**8° NeuroMeeting**

**Riparare il cervello: nuove frontiere terapeutiche**

**Napoli 12 e 13 Maggio 2016**

# La terapia genica

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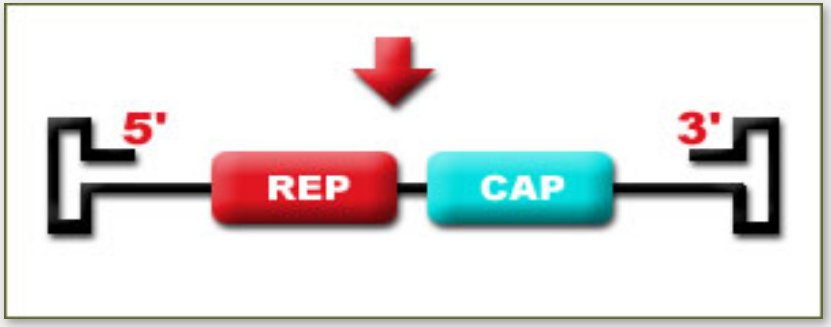
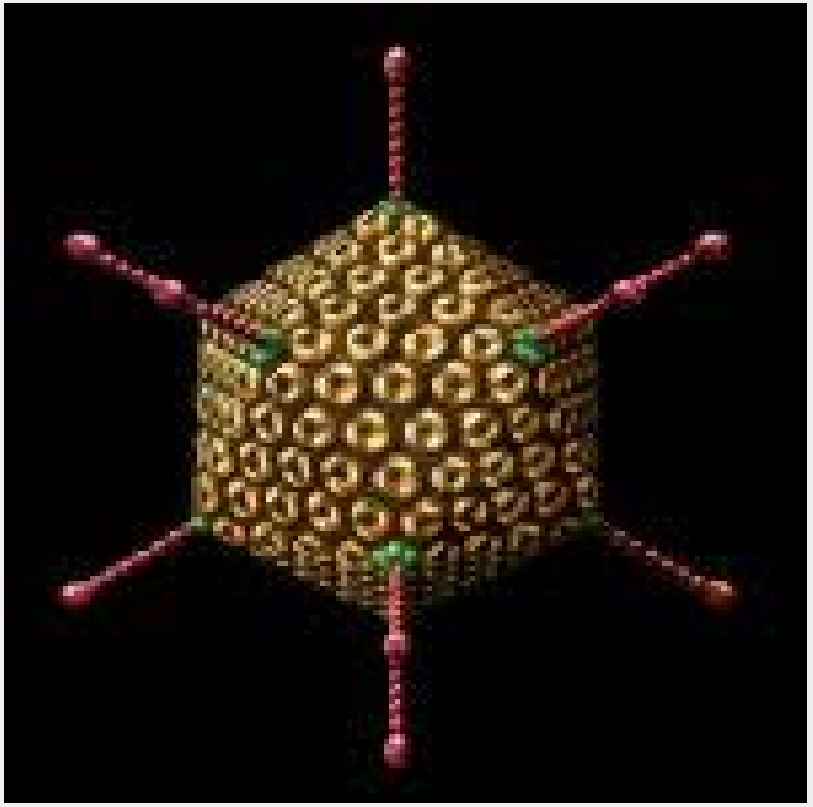
# Gene Therapy

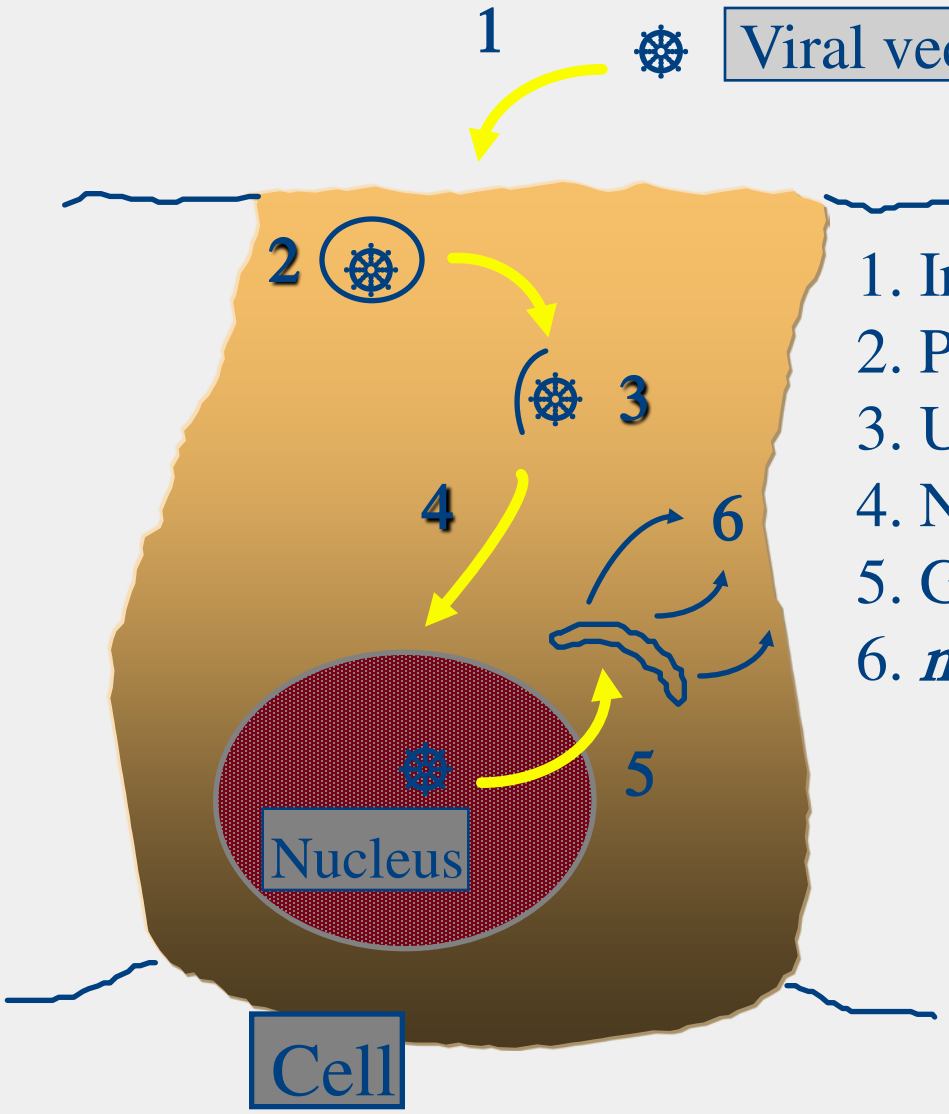
Gene therapy consists of inserting exogen Nucleic acid to specific cell tissues in order to synthesize a biologically active protein

# Viral vector

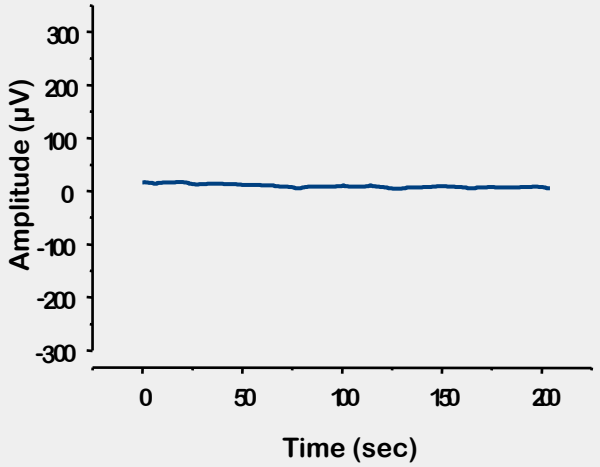
# Gene transfer



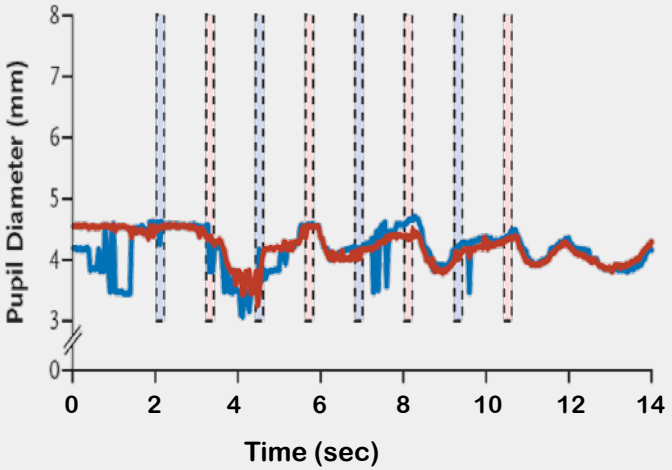

1. Inoculation
2. Penetration
3. Uncoating
4. Nucleus access
5. Gene expression
6. ***maintenance of gene expression***

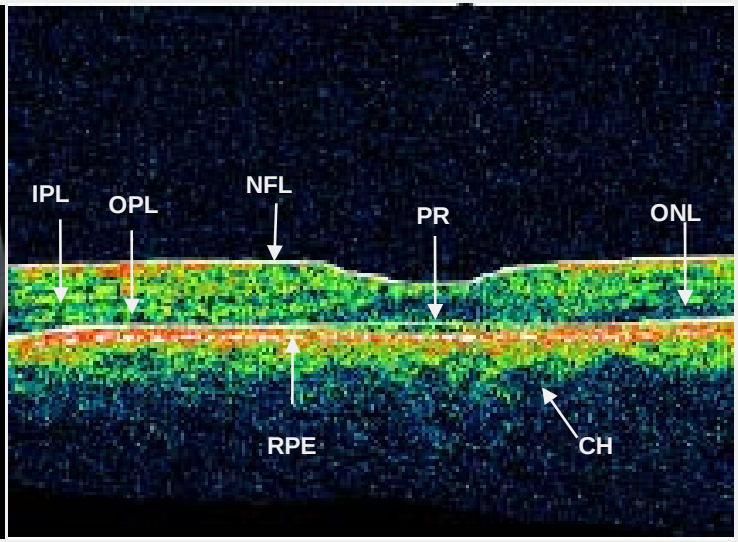
# Clinical Features of Leber Congenital Amaurosis patients

