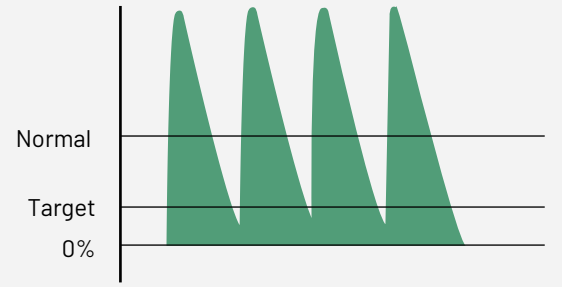
The goal of gene therapy in hemophilia is to significantly reduce bleeding episodes and factor replacement usage. Liver-directed gene therapy for hemophilia with AAV vectors has the potential to maintain endogenous factor levels after a single intervention.[4]

Factor levels by treatment regimen[35]



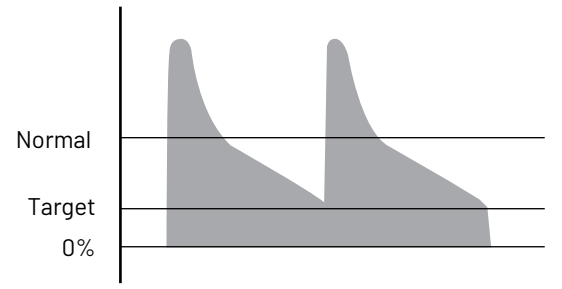
No treatment

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



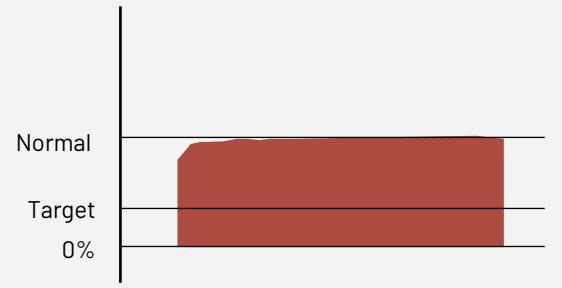
Standard factor therapy

Factor levels spike with every dose, often dipping below adequate levels for prophylaxis.[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 therapies for hemophilia A and B

Significant progress has been made in the development of gene therapies for hemophilia with several candidates that are approved or in clinical development[9,36-38]

Hemophilia A

- Giroctocogene fitelparvovec
- Valoctocogene roxaparvovec

Hemophilia B

- Etranacogene dezaparvovec
- Fidanacogene elaparvovec

Hemophilia A and B are characterized by similar clinical symptoms but result from mutations in different genes, *F8* gene and *F9* gene, respectively.[34] There is a high level of similarity in vector design for hemophilia A and B gene therapies, though size and structural differences of the *F8* and *F9* genes along with the factor products (FVIII and FIX, respectively) may require different considerations.[31,39]

Key considerations for gene therapies in hemophilia

	Hemophilia A	Hemophilia B
Vector design[31,39]	• AAV-based capsids (typically AAV5, AAV6, AAV8), codon optimized, liver-specific promoter	• AAV-based capsids (typically AAV5, AAV8, AAVS3), codon optimized, liver-specific promoter
Transgene[31,39]	• FVIII cDNA (~9 kb) is large and structurally complex, thus needs to be truncated (eg, B-domain deleted FVIII) to be inserted into AAV-based capsids	• Full-length FIX cDNA (1.5 kb) fits into AAV-based capsids without any modifications
Target cells[39]	• Hepatocytes	• Hepatocytes
Native site of factor production[39]	• FVIII is produced in sinusoid endothelial cells of liver	• FIX is produced in hepatocytes in the liver
Highest dose used (vg/kg)[40]	• $6x10^{13}$	• $2x10^{13}$
Clinical data[39-41]	• Currently, up to 6-year follow-up from Phase 1/2 trials. Studies are ongoing • FVIII activity levels appear to decrease over time in some clinical trials • Acceptable safety profile • No occurrence of inhibitors	• Currently, up to 8-year follow-up from Phase 1/2 trials. Studies are ongoing • FIX activity levels remain relatively persistent over time in clinical trials • Acceptable safety profile • No occurrence of inhibitors

vg/kg=vector genomes per kilogram.

