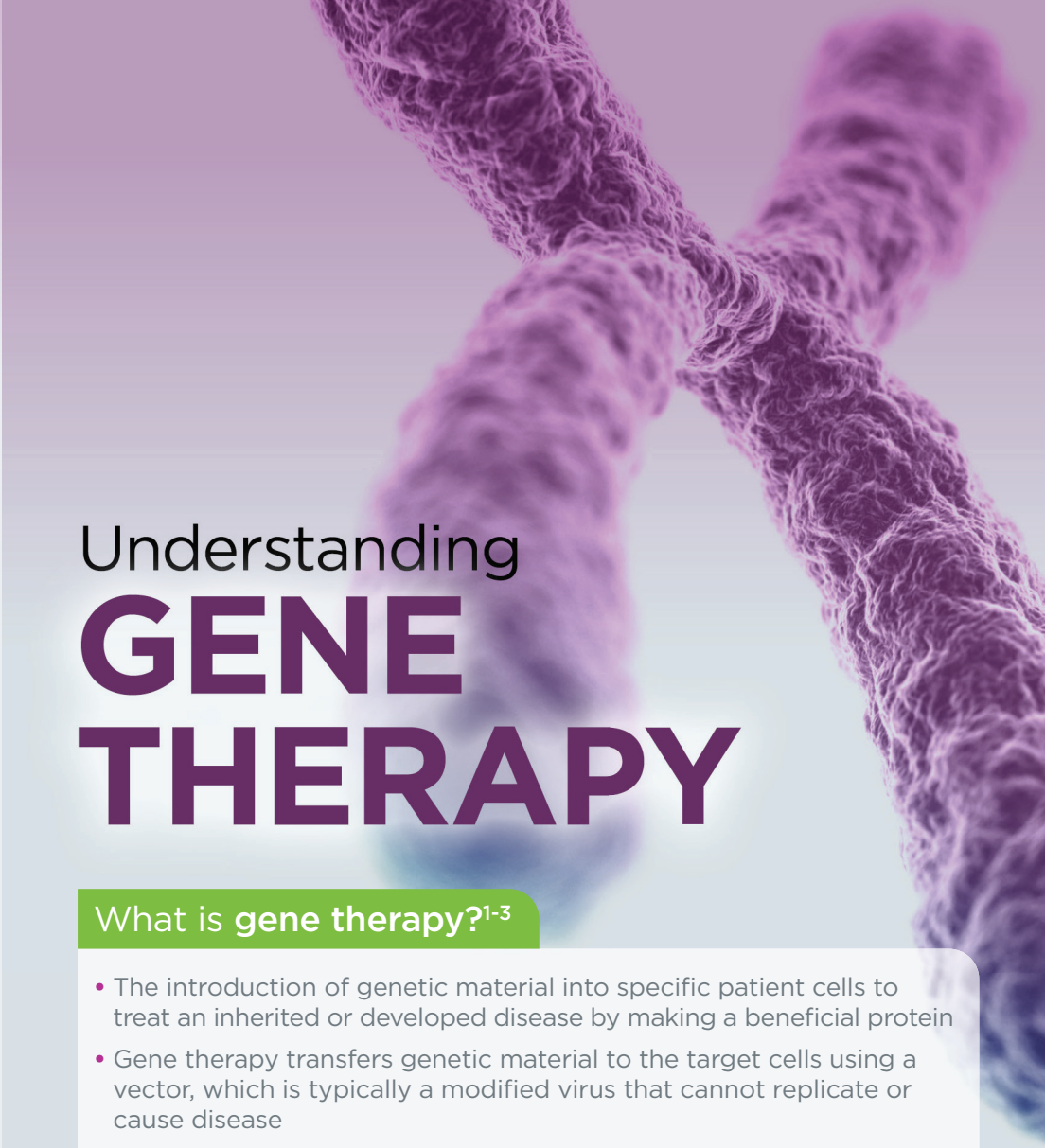

Understanding GENE THERAPY

What is **gene therapy?**[1-3]

- The introduction of genetic material into specific patient cells to treat an inherited or developed disease by making a beneficial protein
- Gene therapy transfers genetic material to the target cells using a vector, which is typically a modified virus that cannot replicate or cause disease
- Once in the patient's cells, the transferred gene produces functioning proteins
- The goal is to alleviate the symptoms or complications of the genetic disease, and possibly offer a cure

Read on to find out more about how gene therapy works.

