Gene Therapy for Usher Syndrome (and other treatments)

Mark E. Pennesi, MD/PhD
Assistant Professor
Paul Casey Translational Clinical Trial Center

Physicians
Dave Wilson, MD
Dick Weleber, MD
Tim Stout, MD/PhD
Mark Pennesi, MD/PhD
Alison Skalet, MD/PhD
Andy Lauer, MD

Reading Center
Sigrid Button
Laura Erker, PhD
Edye Parker
Peter Steinkemp
Ellie Chergarnov
Tomy Tan, PhD

Trial Coordinators
Maureen McBride, MS
Catie Beattie, MS

Technicians
Darius Liseckas
Paula Rauch, BS
Melissa Kramer, MS

Statistician
Dawn Peters, PhD
Usher Syndrome - History

- First reported by Albrecht Von Graefe in 1858 where he described three brothers with deaf-blindness

- Scottish Ophthalmologist Charles Usher described 68 patients in 1912 with retinitis pigmentosa and deafness
Retinal Degeneration in Usher Syndrome

Normal Retina

Usher Syndrome
Anatomy of the Eye and Retina

Rods and Cones die in inherited retinal degenerations
Hair cells and photoreceptors share specialized cilia
Usher Syndrome - Subtypes

**Type I:**
- Retinitis Pigmentosa – very severe
- Profound congenital deafness (cochlear implants)
- Vestibular dysfunction (balance problems)

**Type II:**
- Retinitis Pigmentosa – moderate to severe
- Severe congenital deafness (hearing aids)

**Type III:**
- Retinitis Pigmentosa – moderate to severe
- Progressive deafness and vestibular dysfunction
Genes Involved in Usher Syndrome

**USHER SYNDROME I**
- Myo7A (USH1B) - 46%
- USH1C - 6%
- CDH23 (USH1D) - 27%
- PCDH15 (USH1F) - 13%
- USH1G - 7%
- USH1E (Rare)
- USH1H (Rare)
- USH1J – CIB2

At least 8 genes

**USHER SYNDROME II**
- USH2A - 80%
- USH2B - Rare
- USH2C (GPR98) - 15%
- USH2D (DFNB31) - 5%

At least 3 genes

**USHER SYNDROME III**
- CLRN1
- USH3B – HARS

At least 2 genes
The Usher Interactome

From Brown et al.
Tools to Study Usher Syndrome
Family History

Autosomal Dominant

Autosomal Recessive

X-Linked Recessive
Fundus Photography

Patient with Type I Usher Syndrome
Short Wavelength Autofluorescence

Patient with Type I Usher Syndrome
15 year old with USH1B from mutations of MYO7A
Importance of OCT for detecting Macular Edema

Healthy Eye

Cystoid Macular Edema in an Usher Patient
Segmentation of Retinal Layers

**SD-OCT: Outer Retinal Thickness**

<table>
<thead>
<tr>
<th></th>
<th>Normal group AVG</th>
<th>AMD group AVG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overall average</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total retinal thickness(µm)</td>
<td>307.6±12.8</td>
<td>271.3±11.2</td>
</tr>
<tr>
<td>ORL thickness(µm)</td>
<td>194.5±6.6</td>
<td>175.1±10.1</td>
</tr>
</tbody>
</table>
Adaptive Optics

RTx1 from Imagine Eyes

Individual Cone Photoreceptors

AO image demonstrating normal cone mosaic
Adaptive Optics

Cone Density Map
Full Field Electroretinograms (ERG)

Rod-Driven ERG

Cone-Driven ERG

Fig. 2. Simple diagram of the organization of the retina.
Multifocal Electroretinograms

The mfERG only measures 24 deg!
Multifocal Electroretinograms

Healthy Eye

Usher Syndrome
Octopus Visual Fields

Kinetic Field

Left eye (OS), 2012 1:17:26 PM

Right eye (OD), 2012 10:47:04 AM

OCTOPUS®

OCTOPUS 900, SN 1,152, V 2.2.0 / 2.3.0
Kinetic Perimetry  24yr with USH1B
Faster GATE Algorithm allows 184 points to be sampled and cover entire field
Octopus Visual Fields

