Fiction to Fact:
Gene Therapy for Inherited Blindness

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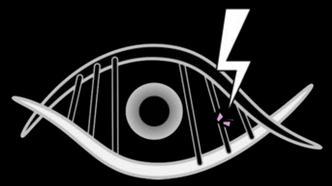

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Children's Hospital of Philadelphia

Center for Retinal and Ocular Therapeutics
PennCAROT

European Reference Network
for rare or low prevalence complex diseases
Network Eye Diseases (ERN-EYE)

Bart P LEROY, MD, PhD

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- **Bayer**: consultancy fees
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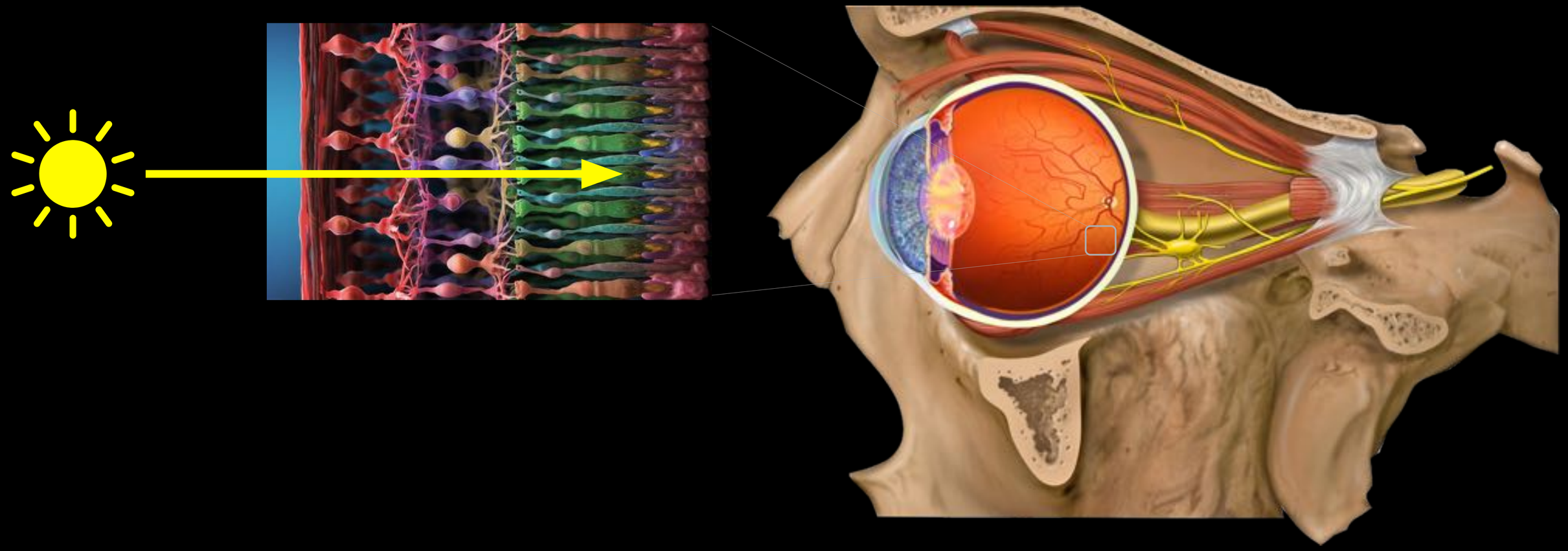
- **MeiraGTx**: trial support
- **Novartis Pharma International & Belgium**: consultancy fees, travel support
- **Oxurion**: consultancy fees
- **ProQR Therapeutics**: consultancy fees, travel support, trial support
- **Spark Therapeutics Inc**: consultancy fees, travel support
- **REGENXBIO**: consultancy fees
- **Vedere Bio**: consultancy fees
- **ViGeneron**: consultancy fees
- No personal financial gain; all consultancy fees paid into Ghent Univ Hosp research accounts

Human Retina



Eye translates light into electricity

Introduction
Retinal Cells & Circuitry

Ganglion cells

Amacrine cells

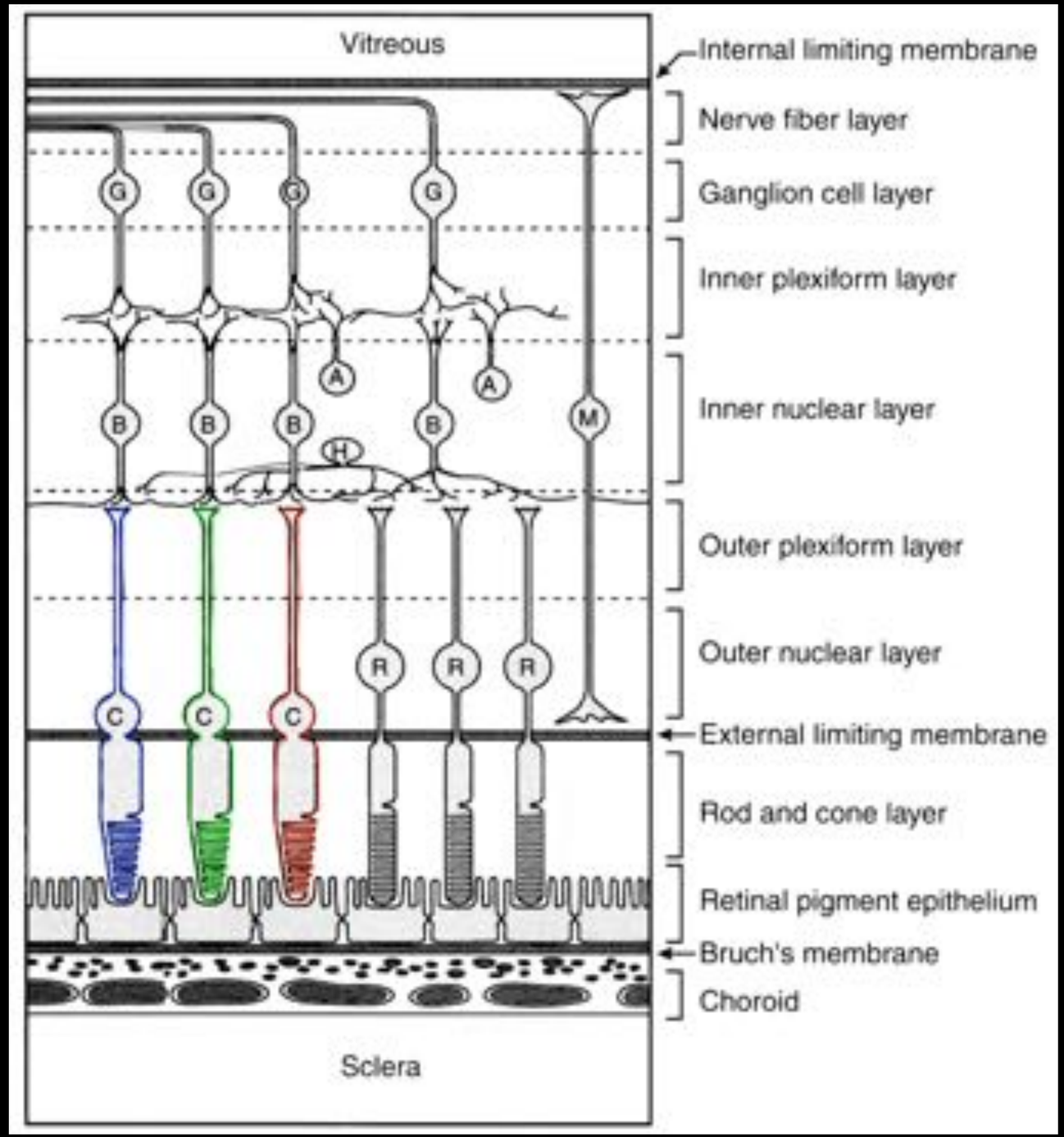
Bipolar cells

Horizontal cells

Photoreceptor cells
(cones & rods)

Retinal pigment epithelium

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



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: 316 genes (280 cloned) (https://sph.uth.edu/retnet)

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

FDA (2017) & EMA (2018) Approval of AAV2-CBA-*RPE65* (voretigene neparvovec - Luxturna®)

