Developing Treatments for Inherited Eye Diseases

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Financial Disclosures

NONE

Off Label Use

NONE
What do patients want?
What do patients want?

• Treatment

Protect what they have.
Get back what they’ve lost.
What do patients want?

- Treatment

  Protect what they have.

  Get back what they’ve lost.

- Diagnosis and Prognosis

  What can they expect?

  Will their kids be affected?
What do patients want?

- Treatment

- Diagnosis and Prognosis

- And, they want it TODAY!
What do patients want?

• REALISTIC HOPE that they will get these things reasonably soon.
What do patients want?

• REALISTIC HOPE that they will get these things reasonably soon.
• Not if, WHEN.
What do patients want?

• **REALISTIC HOPE** that they will get these things reasonably soon.

• Not if, WHEN.

• Realistic Hope requires a plan (the roadmap) and a dedicated group of people committed to carrying out that plan.
Ed’s Roadmap
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• Nonprofit, philanthropic culture.
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• Share ideas freely.

Publish quickly, share detailed methodology when asked.
Ed’s Roadmap

• Nonprofit, philanthropic culture.
• Share ideas freely.
  Publish quickly, share detailed methodology when asked.
• Leave no one behind.
  Work on lots of different diseases (early and late stages) and lots of different genes at the same time.
Ed’s Roadmap

• Reduce waste.

Grant writing, annual reports, institutional overhead, administrative layers.
Ed’s Roadmap

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  Grant writing, annual reports, institutional overhead, administrative layers.

• Replace animal models with cultured cells whenever possible.
  Use cells for efficacy, animals for safety.
Ed’s Roadmap

• Reduce the cost and improve the sensitivity of genetic tests.
  
  Find patients who might wish to join trials.
  
  Find the remaining disease-causing genes.
Ed’s Roadmap

- Reduce the cost and improve the sensitivity of genetic tests.
  - Find patients who might wish to join trials.
  - Find the remaining disease-causing genes.

- Develop philanthropically funded GMP facilities to reduce the costs of therapeutic vectors and cells.
Ed’s Roadmap

• Develop reusable gene therapy strategies.

  Especially genome editing methods for large and/or expression-sensitive genes.
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  Especially genome editing methods for large and/or expression-sensitive genes.

• Develop cell therapies based upon patient-derived stem cells.
  Reduce the risk of immune rejection.
Ed’s Roadmap

• Analyze existing clinical data to determine the best timing and anatomic location for therapy.
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• Focus almost entirely on Phase I-II clinical trials with long but fairly conventional follow-up.
Ed’s Roadmap

• Analyze existing clinical data to determine the best timing and anatomic location for therapy.
• Focus almost entirely on Phase I-II clinical trials with long but fairly conventional follow-up.
• Do everything with a sense of URGENCY.
Achievable Goal #1 for Rare Genetic Diseases

Have clinical trials of gene therapy for DOZENS of genes underway at the same time.
Achievable Goal #2 for Rare Genetic Diseases

Reduce the time between the initial discovery of a new disease gene and the first gene therapy trial in human subjects to . . .

< 24 months
Steven W. Dezii
Translational Vision Research Facility
Overview of Genetic Testing for Usher Syndrome
Usher Cohort
2204 patients, 1751 families

- William Kimberling
- Sam Jacobson
- Jerry Fishman
- Richard Weleber
- Elias Traboulsi
- Elise Heon
- Byron Lam
- Claes Moller
- Sten Andreasson
- Alex Levin
- Christine Kay
- Raymond Iezzi
- Mina Chung
- Alessandro Iannaccone
Usher Cohort
2204 patients, 1751 families

• 1624 probands screened with an MDPD strategy – 52% positive.
• 35 of the “negative” patients sequenced with whole exome sequencing – 15 positive (42%).
• Suggests that this combination has a 72% sensitivity.
Usher Cohort

2204 patients, 1751 families

• USH2A 575
• MYO7A (1B) 215
• CDH23 (1D) 67
• USH3A 40
• PCDH15 (1F) 28
• USH1C 27
• GPR98 (2C) 9
1000 Consecutive Families
71 with Usher Syndrome
58% positive with MDPD test
77% after exome

- USH2A 33
- MYO7A (1B) 6
- CDH23 (1D) 8
- USH3A 0
- PCDH15 (1F) 1
- USH1C 3
- GPR98 (2C) 4
Why the big focus on philanthropy?
Treatment of Genetic Eye Disease
Treatment of Genetic Eye Disease
Treatment of Genetic Eye Disease
Treatment of Genetic Eye Disease
Treatment of Genetic Eye Disease

USH1F  AMD

Rare    Common
Treatment of Genetic Eye Disease

USH1F  |  AMD

Rare   |   Common

Commercial Viability Threshold
Why work on lots of diseases (and different types of treatments) at once?
Leave No One Behind

Disease Progression

- Assistive Technology
- Prostheses/Optogenetics
- Cell Therapy
- Gene Therapy
- Drugs/Neuroprotection
- Family Planning
Leave No One Behind

Gene Therapy

Disease Progression
Use existing clinical data to determine the timing and location of therapy.
ABCA4-associated Retinal Disease

- **Mild** (Stargardt Disease)
- **Moderate** (Cone Rod Dystrophy)
- **Severe** (Retinitis Pigmentosa)

Redrawn from Schindler, et al., *Human Molecular Genetics*, 2010
ABCA4-associated Retinal Disease

- Mild (Stargardt Disease)
- Moderate (Cone Rod Dystrophy)
- Severe (Retinitis Pigmentosa)

Redrawn from Schindler, et al., Human Molecular Genetics, 2010
Age 46

BC
20/20

5 mm Diameter pupil

Relat. Intens.

