Genetic Therapies for Ocular Diseases

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- **4DMT:** consultancy fees
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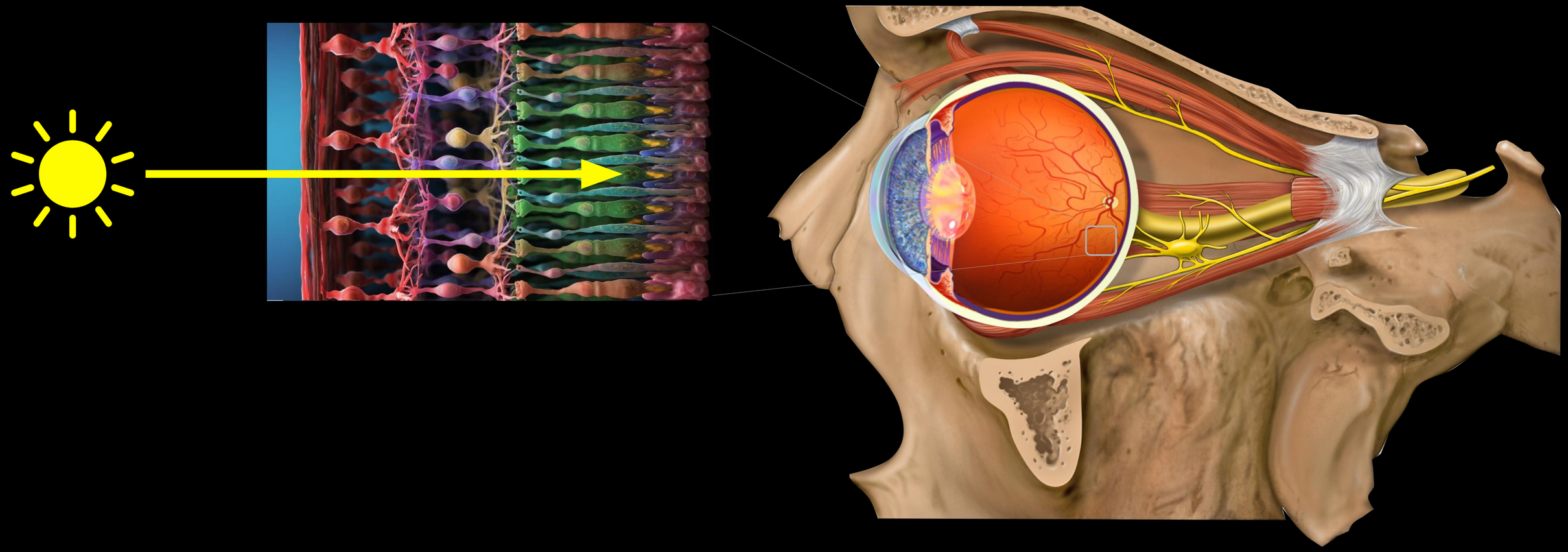
- **Transine Therapeutics**: consultancy fees
- **Vedere Bio I & II**: consultancy fees
- **ViGeneron**: consultancy fees

The Human Eye, Retina & Retinal Disease

Rods, Cones & Retinal Pigment Epithelium (RPE)

Human Retina



Eye translates light into electricity

Introduction
Retinal Cells & Circuitry

Ganglion cells

Amacrine cells

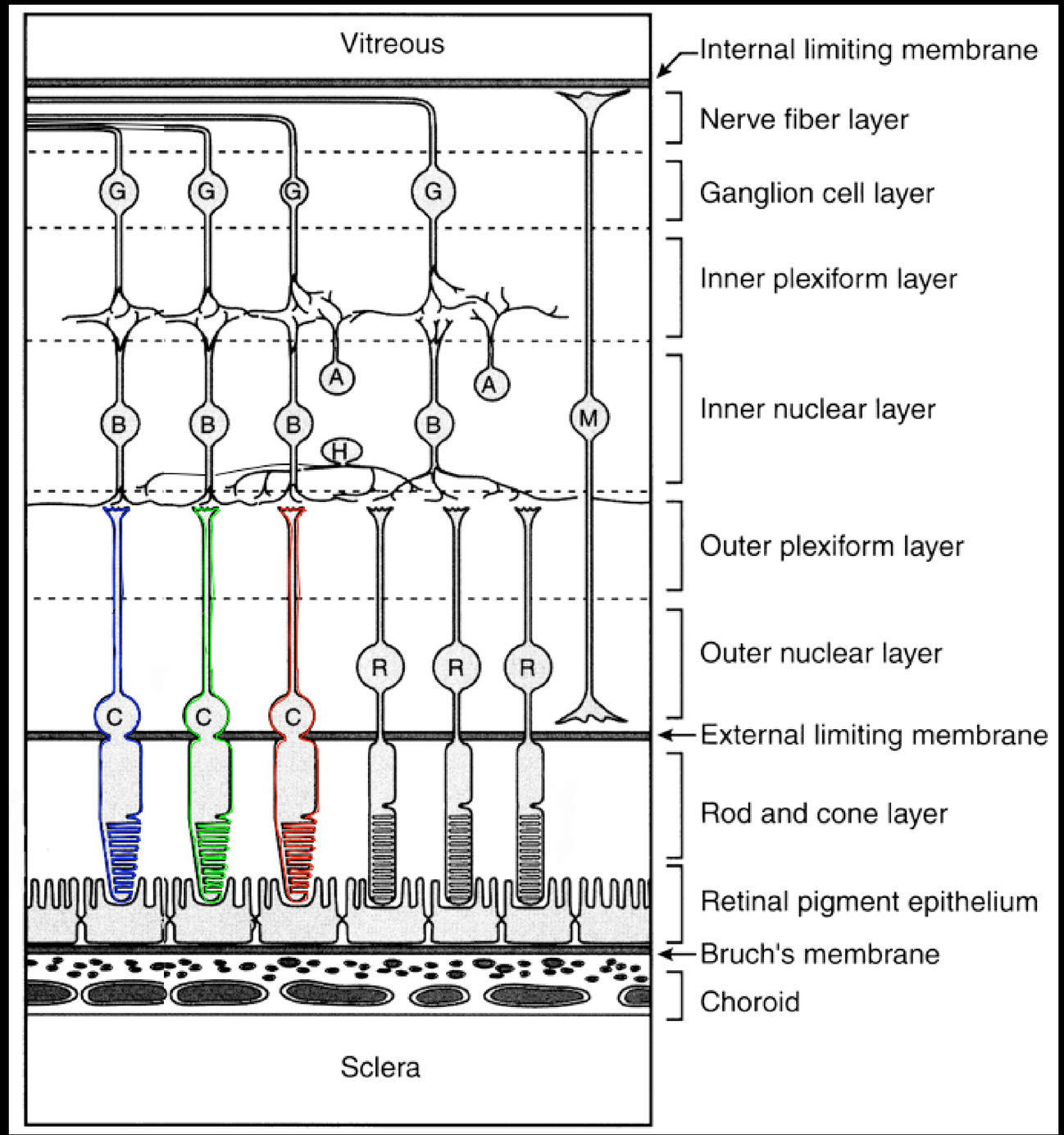
Bipolar cells

Horizontal cells

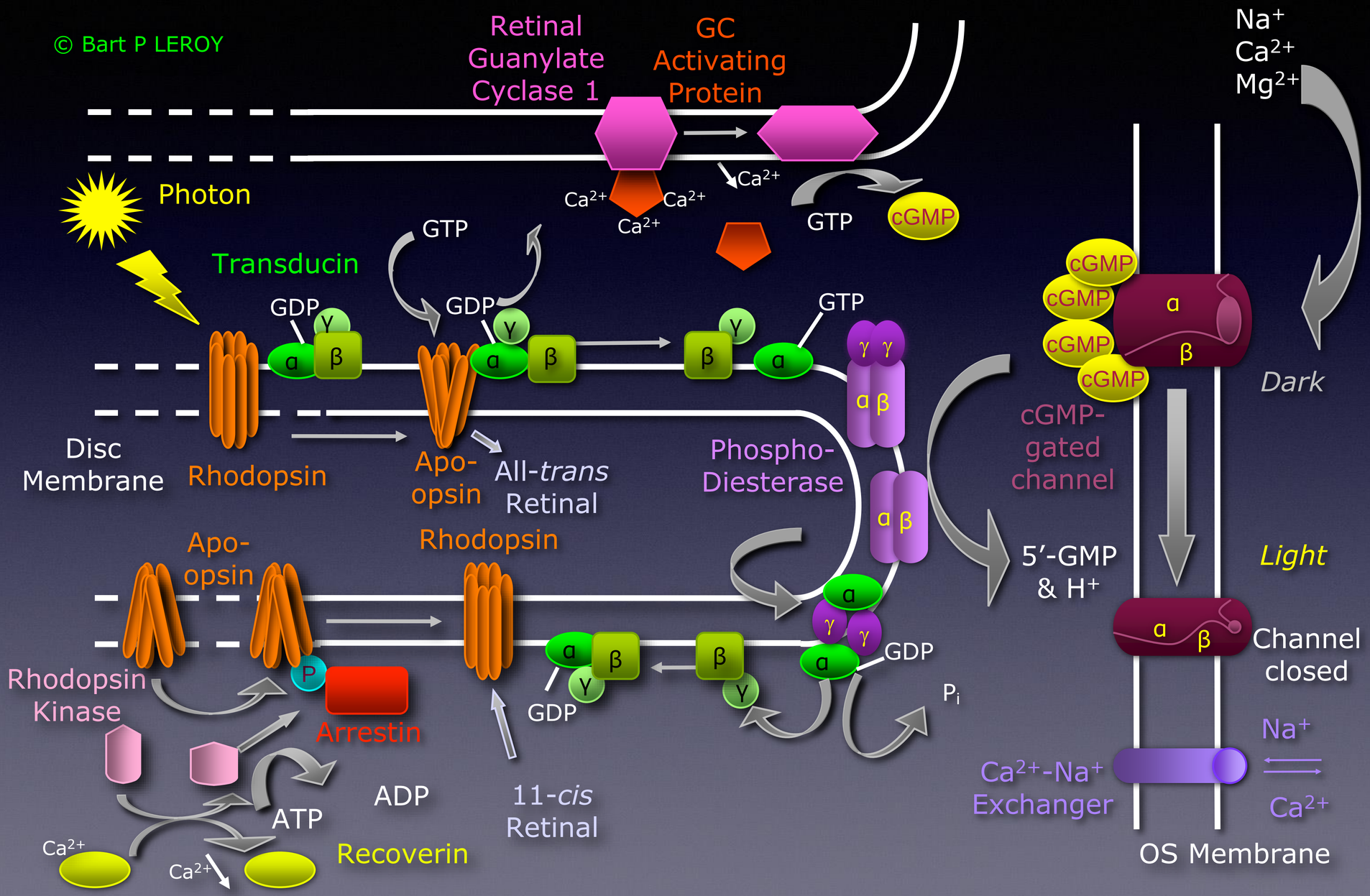
Photoreceptor cells (cones & rods)

Retinal pigment epithelium

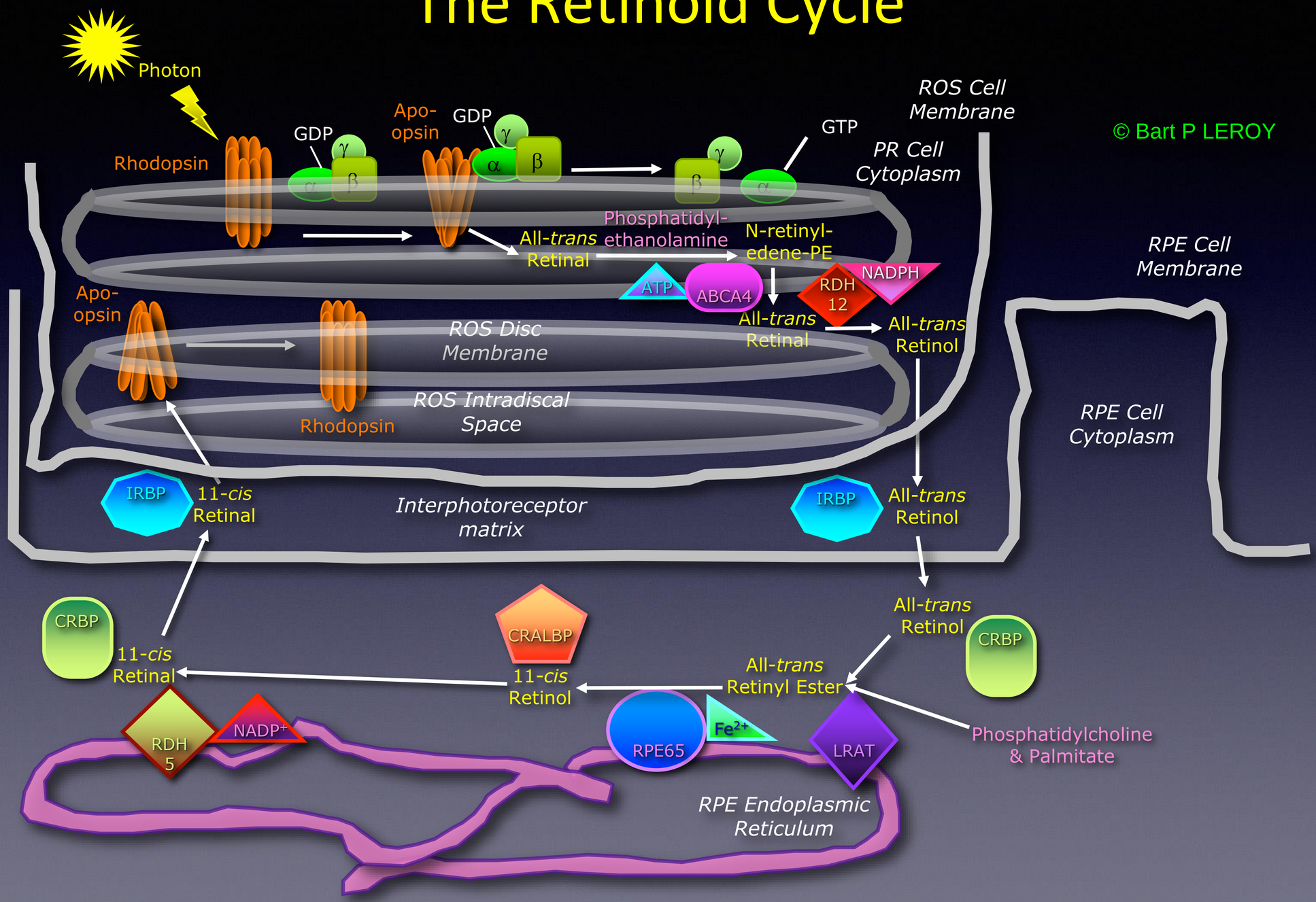
Adapted from ***The Neurology of Vision*** by JD Trobe

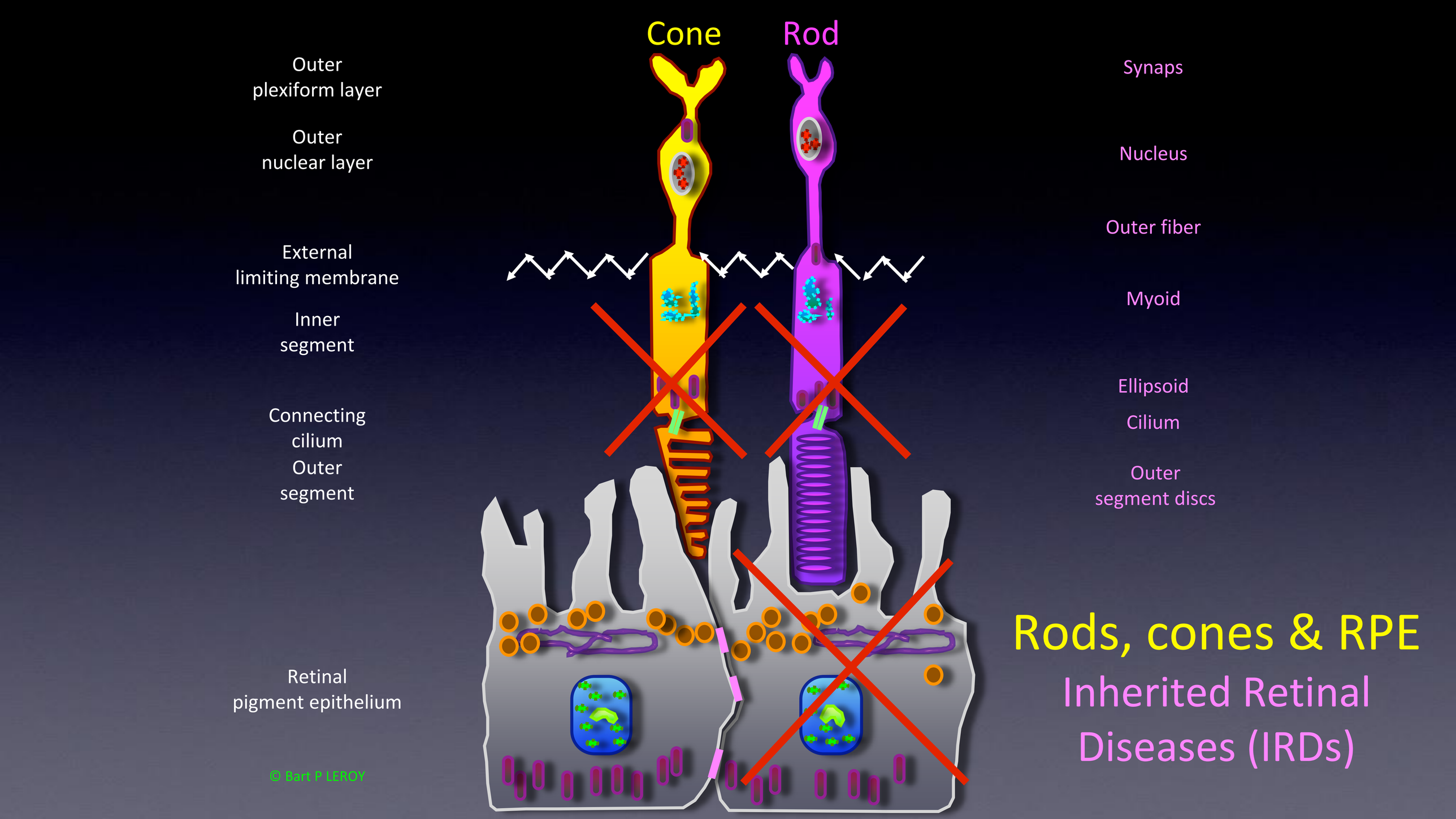


The Phototransduction Cascade



The Retinoid Cycle





Rods, cones & RPE

Inherited Retinal Diseases (IRDs)

The Human Genome, Genotypes & Phenotypes

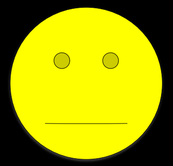
IRD Genetics

Introduction
Basic Genetics

- Humans: 20.338 genes x 2 (= 3.200.000.000 bp (x2))
  - Non-coding genes 22.521
  - Pseudogenes 14.638
  - Gene transcripts 200.310
- Inherited retinal & ON diseases: 317 genes (281 cloned) (https://sph.uth.edu/retnet)

Variants in Genes

Five Classes

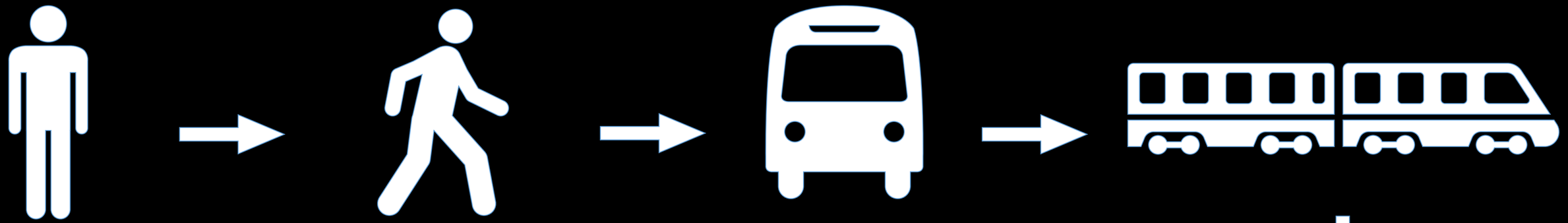
1	2	3	4	5
				
Benign	Likely benign > 90% certainty	Uncertain significance	Likely pathogenic > 95% certainty	Pathogenic
Polymorphism	?	???	?	Mutation

Adapted from Dr Caroline VAN CAUWENBERGH

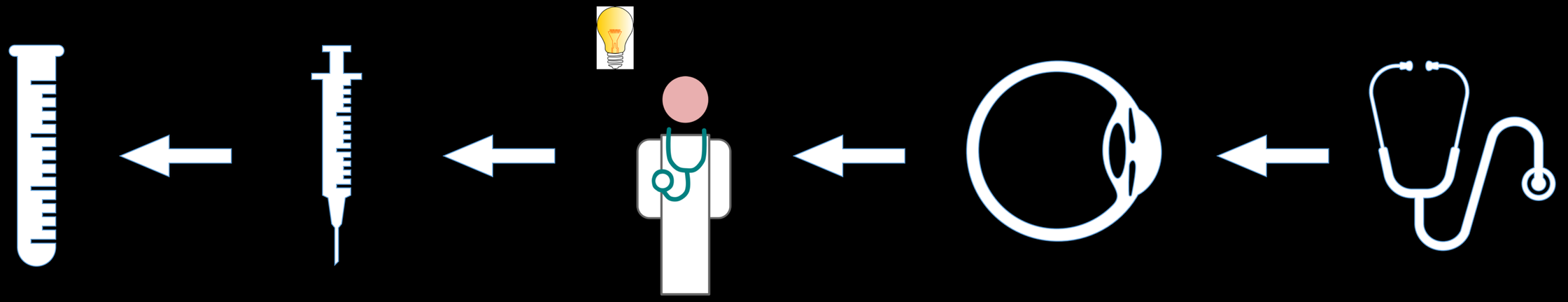
Introduction
Inherited Blindness

- World population: 7.9 x 10^9 individuals
- Blind people: 43.4 x 10^6 individuals (1/3 w/ genetic basis)
- Inherited Retinal Disorders (IRDs): 5.5 x 10^6 individuals (1/1400 individuals)
- Most due to mutations in genes expressed in photoreceptors and/or RPE

Patient Referral Pathway



Referral to ocular genetics specialist essential



Ocular Genetics Evaluation

Approach

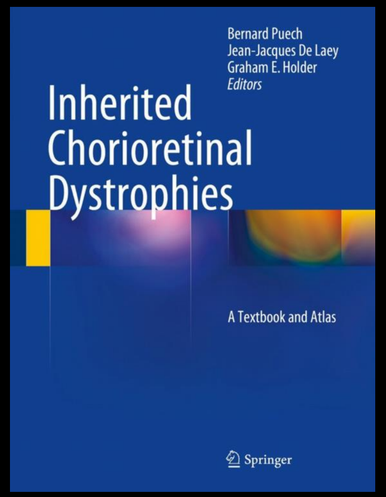
- Ask the right questions in language patient can understand
  - About nature of visual complaints
  - About time of onset of visual symptoms
- Draw a pedigree
- Support clinical Dx w/ specialised imaging, psychophysics & electrophysiology

→ “deep phenotyping”

- Take blood samples & confirm Dx w/ molecular testing “genotyping”

Classification of Inherited Retinal Disease

Towards Precision Medicine



Puech B, De Laey J-J, Holder GE, Editors, Inherited Chorioretinal Dystrophies. Springer Heidelberg New York Dordrecht London, 2014

Phenotypic Classification (examples)

Generalised outer retinal dystrophies
- Rod-cone dystrophies (isolated & syndromic)
- Cone-rod dystrophies (isolated & syndromic)

Stationary retinopathies
- Achromatopsia
- CSNB

Macular dystrophies

Chorioretinal dystrophies
- Choroideraemia
- Gyrate atrophy
- Bietti corneocrystalline chorioretinal dystrophy
- Chorioretinal dystrophy *RPE65*-related

Transretinal dystrophies
- XL retinoschisis

Inner retinal dystrophies or optic neuropathies
- LHON (isolated & syndromic)
- ADOA (isolated & syndromic)

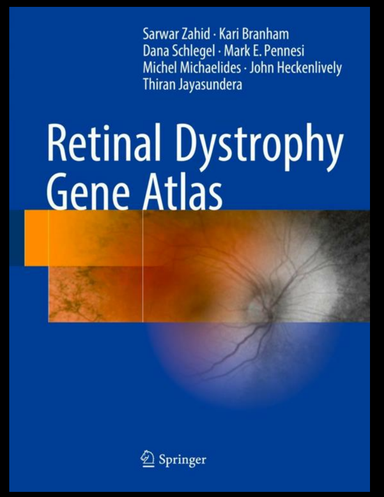
Genotypic Classification (examples)

***ABCA4*-related retinopathy (AR)**
- Maculopathy
- Cone dystrophy
- Cone-rod dystrophy

***RPE65*-related retinopathy**
- AR Leber congenital amaurosis
- AR EORD
- AR Rod-cone dystrophy
- AD Chorioretinal dystrophy (p.Asp477Gly)

***CNGB3*-related retinal disease**
- AR Progressive cone dystrophy
- AR Achromatopsia

***CLN3*-related retinal dystrophies**
- AR Isolated rod-cone dystrophy
- AR Neuronal ceroid lipofuscinosis



Zahid, S *et al.* Retinal Dystrophy Gene Atlas. Springer Heidelberg New York Dordrecht London, 2018

Gene Therapy for IRDs

Strategies, Vectors, Delivery Routes & Trials

Genetic Therapies for Inherited Non-Ocular Diseases

Current Status

Glybera® (alipogene tiparvovec)

AAV1-LPL for *reverse lipoprotein lipase deficiency* (AR); Conditional approval by EMA 2012, withdrawn 2017
Registered trademark of uniQure

