Ushering a new era of gene replacement therapies

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Disclosures

Advisory Board Member Fibro-ossificans Progressiva (Ipsen)

Advisory Board Member Spinal Muscular Atrophy (Novartis)

Advisory Board Member Hypophosphatasia (Alexion)

Primary Investigator PROPEL Study (QED)

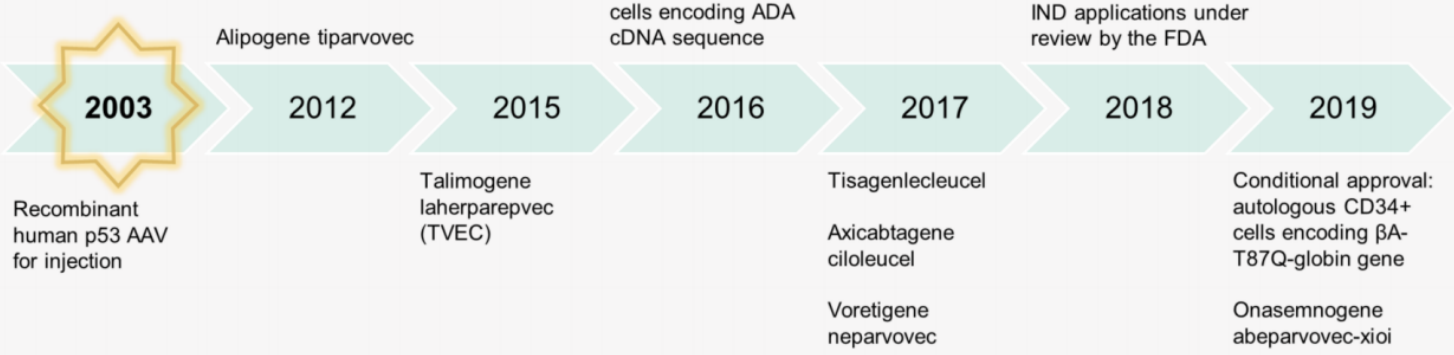
Primary Investigator MOVE Study (Clementia)

Objectives

- 1. Introduce various agents utilized in gene therapy
- 2. Define gene therapy in terms of gene augmentation and inactivation, gene replacement
- 3. Summarise gene replacement therapy and how it can be applied in various diseases

Evolution of Gene Therapies for Rare Diseases

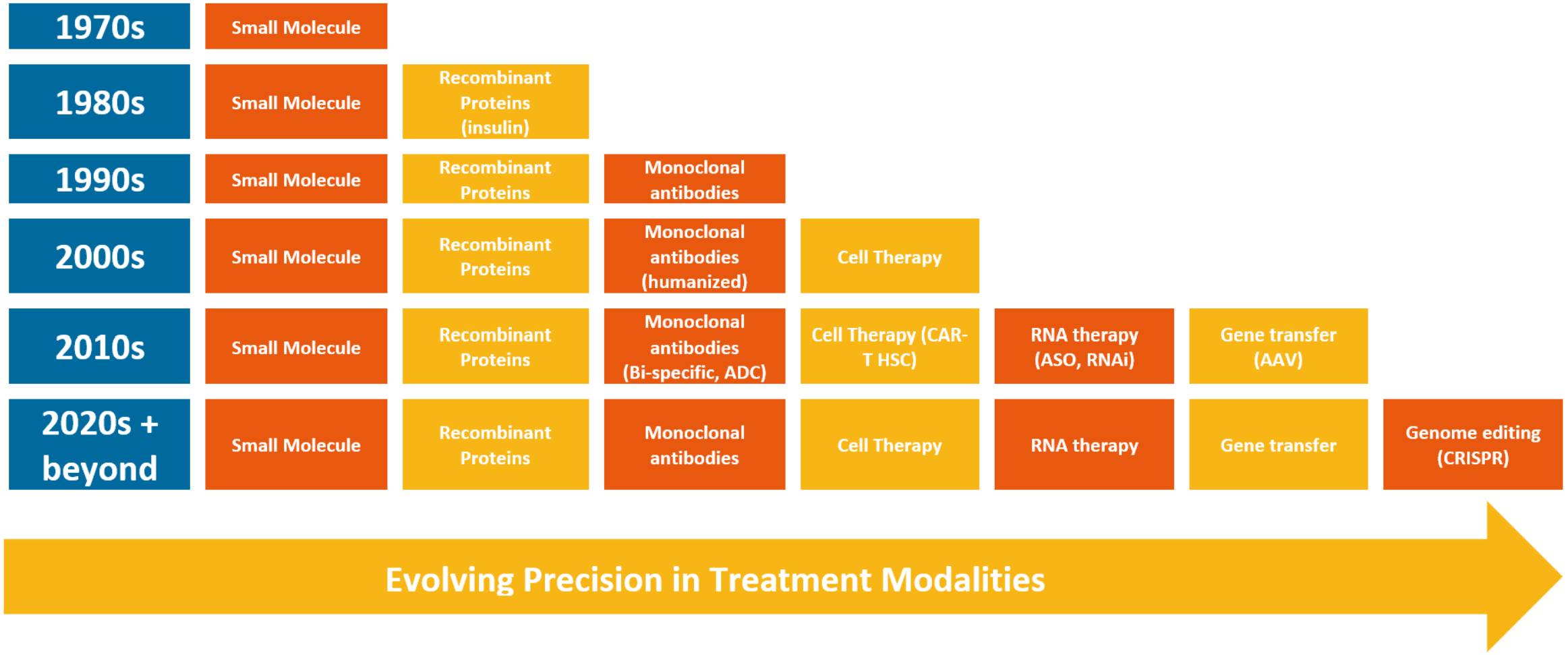
Gene therapy involves the use of nucleic acids (DNA or RNA) to treat or cure the underlying cause of human disorders.



Abbreviation(s): AAV: adeno-associated virus vector; ADA: adenosine deaminase; IND: investigational new drug.

Reference(s): Adapted from: Kaufman KB et al. *EMBO Mol Med.* 2013;5:1642-1661.