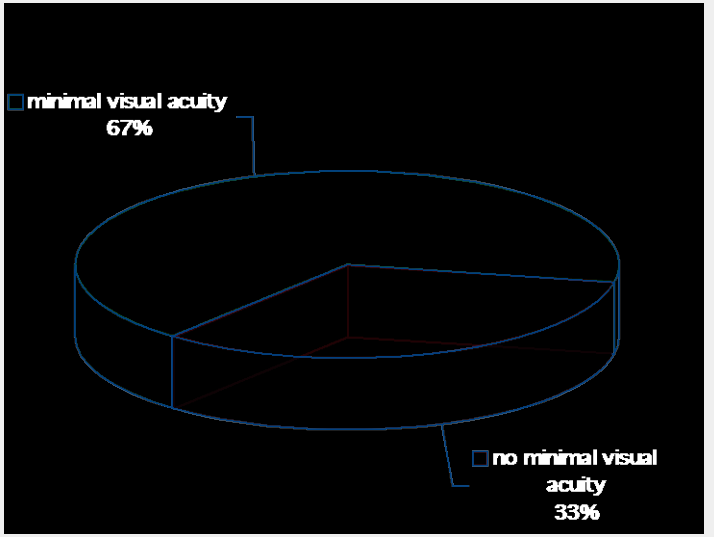
## Electroretinogram




## Pupillometry

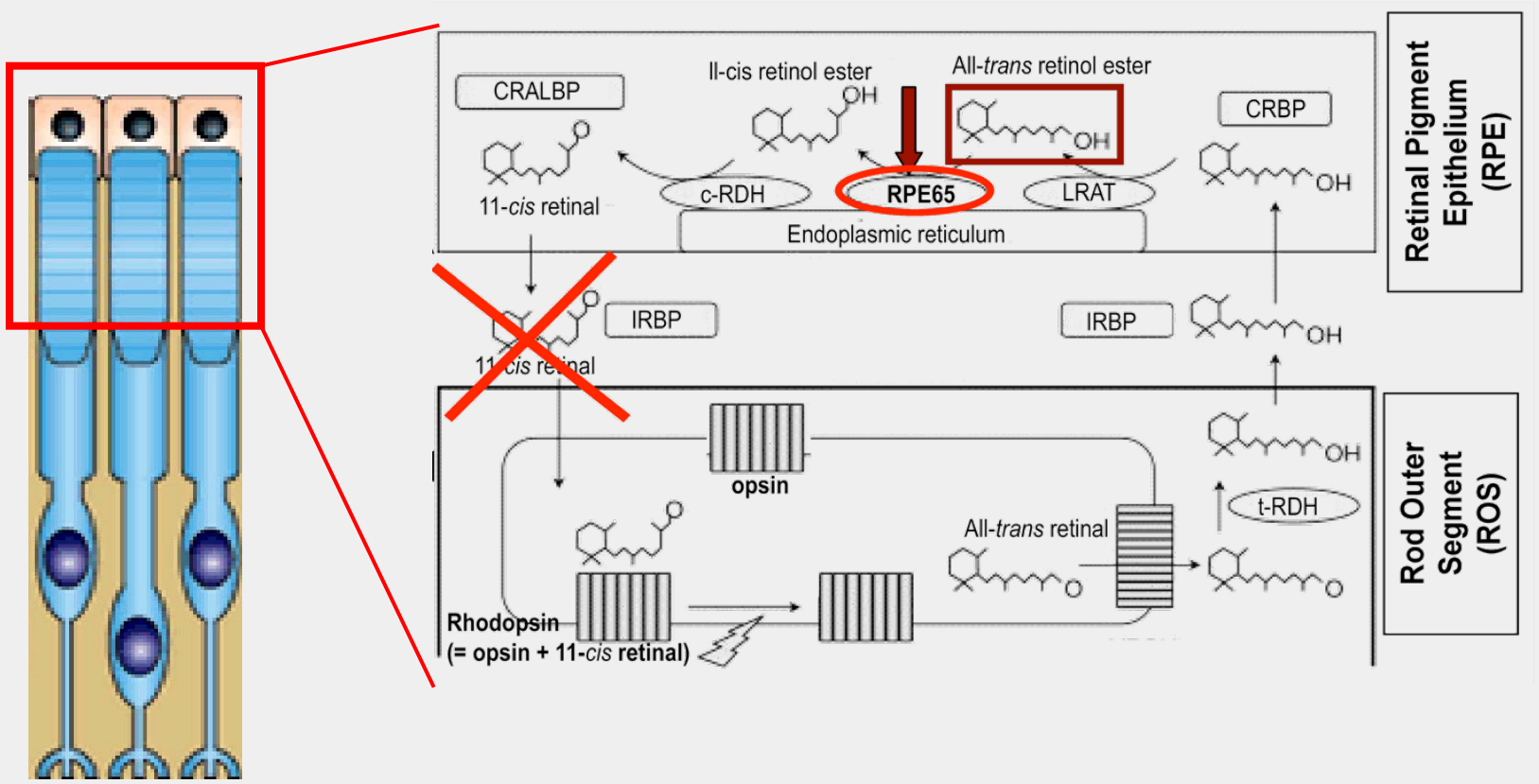



*Simonelli et al IOVS 2007*

# RPE65 is necessary for production of 11-cis-retinol, a component of rhodopsin



# Phase I open-label dose escalation safety study of gene transfer in subjects with LCA due to RPE65 mutation

- GMP Vector manufactured at Center for Cellular and Molecular Therapeutics of Philadelphia

- Patients identified at CHOP, TIGEM, Second University of Naples

- Subretinal administration performed at Children's Hospital of Philadelphia

- Follow-up tests performed at CHOP and at Second University of Naples

# INCLUSION CRITERIA

1. Adhere to protocol and companion protocol for long-term follow-up as evidenced by written informed consent or parental permission and subject assent.

2. Adults and children diagnosed with LCA.

3. Molecular diagnosis of LCA due to RPE65 mutations by a CLIA-approved laboratory.

4. Age eight years old or older at the time of administration.

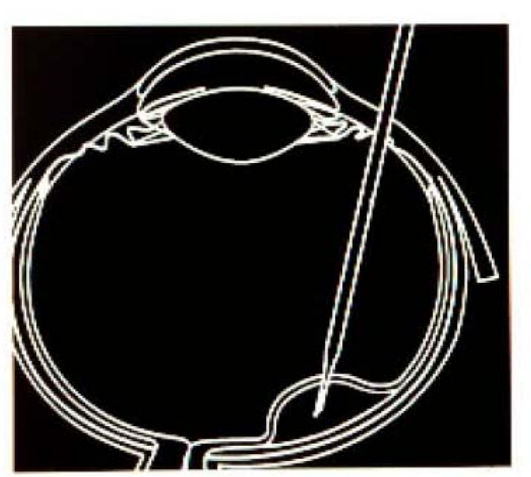
5. Visual acuity ≤ 20/160 or visual field less than $20^{o}$ in the eye to be injected.

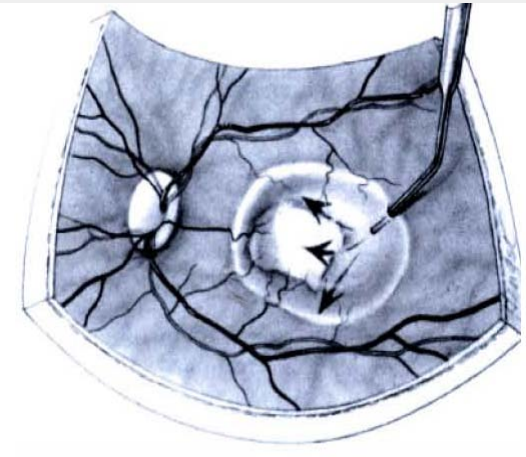
# Summary of patient demographics

| | Low dose | | | Medium dose | | | | | | High dose | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NP01 | NP02 | NP03 | NP04 | CH06* | CH08 | CH09 | CH10 | CH11 | CH12 | CH13 | NP15 |
| Date at administration | Oct 11, 2007 | Dec 13, 2007 | Jan 24, 2008 | April 10, 2008 | May 22, 2008 | July 10, 2008 | Sept 25, 2008 | Nov 18, 2008 | Dec 30, 2008 | March 5, 2009 | April 16, 2009 | June 2, 2009 |
| Age (years) | 26 | 26 | 19 | 17 | 20 | 9 | 8 | 10 | 24 | 44 | 35 | 11 |
| Sex | Female | Male | Female | Male | Female | Male | Male | Male | Female | Female | Male | Male |
| *RPE65* mutations | E102K/ E102K | E102K/ E102K | R234X/ R234X | R91W/ T149N | IVS1+5g>a/ L341S | F530fs/F530fs | R124X/ Lys297del1aggA | IVS1+5g>a/ Phe530del1ttc | V473D/ V473D | K303X/ W431C | IVS1+5g>a/ IVS1+5g>a | D167W/ H313R |
| Injected eye† | Right | Right | Right | Left | Right | Right | Left | Right | Right | Right | Right | Right |

†Eye with worst sight selected for surgery.

# Subretinal Injection of AAV2.hRPE65v2





We did a standard three-port pars plana vitrectomy, with removal of the posterior cortical vitreous.

Patients in the low-dose cohort were injected with $1.5x10^{10}$ vector genomes and those in the medium-dose with $4.8x10^{10}$ vector genomes of AAV2-hRPE65v2 in a volume of 150 µL into the subretinal space.

Patients in the high-dose cohort were injected with $1.5x10^{11}$ vector genomes in a volume of 300 µL.

