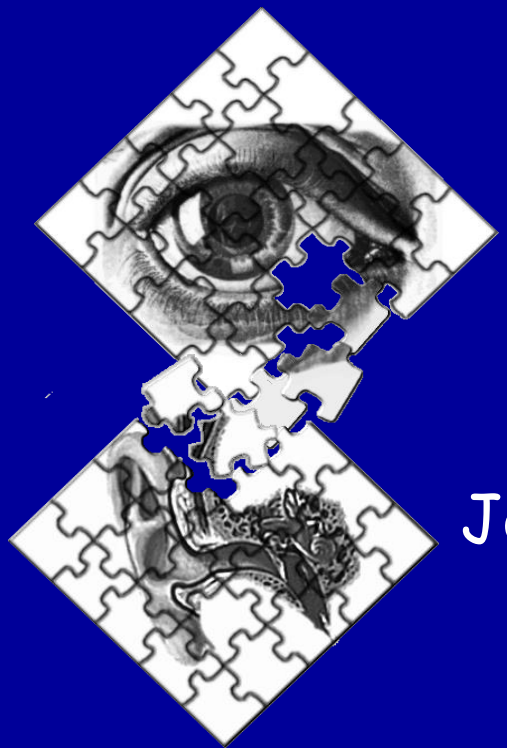
# Ignore the stop: translation read-through of nonsense mutations in Usher syndrome genes

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No STOP

#USH2014

International Symposium on Usher Syndrome | July 10-11, 2014

6th Annual Usher Syndrome Family Conference | July 12, 2014

BOSTON, MASSACHUSETTS, USA

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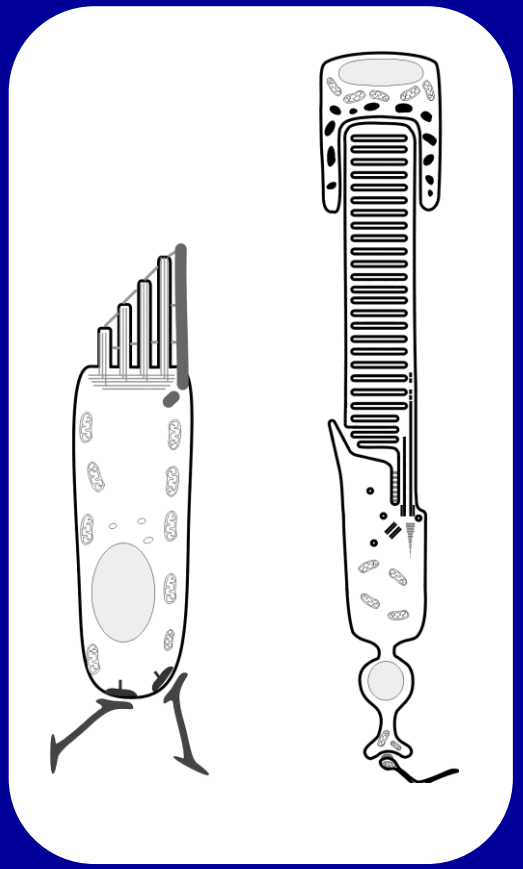
# Human Usher syndrome (USH)

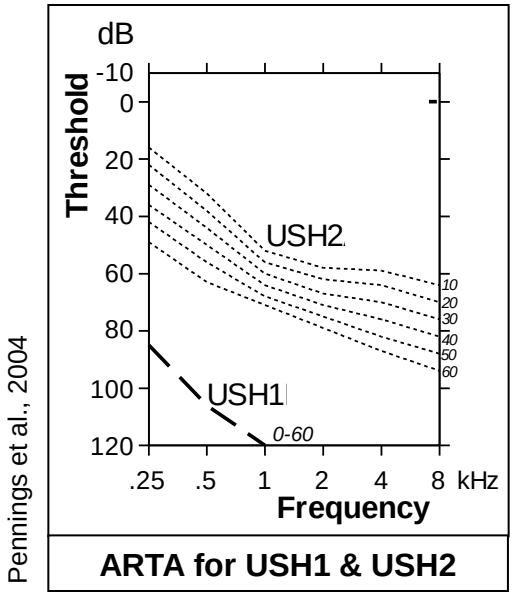
- USH is the most common form of combined hereditary deaf-blindness.
- Prevalence: ~ 1:6,000
- Autosomal recessive disorder
- Symptoms:
  - Hearing impairment
  - Vestibular dysfunction
  - Vision loss - Retinitis pigmentosa
- 3 clinical types (USH1-3) based on severity, age of onset and progression of symptoms.



- 10 USH causing genes are identified.
- USH proteins are members of different protein families.

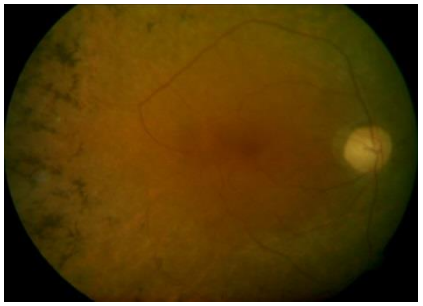
# Diagnosis and treatment of USH patients

## Hearing impairment:
### newborn screening

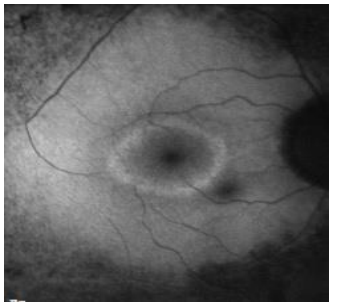



## Vision loss:
### diagnosed in puberty

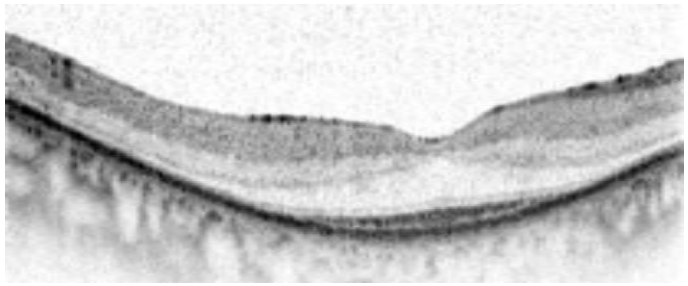
fundus photography



autofluorescence



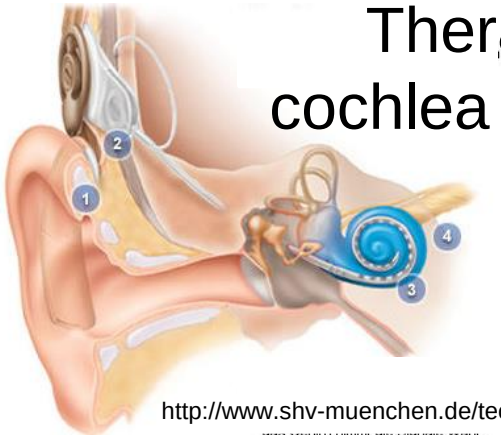
OCT



**USH1C patient**, 35 year male (right eye)

Becker et al., in prep

## Therapy:
## cochlea implant




http://www.shv-muenchen.de/technik/allgemeine-infos/ci

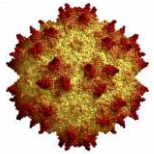
**Currently no therapy for vision loss in USH.**