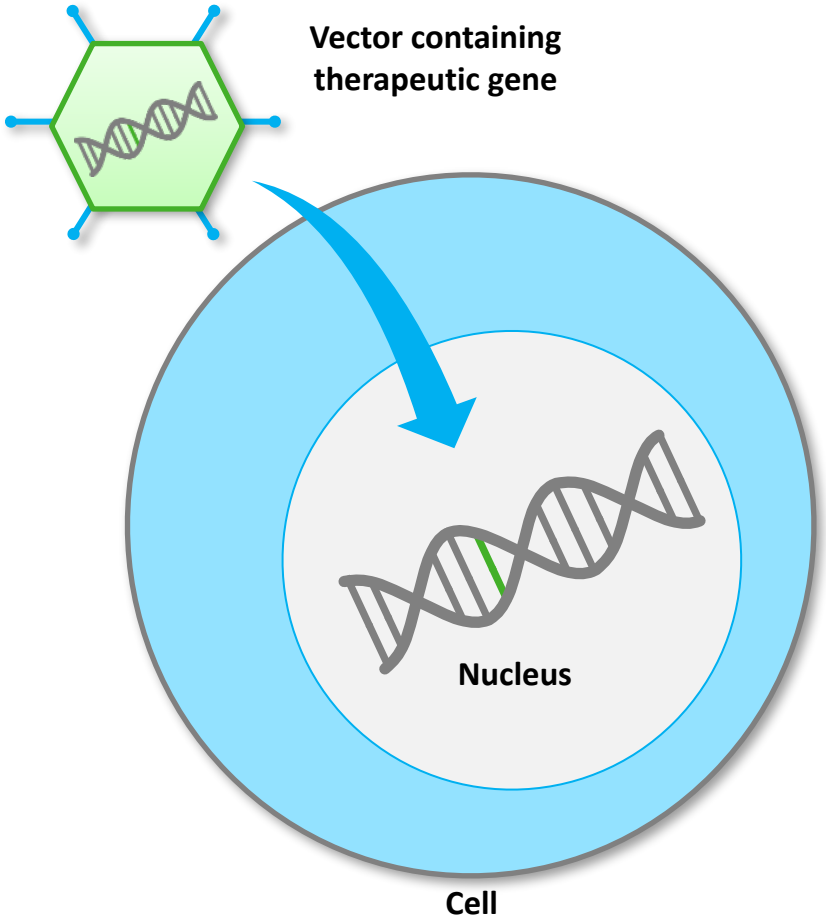
And with understanding and access, our ability to target diseases with precision has continued to improve



Kevin Curran PhD, Rising Tide Biology - link

What is gene therapy?

- Delivery of genetic material into cells to treat disease[1]
- Gene therapy strategies under development to treat[2]:
  - Monogenic inherited diseases
  - Multifactorial/acquired diseases (e.g. heart disease, diabetes, glaucoma)
  - Cancer
- Over 2300 ongoing gene therapy clinical trials in the US[2]
- Currently 3 clinically available gene therapies in the EU[2]



1. https://www.yourgenome.org/facts/what-is-gene-therapy Accessed 21 May, 2019; 2. Hanna et al. *J Mark Access Health Policy* 2017;5(1):1265293.

Gene therapy approaches

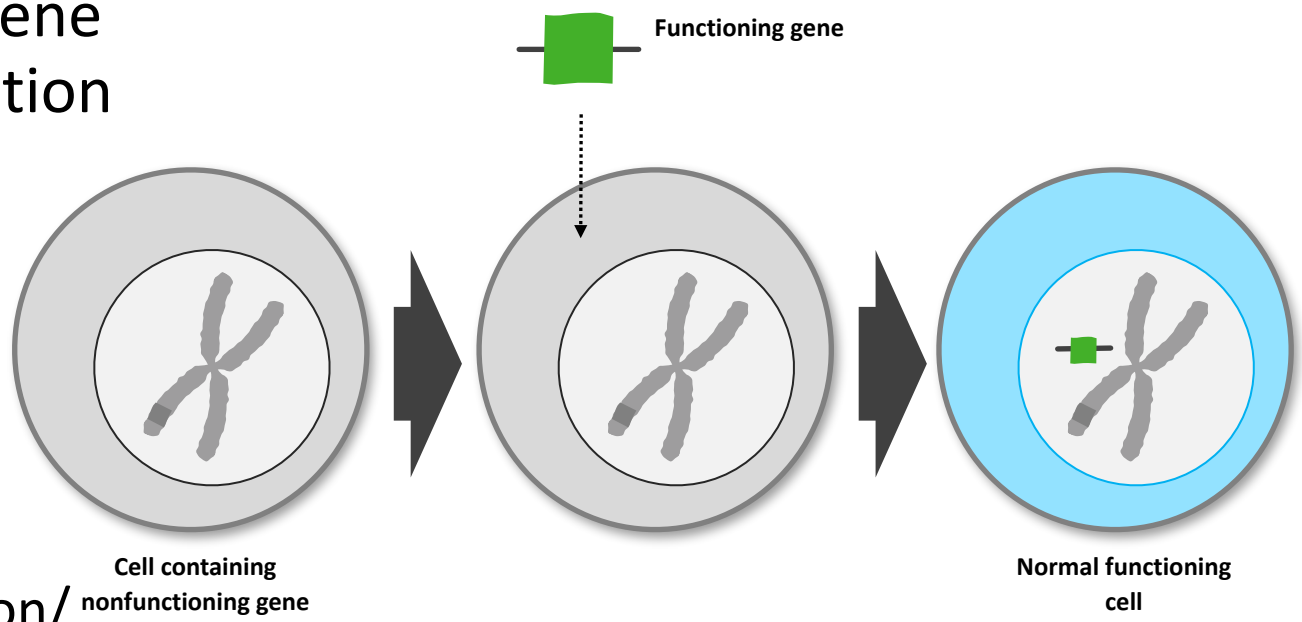
The gene therapy concept can be employed in a variety of strategies[1,2], depending on the underlying condition and its cause

- Gene augmentation
- Gene editing
- Gene inactivation
- Selective toxicity

1. Yourgenome.org. https://www.yourgenome.org/facts/what-is-gene-therapy. Accessed 9/12/17; 2. Yourgenome.org. https://www.yourgenome.org/facts/what-is-genome-editing. Accessed 9/12/17.

