

Human Gene Transfer Protocol #0910-1005:

**Leber Congenital Amaurosis (LCA) using
Adeno-associated Virus Vector to Deliver the
Gene for Human RPE65 to the Retinal Pigment
Epithelium (RPE) [AAV2-hRPE65v2-301]**



CH09, 8yo

Jean Bennett, M.D., PhD

RAC

December 1, 2009

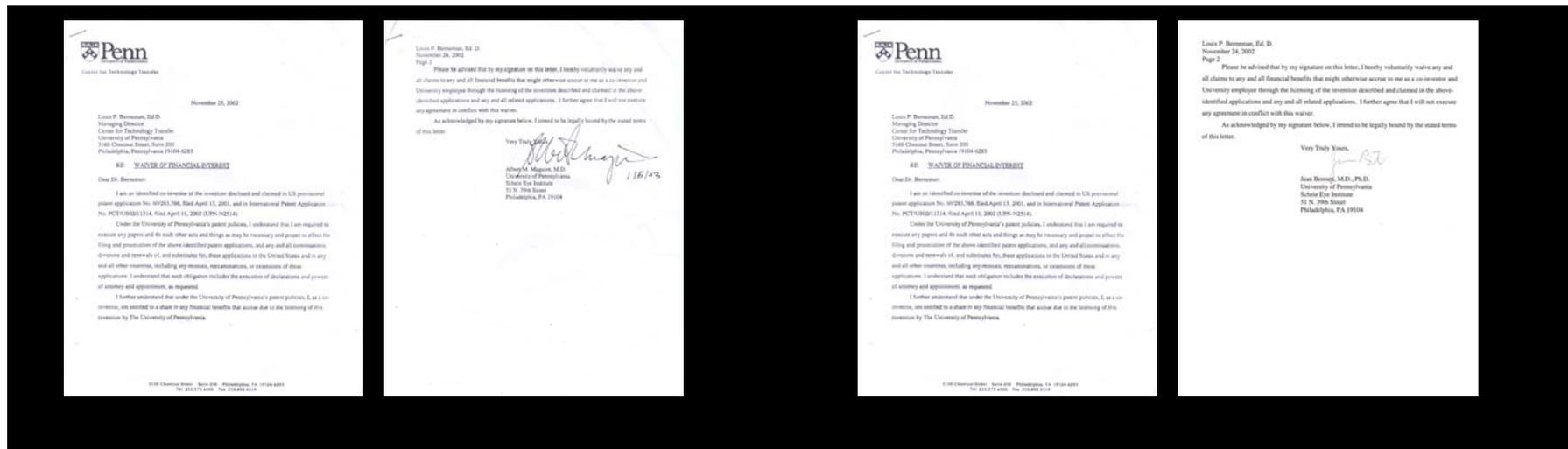
Subjects appearing in this presentation have given media consent and have gone to the media independently

Conflict of Interest Disclosure

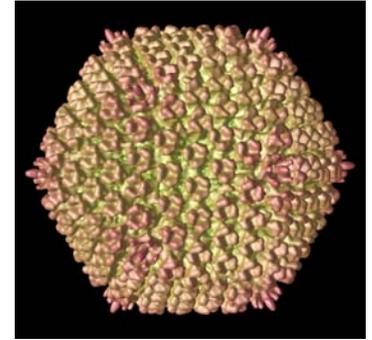
Bennett, J, Jacobson, SG, Maguire, AM, Hauswirth, WW, Aguirre, GD, Acland, GD

“Method of treating or retarding the development of blindness, U.S. Patent (Penn Docket #N2514; 2002), pending.

2002: Bennett & Maguire waived any potential financial gain



Original Phase 1/2 Plan



Inclusion

- Legally blind (visual acuity, visual fields)
- RPE65 mutation(s)
- 8yo and higher
- Able to consent vs. assent + parental permission
- Available for long term follow-up
- Single subretinal injection of AAV2.hRPE65v2 in worst eye

Study Design: Dose escalation study

- (3 doses: 1.5E10, 4.8E10, 1.5E11 vg)
 - Starting (lowest) dose correlates with dose that shows efficacy in dogs
- Volume: 150 μ l
- 3 subjects per dose

Subjects with Leber's Congenital Amaurosis (LCA) treated at CHOP:

		Patient No.	Age	Sex	<u>Follow-Up</u>
Low	Dose	NP01	26	F	↑
		NP02	26	M	
		NP03	19	F	
Medium		NP04	17	M	
		CH06	20	F	
		CH08	9	M	
		CH09	8	M	
		CH10	10	M	
		CH11	24	F	
High 300 ul		CH12	44	F	
		CH13	35	M	
		NP15	11	M	

Visual Function Testing

Subjective:

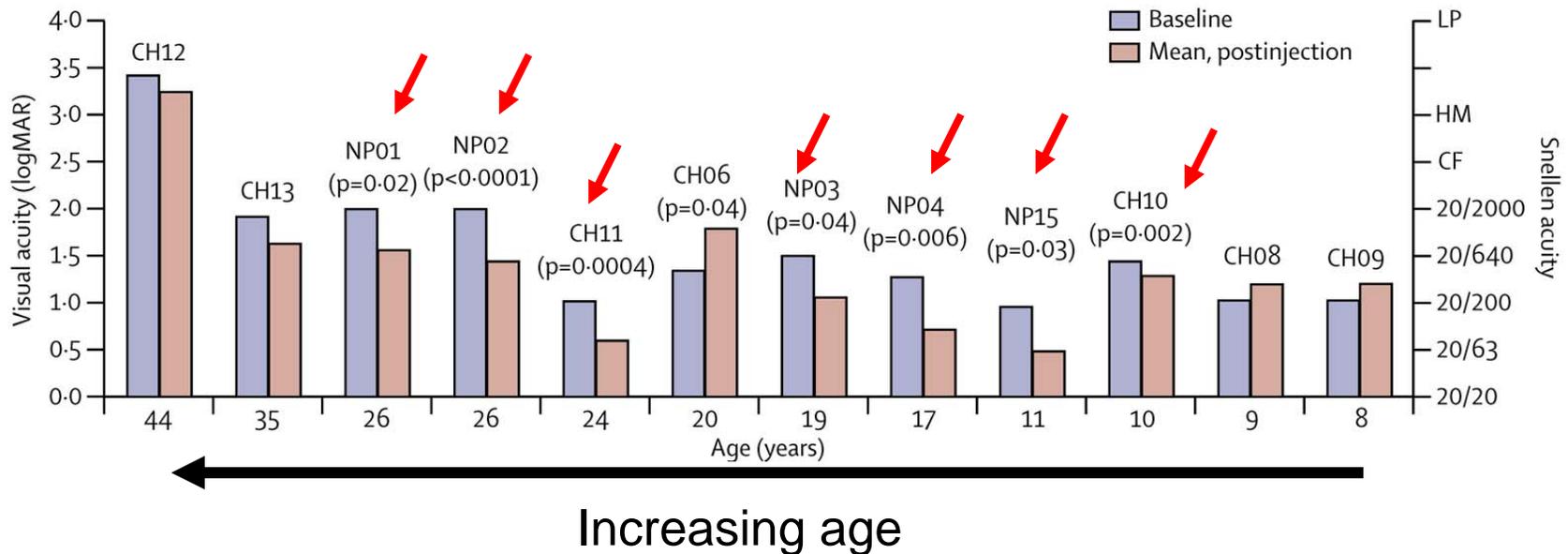
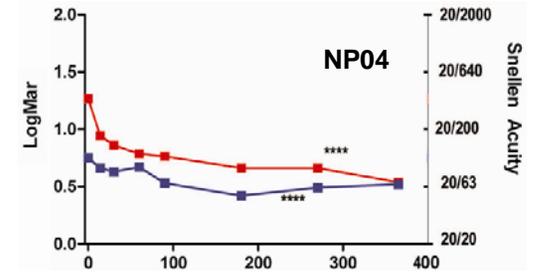
- Visual acuity
- Visual fields
- Full-field sensitivity threshold testing