# Assessment of safety and efficacy: time points before and after surgery

| | Baseline | | | Day | | | | | | | | | | | Year | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **B1** | **B 2** | **Day-3** | **0** | **1** | **2** | **3** | **14** | **30** | **60** | **90** | **180** | **270** | **365** | **1.5** | **2 - 5** |
| **Acceptable time window** (# of days) | < 30 before Day 0 | | | | | | | ±2 | ±5 | ±5 | ±5 | ±30 | ±30 | ±30 | ±30 | ±30 |
| Informed consent process | X | | | | | | | | | | | | | | | |
| History and Physical Examination | X | | | | X | | | | X | | X | | | X | | |
| Pregnancy test (if applicable) | X | | | X | | | | | | | | | | | | |
| Administration of prednisone begins | | | X | | | | | | | | | | | | | |
| Administration of prednisone discontinued | | | | | | | | X | | | | | | | | |
| Vital signs | X | X | | X | X | X | X | X | X | | | | | X | | |
| Blood draw (3 teaspoons) | | | | X | X | | X | | | | | | | X | | |
| Blood draw (6 teaspoons) | | | | | | | | X | X | | X | | | | | |
| Blood draw (10 teaspoons) | X | | | | | | | | | | | | | | | |
| Urine collection | X | | | X | X | | X | X | X | | X | | | X | | |
| Tear collection (*until 2 negative results obtained) | X | | | X | X | X* | | | | | | | | | | |
| Ophthalmic exam (^Day 7 if inflammation present) | X | X | | | X | X | X^ | X | X | X | X | X | X | X | X | X |
| Optical coherence tomography (OCT) | X | | | | | | | | | X | | X | | X | X | X |
| Acuity test | X | X | | | X | X | X | X | X | X | X | X | X | X | X | X |
| Color vision test | X | X | | | | | | | X | X | X | X | X | X | X | X |
| Contrast sensitivity test | | X | | | | | | | | | | X | | X | X | X |
| Electroretinography (ERG) | X | | | | | | | | | X | | | | X | X | X |
| Visual field test | | X | | | | | | | X | X | X | X | X | X | X | X |
| Pupillometry test | | X | | | | | | | X | X | X | X | X | X | X | X |
| Nystagmus testing | | X | | | | | | | | | X | X | X | X | X | X |
| Fundus photography | X | | | X | | | | | X | | | X | | X | X | X |
| Quality of life | X | | | | | | | | | X | | X | | X | X | X |
| Mobility testing | | X | | | | | | | | | X | X | | X | X | X |
| Adverse event recording | X | X | | X | X | X | X | X | X | X | X | X | X | X | X | X |

# RESULTS

# Safety profile

- None of the patients had serious adverse events
- the vector was found in samples of tears and blood only transiently after surgery
- Exposure to subretinal AAV at the doses used did not lead to a harmful immune response
- Patient 2 was found to have a macular hole at day 14 rather stable over time. This did not prevent vision improvement

All 12 patients reported improved vision in dimly lit environments in the injected eyes starting 2 weeks after surgery.