LUXTURNA™
voretigene neparvovec-rzyl
for subretinal injection

Spark™
THERAPEUTICS

1st Ocular Gene Therapy To Be Approved

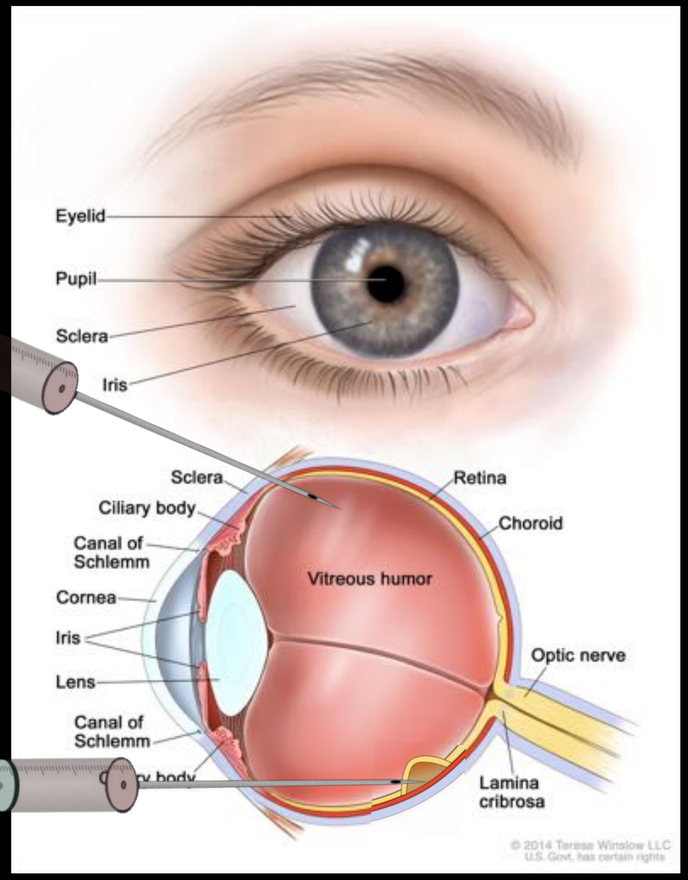
Gene & Genetic Rx for IRDs

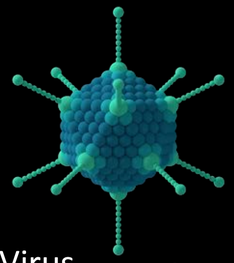
Eye = Ideal Treatment Target

- 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 Rx
Mechanisms

- Gene supplementation > use vector to add correct copy of gene
- Gene silencing > use RNAi to decrease, eradicate or correct protein production
- Gene replacement/correction > use CRISPR/Cas9 to adapt native gene

Rx Options for IRDs

- Gene Therapy
  - *RPE65*-related Inherited Retinal Dystrophy
  - *ND4*-related Leber Hereditary Optic Neuropathy
  - *CEP290*-related Leber Congenital Amaurosis
  - Other Conditions
- Cell Therapy
- Bionic Vision

Rx Options for IRDs

- Gene Therapy
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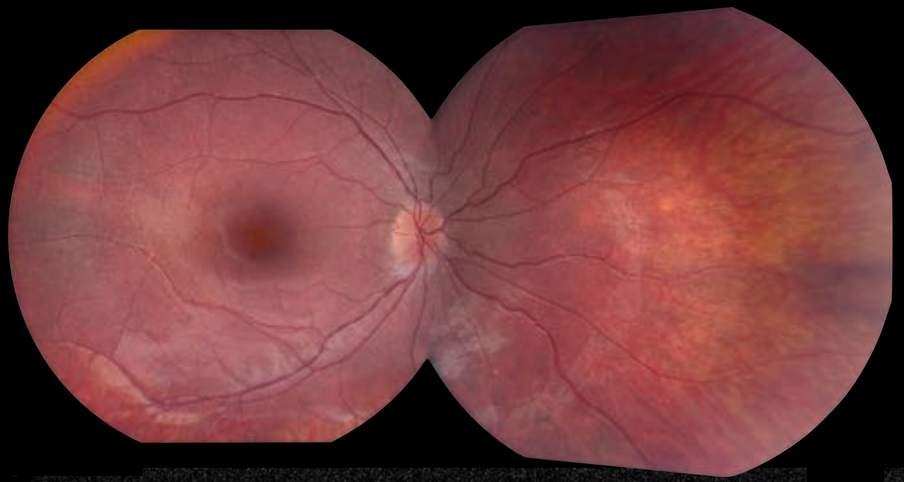
  - Other Conditions
- Cell Therapy
- Bionic Vision