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Types of gene therapy[2,4]

Method	Mechanism
Gene replacement	Compensates for a missing or abnormal gene by adding a functional replacement
Gene silencing	Downregulates expression of an abnormal gene
Gene editing	Edits an abnormal gene to make it functional

Gene therapy **delivery options**[4-6]

Gene therapy can be delivered to a patient's cells outside (ex vivo) or inside (in vivo) the body

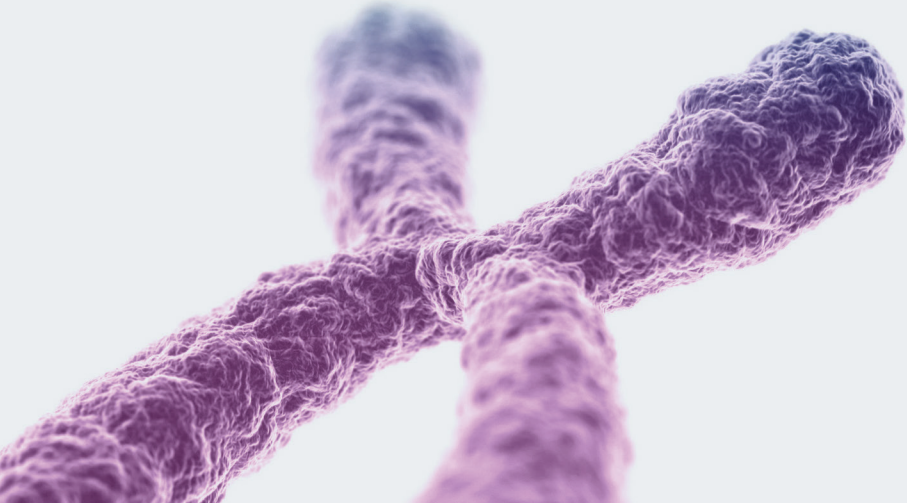
- During ex vivo delivery, the patient's cells are removed from the body, treated with gene therapy, and then reintroduced to the body
- During in vivo delivery, the gene therapy is delivered directly into the patient's body, without the need to remove any cells

Gene therapy can be delivered using nonviral and viral methods

- In gene therapy, a vector is a "vehicle" used to bring outside genetic material into a patient's cells
- Nonviral methods of gene therapy can include direct injection or transfection of DNA or delivery using nonviral encapsulation such as a liposome
- Gene therapy can be delivered by viruses that have had their viral DNA engineered or removed; this prevents the virus from replicating and causing disease

Gene therapy can be delivered by a vector that integrates into the patient's genome (integrating) or one that keeps the therapeutic gene separate from the patient's genome (nonintegrating)

- Integrating viral vectors are useful in cell types that replicate often, so that the therapeutic gene is included in all daughter cells
- Nonintegrating viral vectors are more useful in cell types like those in the brain and liver that do not replicate often



Viral and nonviral vectors used in gene therapy[3-5,7,8]

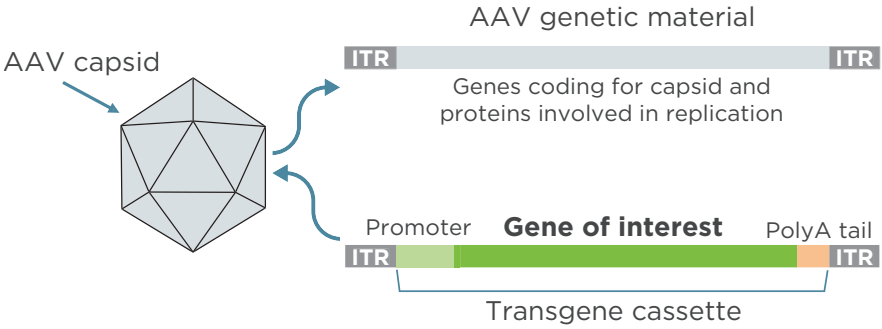
Vector type	Structure	Integrating vs nonintegrating	Advantages	Disadvantages
Liposome		N/A	• Capacity for nearly unlimited transgene size • Lower immunogenicity than viruses	• Inefficient delivery of transgene
Herpes simplex virus		Nonintegrating	• Can carry large transgenes	• May provoke an immune response
Adenovirus		Nonintegrating	• Can carry very large transgenes	• Some patients have antibodies that interfere with gene therapy delivery • Conversely, may provoke a large immune response
Retrovirus/ lentivirus		Integrating	• Can carry large transgenes	• Optimal for ex vivo delivery • Random integration into host genome may have unintended effects on the cell's biology
Adeno-associated virus		Nonintegrating	• Does not cause disease in humans and is less likely to provoke an immune response • Optimal for in vivo delivery to targeted tissues	• Limited to small to medium transgenes • Some patients have antibodies that interfere with gene therapy delivery

