GENE THERAPY FOR REFSUM
Today’s Presenters

**Ryan Butler, PhD**
Assistant Professor at UT Southwestern Medical Center

**Kristie DeMarco**
President and Founder of Global DARE Foundation & ULF Board Member
Today's Agenda

Global DARE Foundation's Mission

Gene Therapy Presentation

Question & Answer Session
Webinar Housekeeping Details

• All participants are in listen only mode

• How to ask a question during the Q&A:
  – Participants following on Zoom can type their questions in the Q&A box at any time during the presentation or by raising their hand at the end to ask a question live.
  – Participants joining by phone can press *9 on their phone to raise their hand.

• Questions will be answered in the following order:
  – Q&A box in Zoom
  – Dial in participants
  – Online participants

• Today’s session will be recorded for later viewing on Global DARE Foundation Website (www.defeatadulttrefsumeverywhere.com)
Global DARE Foundation's mission is to promote world-wide awareness and better quality of life for all who are diagnosed with Adult Refsum Disease.
UPCOMING REFSUM WEBINARS

Global DARE Foundation will be holding additional webinars throughout the summer. Registration can be accessed through our website at https://www.defeatadultrefsumeverywhere.org/dare-events

August 10th 2020, 12:00 PM EST
Overview of the Refsum Disease Patient Registry
Global DARE Foundation and Sanford CoRDS will provide an overview and demonstration of the newly released Refsum Patient Registry
Gene Therapy for Adult Refsum Disease

Dr. Ryan Butler
Departments of Psychiatry and Pediatrics
UT Southwestern Medical Center
The promise of gene therapy is to fix a genetic disease at the source. If you fix the DNA, you’ve solved the problem permanently.
Gene Therapy

• Conceptually began in the early 1970’s (Friedmann and Roblin, 1972)

• An engineered viral capsid and DNA construct

• Gene replacement therapy – transduce a functioning gene to overcome a non- or improperly-functioning gene

• Gene transfer to manipulate gene regulatory systems
Gene therapy

• Over 3000 clinical trials approved as of December 2019 (http://www.abedia.com/wiley/vectors.php)
  • 250 with adeno-associated virus

• At least 27 approved clinical trials for liver-directed gene therapy (Baruteau et al. 2017).
How Does Gene Therapy Work?

1. Identify the defective gene
2. Package a working copy of the gene into an engineered virus
3. Use the virus to deliver the working copy of the gene back into the patient
AADC
compassionate use


Gene therapy for aromatic L-amino acid decarboxylase deficiency.

Hwu WL, Muramatsu S, Tseng SH, Tzen KY, Lee NC, Chien YH, Snyder RO, Byrne BJ, Tai CH, Wu RM.
Gene replacement therapy in humans

• 15 patients with SMA1 (Mendell et al., 2017)
  • 3 received low dose ($6.7 \times 10^{13}$ vg/kg) and 12 received high dose ($2.0 \times 10^{14}$ vg/kg)

• All 15 patients event-free at 20 months of age
Does Gene Therapy Make Sense?

- Example: Hemophilia
  - Current treatment costs >$250K/yr per patient, requiring IV infusions every 2 weeks
  - Gene Therapy could provide lifelong treatment after one IV dose.
  - BioMarin gene therapy drug, valoctocogene roxaparvovec (valrox), 6 out of 7 patients that received the highest dose are bleed-free while all 7 do not need prophylactic drugs any more
Will gene therapy be successful for ARD?

- Gene size
- Accessibility of system
- Appropriate vector for cell target
- Immune response
Gene size

• Self-complementary (sc) vs. single-stranded (ss) vs. too large for gene replacement therapy

• 10-100-fold increase in efficiency of transduction with sc (McCarty et al. 2001)

https://askbio.com/differentiation-advantages/
The PHYH gene provides instructions for making an enzyme called phytanoyl-CoA hydroxylase which is critical for the normal function of peroxisomes.

- Breaks down phytanic acid

- 338 amino acids
Will gene therapy be successful for ARD?

