Human Gene Transfer Protocol #740

A Phase I Safety Study in Subjects with Leber Congenital Amaurosis (LCA) using Adeno-Associated Viral Vector to Deliver the Gene for Human RPE65 into the Retinal Pigment Epithelium (RPE)"

Center for Cellular and Molecular Therapeutics *at* The Children's Hospital *of* Philadelphia

- Location
  - Children's Hospital of Philadelphia (CHOP)
  - Investigators with appointments at University of Pennsylvania
- PI: Albert Maguire, MD
- Sponsor: CCMT

- Scientific Director: Jean Bennett, MD, PhD
- CCMT Vector: Fraser Wright, PhD

Order of Presentation

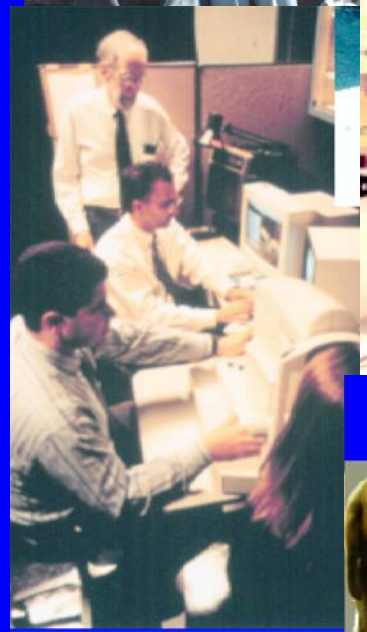
- Jean Bennett, MD, PhD
  - Background: the human disease
  - Pre-clinical proof-of-concept studies
  - Pre-clinical Safety Studies
- Fraser Wright, PhD
  - Vector Manufacture for Human Clinical Trial
- Albert Maguire, M.D.
  - Proposed Human Clinical Trial Involving Pediatric Subjects
  - Subject Selection
  - Trial Design
  - Future Plans
- Chris Rockey
  - Patient Advocate

Original Proof-of-Concept Studies Took Place >5.5 Years Ago (July 2000)

Gregory Acland
Gus Aguirre
Gerri Antonini
Amanda Nickle
Sue Pierce-Kelling
Jharna Ray
Qi Zhang
Cornell and Penn

Tomas Aleman
Artur Cideciyan
Sam Jacobson
U of Pennsylvania

Bill Hauswirth
U of Florida



Albert Maguire
Jean Bennett
Yong Zeng
Vibha Anand
U of Pennsylvania

Lancelot

Funding:
NEI, FFB
RPB and others

An expanding set of collaborators….

- Cornell University
- Gregory Acland
- Gerri Antonini
- Amanda Nickle
- Sue Pearce-Kelling
- Jharna Ray
- Qi Zhang

- University of Florida
- William Hauswirth
- Terence Flotte
- Margaret Humphries
- Shalesh Kaushal
- Sanford Boyce
- Vince Chiodo

University of Washington

Kristof Palczewski

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Jean Bennett
Albert Maguire
Jeannette Bennicelli
Nadine Dejneka
Enrico Surace
Daniel Chung
Nicholas Keiser
Tonia Rex
Shicheng Yang
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Eric Pierce

Edward Pugh
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Samuel Jacobson
Artur Cideciyan
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Gustavo Aguirre
Andras Komaromy
William Beltrand

Geoffrey Aguirre

Yale University

Carolyn Zeiss

University of Iowa

Edwin Stone

Case Western Reserve

Jonathan Jacobs
Lou Dell'Osso

University of Pittsburgh

Richard Hertle

UPenn/CHOP

Katherine High
Fraser Wright
Berndt Hauck
Valder Arruda

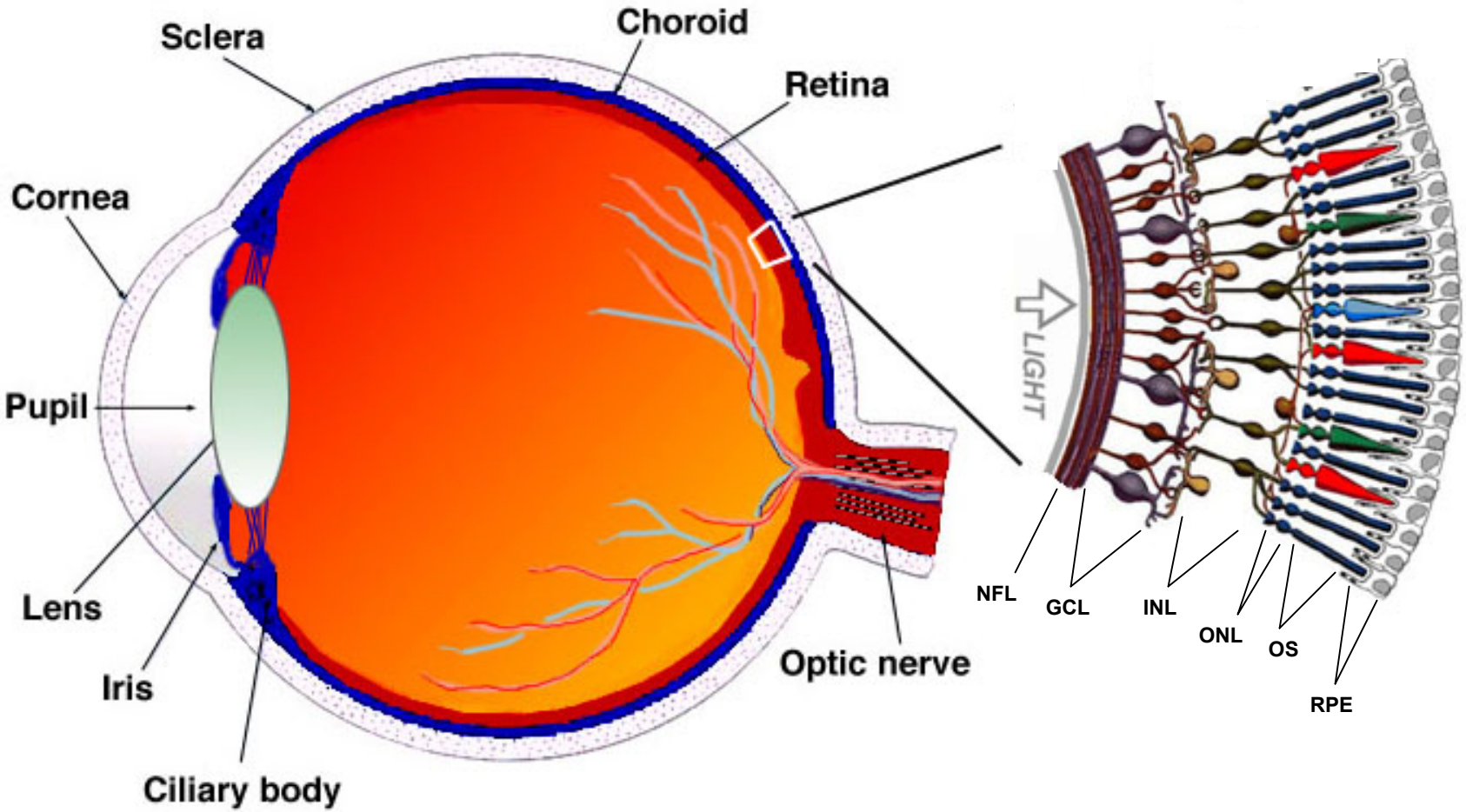
What is Leber Congenital Amaurosis (LCA)?

- **Early onset retinal degeneration**
  - **severe vision loss**
  - **abnormal eye movements**
- **Symptoms are apparent in infancy/early childhood**
- **Mutations in any one of at least 9 different genes can give rise to the disease**
- **No treatment**
- **No cure**

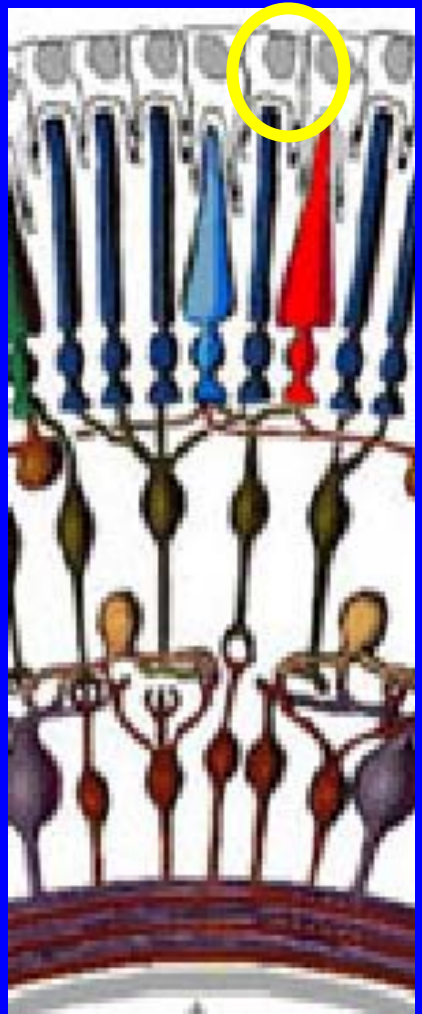
Mutations in the following genes have been found to cause LCA

