Introduction to Gene Therapy and AAV Therapeutics

Noah B. Bissonnette
MacMillan Group Meeting
Literature Review
March 1st, 2022
Outline

What is Gene Therapy?

History of Gene Therapy

AAV Biology

Challenges and Outlook
Outline

What is Gene Therapy?

History of Gene Therapy

AAV Biology

Challenges and Outlook
What is Gene Therapy?

Introduction

“The alteration of cell(s) genome with the goal of providing a therapeutic benefit”

Normal DNA → Folded proteins

Mutated DNA → Misfolded proteins

Monogenic diseases

- Cystic Fibrosis
- Sickle Cell Anaemia
- Hemophilia
- Muscular Dystrophy
- Friedreich’s Ataxia
- Amyotrophic Lateral Sclerosis (ALS)
- Spinal Muscular Atrophy (SMA)
- Huntington’s Disease

An estimated ~6,500 monogenic diseases affect humans

What is Gene Therapy?

Introduction

“The alteration of cell(s) genome with the goal of providing a therapeutic benefit”

Normal DNA

Mutated DNA

Insert or correct

Folded proteins

Misfolded proteins

Folded proteins = Cured monogenic disease
What is Gene Therapy?

Introduction

“The alteration of cell(s) genome with the goal of providing a therapeutic benefit”

Gene Addition

Technologies:

- Adeno-associated viruses (AAVs)
- Retroviruses
- Chimeric antigen receptors (CARs)

Mechanism:

Expression of exogenous DNA

Target:

Modification of somatic cells

What is Gene Therapy?

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Gene Insertion, Modification, and Deletion

Technologies:
- Zinc finger nucleases (ZFNs)
- Transcription activator-like effector nucleases (TALENs)
- CRISPR-Cas9

Mechanism:
- Homology directed repair (HDR) by inducing breaks in endogenous DNA

Target:
- Modification of somatic and germline cells

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What is Gene Therapy?

Retroviral Vectors vs AAVs vs CARs

What is Gene Therapy?

Retroviral Vectors vs AAVs vs CARs

**Retrovirus**
- Oncorecto-, lenți- and spumaviruses
- Can replicate using cell machinery
- Certain subclasses are disease causing
- Packages ~8 kb of ssDNA

**Adeno-Associated Virus**

**CAR-T Cell**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keratin</td>
<td>1.5 kb</td>
</tr>
<tr>
<td>DNA polymerase</td>
<td>2.8 kb</td>
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<tr>
<td>Median protein</td>
<td>26 kb</td>
</tr>
<tr>
<td>Dystrophin</td>
<td>2,400 kb</td>
</tr>
</tbody>
</table>

**What is Gene Therapy?**

*Retroviral Vectors vs AAVs vs CARs*

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**Adeno-Associated Virus**
- Installation of a novel antigen receptor
- Transfection occurs in vitro
- Requires extensive cell culture

**CAR-T Cell**
- Modified patient T-cells

What is Gene Therapy?

Retroviral Vectors vs AAVs vs CARs

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**Adeno-Associated Virus**
- Parvoviridae Dependoparvovirus
- Naturally occurring in humans and primates
- AAVs alone do not cause human disease
- Packages ~4.7 kb of ssDNA

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*AAVs have emerged as the “gold standard” for in-vivo gene addition therapies*

What is Gene Therapy?

History of Gene Therapy

AAV Biology

Challenges and Outlook
Outline

What is Gene Therapy?

History of Gene Therapy

AAV Biology

Challenges and Outlook
Timeline of Development
1965

Adeno-associated viruses are identified by electron microscopy.

Atchison, R. W.; Castro, B. C.; Hammon, W. M.; Science 1965, 149, 754.
Timeline of Development

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Friedmann conceptualizes gene therapy as a treatment for human disease

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1980
Cline (UCLA) illegally attempts the first transfer of foreign genes to treat β-thalassemia

“I don’t know of anyone in the country who has precisely the same type of skills that I have, with knowledge both in the animal systems and in clinical investigations in man. I think that in that sense I must be unique.”
-Martin J. Cline

“It didn’t work in mice, so I’m going to try it in a man”
-Martin J. Cline

“There is very little reason to believe, both from a molecular biology and cell biology standpoint, that an experiment like that would work”
-Philip Leder (NIH) about Cline’s Experiment

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First in human trial of new rAAVs (Safer than wild type AAVs) to treat cystic fibrosis.