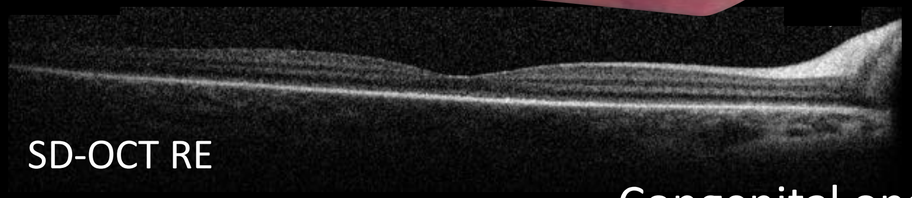
RPE65-related Retinal Dystrophy

Phenotype

F, 4 4/12 yrs
EORD



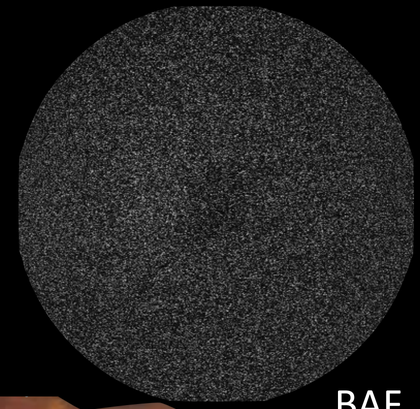
Early Stage
Phenotype



SD-OCT RE

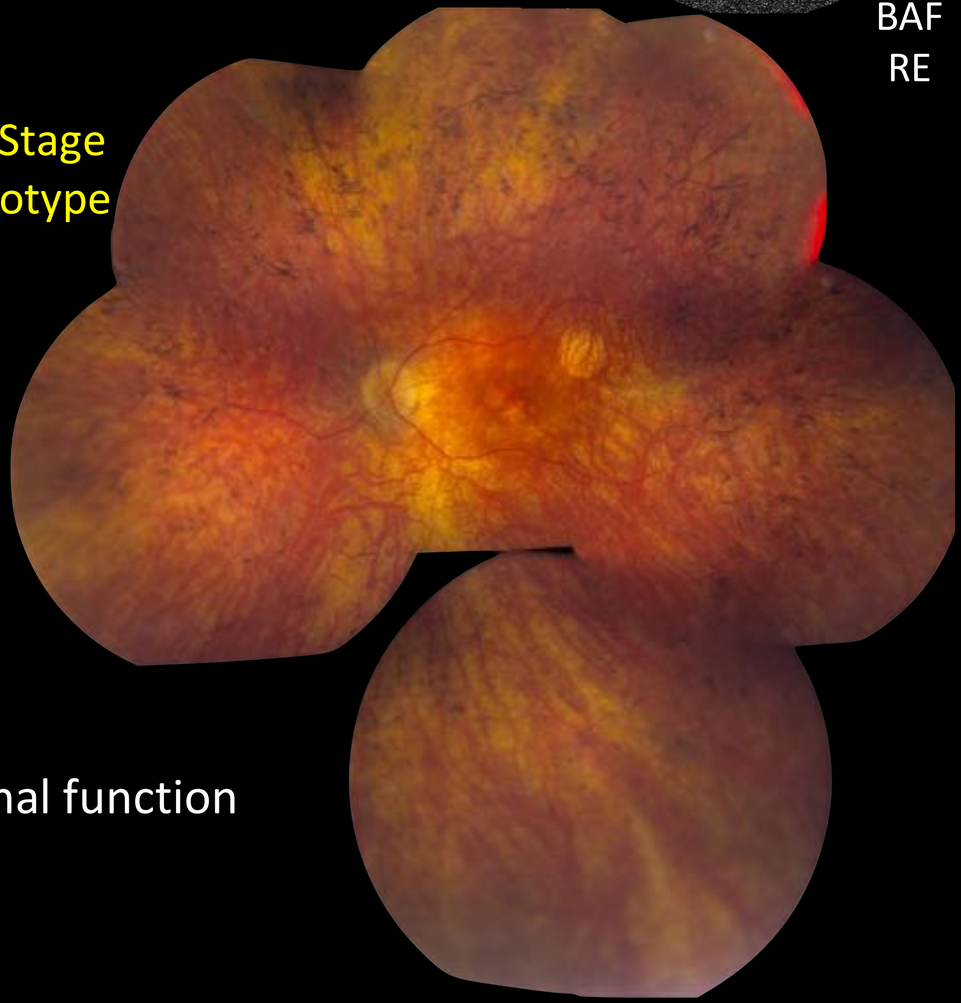
BAF RE

F, 29 yrs
EORD



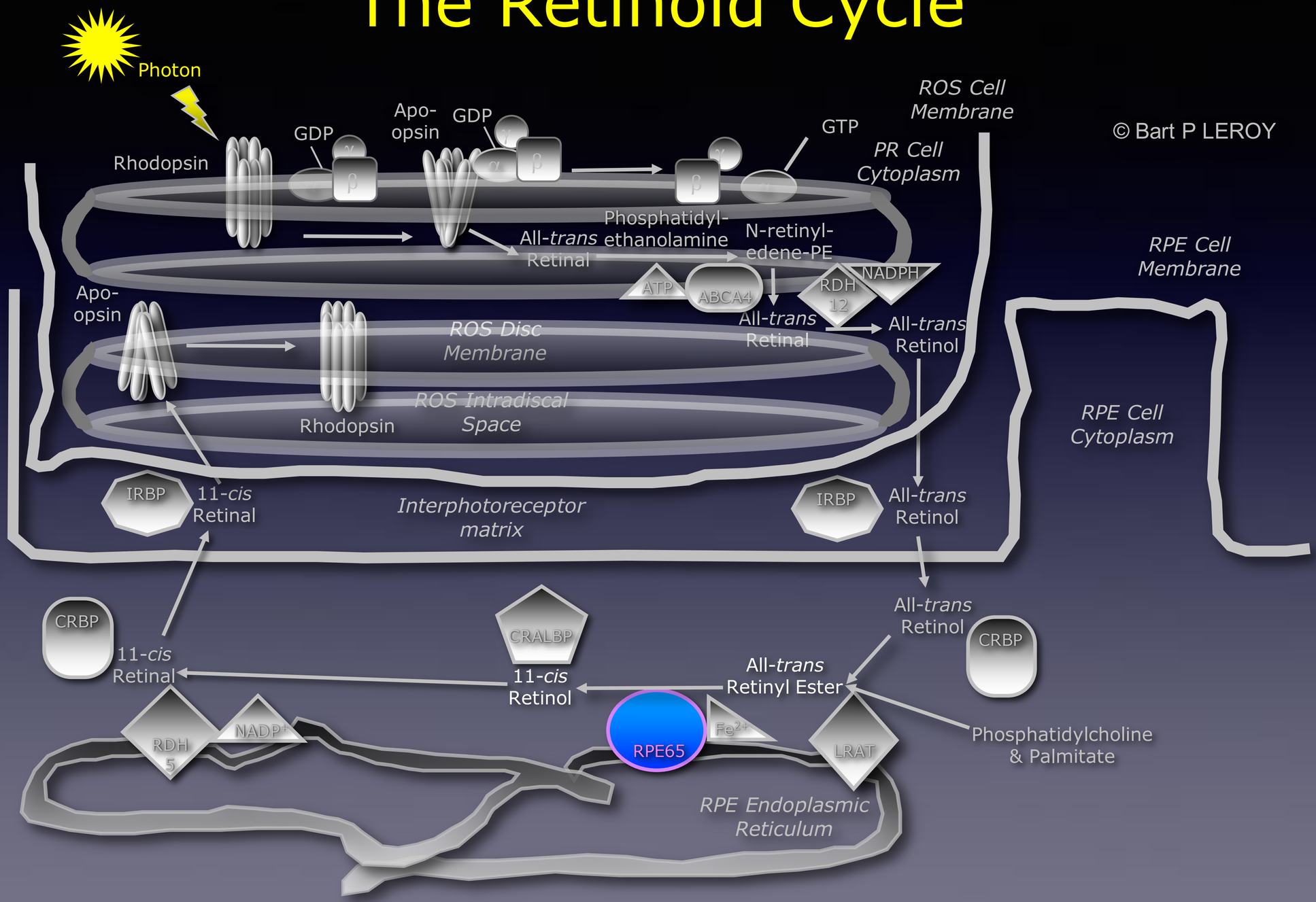
BAF
RE

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

The Retinoid Cycle



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

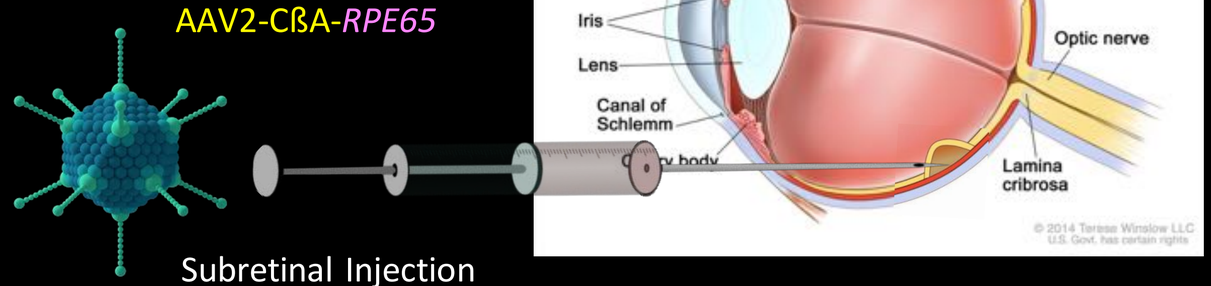
J Bennett, J Wellman, KA Marshall, S McCague, M Ashtari, J DiStefano-Pappas, OU Elci, DC Chung, J Sun, JF Wright, DR Cross, P Aravand, LL Cyckowski, JL Bennicelli, F Mingozzi, A Auricchio, EA Pierce, J Ruggiero, BP Leroy, F Simonelli, KA High, AM Maguire: Safety and durability of effect of contralateral-eye 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



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**)

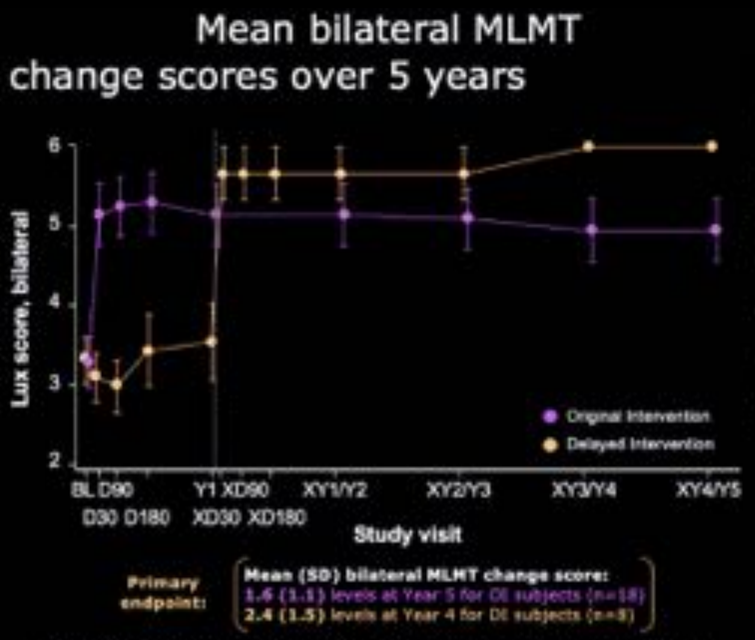
S Russell, *et al.*: Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in subjects with *RPE65*-mediated inherited retinal dystrophy: a randomised, controlled, open-label phase 3 trial, Lancet, 2017

