



Unlocking protein production with **translational read-through** for **rare genetic** diseases

February 2021

Forward-Looking Statements

This presentation contains forward-looking statements, which are generally statements that are not historical facts. Forward-looking statements can be identified by the words "expects," "anticipates," "believes," "intends," "estimates," "plans," "will," "outlook" and similar expressions. Forward-looking statements are based on management's current plans, estimates, assumptions and projections, and speak only as of the date they are made. We undertake no obligation to update any forward-looking statement in light of new information or future events, except as otherwise required by law. Forward-looking statements involve inherent risks and uncertainties, most of which are difficult to predict and are generally beyond our control. Actual results or outcomes may differ materially from those implied by the forward-looking statements as a result of the impact of a number of factors, including: the development of the Company's read-through technology; the approval of the Company's patent applications; the Company's ability to successfully defend its intellectual property or obtain necessary licenses at a cost acceptable to the Company, if at all; the successful implementation of the Company's research and development programs and collaborations; the Company's ability to obtain applicable regulatory approvals for its current and future product candidates; the acceptance by the market of the Company's products should they receive regulatory approval; the timing and success of the Company's preliminary studies, preclinical research, clinical trials, and related regulatory filings; the ability of the Company to consummate additional financings as needed; the impact of global health concerns, such as the COVID-19 global pandemic, on our ability to continue our clinical and preclinical programs and otherwise operate our business effectively; as well as those discussed in more detail in our Annual Report on Form 10-K and our other reports filed with the Securities and Exchange Commission.

Eloxx Pharmaceutical Highlights

- **Experienced Leadership Team**
- **Cash runway extends through the end of 2021**
- **Phase 2 ELX-02 Cystic Fibrosis Program**
 - Evaluating safety, tolerability, PK and PD in cystic fibrosis patients with *G542X* Allele
 - Independent Safety Review Committees have allowed dose escalation to the top dose
 - Expect to report top line data in the first half of 2021, barring any COVID disruptions
 - ELX-02 received U.S. Orphan Drug Designation from FDA and EMA for cystic fibrosis
 - Cystic Fibrosis Medical Advisory Board of leading clinical CF investigators and experts
- **ERSG Pipeline Development**
 - **Kidney; Autosomal Dominant Polycystic Kidney Disease**
 - Preclinical studies demonstrate dose-dependent read-through across most prevalent *PKD1* and *PKD2* alleles
 - Encouraging results of reduced cystogenesis and cyst size
 - **Ocular; Inherited Retinal Disorders such as Usher**
 - Ocular *Oca2* animal model shows POC for intravitreal tolerability and restored protein production
 - *In vitro* sustained release rates consistent with the target range of one to three months

Eloxx Pipeline Overview

	IND ENABLING	PHASE I	PHASE II	PHASE III
ELX-02				
PHASE I SAD / MAD / RENAL	COMPLETED			
PHASE II CYSTIC FIBROSIS (CF)	RESUMED FOLLOWING COVID-19 PAUSE			
PHASE II CYSTINOSIS (CYS)	STRATEGIC HOLD			
New Indications				
ADPKD				
INHERITED RETINAL DISORDERS				

Our Orphan Drug Programs Have Strong Advocacy Support



The Nonsense Mutation Problem

>1,800

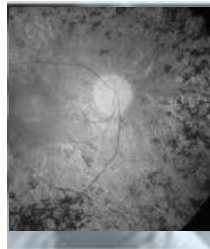
Genetic diseases involve nonsense mutations



Cystic
Fibrosis



Cystinosis



Retinitis
Pigmentosa



Usher
Syndrome



Primary
Ciliary
Dyskinesia



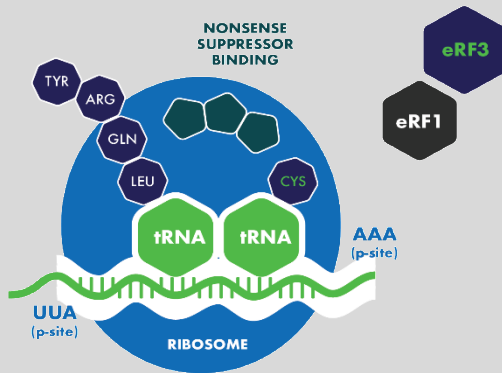
Polycystic
Kidney Disease

- In every genetic disease a subset of patients have nonsense mutations that impair the production of essential proteins
- Translational read-through is directed at restoring the production of full-length proteins by overcoming the premature stop codon and nonsense mediated decay