Adeno-associated viruses (AAVs) are the most common type of vector used for delivery of gene therapies[3,6-8]

- One of the smallest known viruses
- Does not cause any human disease
- Consists of a protein shell (capsid) containing a small, single-stranded DNA genome
  - The genome contains genes allowing for AAV replication, flanked by inverted terminal repeats (ITRs) that are required specifically for replication and packaging of the genome

AAV's genetic material is replaced with the gene of interest to create a recombinant AAV (rAAV)[3,6-8]

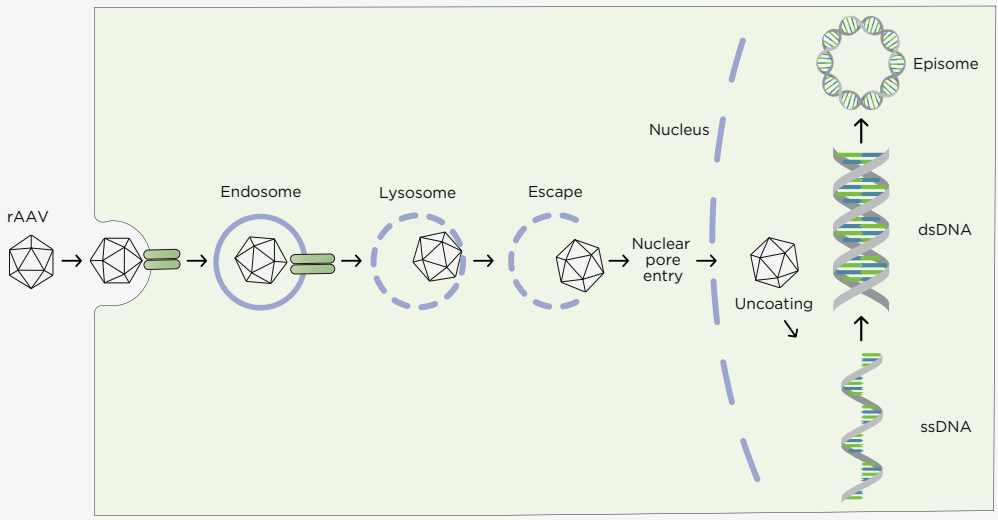
- The ITRs are the only viral DNA retained in order to guide gene replication and packaging during rAAV production



AAV, adeno-associated virus; ITR, inverted terminal repeat.

The **rAAV crosses the target cell membrane** and transports the new gene into the cell nucleus[3,6-8]

- rAAV is recognized by receptors on the cell membrane, triggering internalization of rAAV into the cell
- rAAV is transported uncoated to the cell nucleus and capsid
- The single-stranded DNA is converted to a double strand and forms a circular episome in the cell nucleus, separate from the cell's chromosomes



rAAV, recombinant adeno-associated virus.

How is rAAV targeted to specific types of cells?[3,6-9]

- Many different AAV serotypes have been identified (*see* Serotypes vs tropism)
- Each serotype has the propensity to infect different types of cells via different cell surface receptors
- Tissue-specific gene promoters can also be used to prevent expression of the introduced gene in unwanted cells or tissues