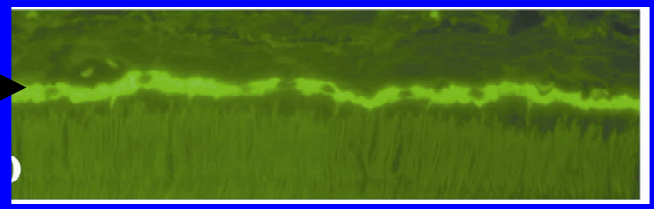
- RPE65
- CRX
- GUCY2D
- AIPL1
- TULP1
- CRB1
- RPGRIP1
- LRAT
- RDH12

http://www.sph.uth.tmc.edu/Retnet/home.htm

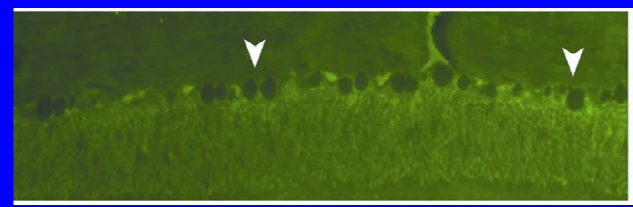

Sclera
Choroid
Retina
Cornea
Pupil
Lens
Iris
Ciliary body
Optic nerve
LIGHT
NFL
GCL
INL
ONL
OS
RPE

RPE65 encodes an enzyme (retinoid isomerase) necessary for production of a vitamin A derivative, 11-cis-retinol, which is necessary for vision





Normal Dog has RPE65 protein in retinal pigment epithelium…



Affected dog does not
(Acland et al 2001
Nat Genet 28:92-5}

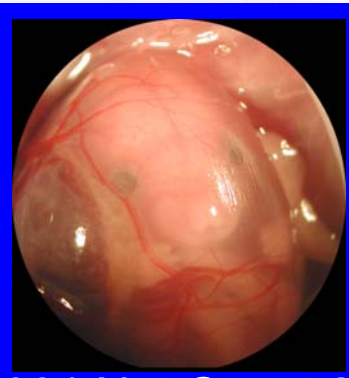
Redmond et al 1998 Nat Genet 20:344-51
Jin et al 2005 Cell 122:449-459

Proof-of-concept studies

Subretinal injections of AAV-RPE65 restored vision in:

- Dogs which have naturally occurring mutations in RPE65

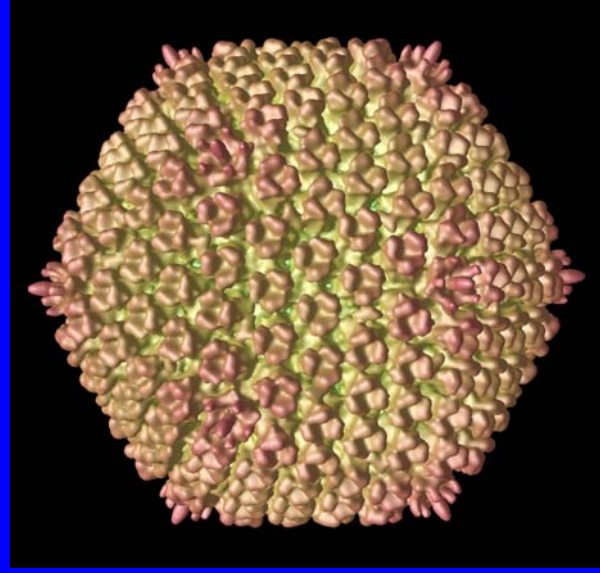
- Mice with “knockout” of Rpe65



Acland et al 2001 Nat Genet 28:92-5
Dejneka and Surace et al 2004 Mol Ther 9:182-8

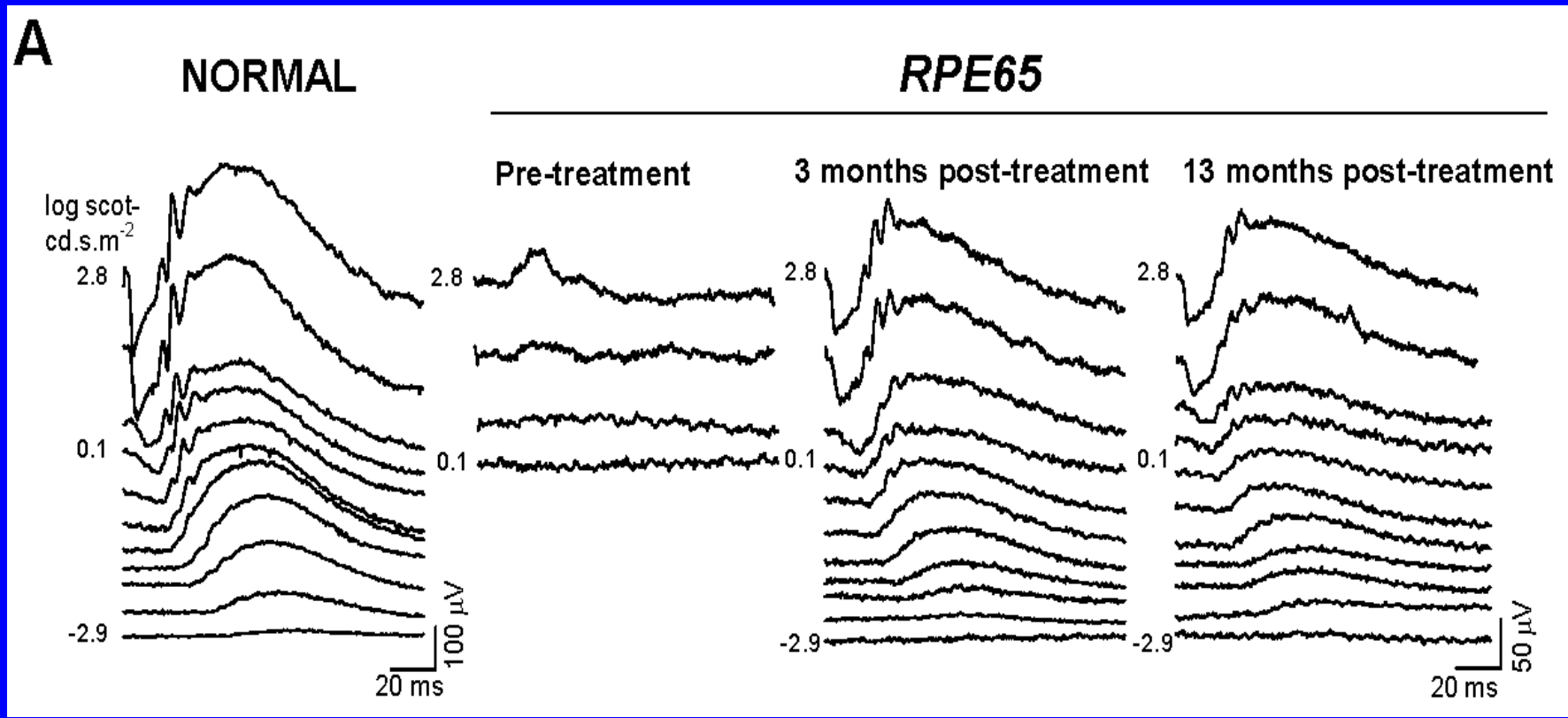
Data show similarity of animal model phenotypes with those of humans with LCA-RPE65
Jacobson et al 2005 PNAS 102:6177-82

The Vector



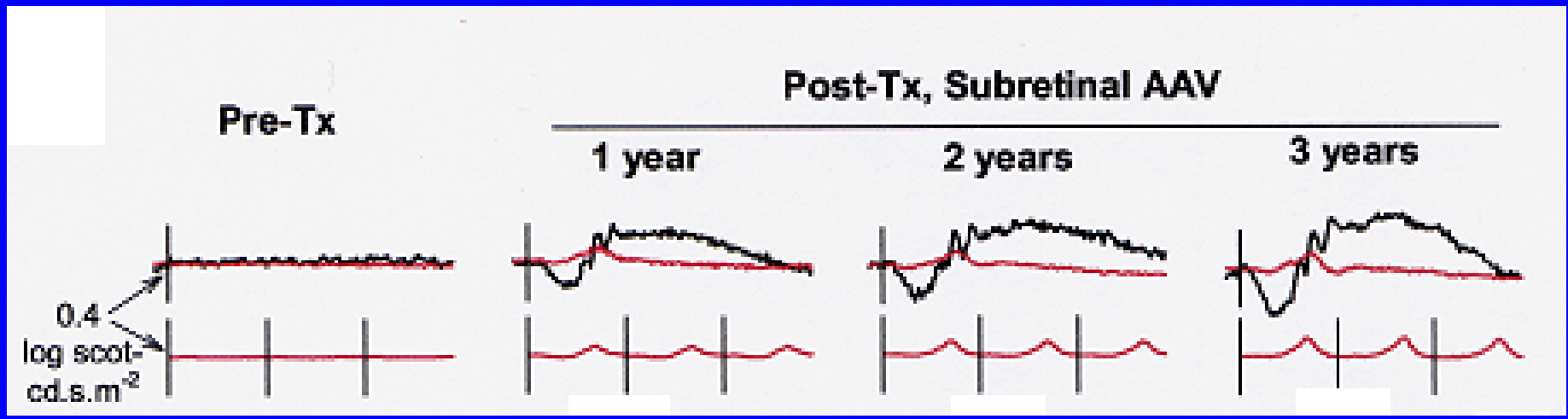
- Adeno-associated virus
- Serotype 2 ITRs and capsid
- Transgene cassette:
  - CMV/Chicken β actin promoter
  - Intron
  - hRPE65 cDNA
  - Poly(A)

