Gene and Cell Therapies Become Real: Big Picture and Examples

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“Gene Therapy Returns to Center Stage”

Nothing to Disclose
Topics to be Covered

- Brief history
- General strategies
- Gene vehicles and routes of delivery
- Examples of ongoing trials for neurologic diseases
- Emerging technologies
Use retrovirus/lentivirus vectors to stably transduce patients’ hematopoietic stem cells (HSCs) with missing gene and re-introduce into patients.
Setbacks

- **1998** - Jesse Gelsinger dies from overdose of adenovirus vector
  - 17 yr old with mild ornithine transcarbamylase deficiency (manageable with diet)
  - Received high dose Ad-OTC into liver and died in a couple of days due to multiorgan failure
  - Issues of consent and conflict of interest, adenovirus toxicity

- **2005** – Baby boy with X-SCID dies from retrovirus vector-induced leukemia
  - “Boy in a bubble” extremely immune deficient
  - Ex vivo delivery of missing gene to hematopoietic stem cells
  - Recovers immune function, but later dies of leukemia
  - New vector designs have dramatically reduced incidence of leukemia

- **2007** – Woman with rheumatoid arthritis dies after AAV-anti-TNF alpha administration
  - Liver and kidney failure within a few weeks after second intra-articular injection
  - Deemed to be due to histoplasmosis infection related to immune suppression from other ongoing therapy, **NOT** related to gene therapy
Why the Renewed Excitement? Some Successes!

Benefit shown in three paradigms with strong safety profile:

1. **Direct injection of adeno-associated virus vector (AAV) for gene replacement (GR):**
   - Leber’s congenital amaurosis: inherited childhood blindness
   - Hemophilia B: a defect in blood clotting factor IX

2. **Ex vivo lentivirus genetic modification of hematopoietic stem cells:**
   - Chronic B cell lymphoma via delivery of chimeric T cells targeted to B cells
   - Immune deficiencies – SCID, Wiskott-Aldrich syndrome (GR)
   - Neurodegeneration – X-linked adrenoleukodystrophy, metachromatic leukodystrophy (GR)

3. **Oligonucleotides:**
   - Familial amyloid polyneuropathy – siRNA to reduce transthyretin produced in liver
Expanded Concept of “Gene Therapy” to Brain

Inject:
intraparenchymal, intraventricular, intrathecal, intravascular, . . .

Mix and match:
plasmid constructs, oligonucleotides, genetically modified (GM) cells, virus vectors
Gene Therapy Strategies for Neurologic Diseases

**Hereditary monogenic diseases:**
- **Recessive** – replace or correct defective genes
  - e.g. re-introduce normal gene, alter splicing pattern to skip mutation, correct endogenous gene
- **Dominant** – suppress defective gene
  - e.g. siRNAs, antisense oligonucleotides (ASOs), inactivate mutant gene

**Unknown etiology:**
- Target symptoms – e.g. supply neurotropic factors for neurodegeneration,
  alter neurotransmission/circuitry, suppress or enhance angiogenesis, provide replacement cells

**Non-hereditary, genetic basis – cancer:**
- e.g. replicating and non-replicating virus vectors armed with chimeric T-cell receptors, pro-drug activating enzymes, cytokines, apoptotic stimuli, anti-angiogenesis factors, etc.
# Vectors for Gene Delivery

## Special Report

<table>
<thead>
<tr>
<th></th>
<th>Retroviruses</th>
<th>Adenoviruses</th>
<th>Adeno-Associated Viruses</th>
<th>Liposomes</th>
<th>“Naked” DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Some Potential Advantages</strong></td>
<td>Integrate genes into host chromosomes, offering chance for long-term stability</td>
<td>Most do not cause serious disease; large capacity for foreign genes</td>
<td>Integrate genes into host chromosomes; cause no known human diseases</td>
<td>Have no viral genes, so do not cause disease</td>
<td>Same as for liposomes; expected to be useful for vaccination</td>
</tr>
<tr>
<td><strong>Some Drawbacks of Existing Vectors</strong></td>
<td>Genes integrate randomly, so might disrupt host genes; many infect only dividing cells</td>
<td>Genes may function transiently, owing to lack of integration or to attack by the immune system</td>
<td>Small capacity for foreign genes</td>
<td>Less efficient than viruses at transferring genes to cells</td>
<td>Inefficient at gene transfer; unstable in most tissues of the body</td>
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</table>
Gene Delivery to Cells via Virus Vectors: Uptake Mechanisms
Adeno-Associated Virus (AAV)