Abnormal Static Field

STGD-RP 184n GATE V
Left eye (OS) / /2012 / 08:43:06
Greyscale (VA)

STGD-RP 184n GATE V
Right eye (OD) / /2012 / 07:58:54
Greyscale (VA)

Programs: CT Standard, WhiteWhite / GATE
Parameters: 31.4 / 4000 as b V 200 ms
Catch trials: 1/102 (2%) ± 0.02 (27%)
Refraction B/C/A: #
Pupil [mm]: #

Questions / repetitions: 642 / 1
Duration: 20.10
RA: 16.1
Intraocular Pressure (IOP) [mmHg]:

Programs: CT Standard, WhiteWhite / GATE
Parameters: 31.4 / 4000 as b V 200 ms
Catch trials: 2/52 (9%) ± 0.02 (12%)
Refraction B/C/A: #
Pupil [mm]: #

Questions / repetitions: 646 / 1
Duration: 24.15
RA: 10.7
Intraocular Pressure (IOP) [mmHg]:

EyeSuite™ Static perimetry, V2.3.0
OCTOPUS 900, SN 1152, V 2.2.0 / 2.3.0

OCTOPUS®

HAAG-STREIT
INTERNATIONAL
Visual Field Modeling in Usher Syndrome

Normal

Usher Syndrome
Combining Structural and Functional Information
Treatments for Retinal Degenerations

• Low Vision Aids
• Micronutrients
• Neuroprotection/Small Molecules
• Transcript Editing (Antisense Oligonucleotides)
  • Cell Based Therapy (Stem Cells)
    • Optogenetics
    • Artificial Retina
  • Gene Therapy
Low Vision Aids

- Proper glasses prescription
- Night vision goggles
- Broad-beam Flashlight
- Magnifiers and minifiers
- Tablet Devices
Micronutrients and Vitamins

• High dose Vitamin A

  Precautions for taking high doses of vitamin A
  • Liver Toxicity
  • Osteoporosis
  • Pregnancy
  • contraindicating in some mutations (such as those seen in Stargardt Disease)

• DHA (docosahexanoic acid)
• Lutein
• ? ß-carotene

• **AVOID**: Vitamin E, Smoking
Neuroprotection

Ciliary Neurotrophic Factor (CNTF)
Results of clinical trials have not been impressive
What is a Stem Cell?

- Totipotent
  - Unlimited capacity to divide
  - Can become any other tissue

- Pluripotent
  - Unlimited capacity to divide
  - Can become a subset of tissues
How stem cells might work...

Release Protective Factors

Differentiate in Retinal Tissue

Image from Eriraku et al. 2011
# Current Stem Cell Trials

<table>
<thead>
<tr>
<th>Sponsor</th>
<th>Cell Derivation</th>
<th>Differentiation</th>
<th>Delivery</th>
<th>Disease</th>
<th>Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACT</td>
<td>Embryonic</td>
<td>RPE</td>
<td>subretinal</td>
<td>Stargardt</td>
<td>UCLA, Bascom, Wills, MEEI, Moorfields, Korea</td>
</tr>
<tr>
<td>ACT</td>
<td>Embryonic</td>
<td>RPE</td>
<td>subretinal</td>
<td>AMD</td>
<td>UCLA, Bascom, Wills, MEEI, Korea</td>
</tr>
<tr>
<td>Pfizer</td>
<td>Embryonic</td>
<td>RPE</td>
<td>subretinal scaffold</td>
<td>AMD</td>
<td>UCL</td>
</tr>
<tr>
<td>Stem Cells Inc.</td>
<td>CNS</td>
<td>subretinal</td>
<td>AMD</td>
<td>RFSW</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bone Marrow</td>
<td>intravitreal</td>
<td>RP, AMD</td>
<td>Univ. Sao Paulo</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bone Marrow</td>
<td>intravitreal</td>
<td>RP</td>
<td>Madihol Univ (Thailand)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bone Marrow</td>
<td>CD34+</td>
<td>intravitreal</td>
<td>RP, DM, AMD</td>
<td>UC Davis</td>
</tr>
</tbody>
</table>

Source: www.clinicaltrials.gov
Self-Formation of Optic Cups and Storable Stratified Neural Retina from Human ESCs