+1.00 +2.00 × 117°
Age 46

BC

20/20

5 mm Diameter pupil

Relat. Intens.
MAK gene transfer vector designs

MAK cDNA = 1800 bp
Replace animal models with cultured cells whenever possible.
Untreated vs Treated lengths in µm

- Untreated: 50 µm, 100 µm
- Treated: 50 µm, 100 µm

Bar chart showing length in µm:
- Untreated: 8 µm
- Treated: 4 µm
Genome editing for very large or expression sensitive genes.
CRISPR/Cas9
(clustered regularly interspaced short palindromic repeats)

Feng Zhang
Keith Joung
George Church
CRISPR-CAS9/HDR for genome editing

Simultaneous delivery of 3 separate AAVs to induce “homologous directed repair” (HDR)
1) Tandem guide RNAs for efficient targeting
2) Mutant Nicking CAS9 for specific cutting
3) Repair template with selection cassette to efficiently select corrected iPSCs
Leave No One Behind

Disease Progression

Cell Therapy
Keratinocytes
iPS cells → EB formation

<table>
<thead>
<tr>
<th>Stage</th>
<th>Media Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>EB media + 1ng/ml Dkk1, +1ng/ml Noggin +1ng/ml Igf1 +0.5ng/ml bFGF</td>
</tr>
<tr>
<td>D5</td>
<td>DF media 1 +10ng/ml Dkk1, +10ng/ml Noggin, +10ng/ml Igf1, +10ng/ml bFGF</td>
</tr>
<tr>
<td>D15</td>
<td>DF media 2 +10ng/ml Dkk1, +10ng/ml Noggin, +10ng/ml Igf1, +10ng/ml bFGF, 10mM DAPT</td>
</tr>
<tr>
<td>D21</td>
<td>DF media 3 +10ng/ml Dkk1, +10ng/ml Noggin, +10ng/ml Igf1, +10ng/ml bFGF, 10mM DAPT, +2ng/ml aFGF</td>
</tr>
<tr>
<td>D30</td>
<td>DF media 4</td>
</tr>
<tr>
<td>D150</td>
<td>120 Days</td>
</tr>
</tbody>
</table>

10 Days

10 Days
45 days

200 µm

iPS cells → EB formation → RPC differentiation → Photoreceptor production

D1 → 5 Days → D5
- EB media +1ng/ml Dkk1, +1ng/ml Noggin +1ng/ml lgf1 +0.5ng/ml bFGF

D10
- DF media 1 +10ng/ml Dkk1, +10ng/ml Noggin, +10ng/ml lgf1, +10ng/ml bFGF

D15 → 5 Days → D21
- DF media 2 +10ng/ml Dkk1 +10ng/ml Noggin +10ng/ml lgf1 +10ng/ml bFGF +10mM DAPT

D30 → 10 Days → D150
- DF media 3 +10ng/ml Dkk1 +10ng/ml Noggin +10ng/ml lgf1 +10ng/ml bFGF +10mM DAPT +2ng/ml aFGF

120 Days
iPS cells → EB formation → RPC differentiation → Photoreceptor production

- **D1** → EB media + 1ng/ml Dkk1, +1ng/ml Noggin +0.5ng/ml bFGF
- **D5** → 10 Days
- **D10** → DF media 1 +10ng/ml Dkk1, +10ng/ml Noggin, +10ng/ml IGF1, +10ng/ml bFGF
- **D15** → 5 Days
- **D20** → DF media 2 +10ng/ml Dkk1, +10ng/ml Noggin, +10ng/ml IGF1, +10ng/ml bFGF, 10mM DAPT
- **D25** → 10 Days
- **D30** → DF media 3 +10ng/ml Dkk1, +10ng/ml Noggin, +10ng/ml IGF1, +10ng/ml bFGF, +2ng/ml aFGF
- **D35** → 120 Days

**200 µm**

**45 days**

**70 days**
150 days
Multi-layer Eyecup-like Structure

400 µm
Multi-layer Eyecup-like Structure

Neurosensoriy Retina

RPE

400 μm
Multi-layer Eyecup-like Structure
rhodopsin kinase GFP
Tucker, et al., eLIFE, 2013
Blood Sample

Skin Biopsy

Evaluate Mutations

Establish Cell Lines

Genetic Testing

Therapy
Blood Sample → Skin Biopsy

Genetic Testing → Establish Cell Lines
Blood Sample → Skin Biopsy

Genetic Testing → Establish Cell Lines

Evaluate Mutations

Create Transplantable Cells

Test Efficacy of Gene and Drug Therapies

Therapy

AAV, Lenti, HSV
Blood Sample

Genetic Testing

Establish Cell Lines

Skin Biopsy

Evaluate Mutations

Create Transplantable Cells

Test Efficacy of Gene and Drug Therapies

Therapy
Acknowledgements

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