- Tiny non-enveloped virus, 25nm capsid
- No disease associated with wild type virus
- ssDNA genome
- Insert capacity ~4.6kb
- Long-term episomal transgene expression
- Excellent transgene expression in brain
- The 1st generation of rAAV is safe in human gene therapy trials (>700 patients in the past 17 years)
Serotypes/AAV Variants

- Over 100 AAV variants
- Some shared tropism/ some different
Disseminated Gene Delivery to the Brain

- Limited propagation of the vector
- Access through CFS through ventricles
- Temporary disruption of blood brain barrier
Schematic Representation of the Eye Structure and Intraocular Injection Routes

Colella & Auricchio, 2012
Primary cell types transduced in mouse brain after I.V. injection

Maguire, 2015
Systemic Injection

- Every cell in the brain is a maximum of 40 microns from endothelial cells

- If BBB can be crossed, systemic delivery would yield widespread gene delivery to brain

- Limitations: circulating Abs to vector and high peripheral transduction of liver, heart etc.

Maguire, 2015
Lentivirus

- Same family as retrovirus
- Enveloped virus, 100 nm diameter
- For safety/broader tropism, coated with VSV-G glycoproteins
- Transduces both dividing and nondividing cells
- HIV-based vector (Gag, pol, accessory genes on separate plasmids for safety)
- Insert capacity ~ 8kb
- Integrates at “hotspots” in host genome
- Excellent long-term transgene expression
- Insertional oncogenesis possible
- Self-inactivating (SIN) lentivirus developed: prevents genome replication from provirus (vector mobilization) as well as reduces U3 promoter activity on cellular genes
Ex Vivo Genetic Modification of Hematopoietic Stem Cells

- Highly purified, high-titre LV
- Cytokines
- HSCs with corrected gene
- Patient with MLD or WAS
- Myeloablation (± immunosuppression adapted to specific disease)
- Intravenous infusion of fresh, transduced cells (high dose of cells)

Leboulch, 2013
Inhibit translation of mRNAs with small antisense RNAs (siRNA, shRNA or artificial miRNA)

3. Inhibit protein translation

With siRNA, ASO or miRNA

- Engage RISC complex to cleave mRNA
- Block movement of translation machinery
- Engage RNases to cleave RNA
Nanoparticles

- Submicron-sized particles comprising various compounds which can bind to nucleic acid and mediate transfection of cells

Examples:
- liposome-polycation-hyaluronic acid + single chain antibody
- polysaccharides
- cholesterol conjugates
Cells as Vehicles for Replacement

Types of cells:
- human/not animal
- embryonic neuroprecursor
- from patient – fibroblasts, astrocytes, mesenchymal stem cells, lymphocytes
- differentiated from induced pluripotent stem cells derived from patient fibroblasts

Genetic/epigenetic modification:
- treat adult cells with growth factors and expose transcription factors to generate pluripotent stem cells (IPSCs) and to differentiate into neurons
- arm with therapeutic replacement transgenes, or "hibernating" oncolytic virus or toxic transgenes for tumors
### Clinical Trials for Gene Therapy for Neurological Disorders

**Table 1** Clinical trials for gene therapy to treat neurological disorders. Data obtained from [http://clinicaltrials.gov/](http://clinicaltrials.gov/). *GDNF* Glial cell-derived neurotrophic factor; *AADC* aromatic l-amino acid decarboxylase; *NGF* nerve growth factor; *SGSH* N-sulphoglucosamine sulphohydrolase; *SUMF1* sulfatase modifying factor 1; *ARSA* arylsulfatase A; *ABCD1* ATP-binding cassette, sub-family D (ALD), member 1; *HSV-TK* herpes simplex virus-thymidine kinase; *CD* cytosine deaminase