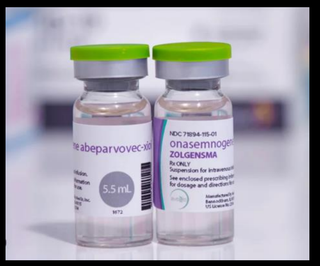
Strimvelis® (GSK269273)

Autologous CD34+ enriched cell fraction containing CD34+ cells transduced w/ retroviral vector that encodes for human ADA cDNA sequence for *severe combined immunodeficiency due to adenosine deaminase deficiency* (AR)
Approval by EMA 2016
Registered trademark of GlaxoSmithKline

SPINRAZA® (nusinersen) injection 12 mg/5 mL

Spinraza® (nusinursen)

Antisense oligonucleotide for *spinal muscular atrophy* (AR)
Approval by FDA 2016 & EMA 2017
Registered trademark of Biogen



Zolgensma® (onasemnogene abeparvovec-xioi)

AAV9-SMN1 for *spinal muscular atrophy* (AR)
Approval by FDA 2019 & Conditional approval by EMA 2020
Registered trademark of AveXis (Novartis)

libmeldy® (atidarsagene autotemcel)

Libmeldy® (atidarsagene autotemcel)

Ex vivo autologous haematopoietic SC gene therapy expressing arylsulfatase 1 for *metachromatic leukodystrophy* (AR)
Approval by EMA 2020
Registered trademark of Orchard Therapeutics

Zynteglo® (betibeglogene autotemcel)

Autologous CD34+ cells encoding ßA-Thr87Gln-globin gene for *adult & pediatric beta-thalassemia* (AR)
Approval by EMA 2019 & FDA 2020
Registered trademark of bluebirdbio

skysona™ (elivaldogene autotemcel) suspension for IV infusion

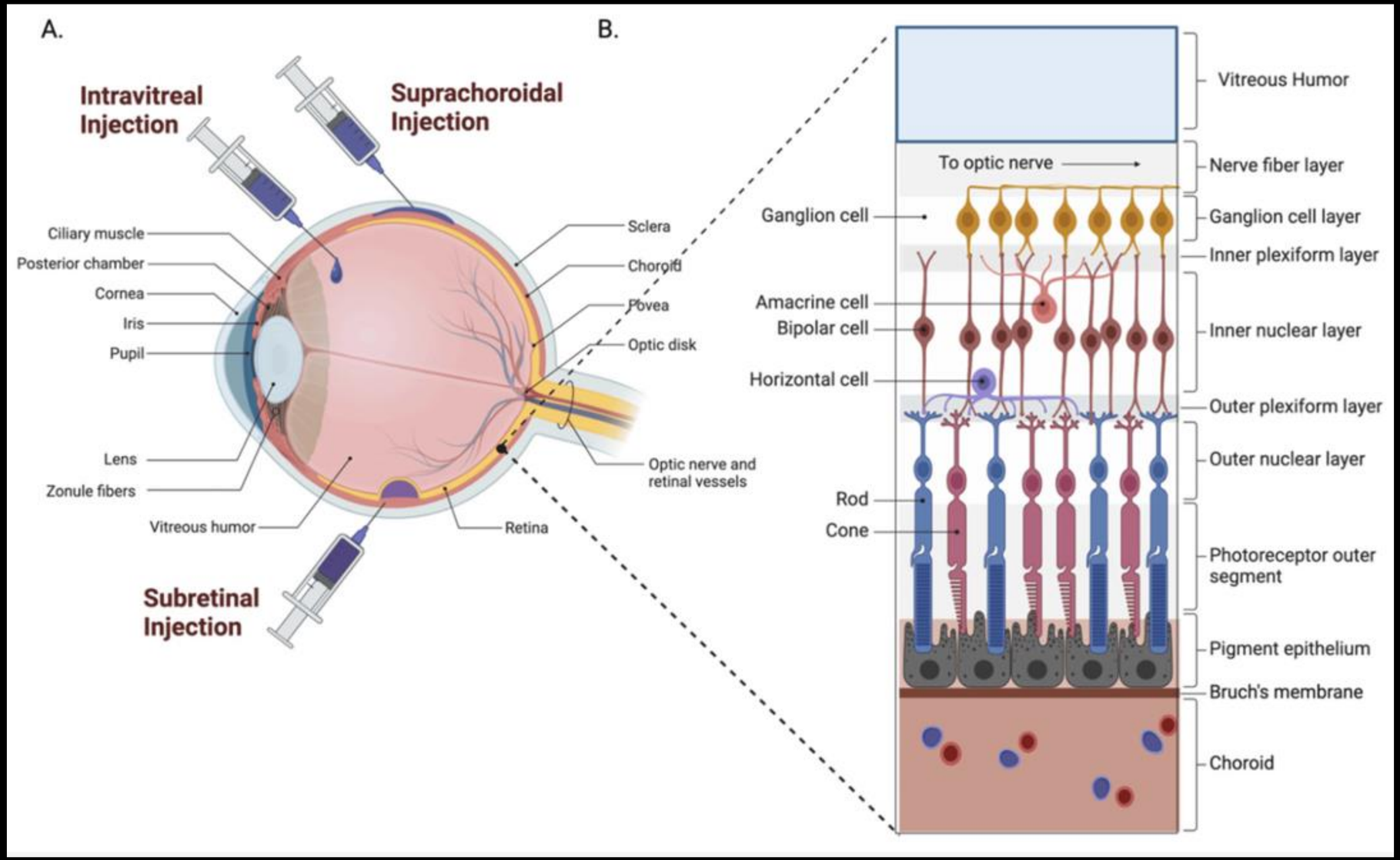
Skysona® (elivaldogene autotemcel)

Gene therapy for *early, active cerebral adrenoleukodystrophy (CALD)* (XL)
Approval by EMA 2019 & FDA 2020
Registered trademark of bluebirdbio

Roctavian® (valoctocogene aroxaparvovec-rvox)

AAV5-SMN1 for *haemophilia A* (XL)
Conditional approval by EMA 2022
Registered trademark of BioMarin

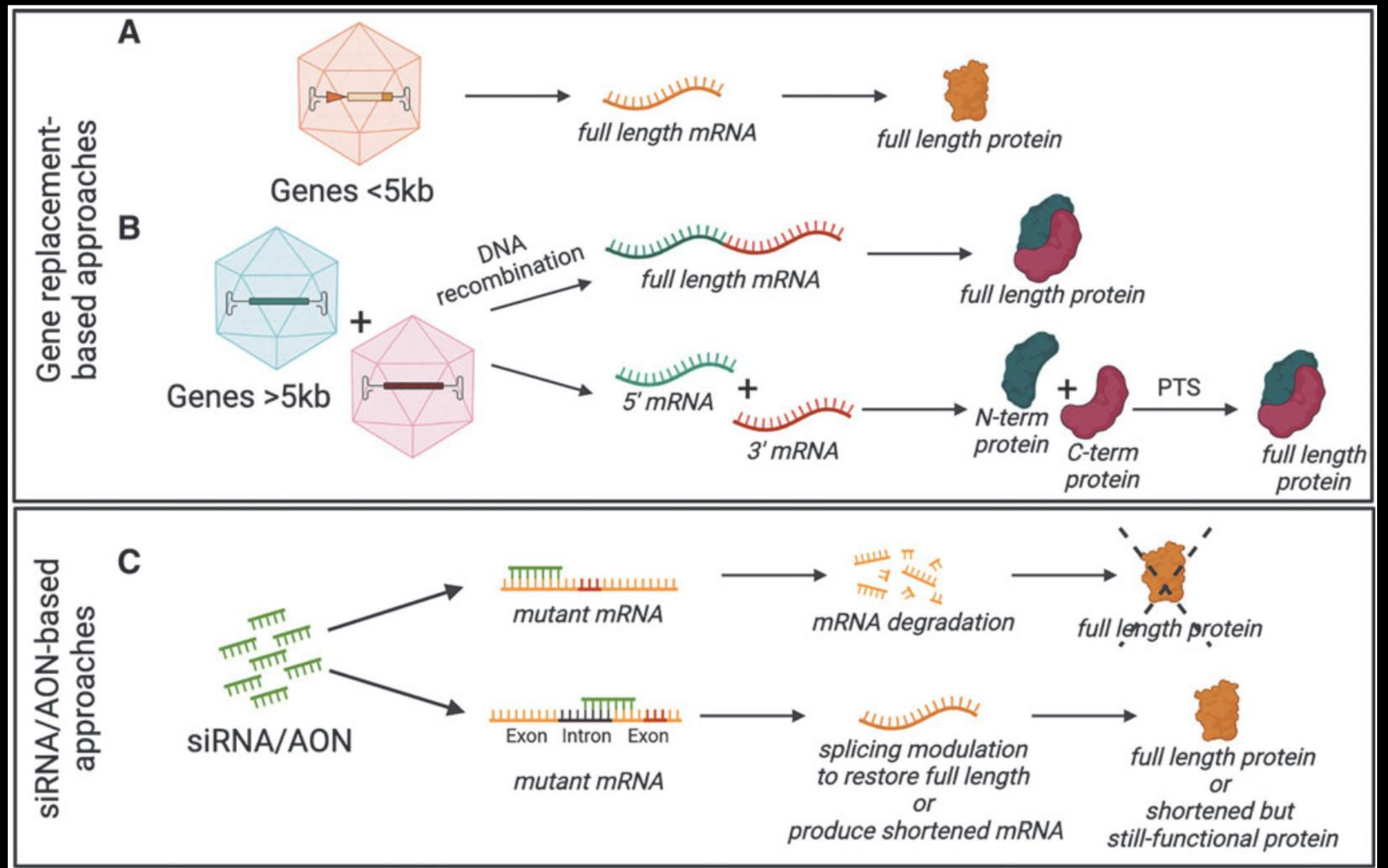
Gene Therapy for IRDs
Administration Routes



From B Tian *et al.*, Pharmaceutics 2022

Genetic Therapies for IRDs

Strategies: Gene Augmentation & AONs

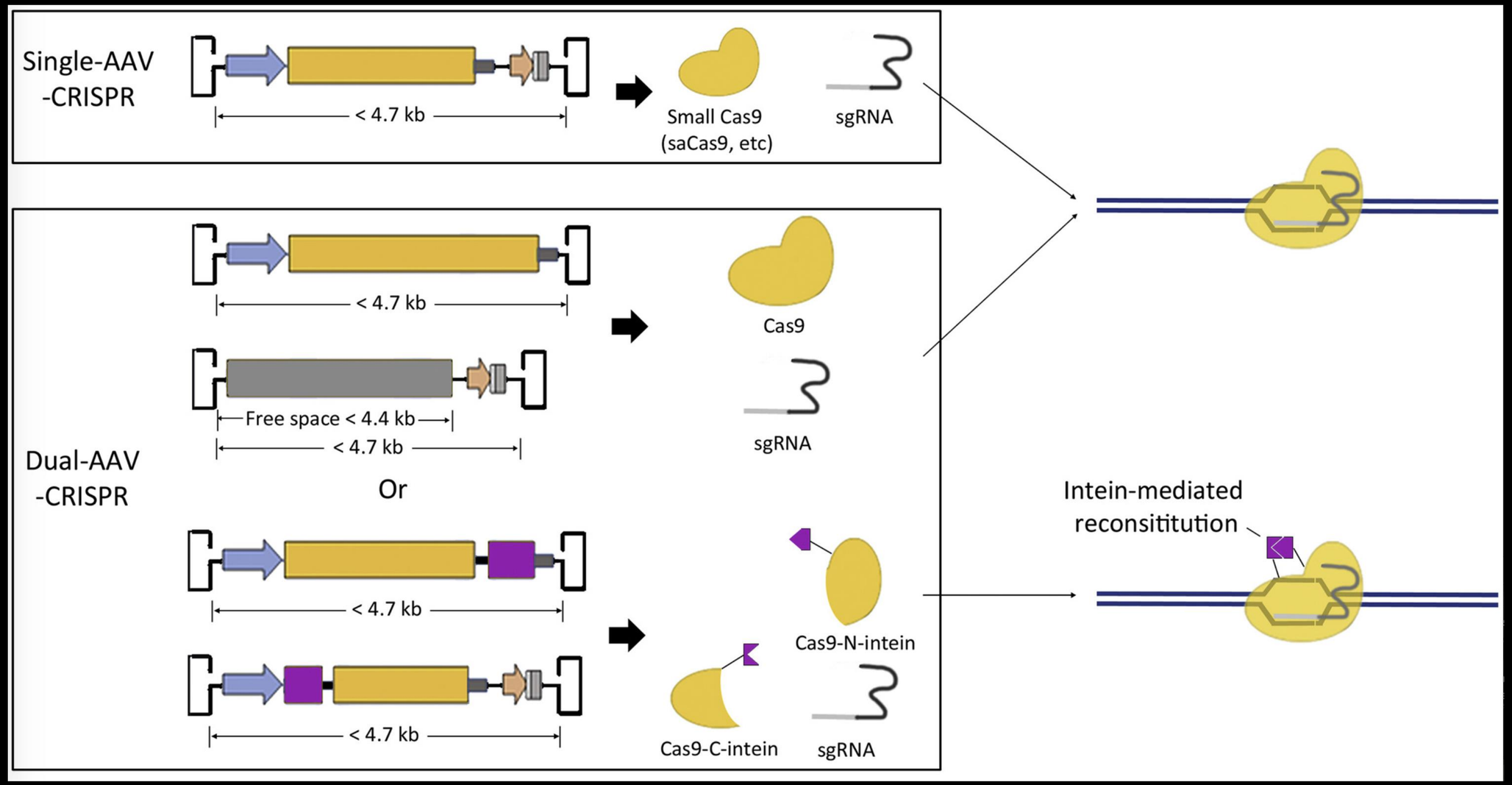


From EJ Simons & I Trapani, Hum Gene Ther 2023

Genetic Therapies for IRDs

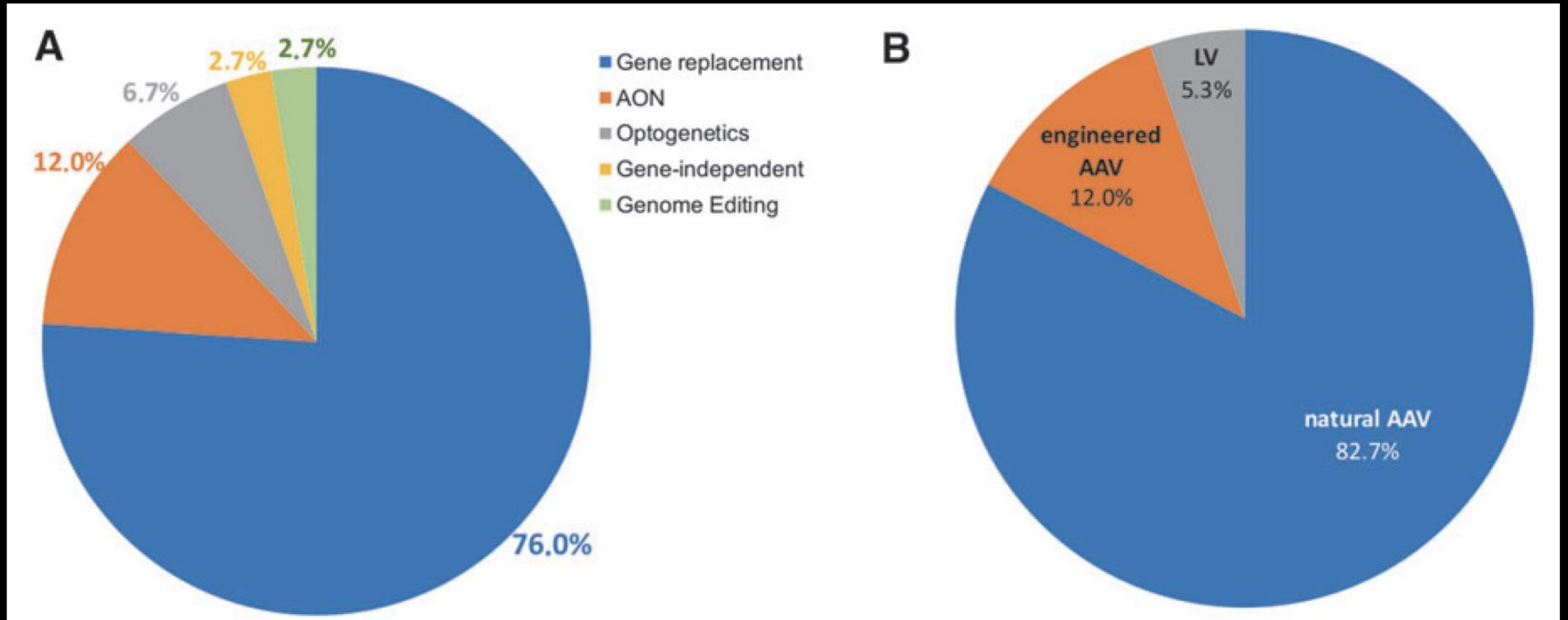
Strategies: CRISPR/Cas 9

From W Yu & Z Wu, Adv Drug Deliv Rev, 2021



Gene Therapy for IRDs
Use of Different Strategies

From EJ Simons & I Trapani,
Hum Gene Ther 2023



Gene therapy approaches **(A)** and viral vectors **(B)** exploited in clinical trials for IRD treatment. IRD, inherited retinal disease.

Ocular Gene Therapy Trials

From B Tian *et al.*, Pharmaceutics 2022

X-linked retinitis pigmentosa	MeiraGT UK II Ltd.	AAV2/5	AAV2/5-RPGR	RPGR coding sequence	One-time subretinal injection	Phase 1/2, 3	NCT03252847, NCT04671433
	NightstaRx Ltd./Biogen	AAV8	BIIB112	RPGR coding sequence	Six-time subretinal injection	Phase 1/2	NCT03116113
	4D Molecular Therapeutics	R100 capsid	4D-125	Codon-optimized *RPGR* gene	One-time intravitreal injection	Phase 1/2	NCT04517149
	Applied Genetic Technologies Corp.	AAV2tYF	AGTC-501 (rAAV2tYF-GRK1-hRPGRco)	G Protein-Coupled Receptor Kinase 1 (*GRK1*) and *RPGR* coding sequences	One-time subretinal injection	Phase 1/2, 2/3	NCT03316560, NCT04850118
Retinitis pigmentosa	Coave Therapeutics	AAV2/5	AAV2/5-hPDE6B	*PDE6B* gene	Subretinal injection	Phase 1/2	NCT03328130
	STZ eye trial	-	rAAV.hPDE6A	*PDE6A* gene	One-time subretinal injection	Phase 1/2	NCT04611503
	King Khaled Eye Specialist Hospital	AAV2	rAAV2-VMD2-hMERTK	VMD2-hMERTK gene vector	Subretinal injection	Phase 1	NCT01482195
	Nanoscope Therapeutics Inc.	AAV2	vMCO-1	Multi-Characteristic Opsin 1 gene expression. cassette	One-time intravitreal injection	Phase 1/2	NCT04919473
	GenSight Biologics	AAV2	GS030 (rAAV2.7m8-CAG-ChrimsonR-tdTomato)-Medical Device	Channel rhodopsin ChrimsonR-tdTomato gene with Visual Interface Stimulating Glasses	One-time intravitreal injection	Phase 1/2	NCT03326336
	Ocugen	AAV5	OCU400	Nuclear Hormone Receptor (NR2E3) gene	One-time subretinal injection	Phase 1/2	NCT03326336
	Nanoscope Therapeutics Inc.	AAV2	vMCO-101	Multi-characteristic opsin (MCO) gene expression cassette	One-time intravitreal injection	Phase 2	NCT04945772
	University of Oxford	AAV2	rAAV2.REP1	Rab-escort Protein 1 (REP1) coding sequence	Subretinal injection	Phase 1/2	NCT01461213
	Spark Therapeutics	AAV2	AAV2-hCHM (human choroideremia gene, same as REP1)	Rab-escort Protein 1 (REP1) coding sequence	Subretinal injection	Phase 1/2	NCT02341807

Ocular Gene Therapy Trials

Adapted from B Tian *et al.*, Pharmaceutics 2022

Choroideremia	Byron Lam	AAV2	AAV2-REP1	Rab-escort Protein 1 (REP1) coding sequence	Subretinal injection	Phase 2	NCT02553135
	4D Molecular Therapeutics	R100	4D-R100	Codon-optimized Rab-escort Protein 1 (REP1) coding sequence	One-time intravitreal injection	Phase 1	NCT04483440
	STZ eye trial	AAV2	rAAV2.REP1	Rab-escort Protein 1 (REP1) coding sequence	One-time subretinal injection	Phase 2	NCT02671539
	Ian M. MacDonald	AAV2	rAAV2.REP1	Rab-escort Protein 1 (REP1) coding sequence	One-time subretinal injection	Phase 1/2	NCT02077361
Leber congenital amaurosis	Spark Therapeutics	AAV2	LUXTURNA, voretigene neparvovec-rzyl (AAV2-hRPE65v2)	RPE65 gene	One-time subretinal injection	Phase 1, 1/2, 5-year follow-up, 3, 15-year follow-up	NCT00516477, NCT01208389, NCT03597399, NCT00999609, NCT03602820
	MeiraGTx UK II Ltd.	AAV2	AAV2/5-OPTIRPE65	RPE65 gene	One-time subretinal injection	Phase 1/2, long-term follow-up	NCT02781480, NCT02946879
	University College, London	AAV2	tgAAG76 (rAAV 2/2.hRPE65p.hRPE65)	RPE65 gene	One-time subretinal injection	Phase 1/2	NCT00643747
	Applied Genetic Technologies Corp	AAV2	rAAV2-CB-hRPE65	RPE65 gene	One-time subretinal injection	Phase 1/2	NCT00749957
Autosomal recessive Leber congenital amaurosis	Atsena Therapeutics Inc.	AAV5	SAR-439483	GUCY2D gene	One-time subretinal injection	Phase 1/2	NCT03920007
Leber Hereditary Optic Neuropathy	GenSight Biologics	AAV2	GS010 (rAAV2/2-ND4)	ND4 gene (mitochondrial)	One-time intravitreal injection	Phase 3	NCT03293524
	Byron Lam	Self-complementary AAV2	scAAV2-P1ND4v2	ND4 gene (mitochondrial)	One-time intravitreal injection	Phase 1	NCT02161380
	MeiraGTx UK II Ltd.	AAV2/8	AAV2/8-hG1.7p.coCNGA3	CNGA3 gene	One-time subretinal injection	Phase 1/2	NCT03758404
Achromatopsia	Applied Genetic Technologies Corp	AAV2	AGTC-402 (rAAV2tYF-PR1.7-hCNGA3)	CNGA3 gene	One-time subretinal injection	Phase 1/2	NCT02935517
	Applied Genetic Technologies Corp	AAV2	AGTC-402 (rAAV2tYF-PR1.7-hCNGA3)	CNGA3 gene	One-time subretinal injection	Phase 1/2	NCT02935517
	Applied Genetic Technologies Corp	AAV2	AGTC-401 (rAAV2tYF-PR1.7-hCNGB3)	CNGB3 gene	One-time subretinal injection	Phase 1/2	NCT02599922
Variant Late-Infantile Neuronal Ceroid Lipofuscinosis	Amicus Therapeutics	Self-complementary AAV9	scAAV9.CB.CLN6	CLN6 Gene	One-time intrathecal injection	Phase 1/2	NCT02725580
X-linked Juvenile Retinoschisis	National Eye Institute (NEI)	AAV8	AAV8-scRS/IRBPhRS	RS1 gene	One-time intravitreal injection	Phase 1/2	NCT02317887
	Genetic Technologies Corp	AAV2	rAAV2tYF-CB-hRS1	RS1 gene	One-time intravitreal injection	Phase 1/2	NCT02416622

Genes & Inherited Retinal Diseases (IRDs)

Leber Congenital Amaurosis (LCA) as a Model

Leber Congenital Amaurosis

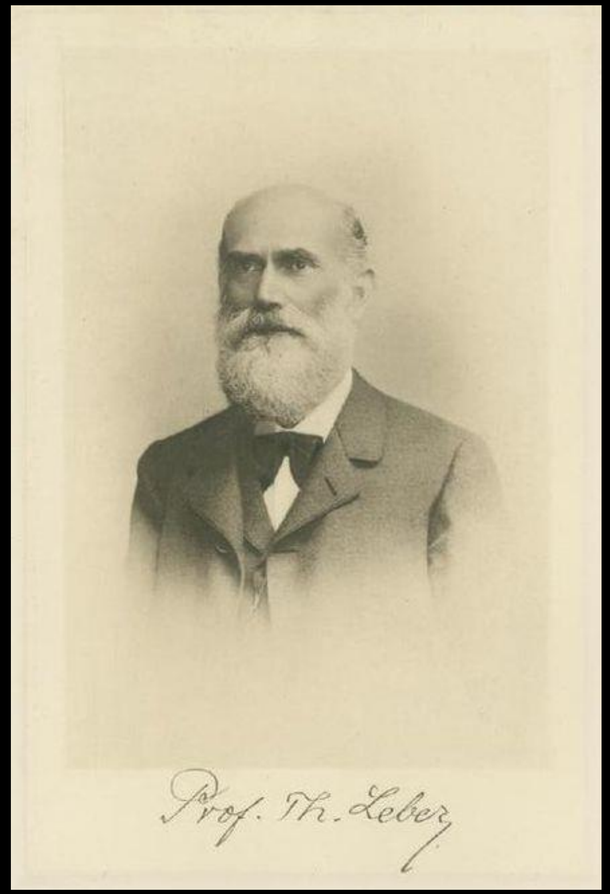
Symptoms & Signs

- No or little sensitivity for visual stimuli from birth
- Variable aspect of retina
- ERG abolished or profoundly abnormal
- Autosomal recessive inheritance