Objective:

- Pupillary light reflex
- Mobility

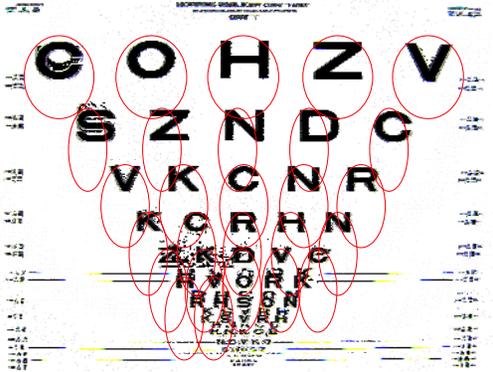
Visual Acuity

- Significant gains in VA in 7/12 subjects
- Gains are stable
- The younger, the better the final result
- Small improvement in uninjected eye



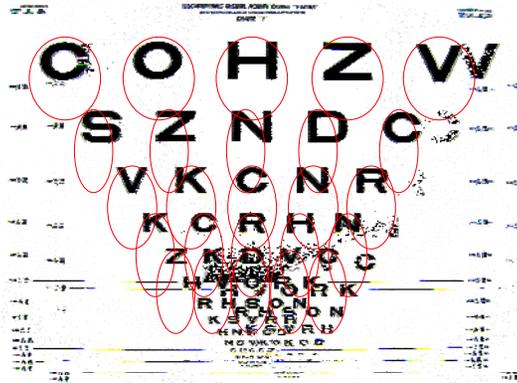
Visual acuity changes are stable

DAY 365



NPO1

DAY 365



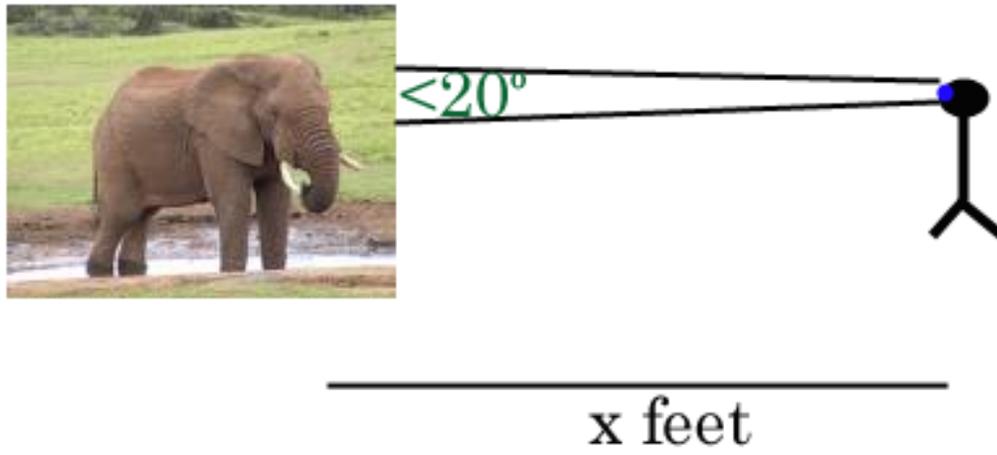
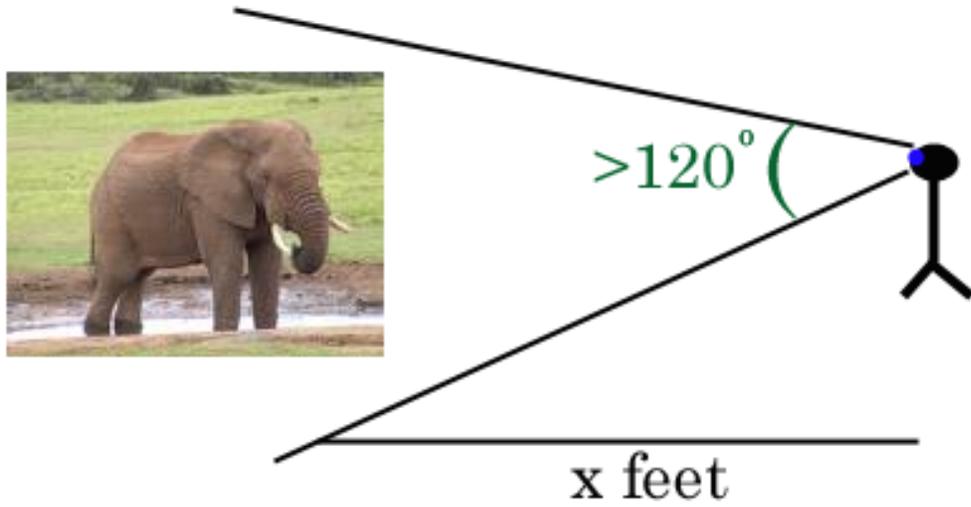
NPO2

Patient	Right			Left		
	Before	Day 30	Day 365	Before	Day 30	Day 365
1	HM	20/1050	20/581	20/1040	20/1100	20/800
2	HM	20/710	20/640	20/500	20/220	20/150

|| P<0.001.
 ** P=0.002.

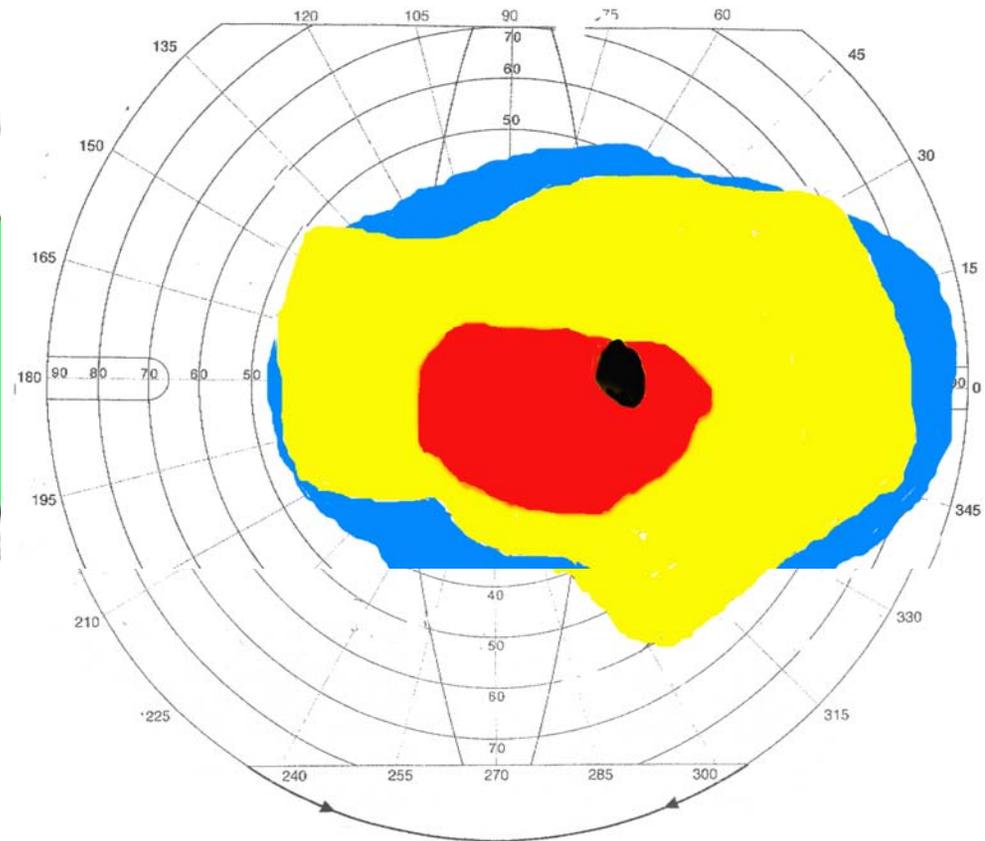
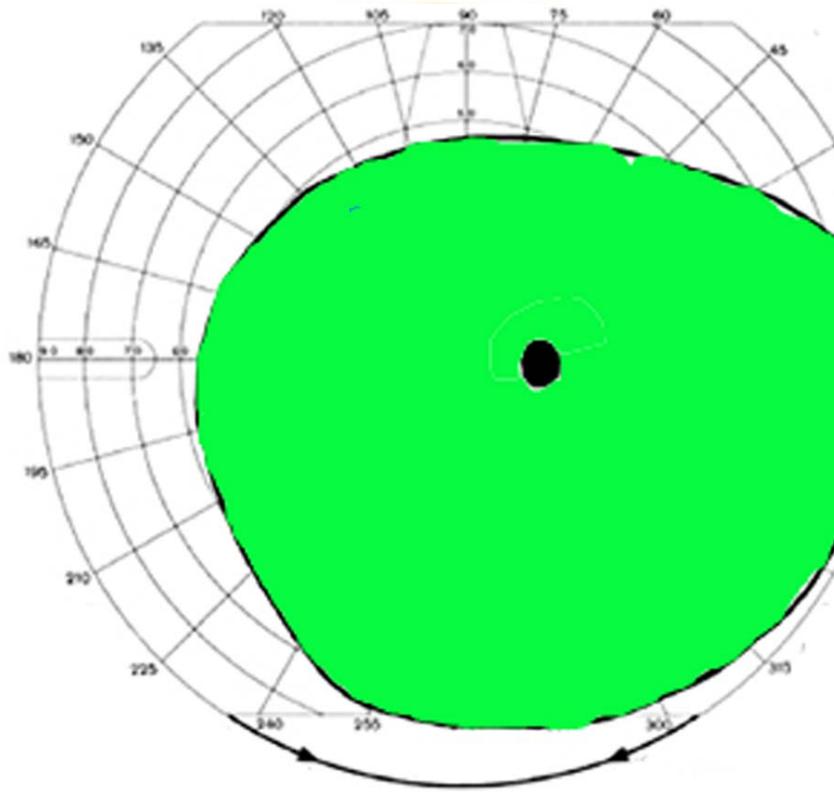
Simonelli et al, Mol Ther, in press