# Currently evaluated gene based strategies by therapy team JGU Mainz:

K. Nagel-W.

- **Gene addition via adeno-associated virus (AAV)**

**Mirjana Becker**



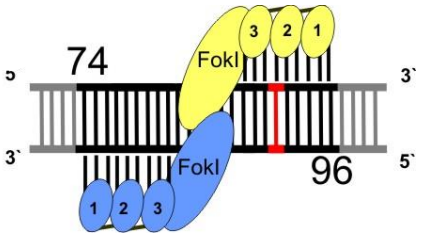
Treatrush
FIGHTING BLINDNESS OF USHER SYNDROME

FAUN

- **Gene editing/correction via homologous recombination mediated by zinc finger nucleases (ZFN) and transcription activator-like effector nucleases (TALENs)**

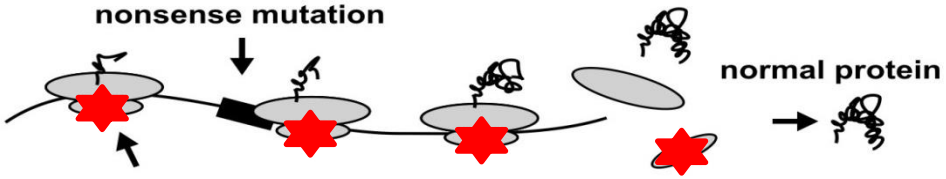
Overlack et al. IOVS 53:4140 (2012)

**Kifai Khan**




FAUN

- **Suppression of a nonsense mutation by translational read-through inducing drugs (TRIDs)**




ur-USH

E-Rare

Bundesministerium für Bildung und Forschung

FAUN

**Fabian Möller (Poster # 17), Inessa Penner, Ananya Samanta**

Goldmann et al. EMBO Mol Med 4:1186 (2012) ; Hum Gene Ther 22:537 (2011); IOVS 51:6671 (2010)

FOUNDATION FIGHTING BLINDNESS

Syscilia

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# Successful gene therapy approaches in LCA2 patients using AAV

Gene Therapy for Leber Congenital Amaurosis Caused by *RPE65* Mutations

*Safety and Efficacy in 15 Children and Adults Followed Up to 3 Years*

*Samuel G. Jacobson, MD, PhD; Artur V. Cideciyan, PhD; Ramakrishna Ratnakaram, MD; Elise Heon, MD; Sharon B. Schwartz, MS, CGC; Alejandro J. Roman, MS; Marc C. Peden, MD; Tomas S. Aleman, MD; Sanford L. Boye, MS; Alexander Sumaroka, PhD; Thomas J. Conlon, PhD; Roberto Calcedo, PhD; Ji-Jing Pang, MD, PhD; Kirsten E. Erger, BS; Melani B. Olivares, BA; Cristina L. Mullins, BA; Malgorzata Swider, PhD; Shalesh Kaushal, MD, PhD; William J. Feuer, MS; Alessandro Iannaccone, MD, MS; Gerald A. Fishman, MD; Edwin M. Stone, MD, PhD; Barry J. Byrne, MD, PhD; William W. Hauswirth, PhD*

Effect of Gene Therapy on Visual Function in Leber's Congenital Amaurosis

James W.B. Bainbridge, Ph.D., F.R.C.Ophth., Alexander J. Smith, Ph.D., Susie S. Barker, Ph.D., Scott Robbie, M.R.C.Ophth., Robert Henderson, M.R.C.Ophth., Kamaljit Balaggan, M.R.C.Ophth., Ananth Viswanathan, M.D., F.R.C.Ophth., Graham E. Holder, Ph.D., Andrew Stockman, Ph.D., Nick Tyler, Ph.D., Simon Petersen-Jones, Ph.D., Shomi S. Bhattacharya, Ph.D., Adrian J. Thrasher, Ph.D., M.R.C.P., F.R.C.P., Fred W. Fitzke, Ph.D., Barrie J. Carter, Ph.D., Gary S. Rubin, Ph.D., Anthony T. Moore, F.R.C.Ophth., and Robin R. Ali, Ph.D.

**Gene addition for RP in USH have some hurdles:**
**- several USH genes are large in size and**
**- most are expressed in different isoform.**

Treatment of Leber Congenital Amaurosis Due to *RPE65* Mutations by Ocular Subretinal Injection of Adeno-Associated Virus Gene Vector: Short-Term Results of a Phase I Trial

William W. Hauswirth,[1,2] Tomas S. Aleman,[3] Shalesh Kaushal,[1] Artur V. Cideciyan,[3] Sharon B. Schwartz,[3] Lili Wang,[4] Thomas J. Conlon,[1] Sanford L. Boye,[1] Terence R. Flotte,[5] Barry J. Byrne,[1,2] and Samuel G. Jacobson[3]

Safety and Durability of Gene Therapy and Measures of Disease Progression after 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.

**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]

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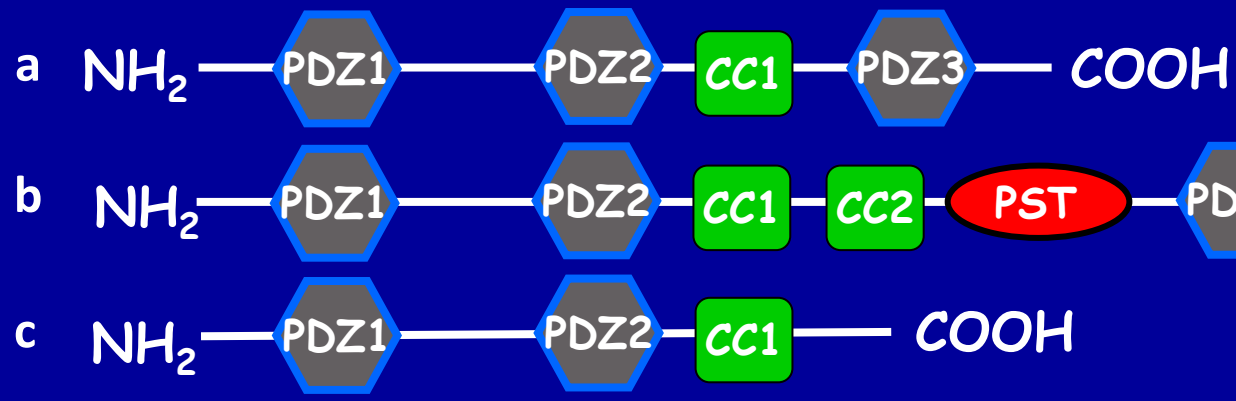
# Sizes and isoforms of USH genes