Therapeutic Effect: No Diminution over Time in Rod-Mediated ERG



Acland et al 2001 Nat Genet 28:92-5
Acland et al 2005 Mol Ther 12:1072-82

Before and yearly post-treatment ERGs

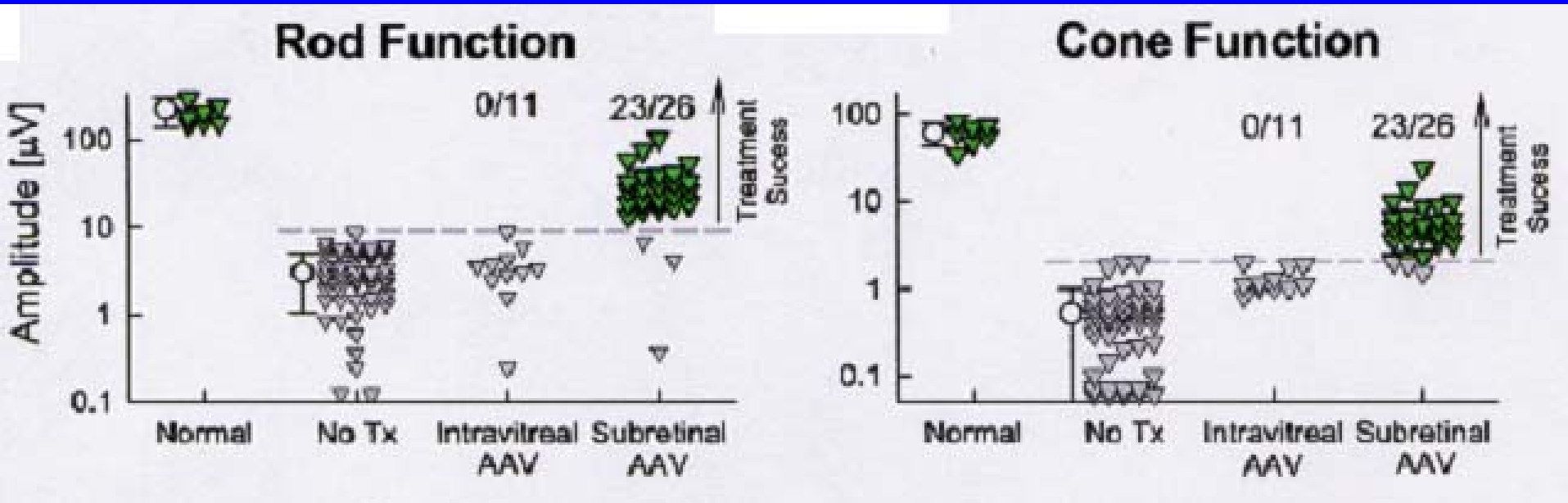


Acland et al 2005 Mol Ther 12:1072-82

Therapy and Visual behavior Twin 4 month old affected dogs: one treated, one untreated

{Large dog is unaffected}

Visual Function Results: Series of 26 dogs



Acland et al 2005 Mol Ther 12:1072-82

Preclinical Toxicity Data

- One dog is presently at 5.5 years post treatment.
  - Healthy from clinical perspective
  - Continues to enjoy vision in treated eye

To date there has been rescue in

- Dogs
  - 34/35 (97%) subretinally injected eyes from affected dogs (30 dogs) treated with >0.1X AAV.hRPE65, ages (2.7 mos – 14.2 mos)
  - 0/16 intravitreally or sub-RPE-injected eyes showed rescue
- Mice
  - 9/13 (69%) affected mice treated *in utero*
  - 24/30 (80%) affected mice treated at postnatal ages 2-4 mos
  - 4/25 (16%) affected mice treated at postnatal ages >15 mos

Dejneka and Surace et al 2004 Mol Ther 9:182-8
Jacobson et al 2005 PNAS 102:6177-82
Acland et al 2001 Nat Genet 28:92-5
Acland et al 2005, Mol Ther 12:1072-82

Preclinical Toxicity Studies

- All animals remained clinically healthy for the duration of the study
- No adverse effects on:
  - Food consumption
  - Body weight
  - Hematology
  - Clinical Chemistry
- Mild (and reversible) ocular inflammatory response after surgery except for 1 eye:

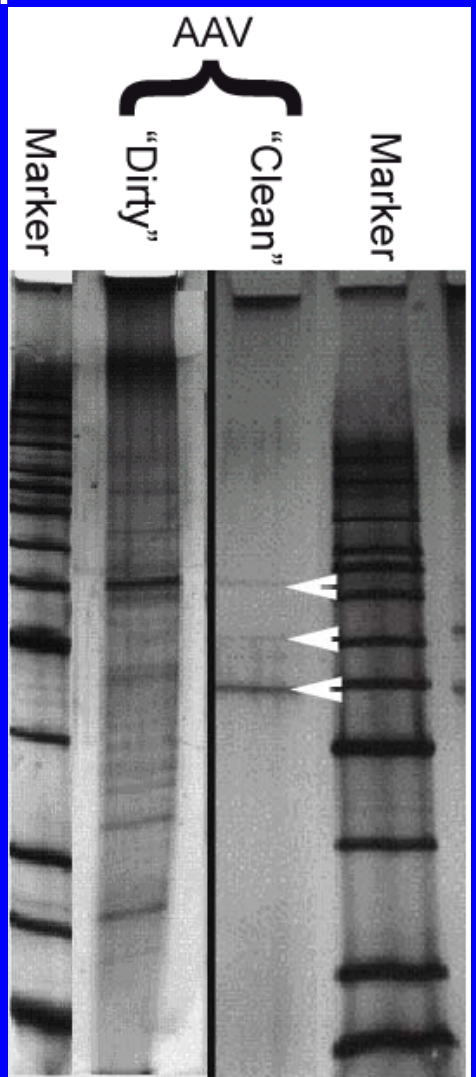
Preclinical Toxicity

Contamination of Vector Prep and Ocular Inflammation

- In Fall 2001, vector preparations were combined to inject a set of 11 dogs (20 eyes)
- A number of the eyes had inflammation after surgery
- All except 1 responded to medical treatment
- Ruled out: Contamination with bacterial pathogens, shedding of vector
- We have not seen significant inflammatory reactions in any of the 3 dogs treated prior, or in any of the >30 dogs treated subsequently with (quality-controlled) AAV.RPE65

Preclinical Toxicity (cont)

Most likely cause of inflammation: Incomplete purification of one of the vector preparations:



Silver-stained protein gel:

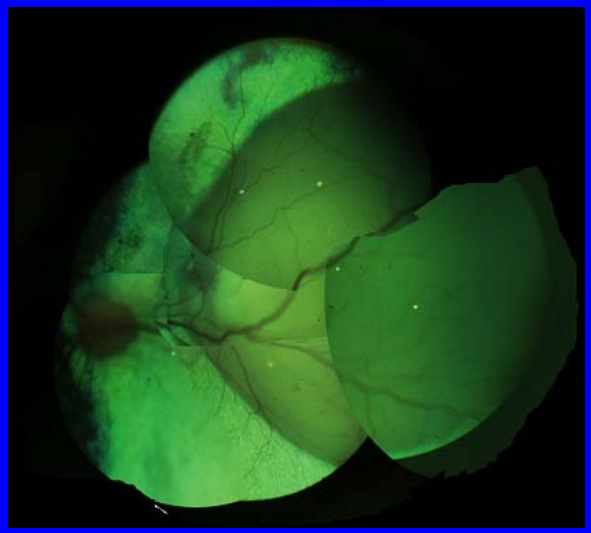
- 3 distinct bands (arrowheads) indicate purified AAV
- These were present in 3 of the 4 vector preps that were combined ("Clean")
- The fourth prep had a smear of bands ("Dirty")