# Outcome measures

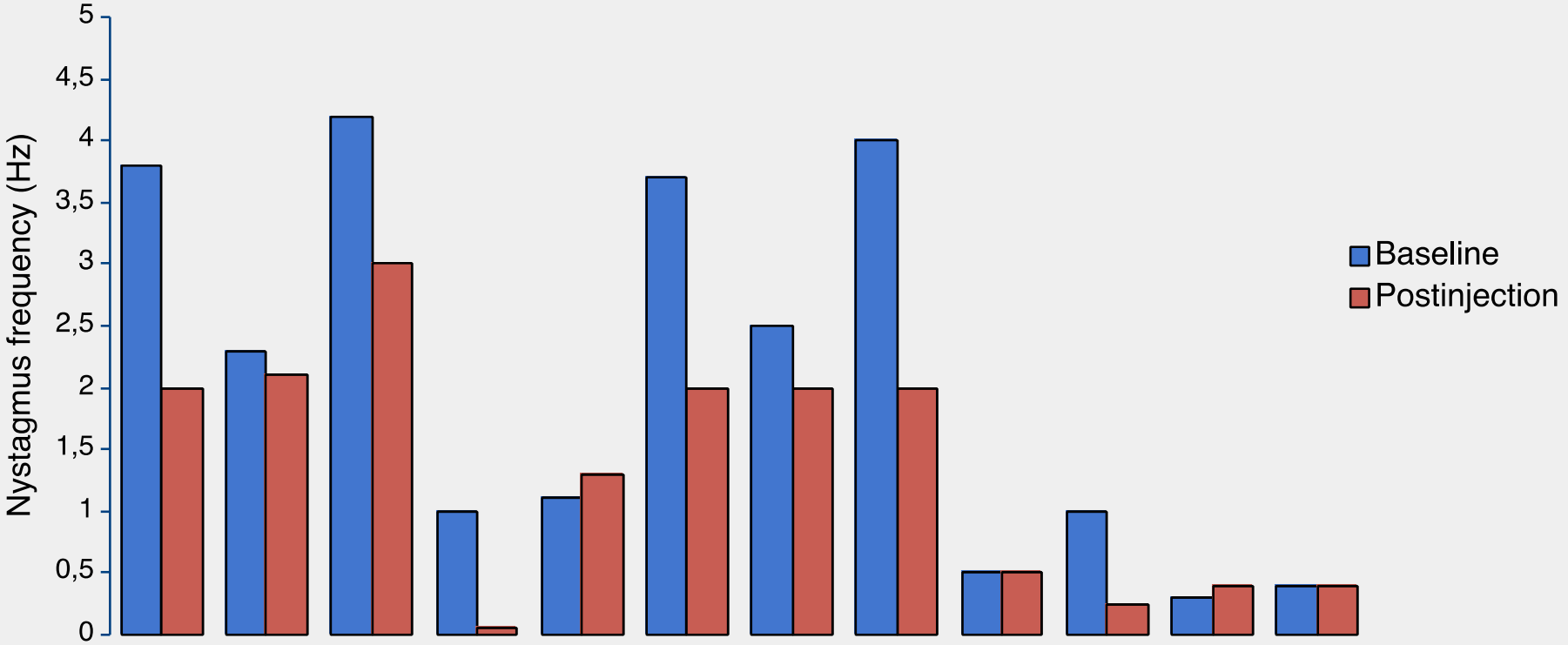
**Objective measures:**

- nystagmus testing
- pupillary light reflex
- Electroretinography

**Subjective measures:**

- standard test of visual acuity
- Goldmann visual field
- mobility testing

# nystagmus testing

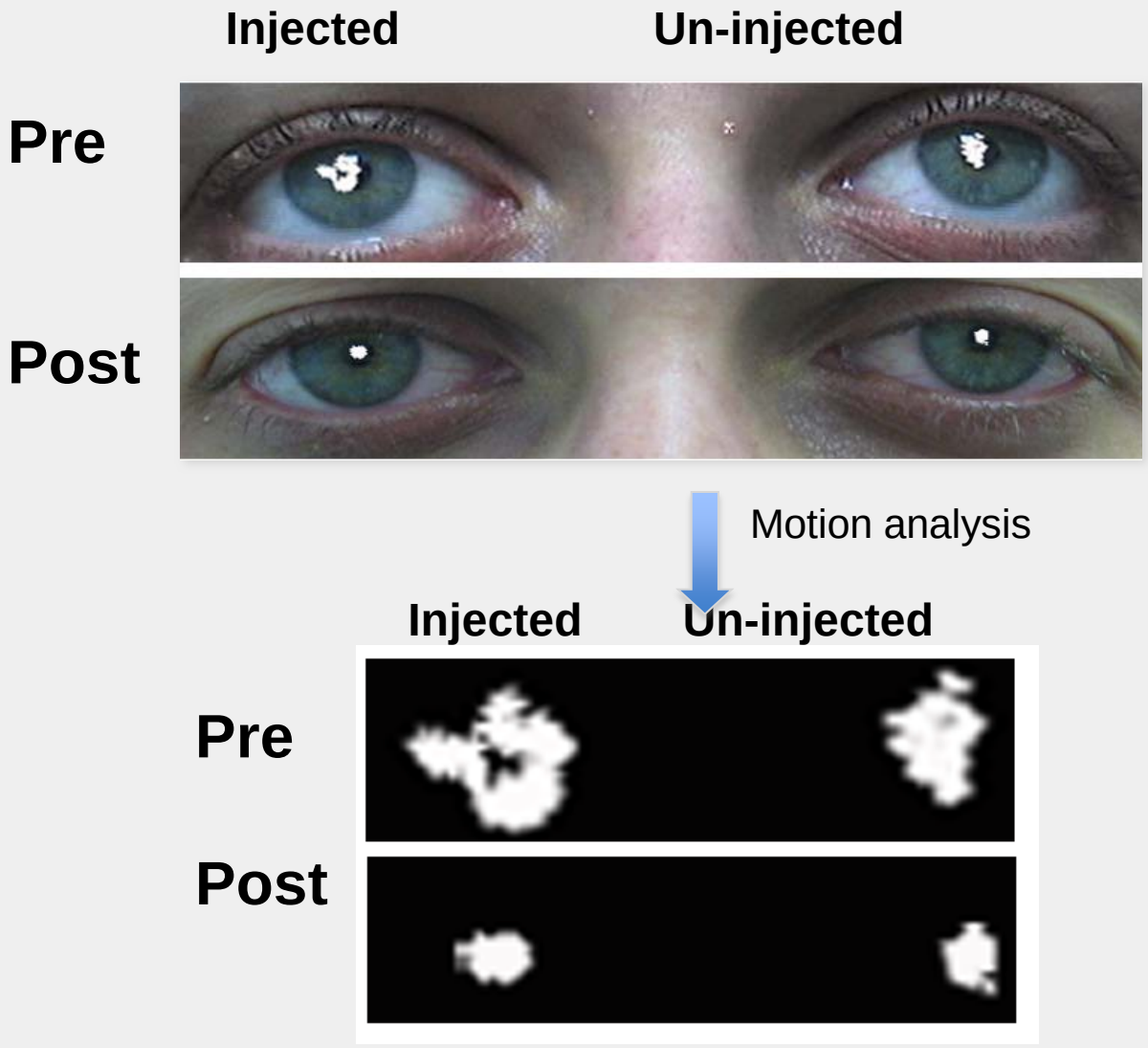


# NP03 - Nystagmus

Before injection

After injection

# NP03: Nystagmus (3 Years Follow-up)

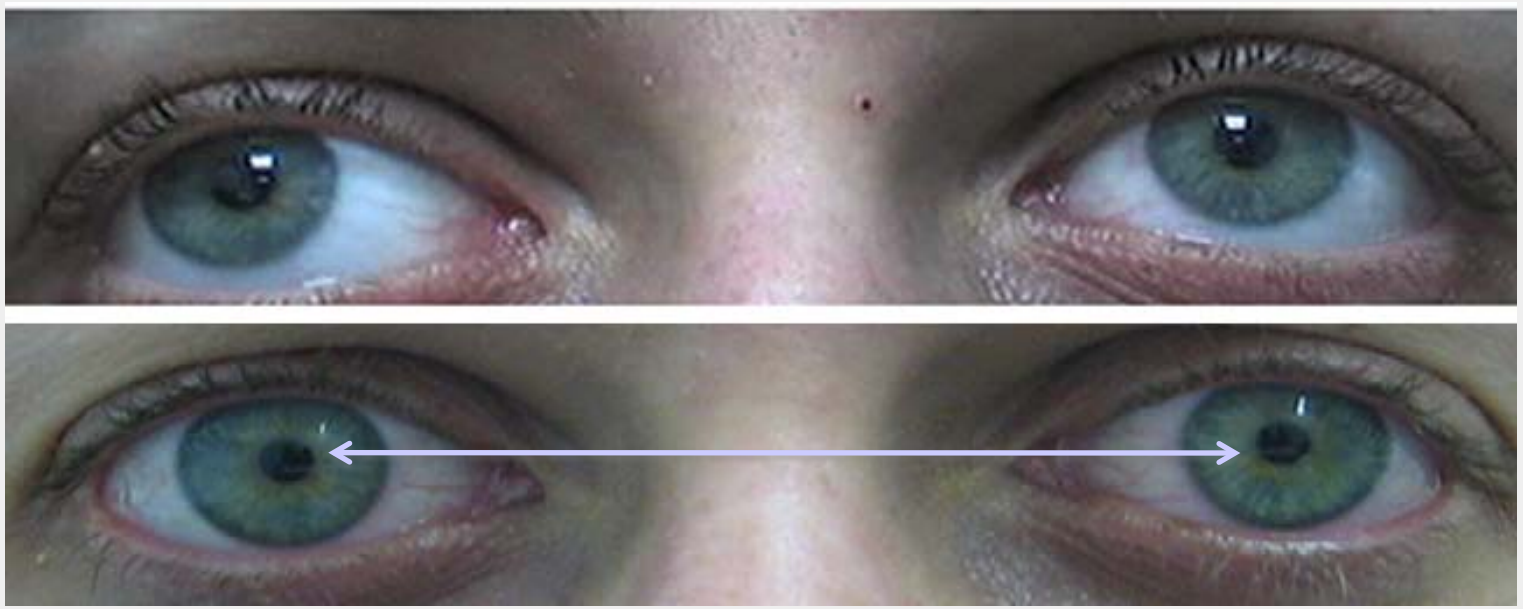



**Smaller excursion of nystagmus after injection in both eyes**

# NP03: Reduction in Exotropia
## (3 Years Follow-up)

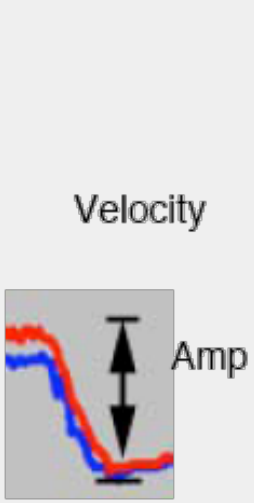
**Pre**

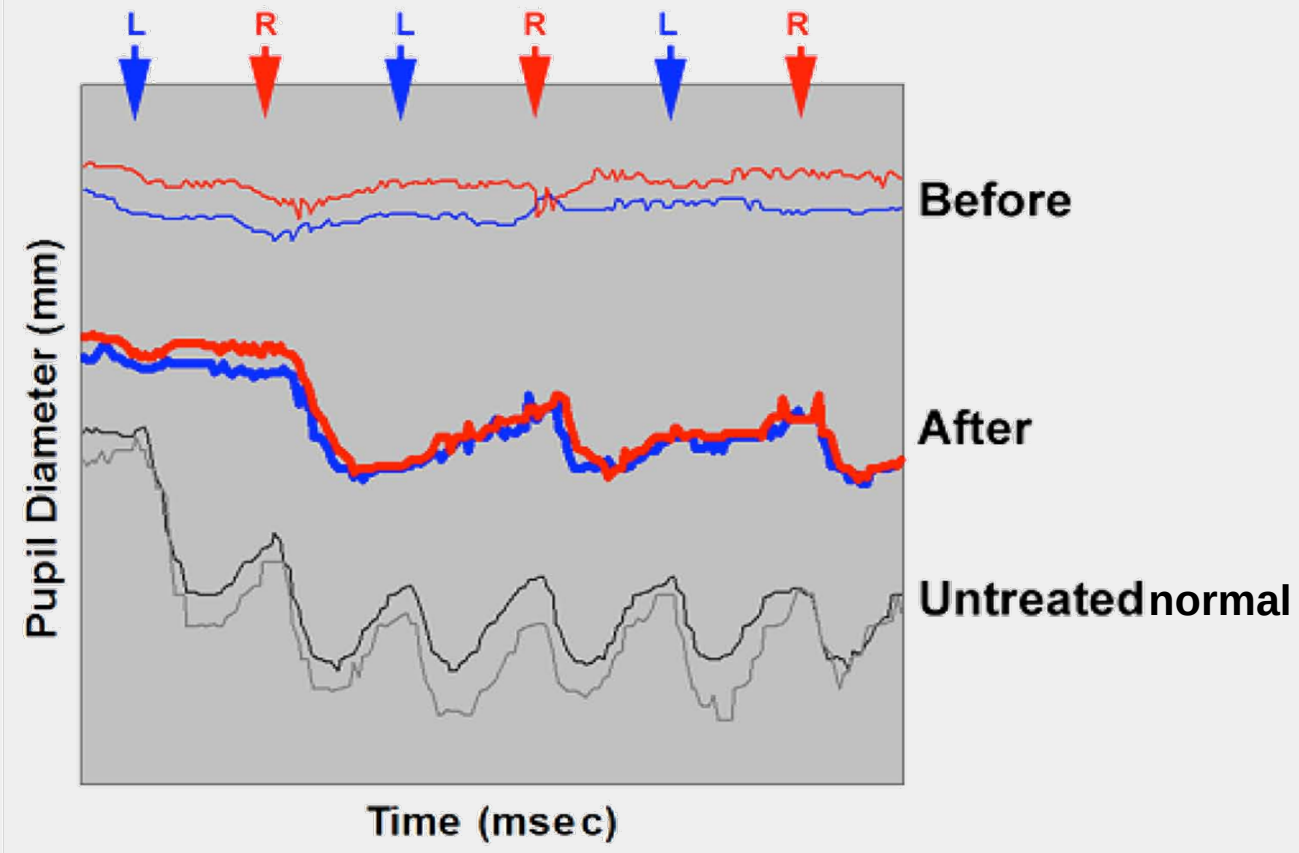
**Post**



**Decreased inter-pupillary distance after injection**

# Pupillary Light Reflex (PLR)

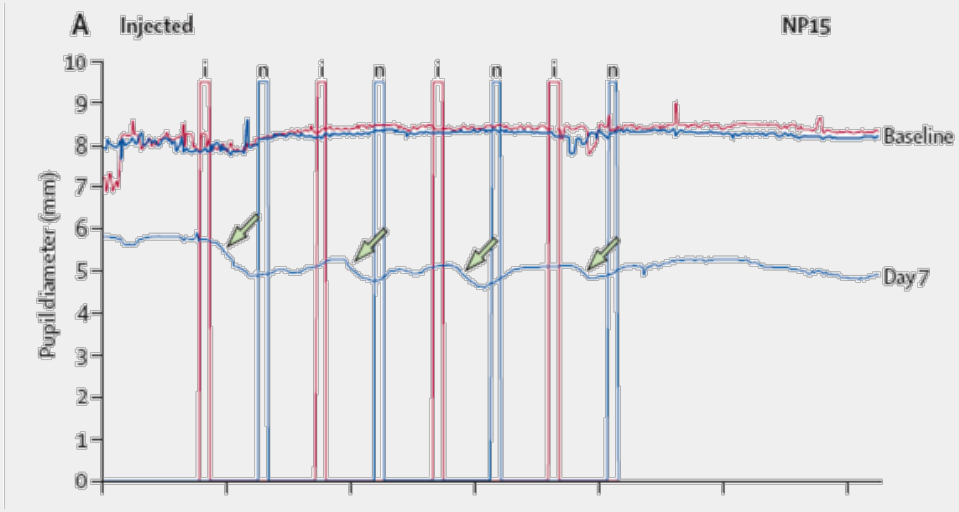


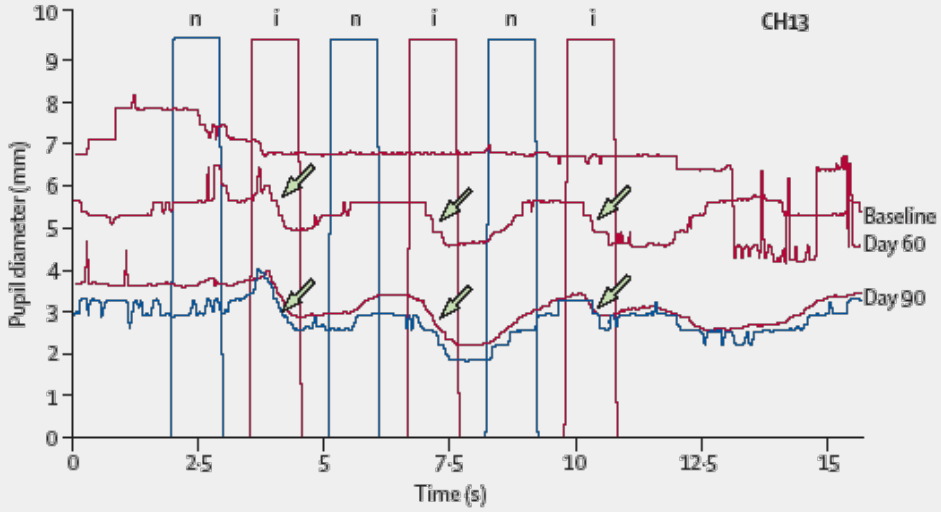




**Pupillometry provides quantitative information about the response of the entire retina to light**

**The improvements in the pupillary responses were easily assessed through measurement of the amplitude and velocity of constriction**