Leber T: Uber retinitis pigmentosa und angeborene amaurose

Graefes Arch Klin Exp Ophthalmol, **15**, 13-20, 1869

LCA is responsible for 18% of legal blindness in children worldwide



Theodor Karl Gustav von Leber
19 Feb 1840 - 17 Apr 1917

Leber Congenital Amaurosis

Symptoms & Signs

- No or little sensitivity for visual stimuli from birth
- Variable aspect of retina
- ERG abolished or profoundly abnormal
- Autosomal recessive inheritance

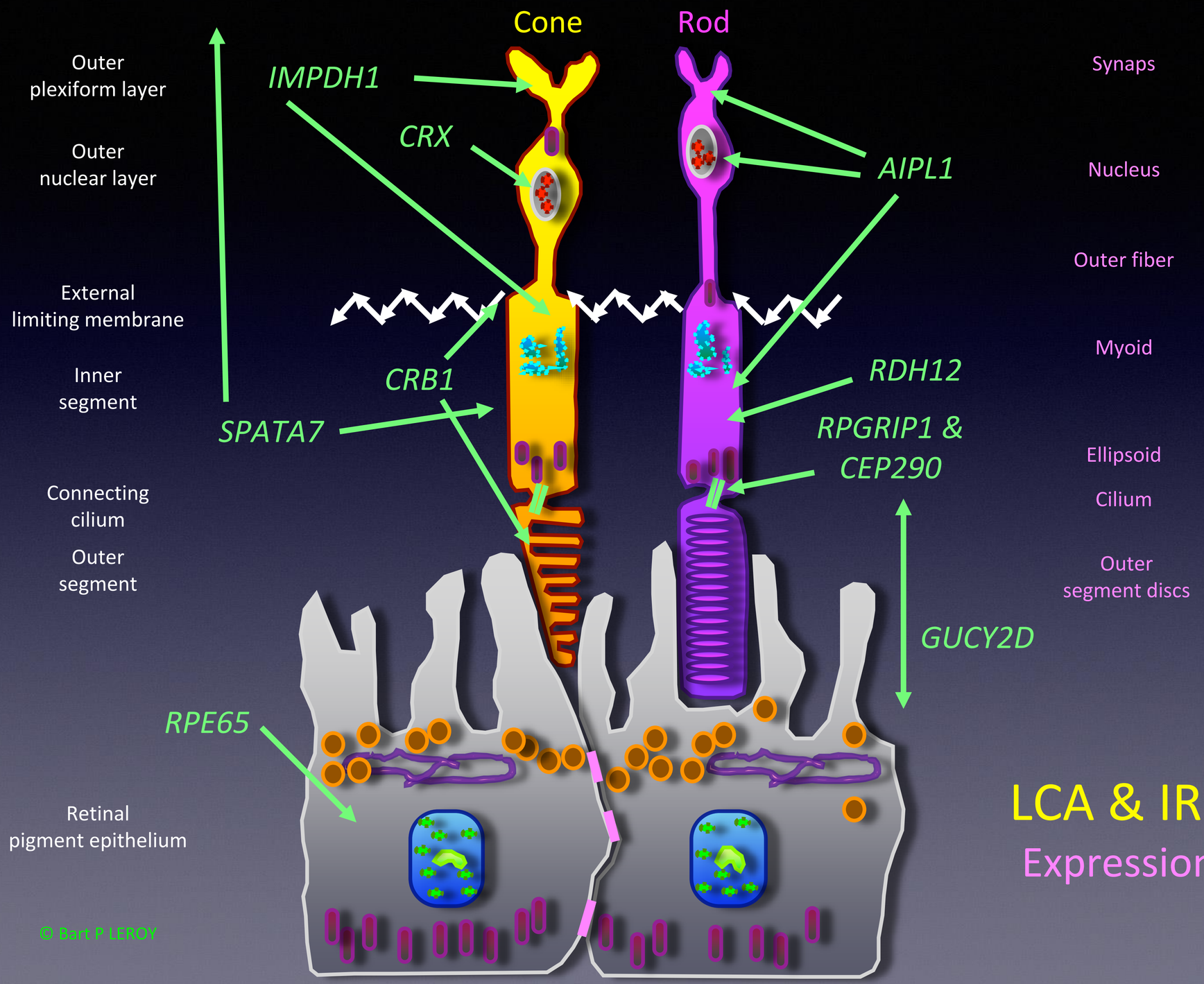
- Hyperopia
- Sluggish pupillary responses
- Oculodigital sign
- Keratoconus
- Occasional photophobia

LCA & EORD Genotypes

- 24 LCA genes:
  - *GUCY2D* on 17p13.1
  - *RPE65* on 1p31
  - *CRX* on 19q13.3
  - *AIPL1* on 17p13.1
  - *CRB1* on 1q31-q32.1
  - *RPGRIP1* on 14q11.2
  - *MERTK* on 2q14.1
  - *RDH12* on 14q24.1
  - *IMPDH1* on 7q31.3-32
  - *TULP1* on 6p21
  - *CEP290* on 12q21-q22
  - *LCA5* on 6q11-q16
  - *SPATA7* on 14q24
  - *OTX2* on 14q21-22
  - *IQCB1* on 3q21.1
  - *PDE6G* on 17q25
  - *KCNJ13* on 2q37.1
  - *RD3* on 1q32
  - *NMNAT1* on 1p36
  - *DTHD1* on 4p14
  - *CAPB4* on Xp11.4
  - *GDF6* on 8q22.1
  - *IFT140* on 16p13.3
  - *PRPH2* on 6p21.1

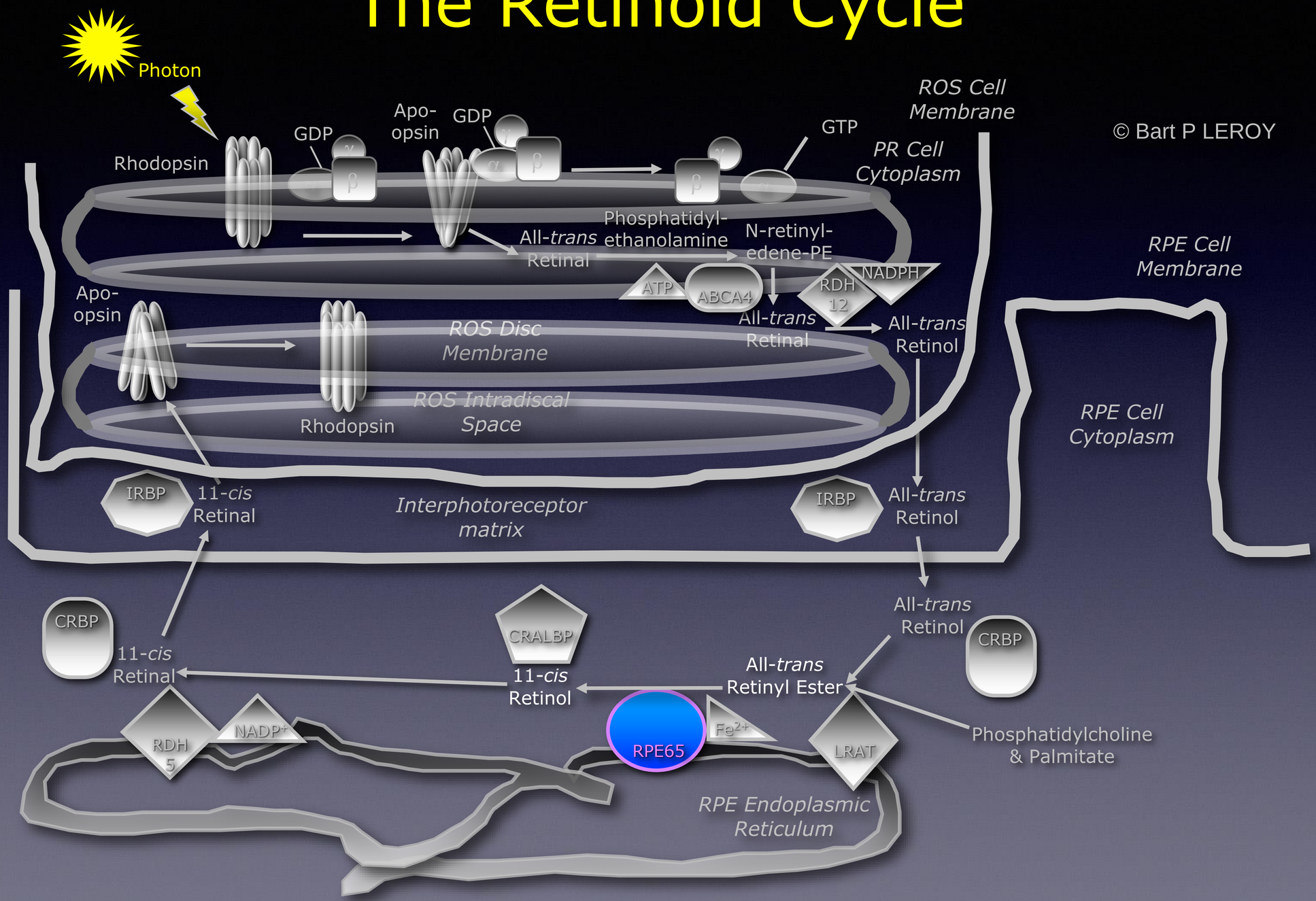
- 6 early-onset RP genes:
  - *RDH12* on 14q23.3
  - *LRAT* on 4q31.2
  - *MERTK* on 2q14.1
  - *TULP1* on 6p21.3
  - *SPATA7* on 14q24
  - *ADAMTS18* on 16q23.1

70% of patients



LCA & IRD Genes
Expression Patterns

The Retinoid Cycle



RPE65-Related IRD
Timeline of Discoveries

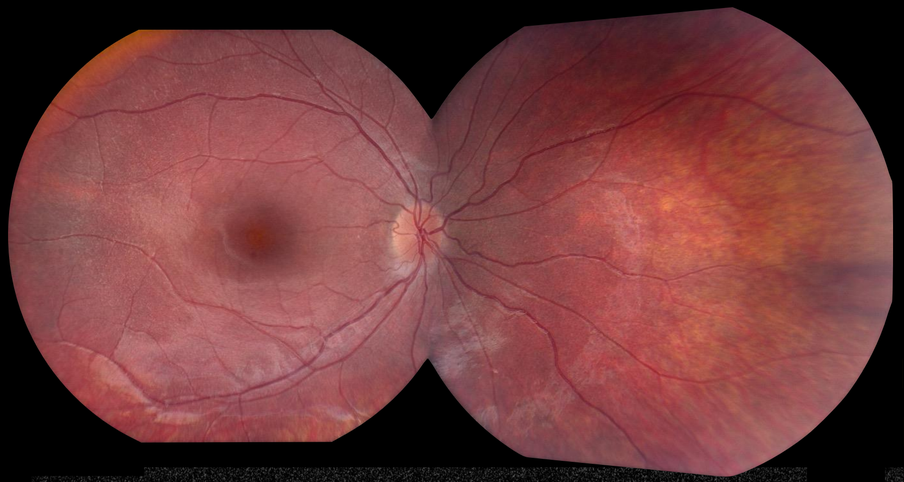
Prof Christian HAMEL
1955-2017

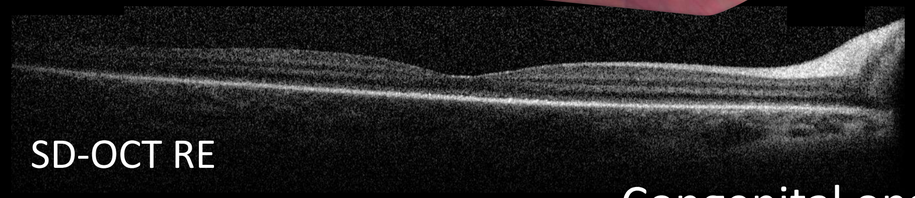
- Discovery of *RPE65* gene: Hamel CP, Jenkins NA, Gilbert DJ, Copeland NG, Redmond, TM: The gene for the retinal pigment epithelium-specific protein RPE65 is localized to human 1p31 and mouse 3, *Genomics*, 20, 509-512, 1994
- Mutations in *RPE65* cause retinal disease:
  - Marlhens F, Bareil C, Griffoin JM, Zrenner E, Amalric P, Eliaou C, Liu SY, Harris E, Redmond TM, Arnaud B, Claustres M, Hamel CP, *Nat Genet*, 17, 139-141, 1997
  - Gu SM, Thompson DA, Srikumari CR, Lorenz B, Finckh U, Nicoletti A, Murthy KR, Rathmann M, Kumaramanickavel G, Denton MJ, Gal A, *Nat Genet*, 17, 194-197, 1997

RPE65-related Retinal Dystrophy

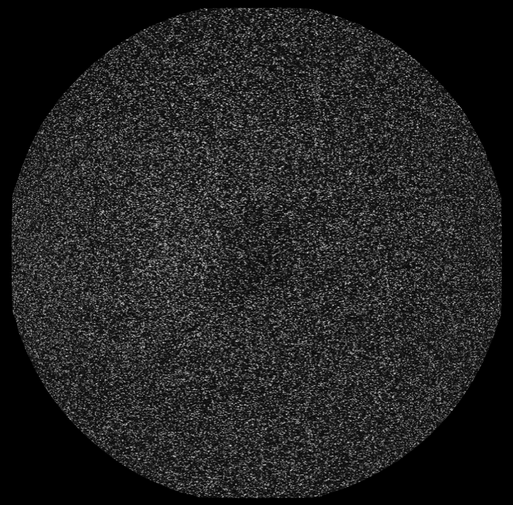
Phenotype

F, 4 4/12 yrs
EORD





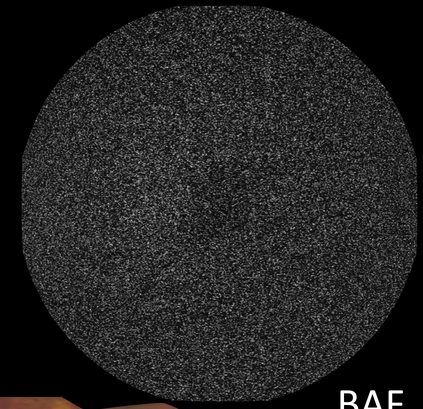
SD-OCT RE



BAF RE

Early Stage
Phenotype

F, 29 yrs
EORD



BAF
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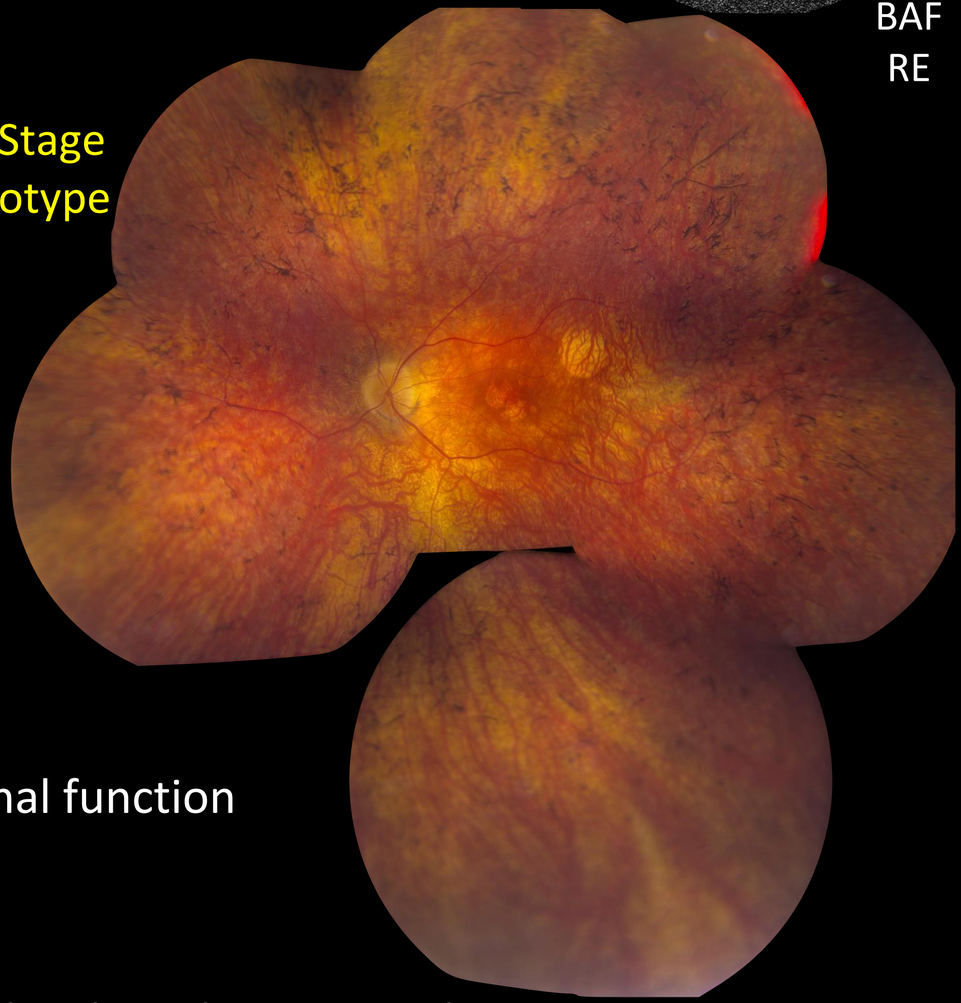
Late Stage
Phenotype



- Congenital onset of night blindness
- Nystagmus often
- Initially retina looks fairly normal
- Many different initial diagnoses
- Later phenotype identical to that of classic RP
- Vascular attenuation suggests early loss of retinal function
- Absence of blue light autofluorescence typical
- Sometimes picked up late w/ Dx of RP
- Progression towards complete blindness; early treatment paramount

RPE65-Related IRD
Unique

- *RPE65* expressed in RPE: retinal pigment epithelium-specific protein 65kDa
- Disproportionately normal outer retinal structure given degree of visual loss
- Window of opportunity to treat

AF Wright, Editorial, NEJM, 372, 1954-1955, 2015

Gene Therapy for *RPE65*-IRD

From Animal Models to Approved Therapy in Humans

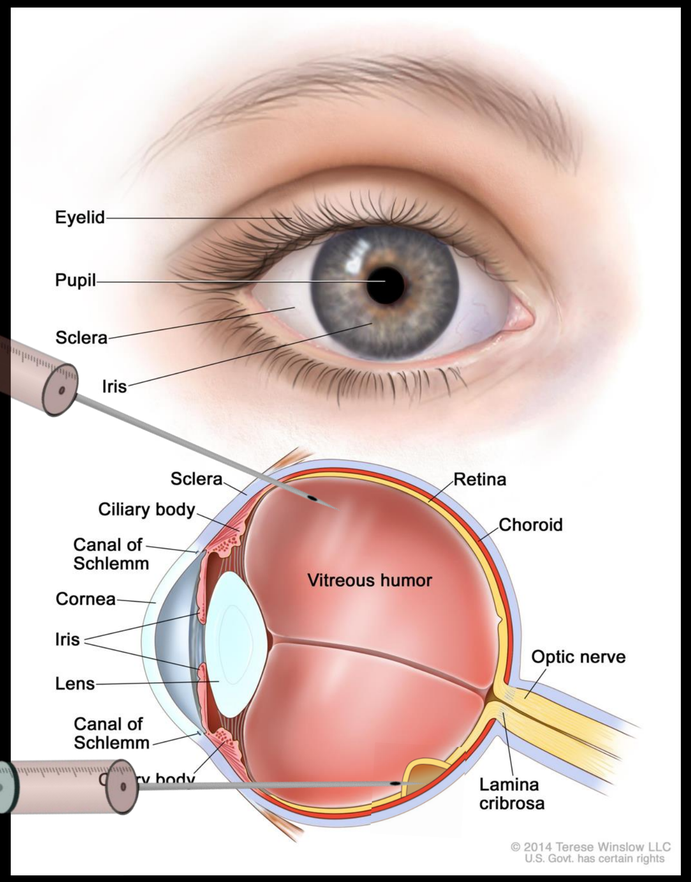
Gene & Genetic Rx for IRDs

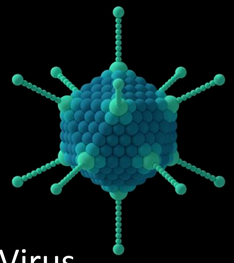
Eye = Ideal Treatment Target

- Accessible for injection
- Allows real-life evaluation
- Immune priviliged

Genetic Rx
Sepofarsen (17-mer AON) directed against *CEP290* pre-mRNA

Intravitreal Injection



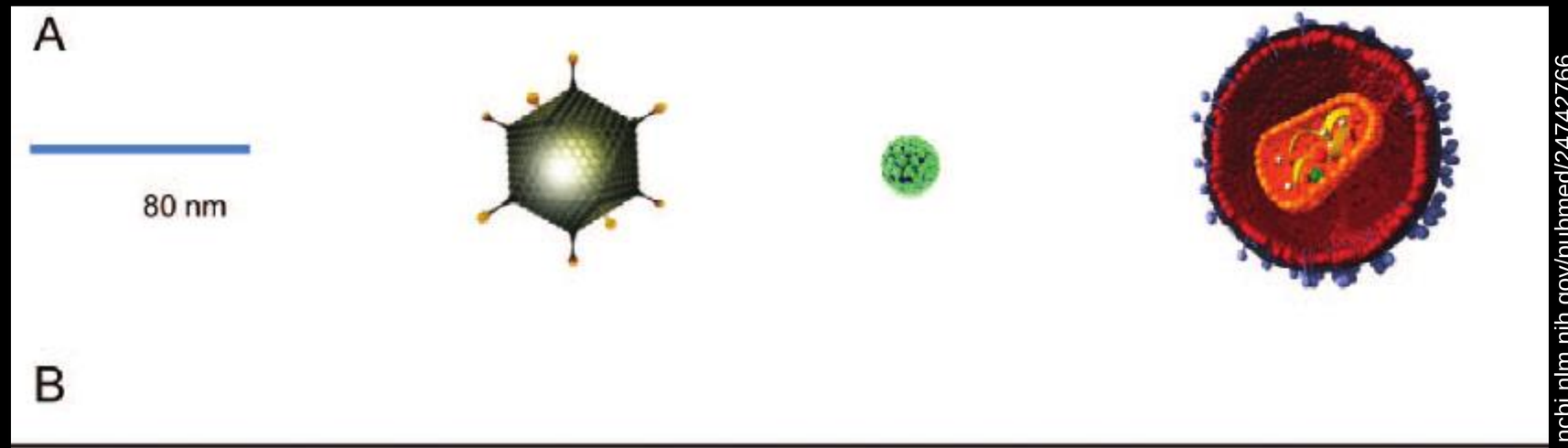


Gene Rx
Adeno-Associated Virus
AAV2-CßA-*RPE65*

Subretinal Injection

Gene Supplementation

Commonly Used Viral Vectors



https://www.ncbi.nlm.nih.gov/pubmed/24742766

Courtesy of
Shannon L Boye, PhD
UFl at Gainesville, FL, USA

	Adenovirus	AAV	Lentivirus
Viral Genome	dsDNA	ss or ds DNA	RNA
Cloning capacity	7.9kb	<5.0 kb	8.0 kb

PROS	Large carrying capacity	Non pathogenic Relatively non immunogenic Genome remains episomal Persistent transgene expression Infects dividing and non dividing cells Serotype variability	Large carrying capacity
CONS	immunogenic	Small carrying capacity	Genome integration Inefficient transduction of non dividing cells

Gene Rx w/ Voretigene Neparvovec

Development = Hacking Path through Jungle with Machete

On the “Path”
to Luxturna Approval

Courtesy of David Mann, Back to the Machete, Aug 3, 2012

Gene Therapy for *RPE65*-related LCA Effective in Briard Dogs

Courtesy of
Jean Bennett, MD, PhD

Briard dog treated w/ subretinal rAAV.*RPE65*

GM Acland *et al*, Nat Genet, 28, 92-95, 2001
GM Acland *et al*, Mol Ther, 16, 458-465, 2005

LCA
Treatment in *RPE65*-related LCA

- Gene Rx for *RPE65*-related LCA successful in dogs (2001)
  - J Bennett & co-workers, Philadelphia, PA, USA

- Gene Rx for *RPE65*-related LCA safe & successful in humans (2008)
  - R Ali & co-workers, London, UK
  - J Bennett & co-workers, Philadelphia, PA, USA
  - WW Hauswirth & co-workers, Philadelphia, PA & Gainesville, FL, USA

LCA
Gene Rx Current Trials

- 8 clinical trials (5 USA, 1 UK, 1 Israel, 1 France) started between 2007 and 2009
- Total of 98 patients
- No vector-related issues
- Trials differed in: subject ages, vector dose, volume (0.15-1.0 ml), promoter, retinal area targeted, outcome measures, etc.