Gene augmentation

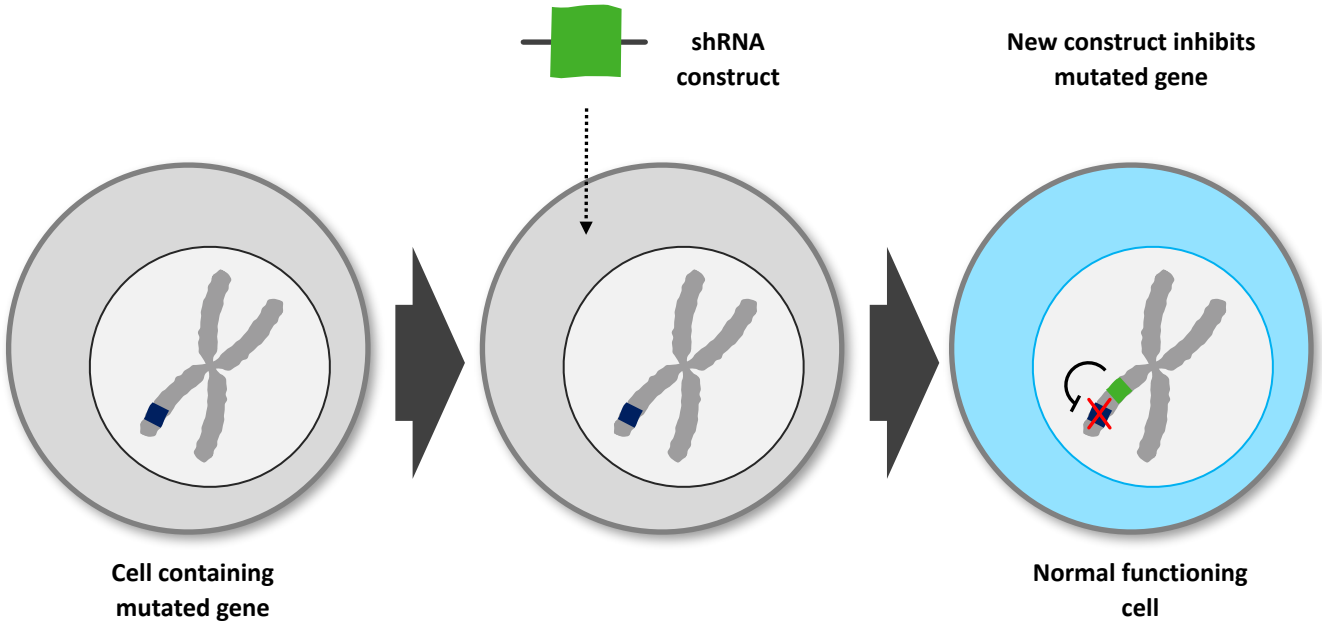
- Insert additional copy of existing gene to restore or enhance normal function
- <u>Appropriate for:</u>
  - Loss-of-function mutations
  - Non-specific beneficial effects (e.g. neuroprotection)
- <u>Considerations:</u>
  - Ineffective in treating gain-of-function/ dominant-negative mutations
  - May have undesirable effects in bystander cells



Yourgenome.org. https://www.yourgenome.org/facts/what-is-gene-therapy. Accessed 9/12/17.

Gene inactivation

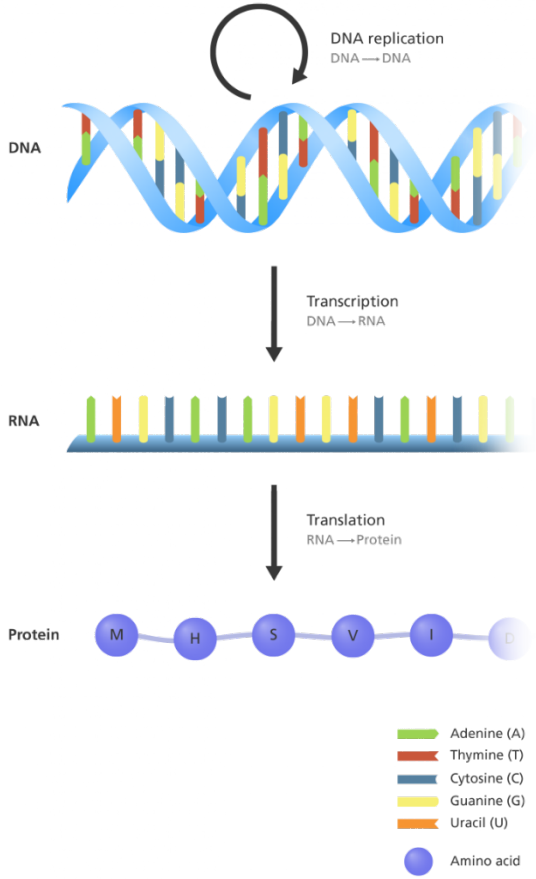
- Block expression of harmful genes by negative regulation
  - e.g. RNA interference
- <u>Appropriate for:</u>
  - Gain-of-function/ dominant-negative mutations
  - Both single-nucleotide mutations and insertions/deletions
- <u>Considerations:</u>
  - May be difficult to maintain targeting specificity



Nielson & Nielson. *Genes (Basel)*. 2013 Sep; 4(3): 457–484.

Antisense oligonucleotide gene therapy

- ASOs are defined as chemically synthesized oligonucleotides, generally 12–30 nucleotides in length, that are designed to bind to RNA by Watson-Crick base pairing rules
- The DNA and RNA code is written using 4 alphabets (nucleotides)



Call your mother for your childhood brownie recipe

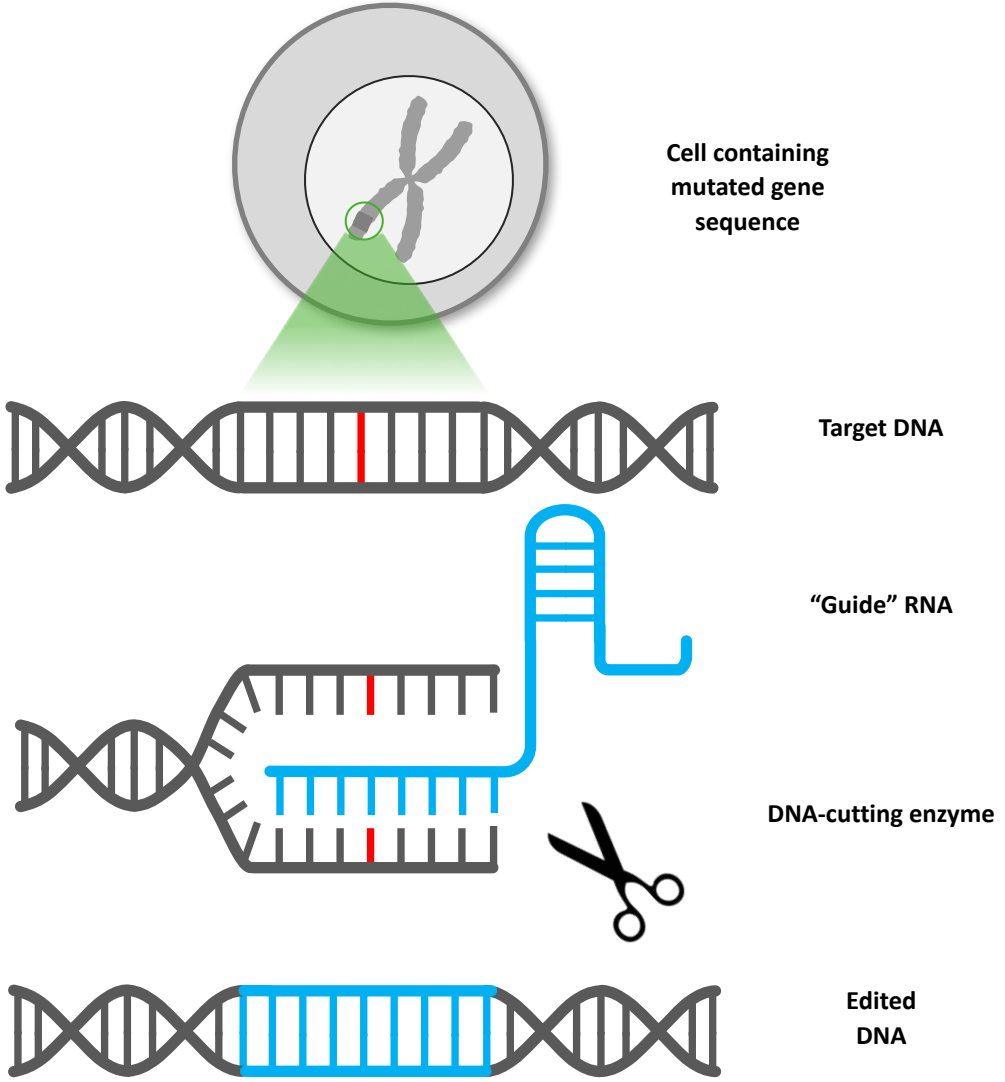
Copy down the recipe while chatting to her on the phone