Modified from:
Acland et al 2005, Mol Ther 12:1072-82

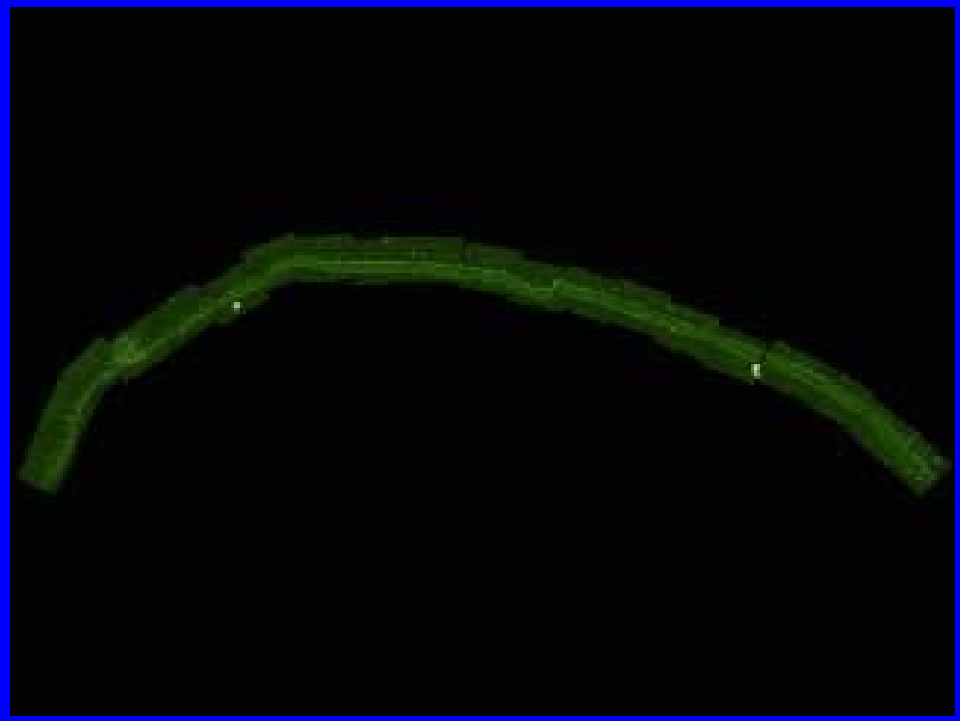
Preclinical Toxicity Data Vector spread beyond retina

- Gonads
  - Transmission from parent to child
    - No evidence in intraocularly treated dogs or mice
    - Results from hemophilia B human clinical trials (K. High et al)
      - 140,000,000,000,000 or 1.4E14vg (1,000X higher than what we propose to inject subretinally) were injected into skeletal muscle -> no vector in semen samples
      - 1.4E14 vg were injected into hepatic artery -> transient detection of vector sequence in semen
- Brain
- Elsewhere

RPE65 protein is delivered specifically to the retinal pigment epithelium of exposed retina



Fundus view immediately after subretinal injection of 11.5 mo old affected dog with AAV.hRPE65

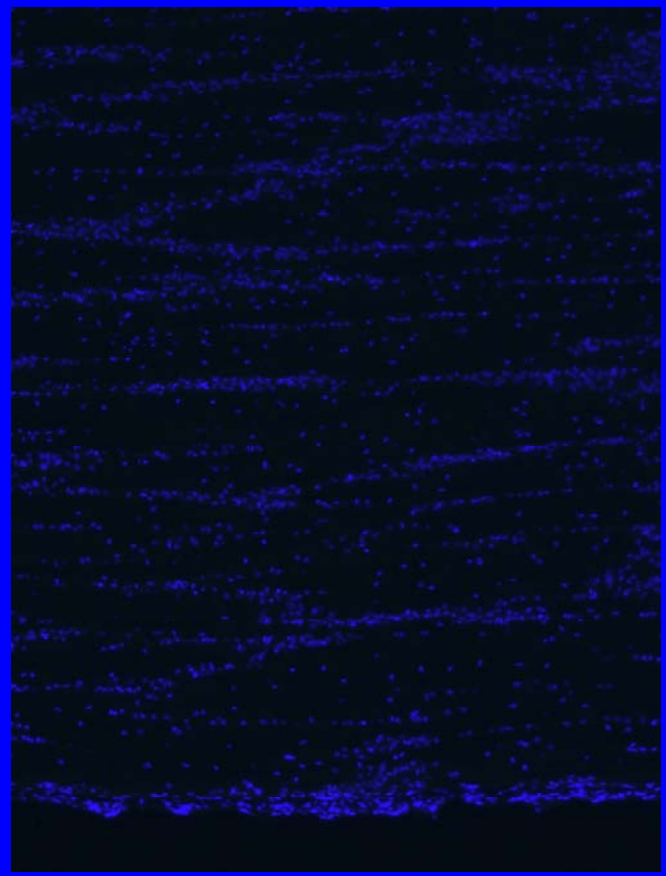


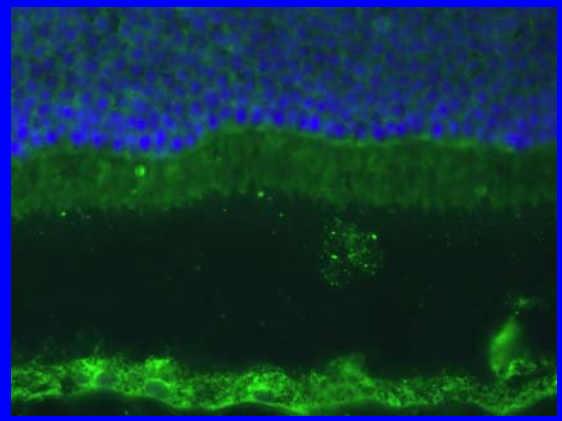
Immunofluorescent detection of RPE65 protein 5.5 months later (age 17 mos) {movie by S Pearce-Kelling et al}

Acland et al 2005 Mol Ther 12:1072-82 (Supplementary); unpublished data

Vector spread beyond the retina

No Evidence of RPE65 Protein in Optic Nerve Tracts of Affected Dogs Treated with AAV.hRPE65





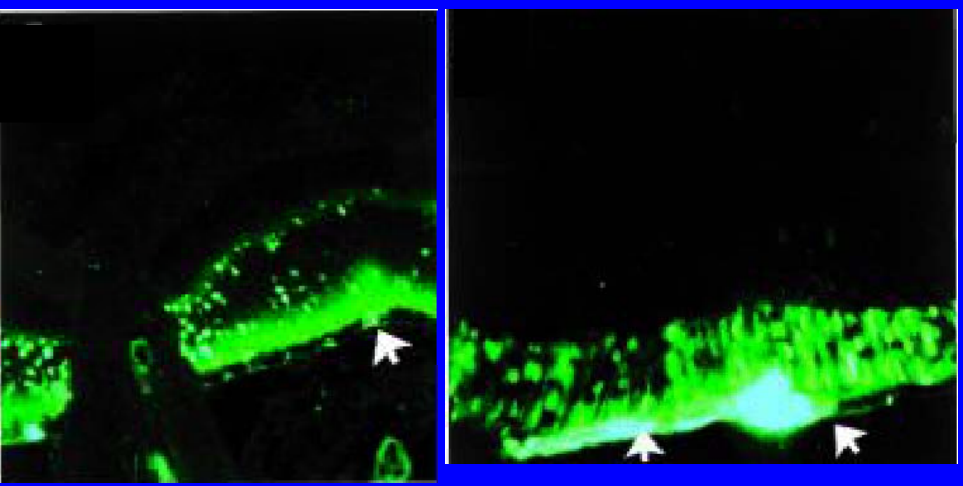
Positive Control: Rpe65-/- mouse treated with subretinal AAV.hRPE65

No RPE65-Specific Immunofluorescence in Optic Chiasm

Preclinical Toxicity Data

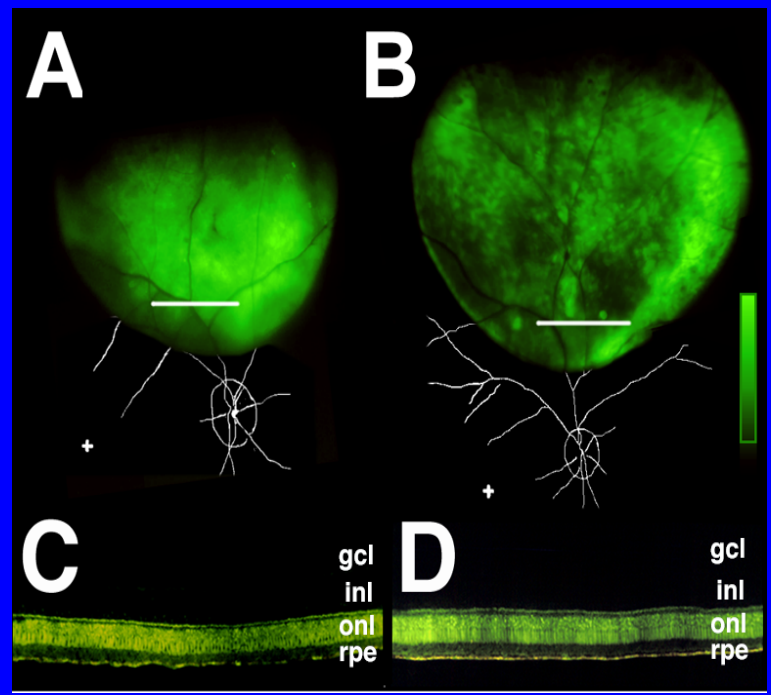
Successful repeat administration in contralateral eye despite small increases in AAV2-specific Abs after injection of the first eye

Mice



Anand et al, Hum Gene Therapy 11:449 (2000)

