Hemophilia Gene Therapy: Key Principles

Pfizer

Contents

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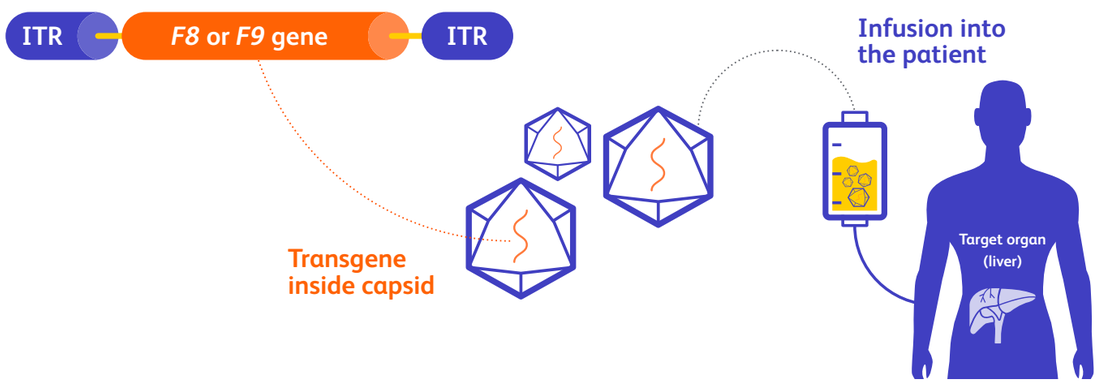
Gene therapy

Gene therapy is the introduction, removal, or change in genetic material—specifically DNA or RNA—into the cells of a patient to treat a specific disease.[1] In its broadest interpretation, the term "gene therapy" may refer to:

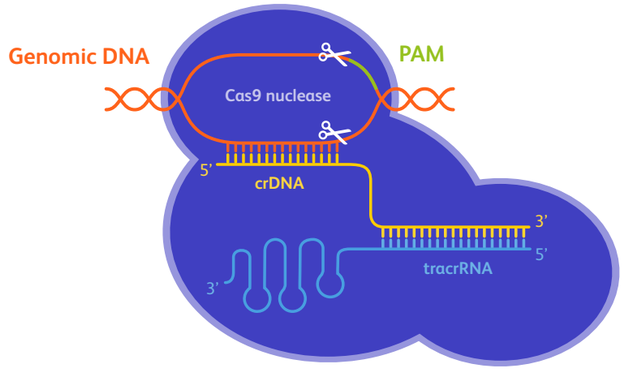
Gene transfer[2] (gene addition[1])

> Gene transfer is the addition of a functional copy of a missing gene or augmentation of a gene that is non-functional into target cells to produce more of a protein[1,2]



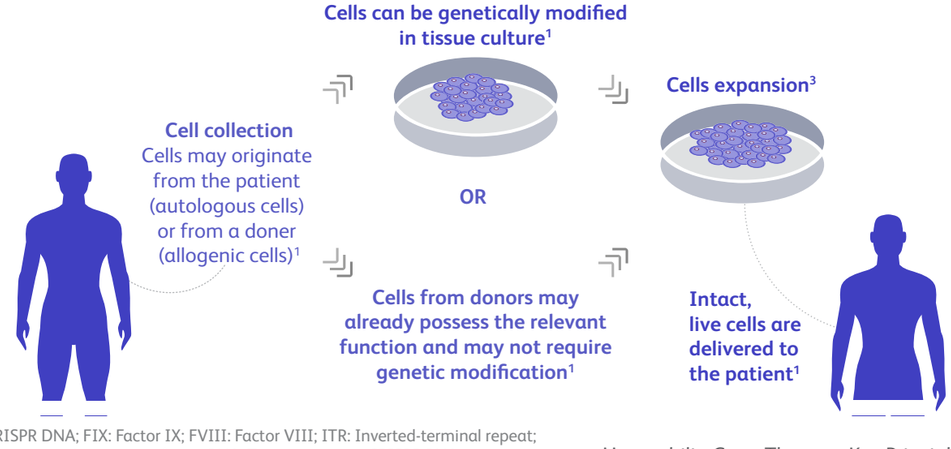
Gene Editing[1]