Bake the brownies

https://www.yourgenome.org/facts/what-is-the-central-dogma

Gene editing

- Modify cellular DNA in place to correct specific mutations
  - e.g. CRISPR/Cas9
- Appropriate for:
  - Single-nucleotide mutations
  - Gain-of-function/dominant-negative mutations
- Considerations:
  - Specific targeting of sequence to edit is difficult; may lead to mutations elsewhere



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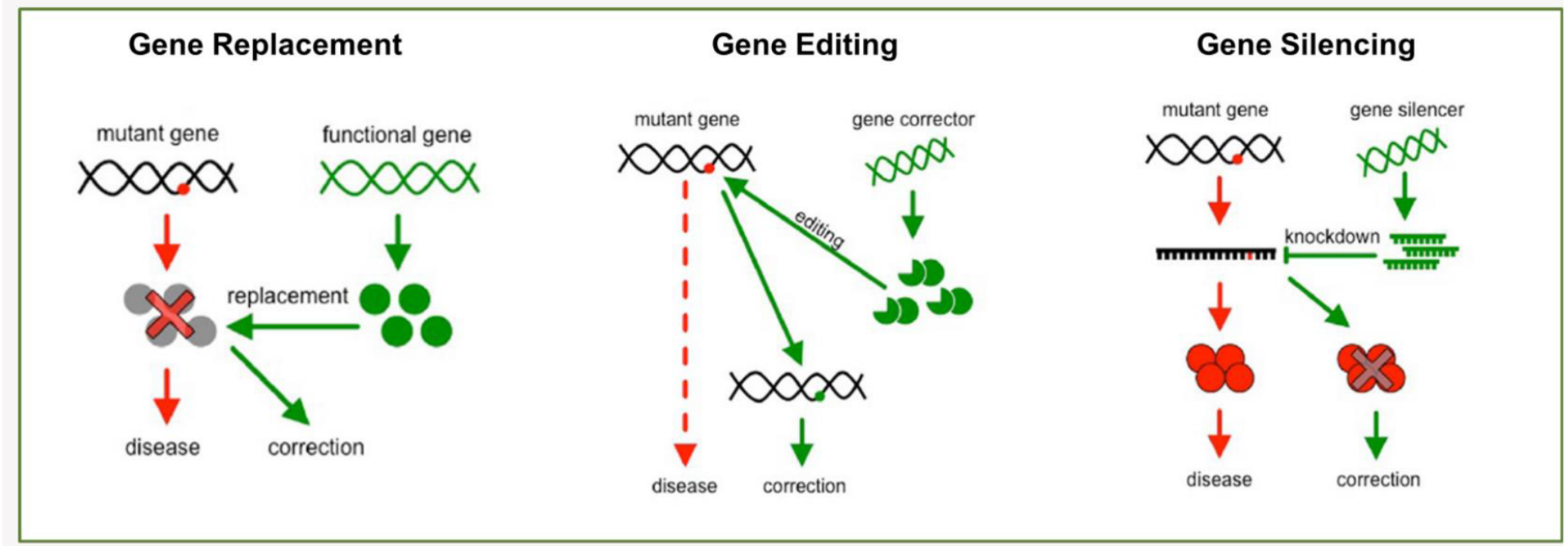
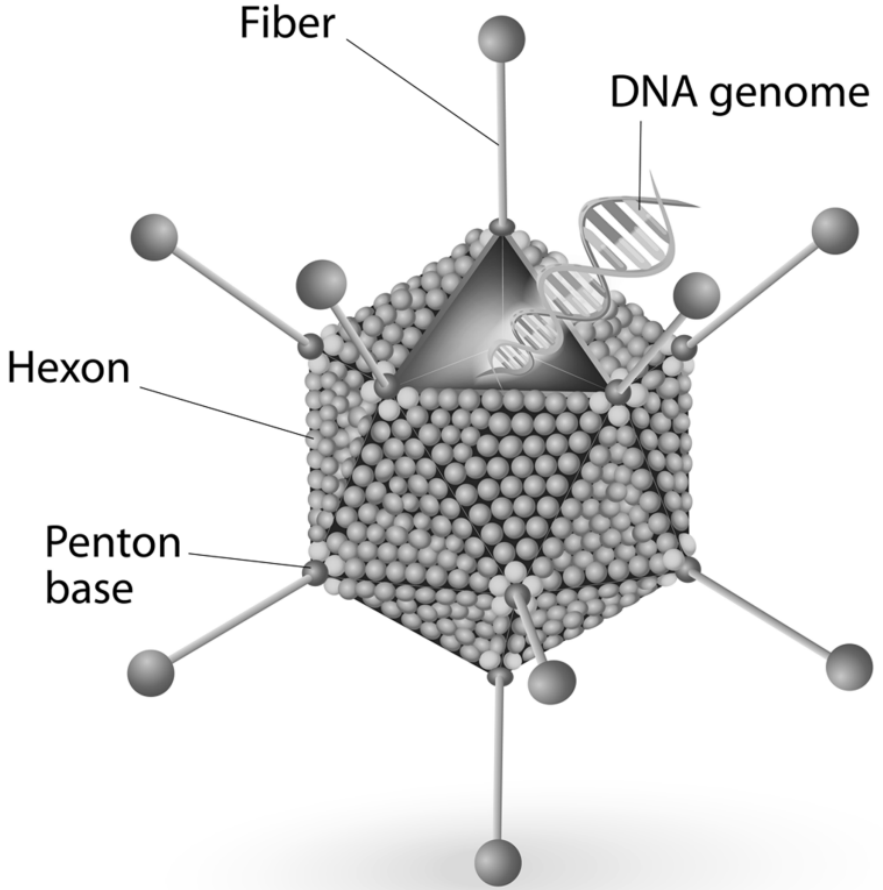