# Pupillary Light Reflex (PLR)



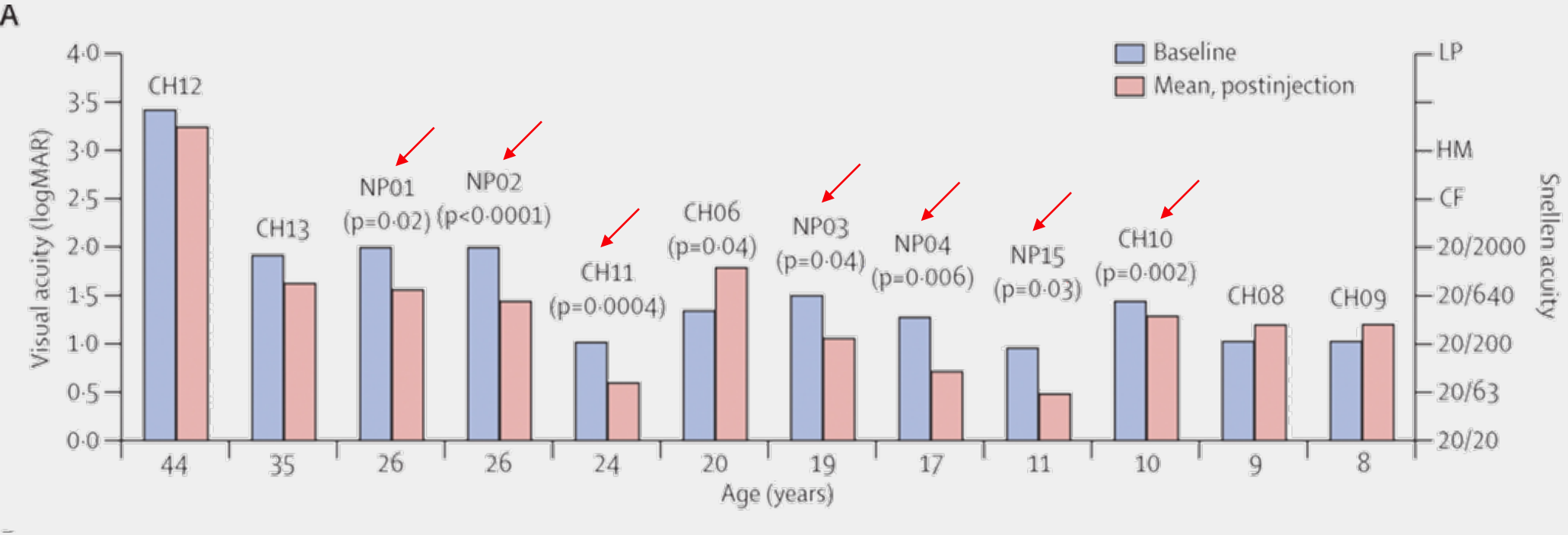




-PLR is restored in the injected eyes in all patients treated

# Electroretinography

- Full field scotopic and photopic electroretinographic responses were flat in all patients before and after the injection.

