Gene Therapy Disease Targets

- Leber’s Congenital Amaurosis, LCA2 (RPE 65)
- Usher’s Syndrome
- Stargardt’s, Recessive ABCA4 Form
- Choroideremia
- X-Linked Retinoschisis

Gene Therapy Challenges

- AAV2 Vector Systemic Safety Issues Have Been Largely Resolved, Ocular Inflammation Still Problematic
- High Cost of Clinical Trials, Will Trials Be Vector or Gene/Disease Specific?
- Over 100 Genetic Subtypes of RP
- Each Disease Requires a Specific Gene
- Agent Must Be Delivered Locally Before Significant Loss of Visual Function
Several Helper Viruses are Required to Incorporate Gene Into Vector; Helper Viruses Cannot Be In Clinical Product

Assays Must Be Developed for GMP, Many Challenges

Initial studies with AAV in the retina have utilized AAV serotype 2. Researchers are now beginning to develop new variants of AAV, based on naturally-occurring AAV serotypes and engineered AAV variants.

Several naturally-occurring serotypes of AAV have been isolated that can transduce retinal cells. Following intravitreal injection, only AAV serotypes 2 and 8 were capable of transducing retinal ganglion cells. Occasional Muller cells were transduced by AAV serotypes 2, 8, and 9. Following subretinal injection, serotypes 2, 5, 7, and 8 efficiently transduced photoreceptors, and serotypes 1, 2, 5, 7, 8, and 9 efficiently transduced RPE cells.

One example of an engineered variant has recently been described that efficiently transduces Muller glia following intravitreal injection, and has been used to rescue an animal model of aggressive, autosomal-dominant retinitis pigmentosa.

The retina is immune-privileged, does not experience a significant inflammation or immune-response when AAV is injected. Immune response to gene therapy vectors is what has caused previous attempts at gene therapy to fail, and is considered a key advantage of gene therapy in the eye. Re-administration has been successful in large animals, indicating that no long-lasting immune response is mounted.

Recent data indicates that the subretinal route may be subject to a greater degree of immune privilege compared to the intravitreal route.

AGTC is currently developing products for three orphan diseases – Alpha-1 Antitrypsin Deficiency (lung & liver), X-linked Retinoschisis (XLRS) and Achromatopsia (ACHM). The U.S. Food and Drug Administration and the European Medicines Agency have granted orphan disease designation to AGTC for each three of the diseases, which means these regulatory agencies will provide a variety of special types of advice and assistance to AGTC as we develop our gene therapy products for these diseases.
SPK-RPE65 improves functional vision in patients with a rare form of a genetic disorder known as RPE65-mediated inherited retinal dystrophies
- Linked to subtypes of Leber congenital amaurosis (LCA type 2) and retinitis pigmentosa (RP type 20)

Intravitreal Subretinal Gene Therapy
- Sub-retinal injection is not difficult but there is a significant learning curve
- Sub-retinal gene therapy in wet AMD with active CNV appears to be safe, but is different from inherited retinal disorders with ‘normal’ macula
- Sub-retinal injection results in outer retinal changes that may persist
- Sub-foveal injection risks macular hole formation — may require surgical adjuncts
- Multiple blebs may be required

AVA-101 for wet AMD potentially reduces the need for frequent injections. AVA-101 is comprised of the AAV2 vector, which contains a gene encoding sFLT-1, a naturally occurring anti-VEGF protein, expressed by the host retinal cells; the sFLT-1 protein inhibits the formation of new blood vessels and reduces vascular permeability by binding and blocking VEGF activity.
**Avalanche AVA-101**
- Company said “In Phase 1 and Phase 2a studies, AVA-101 was shown to be well-tolerated with no significant drug-related safety concerns”
- Analysts have said ~10% inflammation rate, formulation related
- Phase 2 Issues Have Caused Delay in Phase 3
- Merged with Annapurna and renamed Adverum with new stock market symbol

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**Wet AMD**
- ADVM-022 and ADVM-032 are next-generation gene therapy product candidates optimized for intravitreal injection, which are being evaluated as treatments for age-related macular degeneration (wAMD) as well as other retinal conditions associated with VEGF over-expression. wAMD is the leading cause of visual deterioration and legal blindness in patients over 60 years of age, and affects ~1.2 million people in the US alone. Standard of care treatment involves injections of anti-VEGF proteins into the vitreal space, as often as every 4-8 weeks.
- ADVM-022 and ADVM-032 utilize a novel vector allowing for intravitreal delivery of anti-VEGF cDNA to potentially treat wAMD as well as other retinal conditions associated with VEGF over-expression.

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**Color Blindness**
- AVA-322L and AVA-323M are next-generation gene therapy product candidates, which are being evaluated as treatments for color vision deficiency (CVD), commonly known as red-green color blindness. CVD is one of the most common genetic diseases for which there currently are no available treatment options, affecting over 10 million people in the US alone.
- AVA-322L carries the gene for L-opsin and is being developed for the treatment of protan defects.