> Gene editing is the removal, disruption or correction of faulty elements of DNA within the gene[1]



Cell therapy[1]

> Cell therapy is the transfer of intact, live cells into a patient[1]



crDNA: CRISPR DNA; FIX: Factor IX; FVIII: Factor VIII; ITR: Inverted-terminal repeat; PAM: Protospacer adjacent motif; tracrRNA: Trans-activating CRISPR RNA.

Principles of gene therapy

What is gene therapy?

Capsid

- The capsid is the protein shell of a virus that protects the genetic material while interacting with the host environment[4]

Transgene

- The transgene is the exogenous DNA sequence that will be introduced into the genome of a host (e.g. *F8* or *F9* gene)[5]

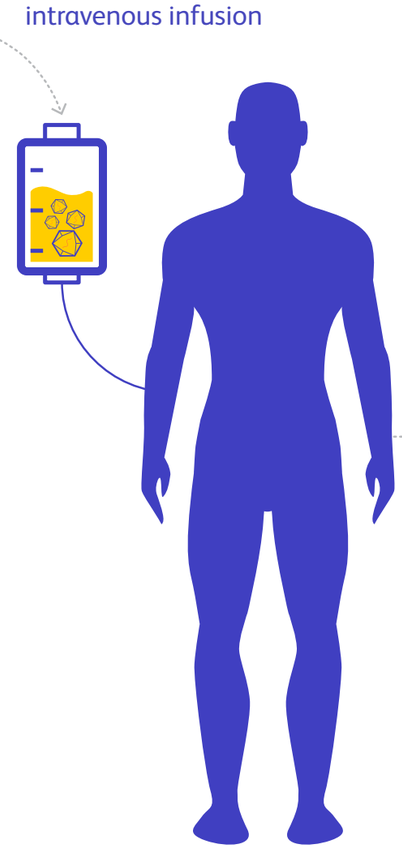
Transgene inside capsid (vector)

- The success of gene therapy depends on effective vehicles for gene transfer, termed 'vectors'[6]
- A vector is a transgene encapsulated into a capsid. Vectors are based on viral platforms but are not viruses[6]
- There are several types of vectors, but two main ones are under investigation in clinical trials:[6,7]

  – Retroviruses (including lentivirus)
  – AAV (adeno-associated virus)

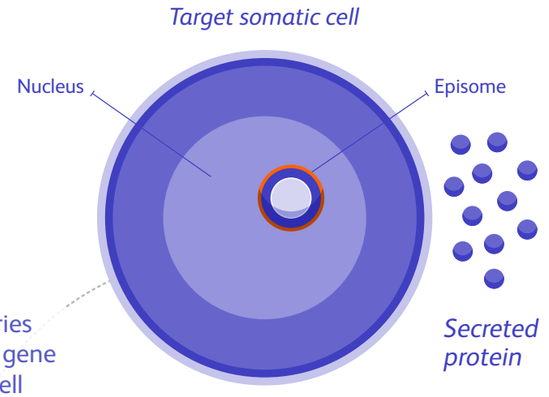
- Recombinant AAV (rAAV) is the vector of choice for hemophilia gene therapy[5]

Gene transfer via intravenous infusion



Vector carries functional gene to target cell (e.g. hepatocytes)

