Gene Therapy: Mechanisms and Potential Disease Applications
Katie Green

What is gene therapy?
The basic concept involves inserting a normal gene into a patient with a disease caused by a defective gene. Strategies include gene replacement and gene addition.

A. Gene replacement
- Mutant gene
- Replacement
- Correction
- New functions
- Editing
- Disease
- Gene therapy
- Gene mutation represented by red dot, prevents appropriate protein production and leads to disease
- Insertion of functional gene copy allows for normal protein production to correct disease

B. Gene addition
- Disease
- Alienation
- Genetic and environmental factors can contribute to disease
- Gene mutation represented by red dot
- Therapeutic gene
- Insertion of functional gene copy allows for normal protein production to correct disease

Gene Delivery Methods

FDA Approved Gene Therapy Treatments
The FDA has approved treatments to target melanoma tumors with a genetically modified herpes simplex viral vector, cancer cells with CAR-T cell vectors, and spinal muscular atrophy with AAV vectors. Other treatments include non-Hodgkin lymphoma with CAR-T cell vectors, inherited retinal disease and RPE65 mutations with AAV vectors, and AAV vectors to treat various genetic disorders.

Vectors: Delivery Vehicles for Gene Insertion
Vectors serve as messengers that infect cells and integrate their genetic material into the host genome, and later use the cellular machinery to produce proteins that they encode.

Types of vectors
- Viral
  - Retroviruses
  - Lentiviruses
  - Adenoviruses
  - Adeno-associated virus (AAV)
  - Recombinant AAV (rAAV)
  - Oncolytic viruses
- Non-viral
  - Naked DNA/RNA
  - Plasmid DNA
  - Bacteria

Integration of Exogenous Genetic Material into Host via AAV Vector
AAV engineered to contain a correct copy of a gene are recognized by host cell glycosylated cell surface receptors, triggering viral internalization by clathrin-mediated endocytosis. AAV moves through the cytosol in an endosome with the help of the cytoskeleton. After escape from the endosome, AAV is transported into the nucleus and uncoated. Alternatively, AAV may undergo proteolysis by a proteasome. Once uncoated in the nucleus, the genetic material is able to integrate into the host genome.

Disease Applications
In order to utilize gene therapy techniques to treat disease, certain conditions must be met. This contributes to the complexities of the technique.

Prerequisites:
- Identify disease and defective gene/factors responsible for disease
- Identify gene mutation
- Establish relation of mutation to disease pathophysiology
- Clone normal healthy gene
- Identify target cell/tissue/organ

Once the prerequisites are met, a vector must be chosen. Each vector has different characteristics, so they must be suited for the specific disease.

<table>
<thead>
<tr>
<th>Vector</th>
<th>Tissue Tropism</th>
<th>Potential Therapeutic Disease Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAV1</td>
<td>Skeletal muscle, lung, CNS, retina, pancreas</td>
<td>HIV, CMV, A</td>
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<tr>
<td>AAV2</td>
<td>Smooth muscle, skeletal muscle, CNS, liver, kidney</td>
<td>AADC-deficiency, Parkinson’s Disease</td>
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<td>AAV3</td>
<td>Hepatocarcinoma, skeletal muscle, inner ear</td>
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<td>AAV4</td>
<td>CNS, retina</td>
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<td>AAV5</td>
<td>Skeletal muscle, CNS, liver, retina</td>
<td>MPS-III B, Hemophilia A, Hemophilia B</td>
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<td>Skeletal muscle, heart, lung, bone marrow</td>
<td>Hemophilia A, Hemophilia B</td>
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<td>AAV7</td>
<td>Skeletal muscle, retina, CNS</td>
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<td>AAV8</td>
<td>Liver, skeletal muscle, CNS, retina, pancreas, heart</td>
<td>Achromatopsia, Hemophilia A, Crigler-Najjar Syndrome, Hemophilia B, HIV, Pompe Disease</td>
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<tr>
<td>AAV9</td>
<td>Liver, heart, brain, skeletal muscle, lung, pancreas, kidney</td>
<td>Batten disease (CLN6), Spinal Muscular Atrophy, Giant Axonal Neuropathy, Duchenne Muscular Dystrophy</td>
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<tr>
<td>AAV10</td>
<td>Liver</td>
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<tr>
<td>LentiV</td>
<td>Transfusion-dependent p-thalassemia, Sickle Cell Disease, Wiskott-Aldrich Syndrome, X-SCID (figure below, part a), ADA-SCID, melanoma (b), CD34-expressing B-cell malignancies</td>
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FDA-approved gene therapy products
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<thead>
<tr>
<th>Product</th>
<th>Manufacturer</th>
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<tr>
<td>Zolgens</td>
<td>GeneSesso</td>
<td>Chimeric antigen receptor T cells in patients with relapsed or refractory minimal residual disease and B cell CD19-positive malignancies</td>
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