Visual Fields



Visual Fields

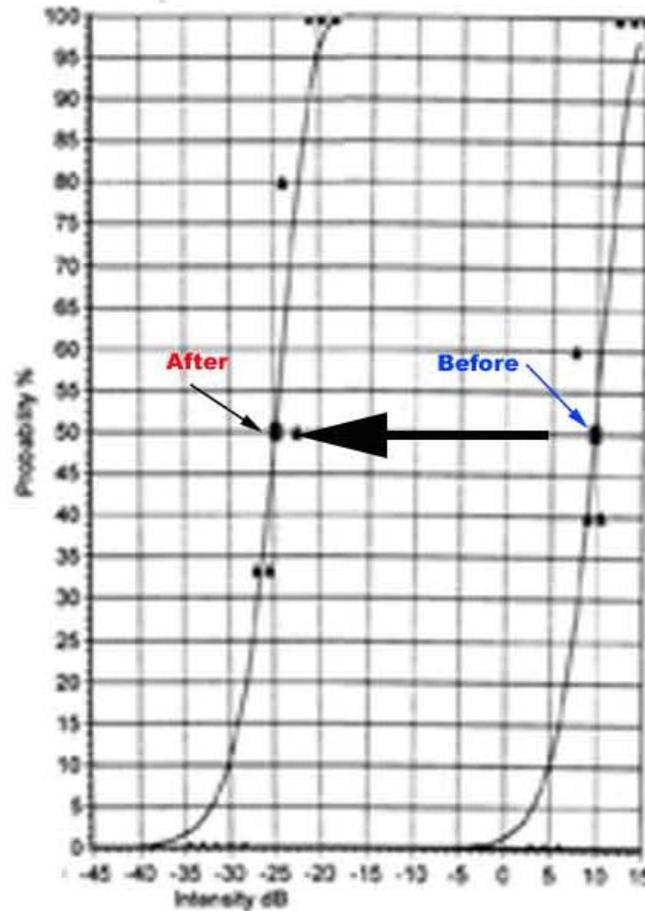
- All subjects have expanded visual fields after injection
- Expansion correlates with the area injected



CH08, Right (Injected) Eye

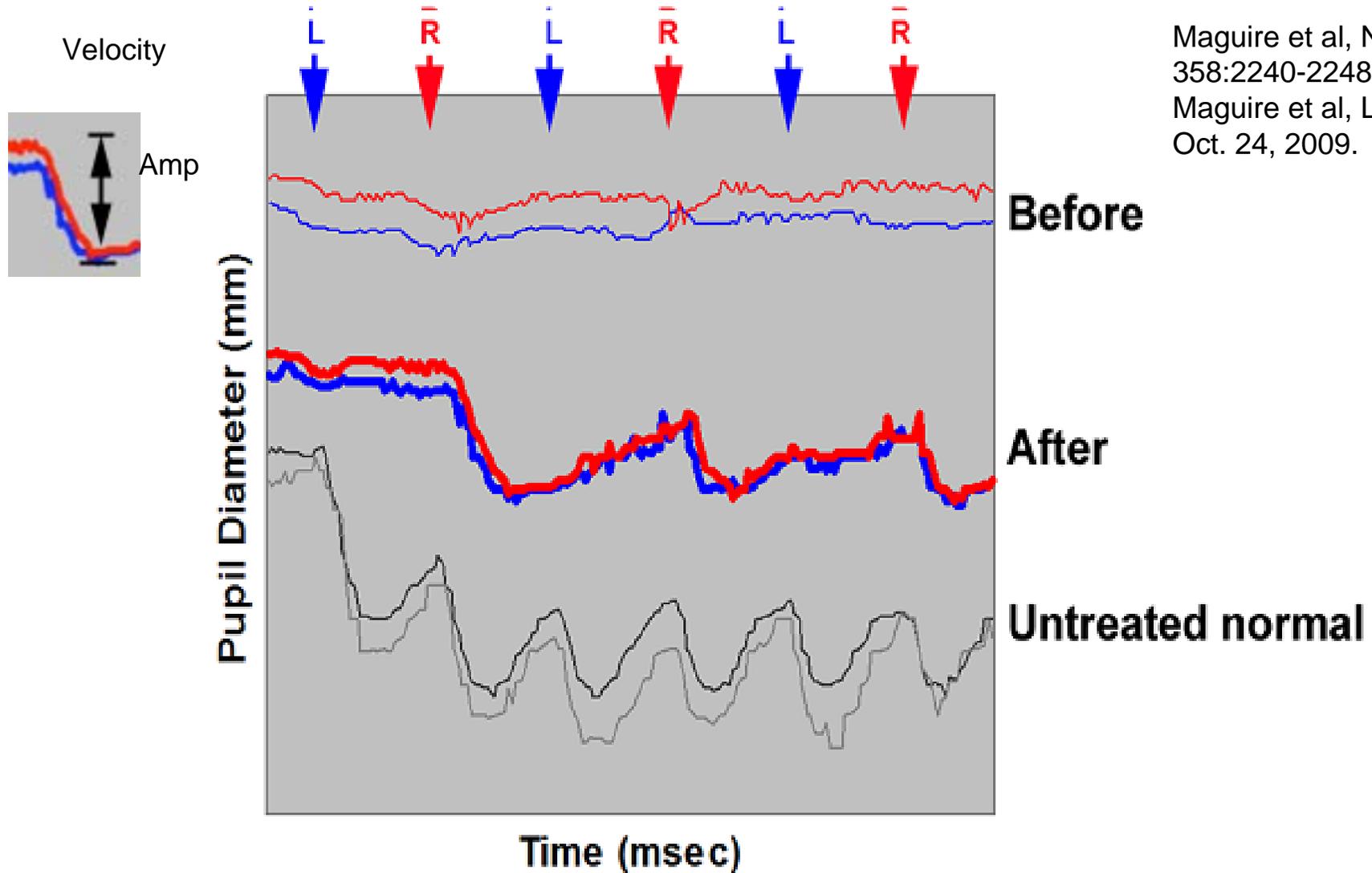
 d30
 d60

Full-Field Light Sensitivity Thresholds (FST's)