Gene Replacement
mutant gene
functional gene
replacement
disease
correction
Gene Editing
mutant gene
gene corrector
editing
disease
correction
Gene Silencing
mutant gene
gene silencer
knockdown
disease
correction

Components of gene therapy

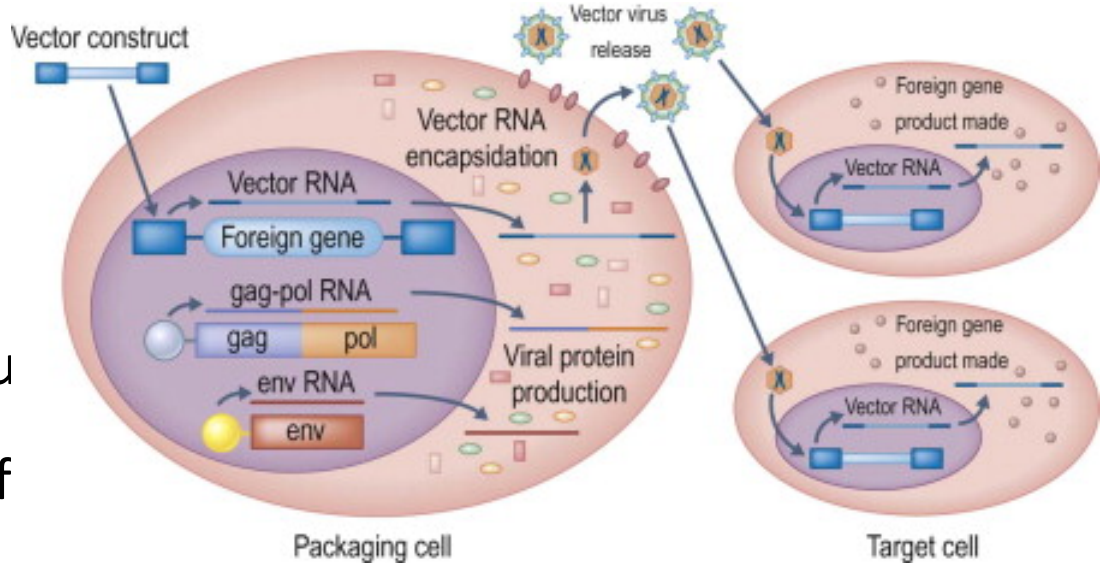
- **Genome**: Genetic material[1]
  - *Transgene*: therapeutic gene
  - *Promoter*: sequence driving gene expression; determines location, timing, levels of gene expressi
    - Tissue specificity
    - Inducible vs. constitutive expression
    - High vs. low level protein production
- **Vector**: Delivery vehicle[2]
  - Chemical carriers
  - Phospholipid coatings (liposomes)
  - ***Viruses – Currently the most prevalent method***



1. Papadakis et al. *Curr Gene Ther*. 2004;4(1):89-113. 2. Nayderossadat et al. *Adv Biomed Res*. 2012; 1: 27.

Viral vectors for gene therapy

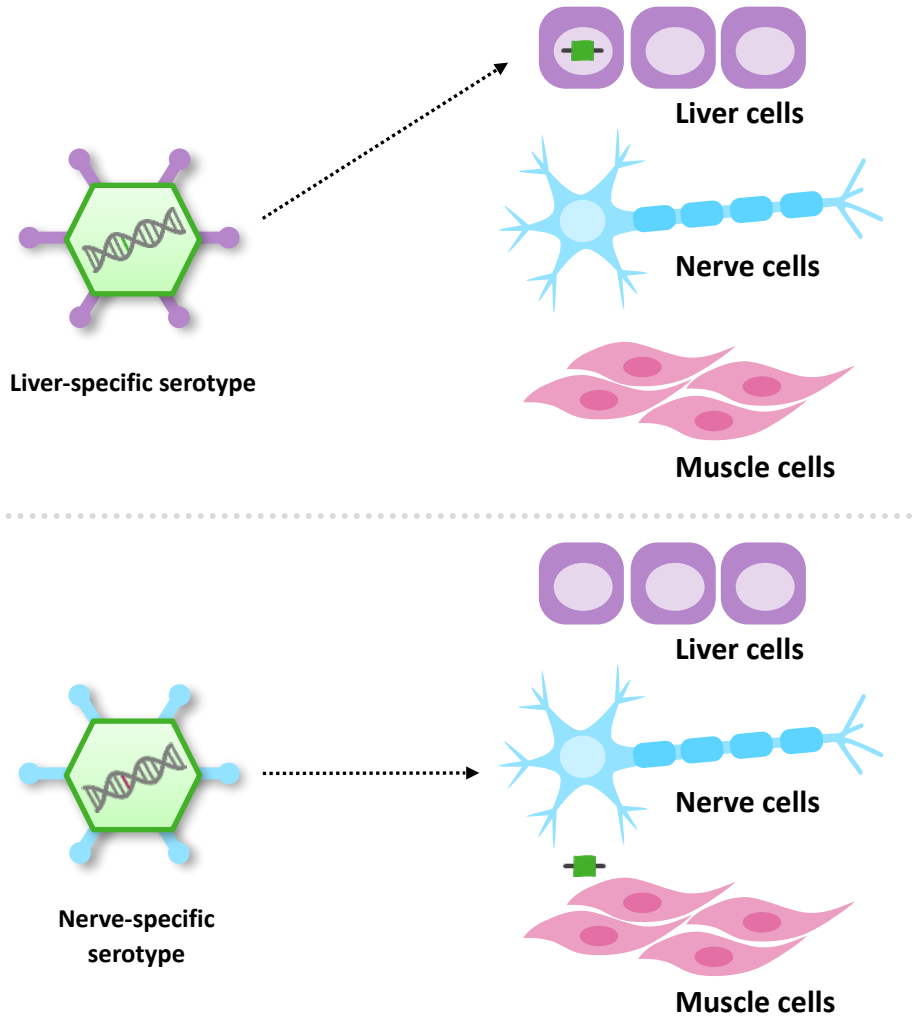
- Viruses are small and infectious parasites characterized by a simple organization, mode of replication and nucleic acid composition.
- Viruses lack the ability to produce energy or synthesize proteins and enzymes
- Gene therapy trials involving adenoviru vectors were used to treat OTC deficiency and resulted in the death of 18-year-old Jesse Gelsinger from a severe immune system reaction and multiple organ system failure.