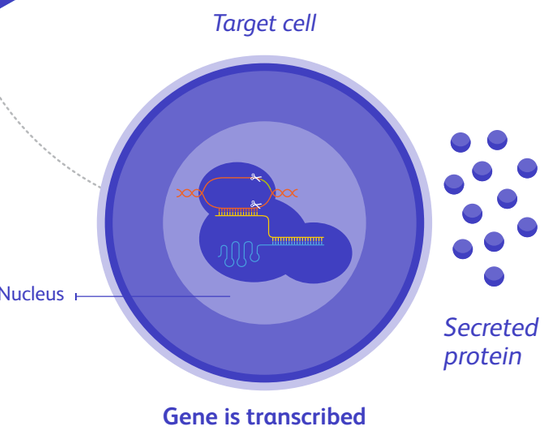
Gene transfer



Gene transfer: Adding a functional gene that is not passed on to daughter cells

- Transgene exists as an episome[8] (a segment of DNA that can exist and replicate autonomously in the nuclear cytoplasm) to replace or supplement a dysfunctional gene.[5,8] Once delivered to the cell, the episome exists in the nuclear cytoplasm[8,9] – the DNA is predominantly non-integrating[8,10]

Gene editing



Gene editing: Permanent removal, disruption or correction of faulty elements of DNA within the gene[1,11]

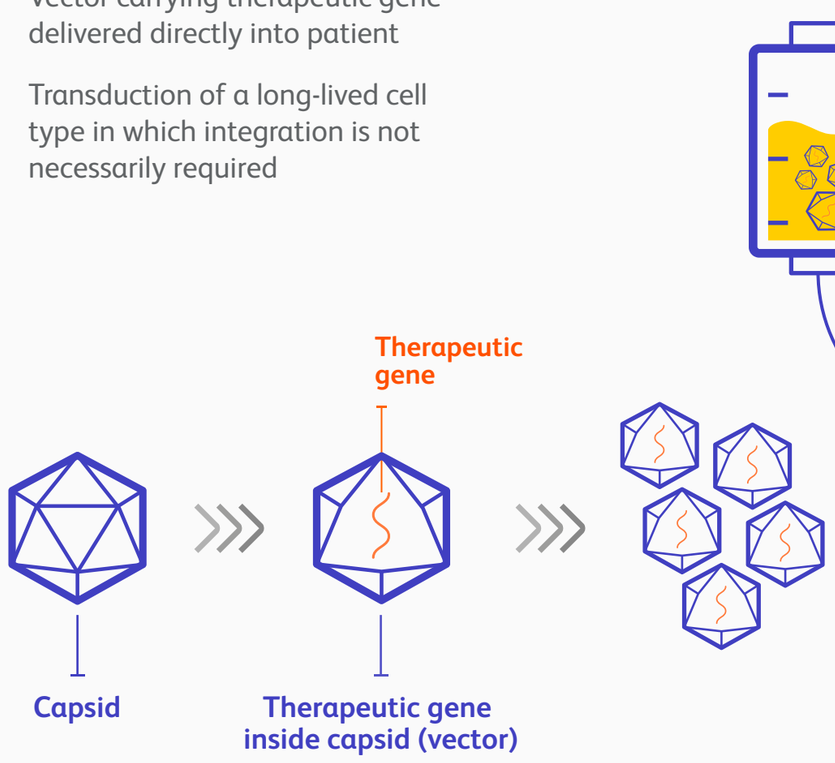
- Organism's DNA changed through the addition, removal, or alteration of genetic material at precise locations in the genome[11]

AAV: Adeno-associated virus.

How is gene therapy administered?