Objective Data: Pupillary Light Reflex (PLR)

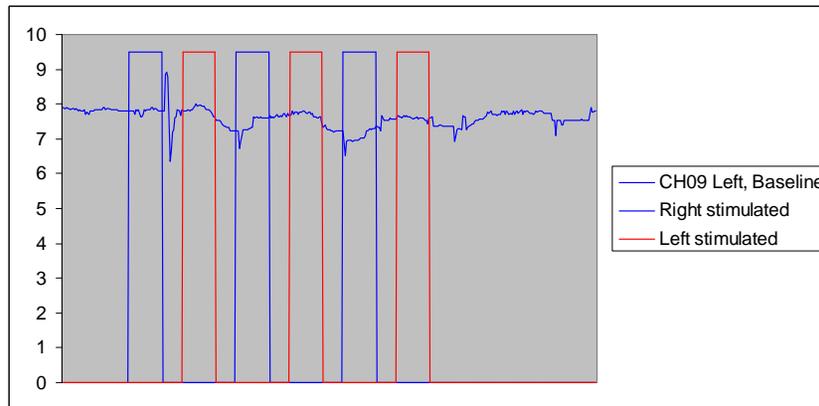
Maguire et al, NEJM
358:2240-2248 (2008);
Maguire et al, Lancet,
Oct. 24, 2009.



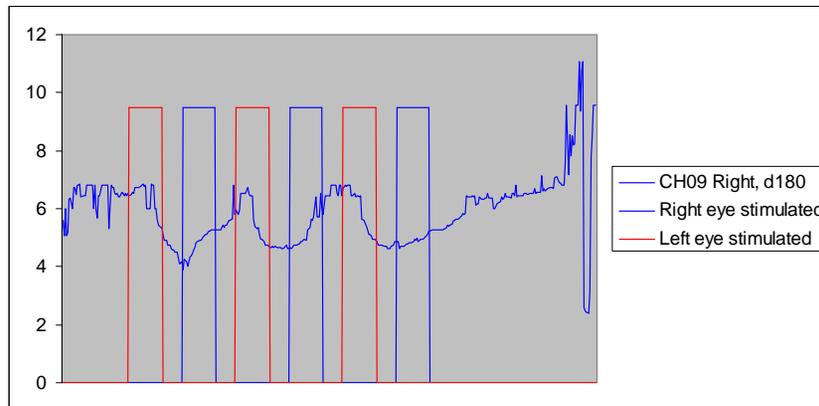
**PLR is restored in the (right) retina exposed to AAV2.hRPE65v2
and not in the uninjected (contralateral) retina**

All subjects showed improvements in pupillary light reflexes

CH09 (8yo): Pupillometry Before and After Left Eye
Injected



Baseline



Day 180
(40,000X more sensitive)