Blood 2000; 95:2499–504

Vectors targeting

- Vector can be engineered to target specific cell/tissue types
- Viral capsid **serotype**[1]
  - Preferentially enters specific cell types
  - Naturally-occurring or artificial
- **Tissue-specific** transgene promoter[2]
  - Gene expression only in the target cell type



1. Vandenberghe & Aurrichio. *Gene Ther*. 2012 Feb;19(2):162-8; 2. Papadakis et al. *Curr Gene Ther*. 2004;4(1):89-113.

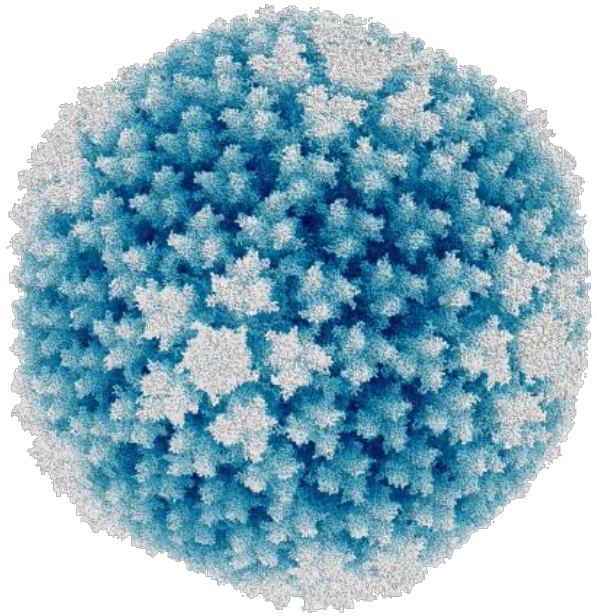
AAV—adeno-associated virus

Pros

- AAV is a small (25 nm), linear single-stranded DNA genome of ~4.7 kb[1]
- Ability to efficiently target various retinal layers
- Ability to spread after subretinal delivery
- Excellent safety profile and low immunogenicity
- AAV introduce their DNA into the nucleus of the cell, but the DNA is not integrated into a chromosome

Cons

- Not efficient at transducing photoreceptors[3]
- Certain forms of the most prevalent IRD are caused by mutations in genes whose cDNA exceeds 5 kb (such as ABCA4, MYO7A, CEP290, STGD, USH1B, LCA10), thus making it hard to treat them with AAV[4]



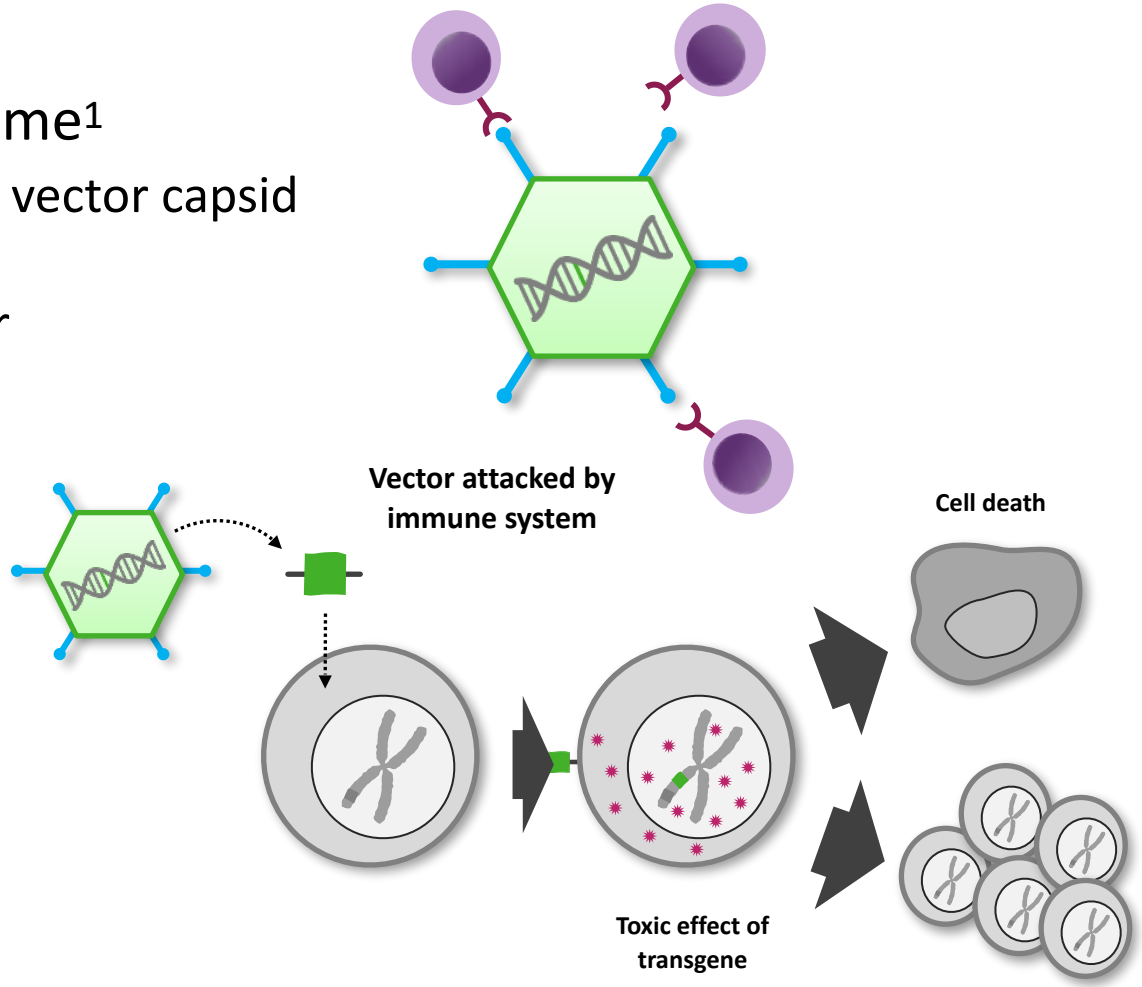
1.Berns and Giraud_1996; 2.Bennett et al., 2012; 3.Bennett_2017; 4.Trapani_2014

Vector Features	Retrovirus	Lentivirus	Adenovirus	AAV	Herpes Virus
Provirus	RNA	RNA	DNA	DNA	RNA
Capacity, kB	~9	~10	~30	~4.6	>30
Integration into the recipient genome	✓	✓	X	Extremely rare	✓
Duration of transgene expression	Long	Long	Transient	Long in post-mitotic cells	Transient
Pre-existing immunity in recipient	X	X	✓	✓	✓
AEs	Insertional mutagenesis	Insertional mutagenesis	Inflammatory response	Mild inflammatory response	Inflammatory response
Germline transmission	May occur	✓	X	May occur	X

Gene therapy potential complications

Immune response and genotoxicity

- Immune system may react to vector or genome[1]
  - Innate immune response, antibodies against to vector capsid proteins
  - Cellular immunity targeting transduced cells for elimination (**clearance**)
- Introduction of foreign DNA can cause negative effects (**genotoxicity**)[2]
  - Insertional mutagenesis
  - Cancer
- Immunosuppression treatment is used prior and during vector administration to reduce immunoresponse to the vector and gene