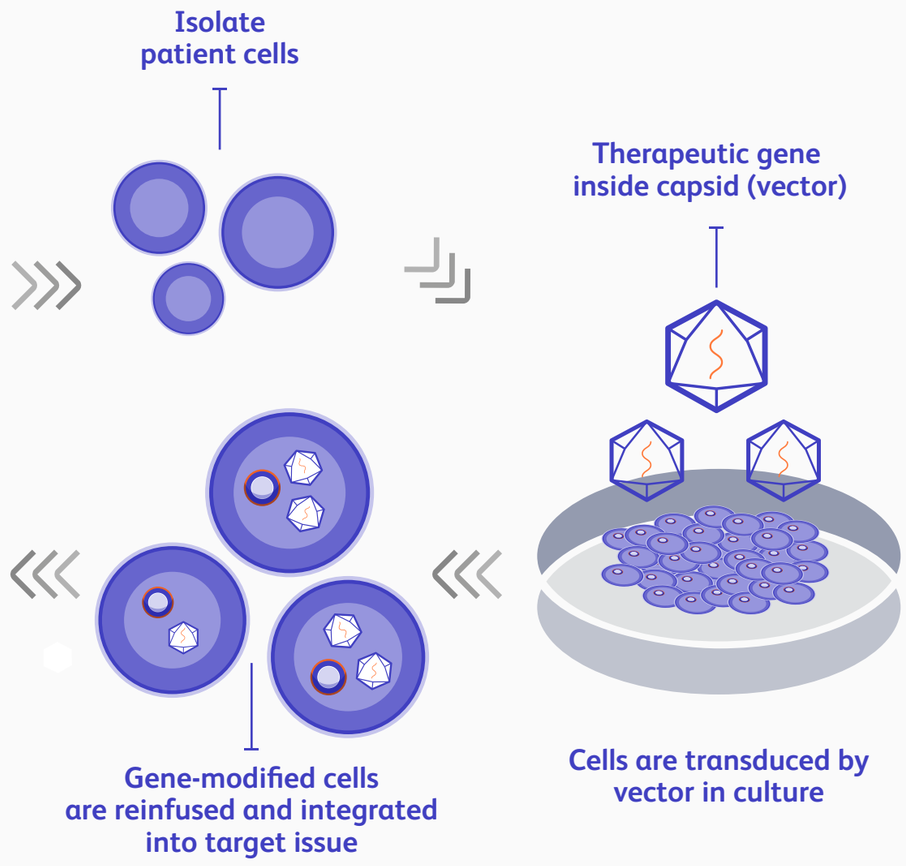
> *In vivo*[7]

- Vector carrying therapeutic gene delivered directly into patient
- Transduction of a long-lived cell type in which integration is not necessarily required