Gene Rx Phase 3

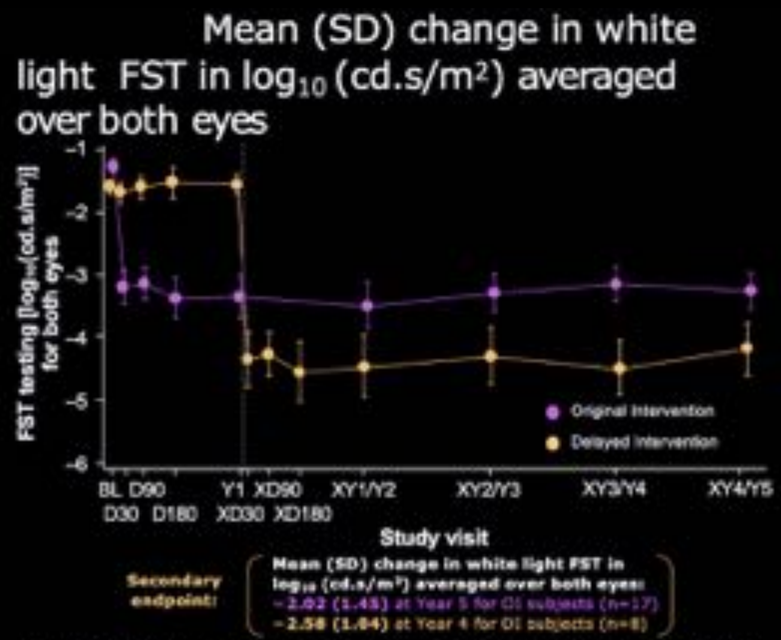
5/4 yrs Results

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

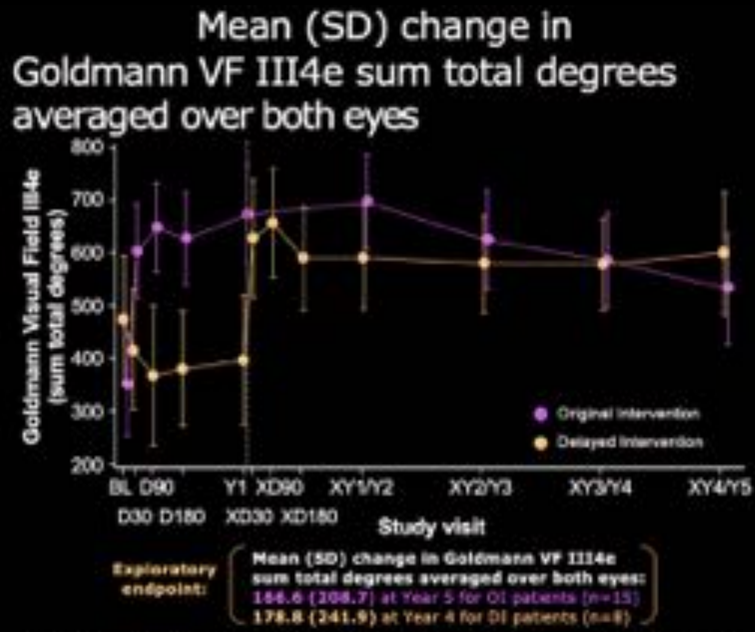
S Russell, *et al.*: Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in subjects with *RPE65*-mediated inherited retinal dystrophy: a randomised, controlled, open-label phase 3 trial, Lancet, 2017



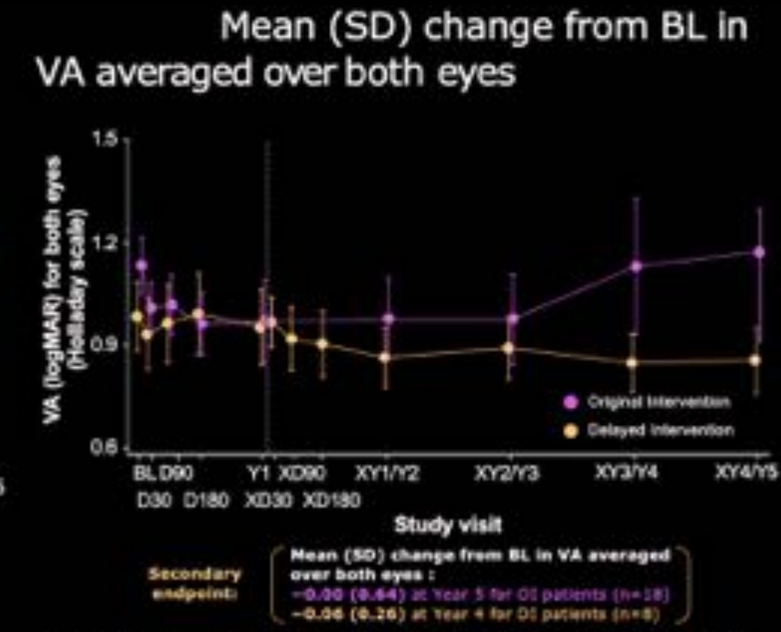
Subjects demonstrated durable improvements in bilateral MLMT change score over 5 years



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



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



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

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

LUXTURNA™
voretigene neparvovec-rzyl
for subretinal injection

Spark™ THERAPEUTICS

Courtesy of Shannon L Boye, PhD

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)

GU & GHU
Dept of Ophthalmology

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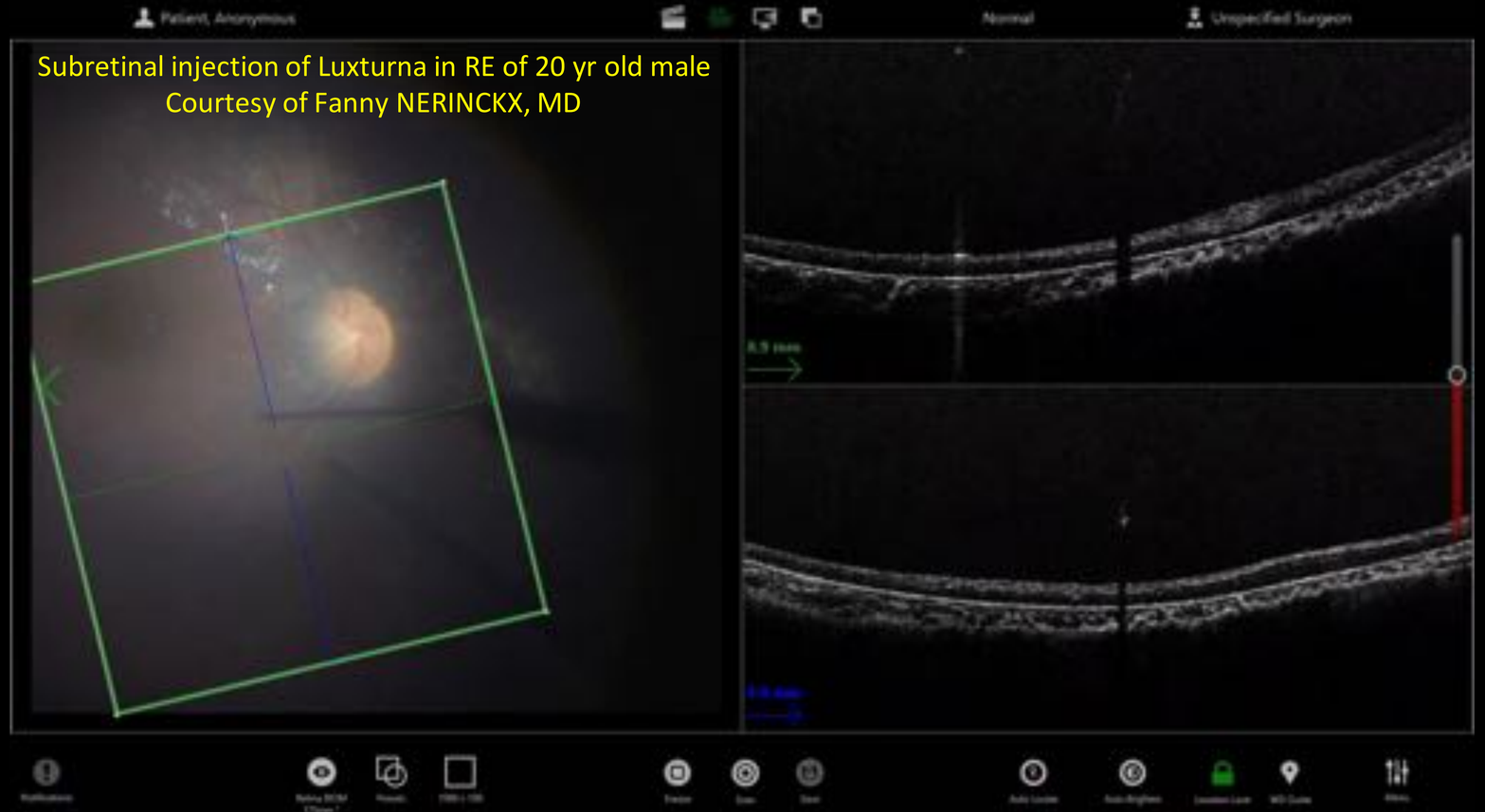
National Referral Center for Ocular Genetics & Gene Therapy