1. Mingozzi et al. *Blood*. 2013; 122(1): 23–36; 2. David & Doherty. *Toxicol Sci.* 2017 Feb;155(2):315-325.

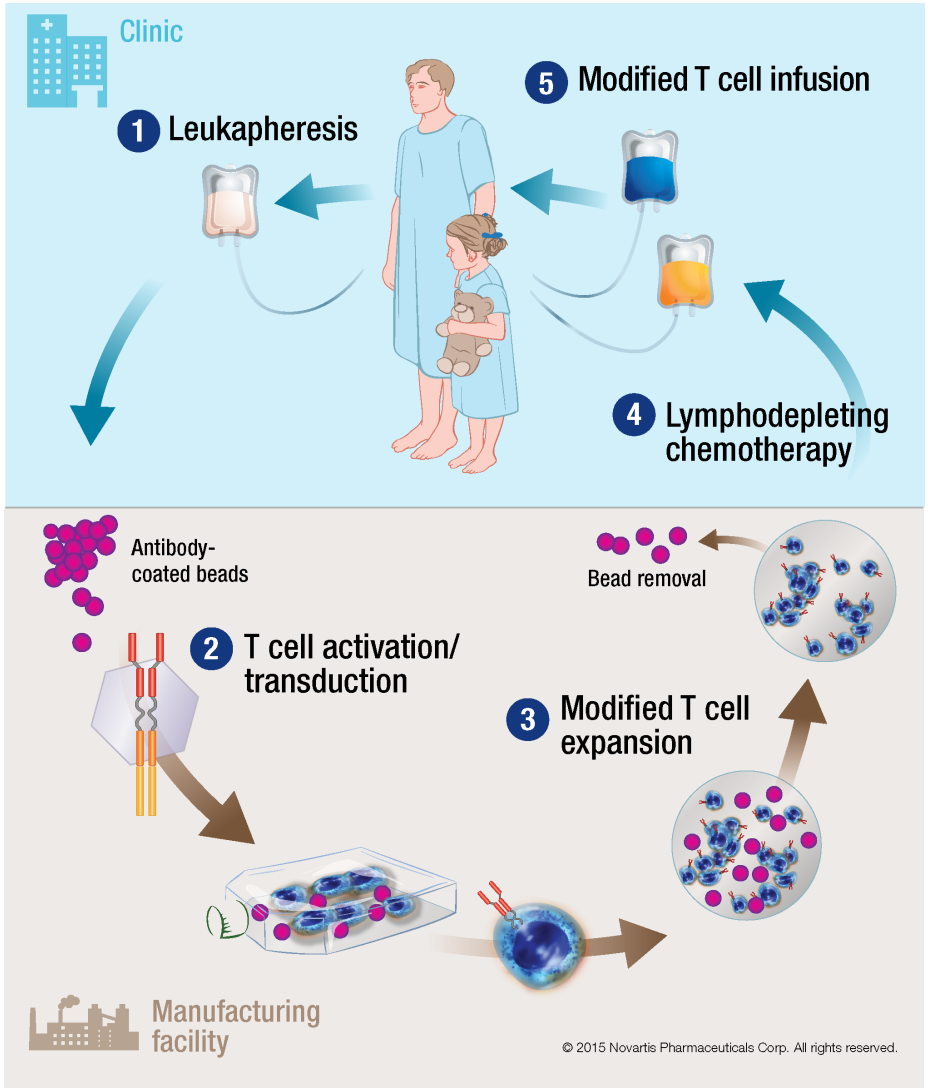
Cell and Gene therapy

- **Cellular therapy:** Using the patient's own cells as therapy *(i.e. cells = T cells)*
- **Gene therapy:** Insertion of genes into a patient's cells, thereby causing these cells to produce a new therapeutic protein

Kenderian SS, et al. *Cancer Res*. 2014;74:6383–6389

Cell therapy for T-cells

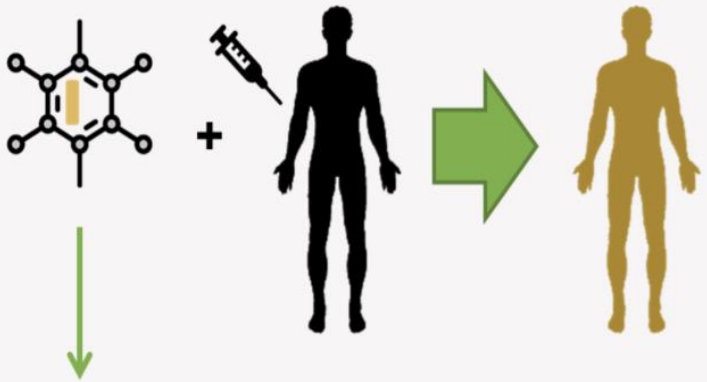
1. **Leukapheresis:** Patient's white blood cells are collected, cryopreserved, and shipped to the manufacturing facility[1-4]
2. **T cell activation/transduction:** T cells are genetically transduced ex vivo with a lentiviral vector encoding the anti-CD19 CAR[1,3-4]
3. **Modified T cell expansion:** Cells undergo ex vivo expansion on magnetic, antibody-coated beads[1-4]
4. **Chemotherapy:** The patient may receive a preparative lymphodepleting regimen before T cell infusion[1-4]
5. **Modified T cell infusion:** Cells are cryopreserved and then shipped back to the clinic and infused into the patient[1-4]



1. Porter DL, et al. N Engl J Med. 2011;365:725-733. 2. Porter DL, et al. J Cancer. 2011;2:331-332. 3. Kalos M, et al. Sci Transl Med. 2011;3:95ra73 4. Levine BL, et al. Mol Ther Methods Clin Dev. 2016;31;4:92-101

In Vivo

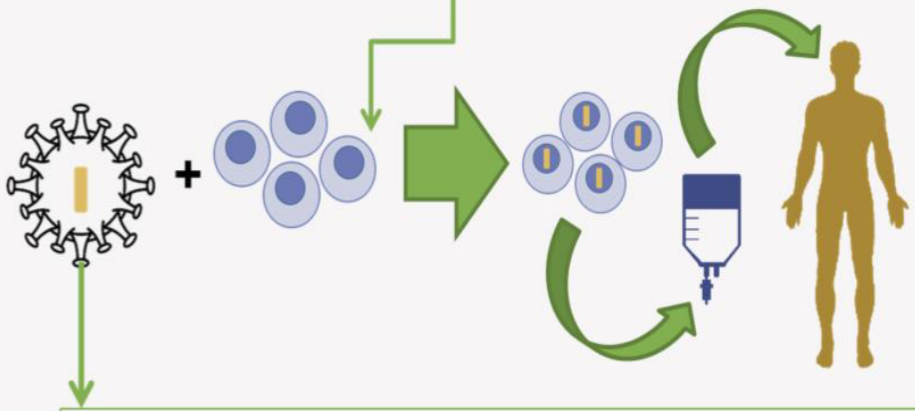
- Vector delivered **directly** into the patient



