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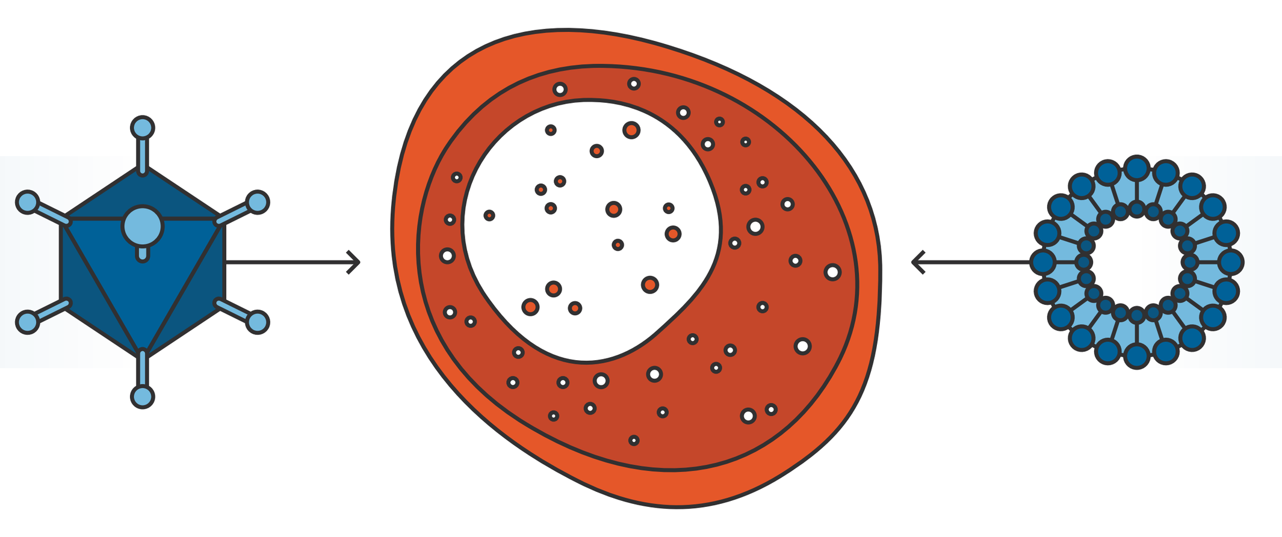
Therapeutic Innovation, Delivered.

Analyzing Gene Therapy Vectors as They Evolve



Gene therapy is growing rapidly as a therapeutic field, spurred by evolutions in the delivery mechanisms used to transfer genetic material to patients' cells with more precision, more safely. **Future success in gene therapy will be dependent on vector improvements to ensure the right cargo is delivered to the right cell at the right time.**

The Main Delivery Systems

The success of a gene therapy depends largely on the **efficiency of the vector to transduce** (when using a viral vector) **or transfect** (in the case of a non-viral method) **the cell.**

VIRAL VECTORS

Frequently used viral vectors include:[1]

Adenovirus (Ad) | Adeno-Assoicated Virus (AAV)
Retrovirus | Lentivirus | Herpesvirus

NON-VIRAL VECTORS

Frequently used non-viral vectors include:[1]

Lipid Nanoparticles (LNPs) | Cationic Polymers
Lipofection | Naked Plasmid DNA

Continually Improving Vector Safety & Efficacy

While there are a number of factors that can impact specific vector safety and performance, **each type of gene delivery method has unique advantages and challenges.**[2, 3, 4]

VIRAL VECTORS

- **High DNA transferring efficiency**
- **Tropism can be tailor made**
- **Increased potential for preexisting and induced immune responses**

NON-VIRAL VECTORS

- **Reduced potential for immune responses (non-toxic and non-infectious)**
- **No limitations in size of transgenic DNA**
- **Less efficient gene transduction**

MODIFIED & NOVEL VEHICLES: Combining the Best of Both Worlds

In the quest to create safer and more efficient vectors, **scientists are attempting to combine the best features of different viruses into hybrid vectors,** and developing novel non-viral delivery methods to improve transfer efficiency without sacrificing safety. **Some examples include:**

HYBRID VIRAL VECTORS

Ad/AAV Hybrid

Combines packaging signals from Ad and integration signals from AAV, creating Ad-like viral particles to reduce chance of contamination of helper Ad virus.[6]

Ad/Retrovirus Hybrid

Combines high transduction efficiency of a gene-deleted adenoviral vector and the long-term genome-integrating potential of a retrovirus like foamy virus (FV), which has the largest packaging capacity of any retrovirus.[3]

EXISTING VECTOR IMPROVEMENTS

Improving Tropism

Viral tropism can be improved by site-directed mutations via various methods to increase binding affinities and specificities to the corresponding receptors.[7]

Phagocytosis-Shielded Lentiviral Vectors

Incorporation of the human phagocytosis inhibitor CD47 into the lentivirus cell membrane protects it from uptake by professional phagocytes and innate immune sensing, thus favoring biodistribution to hepatocytes after systemic administration.[8]

Improving Desirable AAV Traits

In Dec 2019 scientists at Harvard's Wyss Institute and Dyno Therapeutics reported a systematic approach to generate enhanced AAV capsids by mutating one by one each of the 735 amino acids within the AAV2 capsid. **This generated a virus library containing about 200,000 variants and identified capsid changes** that both maintained AAV2's viability and improved its tropism to specific organs in mice.[9]

HYBRID NANOPARTICLES

Multilayered Nanoparticles

Offer an improved loading dose of DNA cellular uptake and more control of the release of the DNA and target delivery compared to other non-viral vectors.[10]