LCA
Gene Rx Trial @ CHOP

- Leber congenital amaurosis (*RPE65*-related)
- *Children's Hospital of Philadelphia (CHOP), Philadelphia, PA, USA & Naples, Italy & Ghent, Belgium* (NCT00516477 & NCT01208389)

Phase 1 & Phase 1 Follow-On; 12 patients; age > 8 yrs; rAAV2-CBA-hRPE65

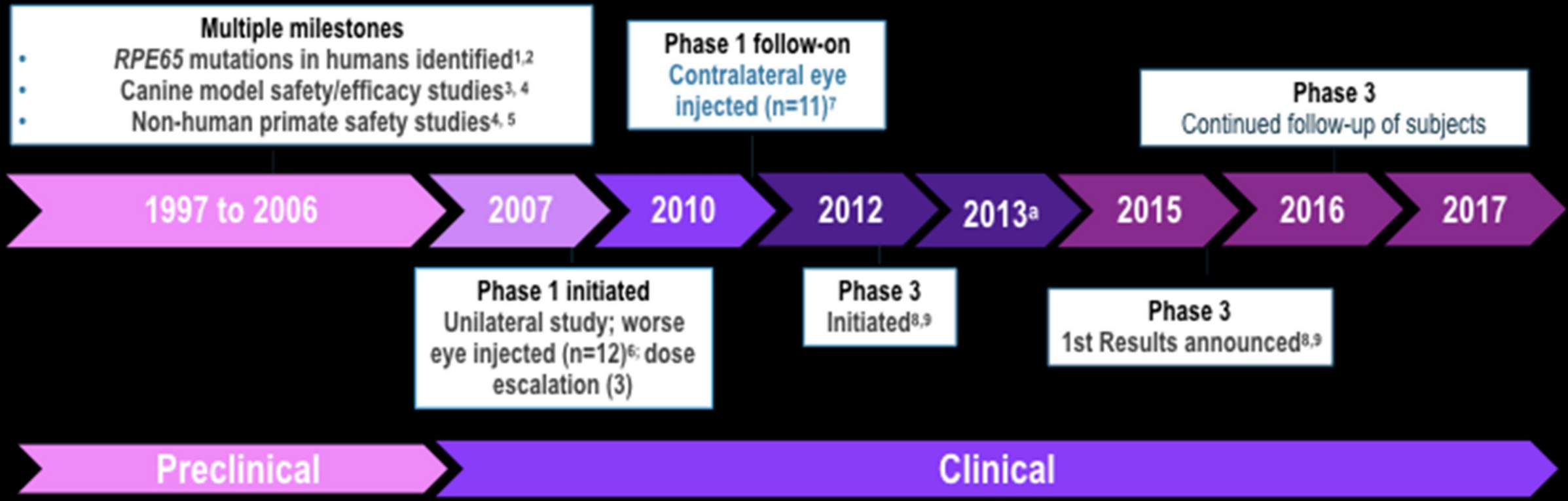
Age-dependent effects of *RPE65* gene therapy for Leber's congenital amaurosis: a phase 1 dose-escalation trial

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 Zelenaia, 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; 374: 1597–605

Gene Rx w/ Voretigene Neparvovec

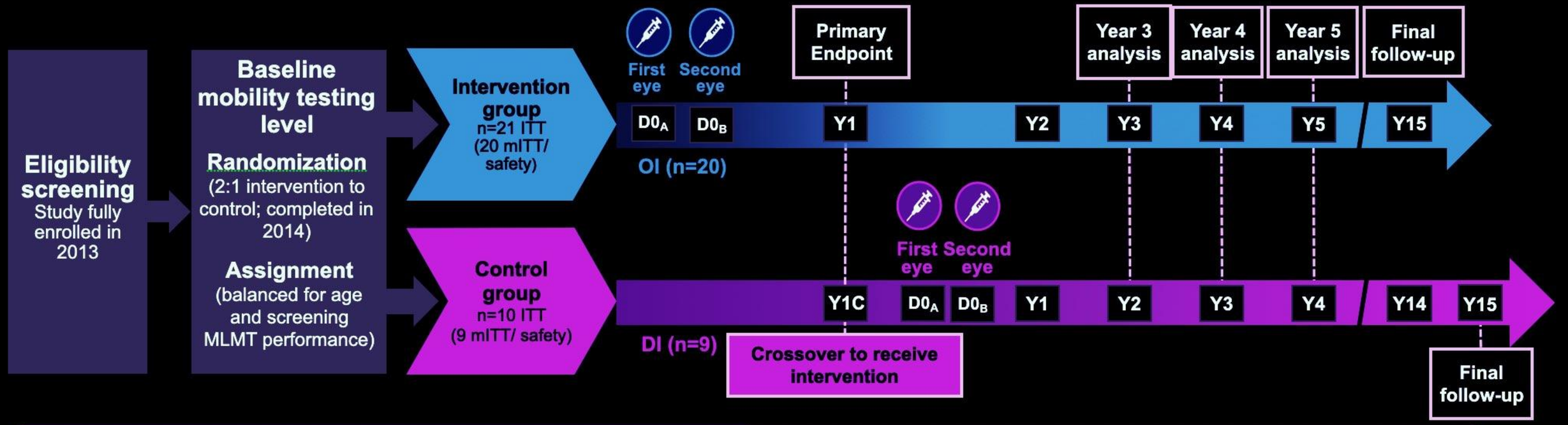
Development History



[a]Spark Therapeutics begins operations

1. Marlhens et al. *Nat Genet*. 1997;17:139-141. **2.** Gu et al. *Nat Genet*. 1997;17:194-197. **3.** Narfström et al. *Invest Ophthalmol Vis Sci*. 2003;44:1663-1672. **4.** Data on File. Spark Therapeutics. **5.** Jacobson et al. *Human Gene Ther*. 2006;17:845-858. **6.** Maguire et al. *Lancet*. 2009;374:1597-1605. **7.** Bennett et al. *Lancet*. 2016 **8.** Russell et al. Abstract presented at: Retina Society 48th Annual Scientific Meeting; October 7-11, 2015; Paris, France. **9.** Maguire et al. Abstract presented at: American Academy of Ophthalmology Meeting 2015; November 14-17, 2015; Las Vegas, NV.

Phase III Trial Design: A Multicenter, Open-label, Randomized, Controlled Crossover Study



Key inclusion criteria:
- Age ≥3 years
- Confirmed *RPE65* mutations
- Sufficient viable retinal cells

Dosing regimen:

1.5×10^{11} vg/eye in 0.3 mL
Eyes treated separately 6–18 days apart

Endpoints:
- Primary: MLMT performance (bilateral)
- Secondary: FST testing, MLMT (assigned first eye), and VA
- Exploratory: Goldmann and Humphrey VFs
- Safety: AEs, physical and ophthalmic examinations, and laboratory tests

Randomization was balanced for age and screening MLMT performance. The second eye was treated within 6–18 days of treating the first eye. The study was conducted at 2 sites in the US. The ITT population was defined as all randomized patients. The mITT population excluded any participant removed from the study on the day of randomization and before any intervention.
AE, adverse event; C, control; D, day; DI, delayed intervention; FST, full-field light sensitivity threshold; ITT, intent-to-treat; mITT, modified intent-to-treat; MLMT, Multi-Luminance Mobility Test; n, number of patients; OI, original intervention; RPE, retinal pigment epithelium; US, United States; VA, visual acuity; VF, visual field; vg, vector genome; Y, year
Russell S, et al. *Lancet*. 2017;390:849-860

S Russell, *et al.*:Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in subjects with *RPE65*-mediated inherited retinal dystrophy: a randomised, controlled, open-label phase 3 trial, Lancet, 2017

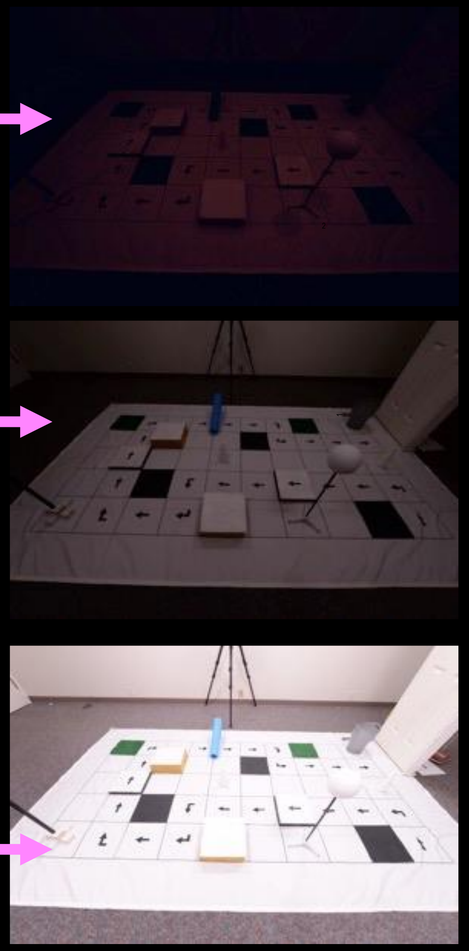
Gene Rx Phase 3
Trial Endpoints

- Primary endpoint: change in bilateral performance in multi-luminance mobility test (MLMT) at 1 year after injection
- MLMT conducted at up to 7 standardized illumination levels
- Secondary endpoints:
  - Full-field light sensitivity threshold (FST)
  - MLMT, assigned first eye
  - Best-corrected visual acuity (BCVA)

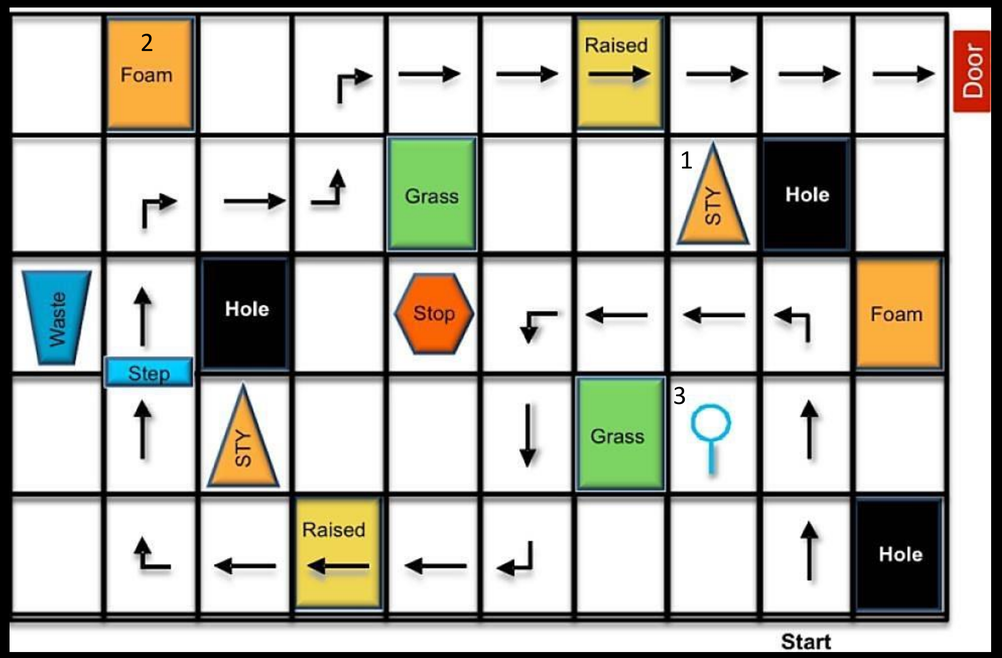
Gene Rx Phase 3

Multi-Luminance Mobility Test

Lux score BE	
6	1 lux Moonless summer night or indoor night-light
5	4 lux Outdoor parking lot at night or Christmas tree lights
4	10 lux An hour after sunset in a city setting or a bus stop at night
3	50 lux Outdoor train station at night or the inside of a stairwell
2	125 lux A half hour before sunrise or the interior of a shopping mall or train or bus at night
1	250 lux Interior of an elevator or office hallway
0	400 lux Office setting



Mobility course layout (1 of 12 standardized templates)

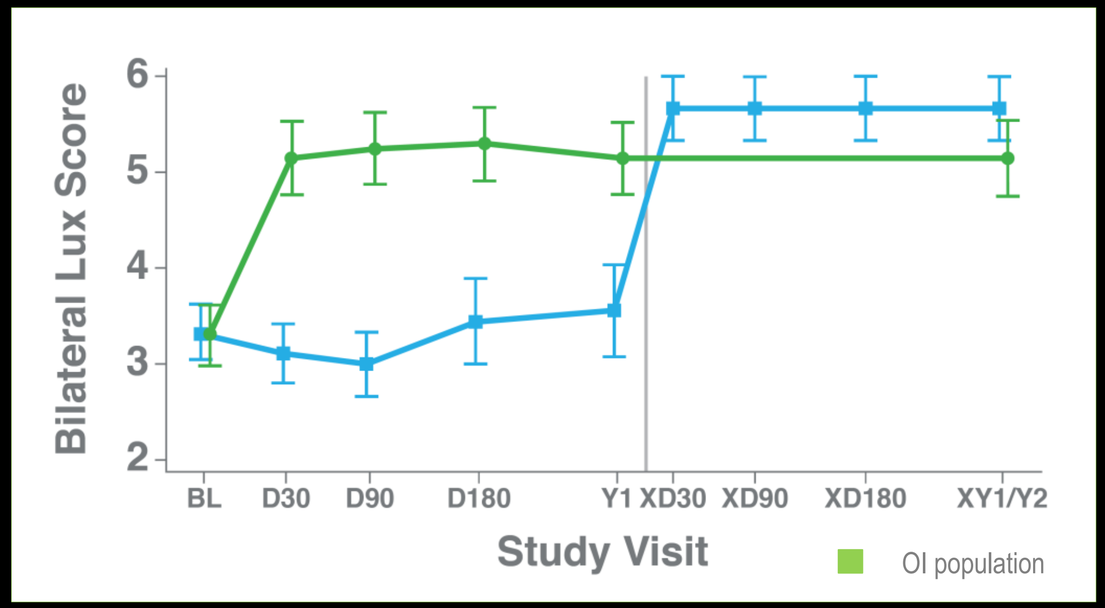


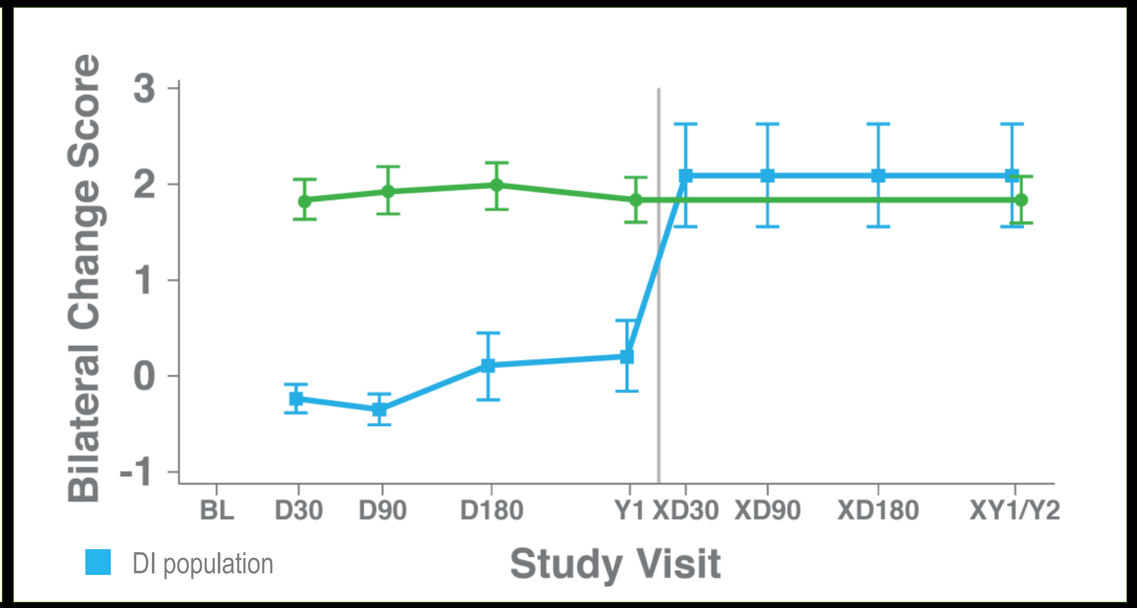
[1]Styrofoam object or cone. [2]Foam is a raised foam block. [3]Waist-high object

S Russell, *et al.*: Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in subjects with *RPE65*-mediated inherited retinal dystrophy: a randomised, controlled, open-label phase 3 trial, Lancet, 2017

Gene Rx Phase 3: Results

Mean Bilateral Multi-Luminance Mobility Test (MLMT) Over Time





Data presented as mean ± SE. For the DI group, change is relative to injection baseline after year 1. BL, baseline; X, crossover; XY1, DI group year 1; Y1, OI group year 1/ DI group baseline; Y2, OI group year 2

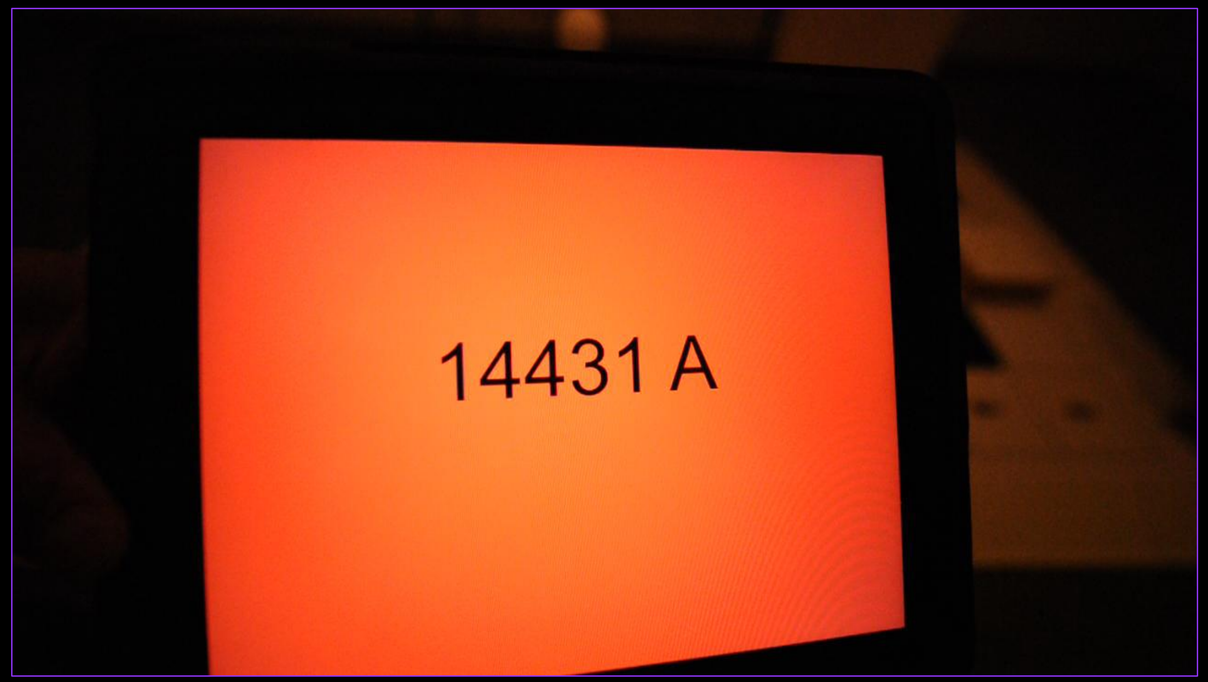
- Mean (SD) bilateral change score for OI subjects (n=20) was 1.9 (1.1) levels at year 2 and 2.1 (1.6) levels for DI subjects (n=9) at year 1

S Russell, *et al.*: Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in subjects with *RPE65*-mediated inherited retinal dystrophy: a randomised, controlled, open-label phase 3 trial, Lancet, 2017

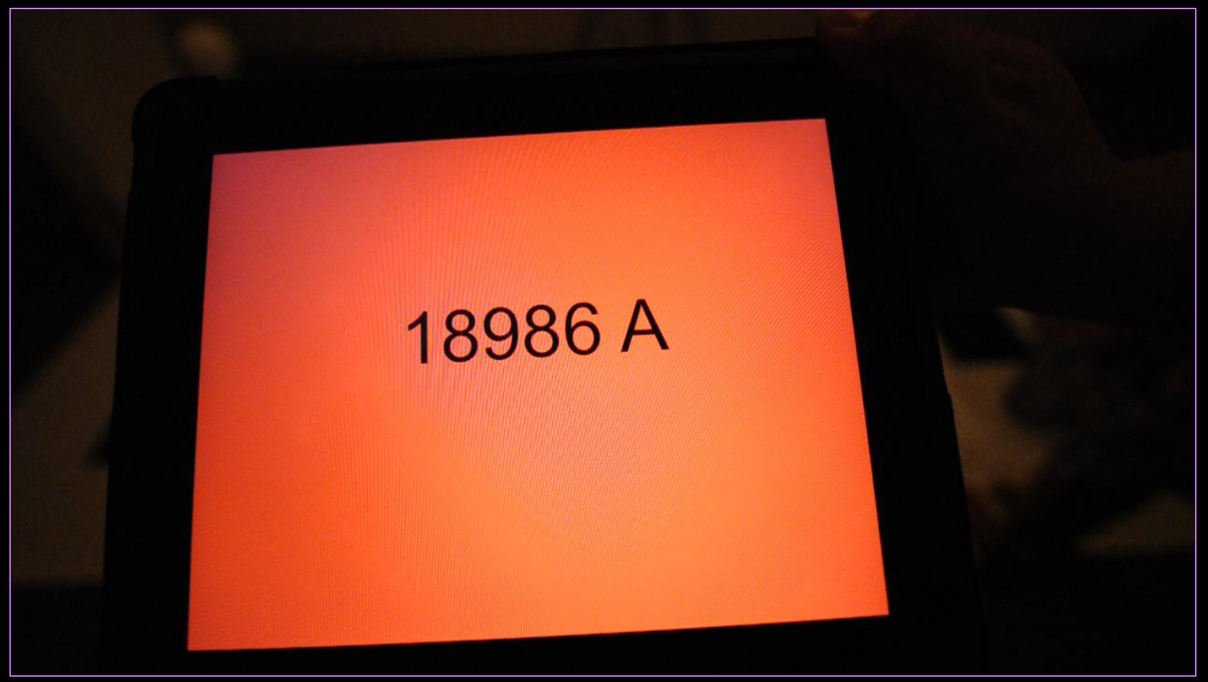
Gene Rx Phase 3: Results

Representative MLMT Videos (Bilateral Testing)

CH-41: baseline visit at 4 lux (**Fail**)



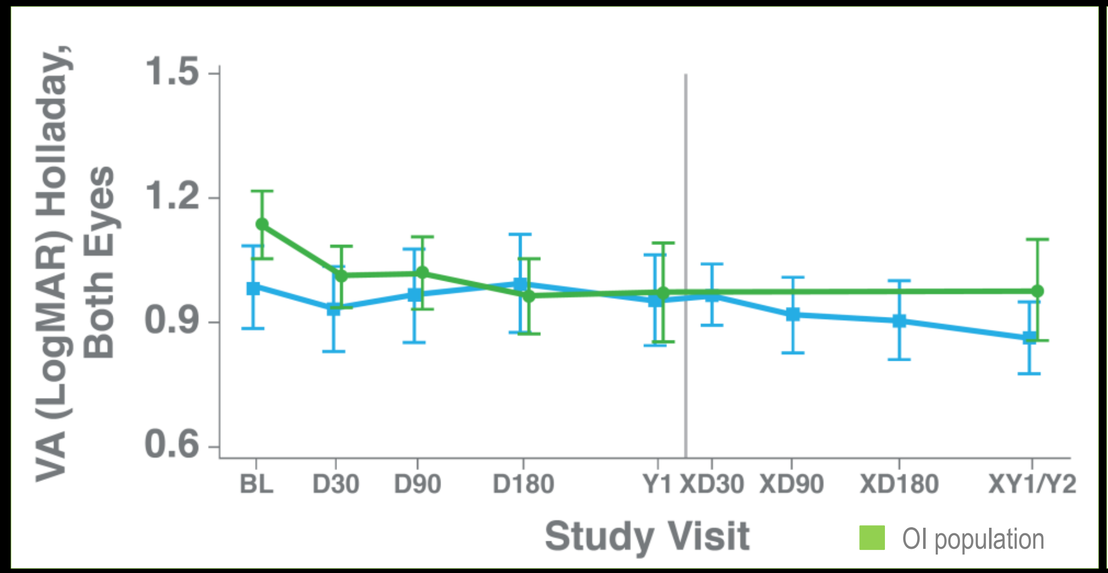
CH-41: 1-year visit after voretigene neparvovec administration at 4 lux (**Pass**)

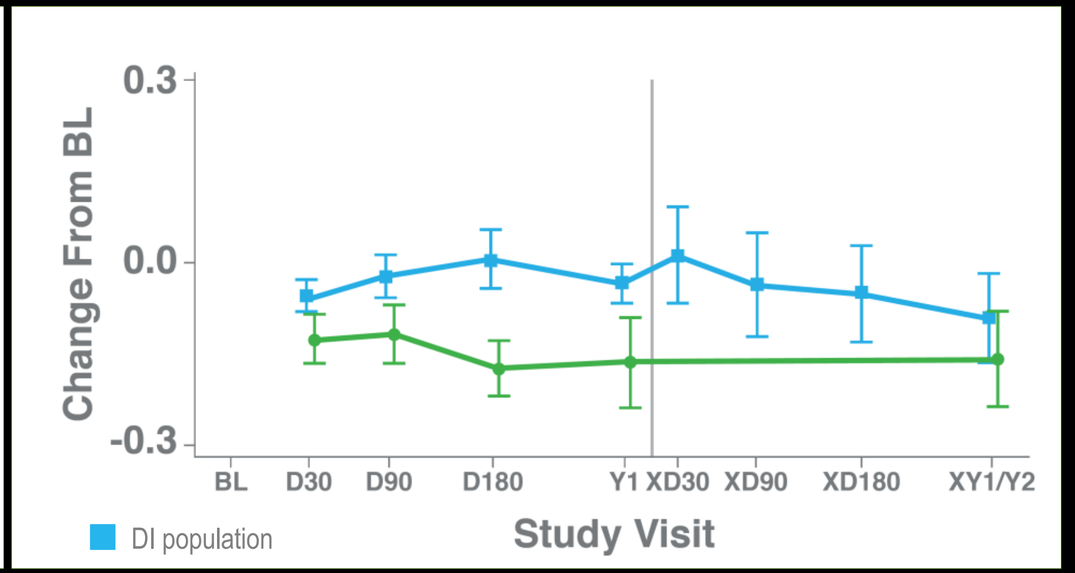


S Russell, *et al.*: Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in subjects with *RPE65*-mediated inherited retinal dystrophy: a randomised, controlled, open-label phase 3 trial, Lancet, 2017

Gene Rx Phase 3: Results

Mean Best-Corrected Visual Acuity (Holladay Scale) Over Time





Data presented as mean ± SE. Off-chart assignments based on scale adapted from JT Holladay, *J Cataract Refract Surg,* 30, 287-290, 2004