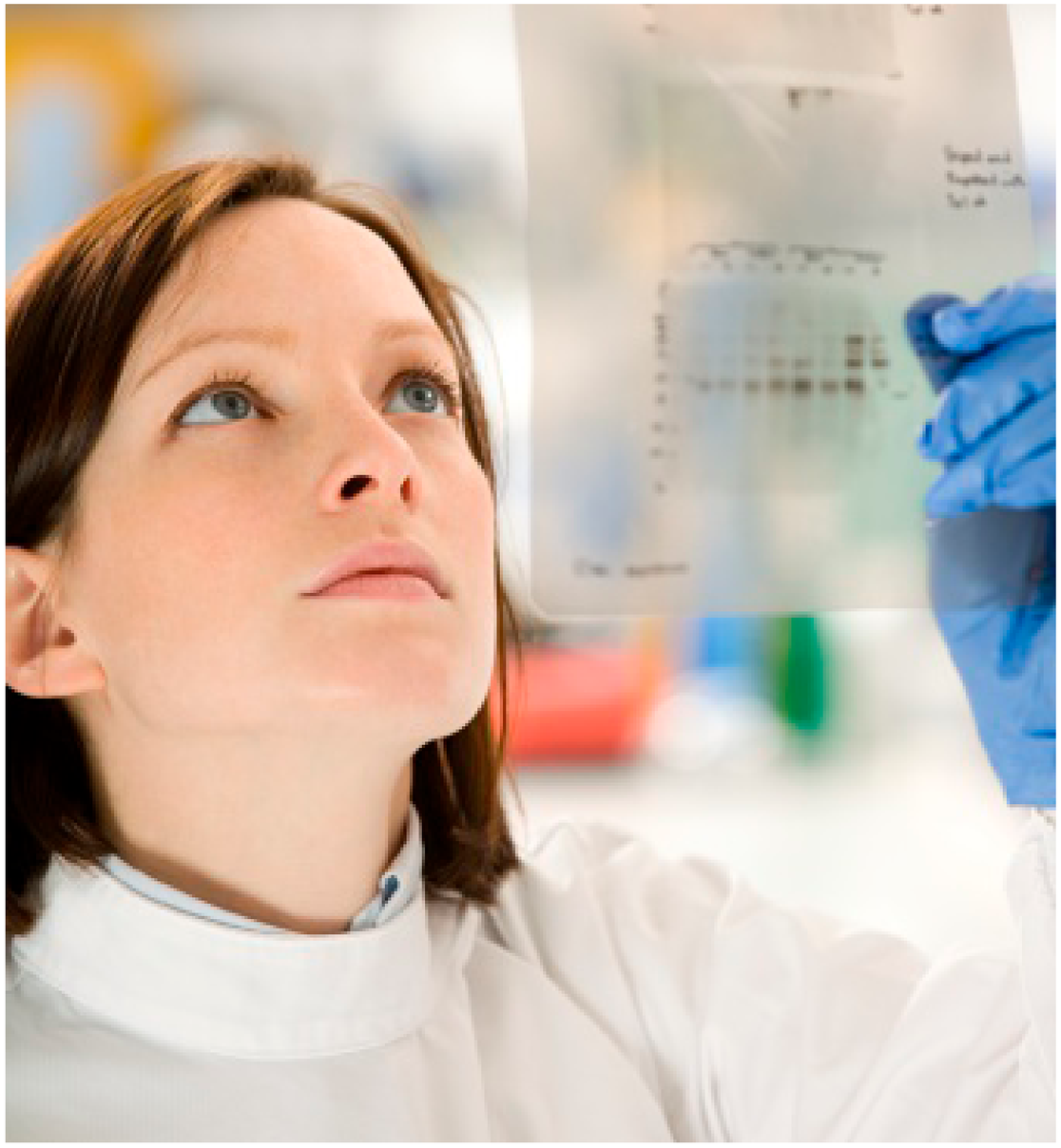


> Throughout this brochure, when referring to ***gene therapy*** in the context of hemophilia, the focus will be on ***in vivo gene transfer***

> *Ex vivo*[7,12]



Rationale for gene therapy in hemophilia



> Gene therapies for hemophilia are currently being studied to determine their safety and efficacy. No gene therapies for hemophilia have been approved for use.

- Both hemophilia A and B are well-characterized diseases, each caused by a mutation in a single gene (*F8* or *F9*) that results in the lack of a single protein (FVIII or FIX, respectively)[10]
- Tight regulation of factor levels is not required to see a potential therapeutic benefit of gene therapy.[10] Even a small increase in circulating levels of FVIII or FIX may modify the bleeding phenotype.[2] Therefore, the delivery of new copies of a single functional gene to a patient, and the initiation of expression of the missing factor to even some degree, may have the potential for sustained therapeutic effect and modification of the patient's bleeding phenotype.[10]
- As observed in clinical practice, coagulation factor levels can have a wide therapeutic window[10]
- Laboratory assays are available to measure plasma factor level[10]
- *F9* and modified *F8* gene sequences are available[13] for packaging into rAAV vectors, which act as the gene-delivery vehicles[14]

rAAV vectors can carry DNA up to a maximum size of ≈5 kb[15]

The *F9* gene is relatively small (1.6 kb)[16]

The *F8* gene is 7 kb, which is too large to insert into an rAAV vector[15]

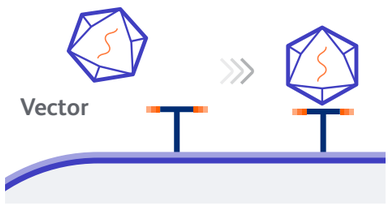
Deleting the B-domain of the *F8* gene takes the size down to ≈4.4 kb, which is small enough for gene therapy vectors[15]

In early hemophilia B gene therapy studies using wild-type *F9* the infusion of a single dose resulted in therapeutic factor expression. However, this research also identified challenges around the immune response[17]

Gene therapy vectors

How does rAAV deliver the transgene to the target tissue?