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

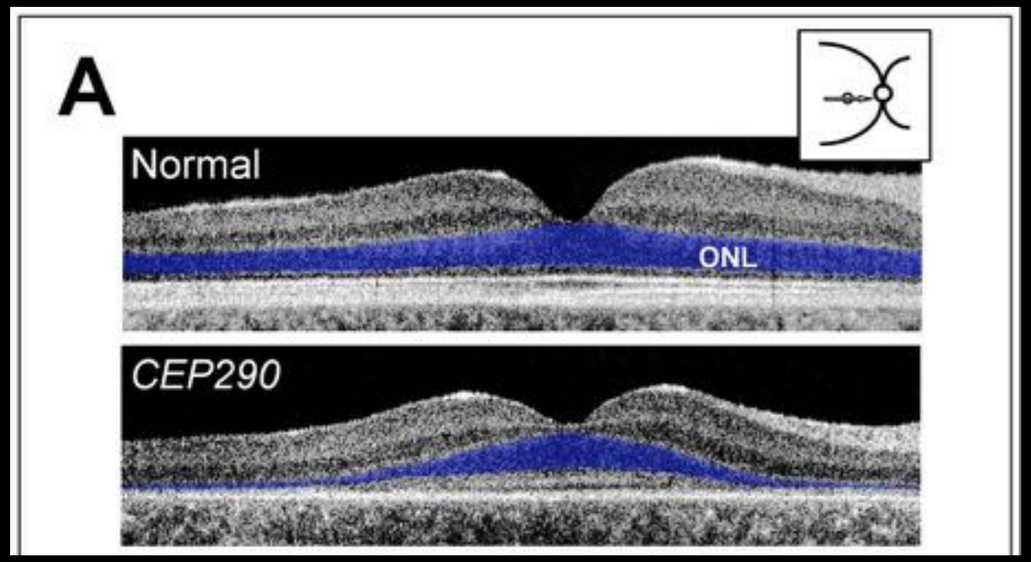


Rx Options for IRDs

- Gene Therapy
  - *RPE65*-related Inherited Retinal Dystrophy
  - *ND4*-related Leber Hereditary Optic Neuropathy
  - *CEP290*-related Leber Congenital Amaurosis
  - Other Conditions
- Cell Therapy
- Bionic Vision

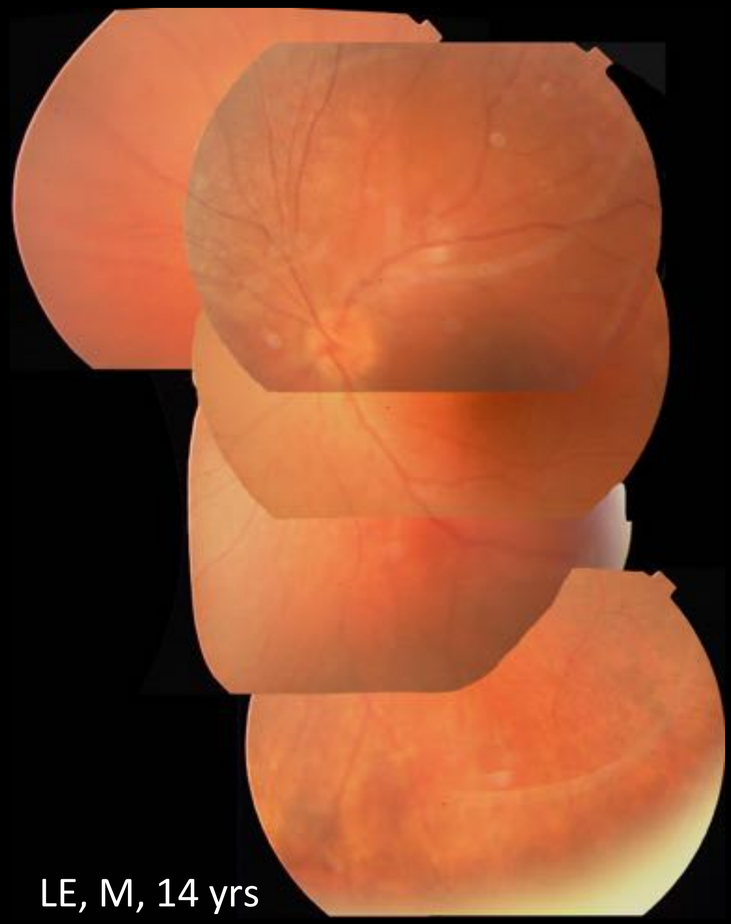
CEP290-LCA10

Severe Phenotype

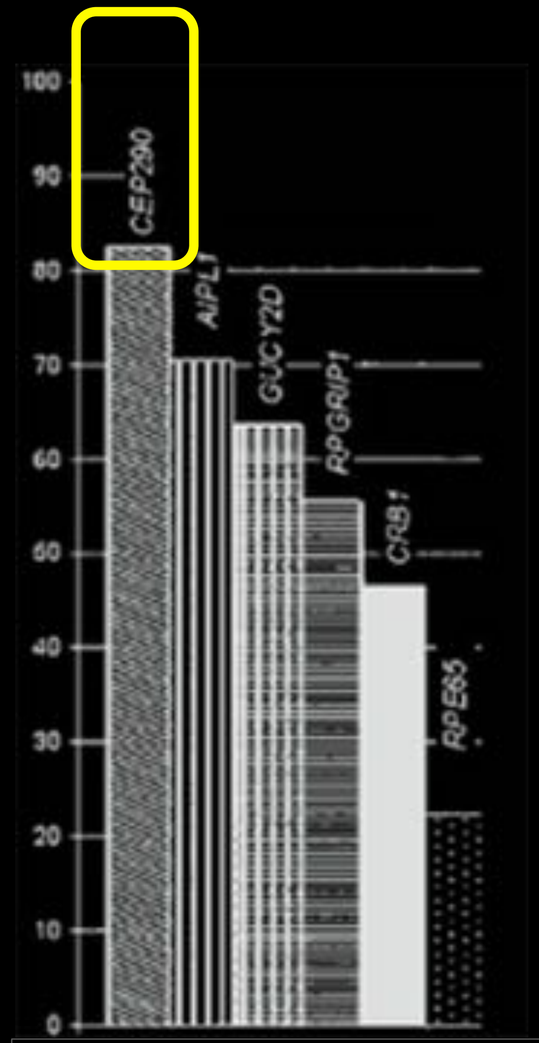


Retained central retinal photoreceptors & RPE disproportionate to low level of vision

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

CEP290-LCA10
Unmet Need

- Bi-allelic mutations in *CEP290* gene
  - Most frequently occurring mutation = c.2991+1655A>G
    - Accounts for up to 21% of all LCA cases & leads to inclusion of cryptic exon X
    - *CEP290* c.2991+1655A>G mutation identified in >50% of LCA10 patients
- Lack of functional CEP290 protein leads to disruption of phototransduction & PR demise
- Currently no approved treatments available

