AAV-mediated gene therapy for rare disorders: New possibilities for treatment of genetic disease

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Howard Hughes Medical Institute
The Children’s Hospital of Philadelphia

HHMI
Two basic strategies to achieve long-term expression

Viral Vector

In vivo
Transduction of a long-lived, post-mitotic cell type, in which case integration not needed

Ex vivo
Integration into a stem cell

Nature Reviews Genetics 2011
Potential complications of gene therapy

- Gene silencing
- *Genotoxicity-integration events
- Phenotoxicity-overexpression of the protein
- Immunotoxicity
- Horizontal transmission
- Vertical transmission
AAV structure and composition

- Highly ordered set of proteins (vector capsid) containing DNA (the active agent)
- Dosage in vector genomes/kg
- Maximum packaging capacity ~5 kb.

<table>
<thead>
<tr>
<th>Element</th>
<th>#/virion</th>
<th>VP 1</th>
<th>VP 2</th>
<th>VP 3</th>
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</thead>
<tbody>
<tr>
<td>ssDNA</td>
<td>1</td>
<td>6</td>
<td>6</td>
<td>48</td>
</tr>
<tr>
<td>ssDNA</td>
<td></td>
<td>735 aa</td>
<td>597 aa</td>
<td>533 aa</td>
</tr>
<tr>
<td>ssDNA</td>
<td>4680 nt</td>
<td>1</td>
<td>6</td>
<td>48</td>
</tr>
</tbody>
</table>

AAV Vector

AAV vectors-viewed from a genetic engineering standpoint

- Vector acts as a highly ordered set of proteins that accesses receptors on cell surface and delivers DNA to nucleus. DNA persists, delivery proteins degraded.

AAV vectors-viewed from an immunologic standpoint

- Engineered from a virus that the immune system has previously encountered. Immune responses to the recombinant virion must be characterized and managed.
Questions

• Biological
  – What host immune response is encountered by a recombinant virion that 1) fails to synthesize viral antigens, but also
  2) fails to synthesize viral genes that normally down-regulate host immune response?

• Medical/therapeutic
  – Additional complexity introduced by tissue-specific aspects of the human immune response
  – Can the host immune response generated by a viral-like particle be controlled so that gene transfer occurs, and the host is unharmed?
The first AAV product was licensed in Europe in 2012
19 July 2012
EMA/882900/2011
Committee for Medicinal Products for Human Use (CHMP)

Assessment Report

Glybera

International Nonproprietary Name: Alipogene tiparvovec

Procedure No. EMEA/H/C/002145
Path to market authorization

- **June 2011**-”The favourable effects of Glybera are uncertain and not considered to be clinically relevant. The unfavourable effects outweigh the possibly favourable effects...the CAT concluded that the overall Benefit Risk for Glybera is negative.” Major findings...
  - no consistent or convincing evidence of long-lasting effect on TG or CM
  - Insufficient evidence of reduction in rate of pancreatitis
  - Uncertainty over efficacy combined with concerns on safety

- **July 2011**-Requested re-examination of the CHMP decision. Ad hoc panel of experts convened. Findings of re-evaluation issued **October 2011** included:
  - Insufficient evidence of persistent LPL activity
  - CAT position-clinically relevant reduction in risk of pancreatitis in at least some patients. Proposed allowing for those with history of pancreatitis.
  - CHMP again concluded that benefit/risk was unfavorable
Path to market authorization

• January 2012, European Commission Standing Committee requests CHMP to assess benefit risk in patients with severe or recurrent pancreatitis

• July 2012
  – “adult patients diagnosed with familial lipoprotein lipase deficiency (LPLD) and suffering from severe or multiple pancreatitis episodes despite dietary fat restrictions. The diagnosis of LPLD has to be confirmed by genetic testing.”
  – Obligation for post-authorization studies, disease registry
  – No major concerns about chemistry and manufacturing of product
AAV-mediated, liver-directed gene transfer is a platform for treatment of a variety of metabolic disorders and plasma protein deficiencies

