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Gene Therapy Series 2: Delivery Systems In Gene Therapy, Including Viral Vectors

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Summary

Continuing with the series of articles on gene therapy, this article will discuss the various delivery methods for gene therapies with a focus on viral vectors.

The article also discusses the classification of gene therapy methods by the site of delivery, and a comparison of the characteristics of viral vectors used in gene therapy.

Examples of public gene therapy companies using different vector delivery systems for gene therapy are also provided.

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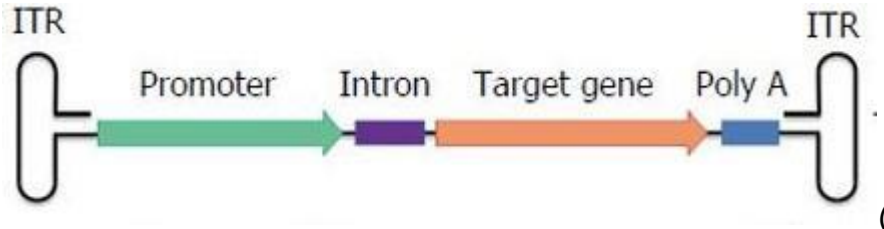
What is transgene?

A transgene is a new therapeutic DNA segment that is to be inserted into the host cell. In addition to the gene needed to make the desired protein, the transgene sequence also contains few other non-coding regions, for example, the enhancer, promoter, and intron.



(An example of a transgene)

Human cells are surrounded by a protective barrier and therefore, getting the transgene containing the normal sequence of a gene into the human cell is not easy, that is why the vectors are important. Vectors are essentially the vehicles that help to carry the transgene inside the human cells.

Why are viruses commonly used as vectors in clinical gene therapy?

Viruses are very good at infecting human cells, that is, they insert their own genetic material inside the human cell. In the process of gene therapy, viruses are manufactured and modified to carry the desired transgene instead of their own DNA, and they are also modified so that they are not infectious and are not able to replicate and spread the disease like viruses normally do. Viral vectors have been utilized in approximately two-thirds of the worldwide clinical trials. While there are advantages of using viral vectors, there are also some disadvantages like some of the genes may be too big to fit inside a viral vector. The second problem is an immune response, that is, the human body will recognize a viral vector as a foreign object and launch an antibody response, which may reduce the efficacy of the gene therapy. Commonly used viral vectors in gene therapy trials include adeno-associated virus (AAV) and lentiviruses.

Non-viral methods of transgene delivery

Although viral vectors are being used in two-third of gene therapy programs, there are some non-viral methods of delivering the transgene as well which include:

1. Physical methods: These include mechanical, electrical, ultrasonic, hydrodynamic, or Laser-based technology, which can be used to temporarily penetrate the host cell membrane and deliver the transgene inside it.

2. Chemical methods: These include positively charged areas that can penetrate the cell membrane or other carrier molecules.

Classification of gene therapy methods by the site of delivery

Before we move on to the more detailed discussion of viral vectors, let us explore some definitions of the classification of gene therapy methods by the site of delivery.

Ex vivo delivery: Ex vivo delivery means outside the body. In this case, the cells are removed from the patient's body, sent to the lab, modified by delivery of the transgene using an appropriate vector, and then infused back into the patient. *Ex vivo* delivery is usually considered a safer method of gene therapy because there is no risk of off-target undesired side effects. Moreover, the risks of immunogenicity against the modified host cells are almost minimal. However, there are also certain limitations because a limited number of cells can be treated at one time. Examples of gene therapy methods using *ex vivo* delivery include CAR-T therapies like Yescarta and Kymriah.

In vivo delivery: By definition, this means inside the body. In this method, the host DNA modification happens inside the body by infusing or injecting the vector containing the transgene systematically into the patient. Although this method is more convenient than the *ex vivo* method, there are some risks also, for example, the viral vector containing the transgene content inserted into some other cells other than those targeted, which may result in off-target side effects. Some of these risks can be mitigated by using a

vector that is specific for a certain type of cell or tissue, as well as choosing a promoter that is specific to the desired tissue. Another risk is the risk of immunogenicity, that is, the body's immune system will launch an antibody response against the viral vector containing the transgene resulting in decreased efficacy. The risk of immunogenicity is lower by using the non-viral vectors and also by using some viral vectors which have comparatively lower immunogenicity. In addition, the patients can be given steroids to dampen the immune response before administering gene therapy. Examples of *ex vivo* gene therapies are hemophilia gene therapies from BioMarin (BMRN) and Spark therapeutics (NASDAQ:ONCE) which target the liver. Currently, *in vivo* gene therapies dominate the early clinical gene therapy space outside oncology.