# Visual acuity



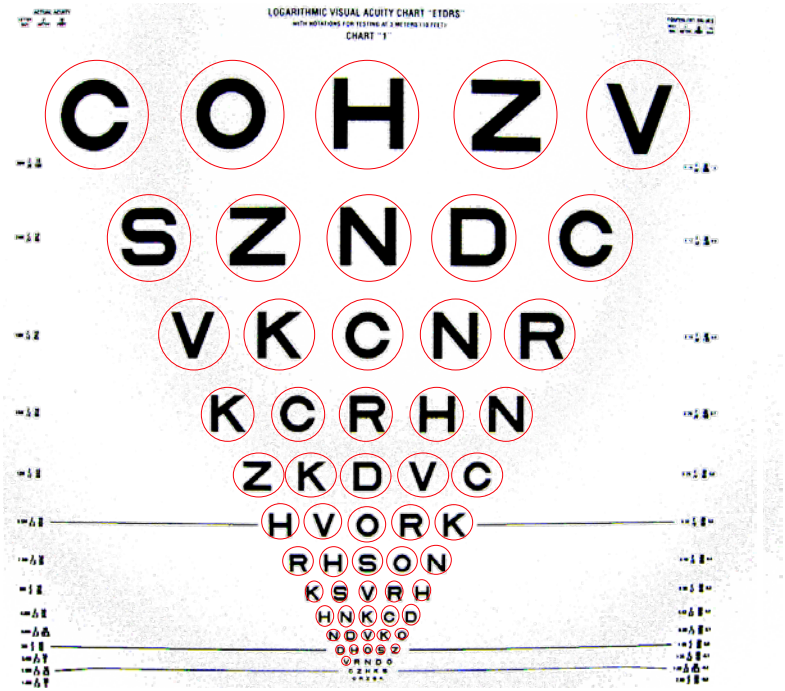

- Significant gains in visual acuity in 7/12 subjects

- Gains are stable

# Visual acuity

*(50 cm)*

**3 years**

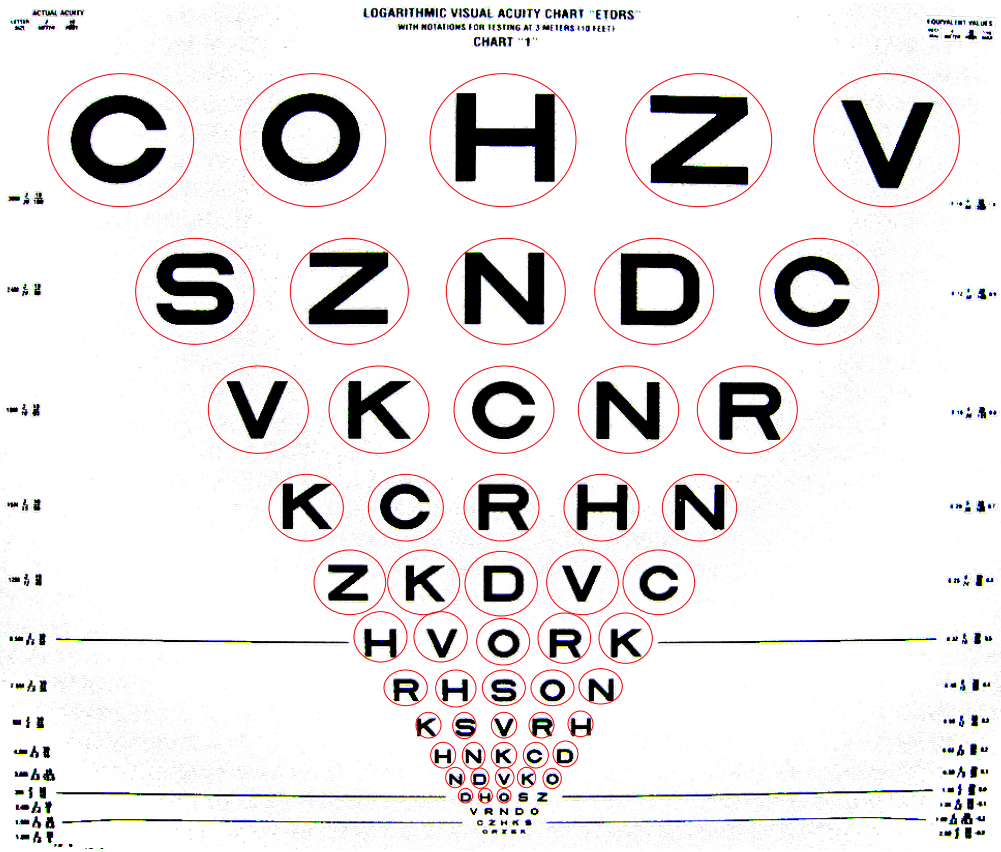



**NPO3**

# Visual acuity

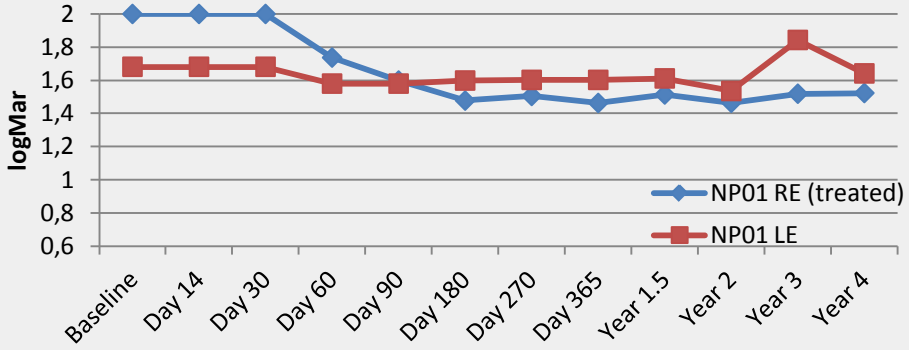
*(2 meters)*

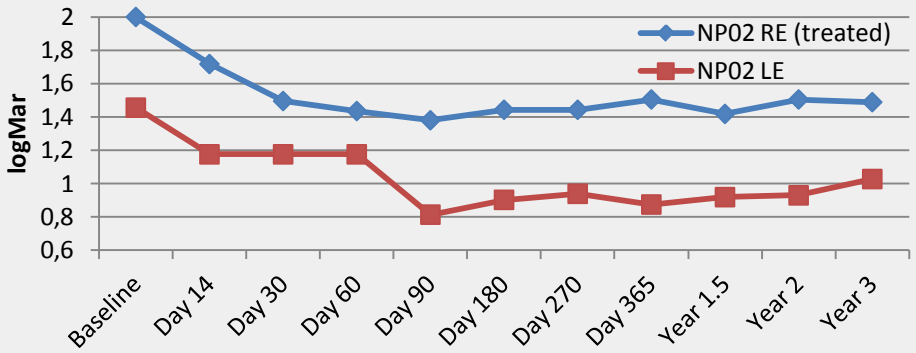
**3years**

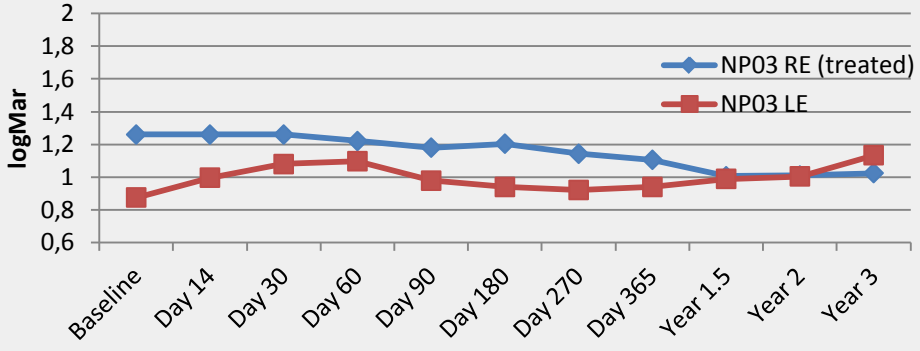


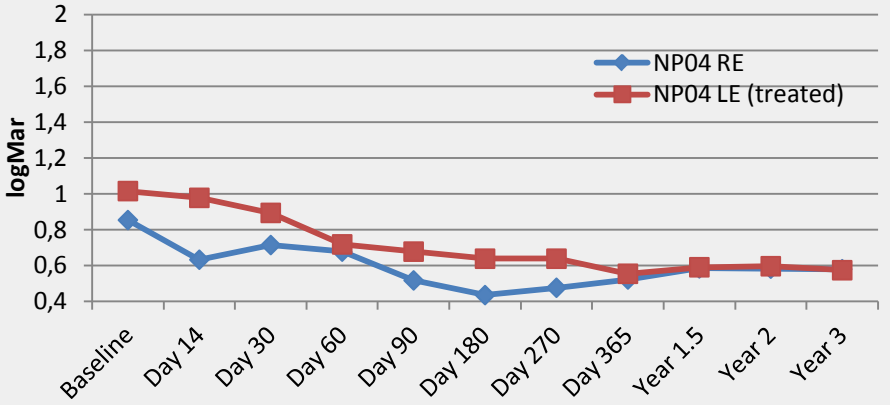

**NP15**

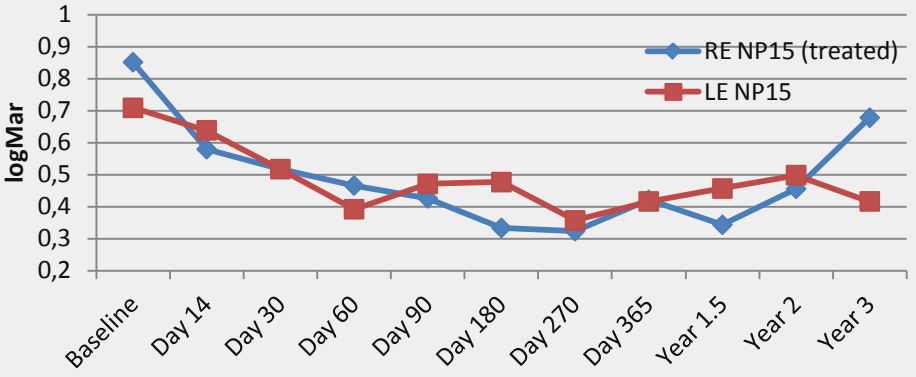
# Longitudinal data analysis of Best Corrected Visual Acuity (BCVA) in all the patients



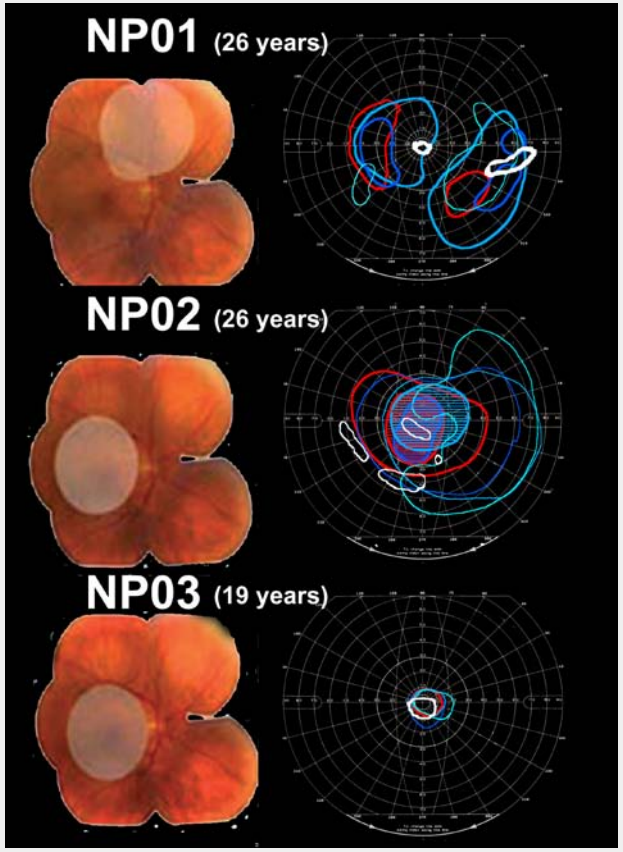



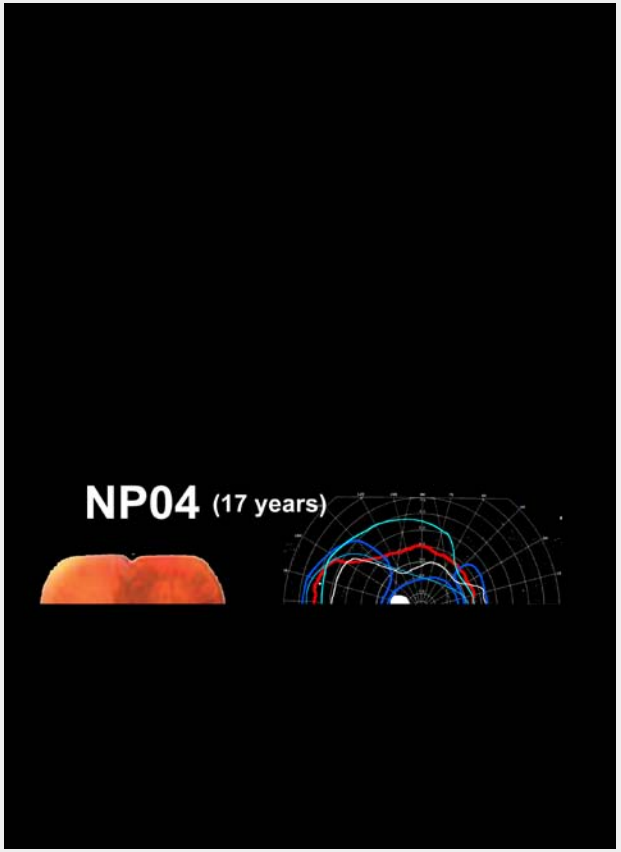




Testa F, Maguire AM, Rossi S, Pierce EA, Melillo P, Marshall K, Banfi S, Surace EM, Sun J, Acerra C, Wright JF, Wellman J, High KA, Auricchio A, Bennett J, Simonelli F, Three-Year Follow-up after Unilateral Subretinal Delivery of Adeno-Associated Virus in Patients with Leber Congenital Amaurosis Type 2. Ophthalmology. 2013