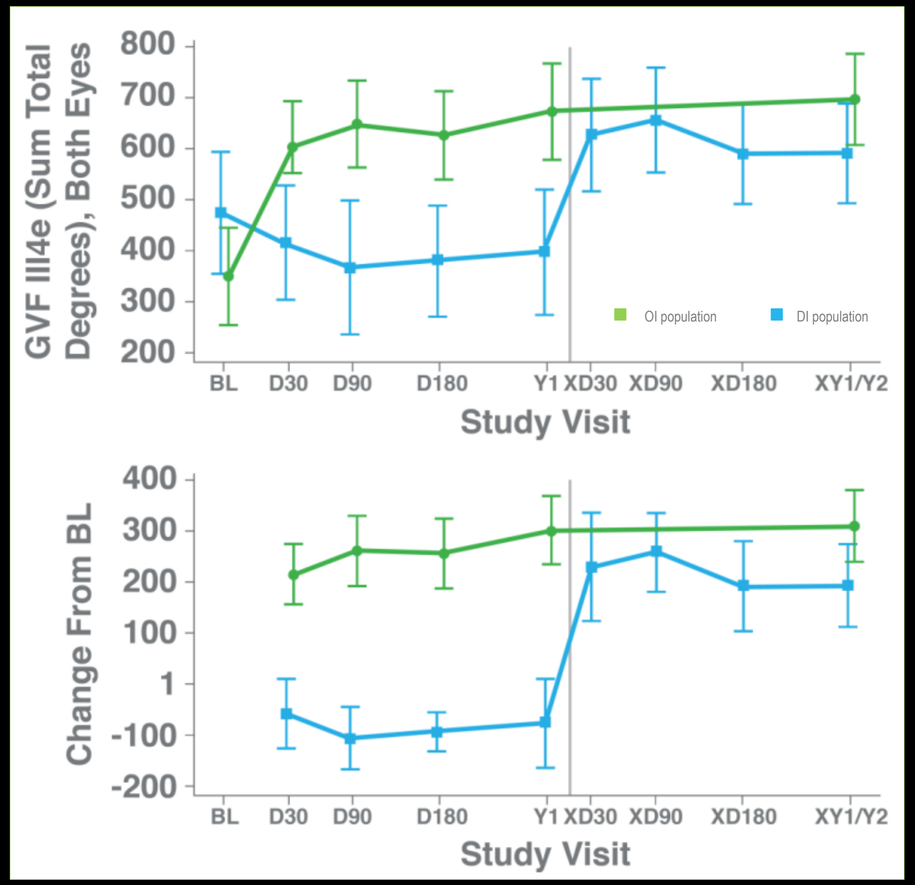
- Mean (SD) logMAR improvement from baseline in VA averaged over both eyes:
  - –0.16 (0.36) (8-letter gain) at year 2 for OI subjects
  - –0.09 (0.22) (4.5-letter gain) at year 1 for DI subjects

S Russell, *et al.*: Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in subjects with *RPE65*-mediated inherited retinal dystrophy: a randomised, controlled, open-label phase 3 trial, Lancet, 2017

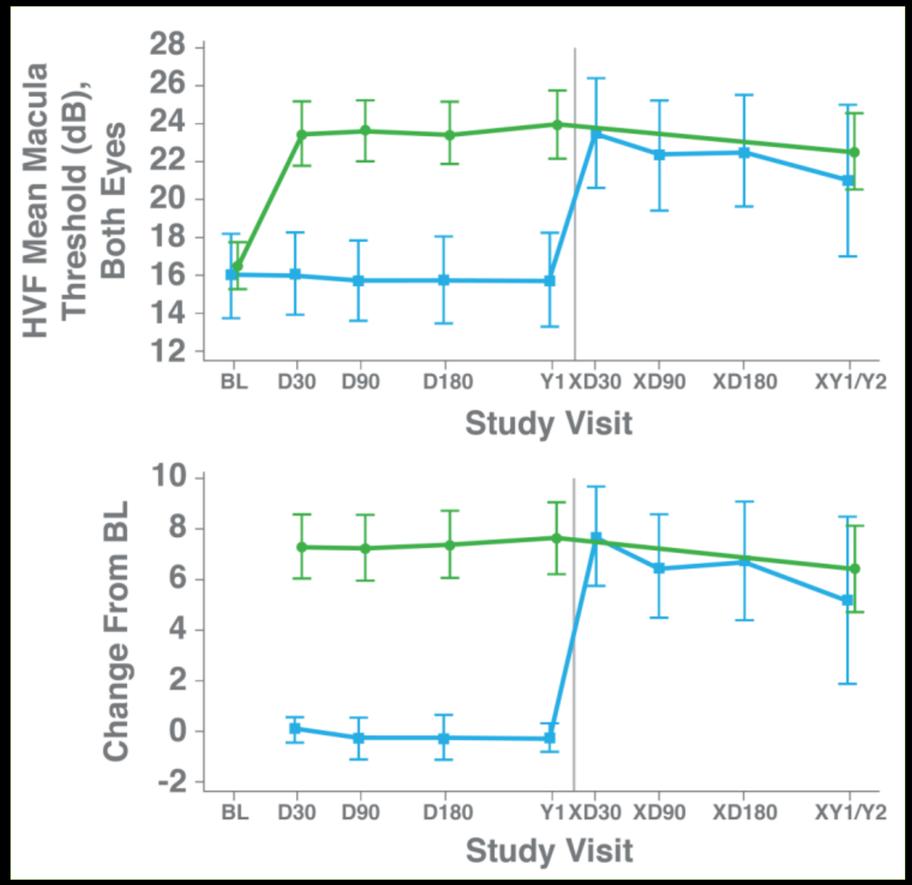
Gene Rx Phase 3: Results

Mean Change in Visual Fields Over Time

Mean Goldmann VF III4e



Mean Humphrey VF
Mean Macula Threshold



S Russell, *et al.*: Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in subjects with *RPE65*-mediated inherited retinal dystrophy: a randomised, controlled, open-label phase 3 trial, Lancet, 2017

Gene Rx Phase 3
Safety

- Most frequently reported ocular treatment-emergent adverse events through 2 yrs after administration of VN (OI & DI population)
  - Increased intra-ocular pressure, 7 events in 5 (17%) subjects
  - Cataract, 5 events in 4 (14%) subjects
  - Retinal tear, 3 events in 3 (10%) subjects
  - Retinal deposits, 3 events in 3 (10%) subjects
- Serious adverse events
  - Two subjects in OI group
    - One experienced an adverse drug reaction related to complications from oral surgery
    - One experienced an adverse drug reaction & convulsions associated with pre-existing complex seizure disorder
  - One subject in DI group
    - One experienced loss of foveal function thought to be related to administration procedure & not to study drug
- No product-related serious adverse events and no deleterious immune responses occurred

S Russell, *et al.*: Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in subjects with *RPE65*-mediated inherited retinal dystrophy: a randomised, controlled, open-label phase 3 trial, Lancet, 2017

Gene Rx Phase 3
Conclusions

- Improvements in MLMT, FST, & VF at year 1 in DI subjects consistent with those seen in OI cohort at 1 yr
- Improvements observed in OI subjects generally maintained at 2 yrs
- Gene augmentation by VN therapy improved functional vision & visual function in subjects with biallelic *RPE65*-mediated IRD as measured by improvements in:
  - Ambulatory navigation
  - Light sensitivity
  - Visual field size

S Russell, *et al.*: Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in subjects with *RPE65*-mediated inherited retinal dystrophy: a randomised, controlled, open-label phase 3 trial, Lancet, 2017

FDA (2017) & EMA (2018) approval of AAV2-CBA-*RPE65* (aka Luxturna®) for treatment of adult & paediatric patients with vision loss due to IRD caused by biallelic mutations in *RPE65*, who have sufficient retinal cells

FDA NEWS RELEASE

FDA approves novel gene therapy to treat patients with a rare form of inherited vision loss

Share | Tweet | Linkedin | Email | Print

For Immediate Release: December 18, 2017

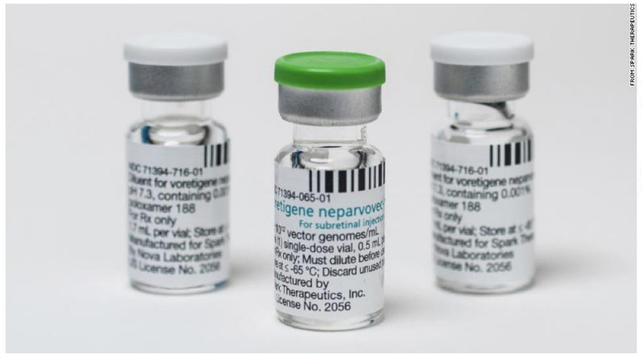
EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

EMA/823783/2018
EMEA/H/C/004451

Luxturna (*voretigene neparvovec*)

An overview of Luxturna and why it is authorised in the EU

LUXTURNA™
voretigene neparvovec-rzyl
for subretinal injection



Spark™ THERAPEUTICS

NOVARTIS

Gene Rx for *RPE65*-Related Retinal Dystrophy

Current Situation Voretigene Neparvovec (Luxturna®)

- USA:
  - FDA Advisory Committee Meeting: unanimously in favour on 12 Oct 2017
  - FDA granted Marketing Authorisation on 21 Dec 2017
  - Voretigene neparvovec (Luxturna®) on the market since March 2018 w/ +/- 9 patients treated
  - Cost $850.000,00 for two eyes (reimbursement by private insurers)
- EU:
  - EMA Committee for Human Medicinal Products meeting w/ Spark Tx on Marketing Licensing Application on 05 Jul 2018
  - EMA Committee for Human Medicinal Products has decided favourably on 21 Sep 2018
  - European Medicines Agency granted Marketing Authorization on 23 Nov 2018
  - Novartis markets voretigene neparvovec (Luxturna®) outside of USA
  - Rx administered at selected superspecialist treatment centers
  - Reimbursement in individual European countries obtained (Belgium on 1 April 2021)

Genotyping

Patient Eligibility Criteria for Voretigene Neparvovec (Luxturna®) Gene Therapy

EU Indication[1]:

"Voretigene neparvovec is an adeno-associated virus vector-based gene therapy indicated for the treatment of adult and pediatric patients with vision loss due to inherited retinal dystrophy caused by confirmed biallelic *RPE65* mutations and who have sufficient viable retinal cells."

Retinal cell viability in practice:

- presence of outer retinal cells on SD-OCT as determined by IRD specialist
- presence of at least Light Perception vision
- some additional measurement of visual function desirable e.g. FST

1. https://www.ema.europa.eu/en/documents/product-information/luxturna-epar-product-information_en.pdf

Gene Rx

Voretigene Neparvovec (Luxturna®)

- Subretinal injection
- 300µl w/ 1,5 x 10^{11} AAV2-CßA-*RPE65*
- Central retina (macula)

AM Maguire, KA High, A Auricchio, EA Pierce, F Testa, F Mingozzi, J Bennicelli, GS Ying, C Acerra, A Fulton, KA Marshall, S Banfi, D Chung, JIW Morgan, B Hauck, O Zelanaia, X Zhu, L Raffini, F Coppieters, E De Baere, KS Shindler, NJ Volpe, EM Surace, S Rossi, A Lyubarsky, TM Redmond, E Stone, J Sun, JF Wright, J Wellman McDonnell, BP Leroy, F Simonelli, J Bennett, Lancet, 374: 1597-1605, 2009

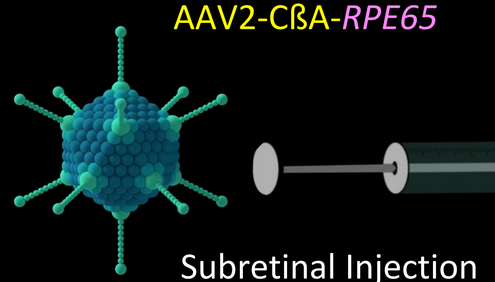
J Bennett, J Wellman, KA Marshall, S McCague, M Ashtari, J DiStefano-Pappas, OU Elci, DC Chung, J Sun, JF Wright, DR Cross, P Aravand, LL Cyckowski, JL Bennicelli, F Mingozzi, A Auricchio, EA Pierce, J Ruggiero, BP Leroy, F Simonelli, KA High, AM Maguire: Safety and durability of effect of contralateral-eye administration of AAV2 gene therapy in patients with childhood-onset blindness caused by *RPE65* mutations: a follow-on phase 1 trial, Lancet, 388, 661-72, 2016

S Russell, J Bennett, JA Wellman, DC Chung, ZF Yu, A Tillman, J Wittes, J Pappas, E Okan, S McCague, D Cross, KA Marshall, J Walshire, TL Kehoe, H Reichert, M Davis, L Raffini, MD; LA George, FP Hudson, L Dingfield, X Zhu, JA Haller, E Stone, EH Sohn, VB Mahajan, W Pfeifer, M Weckmann, CA Johnson, D Gewaily, A Drack, K Wachtel, F Simonelli, BP Leroy, JF Wright, KA High, AM Maguire, Lancet, 390, 849-860, 2017

AM Maguire, S Russell, J Wellman, D Chung, ZF Yu, A Tillman, J Wittes, J Pappas, O Elci, K Marshall, S McCague, H Reichert, M Davis, F Simonelli, BP Leroy, JF Wright, K High, J Bennett, Ophthalmology,126, 1273-1285, 2019

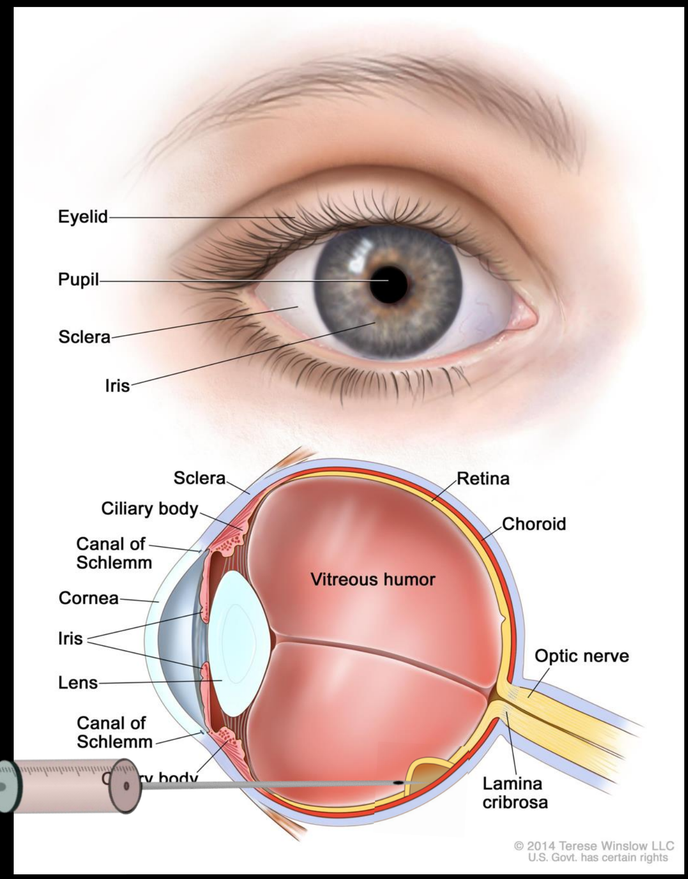
AM Maguire, J Bennett, EM Aleman, BP Leroy, TS Aleman, Mol Ther, 29, 442-463, 2021

AM Maguire, S Russell, DC Chung, ZF Yu, A Tillman, AV Drack, F Simonelli, BP Leroy, KZ Reape, KA High, J Bennett: Durability of Voretigene Neparvovec for Biallelic RPE65-Mediated Inherited Retinal Disease: Phase 3 Results at 3 Years and 4 Years, Ophthalmology, 2021



Adeno-Associated Virus
AAV2-CßA-*RPE65*

Subretinal Injection



So How Long Does Effect of Luxturna® Last?

Data from Phase 3 Trial

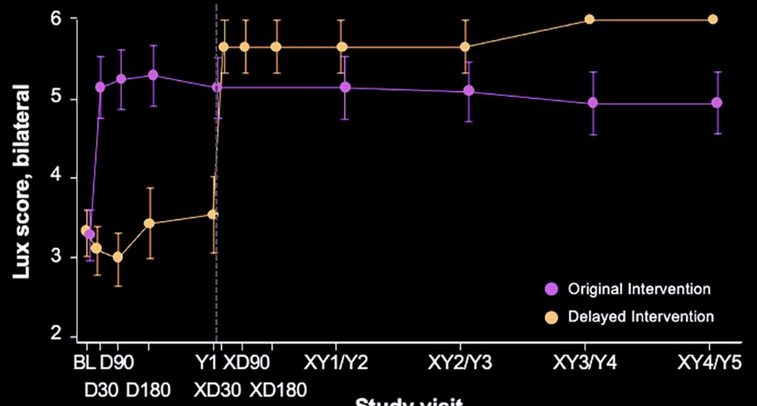
Gene Rx Phase 3

5/4 yrs Results

BP Leroy, et al.: Five-Year Update for the Phase III Voretigene Neparvovec Study in Biallelic *RPE65* Mutation-associated Inherited Retinal Disease, 10th Europaediatrics Congress 2021, Zagreb, Croatia, 07-09/10/2021

S Russell, *et al.*: Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in subjects with *RPE65*-mediated inherited retinal dystrophy: a randomised, controlled, open-label phase 3 trial, Lancet, 2017

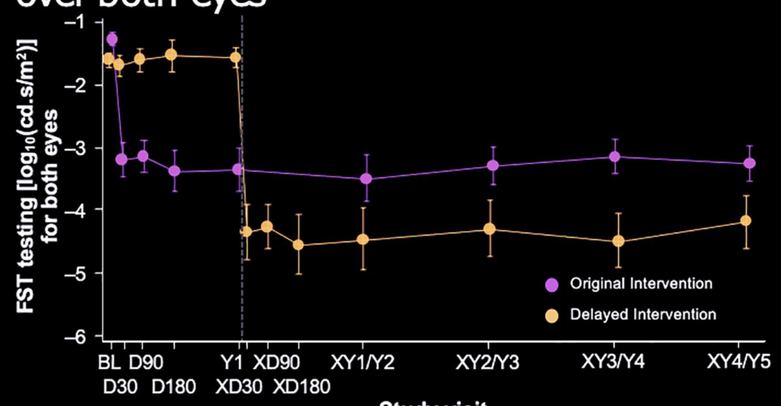
Mean bilateral MLMT change scores over 5 years



Primary endpoint: **Mean (SD) bilateral MLMT change score:**
1.6 (1.1) levels at Year 5 for OI subjects (n=18)
2.4 (1.5) levels at Year 4 for DI subjects (n=8)

Subjects demonstrated durable improvements in bilateral MLMT change score over 5 years

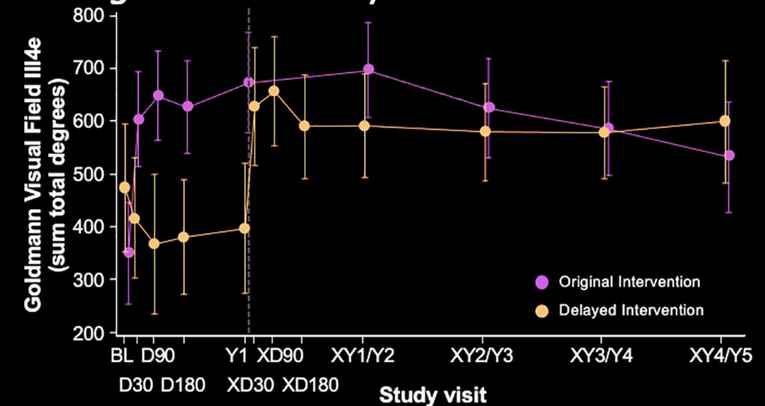
Mean (SD) change in white light FST in log_{10} (cd.s/m²) averaged over both eyes



Secondary endpoint: **Mean (SD) change in white light FST in log_{10} (cd.s/m²) averaged over both eyes:**
−2.02 (1.45) at Year 5 for OI subjects (n=17)
−2.58 (1.04) at Year 4 for DI subjects (n=8)

Over 5 years, light sensitivity (FST) improvement was sustained with voretigene neparvovec treatment

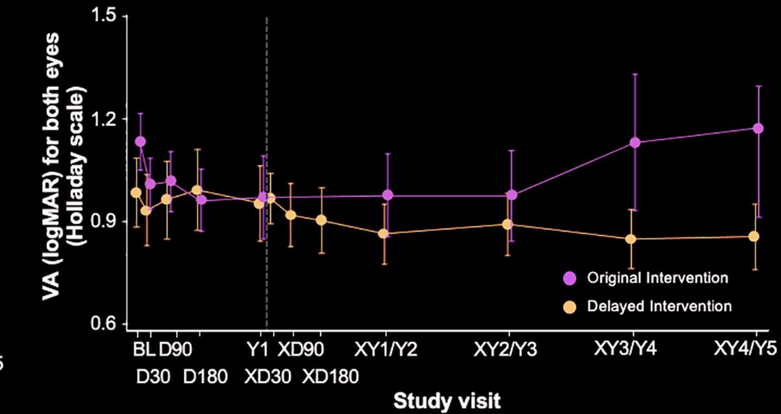
Mean (SD) change in Goldmann VF III4e sum total degrees averaged over both eyes



Exploratory endpoint: **Mean (SD) change in Goldmann VF III4e sum total degrees averaged over both eyes:**
166.6 (208.7) at Year 5 for OI patients (n=15)
178.8 (241.9) at Year 4 for DI patients (n=8)

Improved Goldmann VF at Year 1 was sustained with voretigene neparvovec treatment over 5 years

Mean (SD) change from BL in VA averaged over both eyes



Secondary endpoint: **Mean (SD) change from BL in VA averaged over both eyes :**
−0.00 (0.64) at Year 5 for OI patients (n=18)
−0.06 (0.26) at Year 4 for DI patients (n=8)

VA (Holladay Scale) was maintained with voretigene neparvovec treatment over 5 years

What Have We Learned Since?

Real-World Data

Chorioretinal Atrophy as a New AESI

Data from the Real-World Experience

Multicenter Study > Ophthalmol Retina. 2022 Jan;6(1):58-64. doi: 10.1016/j.oret.2021.03.016. Epub 2021 Apr 8.

Perifoveal Chorioretinal Atrophy after Subretinal Voretigene Neparvovec-rzyl for RPE65-Mediated Leber Congenital Amaurosis

William S Gange [1], Robert A Sisk [2], Cagri G Besirli [3], Thomas C Lee [1], Margaret Havunjian [4], Hillary Schwartz [4], Mark Borchert [1], Jesse D Sengillo [5], Carlos Mendoza [5], Audina M Berrocal [5], Aaron Nagiel [6]

Affiliations + expand

PMID: 33838313 PMCID: PMC8497635 (available on 2023-01-01)
DOI: 10.1016/j.oret.2021.03.016

Abstract

Purpose: To report an anatomic change following subretinal injection of voretigene neparvovec-rzyl (VN) for RPE65-mediated Leber congenital amaurosis.

Design: Multicenter, retrospective chart review.

Participants: Patients who underwent subretinal VN injection at each of 4 participating institutions

Methods: Patients were identified as having perifoveal chorioretinal atrophy if (1) the areas of atrophy were not directly related to the touch-down site of the subretinal cannula; and (2) the area of atrophy progressively enlarged over time. Demographic data, visual acuity, refractive error, fundus photographs, OCT, visual fields, and full-field stimulus threshold (FST) were analyzed.

Main outcome measures: Outcome measures included change in visual acuity, FST, visual fields, and location of atrophy relative to subretinal bleb position.

Results: A total of 18 eyes of 10 patients who underwent subretinal injection of VN were identified as having developed perifoveal chorioretinal atrophy. Eight of 10 patients (80%) developed bilateral atrophy. The mean age was 11.6 years (range, 5-20 years), and 6 patients (60%) were male. Baseline mean logarithm of the minimum angle of resolution visual acuity and FST were 0.82 (standard deviation [SD], 0.51) and -1.3 log cd.s/m^2 (SD, 0.44), respectively. The mean spherical equivalent was -5.7 diopters (D) (range, -11.50 to +1.75 D). Atrophy was identifiable at an average of 4.7 months (SD, 4.3) after surgery and progressively enlarged in all cases up to a mean follow-up period of 11.3 months (range, 4-18 months). Atrophy developed within and outside the area of the subretinal bleb in 10 eyes (55.5%), exclusively within the area of the bleb in 7 eyes (38.9%), and exclusively outside the bleb in 1 eye (5.5%). There was no significant change in visual acuity ($P = 0.45$). There was a consistent improvement in FST with a mean improvement of -3.21 log cd.s/m^2 ($P < 0.0001$). Additionally, all 13 eyes with reliable Goldmann visual fields demonstrated improvement, but 3 eyes (23.1%) demonstrated paracentral scotomas related to the atrophy.

Conclusions: A subset of patients undergoing subretinal VN injection developed progressive perifoveal chorioretinal atrophy after surgery. Further study is necessary to determine what ocular, surgical delivery, and vector-related factors predispose to this complication.

Keywords: Chorioretinal atrophy; Complications; Gene therapy; Leber congenital amaurosis; Luxturna; Outcomes Research; Subretinal injection; Voretigene neparvovec-rzyl.