<table>
<thead>
<tr>
<th>Disease</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propionic acidemias</td>
<td></td>
</tr>
</tbody>
</table>
Hurdles in AAV gene therapy for liver

1997
Obstacle: Large-scale vector manufacturing

2000-2001
Obstacle: Germline issues

2002
Obstacle: Immunotoxicity

2000
Obstacle: Risk of integration

Safety issue: But not seen in dogs, NHP and humans thus far
Coagulation and Hemophilia

- X-linked bleeding disorder caused by the absence of functional coagulation factor VIII (hem A) or factor IX (hem B)

- Characterized by frequent bleeds in joints and soft tissues, and less frequently into other critical closed spaces

- 1 in 5000 male births

- Severe <1%, moderate 1-5%, mild >5%
Long term expression of FIX in hemophilic dog liver and in human muscle tissues

But NOT in human liver

Subject E

- Subject E received $2 \times 10^{12}$ vg/kg
- Identified therapeutic dose, accurately predicted by dog model
- FX activity level gradually lost coincident asymptomatic rise in liver transaminases (AST, ALT)
- Markers for hepatocyte injury
- Returned to baseline over next 9 wks
- Not predicted by animal models
- Raised possibility of immune-mediated destruction of transduced hepatocytes

Herzog et al., HGT 2002
Mingozzi et al., JCI 2003
Mount et al., Blood 2002;
Niemeyer et al., Blood 2009

EXPANSION OF CAPSID-SPECIFIC CD8+ T CELLS

- subject G
- 5 wks: expansion of p74-specific CD8+ T cells
- 20 wks: subsequent contraction
- kinetics of CD8+ T cell expansion parallel transaminitis
- expanded CD8+ T cells specifically lyse HLA-matched target cells in vitro
- secrete IFN-γ after peptide stimulation
- cross-reactivity with corresponding peptide from different AAV serotypes, expected based on high degree of conservation

Neutralizing antibodies to AAV block transduction

Human immune response presents 2 obstacles to AAV-mediated gene transfer to liver

• *Humoral immunity* in the form of neutralizing antibodies to AAV capsid that prevent transduction when vector delivered through the circulation

• A *cellular immune response* to the vector capsid, which if unchecked can destroy the transduced cells and lead to loss of expression of the donated gene
Second AAV-F.IX trial
UCL/SJCRH

- Incorporates solutions to problems identified in the first trial
  - Exclude those with NAb to AAV
  - Add short course of steroids if liver enzymes rise of F.IX level drops
Plasma FIX levels

Low vector dose \((2\times10^{11}\text{vg/kg})\): 1st subject (P1)

- Off prophylaxis >30 months \(\rightarrow\) FIX level @ 1-2%
  - except prior to a minor surgical procedure
- No spontaneous bleeding episodes
  - received vector 30 months ago

\[ \text{hFIX:C (IU/dL)} \]

\[ \text{Days} \]

\[ \text{Factor usage (\% of pre-injection)} \]

\[ \text{NEJM 2011} \]
Further dose escalation to $2 \times 10^{12}$ vg/kg results in capsid T cell expansion and liver enzyme elevation in subject 5.

Capsid IFN-γ ELISPOT

NEJM 2011
Vector administration in Subject 6 ($2 \times 10^{12}$ vg/kg) results in capsid T cell expansion and mild liver enzyme elevation.