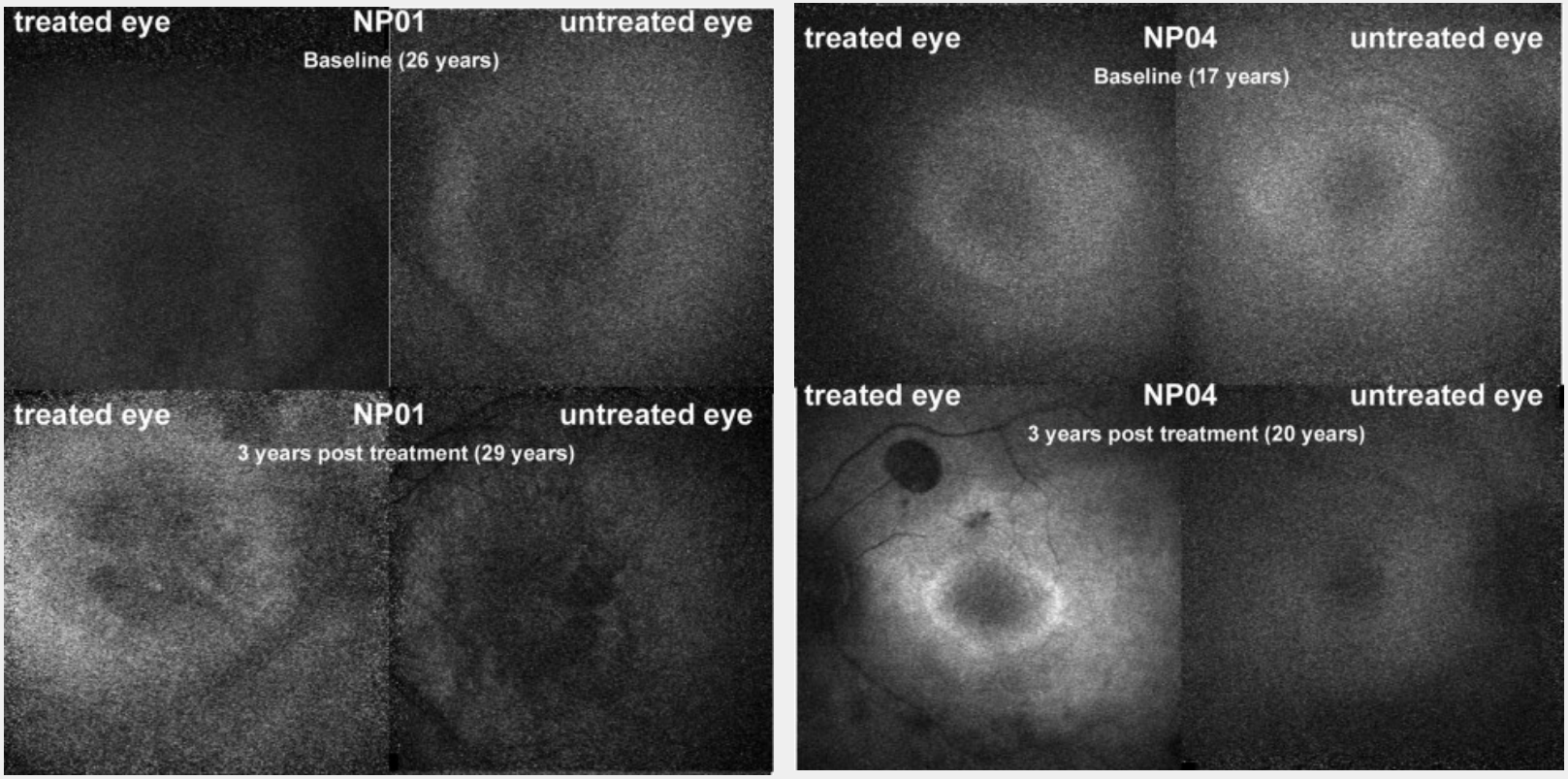
# Visual field



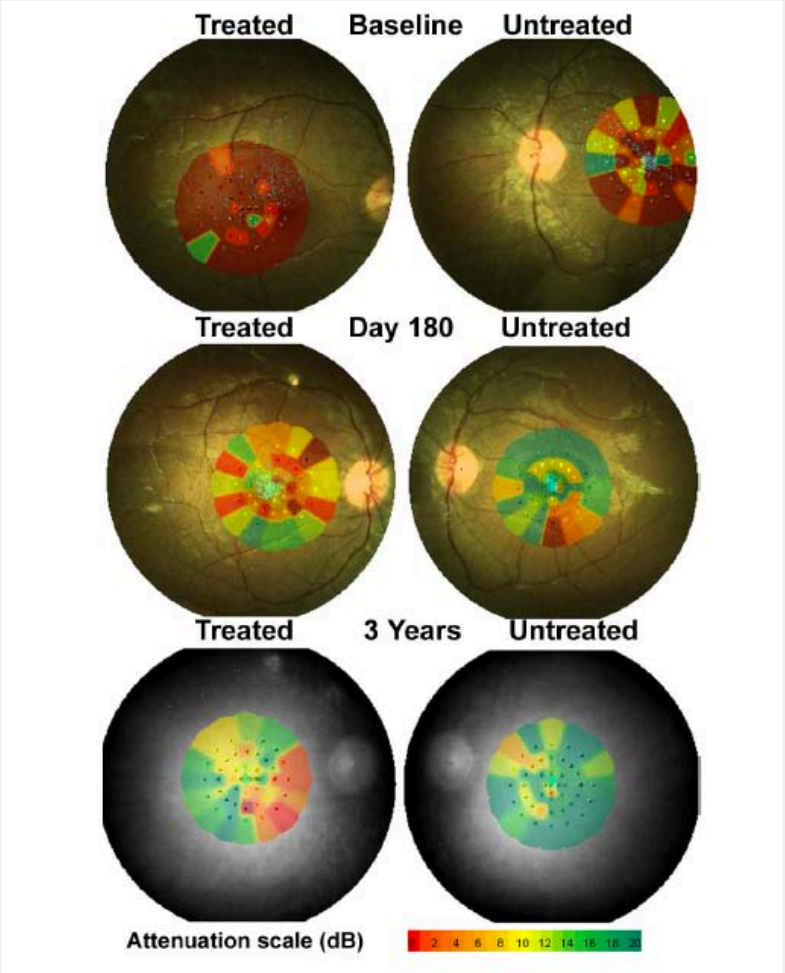

All subjects have expanded visual fields after injection
Expansion correlates with the area injected

# Autofluorescence



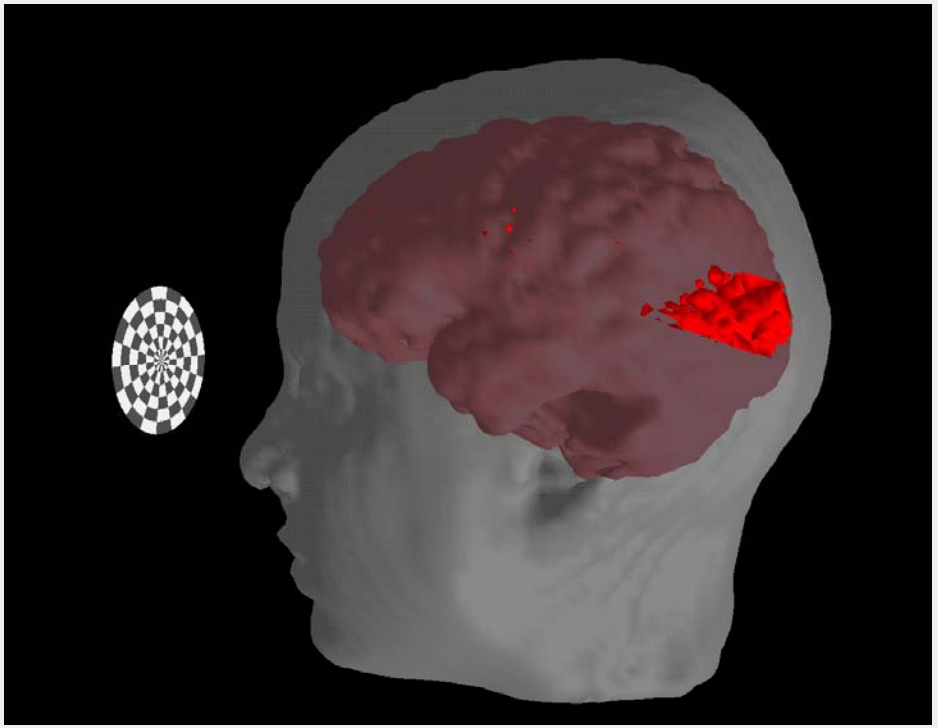

Fundus autofluorescence (FAF) results of patients NP01 and NP04. An increase of FAF is shown in the treated eye compared with baseline and with the untreated eye.

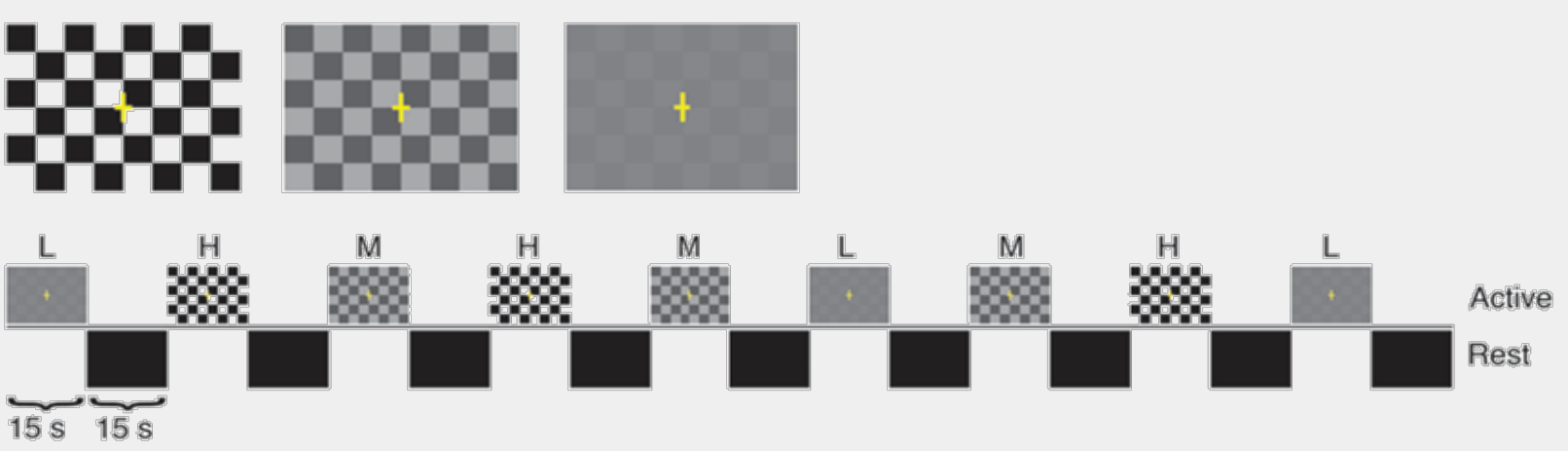
# Microperimetry sensitivity map in NP15 at selected timepoints

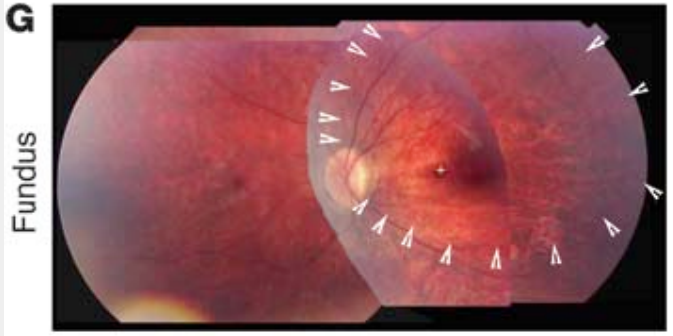


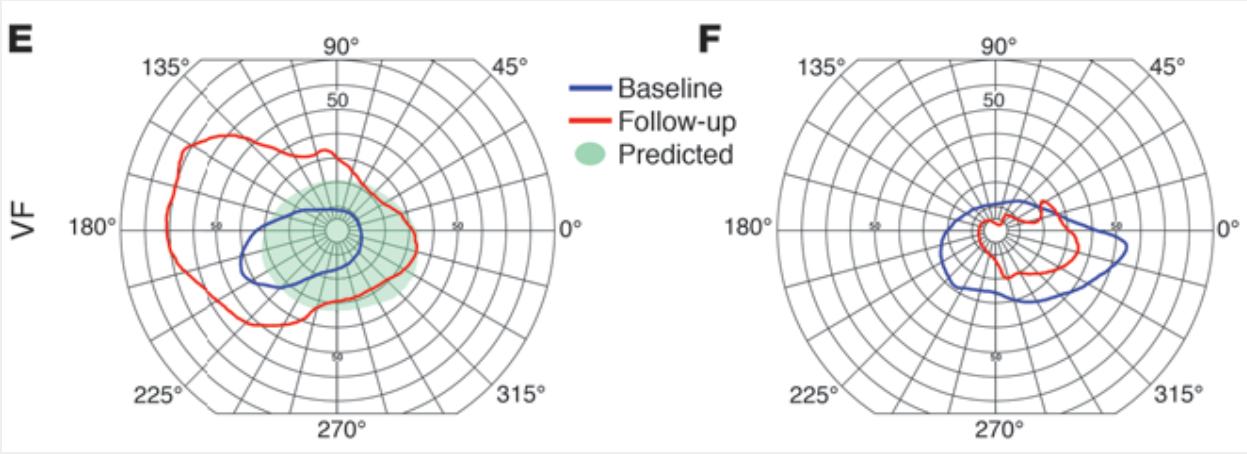

The longitudinal data analysis of microperimetry in NP15 shows a change of fixation from an unstable extrafoveal site to a stable foveal site.