✓ Gene size

☐ Accessibility of system

☐ Appropriate vector for cell target

☐ Immune response
Accessibility of system - liver

- Promising clinical results with AAV8 for the treatment of Hemophilia A and B
- Option for optimized targeting of the liver with organ-specific promoters
Target system

• Route of administration

Saraiva et al. 2016
Will gene therapy be successful for ARD?

✓ Gene size

✓ Accessibility of system

☐ Appropriate vector for cell target

☐ Immune response
**Adeno-associated virus (AAV) characteristics**

- single-stranded DNA human parvovirus
- non-pathogenic, requires helper virus for lytic infection
- able to transduce non-dividing cells and confer long-term transgene expression
- can package up to 4.6 kb in place of viral Rep and Cap genes
- dozens of naturally-occurring serotypes identified with differing tissue tropism
- >100 clinical trials initiated using rAAV with a safe track record.
- In 2012, the first gene therapy product received full regulatory approval in Europe. In 2017, the US approved its first AAV product.
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Optimal Serotype</th>
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<tbody>
<tr>
<td>CNS</td>
<td>AAV1, AAV2, AAV4, AAV5, AAV8, AAV9</td>
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<tr>
<td>Heart</td>
<td>AAV1, AAV8, AAV9</td>
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<tr>
<td>Kidney</td>
<td>AAV2</td>
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<tr>
<td>Liver</td>
<td>AAV7, AAV8, AAV9</td>
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<tr>
<td>Lung</td>
<td>AAV4, AAV5, AAV6, AAV9</td>
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<tr>
<td>Pancreas</td>
<td>AAV8</td>
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<td>Photoreceptor Cells</td>
<td>AAV2, AAV5, AAV8</td>
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<tr>
<td>RPE (Retinal Pigment Epithelium)</td>
<td>AAV1, AAV2, AAV4, AAV5, AAV8</td>
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<tr>
<td>Skeletal Muscle</td>
<td>AAV1, AAV6, AAV7, AAV8, AAV9</td>
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https://www.addgene.org/guides/aav/
How to make recombinant AAV

ITR

Transgene

Rep+Cap

Ad Helper genes

“Producer Cell”

GENE
Genome Design

**Enhancer/Promoter:** primary control of cell specificity and strength

**Gene/Coding Sequence**

**PolyA Signal**

**3’ UTR:** Additional regulatory elements, intron or WPRE can boost expression

**5’ UTR:** Additional regulatory elements, intron can boost expression
Will gene therapy be successful for ARD?

✓ Gene size

✓ Accessibility of system

✓ Appropriate vector for cell target

❑ Immune response
Negative reactions to gene therapy

• Immune response
  • Acute, inflammatory response to capsid
  • Innate immunity to AAV (30%)
  • Prophylactic steroids

• Overexpression of gene protein
Will gene therapy be successful for ARD?

✓ Gene size

✓ Accessibility of system

✓ Appropriate vector for cell target

✓ Immune response
Will gene therapy be successful for ARD?

As close to a winning scenario as there can be
Future work

• Develop the viral vectors: codon-optimized cDNA ordered and will be cloned into existing backbone

• Test in existing mouse model

• Perform safety study in rats
Things to consider for translational studies

- Is it **SAFE**?
- Is it **PRACTICAL**?
- Does it work?
- Does it work the same in a mouse as it does in a human?
- Can I make enough to go into a human?
- How will I administer it? Can I do this in a human?
Additional considerations

• Development of better vectors

• Cost
How Does Gene Therapy Work?

1. Identify the defective gene
2. Package a *working* copy of the gene into an engineered virus
3. Use the virus to deliver the working copy of the gene back into the patient
How this really works…

- Identify the defective gene
- Package a *working* copy of the gene into the virus

Get permission
- FDA
- RAC
- IRB

Test in the Patient

Safety
- Talk to the FDA

Funding
How much does a new drug cost?

Typical cost quoted by industry is $1 billion to bring a new drug to market.

For G.A.N. (funded 100% by Hannah’s Hope Fund)
• ~$1.5 million over 6 years for to treat the mouse and develop a platform for nervous system gene delivery.
• $230,000 for vector (“drug”) manufacture
• ~$700,000 for safety studies (FDA requirement)
• ~$1 million for a small clinical trial in humans
• TOTAL = ~$3.5 million
• The “drug” costs ~$50,000 per patient to make, and it needs to be injected ONCE.