Pupillometry Statistics

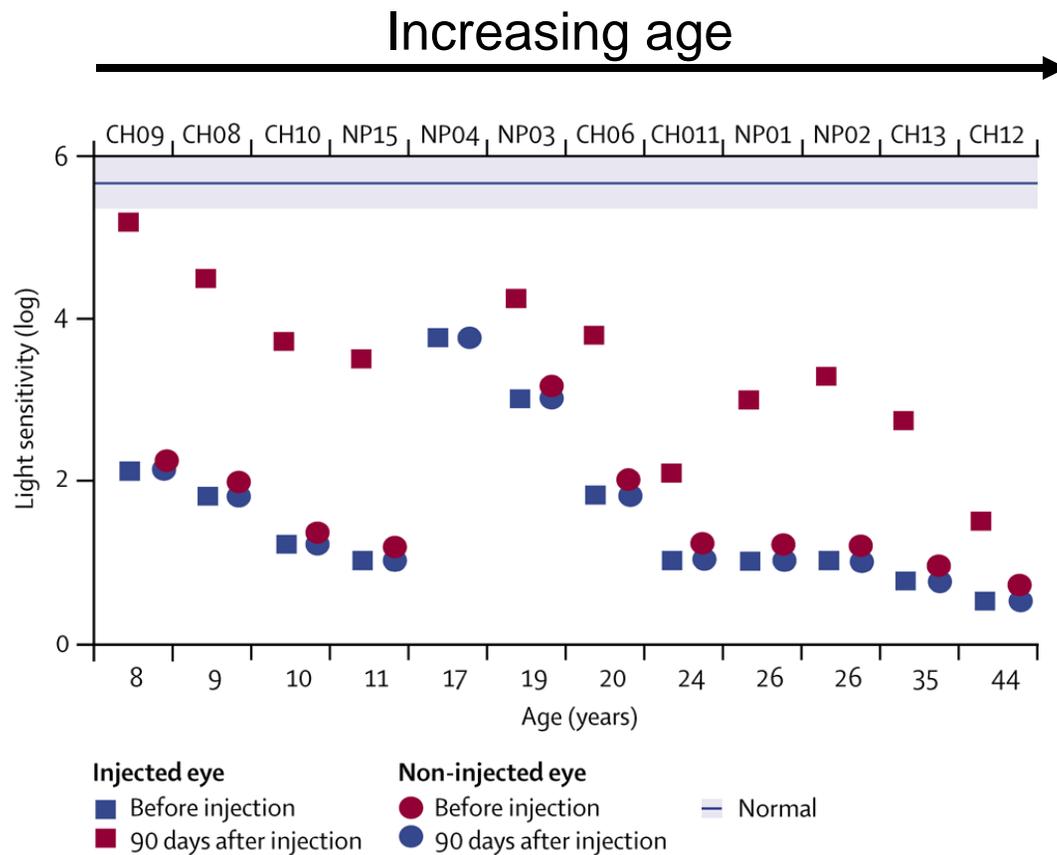
p < 0.003

p < 0.09

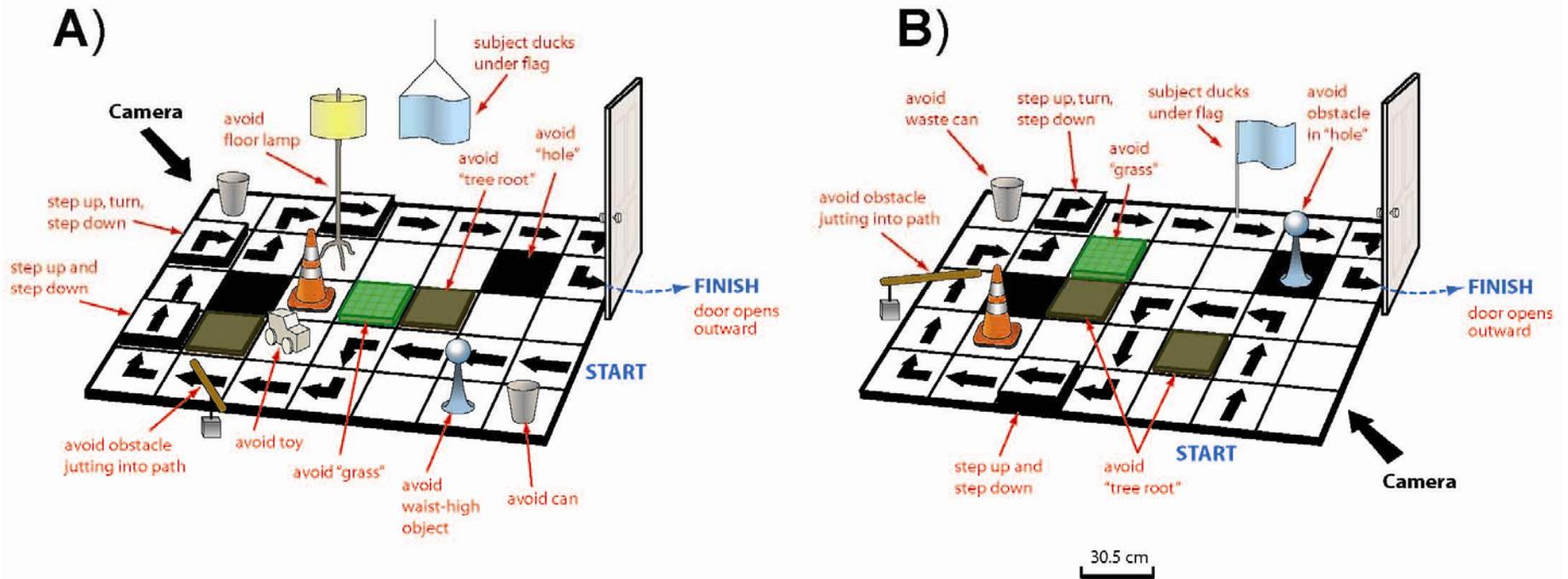
Subject	Baseline Amplitude	Post-Rx Amplitude	p-value	Baseline Velocity	Post-Rx p<0.01 Velocity	p-value
NP01*	0.39 (0.07)	0.64 (0.14)	p=0.003	-0.76 (0.14)	-1.21 (0.19)	p=0.0004
NP02	-0.01 (0.03)	0.46 (0.07)	p<0.001	-0.02 (0.06)	-1.5 (0.24)	p<0.0001
NP03**	0.48 (0.03)	0.80 (0.07)	p = 0.03	-1.7 (0.27)	-2.3 (0.12)	p = 0.09
CH06^	0.27 (0.42)	0.67 (0.01)	p = 0.03	-0.26 (0.40)	-2.0 (0.20)	P=0.001
CH08	-0.14 (0.11)	0.82 (0.13)	p = 0.0002	-0.26 (0.4)	-2.0 (0.2)	p = 0.004
CH09	0.16 (0.28)	1.20 (0.20)	P=0.04	-0.07 (0.28)	-2.58 (0.22)	P=0.001
CH10	0.65 (0.03)	1.83 (0.69)	p=0.07	-2.13 (0.13)	-3.97 (0.54)	p=0.002
CH11	0.2 (0.17)	0.32 (0.07)	p=0.46	-0.61 (0.09)	-0.97 (0.18)	p=0.39
CH12^	0.24 (0.08)	0.96 (0.21)	P=0.001	-0.001 (0.46)		p=0.01
CH13^	0.009 (0.05)	1.04 (0.73)	P=0.06	-0.04 (0.05)	-1.96 (1.29)	p=0.16
NP15^	0.15 (0.84)	0.43 (0.16)	P=0.33	-0.54 (0.21)	-1.4 (0.28)	P=0.03

Light Sensitivity vs. Age

- All individuals gain at least 1 log unit in light sensitivity
- Children gain up to 4 log units
- Improvements were detectable by d8

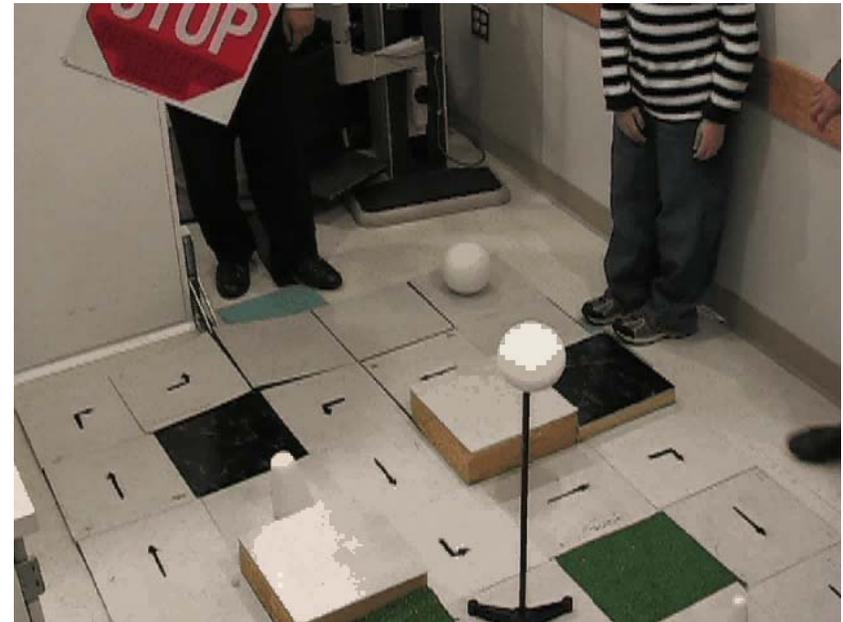


Mobility Course



CH09 (8 yo) 3 Months Post Injection

Navigation in dim light:

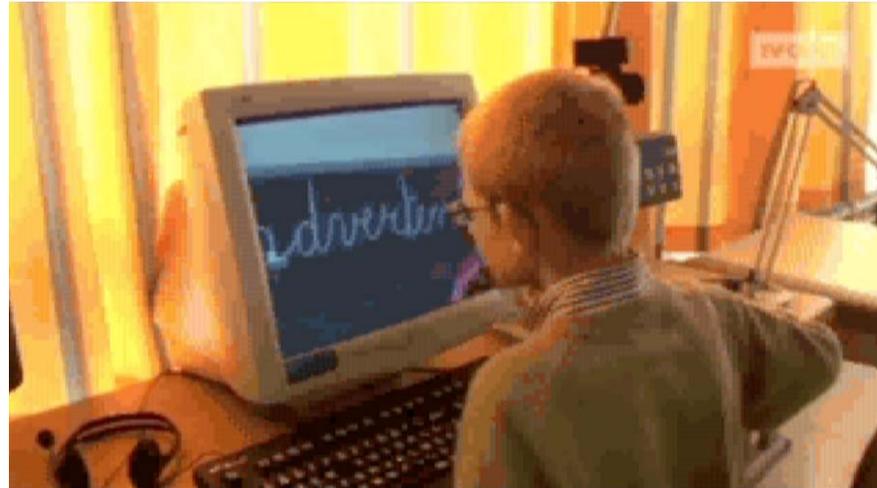


Uninjected Eye

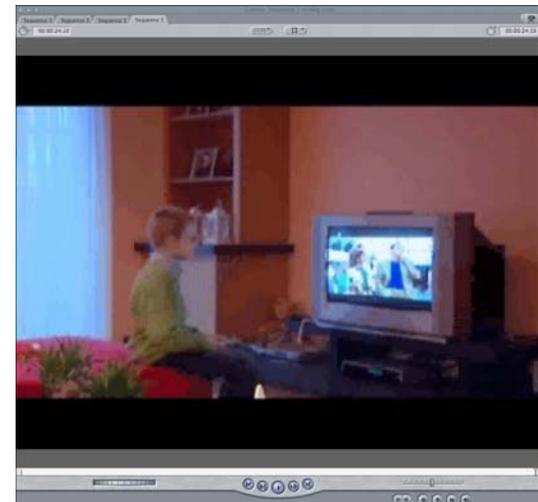
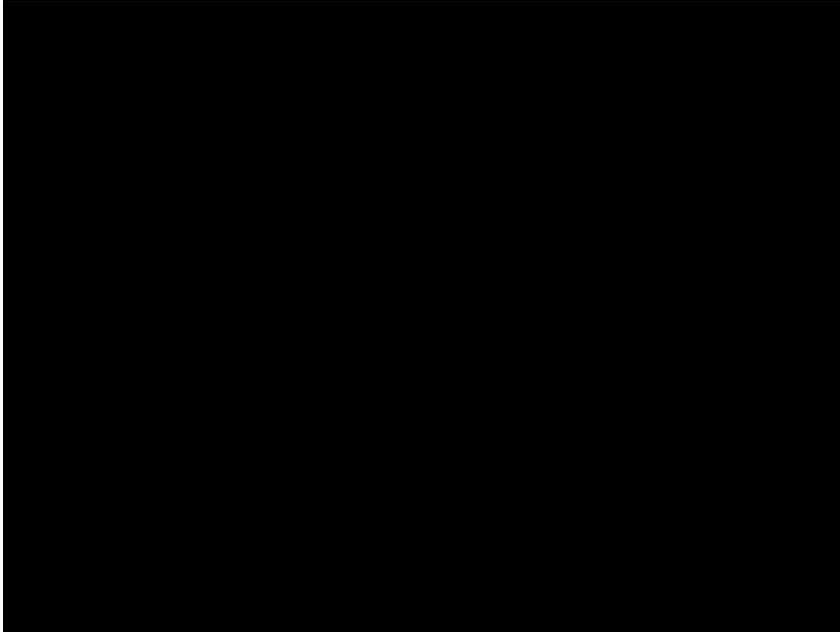
THE LANCET Injected Eye