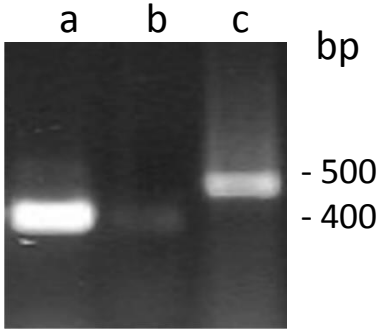
| USH type | Locus | Gene | cDNA | Protein |
|---|---|---|---|---|
| **USH1B** | 11q13.5 | *MYO7A* | **6.6 kb** | Myosin VIIa |
| **USH1C** | 11p14-15 | *USH1C* | 2.6 kb; **isoforms** | Harmonin |
| **USH1D** | 10q21-q22 | *CDH23* | **10.8 kb; isoforms** | Cadherin 23 |
| **USH1E** | 21q21 | -- | | -- |
| **USH1F** | 10q11.2-q21 | *PCDH15* | **9 kb; isoforms** | Protocadherin 15 |
| **USH1G** | 17q24-25 | *SANS* | 1.3 kb; **isoform** | SANS |
| **USH1H** | 15q22-23 | -- | | -- |
| **USH1J** | 15q23-q25.1 | *CIB2* | 561 bp; **isoforms** | CIB2 |
| **USH2A** | 1q41 | *USH2A* | **15.6 kb; isoforms** | USH2A (Usherin) |
| **USH2C** | 5q13 | *GPR98* | **18.9 kb; isoforms** | GPR98 (VLGR1b) |
| **USH2D** | 9q32-q34 | *DFNB31* | 4 kb; **isoforms** | Whirlin |
| **USH3A** | 3q25 | *CLRN1* | 699 bp; i**soforms** | Clarin-1 |
| **atypical USH** | 20q32.3 | *CEP250* | **7.3 kb; isoforms** | Cep250 |
| **atypical USH** | 2p23.2 | *C2orf71* | 3.9 kb | C2orf71 |

# Harmonin (USH1C) in human retina




PDZ-domain: protein-protein interaction; coiled coil domain: dimerization;
PST domain: actin bundeling

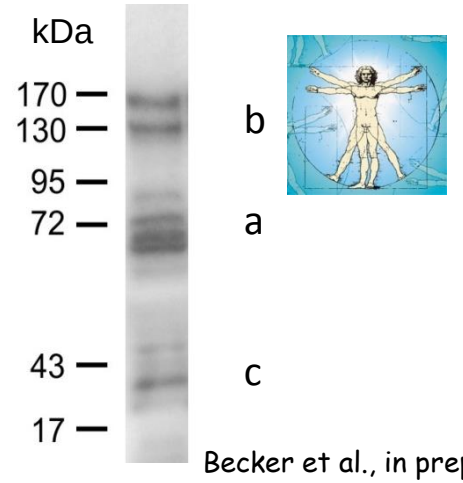
## RT-PCR




## next generation sequencing

| harmonin isoform | RT-PCR | RNAseq |
|---|---|---|
| a1 | ✓ | - |
| a2 | na | ✓ |
| a3 | ✓ | - |
| a4 | ✓ | ✓ |
| a5 | na | ✓ |
| b1-4 | ✓ | ✓ |
| c1 | ✓ | ✓ |
| c3 | ✓ | - |
| c4 | ✓ | - |

## Western blot analysis




Becker et al., in prep.

**_USH1C_ is expressed in various isoforms in human retina.**

# Alternative gene-based therapy strategy?

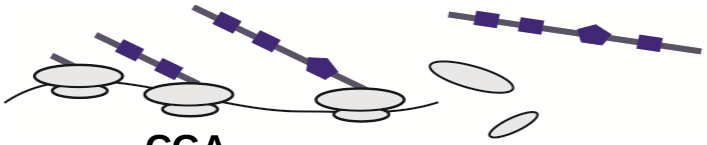
12-20% disease causing nonsense mutations in USH genes.

Strategies for targeting nonsense mutations are promising.

(see Poster # 17 and # 33)

# Read-through of nonsense mutations by translational read-through inducing drugs (TRIDs)

**normal translation**

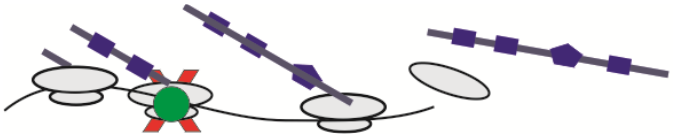



**functional protein**

**nonsense mutation induces premature stop codon**


**non-functional protein**
**Usher syndrome**

**read-through mediated by TRIDs**



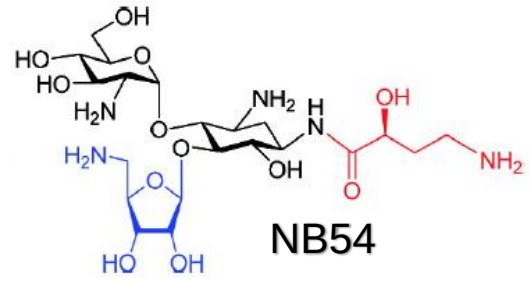

**recovered functional protein**

X nonsense mutation

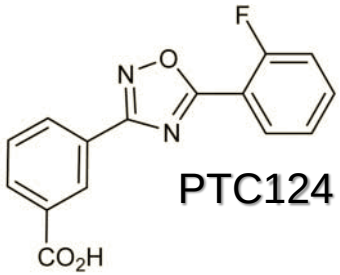
● TRIDs (aminoglycosides, PTC124)

Nagel-Wolfrum et al. (2014)

| TRIDs | example | advantage - disadvantage |
|---|---|---|
| Aminoglycosid antibiotics | Gentamyicin<br>Paromomycin | - clinically applied as antibiotics<br>- high read-through efficacy<br>- oto- and nephrotoxizität |
| Designer aminoglycosids | NB30<br>NB54<br>NB84<br>NB124 | - improved biocompatibility<br>- continues improvement resulted in increased read-through efficacy<br>- not tested in humans |
| Chemical compound | PTC124 | - well tolerated in human<br>- Phase III for patients suffering Duchenne/Becker Muscular Dystrophy and cystic fibrosis<br>- unknown if PTC124 pass blood-retina barrier |

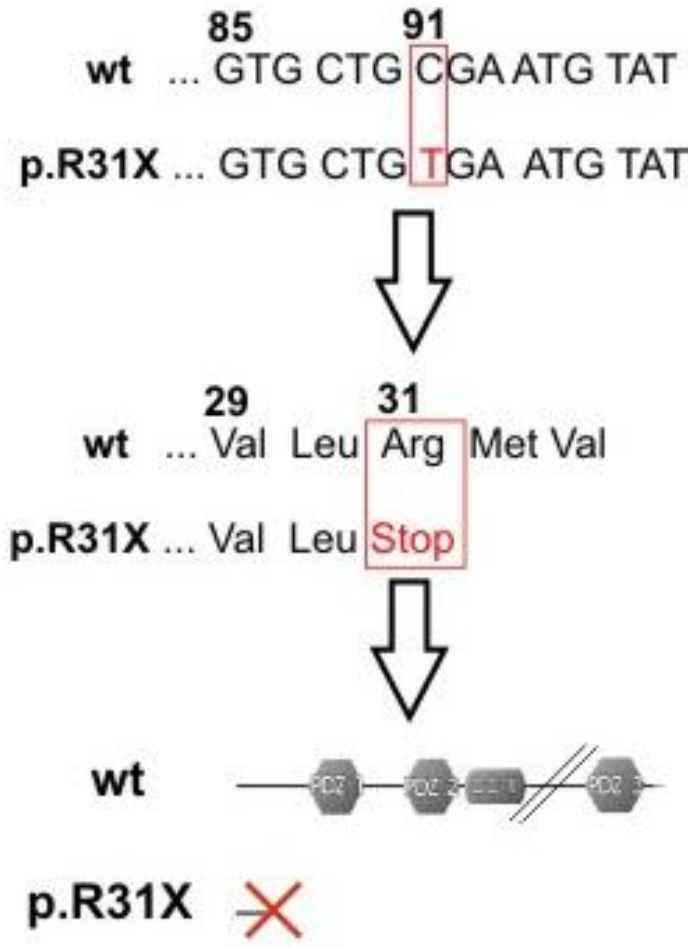


NB54



PTC124

# Proof of principle: p.R31X nonsense mutation in *USH1C*




EMBO Molecular Medicine

Research Article
Comparison of read-through drugs

**A comparative evaluation of NB30, NB54 and PTC124 in translational read-through efficacy for treatment of an *USH1C* nonsense mutation**