* Low cell viability

NEJM 2011
Development of AAV-mediated gene transfer to liver for hemophilia

- Vector Injection
- Short term: Immune Response
  - NAb blocks transduction
  - CD8+ T cell response: Destroys transduced cells
- Long term: Risk of integration
  - Safety issue

Humoral
Cellular
Three AAV-Factor IX trials now open with more in the planning stages

On-going Hemophilia B gene transfer clinical trials:

<table>
<thead>
<tr>
<th>Sponsor</th>
<th>ClinicalTrials.gov Identifier</th>
<th># Subjects</th>
<th>Transgene</th>
<th>Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCL/St. Jude Children's Research Hospital</td>
<td>NCT00979238</td>
<td>10</td>
<td>Codon optimized wild type-sc</td>
<td>AAV8</td>
</tr>
<tr>
<td>Children's Hospital of Philadelphia</td>
<td>NCT01620801</td>
<td>15</td>
<td>Codon optimized wild-type-ss</td>
<td>AAV8</td>
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<tr>
<td>Asklepios Biopharmaceutical, Inc.</td>
<td>NCT01687608</td>
<td>16</td>
<td>Padua-sc</td>
<td>AAV8</td>
</tr>
</tbody>
</table>

Planned Hemophilia B gene transfer clinical trials:

<table>
<thead>
<tr>
<th>Sponsor</th>
<th>ClinicalTrials.gov Identifier</th>
<th># Subjects</th>
<th>Transgene</th>
<th>Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jichi Medical Center</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>Free U. B.</td>
<td>N/A</td>
<td>9</td>
<td>Padua-ds</td>
<td>AAV9</td>
</tr>
</tbody>
</table>
Ocular applications

- Doses needed are small, simplifying manufacturing requirements
- Immune privileged site reduces risks related to immune response
Leber’s congenital amaurosis

- Autosomal recessive
- Early onset retinal degeneration
- Symptoms appear in infancy/early childhood
- No treatment available
- 10-20% cases of LCA are due to mutations in RPE65
- Mouse and dog models of RPE 65 deficiency
# Leber’s Congenital Amaurosis

## Classification of Leber’s

<table>
<thead>
<tr>
<th>Type</th>
<th>Gene</th>
<th>Locus</th>
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<tbody>
<tr>
<td>LCA1</td>
<td>GUCY2D</td>
<td>17p13.1</td>
</tr>
<tr>
<td>LCA2</td>
<td>RPE65</td>
<td>1p31</td>
</tr>
<tr>
<td>LCA3</td>
<td>RDH12</td>
<td>14q23.3</td>
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<tr>
<td>LCA4</td>
<td>AIPL1</td>
<td>17p13.1</td>
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<td>LCA5</td>
<td>6q14</td>
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<td>LCA6</td>
<td>RPGRIp1</td>
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<td>LCA9</td>
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<td>LCA10</td>
<td>CEP290</td>
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<tr>
<td>LCA11</td>
<td>IMPDH1</td>
<td>7q31.3-q32</td>
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</table>

Affects 1 in 76,000

~15% of all LCAs
**RPE65** encodes a protein (isomerase) necessary for production of a vitamin A derivative, 11-cis-retinal, required for vision.
Rescue of vision in Briard dogs with RPE65 mutation

Subretinal injection of AAV2-RPE65

Vision still intact 7 years later

Animal models- rods/cones present but slowly degenerate

<table>
<thead>
<tr>
<th>Animal Model</th>
<th>Efficacy</th>
<th>Age at time of treatment</th>
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<tbody>
<tr>
<td>Dog</td>
<td>34/35</td>
<td>3-14 mos</td>
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<tr>
<td>Mice</td>
<td>24/30</td>
<td>2-4 mos</td>
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<tr>
<td></td>
<td>4/25</td>
<td>15 mos</td>
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</tbody>
</table>

Abundant and robust POC in a large animal model

Acland et al., Nature Genetics 2001
Jacobson et al., PNAS 2005
### Summary of Subjects Enrolled to Date at CHOP:

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP01</td>
<td>26</td>
<td>F</td>
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<tr>
<td>NP02</td>
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<tr>
<td>NP03</td>
<td>19</td>
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<td>NP04</td>
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<td>CH06</td>
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<td>CH08</td>
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<td>CH09</td>
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<td>M</td>
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<tr>
<td>NP15</td>
<td>10</td>
<td>M</td>
</tr>
</tbody>
</table>