www.thelancet.com Vol 374 online 10/24/09

Pre-Treatment



Post-Treatment



Safety: No Severe Adverse Events; No Concerns

Safety and efficacy of gene transfer for Leber's congenital amaurosis

Albert M Maguire, Francesca Simonelli, Eric A Pierce, Edward N Pugh, Jr, Federico Mingozzi, Jeannette Bennicelli, Sandro Banfi, Kathleen A Marshall, Enrico M Surace, Settimio Rossi, Arkady Lyubarsky, Valder R Arruda, Barbara Konkle, Edwin Stone, Junwei Sun, Jonathan Jacobs, Lou Dell'Osso, Richard Hertle, Jian-Xing Ma, T Micahel Redmond, Xiasong Zhu, Bernd Hauck, Olga Zeleniaia, Kenneth S Shindler, Maureen G Maguire, J Fraser Wright, Nicholas J Volpe, Jennifer W McDonnell, Alberto Auricchio, Katherine A High, Jean Bennett

N Engl J Med 2008 May 22;358(21):2240-8, (Epub 2008 Apr 27)

Age-dependent effects of RPE65 gene therapy for Leber's congenital amaurosis: a phase 1 dose-escalation trial

Albert M Maguire, Katherine A High*, Alberto Auricchio, J Fraser Wright, Eric A Pierce, Francesco Testa, Federico Mingozzi, Jeannette L Bennicelli, Gui-shuang Ying, Settimio Rossi, Ann Fulton, Kathleen A Marshall, Sandro Banfi, Daniel C Chung, Jessica I W Morgan, Bernd Hauck, Olga Zeleniaia, Xiaosong Zhu, Leslie Raffini, Frauke Coppieters, Elfride De Baere, Kenneth S Shindler, Nicholas J Volpe, Enrico M Surace, Carmela Acerra, Arkady Lyubarsky, T Michael Redmond, Edwin Stone, Junwei Sun, Jennifer Wellman McDonnell, Bart P Leroy, Francesca Simonelli, Jean Bennett*

Lancet 2009 Nov 7,374(9701):1569-605 (Epub 2009 Oct 23)

Gene therapy for Leber's congenital amaurosis is safe and effective through 1.5 years after vector administration

Francesca Simonelli, Albert M Maguire, Francesco Testa, Eric A Pierce, Federico Mingozzi, Jeannette Bennicelli, Settimio Rossi, Arkady Lyubarsky, Kathleen A Marshall, Sandro Banfi, Enrico M Surace, Junwei Sun, T Micahel Redmond, Xiasong Zhu, Kenneth S Shindler, Gui-shuang Ying, Carmela Ziviello, Carmela Acerra, J Fraser Wright, Jennifer W McDonnell, Katherine A High, Jean Bennett, Alberto Auricchio

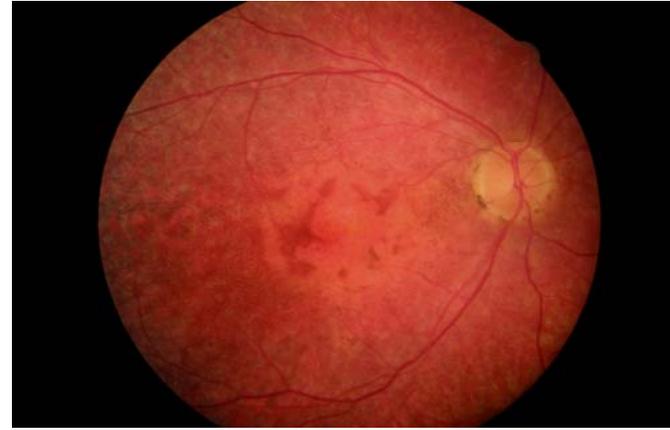
Mol Ther, in press

No Inflammation after AAV delivery in any of the 12 subjects

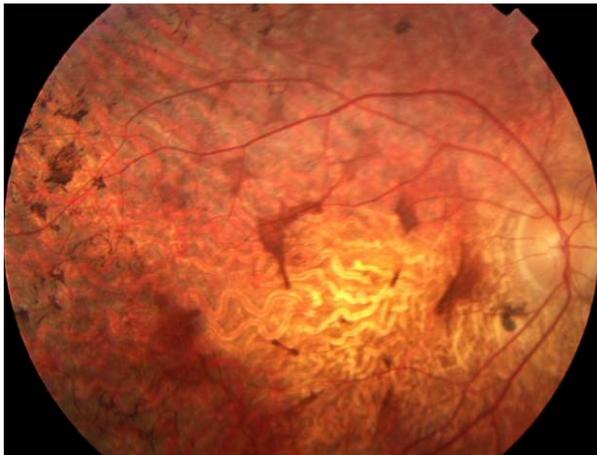
Baseline

d30

NP03
19yo

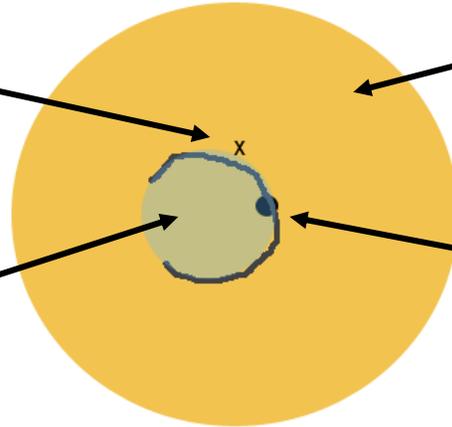


CH13
33yo

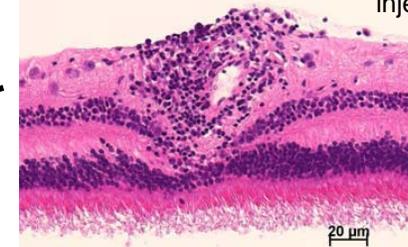


No clinical signs of inflammation after AAV delivery in NHPs (no steroids) using 2X, 5X highest human dose.