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Key terminology

Endocytosis: The highly conserved process by which external substances are internalized by cells.

Endogenous: Produced or synthesized within the organism or system, eg, endogenous factor IX produced from cells in the liver.

Episome: Circular DNA found in the nucleus that generally does not integrate into the chromosome and the episomes in gene therapy do not replicate.

Ex vivo gene therapy: A therapeutic technique in which some of the patient's cells are collected, genetically modified outside the body, and then delivered back into the patient for the treatment of disease.

Expression cassette (or gene expression cassette): The genetic material the gene therapy vector delivers to the target cells. It typically contains the functional gene and other regulatory elements to help direct expression.

Factor VIII (FVIII): FVIII plays a key role in the initiation and propagation of a blood clot. The lack of functional FVIII prevents the formation of a stable platelet plug clot in people with hemophilia A.

Factor IX (FIX): FIX plays a key role in the initiation and propagation of a blood clot. The lack of functional FIX prevents the formation of a stable platelet plug clot in people with hemophilia B.

In vivo gene therapy: Gene therapy that delivers a new or corrected gene directly to cells inside the patient's body.

Inverted terminal repeats (ITRs): Sequences of genetic code (145 base pairs) at each end of AAV vector genetic material. These help the therapeutic genetic materials to circularize.

Monogenic disease: Single-gene disorders, in which the disease is caused by changes in a single gene in the cells.

Neutralizing antibody (NAb): An antibody that defends hosts from a pathogen or infectious particle by neutralizing any effect it has biologically. In gene therapy, these antibodies may preexist in the host from prior exposure to naturally occurring AAVs and/or develop after exposure to the viral vector, inhibiting transduction and reducing clinical efficacy.

Promoter: Short sequences of DNA that instruct the cell when and where to read the gene that follows. Promoters can contain components that make them more likely to work in specific areas (or tissues) of the body, eg, the liver.

Serotype: Different subtypes of AAV are called serotypes. The different serotypes have distinct protein shells that influence delivery of the functional gene, activation of the human immune system, and their ability to enter different types of cells (tropism).

Transcription: The process of turning DNA into mRNA.

Transduction: The process of introducing genetic material into another cell via an infectious agent, eg, a virus or nonpathogenic vector

Transgene (or functional gene): Describes the working gene/genetic material that has been inserted in the gene delivery vehicle (vector).

Tropism: The natural attraction of a virus or vector to receptors present only on certain cells or tissues. Gene therapy researchers exploit tropisms to help different viruses, lipid particles, or other therapeutic carriers reach their targeted cells.

Vector: Delivery vehicles, or carriers, that encapsulate therapeutic genes for delivery to cells. These include both disabled viruses and nonviral vectors, such as lipid particles. Vectors don't replicate; they carry the functional gene to the cells.

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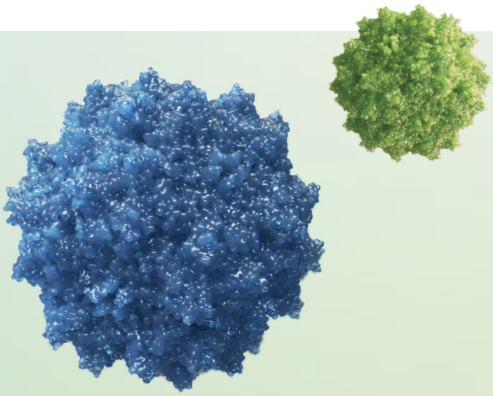
Gene therapy: Key takeaways

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, nonintegrating AAV vectors 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.[2,22,25,26]

- Hereditary retinal dystrophy
- Spinal muscular atrophy
- Hemophilia

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