- Involves the use of **predominantly non-integrating** vectors to deliver the gene to a long-lived or slowly dividing cell, ensuring the expression of that gene for the life of the cell

Ex Vivo

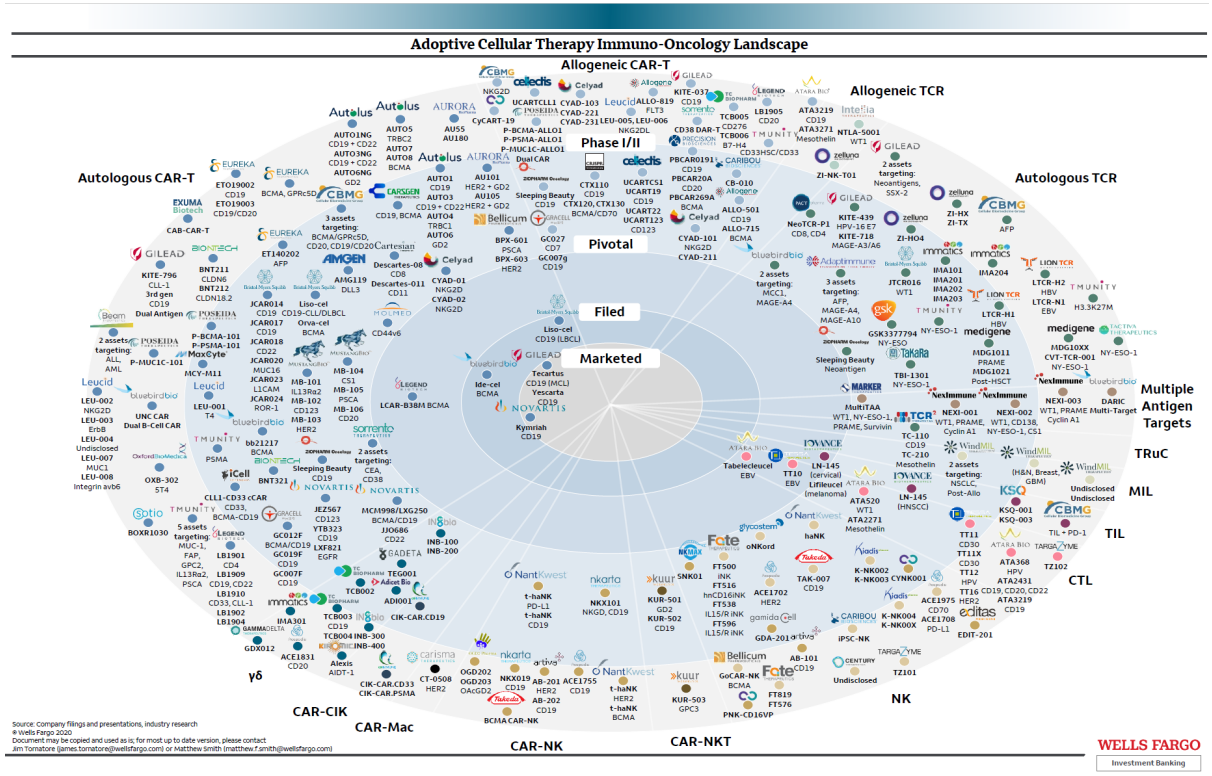
- Vector inserted into **stem cells** and then returned to the patient



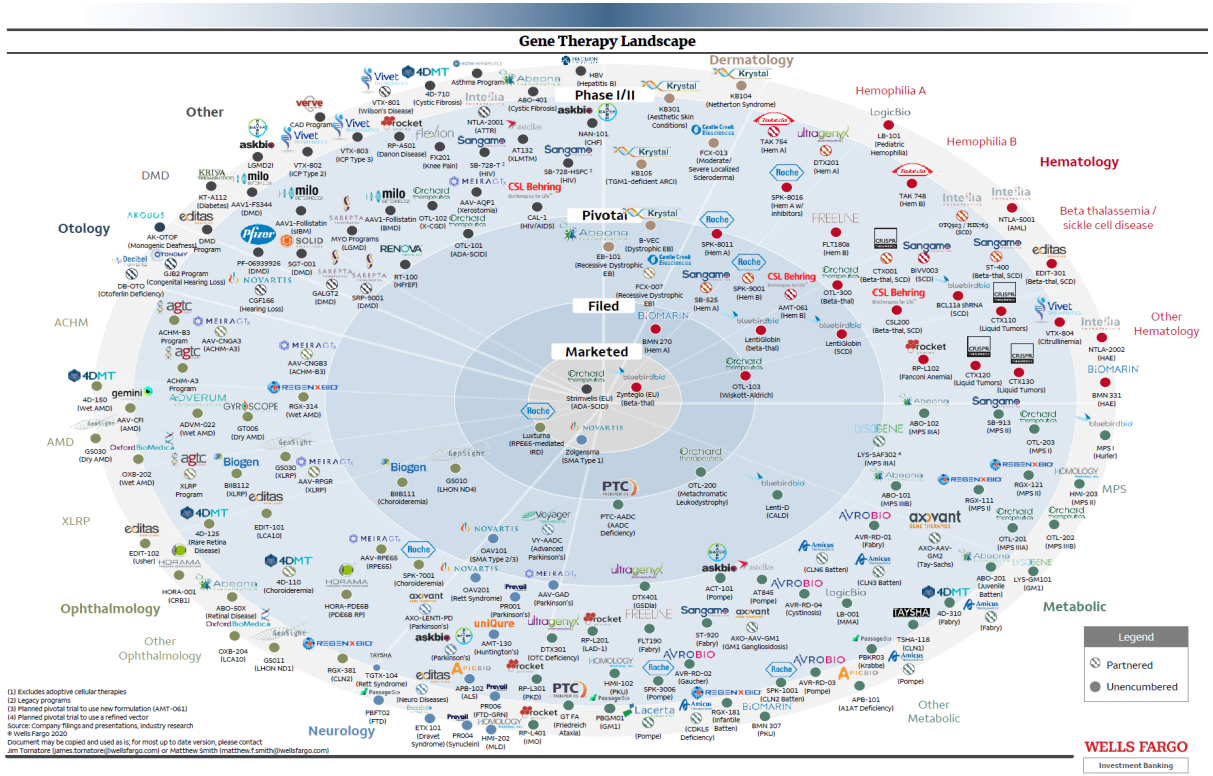
- Involves the use of **predominantly integrating** vectors to deliver the gene into the stem cell so that the gene is passed to every daughter cell

Cell and gene therapies pipelines mostly target rare disease

Adoptive Cellular Therapy[1]



Gene Therapy[1]



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