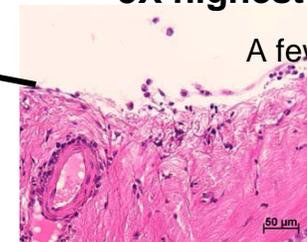
(Composite results from 5 different animals)



Isolated foci (1 vs 3 sites in 2 of 8 AAV2-hRPE65v2-injected eyes)



5X highest human dose



A few inflammatory cells at optic nerve head

2X, 5X highest human dose

Adverse Events: Medical

Likely attributed to oral corticosteroids (prednisone) prescribed from 3 days prior to surgery to d14 following vector administration:

AE	Number of Subjects	Relationship to surgical delivery
Stomach upset (mild)	4	unlikely
Steroid acne (mild)	1	unlikely
Cold-like symptoms (mild)	3	unlikely

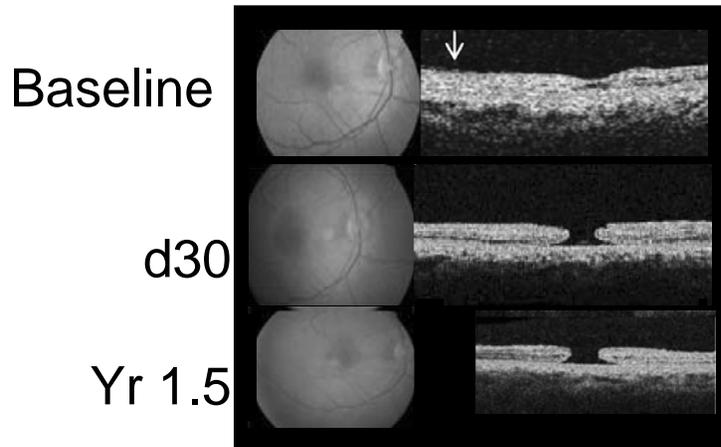
Procedural modifications installed to minimize these complications:

Maximum prednisone dose lowered (60 mg/day -> 40mg/day)

Adverse Events: Surgical

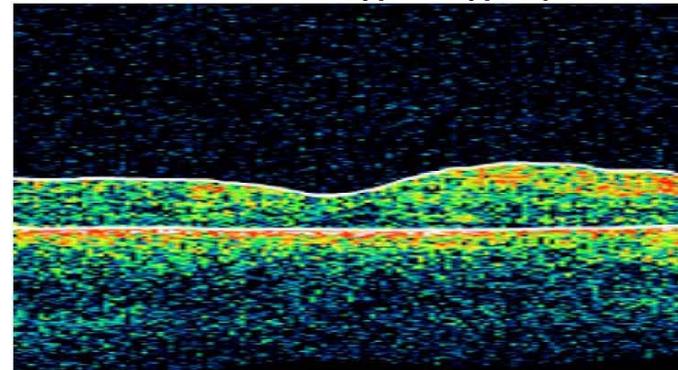
6 Subjects: Conjunctival Hyperemia (immediately post-op)

NP02: Macular Hole



No progression of macular hole over time
Simonelli et al, Mol Ther, in press (2009)

CH10: Foveal dehiscence
observed during surgery.....



No hole observed at d8 (or beyond)
Maguire et al, Lancet 374:1569-1605

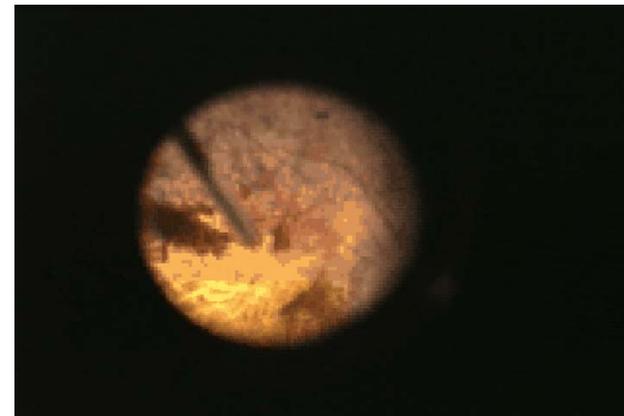
Procedural modifications installed to prevent complications...

- 1) Removal of epiretinal membranes
- 2) Mechanical protection of fovea

Maguire et al, NEJM 358:2240 (2008)
Maguire et al, Lancet 374:1569 (2009)

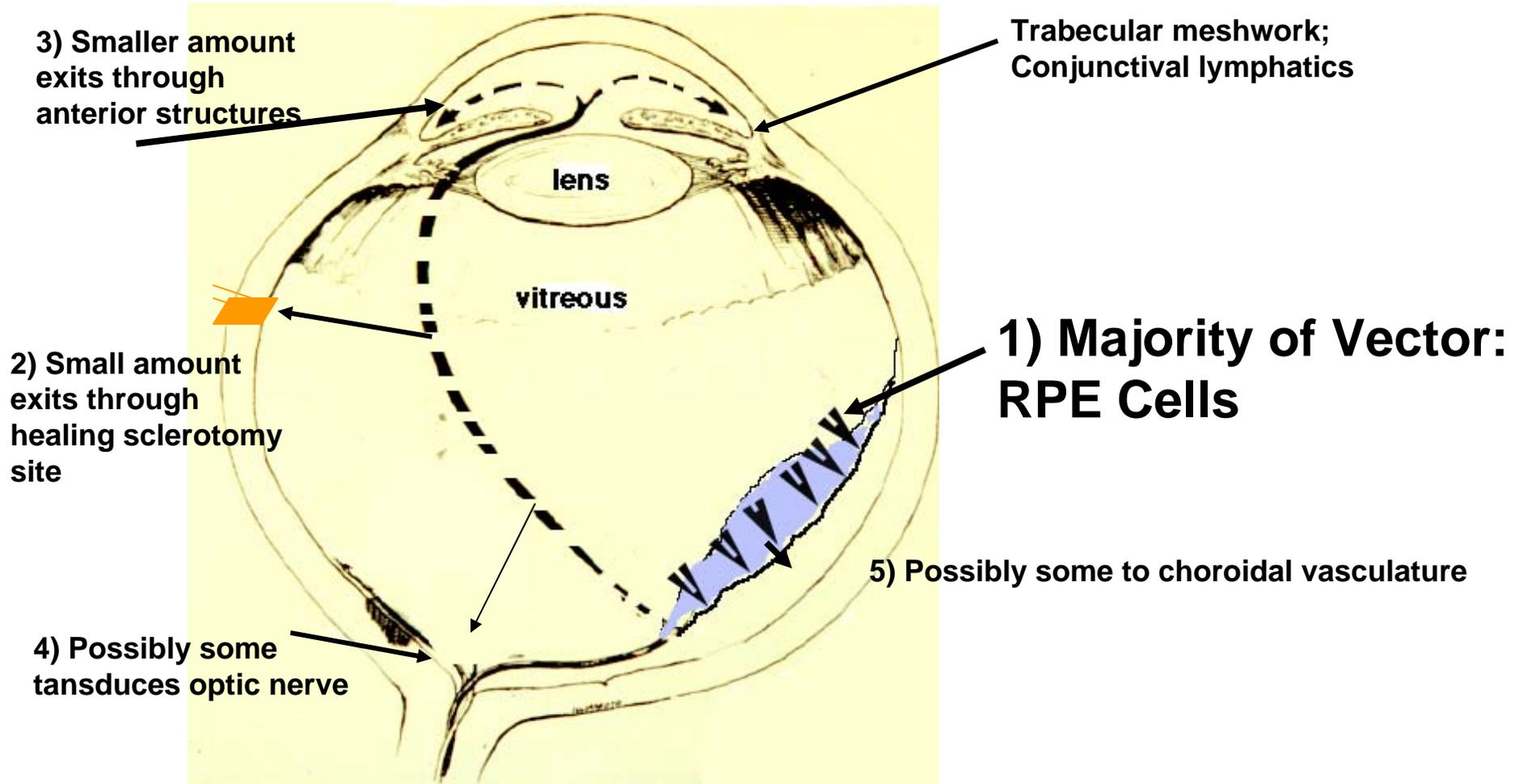
Perfluoron liquid layered over the fovea at
start of procedure →

Perfluoron is removed after the injection



CH13

Biodistribution after Subretinal Injection`



Biodistribution in humans (vector shedding)

PCR detectable sequence
in early post-administration
time period (only) in:

Tears

1/3 low dose

3/6 middle dose

3/3 high dose

Blood:

0/3 low dose

0/6 middle dose

2/3 high dose

Similar to preclinical studies,
minimal evidence of extra-
ocular exposure

Low Dose Cohort

	NP01		NP02		NP03	
	Tear	Blood	Tear	Blood	Tear	Blood
Baseline	Neg	Neg	Neg	Neg	Neg	Neg
Day 0	Neg	Neg	Neg	Neg	Neg	Neg
Day 1	Pos	Neg	Neg	Neg	Neg	Neg
Day 2	Neg	ND	Neg	ND	Neg	ND
Day 3	Neg	ND	Neg	Neg	Neg	Neg