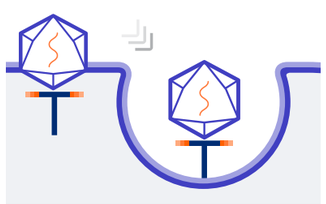
Receptor binding



1. The vector binds or attaches to receptors on the target host cell surface[18,19]

2. A number of AAV serotypes exist. Each serotype includes proteins that bind to surface receptors on specific cell types.[20] Those with specificity for the target organ can be utilized to support delivery of the transgene

Endocytosis

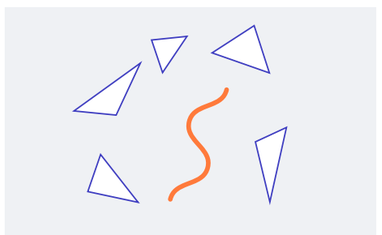


3. Vector taken into target cell by endocytosis[18,19]

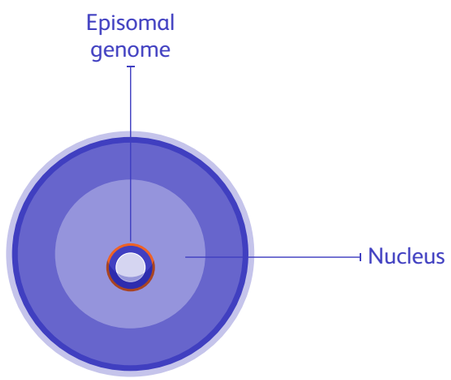
Endosomal escape

4. Vector trafficked from early to late endosomes and delivered to the cell nucleus[19]

Uncoating



5. Uncoating: inside the nucleus, the capsid is removed, releasing the genetic material (transgene)[18,19]



6. The transgene is copied and transcribed.[19] The transgene is mostly maintained episomally as a concatemer of DNA (a DNA molecule made up of multiple copies of the same genome linked together in tandem) and is predominantly non-integrating[8]

Why is rAAV the most commonly used vector to date for hemophilia gene therapy?

- **Lack of pathogenicity:** not associated with known human disease[21]
- **Defective replication:** recombinant AAV vectors have their viral coding sequences removed, retaining only the inverted terminal repeats that allows the therapeutic gene to be packaged inside the viral capsid, so the vector cannot replicate within the patient[21]
- **Predominantly non-integrating:**[21] transgene remains largely outside the host chromosomal DNA and persists as episomes in the nucleus of transduced cells[8]
- **Ability to establish long-term transgene expression:**[17] although lost with each cell division, the expression can be maintained in post-mitotic tissues such as the liver[22]
- **Specific serotypes can be used to ensure targeting to the liver:** capsid proteins can guide the transgene to the target cell / organ[7]

Considerations for capsid and transgene choice

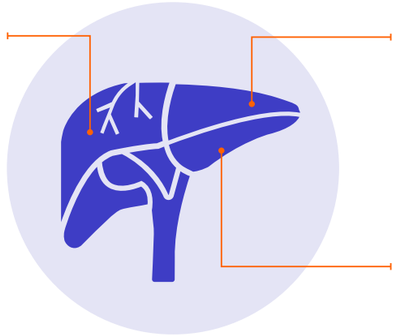
How can we optimize the rAAV vector?

- Enhance different features by designing vectors to contain domains from different AAV serotypes[19,20]
  - These “hybrid” vectors are designed for more efficient and specific delivery to a target cell or tissue[19,20]
- Choose a vector with appropriate tropism – for hemophilia, this should be tropism for hepatocytes[23]

Why is the liver the target for gene therapy for hemophilia?