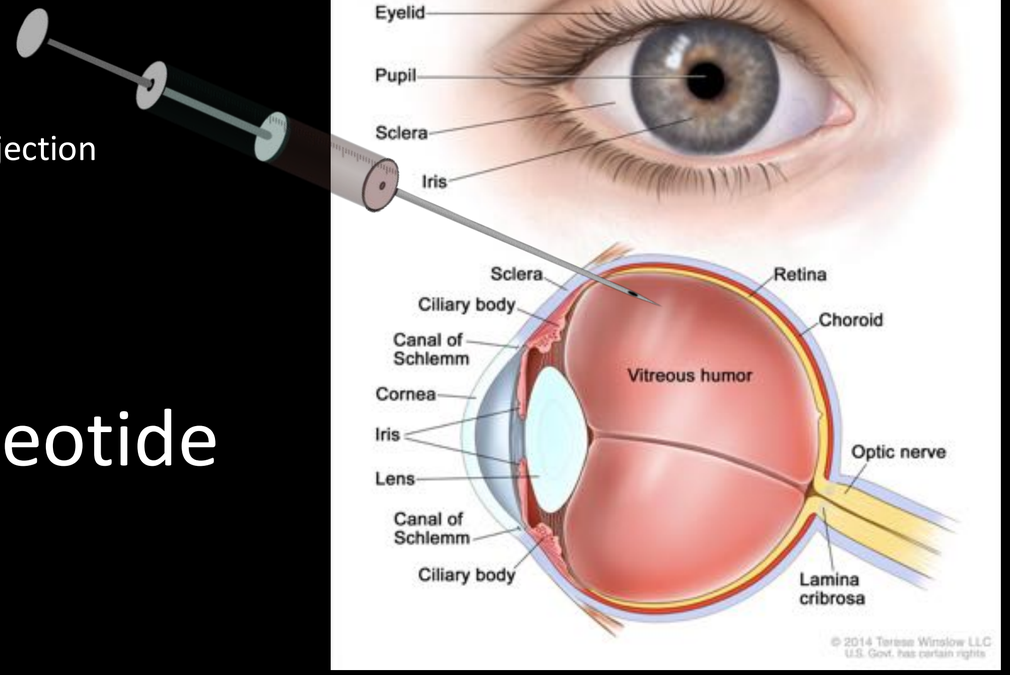
Genetic Rx

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

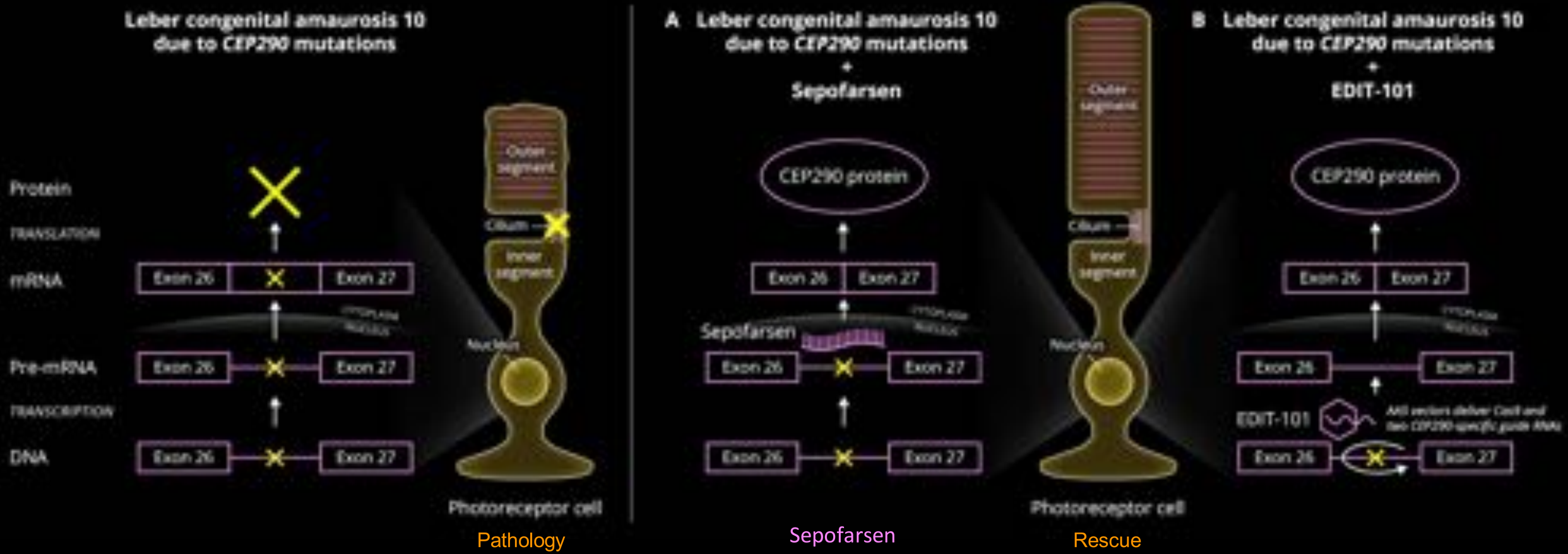
CEP290-LCA10

Splice Correction for p.Cys998X *CEP290* mRNA

CEP290-IRD

Rx w/ Sepofarsen (AON)

Rx w/ EDIT-101 (CRISPR/Cas9)



Pathology

Sepofarsen
= 17-mer antisense oligonucleotide (AON)

