Gene Therapy in Neurology: from benchside promise to clinical practice

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Professor of Neurology and Pediatrics

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Will Society Be Prepared?

New information is being obtained in the field of biochemical genetics at an extremely rapid rate. Thus far, this knowledge has had relatively little effect upon man. More information must be obtained before practical application will be possible, and the technical problems that must be overcome are formidable. However, when these obstacles have been removed this knowledge will greatly influence man’s future, for man then will have the power to shape his own biologic destiny. Such power can be used wisely or unwisely, for the betterment or detriment of mankind.
1990-2003: genome sequence of over 90% of the human genome

1999: Death of Jesse Gelsinger
FDA halted gene therapy human trials
Leukemia as side effect in several SCID patients

2003

2019: The FDA approved onasemnogene abeparvovec for spinal muscular atrophy (SMA)
Return of investors interest and NIH support
Improvement in viral vectors
Management of immune reactions

2020: Nobel Prize in Chemistry for CRISPR/cas9

FIGURE 1 A view of the “hype cycle” the field of gene therapy has traversed
Diseases targeted by gene therapy

Cancer diseases 65% (n=1688)
Monogenic diseases 11.1% (n=287)
Infectious diseases 7% (n=182)
Cardiovascular diseases 6.9% (n=180)
Neurological diseases 1.8% (n=47)
Ocular diseases 1.3% (n=34)
Inflammatory diseases 0.6% (n=15)
Other diseases 2.2% (n=58)
Gene marking 1.9% (n=50)
Healthy volunteers 2.2% (n=56)
Steady increase in trials and proportion of late phase trials

Phases of Gene Therapy Clinical Trials

- Phase I 56.8% (n=1476)
- Phase I/II 20.9% (n=544)
- Phase II 17.1% (n=445)
- Phase II/III 1% (n=25)
- Phase III 3.8% (n=98)
- Phase IV 0.1% (n=3)
- Single subject 0.2% (n=6)
Type of Gene Therapies: *ex vivo* and *in vivo*

- **Ex vivo transduction**
  - Cross correction
  - Replacement

- **In vivo transduction**
  - Cross correction

**Ex vivo**

- **CAR T-Cell Drugs**
- **Zynteglo**: ß-thalassemia
- **Skysona**: cerebral adrenoleukodystrophy
- **Zolgensma**: Spinal Muscular Atrophy (SMA) (2019)
- **Luxturna**: genetic retinal disease *RPE65* (2017)

**In vivo**

- **SRP-9001-delandistrogene moxeparvovec**: Duchenne 2023
Principles of in vivo IV Gene Therapy

- Viral vector carrying promoter + transgene enters the cell
- Viral vector enters the nucleus of target tissue
- Viral vector capsid disintegrates releasing the transgenic material
- The transgenic DNA does not integrate in nDNA
- Transgenic DNA is transcribed into RNA
- The RNA leaves the nucleus and is translated into protein

Cell membrane

<table>
<thead>
<tr>
<th>Cell membrane</th>
<th>Cytoplasm</th>
<th>Nucleus</th>
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<tbody>
<tr>
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</tbody>
</table>

Viral vector

- Enters the target cell
- Enters the nucleus
- Capsid disintegrates
- Transgenic DNA is released
- DNA is transcribed into RNA
- RNA leaves the nucleus
- Translated into protein
AAV-Based Gene Transfer Therapy

ITRs are required for genome replication and packaging; PolyA signals the end of the transgene to the cellular machinery that transcribes (ie, copies) it.

ssDNA = single-stranded DNA

Safety and Transduction
- Non-pathogenic
- Mostly non-integrating
- Target tropism
- High transduction efficiency with IV delivery
- Payload capacity

Tissue expression and specificity
- Full transgene
- Engineered mini- or micro-trans genes

Adeno-associated virus (AAV)

*ITRs are required for genome replication and packaging; †PolyA signals the end of the transgene to the cellular machinery that transcribes (ie, copies) it.

ssDNA = single-stranded DNA
The Rochester Way: Neurology Department Pioneering Patient-Centered Neurological Care and Experimental Therapeutics since 1966

What it takes to go from bench to clinic

- Discoveries from basic and translational research need to be tested in clinical trials to affect patient care

Patient-Centered Research

Clinical Research and Trials Expertise

- Natural History Studies
- Outcome Measures
- Trial readiness

Commitment to Rare Diseases

- There are 7,000 rare diseases
- 30 million Americans living with a rare disease - 1 out of 10 people
- 8 out of 10 are genetic
- 95% of Rare Diseases Lack an FDA Approved Treatment
- 1 of 2 Patients Diagnosed with a Rare Disease is a Child
Traditional Drug Development Path: not well suited for Rare Diseases and Gene Therapy

Drug Discovery and Development: A LONG, RISKY ROAD

Approval does not equal access

- Access to Highly Specialized Centers with institutional Support
- High Pricing: Insurance and Policies variation among payers
- Avoid Health Inequality

Source: Pharmaceutical Research and Manufacturers of America
Gene-Based Therapies for Neuromuscular Diseases