<table>
<thead>
<tr>
<th>Disease</th>
<th>Vector</th>
<th>Transgene</th>
<th>Phase</th>
<th>Status</th>
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</thead>
<tbody>
<tr>
<td>Parkinson’s disease</td>
<td>AAV</td>
<td><em>GDNF</em></td>
<td>1</td>
<td>Recruiting</td>
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<tr>
<td>Parkinson’s disease</td>
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<td><em>AADC</em></td>
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<tr>
<td>Leber’s hereditary optic neuropathy</td>
<td>AAV</td>
<td>Human mitochondrial ND4 gene</td>
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<tr>
<td>Alzheimer’s disease</td>
<td>Fibroblasts</td>
<td><em>NGF</em></td>
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<tr>
<td>Sanfilippo Type A syndrome</td>
<td>AAV</td>
<td><em>SGSH and SUMF1</em></td>
<td>1/2</td>
<td>Completed</td>
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<tr>
<td>Metachromatic leukodystrophy</td>
<td>AAV</td>
<td><em>ARSA enzyme</em></td>
<td>1/2</td>
<td>Recruiting</td>
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<tr>
<td>X-linked adrenoleukodystrophy</td>
<td>Lentivirus <em>in vivo</em> transduced stem cells</td>
<td><em>ABCD1</em></td>
<td>2/3</td>
<td>Recruiting</td>
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<tr>
<td>Glioma</td>
<td>Adenovirus</td>
<td><em>p53</em></td>
<td>1</td>
<td>Completed</td>
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<tr>
<td>Glioma</td>
<td>Oncolytic HSV</td>
<td>—</td>
<td>1</td>
<td>Recruiting</td>
</tr>
<tr>
<td>Glioma</td>
<td>Adenovirus</td>
<td>HSV-TK + ganciclovir</td>
<td>1</td>
<td>Completed</td>
</tr>
<tr>
<td>Glioma</td>
<td>Replicating retrovirus</td>
<td><em>CD</em></td>
<td>1</td>
<td>Recruiting</td>
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<tr>
<td>Familial amyloid polyneuropathy</td>
<td>nanolipoparticle, i.v.</td>
<td>siRNA for transthyretin</td>
<td>1</td>
<td>Completed</td>
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<tr>
<td>Spinal musc. atrophy</td>
<td>scAAV9/i.v./&lt;9 mo</td>
<td><em>SMN1</em></td>
<td>1</td>
<td>Recruiting</td>
</tr>
<tr>
<td>Spinal musc. atrophy</td>
<td>scAAV9/intrathecal/early</td>
<td><em>SMN1</em></td>
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<td>Myotonic dystrophy</td>
<td>ASO (RNA-DNA-RNA)</td>
<td><em>DMPK</em></td>
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<tr>
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<td>AAV9/i.v.</td>
<td><em>ABCD1</em></td>
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<td>Tuberous sclerosis</td>
<td>AAVrh8/i.v.</td>
<td>hamartin</td>
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<td>Planning</td>
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<tr>
<td>NF2</td>
<td>AAV1/intratumoral</td>
<td>caspase-1/ICE</td>
<td></td>
<td>Planning</td>
</tr>
</tbody>
</table>

* X-ALD
Spinal Muscular Atrophy

- Autosomal recessive disorder
- Loss of function mutation in SMN1 gene (encodes survival motor neuron protein)
- Degeneration of alpha-motor neurons in spinal cord – progressive muscle atrophy, paralysis and death
- Leading genetic cause of infant mortality (1/6000 births)

Remarkably, humans are the only species which have a second copy of this gene (SMN2) but it has a variation causing a splicing defect resulting in truncated, inactive protein

Strategies:
- ASO modulation of splice to produce SMN2;
- gene replacement of SMN1;
- grafting of normalized motor neurons derived from induced pluripotent stem (iPS) cells from patient
Schematic of Human SMN Loci

C-to-T in exon 7 inhibits splicing

Lorson et al., 2010
Phenotypic Correction of SMA Mice using scAAV9-SMN1 Delivered Intravascularly

[5 x 10^{11} gc scAAV9-GFP or scAAV9-SMN1 i.v. on P1]

GFP+ in motor neurons (ChAT – red) in lumbar spinal cord at P11

Foust et al., 2010
Phenotypic Correction of SMA Mice with scAAV9-SMN1 Delivered i.v. on P1

Foust et al., 2010
Gene Therapy for Mice with Spinal Muscular Atrophy

MacKenzie et al., 2010

SMA mouse

Spinal cord

Motor neuron

Atrophic muscle fibers

Neurites die back

Dead SMA mouse

scAAV9-SMN

More robust neurite arborization

Rescued SMA mouse

MacKenzie et al., 2010
• **The phase 1 intravenous trial of SMA gene therapy**
  Conducted at Nationwide Children's Hospital in Columbus, Ohio, under the direction of neurologist Jerry Mendell, a longtime MDA research grantee and co-director of the MDA clinic at his institution. (MDA is not, however, funding this trial.)

• **Prospective participants must**
  have type 1 SMA;
  be 9 months old or younger;
  have a mutation in both copies of the SMN1 gene;
  have two copies of the SMN2 gene (no more and no fewer);
  have experienced symptom onset by 6 months of age; and
  have low muscle tone by clinical evaluation.
Metachromatric Leukodystrophy (MLD, also called Arylsulfatase A deficiency) is a lysosomal storage disease which is commonly listed in the family of leukodystrophies as well as among the sphingolipidoses as it affects the metabolism of sphingolipids, which are characterized by the toxic buildup of lipids (fatty materials such as oils) and other storage materials in cells in the white matter of the central nervous system and peripheral nerves.