Rescue

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

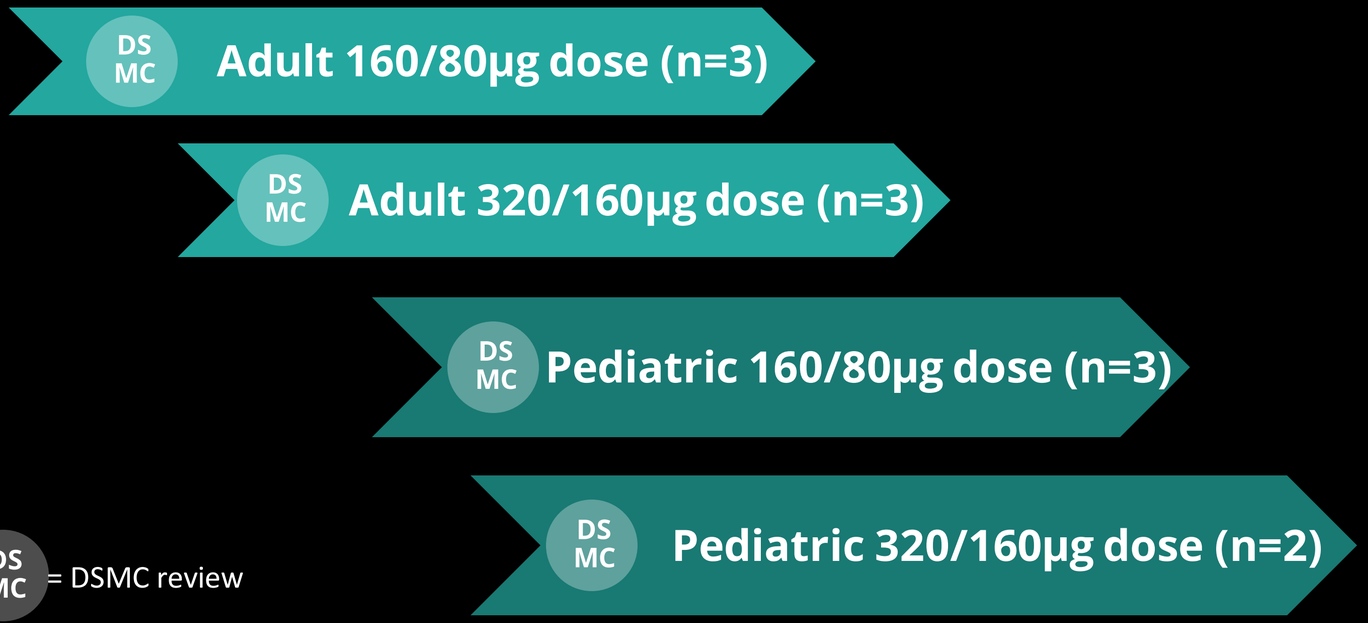
PQ-110-001 Phase 1/2 Trial Design

First-in-Human, Open Label, Multiple Dose, Dose Escalation Trial

Screening baseline

Treatment Period: 12 months
First Treated Eye: Worse seeing eye

Roll-over to extension
+ 2nd eye treatment



DS MC = DSMC review

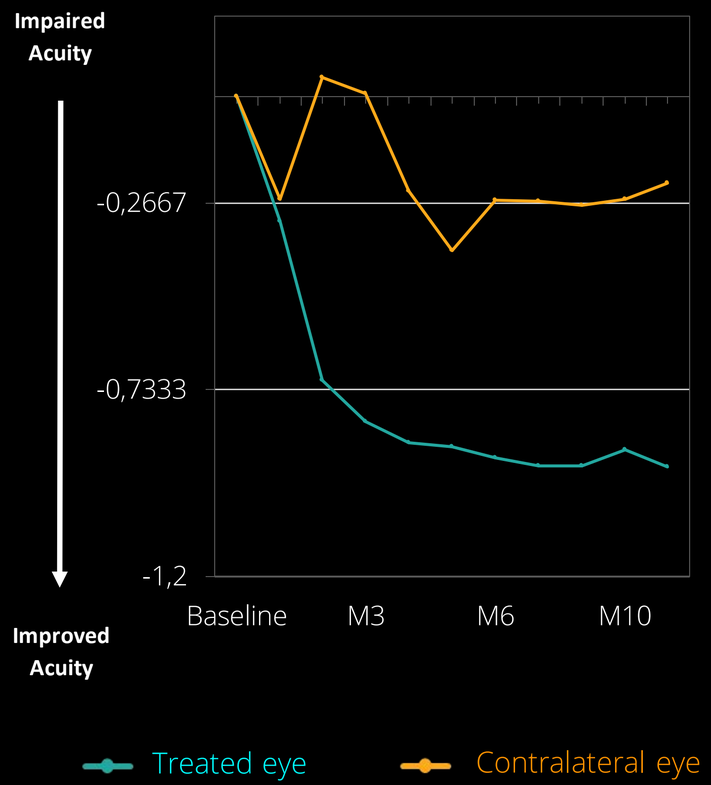
- Enrolled 11 LCA10 patients (age range 8-44) homozygous or compound heterozygous for c.2991+1655A>G (p.Cys998X) mutation
- Up to 4 intravitreal injections to study eye, defined as worse-seeing eye
- Increase retinal sensitivity in 11/11 subjects & clinically meaningful BCVA gains in 7/11 subjects (reported at ARVO 2020)

AON Dx *CEP290* study (NCT03140969) conducted at *Scheie Eye Institute*, UPenn, Philadelphia, PA, USA, *University of Iowa*, Iowa City, IA, USA & Ghent University, Hospital, Ghent, Belgium

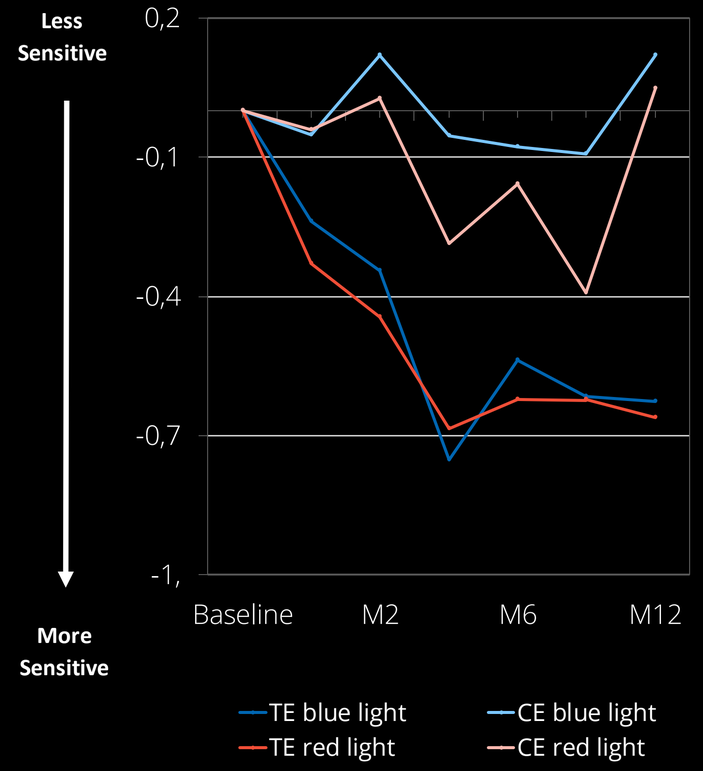
Key Outcome Measures Change Month 12

Target registration dose level: 160µg/80µg (n=6)
Every six-month dosing interval - maintained benefit

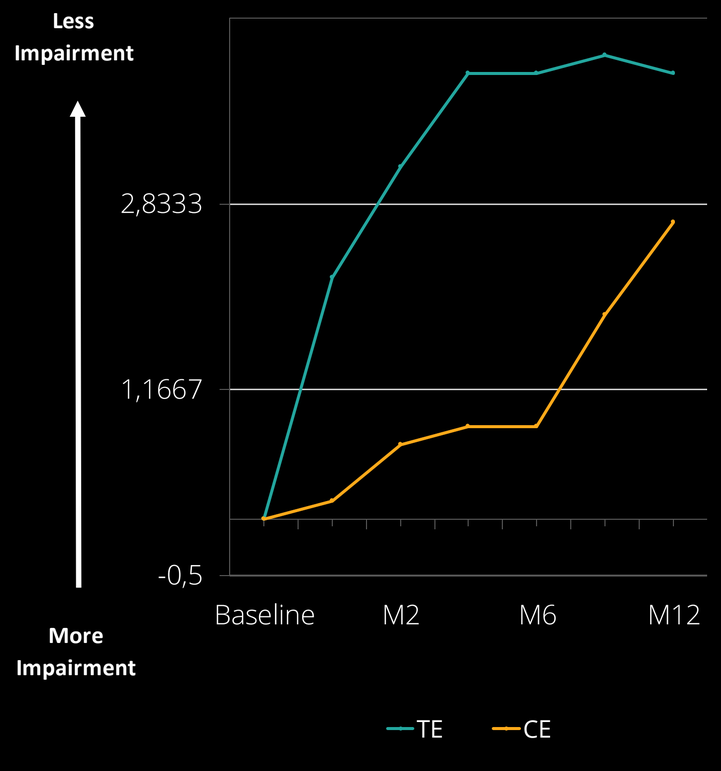
ΔBCVA (LogMAR)



ΔFST (cd/m2)



ΔMobility (Levels)