- Excellent Safety Profile
Subretinal injection of AAV-RPE65

Visual Acuity

Mobility

fMRI

Visual Field

PLR

Sci Transl Med., 2012
Mol Ther., 2010
Lancet, 2009
Pupillary responses in normal and AAV-RPE injected subject

Maguire et al., NEJM 2008
CH09 (8 yo)
3 Months Post Injection

Navigation in dim light:

SUPPLEMENTARY VIDEO 1A

Maquire et al.
"Treatment of Leber Congenital AMAurosis due to RPE65 Mutations in Children and Adults using Adeno-Associated Virus (AAV)-mediated Gene Delivery"

CH09, day 90, Navigation using untreated eye

SUPPLEMENTARY VIDEO 1B

Maquire et al.
"Treatment of Leber Congenital AMAurosis due to RPE65 Mutations in Children and Adults using Adeno-Associated Virus (AAV)-mediated Gene Delivery"

CH09, day 90, Navigation using treated eye

Uninjected Eye

Injected Eye

Lancet 374(9701), 2009
Effect of Gene Therapy on Visual Function in Leber’s Congenital Amaurosis

James W.B. Bainbridge, Ph.D., F.R.C.Ophth., Alexander J. Smith, Ph.D.,
Susie S. Barker, Ph.D., Scott Robbie, M.R.C.Ophth.,
Ananth Viswanathan, M.D., F.R.C.Ophth., Graham E. Holder, Ph.D.,
Andrew Stockman, Ph.D., Nick Tyler, Ph.D., Simon Petersen-Jones, Ph.D.,
Shomi S. Bhattacharya, Ph.D., Adrian J. Thrasher, Ph.D., M.R.C.P., F.R.C.P.,
Fred W. Fitzke, Ph.D., Barrie J. Carter, Ph.D., Gary S. Rubin, Ph.D.,
Anthony T. Moore, F.R.C.Ophth., and Robin R. Ait, Ph.D.

Safety and Efficacy of Gene Transfer for Leber’s Congenital Amaurosis

Albert M. Maguire, M.D., Francesca Simonelli, M.D., Eric A. Pierce, M.D., Ph.D.,
Edward N. Pugh, Jr., Ph.D., Federico Mingozi, Ph.D., Jeannette Bennicelli, Ph.D.,
Sandro Banfi, M.D., Kathleen A. Marshall, C.O.T., Francesco Testa, M.D.,
Enrico M. Surace, D.V.M., Settimio Rossi, M.D., Arkady Lyubarsky, Ph.D.,
Valder R. Arruda, M.D., Barbara Konkle, M.D., Edwin Stone, M.D., Ph.D.,
Junwei Sun, M.S., Jonathan Jacobs, Ph.D., Lou Dell’Osso, Ph.D.,
Richard Hertle, M.D., Jian-xing Ma, M.D., Ph.D., T. Michael Redmond, Ph.D.,
Xiaosong Zhu, M.D., Bernd Hauck, Ph.D., Olga Zelena, Ph.D.,
Kenneth S. Shindler, M.D., Ph.D., Maureen G. Maguire, Ph.D., J. Fraser Wright, Ph.D.,
Nicholas J. Volpe, M.D., Jennifer Willirnann McDonnell, M.S., Alberto Auricchio, M.D.,
Katherine A. High, M.D., and Jean Bennett, M.D., Ph.D.