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1999
Jesse Gelsinger dies 4 days after being dosed with an experimental gene therapy for ornithine transcarbamylase (OTC) deficiency.

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Adeno-associated viruses are identified by electron microscopy
Timeline of Development

Jesse Gelsinger

Suffered from a very mild form of OTC deficiency
Unable to metabolize ammonia
18th patient in the study
Received the largest dose \(6 \times 10^9\) vg/kg
Trigged a massive immune response leading to organ failure
Timeline of Development

Jesse Gelsinger

Suffered from a very mild form of OTC deficiency

Unable to metabolize ammonia

18th patient in the study

Received the largest dose (6 x 10^9 vg/kg)

Trigged a massive immune response leading to organ failure

James Wilson, M.D. Ph.D.

Found to have violated FDA guidelines

Included Jesse in the study despite high ammonia levels

Failed to disclose the deaths of monkeys in earlier phases

University didn’t properly report 2 patient’s side effects

Barred from clinical trials for 10 years
Wilson continued his research and is now considered the “premier” authority in AAV gene therapy
42 companies, >100 clinical trials
Timeline of Development

2002-2004
New, safer AAVs (7-9) are developed by Wilson (UPenn) enabling lower dosing requirements

Gao, G.; et. al.; *PNAS* 2003, 100, 6081.
Timeline of Development

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Clinical efficacy is achieved for the first time for the AAV treatment of inherited blindness

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Luxturna (Spark Therapeutics) becomes the first FDA approved gene therapy (AAV2).
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- **2017-2022**
  21 more gene/cell therapy treatments are approved by the FDA. As of 2021, >3,000 patients have received AAVs therapies through clinical trials.

AAV Biology

AAV composition

AAV Vector
AAV Biology

AAV composition

Capsid
- Oligomer of 3 proteins
- Protective “shell”
- Different serotypes enable targeting

AAV Vector
**AAV Biology**

**AAV composition**

- **Capsid**
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- **Gene**
  - Rep and cap in natural AAVs
  - Therapeutic DNA in rAAVs
  - 4.7kb maximum size

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Promoter
- Not found in natural AAVs
- CMV or chicken β-actin promoter
- Improves gene expression
**AAV Biology**

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**Inverted Terminal Repeats (ITRs)**
- Essential for downstream expression
- Enables circularization (episomal DNA)
- Only conserved ssDNA in rAAVs

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**AAV Vector**

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- “ITRs”
- “Promoter”
- “Gene”

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- “Capsid”
AAV Biology

Mechanism of gene addition

- Recognized by receptors
- Internalized by clathrin vesicles

AAV Biology

Mechanism of gene addition

- Lower pH enables vesicle escape
  - Enters nucleus or consumed

AAV Biology

Mechanism of gene addition

Uncoating releases ssDNA
- Complementary strand synthesized
- Circularized to episomal DNA

AAV Biology

Immune response to rAAVs


AAV Biology

Immune response to rAAVs

Found in 30 to 60% of the population


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AAV Biology

Immune response to rAAVs

Immunosuppressant drugs can significantly reduce immune response

More problematic in disease states with no residual protein expression

Found in 30 to 60% of the population


AAV Biology

AAV serotypes

"Capsid"

"ITRs"

"Promoter"

"Gene"

AAV Vector
AAV Biology

AAV serotypes

“Capsid”

“Gene”

“Promoter”

“ITRs”

AAV Vector

Novel capsid design enables discovery process

Optimizing the “cap” changes localization
AAV Biology

AAV serotypes

Target-Organs for AAV

AAV1

Brain

AAV2

AAV9

Submandibular glands

AAV12

Lumbar cistern

AAV10

Spleen

AAV11

Smooth muscle cell

AAV2

Skeletal muscle

AAV2

Lung

AAV5

Heart

AAV4

AAV8

Liver

AAV6

AAV7

AAV8

AAV8
AAV Biology

AAV serotypes and immune response

An estimated 30 to 60% of the population contains AAV NAbs

Novel AAV capsids may escape NAbs

% of samples with >50% inhibition of vector transduction
n = 889

AAV Biology

Discovery of AAV serotypes

Directed Evolution

AAV Biology

Discovery of AAV serotypes

Rational Design

AAV Biology

Discovery of AAV serotypes

In Silico Design

AAV Biology

Discovery of AAV serotypes

Naturally Occurring AAVs

**AAV Biology**

**AAV production**

- **HEK293 cells**
- **Transfection**
- **Transfected HEK293 cells**
- **Lysis**
- **Chromatography**
- **Mixture of filled and empty AAVs**

**500 L Bioreactor**

**On-scale AAV production is a major challenge to the field**

Outline

What is Gene Therapy?