Liposome-Polycation-DNA (LPD) Nanoparticles

Have demonstrated promise to be potent vaccine carriers and/or adjuvant for many antigens.[11]

NOVEL NON-VIRAL DELIVERY METHODS

Researchers are also exploring new methods to improve the transduction efficiency of non-viral gene delivery with **physical methods including:**[3]

Electroporation | Hydrodynamic | Magnetofection

And **chemical systems including:**

Cationic Lipids

Show advantages such as low toxicity and antigenicity and long-term expression with less risk of insertional oncogenesis.[3]

Keeping Safety at the Forefront of Gene Delivery Innovation

Exciting progress is being made to optimize gene carrier formulations, but it is important to remember that **the immunogenic profile of these modified and novel vehicles must be fully assessed.** This includes evaluation of:

PRE-EXISTING ANTIBODIES

Seroprevalence studies show that **UP TO 90%** **of human populations** have been exposed to AAVs, resulting in capsid-directed humoral immunity.[8]

One potential consequence of prior AAV exposure is the presence of pre-existing neutralizing antibodies (NAbs) which may limit transduction efficiency.

ADVERSE EVENT MONITORING THROUGHOUT TREATMENT

The first human gene therapy trial for SCID-X, which began in 1999, **used a murine γ-retroviral vector for gene transfer** which led to the development of leukemia in **5 OF 20 PATIENTS treated.**[13]

Thanks to the great work of researchers to improve vector safety, the latest clinical trial of gene therapy for SCID-X1, which used a lentiviral vector with a preconditioning course of busulfan, is showing very promising results with no immediate treatment side effects.[14]

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Sources

1. Edelstein M, ed. Gene Types Transferred in Gene Therapy Clinical Trials. Gene Therapy Clinical Trials Worldwide. http://www.abedia.com/wiley/vectors.php. Published 2018. Accessed December 2, 2019.
2. Ramamoorth M, Narvekar A. Non viral vectors in gene therapy- an overview. US National Library of Medicine National Institutes of Health. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4347098/. Published January 2015. Accessed December 2, 2019.
3. Nayerossadat N, Maedeh T, Ali PA. Viral and nonviral delivery systems for gene delivery. US National Library of Medicine National Institutes of Health. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3507026/. Published July 2012. Accessed December 2, 2019.
4. Li J, Liang H, Liu J, Wang Z. Poly (amidoamine) (PAMAM) dendrimer mediated delivery of drug and pDNA/siRNA for cancer therapy. ResearchGate. https://www.researchgate.net/figure/Advantages-and-disadvantages-of-viral-vectors-and-non-viral-vectors-for-gene-therapy_fig3_325250926. Published June 2018. Accessed December 2, 2019.
5. Sallach J, Di Pasquale G, Larcher F, et al. Tropism-modified AAV vectors overcome barriers to successful cutaneous therapy. US National Library of Medicine National Institutes of Health. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4015237/. Published May 2014. Accessed December 2, 2019.
6. Yang B. HSV/AAV and AAV/Ad hybrid viral vectors for gene therapy. Discovery Medicine. http://www.discoverymedicine.com/Benjamin-Yang/2009/05/17/hsvaav-and-aavad-hybrid-viral-vectors-for-gene-therapy/. Published May 17, 2009. Accessed December 2, 2019.
7. Büning H, Srivastava A. Capsid Modifications for Targeting and Improving the Efficacy of AAV Vectors. US National Library of Medicine National Institutes of Health. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6378346/. Published March 15, 2019. Accessed December 2, 2019.
8. Milani M, Annoni A, Moalli F, et al. Phagocytosis-shielded lentiviral vectors improve liver gene therapy in nonhuman primates. Science Translational Medicine. https://stm.sciencemag.org/content/11/493/eaav7325. Published May 22, 2019. Accessed December 2, 2019.
9. Ogden J, Kelsic E, Sinai S, Church G. Comprehensive AAV capsid fitness landscape reveals a viral gene and enables machine-guided design. Science. https://science.sciencemag.org/content/366/6469/1139. Published November 29, 2019. Accessed December 2, 2019.
10. Dizaj SM, Jafari S, Khosroushahi AY. A sight on the current nanoparticle-based gene delivery vectors. US National Library of Medicine National Institutes of Health. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4046008/. Published May 21, 2014. Accessed December 2, 2019.
11. Cui Z, Huang L. Liposome-polycation-DNA (LPD) particle as a carrier and adjuvant for protein-based vaccines: therapeutic effect against cervical cancer. US National Library of Medicine National Institutes of Health. https://www.ncbi.nlm.nih.gov/pubmed/15846491. Published December 2005. Accessed December 2, 2019.
12. Long BR, Sandza K, Holcomb J, et al. The Impact of Pre-existing Immunity on the Non-clinical Pharmacodynamics of AAV5-Based Gene Therapy. US National Library of Medicine National Institutes of Health. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6513774/. Published April 11, 2019. Accessed December 2, 2019.
13. Kumar SRP, Markusic DM, Biswas M, High KA, Herzog RW. Clinical development of gene therapy: results and lessons from recent successes. Science Direct. https://www.sciencedirect.com/science/article/pii/S2329050116301772#bib20. Published January 2016. Accessed December 2, 2019.
14. Mamcarz E, Biswal S, Ramalingam SS, Kapur J. Lentiviral Gene Therapy Combined with Low-Dose Busulfan in Infants with SCID-X1: NEJM. New England Journal of Medicine. https://www.nejm.org/doi/full/10.1056/NEJMoa1815408. Published April 18, 2019. Accessed December 2, 2019.