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**Juvenile X-linked Retinoschisis**
- AVA-311 is being developed for the treatment of Juvenile X-linked Retinoschisis (XLRS), an inherited retinal disease caused by mutations in the RS1 gene located on the X chromosome, therefore occurring almost exclusively in males. AVA-311 is comprised of an optimized AAV vector to deliver the RS1 gene into the eye via intravitreal injection.
- In May 2014, Adverum signed a collaboration agreement with Regeneron that includes the development of AVA-311.

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**X-linked Juvenile Retinoschisis**
- X-linked juvenile retinoschisis (XLRS) is caused by changes in the RS1 gene. These changes cause abnormal function of the eye protein retinoschisin. Without normal retinoschisin, the layers of the retina split and vision is lost. NEI team will try to introduce a healthy RS1 gene, to see if this helps retinal cells make healthy retinoschisin. The gene and virus package is known as a gene transfer vector (AAV-RS1 vector).

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**Other XLRS Trials**
- NEI- Paul Sieving MD
- AGTC, Applied Genetic Technologies Corp
LCA UPenn Trial
- University of Pennsylvania and the Children's Hospital of Philadelphia: Jean E. Bennett, MD PhD, Albert Maguire MD, Katherine High MD, and J. Fraser Wright, PhD.
- Improved visual acuity, pupillometry, visual field, light sensitivity, mobility, and functional MRI. In the Phase 1/2 clinical trial, children as young as 8 years old were treated; younger patients had better results than older patients.
- Spark therapeutics was spun out of the work done at the UPenn in 2013. Spark was granted a "breakthrough-therapy" designation by the FDA and in November 2014 the company was running a phase III trial of their gene therapy product SPK-RPE65. Spark planned to submit results to the FDA in 2016.

LCA Moorfields Trial
- The first gene therapy trial for LCA took place in 2007 at Moorfield Eye Hospital and University College London's Institute of Ophthalmology. The first operation was carried out on a 23-year-old British male in early 2007. A relatively large amount of fluid (up to 1 milliliter) was injected underneath the retina. The treatment was well tolerated with no serious adverse events. There was no significant change in visual acuity, peripheral visual fields, or electrophotography. However, one patient had significant improvement in visual function on microperimetry and on dark-adapted perimetry. This patient also showed improvement in subjective visual mobility.

LCA Florida Trial
- Conducted under the leadership of Dr. Samuel Jacobson and Dr. William Hauswirth, funding support from the National Eye Institute. Trial treated adults over the age of 18. Unlike the UPenn trial, the injection was not subfoveal, but extra-foveal. Patients had the best vision in the injected area, rather than the fovea. This led to the development of a so-called "pseudo-fovea" that corresponded to the treated area.

Choroideremia
- In October 2011, the first clinical trial was announced for the treatment of choroideremia. Dr. Robert MacLaren at University of Oxford, lead the trial, co-developed treatment with Dr. Miguel Seabra of the Imperial College, London. Phase 1/2 trial used subretinal AAV to restore the REP gene in affected patients. Initial results of the trial were reported in January 2014 as promising as all six patients had better vision.

Achromatopsia
- Non-randomized, open-label, Phase 1/2 study of the safety and efficacy of rAAV21YF-PR1.7-1xCNGB3 administered to one eye by subretinal injection in individuals with achromatopsia caused by mutations in the CNGB3 gene.

Gene Therapy Challenges
- Inflammation with Intravitreal Injection Much Greater Than With Subretinal Injection
- Transfection Occurs Near Injection Site; Injection Must Submacular
- Hydraulic Macular Hole Can Be Caused by Subretinal Injection
RetroSense's lead candidate, RST-001 employs a photosensitivity gene, channelrhodopsin-2, to create new photosensors in retinal cells and restore vision (very limited vision, Steve Charles editorial comment) in retinal degenerative conditions such as RP and advanced dry-AMD.

Channelrhodopsin-2 is supported by a strong body of published literature on its efficacy and safety in animal models. Numerous studies have demonstrated the ability of channelrhodopsin-2 to restore light perception and vision in animals with naturally occurring or induced blindness due to loss of photoreceptors. In primate studies, the administration of channelrhodopsin-2 was well tolerated. This approach to vision restoration was pioneered by Dr. Zhuo-Hua Pan at Wayne State University and Dr. Alex Dizhoor at Salus University.

RetroSense is currently at the pre-clinical stage of development and working toward human clinical trials. RST-001 will be developed initially for retinitis pigmentosa, with advanced dry-AMD as a follow-on indication.