Monkeys



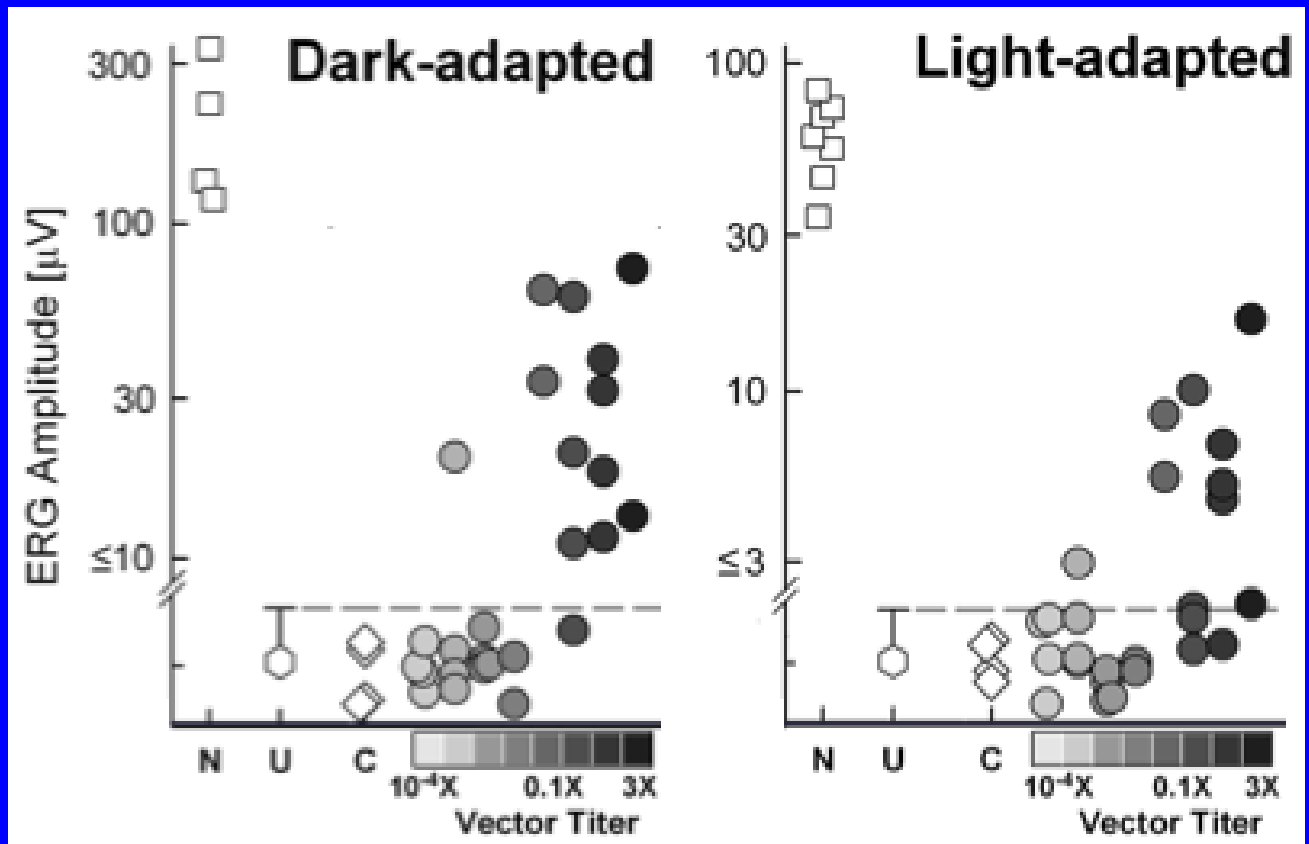
Bennett et al, PNAS 96:9920 (1999)

Determination of Dose Levels

- Cumulative experience with 29 eyes of affected dogs treated with subretinal AAV.hRPE65; Dose range = ~10^{10}-10^{12} vector genomes (vg)) {Acland et al 2005 Mol Ther 12:1072}
- Dosing study in 15 affected dogs over a 4.5 log unit range of doses of AAV.hRPE65 (0.0001X to 3X dose), where 1x = 1.5 X 10^{12} vg

Determination of Dose Levels (cont.)

- All eyes receiving > 0.1X dose showed significant ERG responses
- One eye receiving 0.001X dose showed ERG responses
- We propose to begin our dose series at the 0.001X dose



Jacobson et al,
RAC June 2005

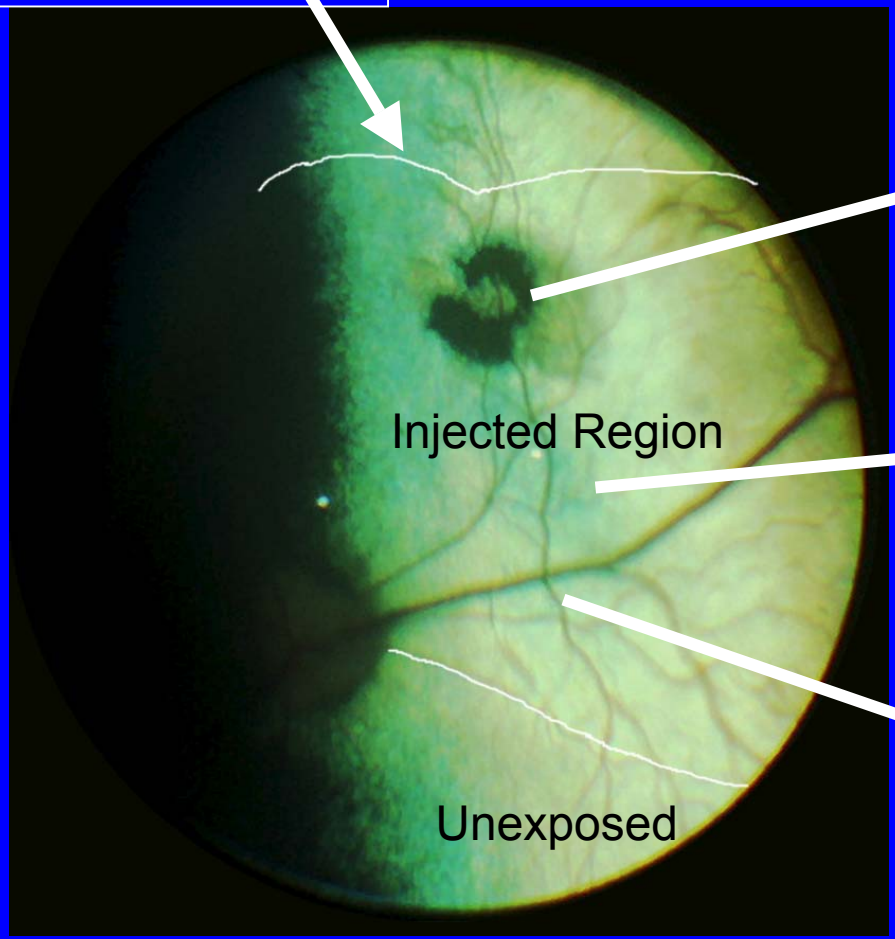
Dose and Inflammation

- Affected dogs and (unaffected) monkeys
- Inflammation scored for different ocular compartments
- The inflammatory changes in both monkeys and dogs were comparable to those noted after injection of vehicle
- Trend of increased ocular inflammation with dose, with the 3X dose resulting in the most inflammation
- Only mild ocular inflammatory changes were observed in NHPs even after injection of the highest (3X) dose and all of these resolved after 1 month.
- Slightly higher inflammatory changes were noted in the equivalent canine study, however these mostly resolved after 2 months {Note: ocular surgical inflammation in dogs >>> than most other species}

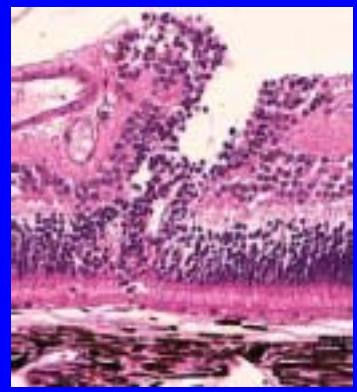
Preclinical Toxicity Data

Histopathological changes 3 mos after subretinal AAV.hRPE65

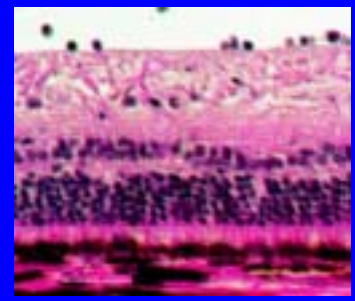
Demarcation Line



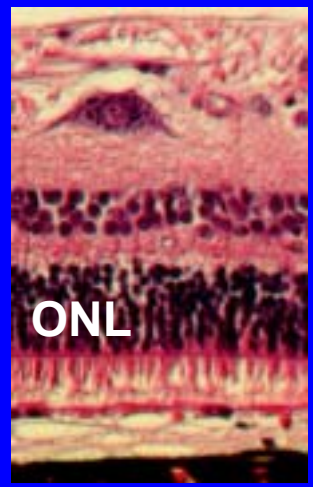
1X Dose



Trauma at Injection Site



Inflammatory Cells



Thinning of ONL

GLP Preclinical Toxicity Study

- Safety and biodistribution of the vector that will be used in the CCMT-LCA clinical trial will be the focus of a GLP preclinical toxicity study using early and late timepoints