PQ-110-001 Phase 1/2 Trial Design

First-in-Human, Open Label, Multiple Dose, Dose Escalation Trial

Screening
Baseline

Treatment Period: 12 months
First Treated Eye: Worse Seeing Eye

Roll-over to Extension
+ 2nd eye Treatment

Extension Study

DSMC **Adult 160/80µg dose (n=3)**

DSMC **Adult 320/160µg dose (n=3)**

DSMC **Pediatric 160/80µg dose (n=3)**

DSMC **Pediatric 320/160µg dose (n=2)**

Extension trial 160/80µg dose

Analysis 3-month Data + 2nd eye

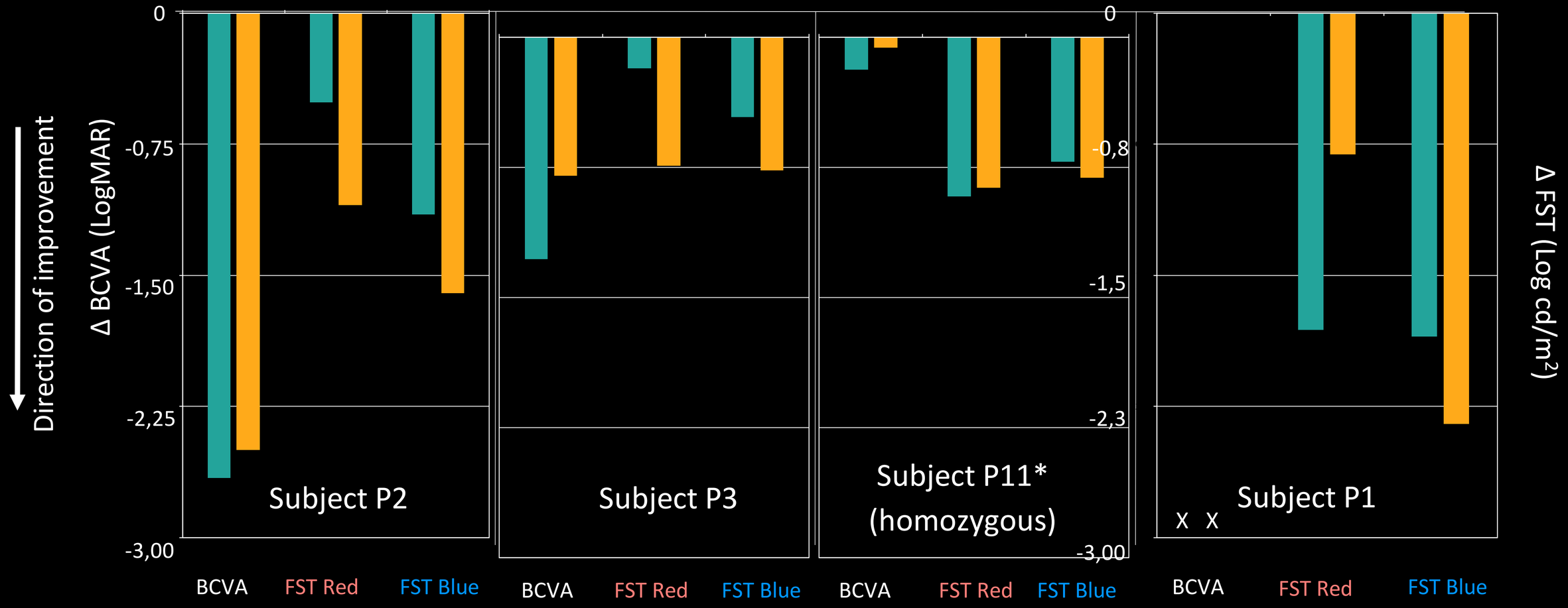
DSMC = DSMC review

inSight

Phase 1/2 Extension Study

Change from Baseline to 3 Mths Post Dosing

Consistent Treatment Response in Both Eyes



*= 6 month value of 2nd eye as 3 month visit was missed due to COVID-19

Preliminary data – July 2020

AON Therapy for *CEP290*-IRD

Conclusions

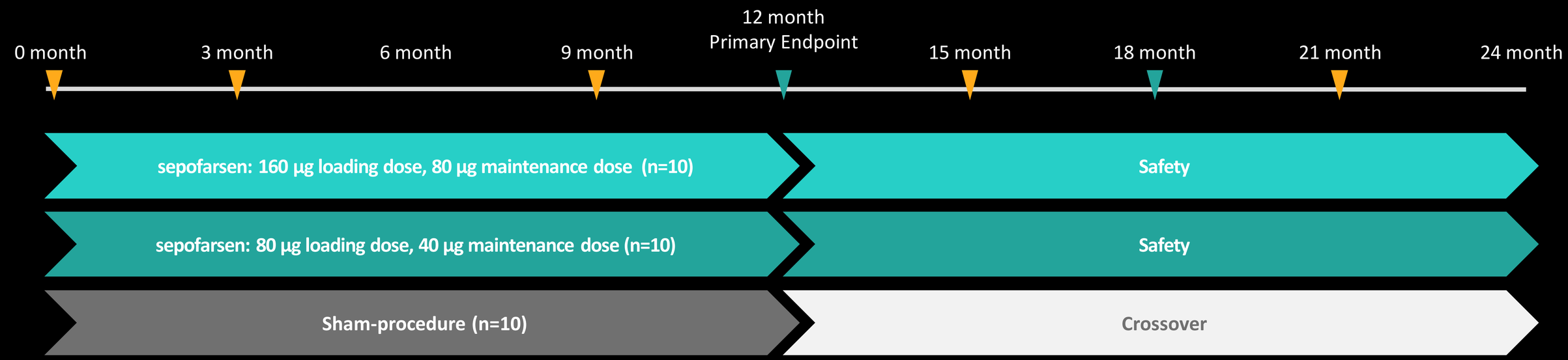
- Safety profile in target dose of 160/80 µg is consistent w/ Ph1b/2 safety data
  * available data confirm manageable safety profile of IVT sepofarsen

- Efficacy data in 2nd eye strongly corroborate clinically meaningful vision improvements observed in Ph1b/2 trial
  * 4 out of 4 second eyes responded to Rx (in BCVA or retinal sensitivity) to a similar extent as compared to initially treated eyes

- Further analyses are expected on ongoing extension trial (INSIGHT; NCT03913130) & phase 2/3 trial (ILLUMINATE; NCT03913143)

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

Actively Enrolling



= Dose 1st eye *= Dose 2nd eye*

- Double-masked, randomized, controlled, 12-month, multiple dose study
- Could serve as the sole registration trial
- Sites in North America and select EU countries

- 30+ patients >8 years old
- Multiple IVT injections in both eyes
- First patient dosed in April 2019
- Primary (registration) endpoint:
  - Visual Acuity (ETDRS, BRVT)

- Key secondary endpoints
  - Multiluminance mobility test score (MLMT)
  - Full field stimulus testing (FST)
  - Ocular instability (OCI)
  - Optical coherence tomography (OCT)

Illuminate

Gene Therapy in Ghent

Other Trials

- mtND4-LHON: Gensight: AAV2 lenadogene nolparvovec (Lumevoq) (NCT03406104)
- *RPGR*-XLRP: MeiraGTx/Janssen: AAV5.hRPGR Phase 3 (NCT03252847)
- *CNGA3*-related achromatopsia: MeiraGTx/Janssen: AAV8-hG1.7-hCNGA3 Phase 1/2 (NCT03758404)
- *CNGB3*-related achromatopsia: MeiraGTx/Janssen: AAV8-hCAR-hCNGB3 Phase 1/2 (NCT03278873)

Gene Therapy: Future

Conclusions

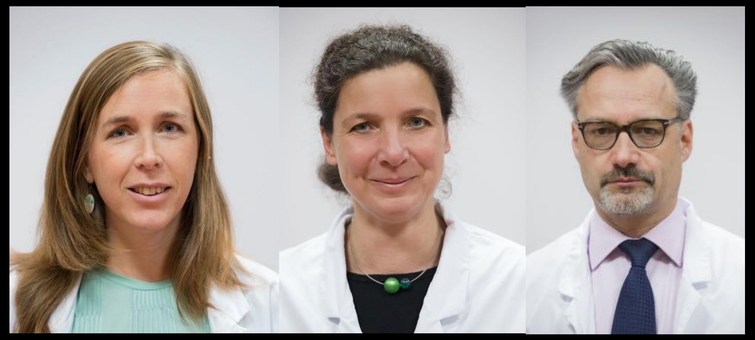
- Dept of Ophthalmology @ GUH is National Belgian Referral Centre
- The future for ocular gene therapy is bright:
  - Luxturna is first approved ocular gene therapy
  - Ongoing clinical trials:
    - Gene augmentation therapies for LHON, CHM, XLRP, XLRS, ACHR, …
    - Antisense OligoNucleotide (AON) therapies for *CEP290*-LCA10, *USH2A*-IRD
    - AAV5-based CRISPR/Cas9 therapy for *CEP290*-LCA10
    - Optogenetic approaches w/ e.g. channelrhodopsins from algae

Need to Improve Patient Identification

Ocular Genetics & Gene Rx in Ghent

Team

Ophthalmic Genetics & Visual Function Team



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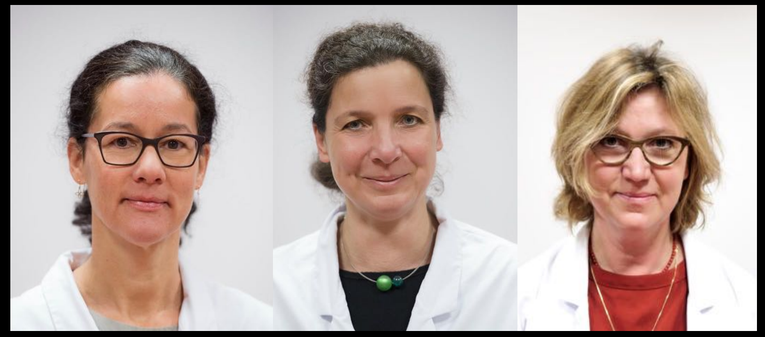

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Gene Rx for RPE65-IRD @ GU & GHU

Patient's Experience