FVIII and FIX are naturally produced in the liver[9,24]

- FVIII is naturally generated by liver sinusoidal endothelial cells[9,24]
- FIX is naturally generated by hepatocytes[9,24]



Post-mitotic hepatocytes are long-lived[24]

Specific AAV serotypes can support transduction of the liver cells (e.g. AAV2, AAV5, AAV8 or AAV9)[20]

How can we optimize the transgene?

- Use of high-specific-activity gene variants (e.g. FIX Padua)[24]
- Design the transgene to optimize its size (to meet packaging capacity restrictions) – for example, by using B-domain-deleted FVIII[24]
- Codon and promoter optimization can be used to increase gene expression[24]

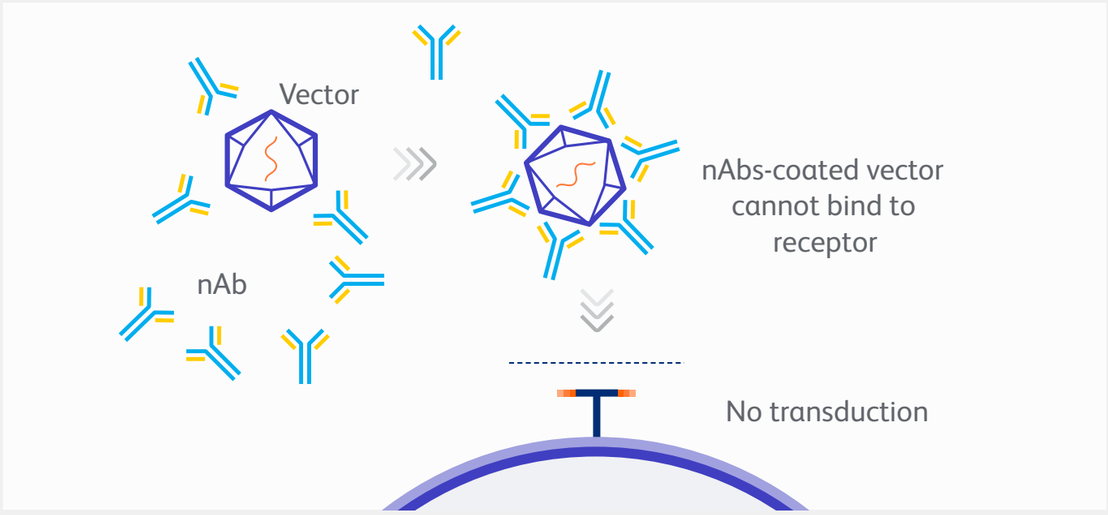
Considerations for effective transduction

Why is immunity an important challenge in gene therapy?

- Some people have pre-existing antibodies[8] to AAV from naturally occurring infections and exposure to wild-type AAV*
- Vector components and the transgene may be seen as ‘foreign’ by the immune system, potentially resulting in an immune response[5,22,25]

What is the impact of pre-existing immunity on gene therapy?

- The pre-existing AAV-specific antibodies that may result from prior AAV infections[21,26] can be neutralizing (nAbs) or non-neutralizing[26]
- nAbs can bind to capsids and **may prevent transduction**[22,27]
- Since any immune response against the vector may have an impact on the expected therapeutic effect, many early gene therapy trials recruited only seronegative patients[21,22]