Abramson Research

3615 Civic Center Blvd
Philadelphia, PA 19104

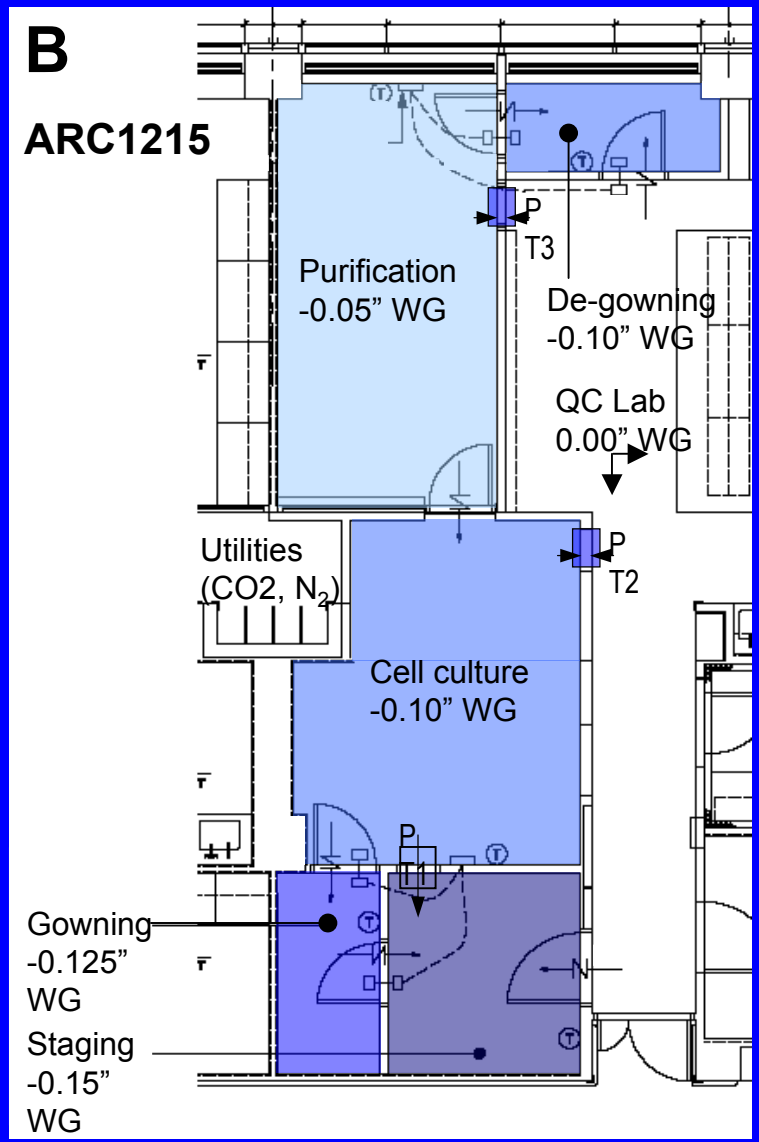
Center for Cellular and Molecular Therapeutics *at* The Children's Hospital *of* Philadelphia

Children's Hospital of Philadelphia (CHOP)
- Premier pediatric hospital
- ~ 8,000 staff (multiple buildings / locations)

Clinical Vector Core (CVC)
- Part of the Center for Cellular and Molecular Therapeutics
- Commitment to translational research

A

B



Overview of AAV2 Vector Biosynthesis Method

1. Initiation and propagation of HEK293 cells from a Master Cell Bank vial

↓

2. Seeding of HEK293 cells in roller bottles for AAV2 vector biosynthesis

↓

3. Transfection of HEK293 cells for AAV2 vector biosynthesis

↓

4. Post transfection medium exchange in serum free medium

Overview of rAAV2 Purification Process

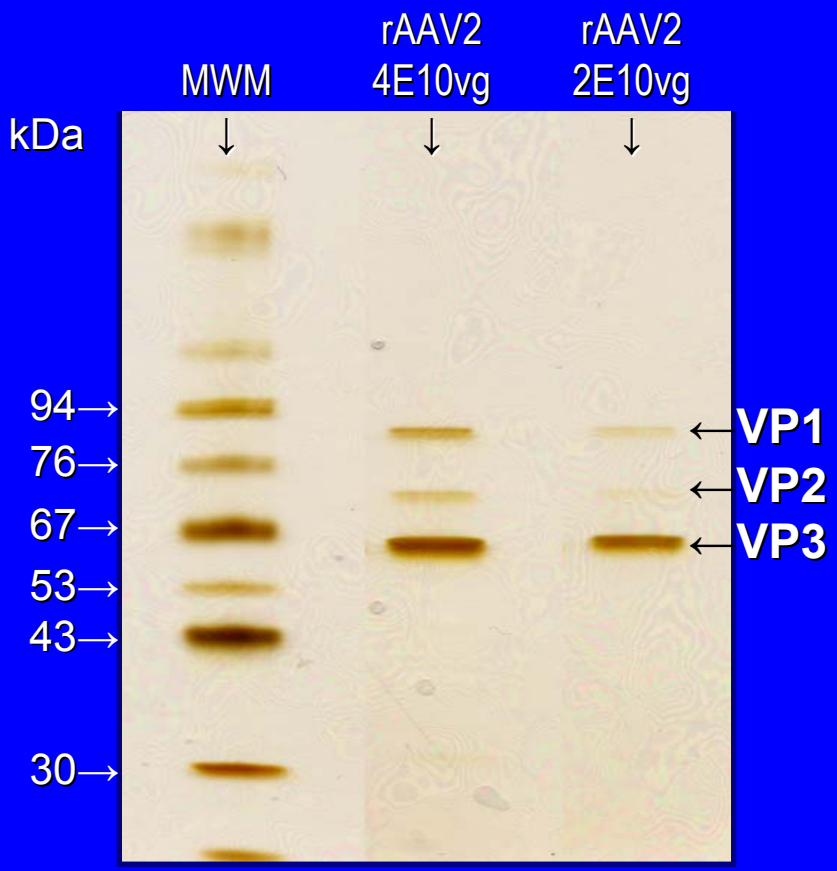
1. AAV2 vector harvest concentration and diafiltration by TFF
   ↓
2. Concentrated harvest lysis by microfluidization and clarification by filtration
   ↓
3. Vector purification and nuclease digestion by cation exchange chromatography
   ↓
4. Vector purification by gradient centrifugation
   ↓
5. Vector buffer exchange, formulation and 0.2micron filtration (Bulk Product)
   ↓
6. Final 0.2 µm filtration and vial fill (CMO)

AAV vector QC testing / characterization

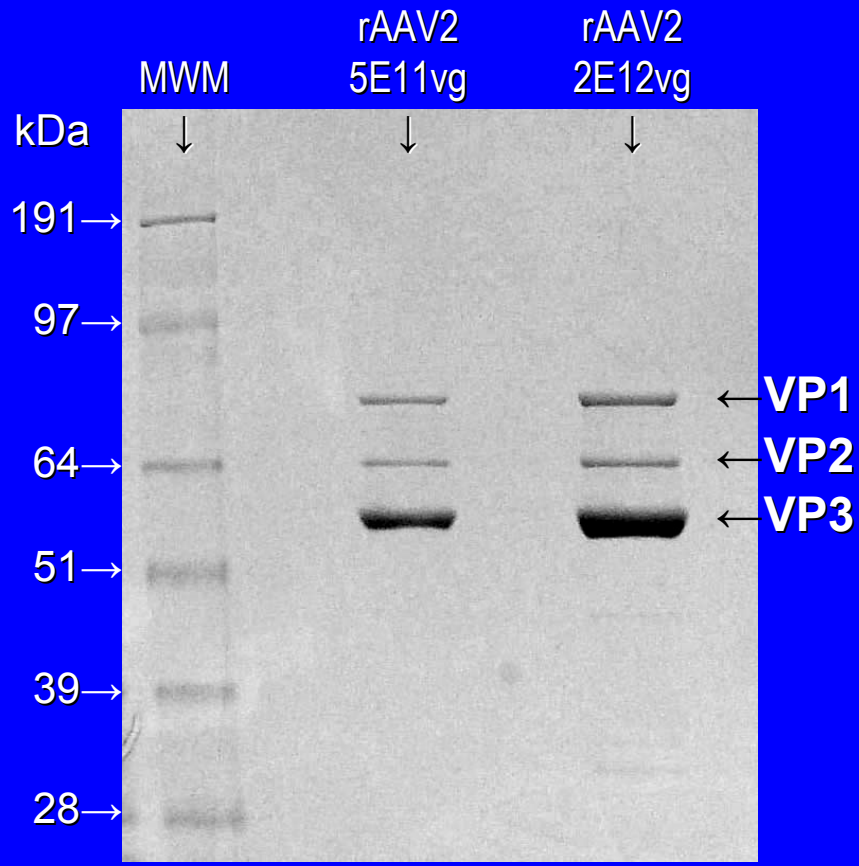
Identity:	***Method:***
AAV capsid protein	SDS-PAGE silver staining / WB
vector genome	Restriction digest / SB
Purity:	
Protein identity and impurities	SDS-PAGE ilver staining / densitometry
Residual plasmid DNA	Q-PCR
Residual mammalian DNA	Q-PCR
Residual cesium chloride	Mass spectrometry
Residual Benzonase	ELISA
Potency:	
vector genomes	Q-PCR
in vitro transduction	Vector transduction / transgene ELISA
Safety:	
Adventitious viral agents	*In vitro* and *in vivo* adventitious agents
Mycoplasma	Agar isolation / Vero cell culture (PTC method)
WT AAV	Infectious titer (+Ad)
USP sterility	Direct transfer in broth
Endotoxin	Kinetic chromogenic
pH	Potentiometry
Osmolality	Osmometry
Aggregation	Dynamic light scattering
Appearance	Visual Inspection