**CRISPR/Cas9 Gene Editing**

- *In Vivo* CRISPR/Cas9 Gene Editing Corrects Retinal Dystrophy in the S334ter-3 Rat Model of Autosomal Dominant Retinitis Pigmentosa
- Benjamin Bakondi, Wenjian Lv, Bin Lu, Melissa K Jones, Yuchun Tsai, Kevin J Kim, Rachelle Levy, Aslam Abbasi Akhtar, Joshua J Breunig, Clive N Svendsen and Shaomei Wang

**Stem Cells**

- Sources
  - Induced Pluripotential Stem Cells
  - Embryonic Stem Cells
  - Mesenchymal Stem Cells (not viable for RPE or retina)
  - RPE + Possibly Photoreceptor Only, Not Retina (over 100 cell types, complex connectivity, and T.7M nerve fibers)
  - RPE & Choriocapillaris RPE & Retina Are Inter-Dependent; If Either One is Dysfunctional or Atrophic, the Other Undergoes Atrophy
  - Trans-Synaptic Degeneration of Horizontal, Bipolar, Amacrine, and Ganglion Cells Follows Photoreceptor Apoptosis
  - Stem Cells May Ultimately Be Useful But Many Complex Problems to Solve

**RPE Cell Replacement**

**Stem Cells for Trophic Factors**
Stem Cell Issues
- Sterility w/o viral/prion/bacterial contamination crucial during entire lengthy (~120 days) differentiation process
- Must be authentic RPE or photoreceptor cells
- eSC (embryonic stem cells)
  - Rejection, MHC compatibility crucial, cannot immune suppress elderly AMD patients
  - Ethical issues
- iPSC (induced pluripotential stem cells)
  - Recurrent disease possible because patient’s stem cells have their AMD genes; an opportunity for CRISPR cas9 gene editing

RPE Stem Cells for Geographic Atrophy (dry AMD)
- Photoreceptors, Bipolar Cells, and Ganglion Cells Degenerate (in that order) After RPE Loss, Therefore Stem Cell Derived RPE Cells Must Be Implanted on Diseased RPE, Not Absent RPE
- Injections of Cell Suspensions are Not Effective, Biodegradable Scaffold with a Monolayer of RPE Cells Must Be Used

<table>
<thead>
<tr>
<th>Retinal Disease Cell Source</th>
<th>Sponsor</th>
<th>Trial Design</th>
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</thead>
<tbody>
<tr>
<td>Stargardt’s Macular Dystrophy</td>
<td>Advanced Cell Technology</td>
<td>Phase I/II, Open-label, Multi-Center, Prospective Study</td>
</tr>
<tr>
<td>Dry AMD</td>
<td>Advanced Cell Technology</td>
<td>Phase I/II, Open-label, Multi-Center, Prospective Study</td>
</tr>
<tr>
<td>Acute Wet AMD</td>
<td>Pfizer, University College London</td>
<td>Phase I, Not started</td>
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<tr>
<td>Age-related Macular Degeneration (AMD)</td>
<td>StemCells, Inc.</td>
<td>Not started</td>
</tr>
</tbody>
</table>

Table 1: Molecular and functional criteria of authentic human retinal pigment epithelium (RPE) cells

<table>
<thead>
<tr>
<th>Feature</th>
<th>Testable criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPE signature gene set (fetal and adult)</td>
<td>Maintenance of expression of the RPE signature set of genes, including genes for melanogenesis, channel proteins, tight junction proteins, visual cycle, response to sensory perception, oxidoreductase activity, phagocytic activity, and transporter activity</td>
</tr>
<tr>
<td>Micro-RNAs (fetal and adult)</td>
<td>Absence of expression of genes associated with early development, including genes marking ES or induced pluripotent stem cells or neuroectodermal cells of the optic-iris-epithelial cap (OCX1, OCX2, NAKO1, KLIF4, MYC, LRRD1 high levels of RAN8, MTP)</td>
</tr>
<tr>
<td>Pumplin, fetal, and non-epithelial genes</td>
<td>Absence of expression of organ-specific genes but presence of expression of tumour suppressor genes</td>
</tr>
</tbody>
</table>

RPE derived from iP7 cells should not have an agenetic memory of their tissue origin except when derived from RPE or neuroectodermal progenitors. Characteristics of promoter choice, splicing patterns, and post-translational modifications such as phosphorylation, sumoylation, or ubiquination (dependent on ES origins and de-ubiquitination) likely, cells should be post-mitotic, i.e., not express ES or incorporate BrdU.
Iris RPE cells do not support the visual cycle (all-trans to 11-cis rhodopsin reisomerization).

Peripheral or nasal RPE cells do not support macular photoreceptor viability, either because of epigenetic microenvironment or terminal differentiation.

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Problems with Alternative RPE Cell Sources

- Rapid, chaotic rewiring of inner retina occurs after photoreceptor death (Marc).
- Glial “seal” between inner and outer retina forms rapidly after photoreceptor death (Robert Marc).

Issues Common to RPE Stem Cells, Retinal Prosthesis

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