*Tobias Goldmann[1,†], Nora Overlack[1], Fabian Möller[1], Valery Belakhov[2], Michiel van Wyk[1,‡], Timor Baasov[2], Uwe Wolfrum[1,§], Kerstin Nagel-Wolfrum[1,§*]*

HUMAN GENE THERAPY 22:537–547 (May 2011)

DOI: 10.1089/hum.2010.067

**Research Articles**

**PTC124-Mediated Translational Readthrough of a Nonsense Mutation Causing Usher Syndrome Type 1C**

T. Goldmann, N. Overlack, U. Wolfrum,* and K. Nagel-Wolfrum*

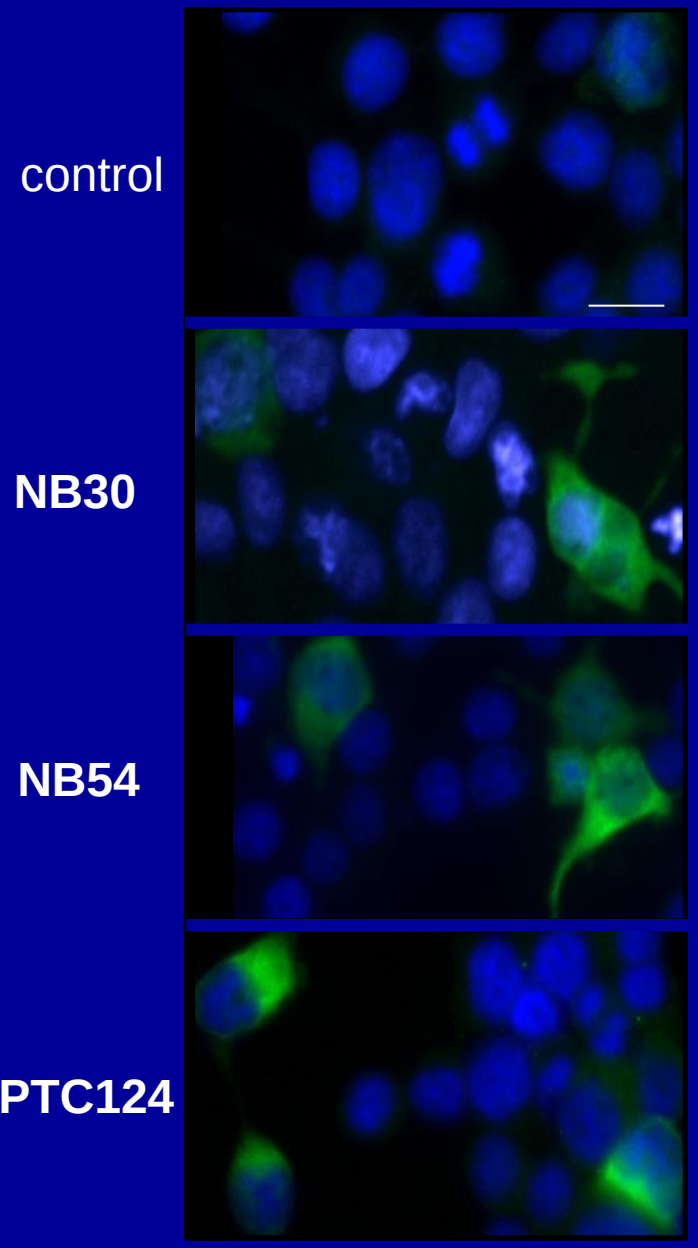
Physiology and Pharmacology

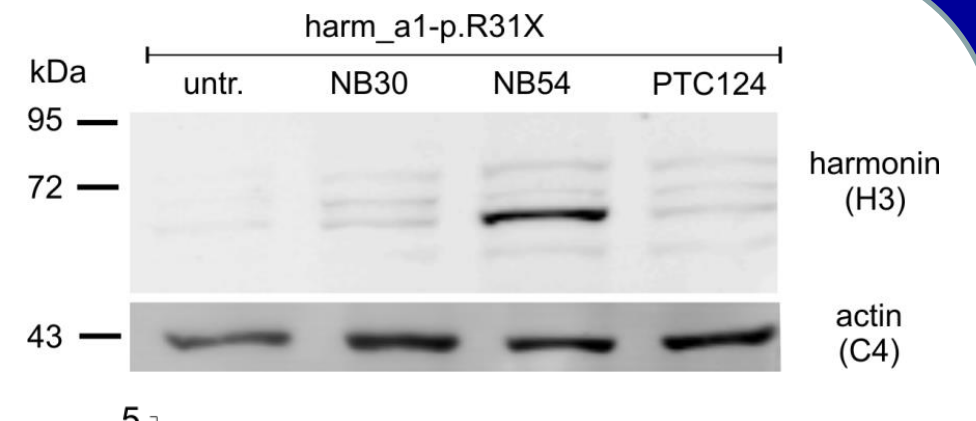
**Beneficial Read-Through of a *USH1C* Nonsense Mutation by Designed Aminoglycoside NB30 in the Retina**

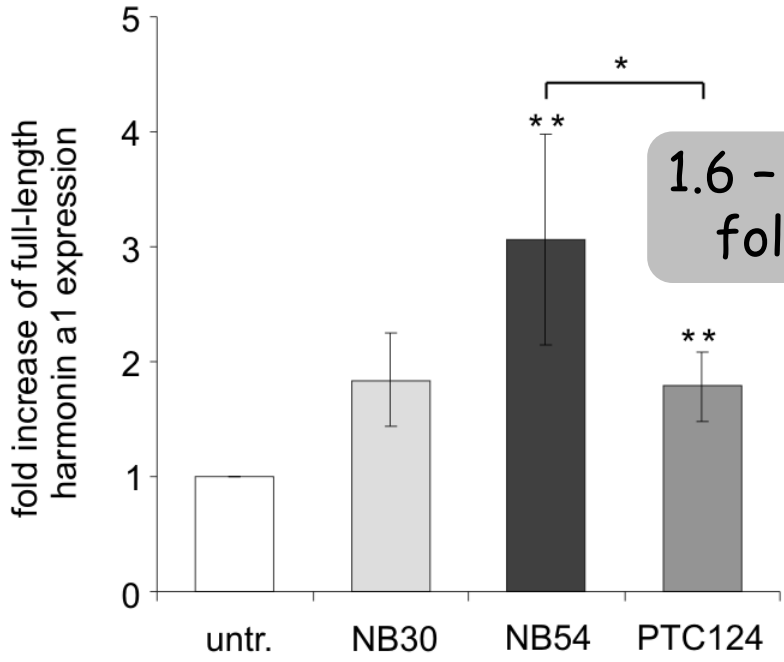
*Tobias Goldmann,[1] Annie Rebibo-Sabbah,[2] Nora Overlack,[1] Igor Nudelman,[3] Valery Belakhov,[3] Timor Baasov,[3] Tamar Ben-Yosef,[2] Uwe Wolfrum,[1,4] and Kerstin Nagel-Wolfrum[1,4]*