In situ delivery: By definition, this means inside the body using a site-specific injection. This describes a more specific type of *in vivo* gene therapy where the transgene is administered directly to the target tissue. In this case, the local administration of the viral vector limits systemic exposure and reduces the immunogenicity and off-target side effects. An example is Spark Therapeutics' Luxturna to treat a form of inherited blindness which is given as a local injection in the eye.

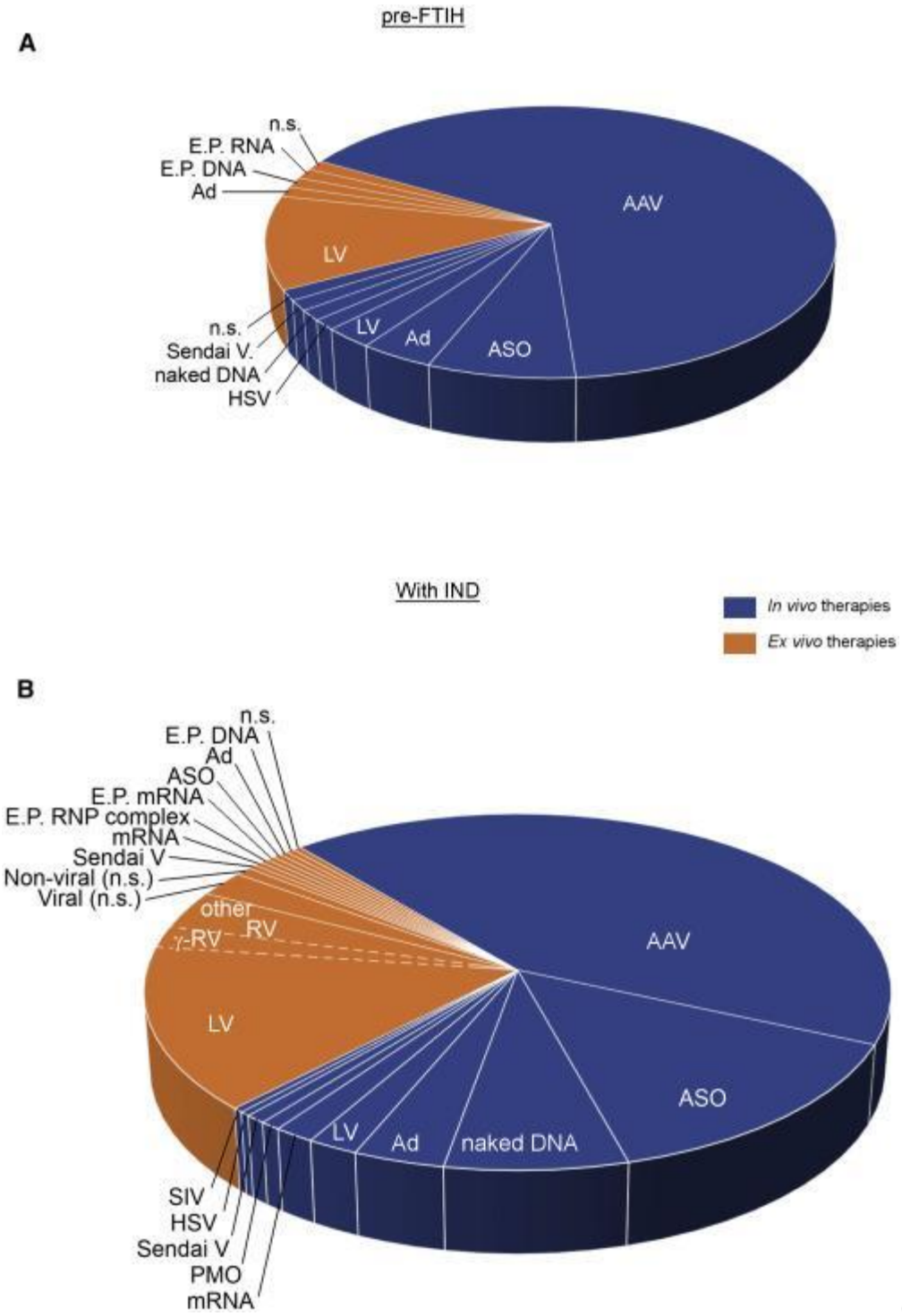
A detailed discussion of viral vectors

Viral vectors can also be subdivided into two categories: integrating and non-integrating. Integrating viral vectors combine inside the therapeutic transgene into the host DNA and make a permanent change, that will also transfer to any daughter cells. This leads to long-term expression of the corrective gene.