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AAV Biology

Challenges and Outlook
Current Challenges and Outlook

Approved therapeutics

Inherited blindness
2017 FDA approval
AAV 2

Lipoprotein lipase deficiency
2012 EMA approval
AAV 1
(Removed from market)

Spinal muscular atrophy
2019 FDA approval
AAV9

Eye, liver, muscle and CNS are major targets
AAV2 is the most common serotype
Current Challenges and Outlook

Approved therapeutics

**Inherited blindness**
- 2017 FDA approval
- AAV 2

**Lipoprotein lipase deficiency**
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  (Removed from market)

**Spinal muscular atrophy**
- 2019 FDA approval
- AAV9

101 active clinical trials
- Eye, liver, muscle and CNS are major targets
- AAV2 is the most common serotype
Current Challenges and Outlook

Case study: Leber's Congenital Amaurosis

Leber's Congenital Amaurosis
Autosomal recessive inherited blindness

Current Challenges and Outlook

Case study: Leber's Congenital Amaurosis

Leber's Congenital Amaurosis

Autosomal recessive inherited blindness

Affects 3 out of 100,000 births

Accounts for ~20% of cases of inherited blindness

25 distinct mutation patterns identified

8% of patients suffer from RPE65 mutation

Fundus photos revealing punctuate yellow dots and pigment mottling associated with LCA

Current Challenges and Outlook

Case study: Leber's Congenital Amaurosis

[Chemical structure image]

Rod outer segment

Retinal pigment epithelium

Current Challenges and Outlook

Case study: Leber’s Congenital Amaurosis

Rhodopsin
Rod outer segment
Retinal pigment epithelium

Current Challenges and Outlook

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Current Challenges and Outlook

Case study: Leber's Congenital Amaurosis

trans-RDH

Rod outer segment

Retinal pigment epithelium

RPE65

Current Challenges and Outlook

Case study: Leber's Congenital Amaurosis

\[ \text{trans-RDH} \rightarrow \text{R}^1\text{Me} \]

\[ \text{Retinal pigment epithelium} \]

\[ \text{RPE65} \]

\[ \text{11-cis-RDH} \]

Current Challenges and Outlook
Case study: Leber's Congenital Amaurosis

Current Challenges and Outlook

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Current Challenges and Outlook

Case study: Leber’s Congenital Amaurosis

“Subtle differences in manufacture processes, final formulation, design of expression cassette, or adjuvant immunomodulatory regimes could potentially affect long-term efficacy”

Current Challenges and Outlook

Case Study: Economics of “regenerative therapies”

Gene and cell therapies are extremely expensive

Number of patients eligible for treatment annually
Current Challenges and Outlook

Case Study: Economics of “regenerative therapies”

- An estimated ~50 new cell and gene therapies could be launched by ~2030
- 650,000 individuals will most likely receive a treatment within the next 10 years
- Likely to become a $24.4 billion (annual) market by 2030

Quinn, C.; et. al.; Value Health. 2019, 22, 621.
Bainbridge, J. W.; et. al.; Drug Discov. 2022, 27, 17.
Current Challenges and Outlook

Case Study: Economics of “regenerative therapies”

Novel Payment Models and Regulation
Outcome based payments
Amortized pricing

Affordable Regenerative Therapies

Reducing Costs
Improvements in on-scale production

Inherent Drivers
Rare diseases have limited patient populations

Quinn, C.; et. al.; Value Health. 2019, 22, 621.
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Introduction to Gene Therapy and AAV Therapeutics

Summary

Promising therapy for curing monogenetic diseases

AAV Vector

Improvements in evading the immune system (NAbs in particular) enable dosing larger populations

Further understanding of basic AAV biology (Novel capsids)

High cost of manufacturing must be reduced to increase adoption
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