Chorioretinal Atrophy After Gene Rx for *RPE65*-LCA

Conclusions

- Several patients show chorioretinal atrophy of 3 different types:
  - at injection sites
  - within treatment area
  - beyond treatment area
- Potential causes require further study

RPE65-related Retinal Dystrophy

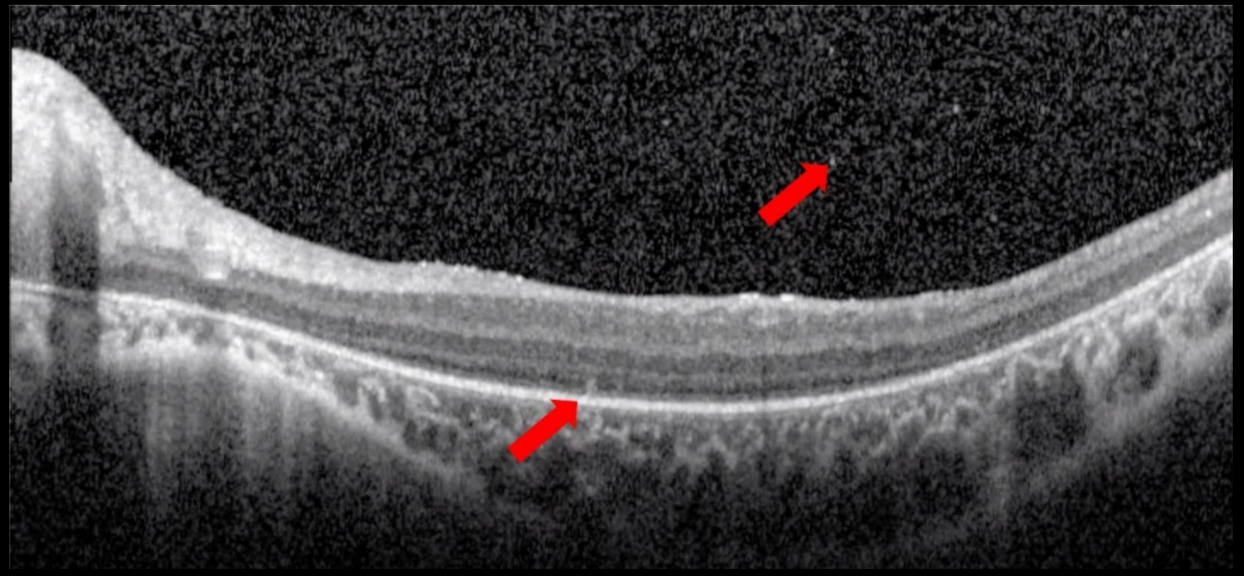
Need for Tight Control of Inflammation

- Tight control of retinal and vitreal inflammation required:

Bucher *et al.* Immune responses to retinal gene therapy using adeno-associated viral vectors - Implications for treatment success and safety, Progress in Retinal & Eye Research, https://doi.org/10.1016/j.preteyeres.2020.100915

- Even if retinal and vitreal inflammation are mild: use high doses of local steroids

From Bucher *et al.*, PRER, 2020



Genotyping

Patient Eligibility Criteria for Voretigene Neparvovec Gene Therapy

EU Indication[1]:

"Voretigene neparvovec is an adeno-associated virus vector-based gene therapy indicated for the treatment of adult and pediatric patients with vision loss due to inherited retinal dystrophy caused by confirmed biallelic *RPE65* mutations and who have sufficient viable retinal cells."

Retinal cell viability in practice:

- presence of outer retinal cells on SD-OCT as determined by IRD specialist
- presence of at least Light Perception vision
- some additional measurement of visual function desirable e.g. FST

1. https://www.ema.europa.eu/en/documents/product-information/luxturna-epar-product-information_en.pdf

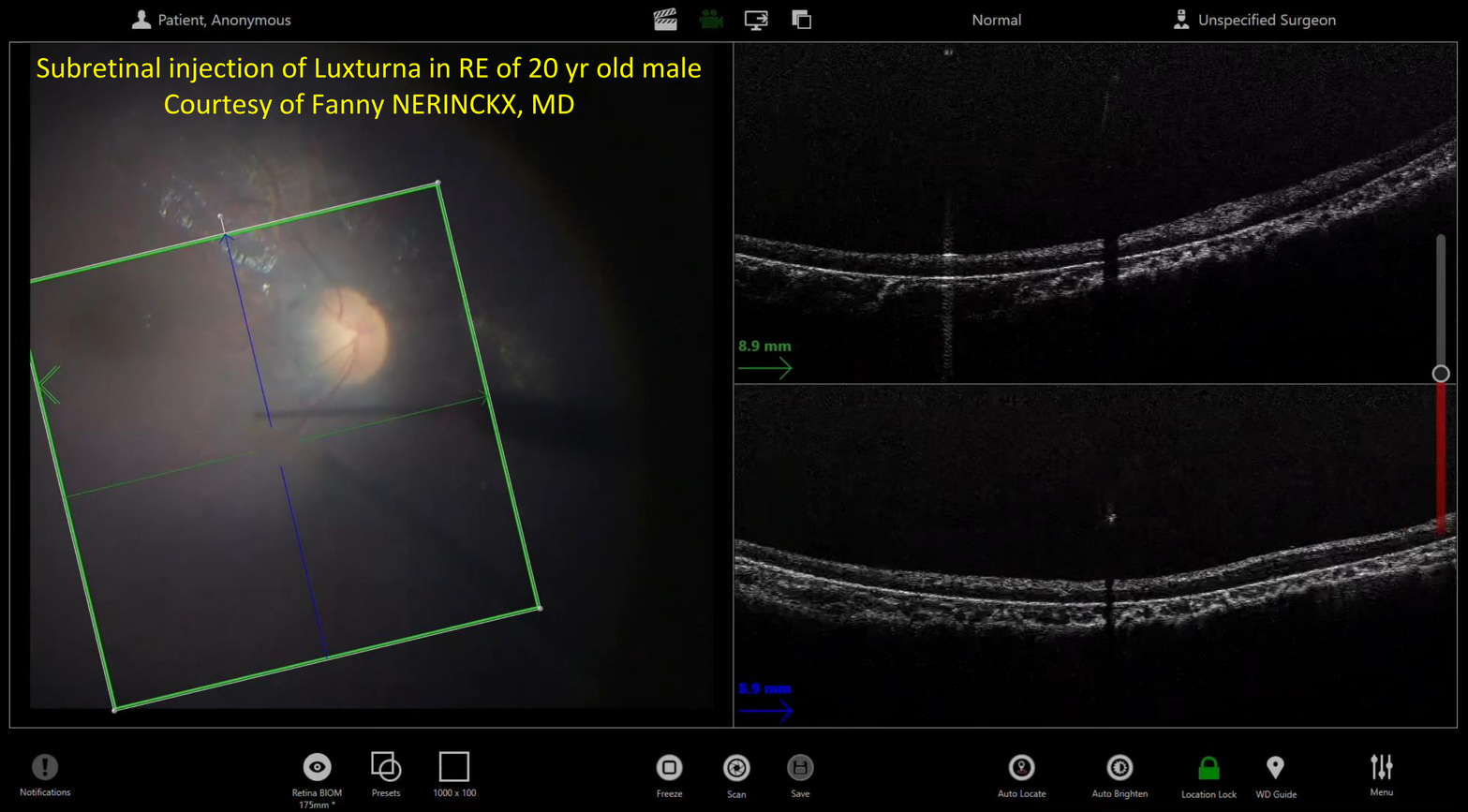
GU & GHU
Dept of Ophthalmology

UZ GENT

UNIVERSITEIT GENT

National Referral Center for Ocular Genetics & Gene Therapy

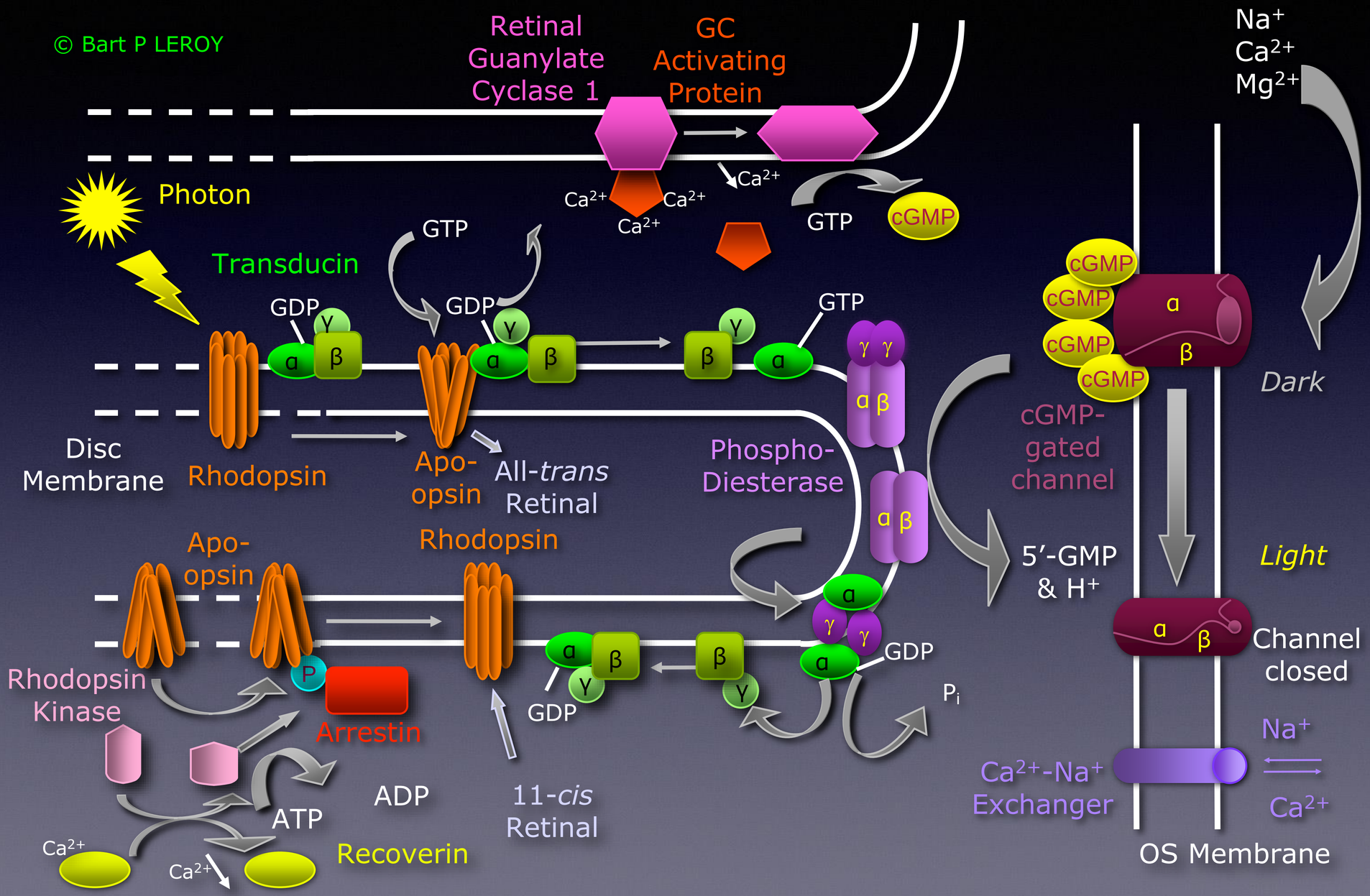
- *RPE65*-related Inherited Retinal Dystrophy
- *ND4*-related Leber Hereditary Optic Neuropathy
- *CEP290*-related Leber Congenital Amaurosis
- *RPGR*-related XLRP
- *CNGA3*- & *CNGB3*-related Achromatopsia

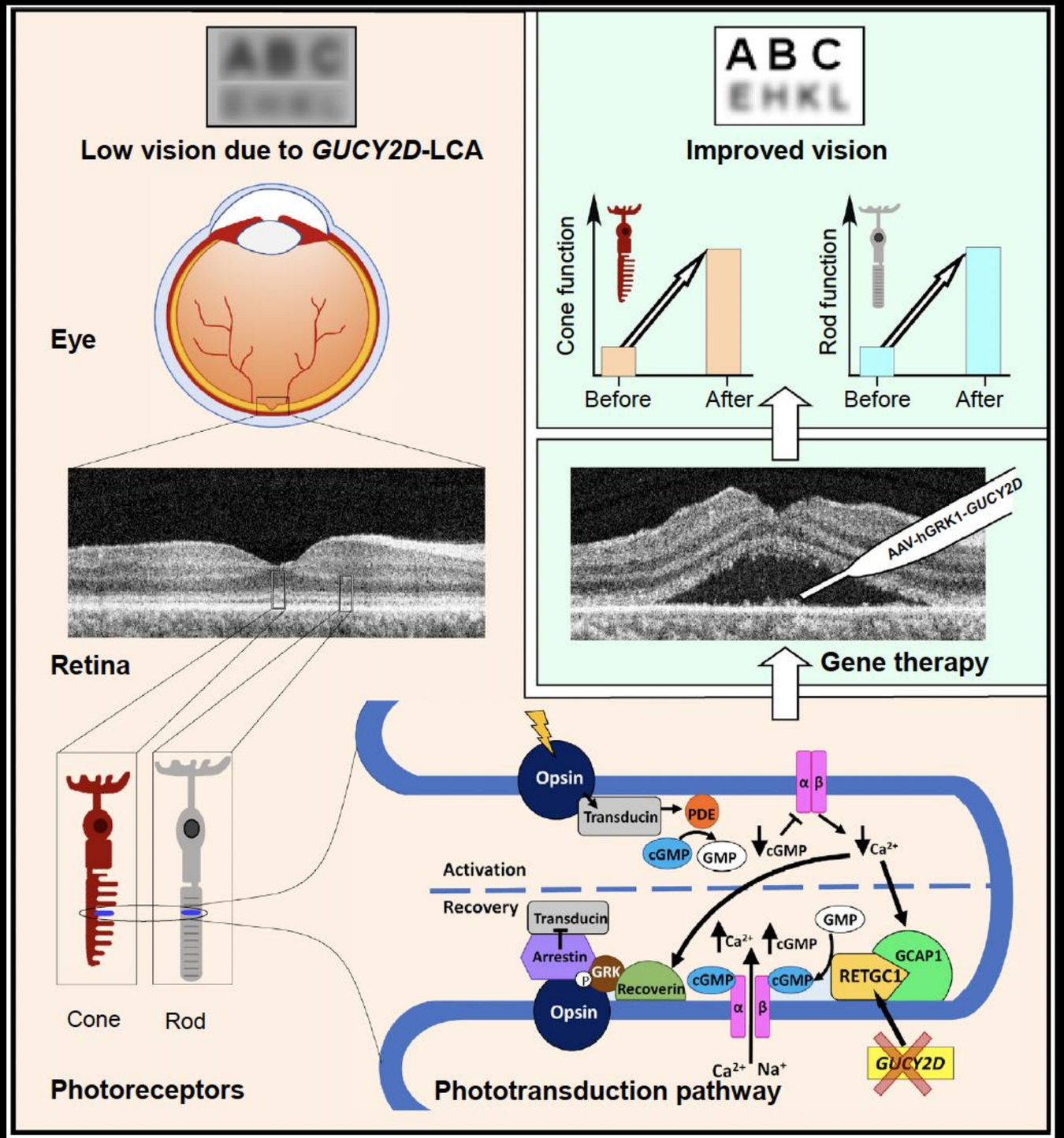


Gene Augmentation Therapy with AAV5

GUCY2D-IRD

The Phototransduction Cascade





Samuel G. Jacobson, Artur V. Cideciyan, Allen C. Ho, ..., Sanford L. Boye, Alexander M. Dizhoor, Shannon E. Boye

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Highlights

Blindness from genetic disorders of the retina has been incurable for centuries

The first photoreceptor-based childhood blindness (*GUCY2D*-LCA) has now been treated

Proof of safety and efficacy of subretinal gene therapy in *GUCY2D*-LCA is reported

iScience

CellPress
OPEN ACCESS

Article

Safety and improved efficacy signals following gene therapy in childhood blindness caused by *GUCY2D* mutations

Samuel G. Jacobson,[1,6,*] Artur V. Cideciyan,[1] Allen C. Ho,[2] Igor V. Peshenko,[3] Alexandra V. Garafalo,[1] Alejandro J. Roman,[1] Alexander Sumaroka,[1] Vivian Wu,[1] Arun K. Krishnan,[1] Rebecca Sheplock,[1] Sanford L. Boye,[4] Alexander M. Dizhoor,[3] and Shannon E. Boye[5]

SUMMARY

A first-in-human clinical trial of gene therapy in Leber congenital amaurosis due to mutations in the *GUCY2D* gene is underway, and early results are summarized. A recombinant adeno-associated virus serotype 5 (rAAV5) vector carrying the human *GUCY2D* gene was delivered by subretinal injection to one eye in three adult patients with severe visual loss, nystagmus, but preserved retinal structure. Safety and efficacy parameters were monitored for 9 months post-operatively. No systemic toxicity was detected; there were no serious adverse events, and ocular adverse events resolved. P1 and P2 showed statistically significant rod photoreceptor vision improvement by full-field stimulus testing in the treated eye. P1 also showed improvement in pupillary responses. Visual acuity remained stable from baseline in P1 and P2. P3, however, showed a gain of 0.3 logMAR in the treated eye, indicating greater cone-photoreceptor function. The results show safety and both rod- and cone-mediated efficacy of this therapy.

Dr Shannon BOYE
A/Prof @ University of Florida,
Gainesville, FL, USA

ATSENA THERAPEUTICS

News

PRESS RELEASES

Atsena Therapeutics Receives FDA Regenerative Medicine Advanced Therapy (RMAT) Designation for ATSN-101 Gene Therapy for GUCY2D-associated Leber Congenital Amaurosis (LCA1)

November 14, 2023

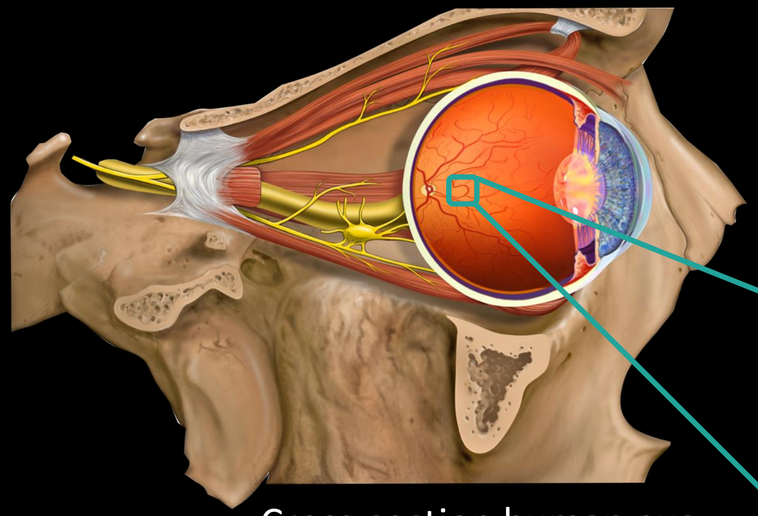
Dr Shannon BOYE
A/Prof @ University of Florida,
Gainesville, FL, USA

Antisense OligoNucleotide (AON) Therapy as an Alternative

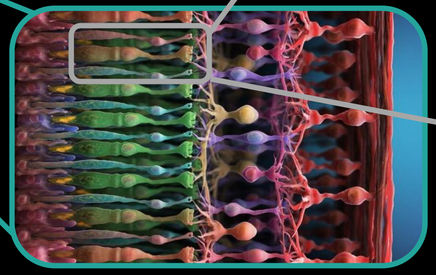
Sepofarsen for *CEP290*-IRD

CEP290 -Related Leber Congenital Amaurosis (LCA10)

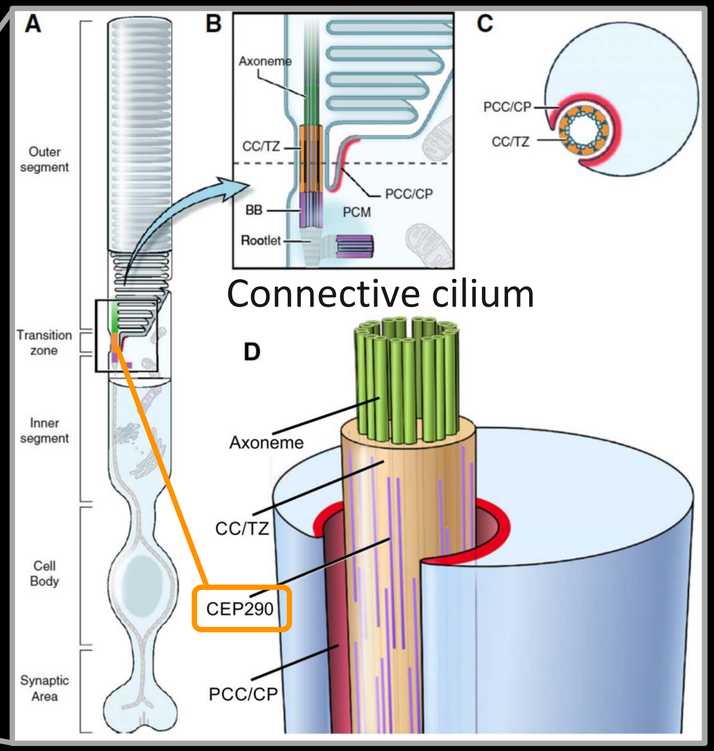
- Rare (prevalence of <1 per 100,000), autosomal recessive retinal disease
- CEP290 is involved in cilium formation and intracellular protein trafficking



Cross-section human eye



Retinal layers

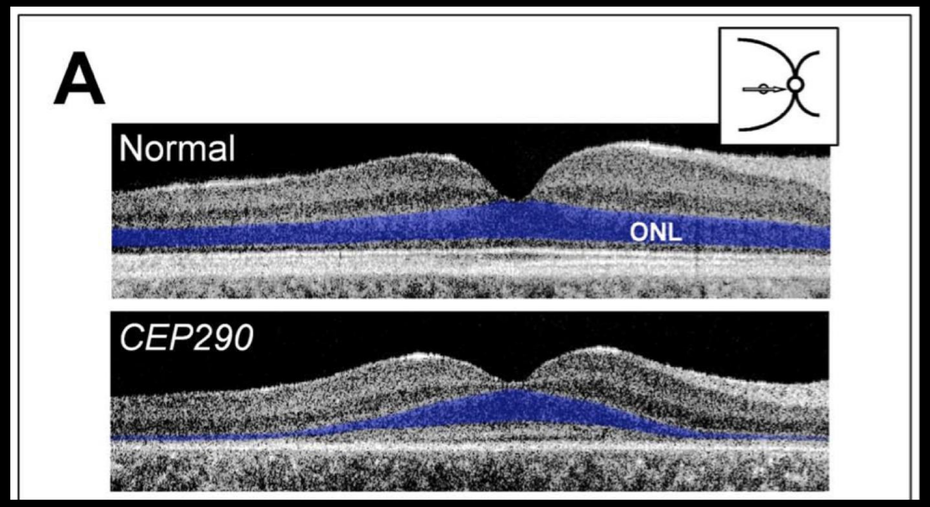


Photoreceptor cell[1]

Cideciyan AV, Jacobson SG. *Invest Ophthalmol Vis Sci,* 2019, 60, 1680-1695

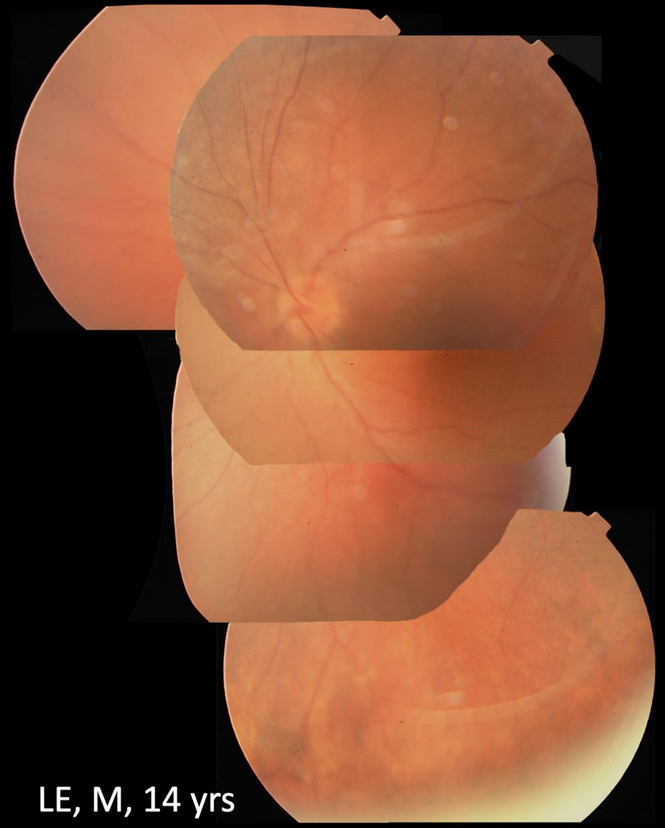
CEP290-LCA10

Severe Phenotype

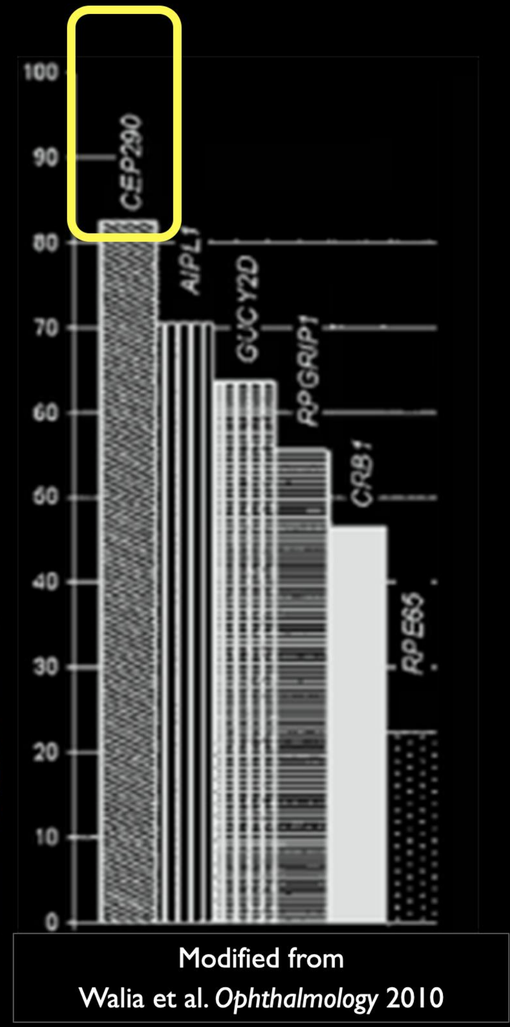


Retained central retinal photoreceptors & RPE disproportionate to low level of vision