Middle Dose Cohort

	NP04		CH06		CH08	
	Tear	Blood	Tear	Blood	Tear	Blood
Baseline	Neg	Neg	Neg	Neg	Neg	Neg
Day 0	Neg	Neg	Neg	Neg	Neg	Neg
Day 1	Neg	Neg	Neg	Neg	Neg	Neg
Day 2	Pos	ND	Neg	ND	Neg	ND
Day 3	Pos	Neg	Neg	Neg	Neg	Neg
Day 4	Pos	Neg				
Day 6	Neg	Neg				
Day 8	Neg	Neg				
Day 9	Neg	Neg				

	CH09		CH10		CH11	
	Tear	Blood	Tear	Blood	Tear	Blood
Baseline	Neg	Neg	Neg	Neg	Neg	Neg
Day 0	Neg	Neg	Neg	Neg	Neg	Neg
Day 1	Pos	Neg	Neg	Neg	Pos	Neg
Day 2	Neg	ND	Neg	ND	Neg	ND
Day 3	Neg	Neg	Neg	Neg	Neg	Neg

High Dose Cohort

	CH12		CH13		NP15	
	Tear	Blood	Tear	Blood	Tear	Blood
Baseline	Neg	Neg	Neg	Neg	Neg	Neg
Day 0	Neg	Neg	Neg	Neg	Neg	Neg
Day 1	Neg	Neg	Pos	Pos PBMC	Pos NQ	Neg
Day 2	Pos	ND	Pos Both eyes Uninjected NQ	ND	Pos NQ	ND
Day 3	Pos	Pos PBMC NQ	Neg	Pos Serum NQ	Neg	Neg
Day 8	ND	Neg	ND	ND	Neg	ND
Day 14	ND	Neg	Neg	Neg		
Day 30	ND	Neg	Neg	Neg		

Lancet 2009 Nov 7,374(9701):1569-605

Biodistribution in Preclinical Studies (vector shedding)

Quantitative PCR detectable
sequence at 3 months in:

Blood:
0/5

Animal	Spleen/% found in eye	Liver/% found in eye	Eye
1101	1539/0.15	415/0.04	>1,000,000
2101	1044/0.02	166/0.003	>5,000,000
3101	1088/0.16	121/0.002	>700,000
4101	244/0.005	61/0.001	>5,000,000
5101 (bilateral injections)	72/0.002	20/0/0.00007	>3,000,000

Similar to preclinical studies,
minimal evidence of extra-ocular
exposure in human studies

Bennett et al, unpublished data

Summary of Immunology Data

# Subjects/Total	Response
0/12	T-cell response: AAV2 capsid
2/12*	T-cell response: RPE65 protein
0/12	Abs vs RPE65 protein

* One sample positive at baseline; another at d30

Maguire et al, Lancet 374:1569-1605

# Affected Dogs/Total	Response
0/9	T-cell response: AAV2 capsid
0/9	T-cell response: RPE65 protein
0/9	Abs vs RPE65 protein

Bennicelli et al, Mol Ther 16:458 (2008) and Bennett, Mingozi, Amado et al, unpublished data

Summary of Immunology Data (cont.): Neutralizing Abs vs AAV2 Capsid

# Subjects/Total	Response
5/12	No change
2/12	Increase (0.5 log) followed by return to baseline
4/12	Increased followed by a decrease (but elevated 0.5 log compared to baseline)
1/12	Decrease (0.5 log) compared to baseline

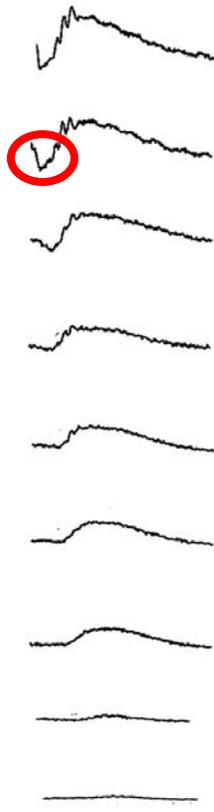
Maguire et al, Lancet 374:1569-1605

# Affected Dogs/Total	Response
1/8	No change
0/8	Increase followed by return to baseline
7/8	Increased (by 1.5 – 3 log) followed by a decrease (but elevated above baseline)
0/8	Decrease (0.5 log) compared to baseline

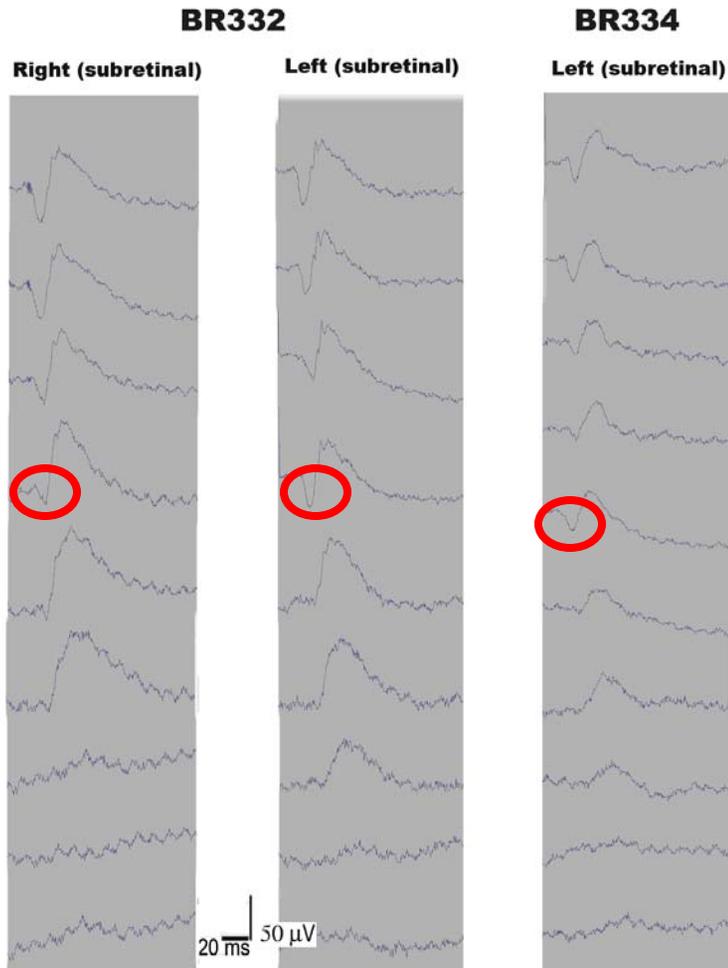
Bennicelli et al, Mol Ther 16:458 (2008) and Bennett, Mingozi, Amado et al, unpublished data

More sensitive responses than our “gold standard” (Lancelot)

Acland et al
Nat Genet 28:92 (2001)



3 Month Comparative Data



Bennicelli et al,
Mol Ther 16:458 (2008)

“a” waves are detectable at 1-2 log units lower light intensity than they were in Lancelot

Summary

- Gene therapy firsts:
 - for a non-lethal disease in pediatric subjects
 - for inherited retinal degeneration in older individuals
- High level of safety
- All 12 individuals enjoy improved vision
- Several subjects are arguably no longer legally blind
- Stable and safe recovery of vision
- Extent of recovery is age-dependant
- Animal study results provided framework for an optimal risk:benefit ratio in the human protocol

Lancet 2009 Nov 7,374(9701):1569-605

We are grateful for support from:

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- Scheie Eye Institute
- Research to Prevent Blindness
- Macula Vision Foundation
- Paul and Evanina Mackall Foundation Trust
- Italian Telethon Foundation
- National Center for Research Resources
- Howard Hughes Medical Institute.



The Children's Hospital
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