The buildup of storage materials impairs the growth or development of the myelin sheath, the fatty covering that acts as an insulator around nerve fibers.

The prognosis for MLD is poor. Most children within the infantile form die by age 5. Symptoms of the juvenile form progress with death occurring 10 to 20 years following onset. Those persons affected by the adult form typically die within 6-14 years following onset of symptoms.
Clinical Follow-Up of MLD Patients after HSC-GT

Biffi et al., 2013
Monocyte/Macrophage Ontogeny –
Monocytes Migrate into Damaged Brain and Become Macrophages

Sanberg et al., 2010
Distribution of Human CD34-Derived Cells Introduced I.V. in the Brain of NODSCID Mice

Asheuer et al., 2004
Cross Correction or Bystander Effect

Normal or Genetically Modified Cells

Enzyme-Deficient Cells
Nerve function compromised by protein aggregation and/or amyloid fibril formation.

Niemietz et al., 2015
siRNA-Mediated Gene Silencing

Niemietz et al., 2015
Efficacy of ALN-TTR01 in Patients with Transthyretin Amyloidosis

Coelho et al., 2013
siRNA Therapy for FAP

- Alnylam demonstrates continued commitment to transthyretin-mediated amyloidosis patients with advancement of ALN-TTRsc02, an investigational RNAi therapeutic with potential for low volume, once quarterly, subcutaneous dose regimen
Genome-Editing Nucleases – the Future?

- Zinc Finger Nucleases (ZFNs)
- TALE Nucleases (TALENs)
- Meganucleases
- CRISPR RNA-Guided Nucleases

K. Joung
Alter DNA genome with ZFNs, TALENs or CRISPR

1. Alter DNA genome

   a. Disrupt
   b. Repair
   c. Add or delete
Targeted Genome Editing using a Small Guide RNA (sgRNA): Cas9 Complex

Richter et al., 2013
New Methods on the Horizon

- exo-AAVs (AAV in extracellular vesicles) and ancestral AAV (Anc80) – less immunogenic
- Synthetic vectors
- Drug-regulated transgene expression
- Cell-specific targeting ligands and promoters (neg. miRNAs)
- New genetically modified, patient-derived cells from iPSCs
Safety Issues in Gene Therapy Clinical Trials

1. Vector delivery/toxicity
   - Too much vector may be directly toxic to cells, e.g. adenovirus vector caused death of OTC deficiency patient
   - vector or transgene may cause a toxic immune response, e.g. may induce immune reaction to factor IX in hemophilia patients such that they can no longer receive protein replacement therapy
   - route of delivery may be damaging, e.g. injection of AAV into the spinal cord of ALS patients may damage the cord
   - transgene may have untoward effects, e.g. NGF delivery to Alzheimer diseased brain may cause sprouting and misconnection of neurons
   - retrovirus vectors can cause insertional mutagenesis or oncogenesis
   - replication-conditional vectors can cause damage to normal tissue via immune inflammation and cell death, and may activate latent viruses
2. Genetic modification of germ line and transmission to future generations
   - so far no evidence of this for vectors in use

3. Generation of new epidemiologic viral agents – so far no evidence
   - recombination of viral elements can potentially yield replication-competent virus novel to humans, e.g. human MoMLV retrovirus with oncolytic potential
     - replication-selective vectors which place essential virus genes under tissue specific promoters can change the tropism of a replicating virus, e.g. adenovirus vectors in which essential genes are placed under prostate-specific promoters could cause sterility in male relatives
     - species specific virus may “jump” species, e.g. fowl pox vaccinia-type virus or Newcastle disease virus creating new pathogen for humans
Gene Therapy is Becoming a Medical Reality

• Three vector systems have a proven track/safety record and have produced benefit in the nervous system
  – AAV delivered either directly into the NS or systemically
  – Lentivirus for *ex vivo* gene replacement in hematopoietic stem cells which migrate into damaged brain
  – Modified siRNA/ASOs delivered systemically

• Benefit in eye and brain neurodegeneration – earlier treatment better

• Systems coming into place to support clinical trials:
  – New pharm/biotechs: Voyager, Novartis, Glaxo-Smith, Biogen, SPARC, Bluebird, to name a few gene vehicles and routes of delivery
  – One approved gene therapy product in Europe – Glybera (rAAV1 to treat lipoprotein lipase deficiency)

• New technologies on the horizon – expect some ups and downs on an upward incline