Gene encompasses 54 exons w/ open reading frame of 7,440 bp) that exceeds typical cargo size (4.7 kb) of rAAV



LE, M, 14 yrs
Compound HeZ
p.Cys998X &
p.Glu1956GlyfsX9



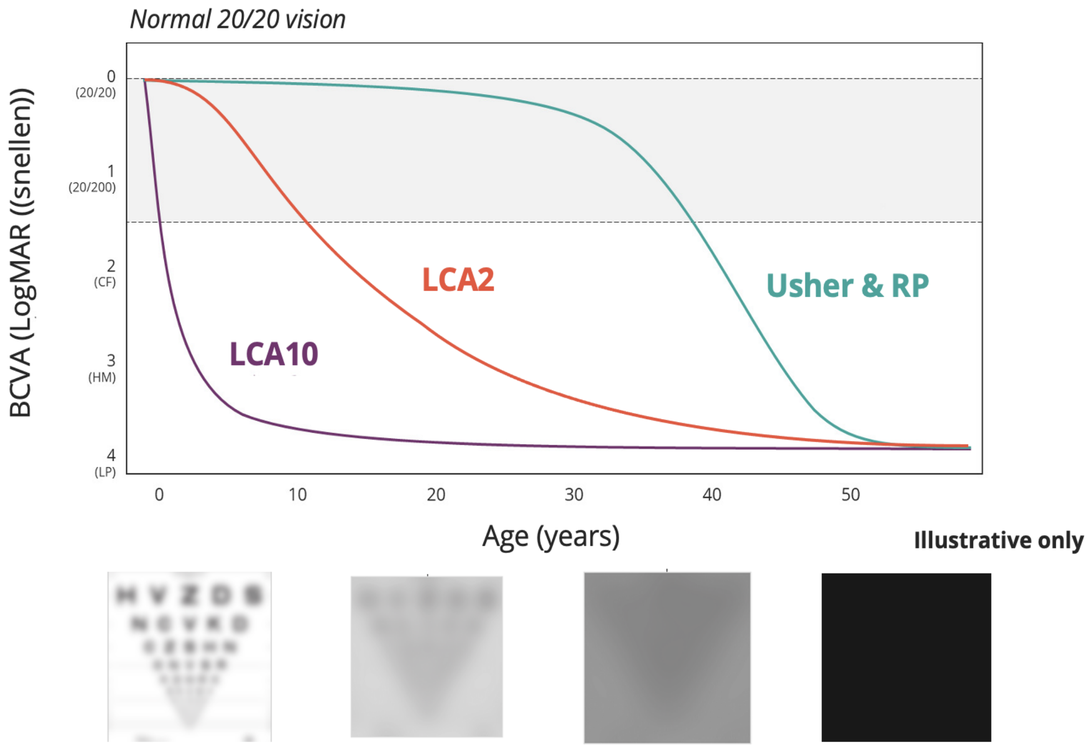
Modified from
Walia et al. *Ophthalmology* 2010

Percentage of patients w/ very severe vision loss w/ best-corrected visual acuities= CF, HM, LP & NLP

High unmet medical need in LCA10

- Autosomal recessive retinal disease leading to severe and early vision loss
- Caused by mutations in the *CEP290* gene
- c.2991+1655A>G variant accounts for approximately 2,000 patients in the Western world
- The vision loss associated with LCA10 impacts quality of life of individuals living with the disease

A severe and early onset vision loss in LCA10 vs. other IRDs



There are currently no approved therapies for LCA10

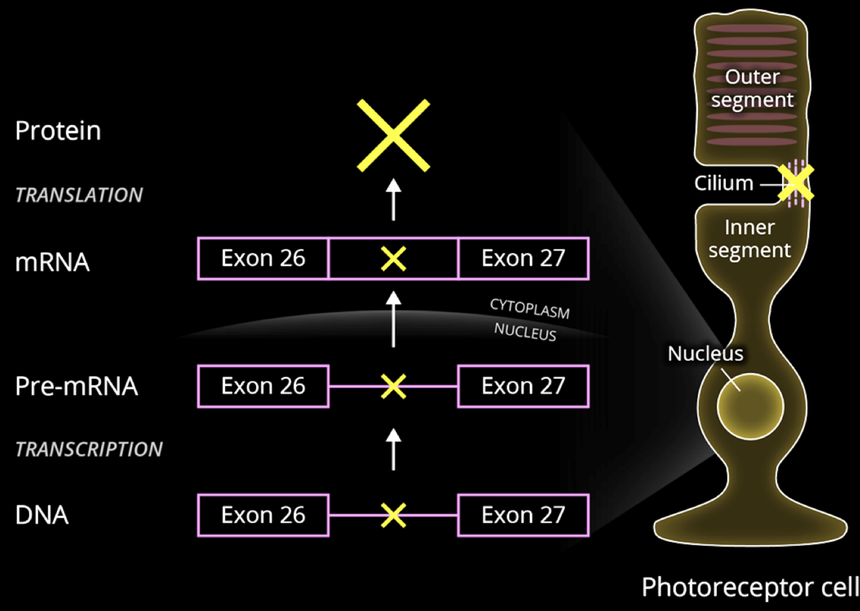
LCA, Leber congenital amaurosis; 1. Chacon-Camacho OF, Zenteno JC. *World J Clin Cases*. 2015;3(2):112–24; 2. Cideciyan AV, Jacobson SG. *Invest Ophthalmol Vis Sci.* 2019;60(5):1680–95; 3. Jacobson SG, et al. *Invest Ophthalmol Vis Sci*. 2017;58(5):2609–22; 4. Leroy BP, et al. *Retina* 2021;41(5):898-907.

CEP290-LCA10

Splice Correction for p.Cys998X *CEP290* mRNA

CEP290-IRD

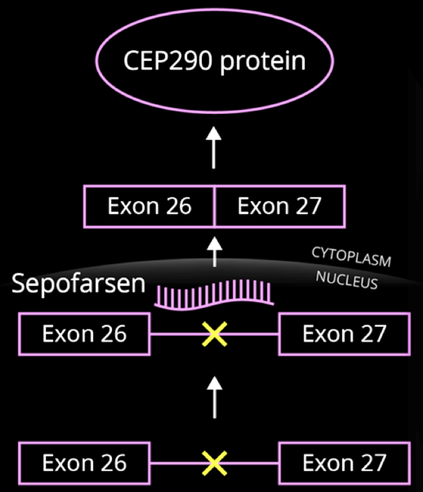
Leber congenital amaurosis 10 due to *CEP290* mutations



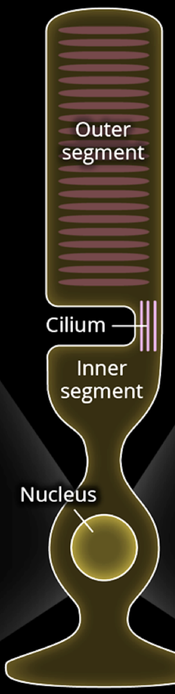
Pathology

Rx w/ Sepofarsen (AON)

A Leber congenital amaurosis 10 due to *CEP290* mutations + Sepofarsen



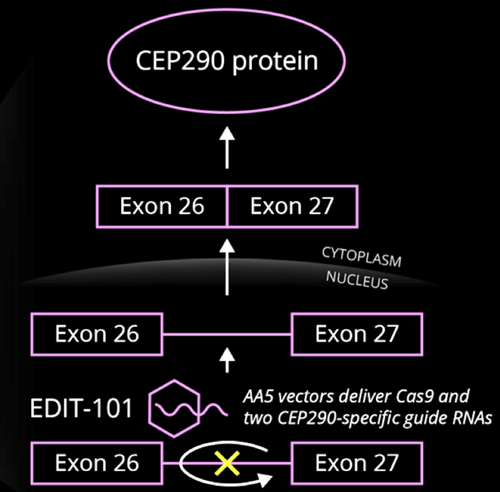
Sepofarsen
= 17-mer antisense oligonucleotide (AON)



Rescue

Rx w/ EDIT-101 (CRISPR/Cas9)

B Leber congenital amaurosis 10 due to *CEP290* mutations + EDIT-101



Adapted from BP Leroy, DG Birch, JL Duncan, BL Lam, RK Koenekoop, FBO Porto, SR Russell, A Girach, Retina, 41, 898-907, 2021

Genetic Rx

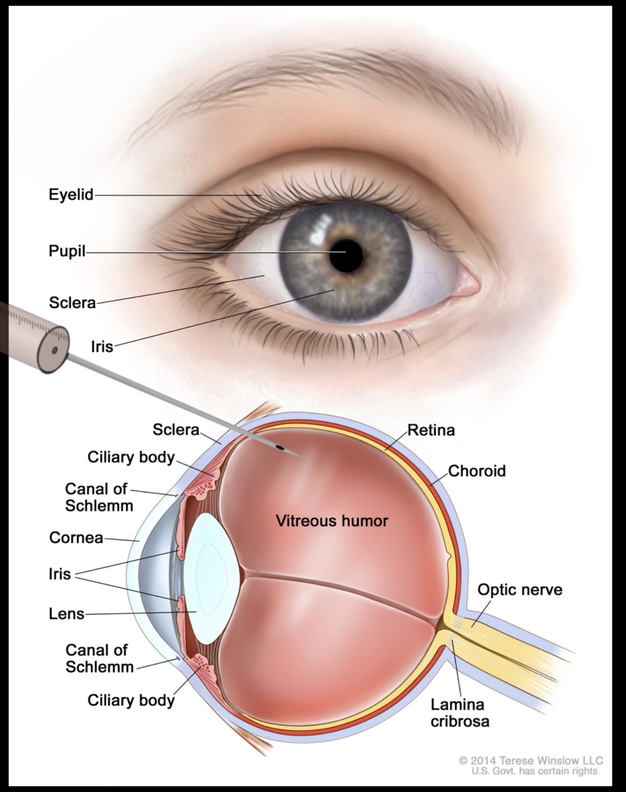
Sepofarsen (17-mer AON)

AV Cideciyan, SG Jacobson, A Drack, AC Ho, J Charng, AV Garafalo, AJ Roman, A Sumaroka, IC Han, MD Hochstedtler, W Pfeiffer, EH Sohn, M Taiel, MR Schwartz, P Biasutto, W de Wit, ME Cheetham, P Adamson, DM Rodman, G Platenburg, MD Tome, I Balikova, F Nerinckx, J De Zaeytijd, C Van Cauwenbergh, BP Leroy, SR Russell, Nat Med, 25, 225-228, 2019

BP Leroy, SR Russell, AV Drack, AV Cideciyan, SG Jacobson, AC Ho, C Van Cauwenbergh, J De Zaeytijd, AK Krishnan, W den Hollander, A Hollestein-Havelaar, MR Schwartz, A Girach: Safety and efficacy of sepofarsen in the second treated eye in the Phase 1b/2 extension trial in Leber congenital amaurosis due to mutations in the CEP290 gene (Insight Trial), EURETINA 2021 Virtual Meeting, 09-12/09/2021

Sepofarsen (17-mer AON) directed against *CEP290* pre-mRNA

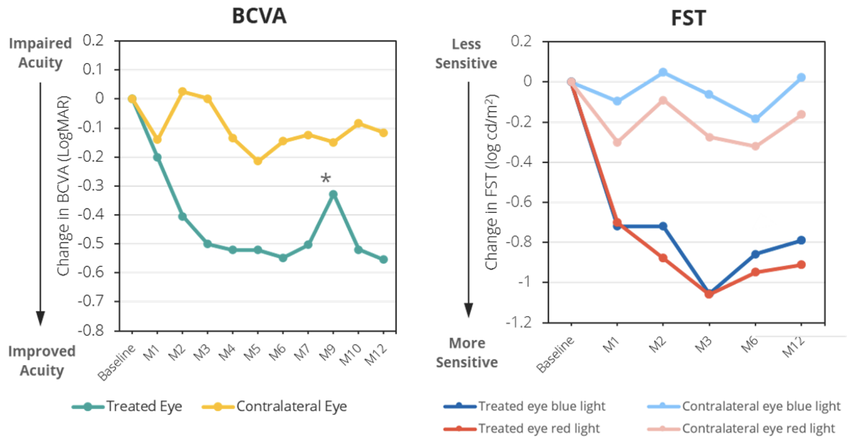
Intravitreal Injection



- Intravitreal injection - broad distribution
- Sepofarsen is 17-mer antisense oligonucleotide (160/80 or 80/40 µg in 50 µl)
- Effect not permanent - thus reversible

Efficacy in the Phase 1/2 maintained in the *InSight* extension trial

Phase 1/2 study[1] (n=11)



- Mean BCVA improvement of more than 25 letters at M12
- Retinal sensitivity improvement (FST)

InSight extension study[2] (n=9)

inSight

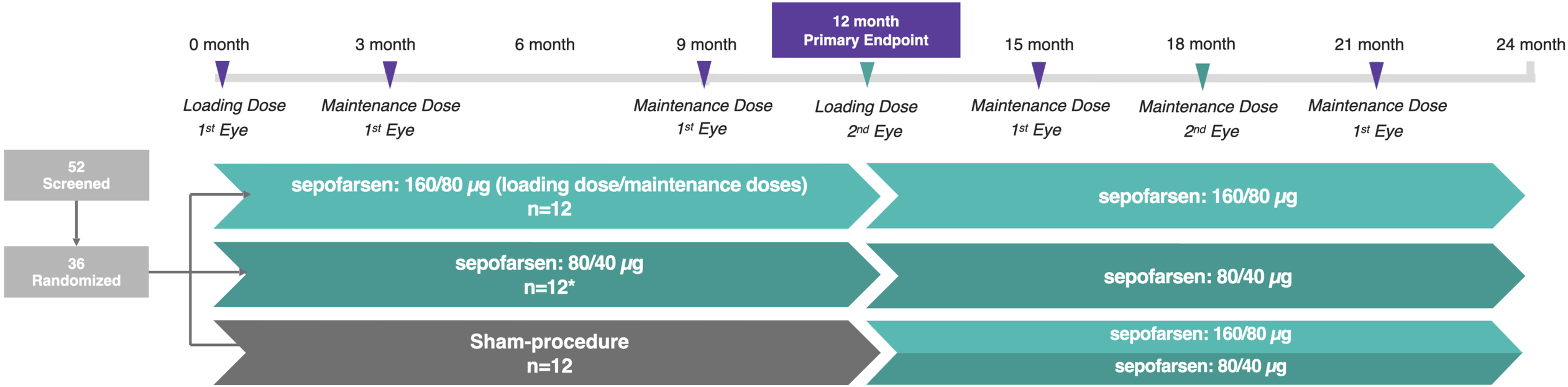
Up to approximately 4 years of follow-up

- Continued good tolerability
- Sustained improvements in the first treated eye
- Similar response in the second treated eye

1. Russell, SR et al. *Invest. Ophthalmol. Vis. Sci.* 2020;61(7):866; 2. Russel, SR et al. *Invest. Ophthalmol. Vis. Sci.* 2022

Sepofarsen Pivotal Phase 2/3 Trial Design

All 36 participants at 14 sites in 9 countries at the 12M timepoint



Key inclusion criteria:

- LCA10 due to the c.2991+1655A>G mutation in the *CEP290* gene
- Age ≥ 8 years
- BCVA = 0.4 to 3.0 logMAR (20/50-HM)

Study design:

- Multicenter, Randomized, Double-Masked, Sham controlled phase 2/3 study

Primary Endpoint:

- Change from baseline in BCVA (logMAR) at Month 12

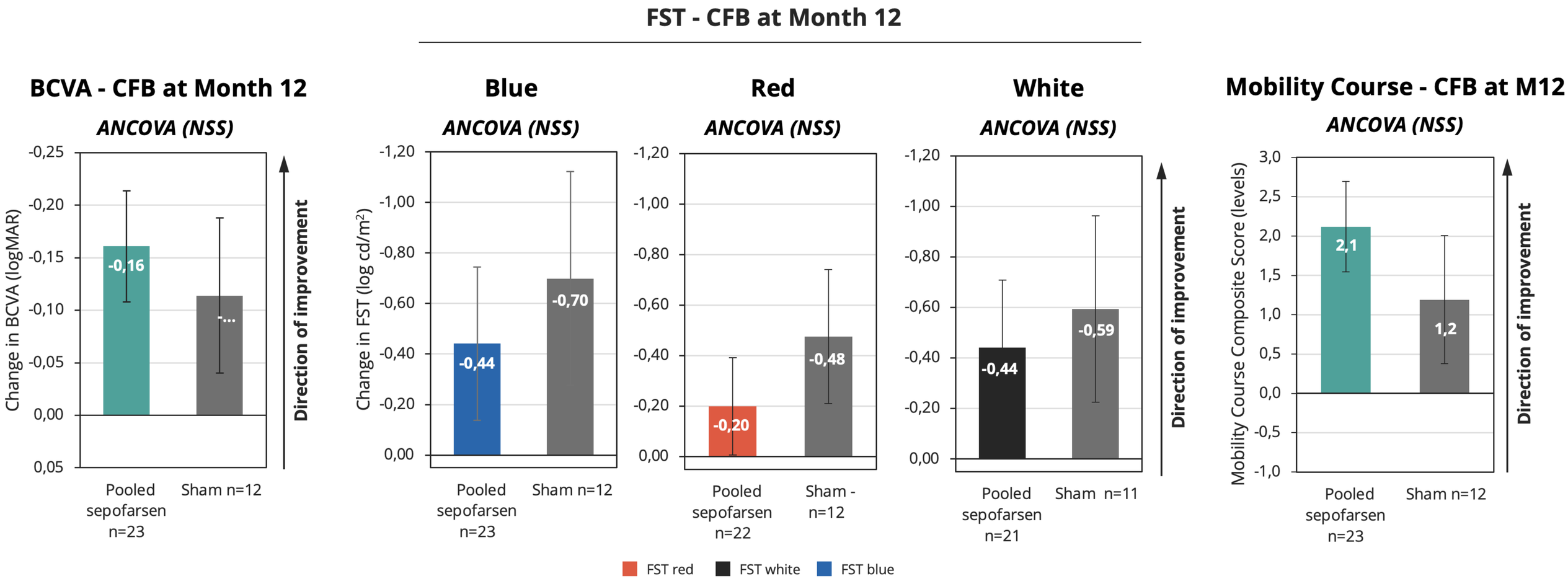
Secondary Endpoints:

- Mobility course
- Full field stimulus testing (FST)
- Optical coherence tomography (OCT)

*One participant was subsequently found to be a Light Perception patient and has been excluded from the post-hoc analyses

Key efficacy outcomes – Pooled sepofarsen group

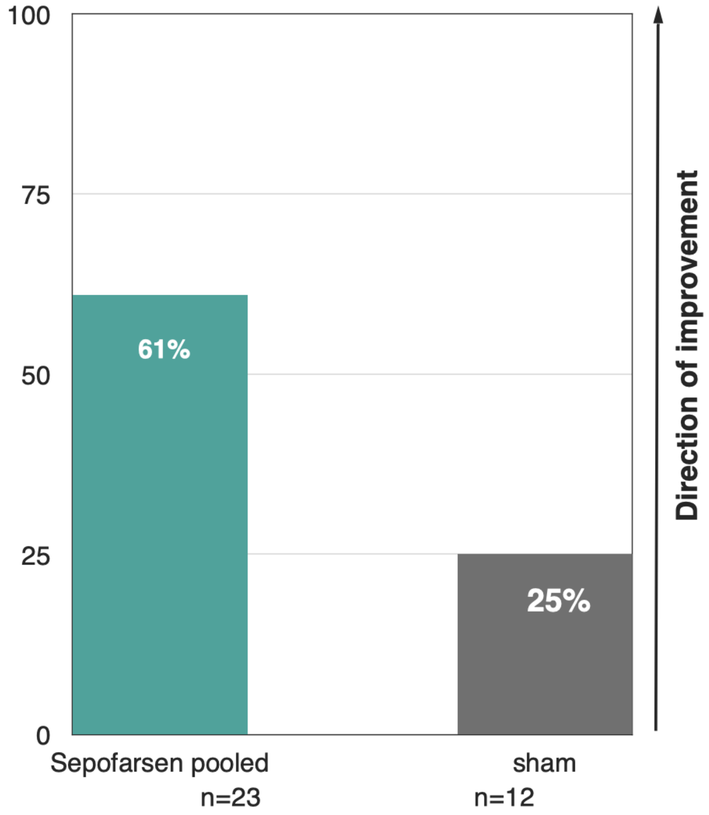
Study did not meet its primary or key secondary endpoints



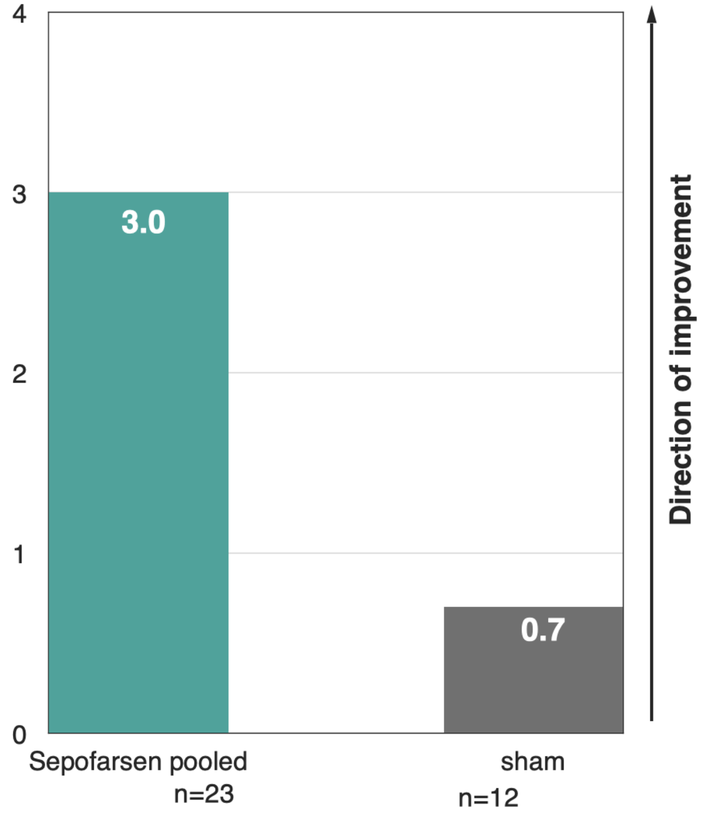
BCVA, Best corrected visual acuity; CFB, Change from baseline; FST, full-field stimulus test; NSS, Not Statistically Significant

Sepofarsen Treated Patients Self-Report an Improvement in Vision on 2 Separate PROs

PGI-C - % of subjects self-reporting an improvement CFB at Month 12



VFQ-25 – composite score CFB at Month 12



Single question PGI-C

- 14/23 (61%) patients on sepofarsen self reported an improvement in their vision
- 3/12 (25%) of patients in sham reported an improvement in vision

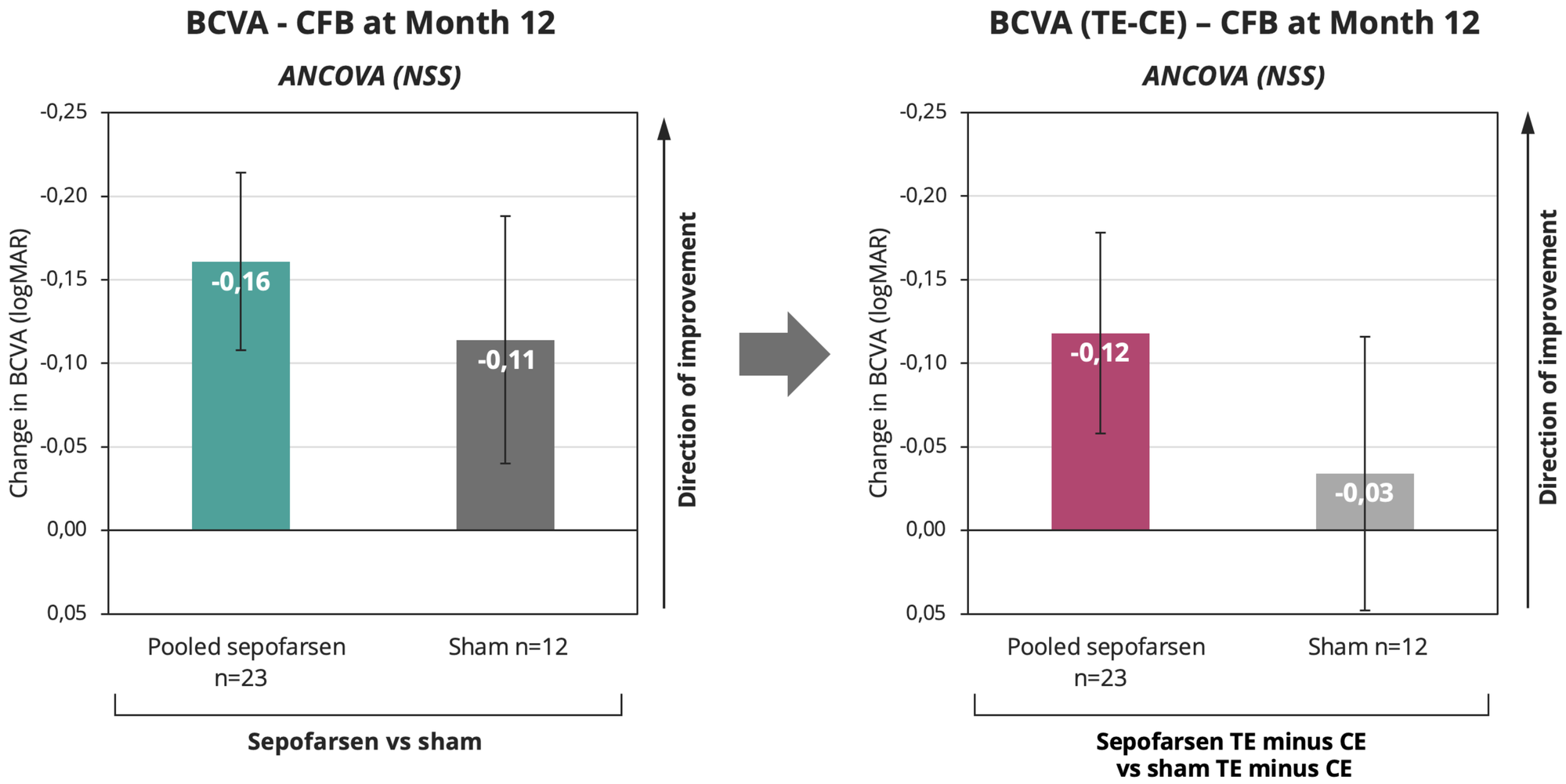
VFQ-25 composite score

- Vision subscales indicated a more pronounced benefit in sepofarsen

PGI-C and VFQ-25 were pre-specified analyses

In the TE minus CE analysis, the sepofarsen response is maintained but no longer seen in the sham group