- Spinal Muscular Atrophy
- Duchenne Muscular Dystrophy
- Myotonic Dystrophy
- Fascioscapular muscular dystrophy (FSHD)
- Limb-Girdle-Muscular Dystrophies
- Pompe Disease
- Hereditary Neuropathy: Charcot-Marie-Tooth (CMT)
- ALS
- Giant Axonal Neuropathy
First Patient treated with Gene Therapy for Spinal Muscular Atrophy at URMC
December 18, 2018
How SMA Changed Everything

Bo Lee, M.D.
Assistant Professor of Neurology and Pediatrics
SPINAL MUSCULAR ATROPHY

PARENTS
- father: genetic carrier of SMA
- mother: genetic carrier of SMA

OFFSPRING
- healthy child: 25%
- SMA carrier: 50%
- SMA: 25%

SMN2 gene
- pre-mRNA
- mature mRNA
- SMN protein
## Classification of Spinal Muscular Atrophy

<table>
<thead>
<tr>
<th>Age of Onset</th>
<th>Highest Achieved Motor Milestone</th>
<th>Life Expectancy*</th>
<th>Proportion of Patients With SMA</th>
<th>SMN2 Copies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 0</td>
<td>Birth</td>
<td>Never sitting</td>
<td>&lt;6 months</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Type I</td>
<td>&lt;6 months</td>
<td>Never sitting</td>
<td>8-24 months</td>
<td>50%-60%</td>
</tr>
<tr>
<td>Type II</td>
<td>6-18 months</td>
<td>Sitting</td>
<td>20s-30s</td>
<td>30%</td>
</tr>
<tr>
<td>Type III</td>
<td>18 months-30 years</td>
<td>Walking</td>
<td>Normal</td>
<td>10%</td>
</tr>
<tr>
<td>Type IV</td>
<td>&gt; 30 years</td>
<td>Walking</td>
<td>Normal</td>
<td>5%</td>
</tr>
</tbody>
</table>

*Without disease-modifying treatment or mechanical ventilation.

- Leading genetic cause of infant death
- Estimated incidence of 1 in 10,000 live births in the US

Natural History of SMA Type 1

90% of SMA Type 1 patients will not survive to the age of 2

% Event-free survival

Age (mos)

Holds head steady alone; brings hands to mouth
Rolls over in both directions
Sits alone; crawls
Cruises; may stand alone
Walks alone; may run and walk up stairs; eats with a spoon
Climb furniture alone; kicks and throws a ball

50% survival* 10.5 mos
25% survival* 13.6 mos
8% survival* 20 mos

*Milestone for a healthy infant
SMA Type 1 survival rates

*Survival = no death, or no need for ≥16-hr/day ventilation continuously for ≥2 weeks, in the absence of an acute reversible illness
Gene therapy for SMA: Onasemnogene abeparvovec

Able to deliver across the blood-brain barrier and into the spinal cord

Designed not to integrate into genome of the patient

Recombinant AAV9 Capsid Shell

Mutated AAV2 ITR  CMV Enhancer  CB Promoter  SV40 Intron  Human SMN cDNA  BGH Poly A  AAV2 ITR

Continuous Promoter
- Hybrid CMV enhancer and CB promoter activates the transgene to allow for continuous and sustained SMN protein expression

Human SMN Transgene
- Full copy of a stable, functioning human SMN gene that is introduced into the cell’s nucleus

Self-Complementary AAV Inverted Terminal Repeats (scAAV ITR)
- The scAAV ITR increases the speed at which the double-stranded transgene is transcribed and the resulting protein is produced
Single-Dose Gene-Replacement Therapy for Spinal Muscular Atrophy

Jerry R. Mendell, M.D., Samiah Al-Zaidy, M.D., Richard Shell, M.D., W. Dave Arnold, M.D., Louise R. Rodino-Klapac, Ph.D., Thomas W. Prior, Ph.D., Linda Lowes, PT, Ph.D., Lindsay Alfano, D.P.T., Katherine Berry, P.T., Kathleen Church, M.S.W., John T. Kissel, M.D., Sukumar Nagendran, M.D., et al.
48 States Currently Screen for SMA | 99% of Newborn Babies in the U.S. are Screened
NYS Newborn Screening Program (1 October 2018 – 30 September 2021)

Newborn screened at birth
~650,000

+ Screen SMN1 / SMN2 reported
N = 34

Median DOL = 7

Outcomes gathered for improvements

Neuromuscular Specialty Care Center

Initial consult
Median DOL = 9
No false positives

Dx confirmation

Insurance Prior Auth

Treatment
N = 32
Median DOL = 34.5
Pediatric Neuromuscular Care Model: How SMA Changed Everything

OLD MODEL (prior to 2016)
- Clinical Diagnosis and Genetic Confirmation
- Diagnostic Delay
- Multidisciplinary supportive care, no disease modifying treatments

NEW MODEL
- Diagnosis through NBS
- Deliver gene therapy: “Time is muscle”
- Manage adverse events
- High cost drugs
- New expertise, new infrastructure, new collaborations
Duchenne Muscular Dystrophy and Gene Therapy