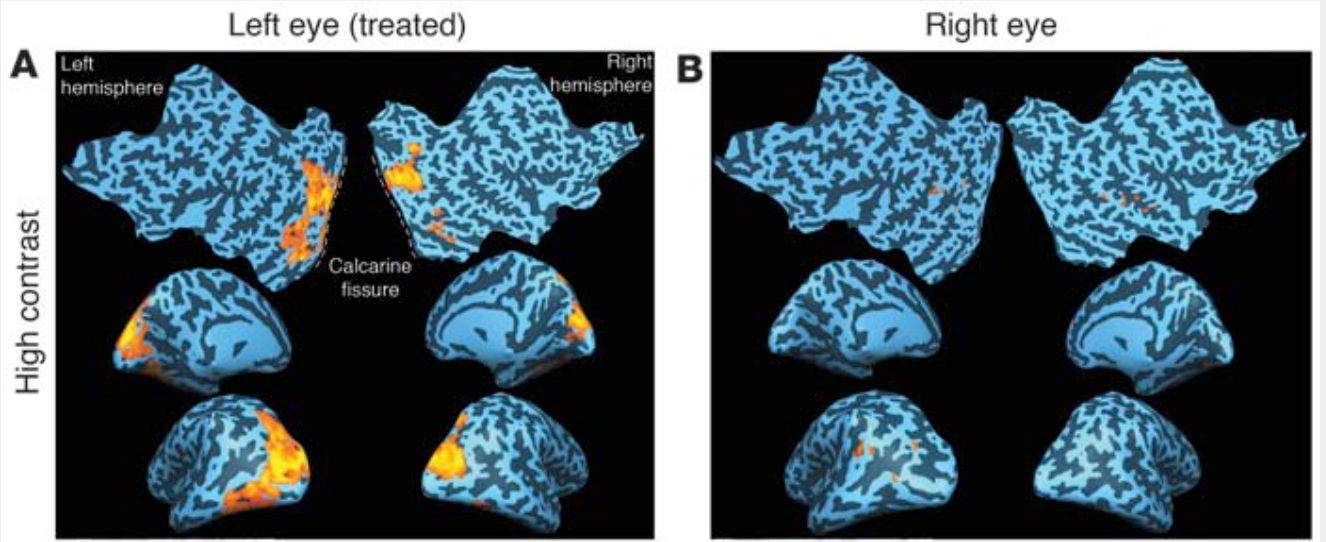
# Functional MRI

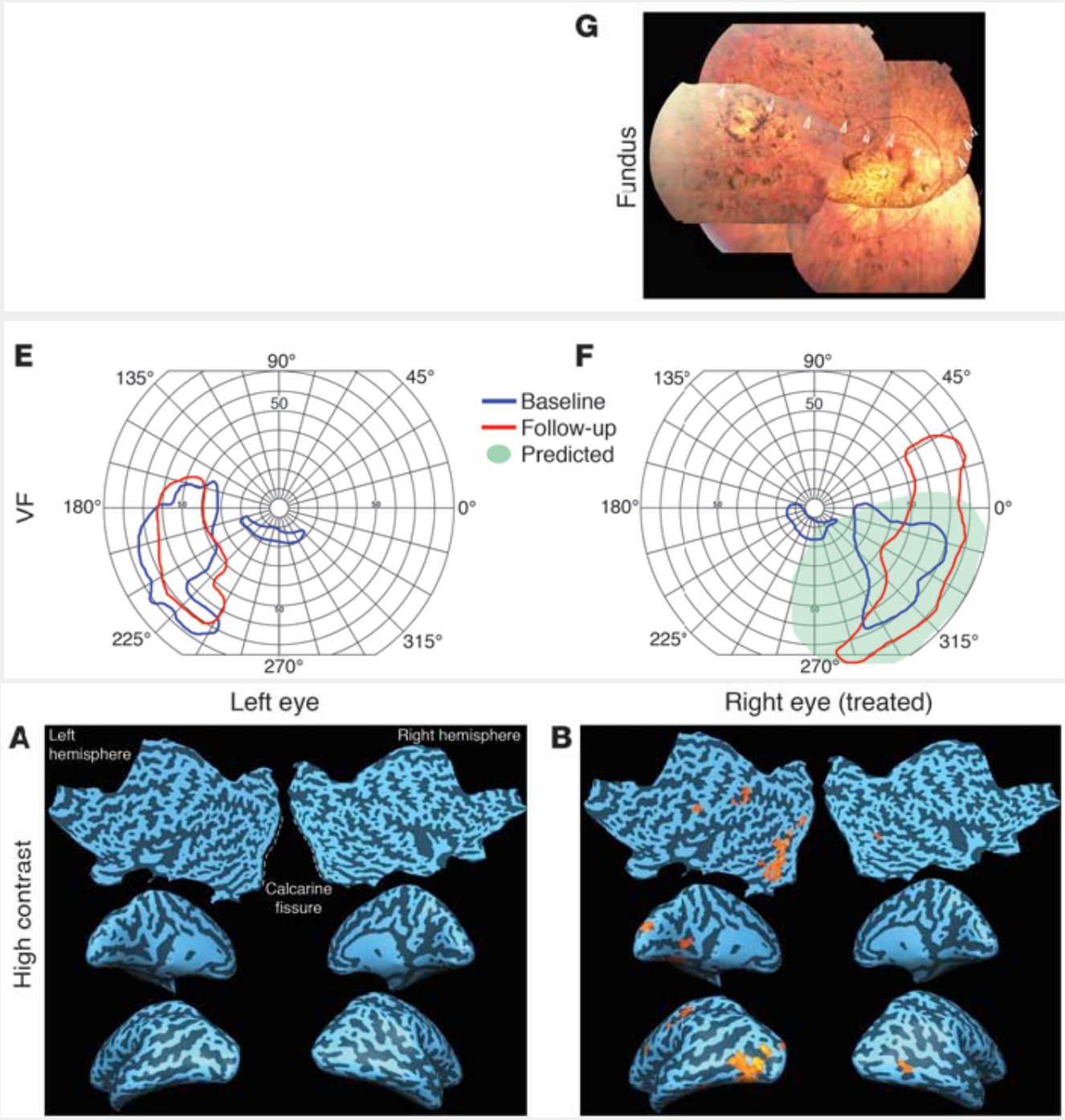





G
Fundus


E
F
VF
90°
135°
45°
180°
0°
225°
315°
270°
50
Baseline
Follow-up
Predicted


Left eye (treated)
Right eye
A
B
High contrast
Left hemisphere
Right hemisphere
Calcarine fissure


G
Fundus
E
F
VF
90°
135°
45°
180°
0°
225°
315°
270°
50
Baseline
Follow-up
Predicted
Left eye
Right eye (treated)
A
B
High contrast
Left hemisphere
Right hemisphere
Calcarine fissure

# CONCLUSION

- **There was no evidence of adverse effects of gene transfer on retinal function**

- **There was no evidence of systemic toxicity in the long-term study**

# CONCLUSION

- **Most of the improvement occurs in the first six months after injection in all subjects**
- **The improvement is maintained in the treated patients up to 3 years post injection**
- **The visual recovery noted in the children confirms that the efficacy will be improved if treatment is applied before retinal degeneration has progressed.**

# CONCLUSION

**Gene therapy of LCA patients with RPE65 mutations appears safe and results in stable improvement in retinal/visual function after a single vector application**

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

# 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 Mingozzi, 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 Zelenaia, 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 Wellman McDonnell, M.S., Alberto Auricchio, M.D., Katherine A. High, M.D., and Jean Bennett, M.D., Ph.D.

*Molecular Therapy*

*MT Open*

*original article*

# Gene Therapy for Leber's Congenital Amaurosis is Safe and Effective Through 1.5 Years After Vector Administration

Francesca Simonelli[1,2], Albert M Maguire[3–5], Francesco Testa[1], Eric A Pierce[3,5], Federico Mingozzi[4], Jeannette L Bennicelli[3,5], Settimio Rossi[1], Kathleen Marshall[4], Sandro Banfi[2], Enrico M Surace[2], Junwei Sun[4], T Michael Redmond[6], Xiaosong Zhu[4], Kenneth S Shindler[3,5], Gui-Shuang Ying[3], Carmela Ziviello[2,7], Carmela Acerra[1,2,4], J Fraser Wright[4,5], Jennifer Wellman McDonnell[4], Katherine A High[4,5,8], Jean Bennett[3,4] and Alberto Auricchio[2,9]

THE LANCET

Articles

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

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The Journal of Clinical Investigation

Technical advance

# The human visual cortex responds to gene therapy–mediated recovery of retinal function

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RESEARCH ARTICLE

Science Translational Medicine
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GENE THERAPY

# AAV2 Gene Therapy Readministration in Three Adults with Congenital Blindness

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# Three-Year Follow-up after Unilateral Subretinal Delivery of Adeno-Associated Virus in Patients with Leber Congenital Amaurosis Type 2

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