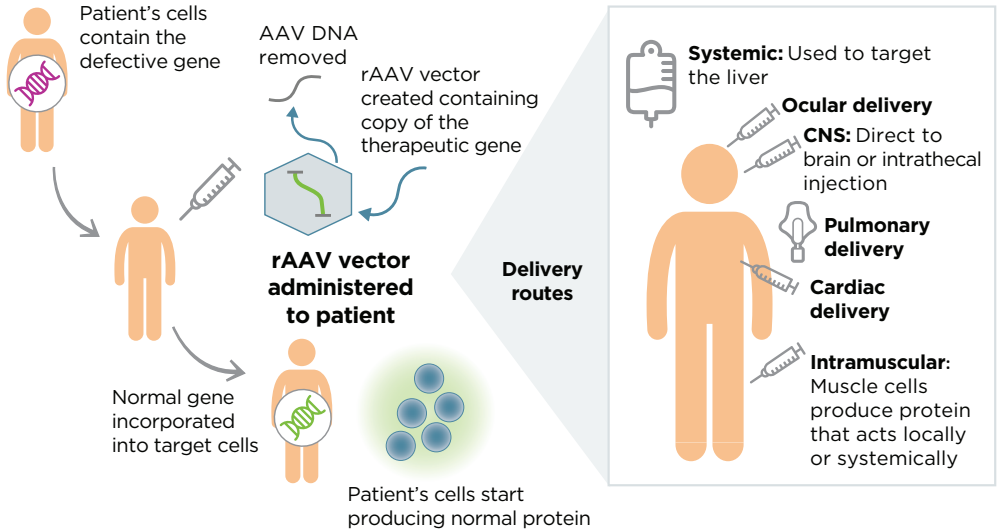
Serotypes vs tropism[6,7]

A **serotype** classifies viral strains by their surface proteins. Different serotypes can vary in the cellular receptors with which they interact. These receptors, along with variations in viral capsid structure, determine the **tropism**, or target cells/tissue, of each serotype.

Common AAV serotypes and their target tissues[3,6,7]

Serotype	Target tissue
AAV1	Lung, muscle, eye
AAV2	Liver, muscle, brain, eye
AAV3	Inner ear
AAV4	Eye
AAV5	Lung, muscle, liver, eye, brain
AAV6	Lung, heart, bone marrow, liver, muscle
AAV7	Muscle
AAV8	Liver, eye, muscle, heart, pancreas
AAV9	Heart, brain, spinal cord, muscle, lung, liver
AAVrh.10	Brain, liver
AAVhu.37	Liver
AAVrh.74	Muscle

Delivery of the rAAV vector into the patient's cells[3]



AAV, adeno-associated virus; rAAV, recombinant adeno-associated virus; CNS, central nervous system.

Challenges of gene therapy[6,10,11]

- Gene therapies are very expensive to manufacture and difficult to produce at high volumes
- The rAAV vector cannot deliver large genes (size limit: ~5 kilobases); as a result, some genetic diseases are more challenging to treat with AAV
- The patient's immune system may inhibit gene therapy via
  - Pre-existing antibodies against a given serotype of AAV
  - An immune response to the rAAV treatment, preventing effective re-administration

LOOKING TO THE FUTURE of gene therapy[3,6,7]

- More than 50 rAAVs are in clinical development for a wide variety of genetic diseases
- Engineering and novel AAV discovery are likely to provide AAV vectors with specialized functions on demand
- There is hope that these rapidly progressing developments will result in exciting new therapeutic strategies for various new indications, helping many more patients whose lives are affected by genetic diseases

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1. American Society of Gene and Cell Therapy. Different approaches. https://www.asgct.org/education/different-approaches. Accessed April 8, 2020. **2.** US National Library of Medicine. Help me understand genetics: gene therapy. February 11, 2020. https://ghr.nlm.nih.gov/primer/therapy/genetherapy. Accessed April 8, 2020. **3.** Naso MF, et al. *Bio Drugs*. 2017;31(4):317–334. **4.** Maeder ML, et al. *Mol Ther*. 2016;24(3):430-446. **5.** Petrich J, et al. *J Pharm Pract*. 2019;897190019854962. **6.** Wang D, et al. *Nat Rev Drug Discov*. 2019;18(5):358-378. **7.** Balakrishnan B, et al. *Curr Gene Ther*. 2014;14(2):86-100. **8.** Kotterman MA, et al. *Annu Rev Biomed Eng*. 2015;17:63–89. **9.** Herrmann A-K, Grimm D. *J Mol Biol.* 2018;430(17):2626-2640. **10.** US Department of Health and Human Services, US Food and Drug Administration. Human gene therapy for rare diseases: guidance for industry. January 2020. https://www.fda.gov/media/113807/download. Accessed April 8, 2020. **11.** Nidetz NF, et al. *Pharmacol Ther.* 2020;207:107453.

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