Goldmann et al. (2012) EMBO Mol Med 4:1186-99

# TRIDs induced read-through of *USH1C*



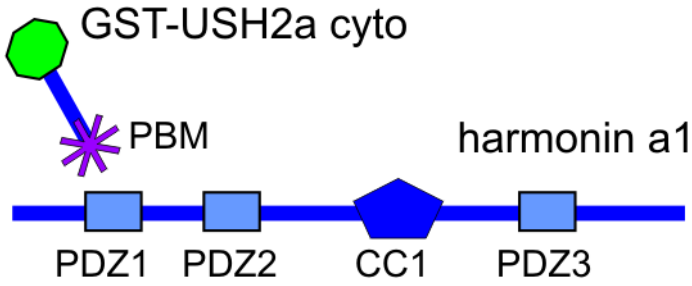



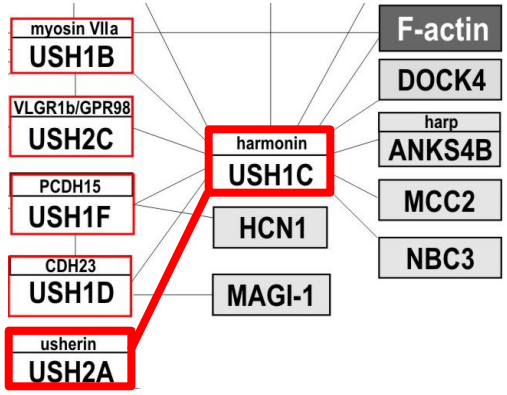




1.6 - 2.3
fold increase

Goldmann et al., 2010; 2011; 2012

# Recovery of harmonin´s scaffold function following TRIDs application



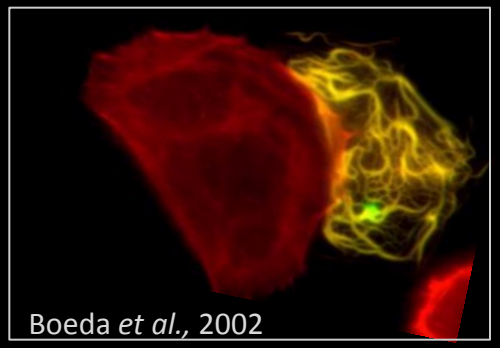

**GST pull down**

| | | | pm | NB30 | NB54 | PTC124 |
|---|---|---|---|---|---|---|
| harm_a1-p.R31X | - | + | + | + | + | + |
| GST-USH2a cyto | + | + | + | + | + | + |
| ~80 kDa | | | | | | |

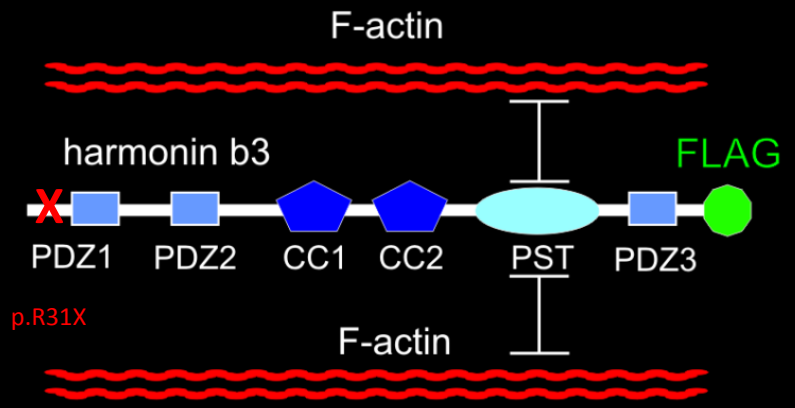
Goldmann et al. (2012) EMBO Mol Med 4:1186-99