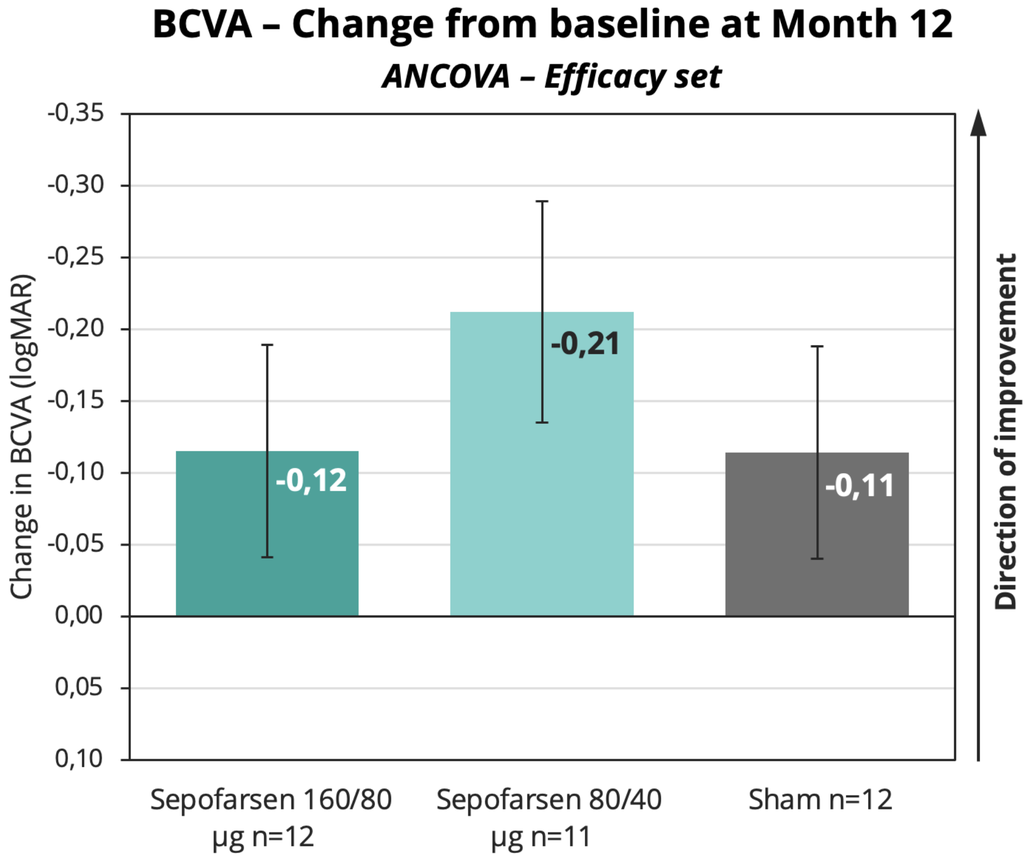
BCVA at Month 12 – Post hoc analyses

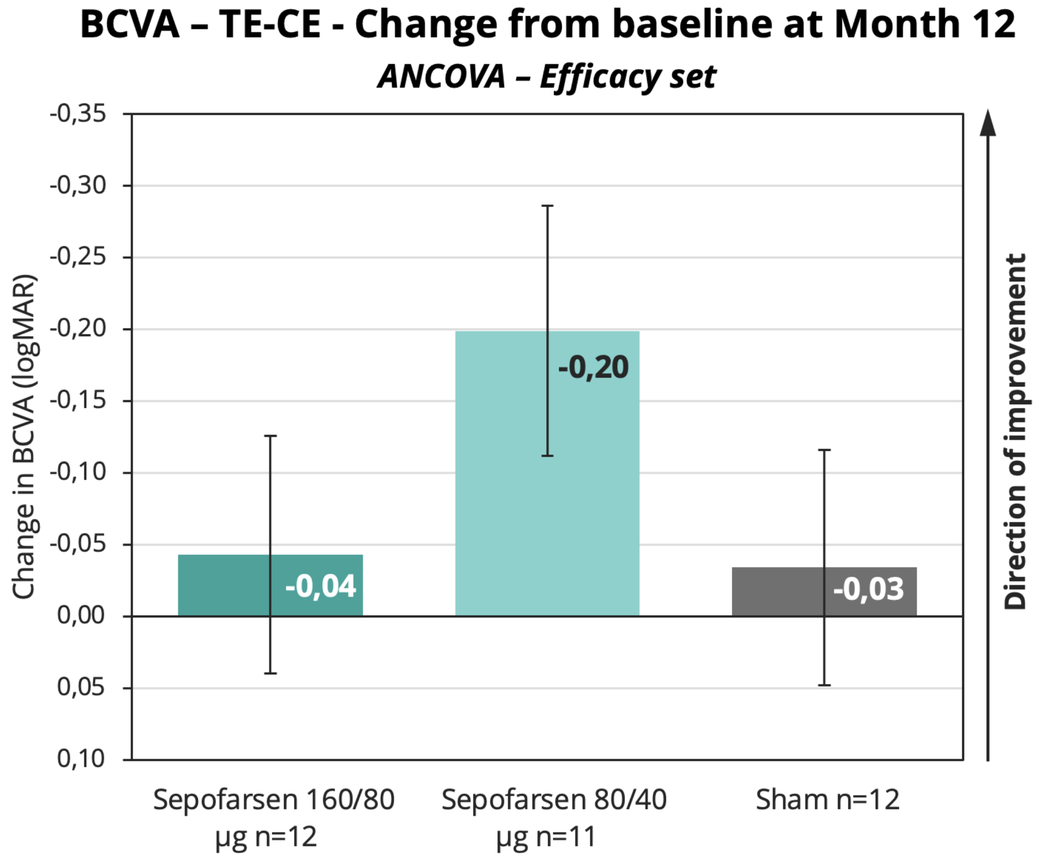


BCVA, Best corrected visual acuity; CE, Contralateral eye; CFB, Change from baseline; NSS, Not Statistically Significant; TE, Treated eye

BCVA – TE vs Sham Analysis vs TE-CE Analysis

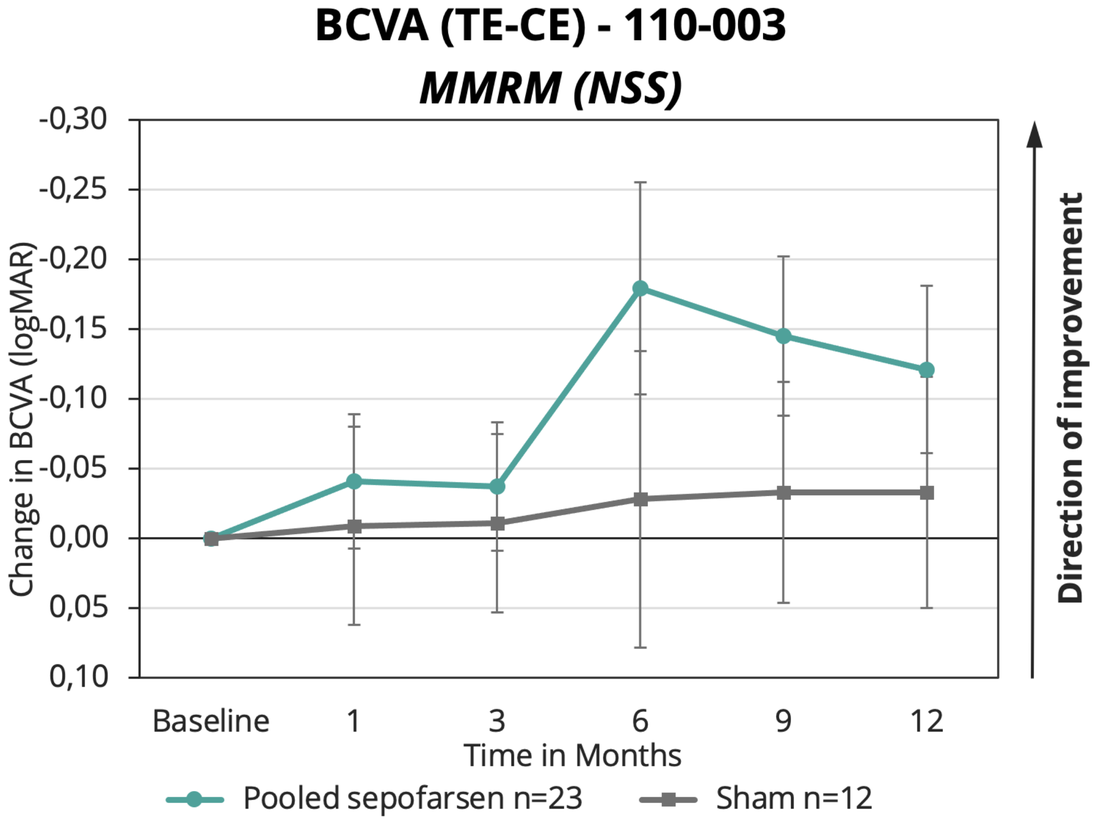
80/40 µg group demonstrates a -0.2 logMAR improvement at M12 in both analyses

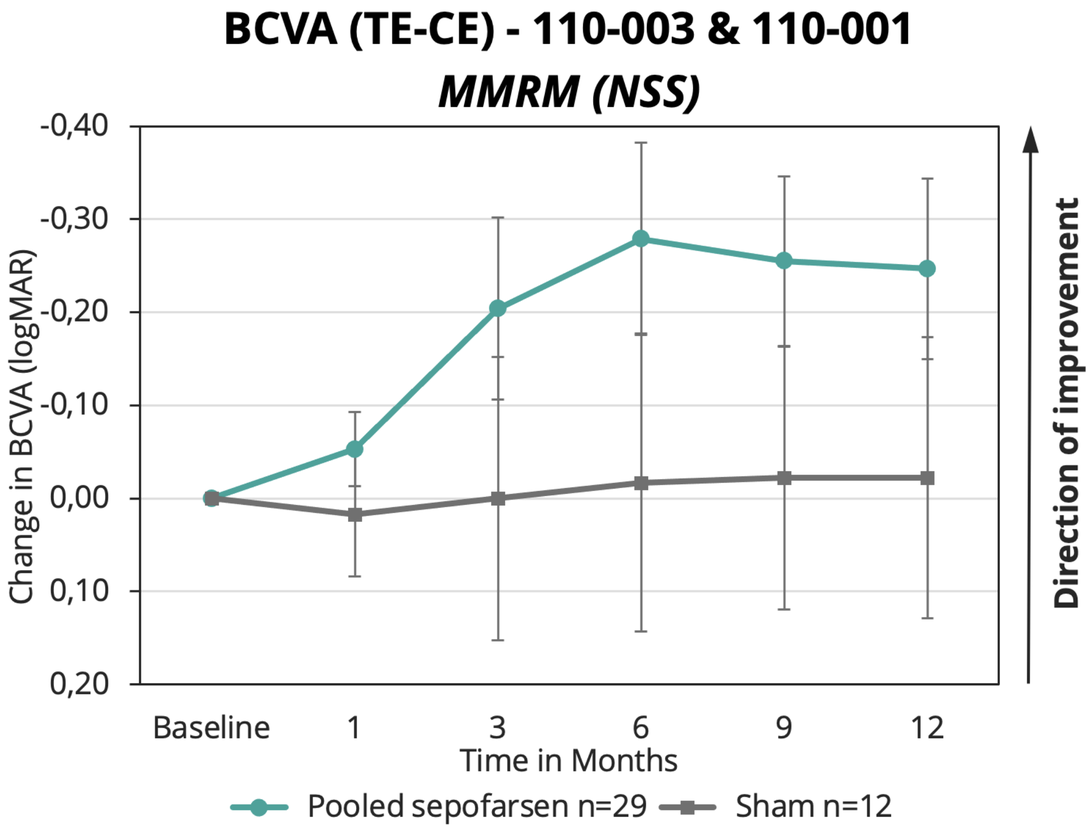




ANOVA: Analysis of Variance; BCVA: Best Corrected Visual Acuity; CE: Contralateral Eye; TE: Treatment Eye

Meta analyses using TE minus CE approach shows a benefit of -0.2 LogMAR with sepofarsen

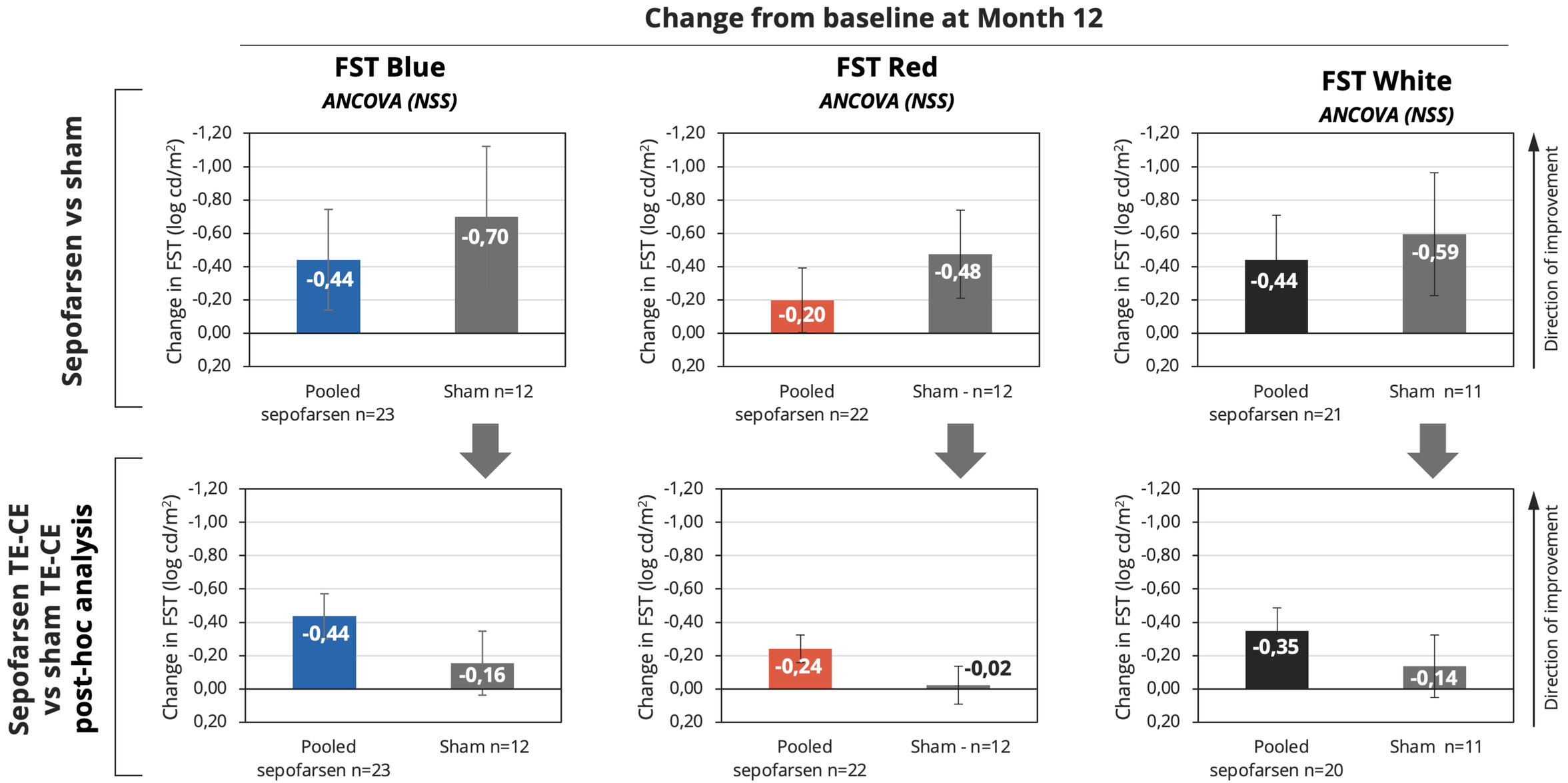




1. Russell SR, et al. Nat Med. 2022 Apr 4. Epub ahead of print

BCVA, Best corrected visual acuity; CE, Contralateral eye; CFB, Change from baseline; MMRM: Mixed Model Repeated Measures; NSS, Not Statistically Significant; TE, Treated eye

The TE minus CE analysis confirms the effect of sepofarsen in FST; but no longer seen in the Sham group



CE, Contralateral eye; CFB, Change from baseline; FST, full-field stimulus test; NSS, Not Statistically Significant; TE, Treated eye

Sepofarsen is well tolerated at M12

- Consistent with the prior studies
- Similar numbers of TEAEs in each treatment group (including sham):
  - The majority were mild in severity
  - Fewer incidences of cataract than in previous studies
  - Cataract, retinal thinning (1 SAE) and cystoid macular oedema were closely monitored.
- 3 other SAEs not related to the treatment (glaucoma linked to patient medical history, acute alcohol intoxication and epileptic seizure)

	Sepofarsen 160/80 µg (n=12)	Sepofarsen 80/40 µg (n=12)	Sham (n=12)
Any Ocular TEAE, n (%)	12 (100.0%)	12 (100.0%)	9 (75.0%)
Mild	7 (58.3%)	10 (83.3%)	7 (58.3%)
Moderate	2 (16.7%)	2 (16.7%)	2 (16.7%)
Severe	2 (16.7%)	0 (0.0%)	0 (0.0%)
Cataract Events (11 of 36 past medical history)			
Mild	2 (16.6%)	0 (0.0%)	1 (8.3%)
Moderate	0 (0.0%)	1 (8.3%)	***0 (0.0%)
Severe	1 (0.0%)	0 (0.0%)	0 (0.0%)
Cystoid Macular Edema (CME) Events (4 of 36 past medical history)			
Mild	0 (0.0%)	2 (16.7%)	0 (0.0%)
Moderate	0 (0.0%)	1 (8.3%)	1 (8.3%)
Severe	1 (8.3%)	0 (0.0%)	0 (0.0%)
Retinal Thinning Events (5 of 36 past medical history)			
Mild	**0 (0.0%)	0 (0.0%)	0 (0.0%)
Moderate	*2 (16.6%)	0 (0.0%)	1 (8.3%)
Severe	0 (0.0%)	0 (0.0%)	0 (0.0%)

** 1 Retinal thinning considered a SUSAR and participant discontinued therapy; **1 additional subject had retinal thinning post Data Lock Point; *** 1 additional subject posterior capsule opacification at Month 12 in treated CE*

TEAEs, Treatment emergent adverse events; SAEs, Serious adverse events

PQ-110-003 (Sepofarsen) Phase 2/3 Illuminate Trial

A Story of a Suboptimal Comparison

- First year results: Illuminate did not meet primary endpoint of Best-Corrected Visual Acuity (BCVA) at Month 12 compared to sham procedure control group
- Traditional analysis approach of TE vs sham is difficult to show Tx effect due to high variability & small N
- However, when adjusting TE & sham eyes by subtracting effects of their corresponding CE, a numeric treatment difference between sepofarsen & sham is observed
  - Consistent w/ Phase 1b/2 study results
  - Individual participants demonstrated improvement from baseline in multiple endpoints
  - Responses also seen in year 2 when 2nd eye/sham was treated
- Overall good safety profile: no intraocular inflammation, no systemic effects
- EMA & FDA recommended setting up another phase 2/3 trial prior to submitting Marketing Authorization Application

08.11.2022

ProQR to Focus Exclusively on Axiomer RNA-Editing Technology and Partner Ophthalmology Programs

ProQR Therapeutics provided an update on its ophthalmology programs following feedback from the European Medicines Agency (EMA) related to sepofarsen and will now focus exclusively on its Axiomer RNA-editing technology platform.

Following the results from the sepofarsen Illuminate trial, the EMA has recommended an additional clinical trial be conductedfor sepofarsen prior to submitting a Marketing Authorisation Application (MAA). In light of this feedback and in order to continue advancement of the portfolio of ophthalmic product candidates, including sepofarsen for LCA10 and ultevursen (QR-421a) for USH2A-mediated Usher syndrome and retinitis pigmentosa, the company will seek to identify a strategic partner to take the ophthalmology portfolio forward.

To preserve operating capital, and until a partner is found that can fund the clinical programs moving forward, the current ongoing trials of sepofarsen and ultevursen—including Illuminate, Insight, and Brighten for sepofarsen, along with Sirius and Helia for ultevursen—will be wound down. For people currently participating in these trials, ProQR will offer continued access to currently available sepofarsen or ultevursen.

Genetic Therapy for IRDs

Conclusions

Rx for Genetic Retinal Disease

Need For Genotyping

- Need for genotyping enormous:
  - Frequency of inherited retinal disease = 1/2500
  - World population = 7.900.000.000
  - 3.160.000 patients
- Gene-specific Rx feasible for everyone?

Genetic Therapies for IRDs

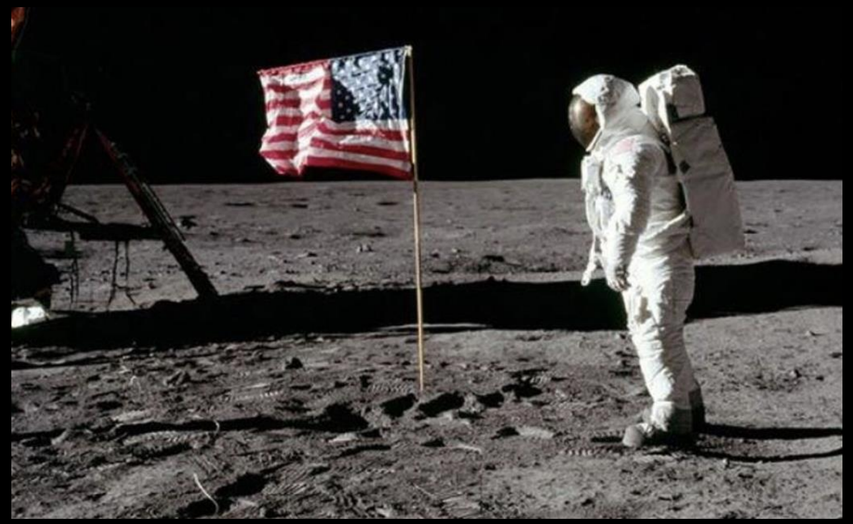
Overall Conclusions

- Very recent field (+/- 20 yrs)
- Gene Rx efforts are mushrooming (Luxturna® is 1st of many)
- Genetic Rx requires intact target cells, works but is not perfect
- A lot remains to be learned
- A difficult path lies ahead, but future is bright
- Urgent need to improve patient identification through systematic genotyping
- Better understanding of CRA & inflammation required

Genetic Therapies for IRDs

Overall Conclusions

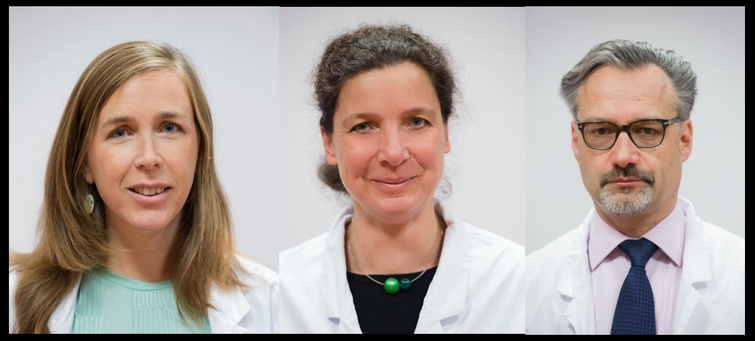
- Putting Man on the Moon was not a walk in the park
- Together, we can bring innovative Rx to IRD patients



20 Jul 1969 NASA's Apollo 11 landed on the Moon
w/ Neil Armstrong, Buzz Aldrin & Michael Collins aboard

Ghent Ocular Genetics Team

Ophthalmic Genetics & Visual Function Team



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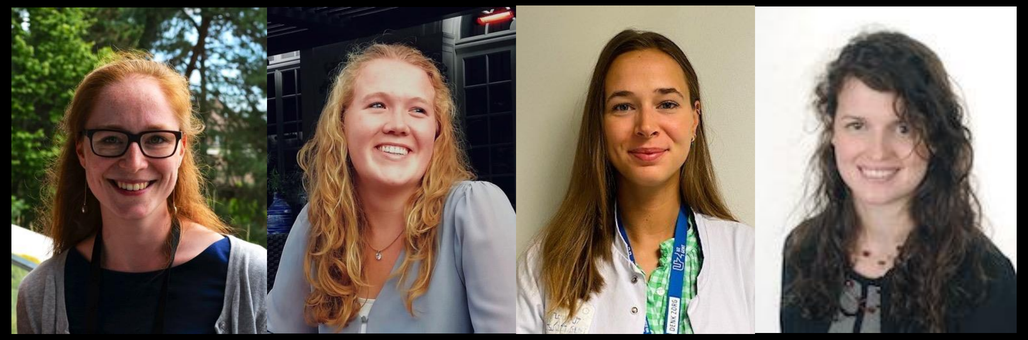

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