Non-integrating viral vectors transfer the transgene in the nucleus but not into the host DNA. The transgene exists inside the host cell but does not combine with the host DNA. Because the corrective gene is not integrated into the host DNA, it is not passed on to their daughter cells. Examples include AAV, adenovirus, and herpesvirus.

Two of the most commonly used viruses as vectors in gene therapy include adeno-associated virus (AAV) and lentiviruses (LV).

AAV-based transgene delivery is the most common form of *in vivo* gene delivery. In the *ex vivo* delivery, lentivirus vectors dominate the delivery approaches. In the overall landscape, currently, AAV based *in vivo* therapies dominate the gene therapy space outside oncology. Some examples of AAV based gene therapies that have already reached the market include Glybera.



(Analysis of Delivery Methods in the Early Clinical Gene Therapy, GT Pipeline. Source: Molecular Therapy Journal)

From the above figure:

Fig. A: 81 GTs are awaiting FTIH (pre-FTIH), with 84% (68) *in vivo* therapies, 65% (53) *in vivo* AAV therapies, and 11% (9) LV-mediated *ex vivo* therapies. *Fig. B:* 255 GTs in phase 1/2 clinical trial (with IND) are made up of 73% (187) *in vivo* therapies, 43% (109) *in vivo* AAV therapies, and 15% (38) LV-mediated *ex vivo* therapies. FTIH, the first time in human; GT, gene therapy; n.s., non-specified.

The table given below summarizes the characteristics of viruses used in gene therapy:

Viral Vector	**Size**	**DNA/Insert size**	**Infection**	**Expression**	**Potential limitations**
Retrovirus	7-11 kb (ssRNA)	8 kb	Dividing cells	Stable	Insertional mutagenesis potential
Lentivirus	8 kb (ssRNA)	9 kb	Dividing and non-dividing cells	Stable	Insertional mutagenesis potential
Adenovirus	36 kb (dsDNA)	8 kb	Dividing and non-dividing cells	Transient	Strong antiviral immune response limits repeat administration
Adeno-associated Virus, AAV	8.5 kb (ssDNA)	5 kb	Dividing and non-dividing cells	Stable: integration in one spot of the host genome	Required helper virus for replication: Difficult to produce pure stocks of AAV free of helper virus
Herpes Simplex Virus, HSV	150 kb (dsDNA)	30-40 kb	Dividing and non-dividing cells	Transient	No gene expression during latent infection

Vaccinia virus	190 kb (dsDNA)	25 kb	Dividing cells	Transient	Potential cytopathic effects

(Source: Guggenheim's Gene Therapy Primer)

Since AAV is the most common vector for transgene delivery of gene therapy, let's dig deeper into various AAV types. Different AAV types are believed to be optimized for targeting diseases related to various organs and are summarized in the table given below.

Tissue	Optimal Serotype
CNS	AAV1, AAV2, AAV4, AAV5, AAV8, AAV9
Heart	AAV1, AAV8, AAV9
Kidney	AAV2
Liver	AAV7, AAV8, AAV9
Lung	AAV4, AAV5, AAV6, AAV9
Pancreas	AAV8
Photoreceptor Cells	AAV2, AAV5, AAV8
RPE (Retinal Pigment Epithelium)	AAV1, AAV2, AAV4, AAV5, AAV8
Skeletal Muscle	AAV1, AAV6, AAV7, AAV8, AAV9

(Source)

Examples of some public companies using different delivery systems for transgene delivery include:

- *AAV:* AXO-AAV-GM1 and AXO-AAV-GM2 by Axovant Gene Therapies (AXGT). Amicus Therapeutics' (FOLD) preclinical GT program targeting Fabry's disease, Pompe's disease, Niemann Pick's disease, etc., VY-AADC targeting Parkinson's disease by Voyager Therapeutics (VYGR).
- *Lentivirus:* LentiGlobin by bluebird bio (BLUE), AXO-LENTI-PD by Axovant Gene Therapies, AVR-RD-01 for Fabry's disease by AVROBIO (AVRO).

- *Herpes Simplex, type 1, HSV-1:* KB103 targeting recessive epidermolysis bullosa by Krystal Biotech (KRYS).
- *Retrovirus:* TOCA-511 and TOCA-FC by Tocagen (TOCA) in oncology.
- *Electroporation:* MaxCyte (Lon: MXCT).

In the third article in the gene therapy series, I will discuss the manufacturing process for gene therapy (it is very complex, but I will make it as simplified as possible). After this, I will move on to discussing the competitive landscape for gene therapies in different diseases (one article on each genetic disease), starting with Duchenne muscular dystrophy (DMD).

Disclaimer

I am/we are long TOCA, AXGT, AVRO, FOLD, BLUE.