# Recovery of harmonin b´s actin filament bundling activity following TRIDs application

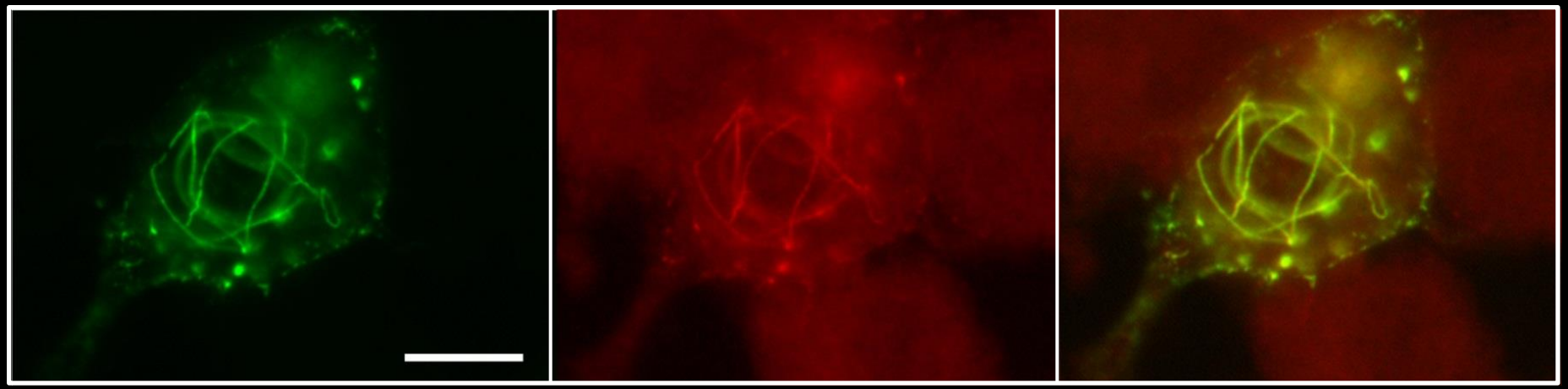
harmonin b-FLAG




anti-FLAG,
F-actin,
merged




harmonin b_p.R31X-FLAG + NB54



anti-FLAG F-actin merged

Goldmann et al. (2011) Hum Gen Ther; (2012) EMBO Mol Med

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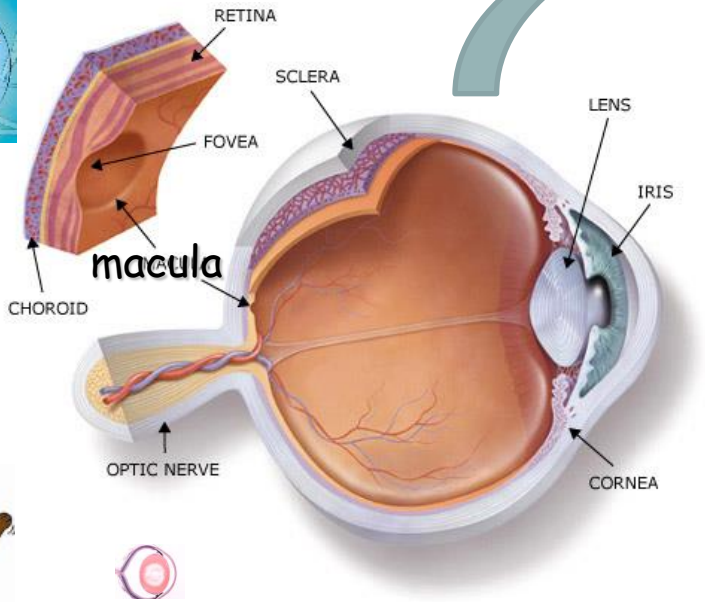
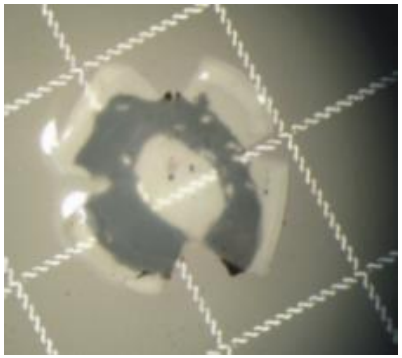
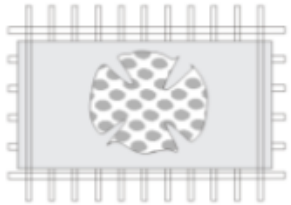
# TRIDs biocompatibility of human retinal cells



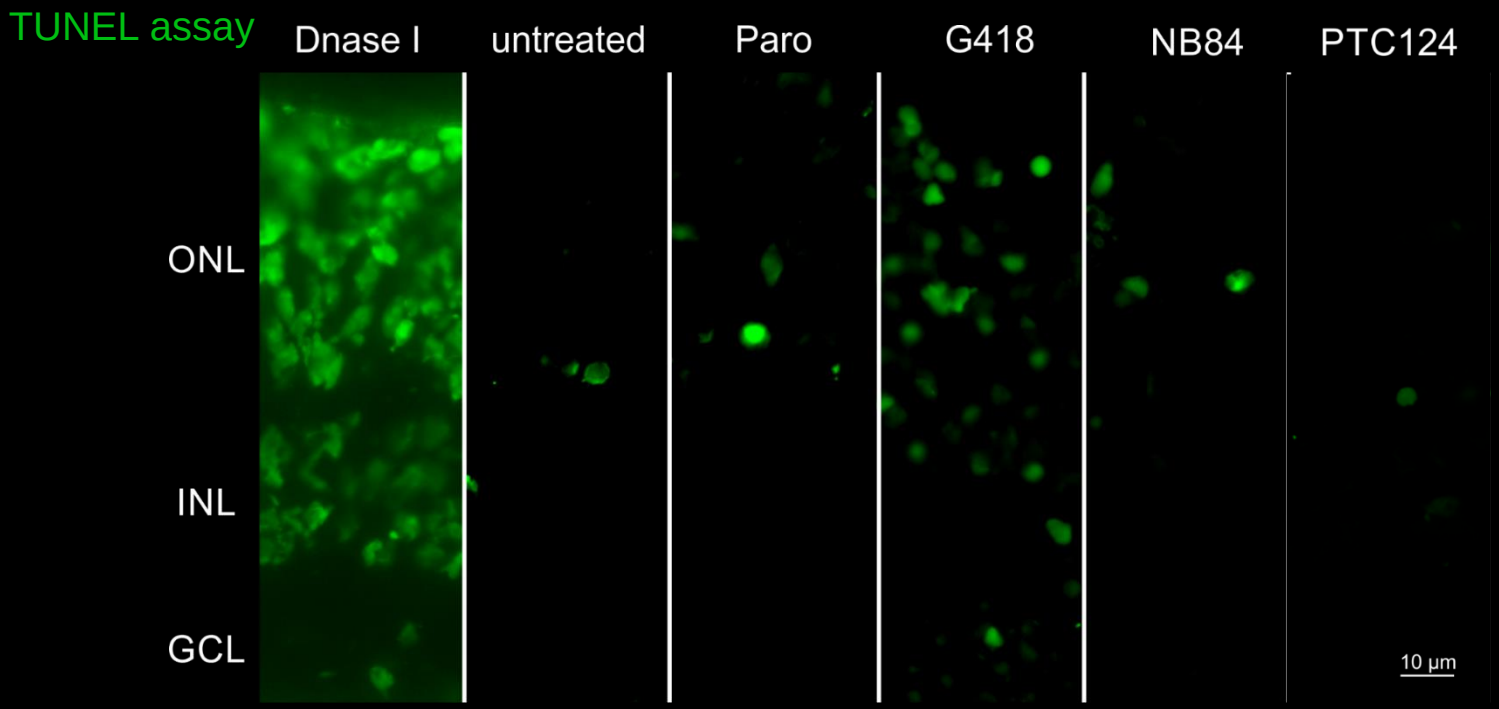


human and mouse
organotypic culture

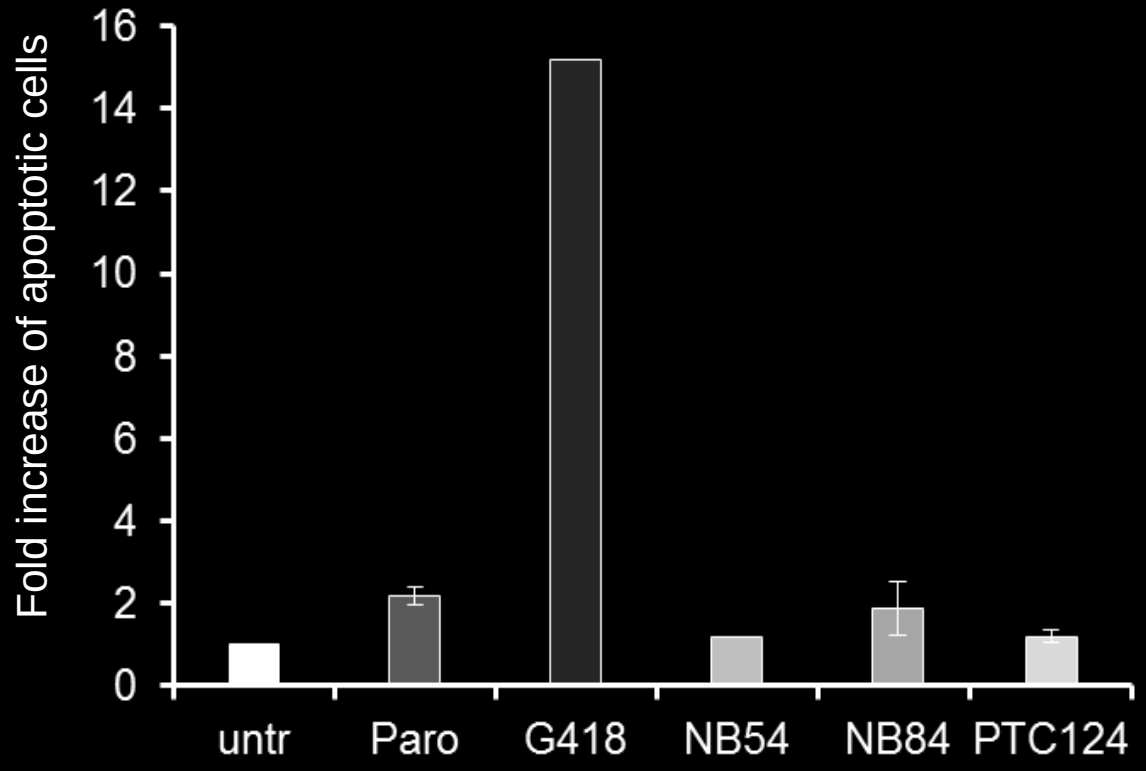
- TRIDs application, culture retina for 3 days, analyses of biocompatibility/toxicity:
  - histology (molecular markers)
  - apoptosis (TUNEL)