Analysis of AAV2 vector purity by SDS-PAGE (Silver and CB staining)

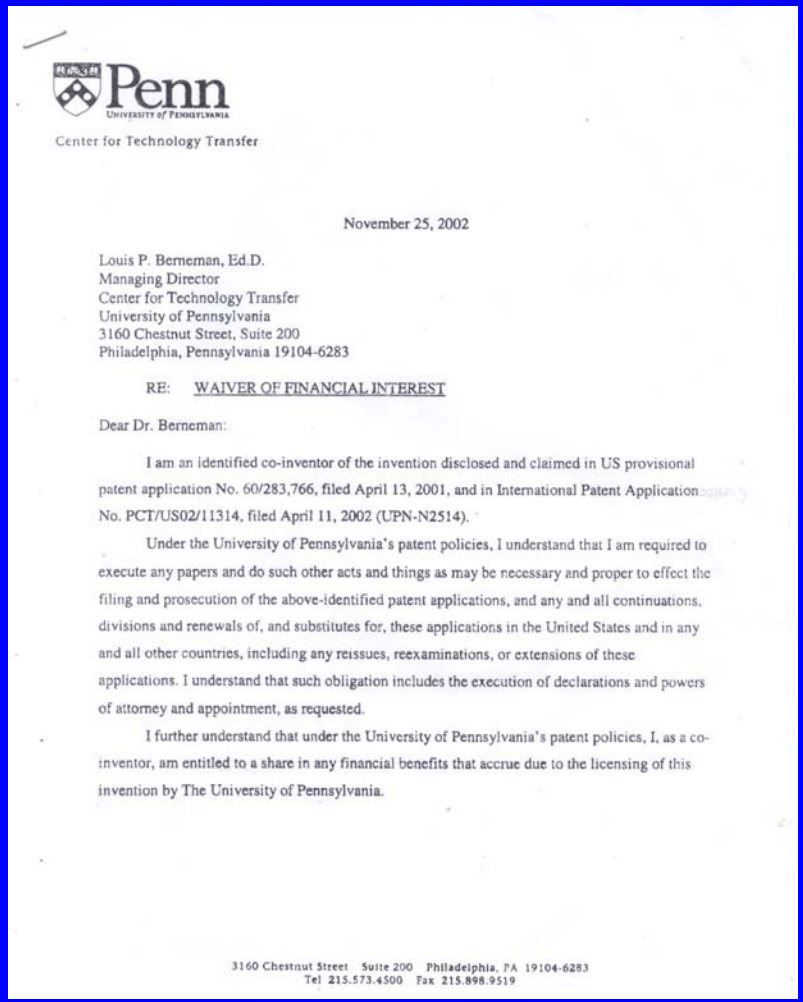
Silver staining:



CB staining:



Waiver of Financial Interest



Penn
University of Pennsylvania

Center for Technology Transfer

November 25, 2002

Louis P. Berneman, Ed.D.
Managing Director
Center for Technology Transfer
University of Pennsylvania
3160 Chestnut Street, Suite 200
Philadelphia, Pennsylvania 19104-6283

RE: WAIVER OF FINANCIAL INTEREST

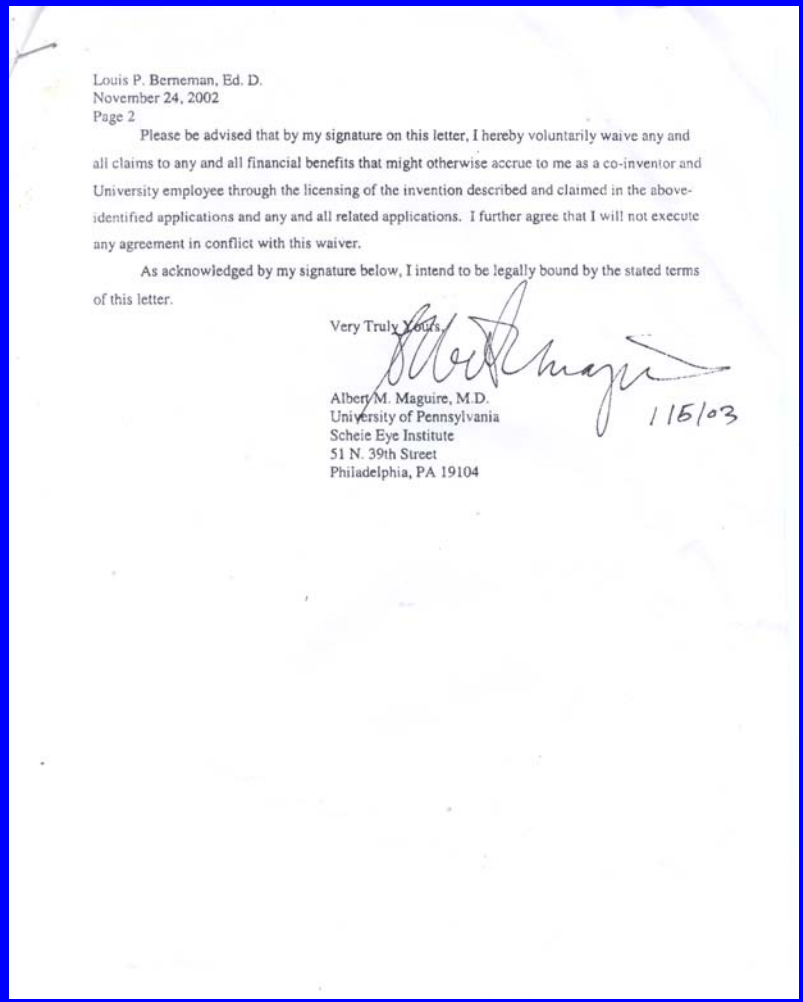
Dear Dr. Berneman:

I am an identified co-inventor of the invention disclosed and claimed in US provisional patent application No. 60/283,766, filed April 13, 2001, and in International Patent Application No. PCT/US02/11314, filed April 11, 2002 (UPN-N2514).

Under the University of Pennsylvania's patent policies, I understand that I am required to execute any papers and do such other acts and things as may be necessary and proper to effect the filing and prosecution of the above-identified patent applications, and any and all continuations, divisions and renewals of, and substitutes for, these applications in the United States and in any and all other countries, including any reissues, reexaminations, or extensions of these applications. I understand that such obligation includes the execution of declarations and powers of attorney and appointment, as requested.

I further understand that under the University of Pennsylvania's patent policies, I, as a co-inventor, am entitled to a share in any financial benefits that accrue due to the licensing of this invention by The University of Pennsylvania.

3160 Chestnut Street Suite 200 Philadelphia, PA 19104-6283
Tel 215.573.4500 Fax 215.898.9519



Louis P. Berneman, Ed. D.
November 24, 2002
Page 2

Please be advised that by my signature on this letter, I hereby voluntarily waive any and all claims to any and all financial benefits that might otherwise accrue to me as a co-inventor and University employee through the licensing of the invention described and claimed in the above-identified applications and any and all related applications. I further agree that I will not execute any agreement in conflict with this waiver.

As acknowledged by my signature below, I intend to be legally bound by the stated terms of this letter.

Very Truly Yours,

Albert M. Maguire, M.D.
University of Pennsylvania
Scheie Eye Institute
51 N. 39th Street
Philadelphia, PA 19104

11/5/03

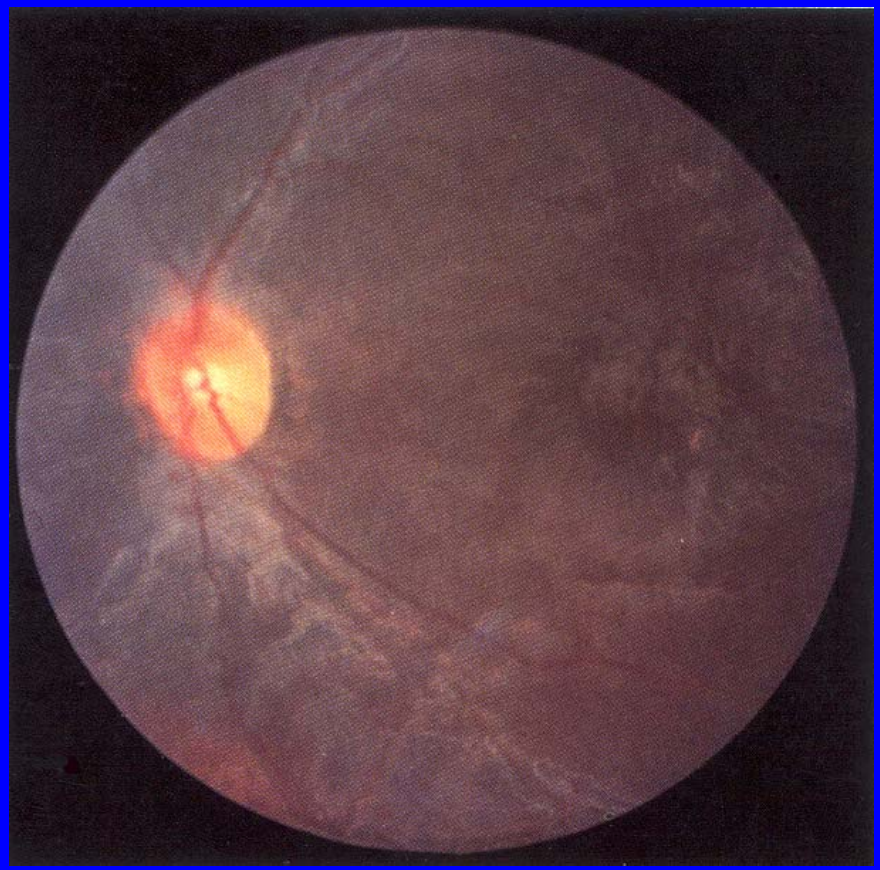
LCA- general considerations

- Incurable
- Loss of function (non-lethal)
- Rare

LCA: Pediatric disease

- Presents in infancy
- Deteriorates with age
- Nil function in later adulthood
- Blind adaptations in childhood