Samuel Mackenzie, M.D., Ph.D.
Assistant Professor of Neurology, Neuroscience and Pediatrics
Motor Signs and Symptoms of DMD

- Delayed walking
- Waddling gait, toe walking
- Difficulty walking, running, jumping, and negotiating stairs
- Lordosis
- Frequent falls
- Gowers sign
- Enlarged calves

Timed rise from supine

Gowers sign
Damage Starts In Utero, Is Progressive, and Leads to Early Death

Birth – 2 years of age

- Birth
  - Creatine Kinase > 2,000 (U/L)
- Poor head control
- Can’t sit without support
- Standing, cruising late
- Walk
  - 15 – 18 months
- Autistic spectrum, delayed speech

3 – 4 yoa
- Increased CK
- Genetic diagnosis
- Motor delay
- Toe walking
- Difficulty stair climbing
- Positive Gowers’ sign

5 – 7 yoa
- Walking difficulty
- Tires easier
- Wheelchair at times
- Frequent falls

8 – 11 yoa
- Loss of ambulation
- Full-time wheelchair use
- Increased arms weakness

Early Teens
- Reduced forced vital capacity
- Need for ventilatory support
- Reduced activities of daily living

Teens
- Cardiac dysfunction
- Heart failure
- Death

Teens to Twenties
Children with Duchenne Suffer Irreparable Harm Highlighting Urgent Need for Effective Therapies

Based on US incidence and prevalence

- ~ 400 lose ambulation each year
- 2,000 more will lose ambulation over 5 years
- Median survival 28 years
- > 400 patients in US die each year
- 2,228 patients will die over 5 years

McDonald et al. 2018; Passamano et al. 2012; Broomfield et al. 2021; Paramsothy et al. 2022
Natural History Demonstrates that Large Portions of Dystrophin Protein Are Less Critical

- First discovered by Professor Kay Davies in 1990
  - Mild Becker muscular dystrophy (BMD) patient who, surprisingly, could still ambulate at age 61
- Genetic testing confirmed patient missing nearly half their dystrophin protein
  - Missing 46% of dystrophin coding region (Del 17 – 48)\(^1\), specifically large stretch of spectrin-like repeats in middle

\(^1\) England et al. 1990

Evidence includes 2 additional mildly affected individuals in the pedigree
Gene Therapy for Duchenne

**Tissue Expression and Specificity**

Function proved to greatly prevent muscle damage in transgenic *mdx* mice and NHP

**Safety and Transduction**
- non-pathogenic
- mostly non-integrating
- strong muscle and heart tropism
- high transduction efficiency with IV delivery
- Payload capacity

**Vector**

**PROMOTER**
- Sarepta Therapeutics: MHCK7
- Pfizer: Muscle specific promoter
- Solid Biosciences: CK8
- Genethon: Spc.12

**TRANSGENE**
- ABD1
- H1, R1, R2, R3, H2, R24, H4, CRD

**AAV SEROTYPE**
- m14
- 9
- 9
- 8
SRP-9001: AAV-Based Investigational Gene Transfer Therapy for Treatment of DMD$^1,2$

<table>
<thead>
<tr>
<th>Expression levels and specificity$^1,3$</th>
<th>Functional impact$^1,4$</th>
<th>Safety profile and transduction efficiency$^1,3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRP-9001 MHCK7$^5$</td>
<td>SRP-9001 dystrophin$^5$</td>
<td>rAAVrh74$^5$</td>
</tr>
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*ITRs are required for genome replication and packaging; †PolyA signals the end of the transgene to the cellular machinery that transcribes (i.e., copies) it.

AAV = adeno-associated virus; AAVrh74 = recombinant human adeno-associated virus 74; ITR = information transfer rate; ssDNA = single-stranded DNA

The achievements of Kunkel and his colleagues have introduced a new era of research that should be useful and exhilarating.

Dr. Rowland Editorial: “Dystrophin”, NEJM 1988

Louis Kunkel and Jerry Lewis announcing the identification of the dystrophin gene Oct. 16, 1986

ELEVYDIS first gene therapy for Duchenne Dystrophy approved June 22, 2023
18 months post-gene therapy (age 7)

First patient dosed in Phase 3 trial at Rochester

Videos courtesy of Jerry Mendell, MD. With permission
World Rare Disease Day

RARE DISEASE TYPES

7,000+
Identified rare diseases, with more being discovered every day.

THE CAUSE

80%
of rare diseases are caused by faulty genes.

RARE DISEASE EFFECT

30 MILLION
AMERICANS

350 MILLION
WORLDWIDE

If all of the people with rare diseases lived in one country, it would be the world’s 3rd most populous country.
What makes a condition a good candidate for gene therapy?

- Small gene
- “Loss of function” condition
- Severe consequences if untreated
- Rare but not too rare
TANGO2-deficiency disorder

- Small gene
- "Loss of function" condition
- Severe consequences if untreated
- Rare but not too rare
A research roadmap

- Retrospective studies of patients
- Natural history studies
- Understanding disease mechanisms
- Development of disease models
- Outcome measure development
- Therapeutic testing (safety, efficacy) in animals
- Clinical trials
- Long-term follow-up studies
THANK YOU

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