Human RPE65 Gene Therapy for Leber Congenital Amaurosis: Persistence of Early Visual Improvements and Safety at 1 Year

Artur V. Cideciyan,1 William W. Hausrith,2,3 Tomas S. Aleman,1 Shalesh Kaushal,2 Sharon B. Schwartz,1
Sanford L. Boye,2 Elizabeth A.M. Windsor,1 Thomas J. Conlon,2 Alexander Sumaroka,1
Ji-jing Pang,2 Alejandro J. Roman,1 Barry J. Byrne,3 and Samuel G. Jacobson1
Clinical Study #AAV2-hRPE65v2-102
(Phase 1/2 Follow-on, Second Eye Administration) - CHOP

Part One
(3 adults)

Part Two
(up to 9 subjects)

Regulatory Submissions

November 2010

May 2011

June to September 2011

October 2011

October 2012

(3 to 4 months)

(6 months)

(3 to 4 months)

(12 months)

NIH/OBA RAC Submission (exempt)

CHOP IRB & IBC Review and Approval

DSMB Review and Approval

Submission to IND 13408

8 week stagger to monitor for immune responses to re-administration

Vector administrations occurred in November 2010, January 2011, and March 2011

DSMB review of Part One subjects through Week 8

Submission to CHOP IRB to release Part Two consent and assent documents

Stagger reduced to 3 weeks

Eleven of twelve subjects have received 2nd eye injection (one subject is currently not eligible due to glaucoma in the un-injected eye)

Sci Transl Med 2012
Mobility Testing Course Diagram

Lancet 374(9701), 2009
Science Translational Medicine 2012
Other eye trials now in progress

<table>
<thead>
<tr>
<th>Disease</th>
<th>Vector</th>
<th>Sponsor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age-related macular degeneration</td>
<td>AAV</td>
<td>Genzyme</td>
</tr>
<tr>
<td>Age-related MD</td>
<td>AAV</td>
<td>Avalanche</td>
</tr>
<tr>
<td>Choroideremia</td>
<td>AAV</td>
<td>Oxford University</td>
</tr>
<tr>
<td>Retinal disease 2° MERTK</td>
<td>AAV</td>
<td>King Khaled Eye Hospital, Saudi Arabia</td>
</tr>
<tr>
<td>Stargardt’s disease</td>
<td>Lentiviral vector</td>
<td>Oxford BioMedica</td>
</tr>
</tbody>
</table>
Biotech/Pharmaceutical Companies with Significant Ocular Gene Therapy Programs
Rhodopsin cloned

1st Rhodopsin mutation identified

RPE65 cloned

1st human RPE65 mutation in LCA

RPE 65 mutation in dog

Dog Model Lancelot

Mapped
Identified

Mapped and Identified Retinal Disease Genes 1980 - 2008

From RetNet
In vivo gene transfer with AAV

• Proof-of-concept established for delivery to liver, to subretinal space, and to focal areas of CNS.

• Each target tissue is a separate series of problems, but once solved, provides a platform
Dr. Jean Bennett, Penn Dept. of Ophthalmology
Dr. Albert Maguire
Acknowledgments

• CHOP
  – Federico Mingozi
  – Hojun Li
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  – Gary Pien
  – Etien Basner-Tschkarajan
  – Daniel Hui
  – John Finn
  – Shangzhen Zhou
  – Marcela Maus
  – Shangzhen Zhou
  – Xavier Anguela
  – Rajiv Sharma

• UNC-Chapel Hill
  – Tim Nichols

• Iowa
  – Steve Russell

• Penn/LCA2 trial
  – Jean Bennett
  – Albert Maguire

• SUN
  – Francesca Simonelli

• CHOP
  – Fraser Wright
  – Paris Margaritis
  – Valder Arruda
  – Katie Manno
  – Jen Wellman
  – Greg Podsakoff

• Wistar/Penn
  – Gundi Ertl

• AAV2 clinical trial collaborators
  – Stanford-Kay, Nakai, Glader
  – Avigen-Couto, Pierce
  – Pitt-Margaret Ragni
  – Sydney-Rasko
  – Campinas-Ozello, DiPaula

• St. Jude/UCL/Royal Free
  – Nathwani, Tuddenham, Nienhuis, Davidoff, Reiss