Reidel et al., 2006, 2008;
Goldmann 2010, 2011, 2012

# No increase of apoptotic cells in human retinal explants following NB54 and PTC124 application



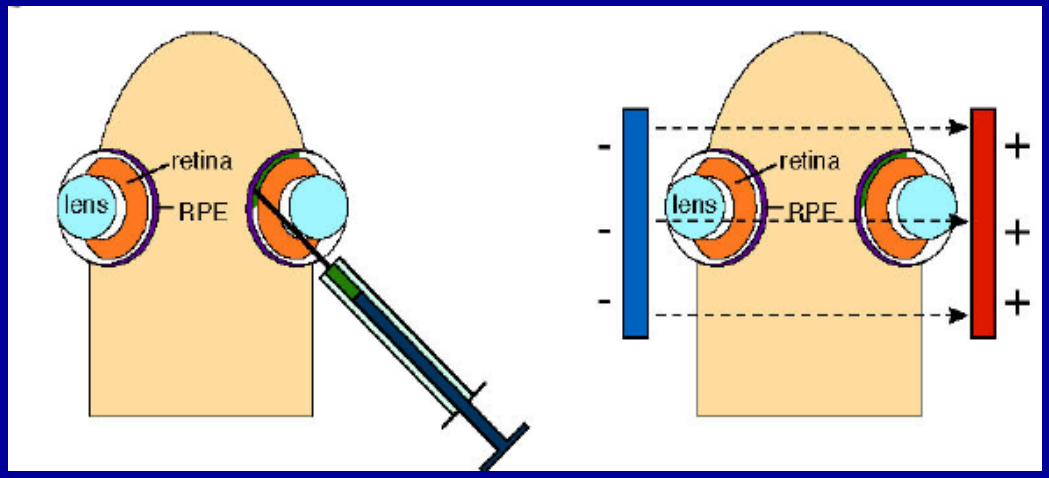
# No increase of apoptotic cells in human retinal explants following NB54 and PTC124 application



# TRIDs induced read-through *in vivo*

## Problem: no USH animal model carries a nonsense-mutation

Currently applied method:




Matsuda & Cepko (2006)

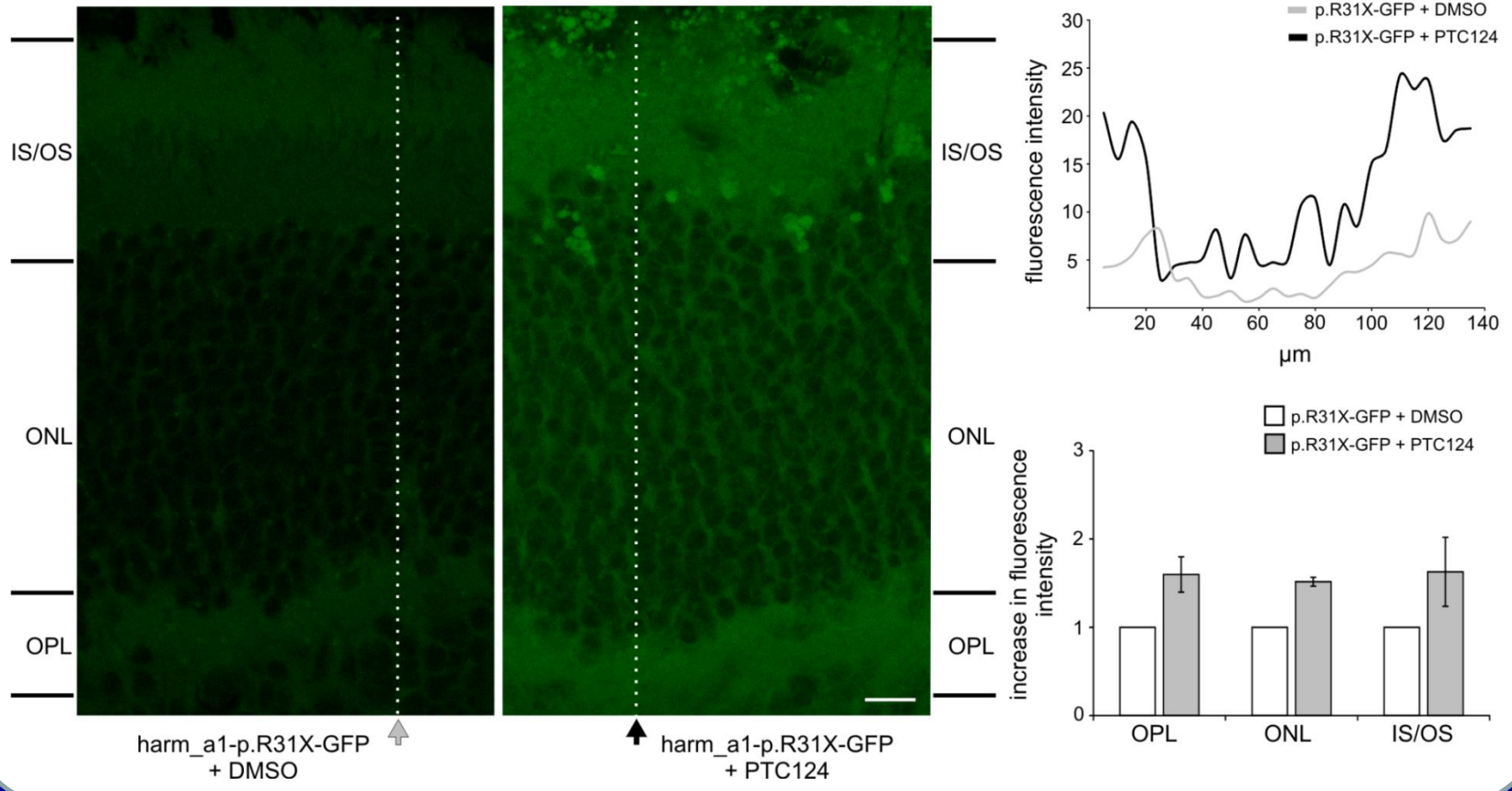
Subretinal injection of an harmonin-p.R31X construct