• We have several existing industry partners. In addition, proof-of-concept data with Foundation support would go a long way to establishing new partners.
AAV Vector Core with GMP Facility is Now Operational

- Hired and trained facility manager and 11 staff
- Renovated and recertified GMP manufacturing facility
- Manufacturing process is developed and locked.
- Four 50L scale research AAV batches have been produced.
- Facility is on track to begin production on 1\textsuperscript{st} GMP 500L scale batch on Sept. 14.
**Our Pipeline of Diseases**

<table>
<thead>
<tr>
<th>Discovery</th>
<th>IND-Enabling</th>
<th>Clinical Trial</th>
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<tbody>
<tr>
<td>Angelman Syndrome (UBE3A)</td>
<td>Multiple Sulfatase Deficiency (SUMF1)</td>
<td>Giant Axonal Neuropathy (GAN)</td>
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<tr>
<td>Rett Syndrome (MeCP2)</td>
<td>Krabbe (GALC)</td>
<td>CLN1 Batten (active IND)</td>
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<tr>
<td>SPG50</td>
<td>Tay-Sachs (HexAB)</td>
<td>(IND submission late 2021)</td>
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<tr>
<td>ULD</td>
<td>Sandhoff (HexAB)</td>
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<td>Lafora Disease</td>
<td>Aspartylglucosaminuria (AGA)</td>
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<tr>
<td>SLC13A5</td>
<td>Charcot-Marie Tooth type 4J (FIG4)</td>
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<tr>
<td>APBD</td>
<td>CLN7 Batten (MFSD8) **</td>
<td>(IND submission Q3/Q4 2020)</td>
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<tr>
<td>Peroxisome Biogenesis Disorders</td>
<td>CLN5 Batten</td>
<td>(IND submission Q2/Q3 2021)</td>
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<td>Leigh Syndrome (SURF1)</td>
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<td>SLC6A1</td>
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Thank you!

- Butler Lab
  - Hye Ri Kang, Ph.D.
  - Aymun Rahim

- Gray Lab
  - Steve Gray, Ph.D.
  - Erik Lykken, Ph.D.
  - Casy Wilder, Ph.D.

- Department of Psychiatry/Neuroscience
  - Steve Shabel, Ph.D.
  - Helen Lai, Ph.D.
  - Carol Tamminga, MD.
  - David Self, Ph.D.

- GFPD
  - Mousumi Bose, Ph.D.

- UCHC
  - Stormy Chamberlain, Ph.D.

- McGill
  - Nancy Braverman, Ph.D.

- Jackson Laboratories
  - Cat Lutz, Ph.D.
# New collaborators

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<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Position</th>
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<tbody>
<tr>
<td>Florian Eichler, MD</td>
<td>Massachusetts General Hospital (MGH) and Harvard Medical School</td>
<td>Associate Professor of Neurology</td>
</tr>
<tr>
<td>Ronald Wanders, PhD</td>
<td>Academic Medical Center, UMC Amsterdam</td>
<td>Professor Emeritus Clinical Enzymology of Metabolic Disease</td>
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<td>Sacha Ferdinandussa, PhD</td>
<td>Academic Medical Center, UMC Amsterdam</td>
<td>Clinical Laboratory Geneticist</td>
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<tr>
<td>Nancy Braverman, MD, MS</td>
<td>McGill University</td>
<td>Professor, Dept of Human Genetics and Pediatrics</td>
</tr>
<tr>
<td>Joseph Hacia, PhD</td>
<td>University of Southern California</td>
<td>Associate Professor of Biochemistry and Molecular Medicine</td>
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<td>Paul Watkins, MD, PhD</td>
<td>John Hopkins University School of Medicine</td>
<td>Professor Emeritus of Neurology</td>
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<td>Academic Medical Center, UMC Amsterdam</td>
<td>Professor in Ophthalmogenetics</td>
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Q&A

For more information contact:

- Global DARE Foundation
- info@globaldarefoundation.org
- www.defeatadultrefsumeverywhere.org