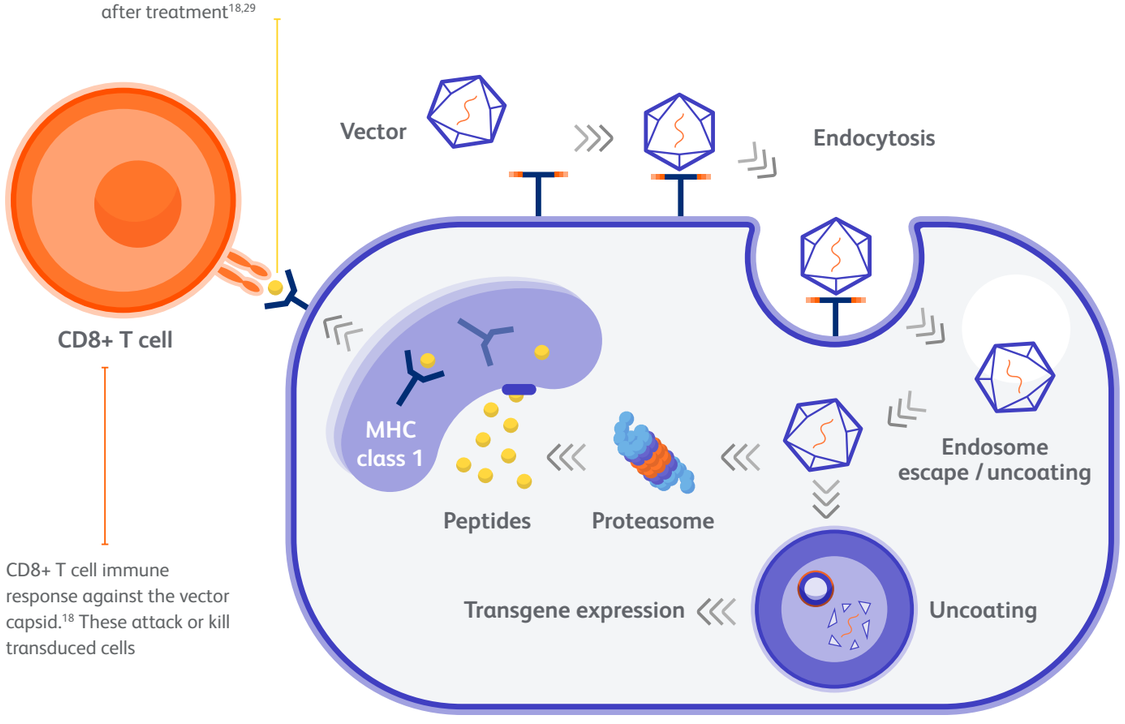
*Estimates of prevalence vary for each AAV serotype depending on the study. It also depends on the titer cutoff used to define seropositivity and the assay utilized in the study – assays to measure nAbs have not yet been standardized.[28]

Following gene therapy administration using current approaches, a potent humoral immune response develops, blocking further rAAV delivery with the same serotype – rAAV infusion is therefore presently limited to a single dose[25]

Considerations for gene expression

What is the impact of the cellular immune response?

Activated T cells can destroy transduced hepatocytes, resulting in a loss of gene expression[29]



Future considerations

What might the future hold?

Patient eligibility and expectations of treatment

- Observed interpatient variability in attaining and sustaining expression levels long-term should be considered, since not all patients will achieve the same increase in factor levels[17,30]
- Transduction of a pediatric liver may lead to a dilutive effect due to rapid hepatocyte division at this age.[22] Episomal DNA is predominantly non-integrating and will eventually be diluted over time as the transduced cell undergoes repeated rounds of replication, with the rate of loss of transgene expression depending on the rate of cell division.[8]
- Currently, gene therapy can be administered only once because of immune responses[31] – patients should be made aware of this potential limitation
- Patient expectations of gene therapy and outcomes should be considered:
  - The coreHEM initiative identified expectations of varied stakeholders about gene therapy, including their expectations around frequency of bleeds, duration of expression, factor activity levels, and chronic pain[32,*]

Long-term efficacy and safety

- As a field of research, clinical gene therapy is still in its early stages
- Long-term follow-up and postmarketing surveillance of gene therapy products will need to be established[33]

Large-scale manufacturing

- Large-scale manufacturing technologies need to be established in accordance with current good manufacturing practice (cGMP) regulations to yield the purified vector quantities required[34]

*Stakeholders included patients, clinicians, researchers, regulators, research agencies, health technology assessors, payers, and drug developers[32]

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