Tokushige Nakano,1,2,4,5 Satoshi Ando,1,2,4 Nozomu Takata,1 Masako Kawada,1 Keiko Muguruma,1 Kiyotoshi Sekiguchi, Koichi Saito,4 Shigenobu Yonemura,3 Mototsugu Eiraku,1,2 and Yoshiki Sasai1,2,5,*

1Organogenesis and Neurogenesis Group
2Division of Human Stem Cell Technology
3Electron Microscopy Laboratory
RIKEN Center for Developmental Biology, Kobe 650-0047, Japan
4Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka 554-8558, Japan
5Department of Medical Embryology, Graduate School of Medicine, Kyoto University, Kyoto 606-8501, Japan
6Laboratory of Extracellular Matrix Biochemistry, Institute for Protein Research, Osaka University, Suita 565-0871, Japan
*Correspondence: yoshikisasai@cdb.riken.jp
hESC grown in 3D Matrigel with SFEBq media

FACS for RX:Venus positive cells

Transfect venus under RX promoter
A Novel Approach for Subretinal Implantation of Ultrathin Substrates Containing Stem Cell-Derived Retinal Pigment Epithelium Monolayer

Yuntao Hu a,e Laura Liu a,f Bo Lu c Danhong Zhu a,b Ramiro Ribeiro a,g Bruno Diniz a,h Padmaja B. Thomas a Ashish K. Ahuja a David R. Hinton a,b Yu-Chong Tai c Sherry T. Hikita d Lincoln V. Johnson d Dennis O. Clegg d Biju B. Thomas a Mark S. Humayun a


**Fig. 1.** Diagrammatic sketch of the implantation tool. The device consists of a thin parylene plate (A) containing barriers (B) arranged in the form of a ‘U’. The substrate for implantation is placed in the middle of the U-shaped area (substrate chamber).

**Fig. 2.** The ultrathin substrate containing hESC-RPE cells. Images were taken before (a) and after (b) subretinal implantation. Considerable cell loss can be observed along the edges of the substrate (arrows).

4-µm-thick parylene substrates containing a monolayer of human embryonic stem cell-derived RPE
Implantable Retinal Silicon Chips

Argus II Retinal Implant
Gene Therapy
What is gene therapy?

Normal DNA → Protein → Cells

Abnormal DNA → Mutation → Mutant Protein

Gene Therapy Approach
- Replace missing protein
- Decrease bad protein
How to Deliver Gene Therapy?

- **Vector** – a mechanism to deliver DNA to a cell

  - Bare DNA
  - Modified Virus
  - Nanoparticles
Gene Therapy

Gene therapy using an adenovirus vector
Gene Therapy - Subretinal Injection

450µL
Recovery of Subretinal Bleb

- Immediately after injection
- Bleb resorbed
Gene Therapy Trials at Casey Eye Institute

**Current**
- Leber Congenital Amaurosis Type 2 (*RPE65*)
- Stargardt Disease (*ABCA4*)
- Usher Syndrome Type 1B (*MYO7A*)
- Retinostat (Endostatin/Angiostatin for NVAMD)

**Future/Planned Trials**
- X-Linked Retinoschisis (*RS1*)
- Achromatopsia (*CNGB3*)

**Gene Therapy Trials at Other Centers**
- LCA (*RPE65*) – University of Pennsylvania, CHOP, Moorfields, Israel
- Choroideremia (*REP1*) – University of Oxford
- Retinitis Pigmentosa (*MERTK*) – King Khalid Eye Specialist Hospital
UshStat
(For Type 1B Usher Syndrome)
Type 1B Usher Syndrome

Genetic Defect
• Autosomal Recessive
• Mutations in *MYO7A*

Clinical Features
• Severe Early Onset Rod-cone dystrophy
• Severe Congenital Deafness
• Balance problems
Usher Syndrome – Type 1B

61 yr Female, Va = 20/40 OU
Severe Deafness
Usher Syndrome – Type 1B

61 yr Female, Va = 20/40 OU
A Phase I/IIa Dose Escalation Safety Study of Subretinally Injected USHStat™, Administered to Patients with Usher Syndrome Type 1B