Disease Progression



13yo

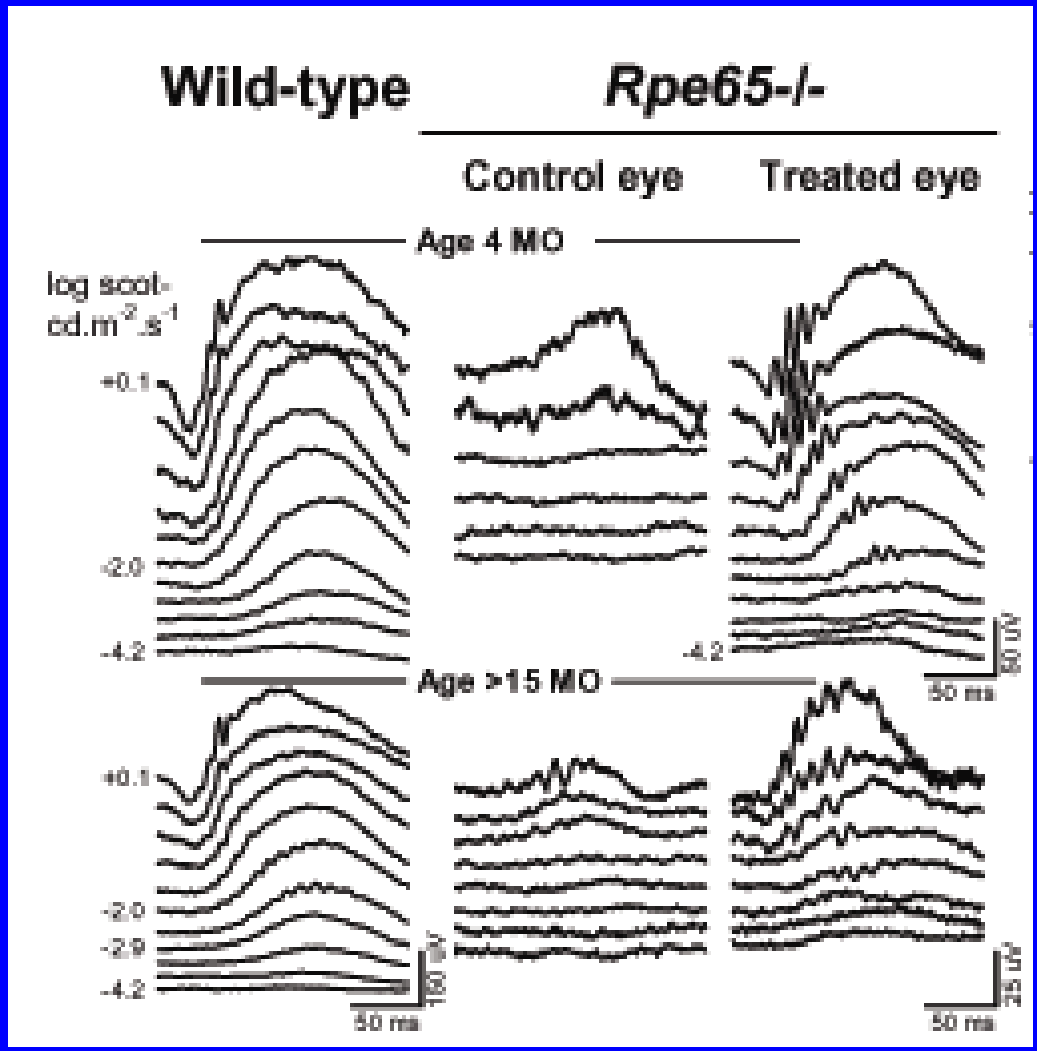
27yo

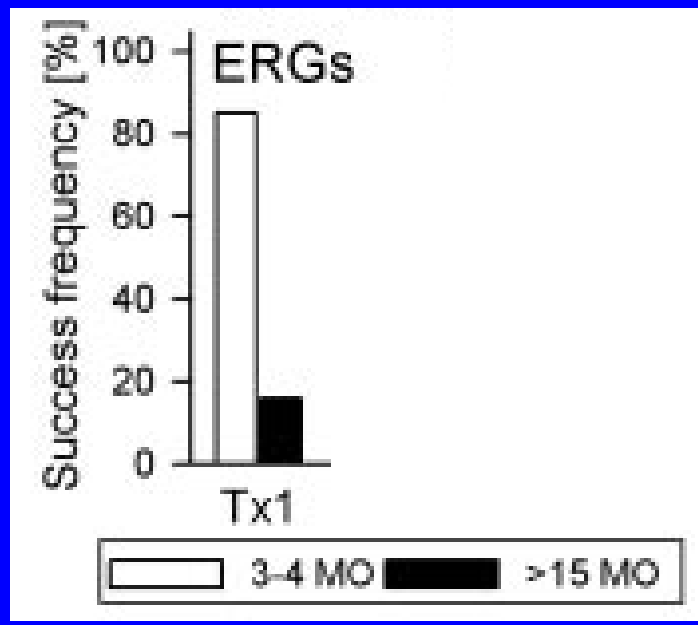
From: J.R. Heckenlively, Retinitis Pigmentosa, JB Lippincott Co, Philadelphia, 1988

LCA/RPE65: Animal studies

- Pediatric model (especially canine)
- Outcome versus age

Gene therapy in Rpe65-/- mice with advanced age/disease leads to limited visual restoration





Modified from Jacobson et al 2005 PNAS 102:6177-82

Issues: Age

- Viability of tissue decreases with time
  - Potential benefit
  - Information derived
- Amount of treatable tissue decreases with time
  - Surgical implications

Greater than minimal risk; prospect of direct benefit

- Risk of mortality from use of general anesthesia (GA)
  - Risk of death for ASA Physical Status (ASA PS) 1 and 2 patients is estimated at 4 per million. At CHOP the risk is even lower. We are unaware of any deaths during the past 20 years among ASA PS 1 or 2 patients undergoing minor, day surgical procedures.
- Risk of morbidity from potential complications to treated organ.
  - Worst possible morbidity would be complete loss of vision of one eye. The functional impact would be small, however, as these children are legally blind at baseline and have limited use of their impaired vision to perform activities of daily living.
- Prospect of Direct Benefit (21CFR§50.52)
  - Based on the pre-clinical data, we believe the above risks are justified by the anticipated direct benefit to child subjects, and the relation of the anticipated benefit to the risks is at least as favorable as available alternative approaches (none).

Study design: Phase 1 Dose Escalation

- Phase 1 safety study
- Dose escalation
- 3x3 groups, N=9 subjects
- Lowest dose shows evidence of efficacy in animal studies
- Time intervals based on vector expression

Study Design: Enrollment

- Legally blind (visual acuity)
- RPE65 mutation
- 8-18 y.o.
- Able to consent/assent
- Available for long term follow-up

Consent/Assent Process

- Parental consent+subject assent -or- subject consent
- Reference materials
- Anesthesiologist representative
- Risk documentation

Study Design: Exclusion

- Ocular
- Systemic
- Psychological

Assessments

- Ophthalmic function
  - Psychophysical (visual acuity/visual field)
  - Physiologic (ERG, pupil reactivity)
- Ophthalmic structure
- Systemic
- Biodistribution
- Other (Quality of Life)

Issue: Age

- Anatomic (surgical)
  - Growth ~90% by 3 years old
- Functional (visual development)
  - Amblyopia risk until 8 years old
- Biologic (disease)
  - continuum vis-à-vis retinal degeneration
  - 18 years old = arbitrary

Comparative Risk/Benefit as a Function of Age

- Benefits

	6mos -3 yo	**3-8 yo**	**8-18 yo**	**>18 yo**
Visual function	+++	++	+/-	-

- Risks

Amblyopia	++	+	-	-
Anatomic/ Surgical	+	-	-	+/- [eg cataract, area of bleb]