Electroporation

6 weeks later:
subretinal injection of TRIDs

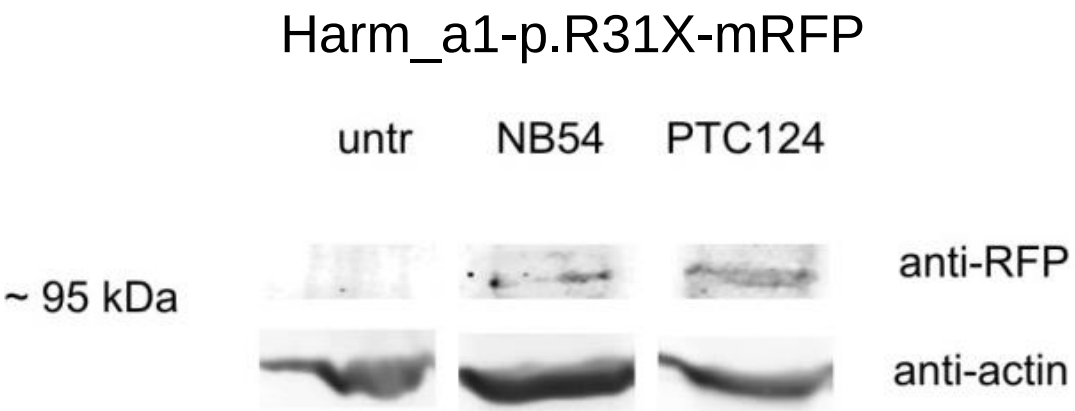
3 days later: detection of recovered protein expression:
immunofluorescence and Western blot analyses

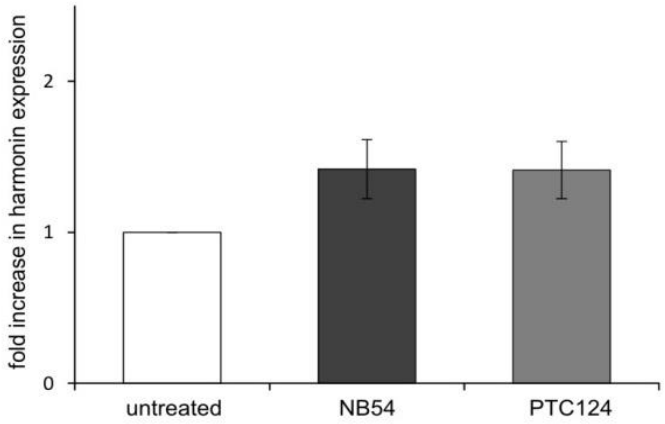
# Recovered harmonin expression *in vivo* in the murine retina




Goldmann et al. (2012) EMBO Mol Med 4:1186-99

# Recovered harmonin expression *in vivo* in the murine retina

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# Summary

- Designer aminoglycosides (NBs) and PTC124 have an excellent retinal biocompatibility.
- Read-through of *USH1C* has been demonstrated in cell culture, organotypic retina cultures and *in vivo*.
- TRIDs induce the read-through of various nonsense mutations in cell culture causing USH, including:

  *USH1C*, *USH1D*, *USH1F*, *USH2A*, *USH2C*, *CLRN*.

**TRIDs have a high potential as therapeutics for the treatment of retinal degenerations caused by nonsense mutations.**

# Work in Progress - Prospects

- Animal model for USH nonsense mutation

  *Nphp4*$^{nmf192/nmf192}$ rd/ciliopathy mouse model;
  in frame nonsense mutations (Won et al. 2011)

  Larger animal model: transgenic USH (mini)pig carrying a nonsense mutation.

- Evaluation of TRIDs formulation and their delivery modes into the eye.
  - **systemic** (intravenous, intraperitoneal injection, oral)
  - **local applications** (subretinal injection, eye pump, intravitreal depots, eye drops, )

  e.g. START PTC124 formulation for topic application.

  Gregory-Evens et al. (2014) *J Clin Invest.* 124:111–116

# The USH therapy team, MZ

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- I. Penner
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- U. Wolfrum
- S. Bolz, E. Sehn, G. Stern-Schneider, U. Maas

# Thank you!

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Poster #33

PRO RETINA
FAUN
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SEVENTH FRAMEWORK PROGRAMME
Bundesministerium für Bildung und Forschung
Eur-USH
E-Rare
Syscilia
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# EUR-USH interconnects diagnostic, basic research and therapeutic applications

## Poster #33


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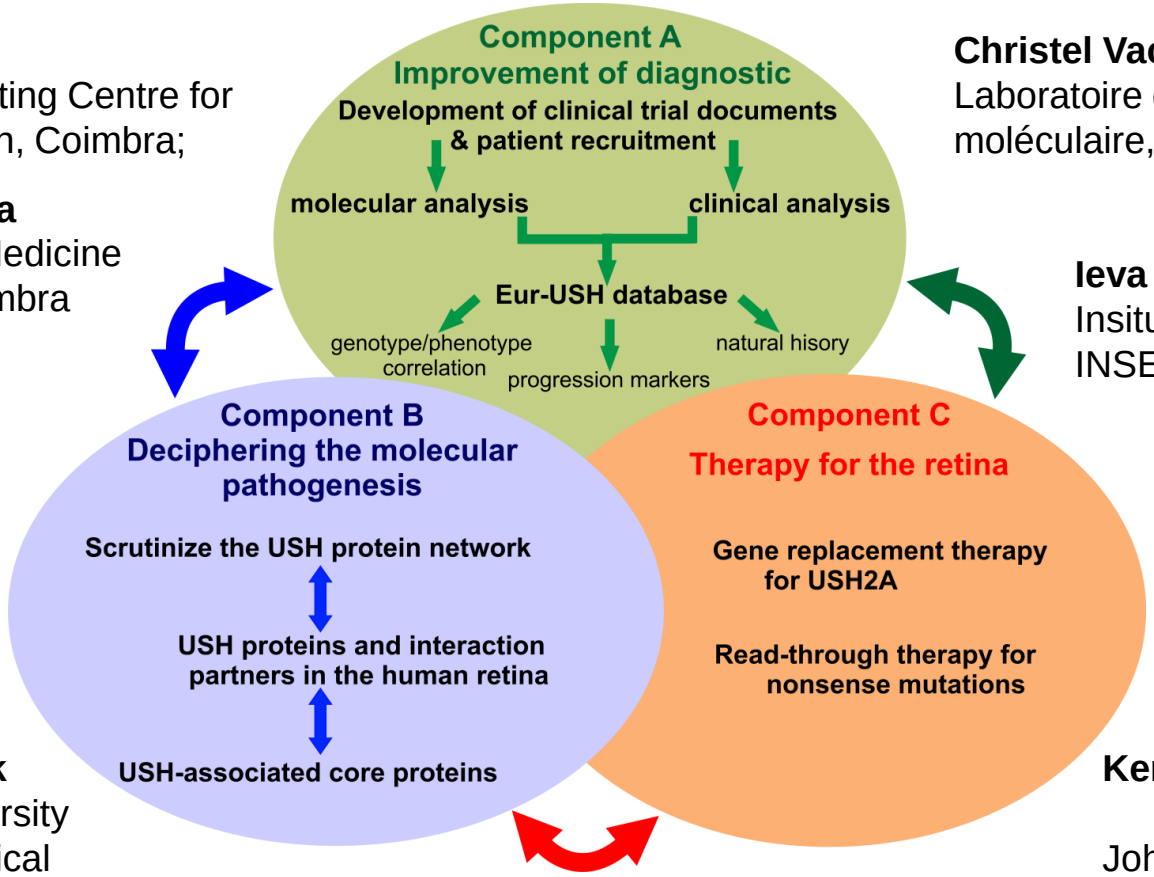

# Young investigator consortium: EUR-USH


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