**Sponsor:** Oxford Biomedica UK / Sanofi

**Primary Investigator:** Richard Weleber MD

**Design:** Phase I/IIa dose escalation study

**Sites:** Casey Eye Institute, OHSU
          Hospitalier Nationale d’Ophthalmologie des Quinze-Vingts

**Vector:** non-primate lentiviral vector based on EIAV

**Delivery:** Subretinal injection
Based on Equine Infectious Anemia Virus (EIAV)
- Wild-type virus causes transient anemia in horses, non-pathogenic in humans
- Ushstat\textsuperscript{tm} vector contains <10% of original viral genome
- Non-replicating, but does integrate

Safety/Transfection Studies in Non-human Primates
- Minimal inflammation
- Low risk for insertional mutagenesis
Ushstat – Study Design

Dosage

MTD

Highest

Med

Lowest

Cohort 1  n=3

Cohort 2  n=3

Cohort 4  N=3

Cohort 5  N=6

Age:  
≥18 years  
≥18 years  
≥18 years  
≥18 years  
≥6 years

Rod ERG:  
Unrecordable  

Visual Field:  
Legal Blindness  

Severely decreased  
<30% normal

DSMB Review

STOP

STOP

STOP

STOP

STOP

18 Patients Total
Ushstat Inclusion Criteria

**All Patients:** Two confirmed *MYO7A* mutations

**Cohorts 1, 2, 3**
- ≥18 years
- Constriction of Kinetic Visual Field that meets criteria for legal blindness
- No detectable rod ERG

**Cohort 4**
- ≥18 years
- Kinetic Visual Loss - ≥30% reduction sensitivity volume
- Evidence of severe rod/cone dysfunction on ERG

**Cohort 5**
- ≥6 years
- Kinetic Visual Loss - ≥30% reduction sensitivity volume
- Evidence of severe rod/cone dysfunction on ERG
Ushstat Endpoints

Primary – Safety
- Visual Acuity
- Examination
- Static and Kinetic Visual Field
- OCT
- Laboratory Parameters

Secondary – Efficacy
- BCVA
- Kinetic and Static Perimetry
- ERG
- OCT
- Adaptive Optics
Recruitment

• First patient dosed April 17, 2012
• Second patient dosed June 28, 2012
• Third patient dosed Oct 4, 2012
• Fourth patient dosed February 16, 2013
• Fifth patient planned, but trial currently on hold
Ushstat – Study Design

Dosage

- MTD
- Highest
- Med
- Lowest

Cohort 1: n=3
Cohort 2: n=3
Cohort 3: +/- n=3
Cohort 4: N=3
Cohort 5: N=6

Age:
- ≥18 years
- ≥18 years
- ≥18 years
- ≥18 years
- ≥6 years

Rod ERG:
- Unrecordable
- Severely decreased

Visual Field:
- Legal Blindness
- <30% normal

• 4 patients treated (3 from Cohort 1, 1 from Cohort 2)
• No serious adverse events
Conclusions

• Multiple gene therapy trials are underway with more planned

• Preliminary safety reports are encouraging
Oregon Retinal Degeneration Center at the Casey Eye Institute

Physicians/Scientists
Dave Wilson, MD
Richard Weleber, MD
Tim Stout, MD/PhD
Alison Skalet, MD/PhD
John Chiang, PhD

Research Administrator
Laura Erker, PhD

Genetic Counselors
Rebecca Clark, MS
Catie Beattie, MS, CGC

ERG Technicians
Melissa Krahmer, MS
Paula Rauch, BS

Study Coordinator
Maureen Toomey, BS
Catie Beattie, MS, CGC

Technicians
Ellie Chegarnov
Darius Liseckas

Administrative
Jacqueline Holodak
Carolyn Weleber

Lab Technicians
Anastasiya Maricle, MS
Keith Michaels, BS

Collaborators
AGTC
Jeff Chulay, Bill Hauswirth, PhD
Oxford Biomedica
Stuart Naylor, Scott Ellis
Hospitalier d’Ophthalmologie des Quinze-Vingts
Jose Sahel, MD

Grant Support
• Hear See Hope
• NIH/NEI 1 K08 EY021186-01
• Foundation Fighting Blindness – CDA
• Research to Prevent Blindness – CDA
• Research to Prevent Blindness – Unrestricted grant to CEI
• Foundation Fighting Blindness – Center Grant