Unmet need for patients with nonsense mediated disease across multiple indications

Eloxx Small Molecule ERSG Solutions

Normal



NORMAL TRANSLATION

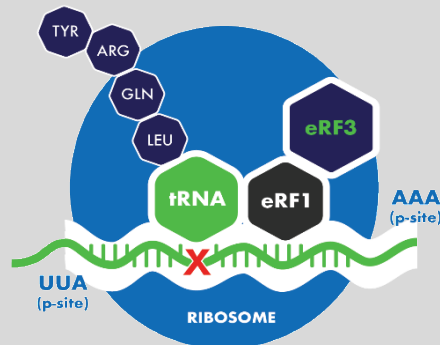


FULL LENGTH PROTEIN

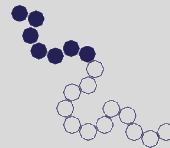


mRNA TRANSCRIPT AVAILABLE
FOR MORE TRANSLATION

Nonsense



NONSENSE MUTATION

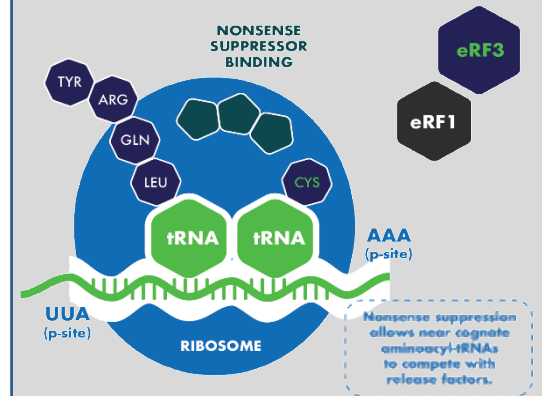


TRUNCATED PROTEIN



mRNA DEGRADATION

Read-through



NONSENSE MUTATION READTHROUGH



RESCUED FULL LENGTH PROTEIN



mRNA TRANSCRIPT STABILIZED

ELX-02: Phase 1 Program Completed

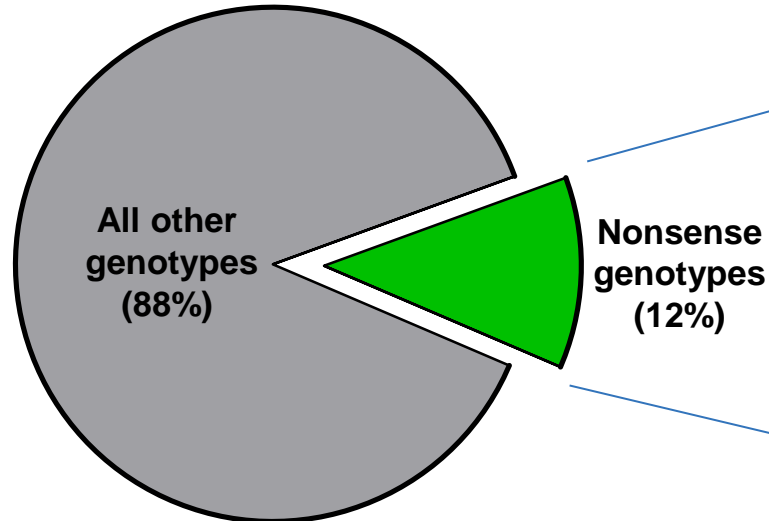
SAD (single ascending dose)	MAD (multiple ascending dose)	Renal Impairment
<ul style="list-style-type: none">✓ Submission of CSR to regulators✓ Published in <i>Clin. Pharm. Drug Dev.</i> 2019 Jan 16.✓ PK presented at ECFS 2019✓ Full data presented at NACFC Oct. 31- Nov. 2, 2019	<ul style="list-style-type: none">✓ Full data presented at NACFC Oct/Nov 2019✓ Scientific manuscript published in the <i>Journal of Clinical Pharmacology in Drug Development</i> in January 2021	<ul style="list-style-type: none">✓ Full data presented at ASN Kidney Week Nov. 5-10, 2019✓ Scientific manuscript published in the <i>Journal of Clinical Pharmacology</i> in January 2021

Phase 1 Program Conclusions

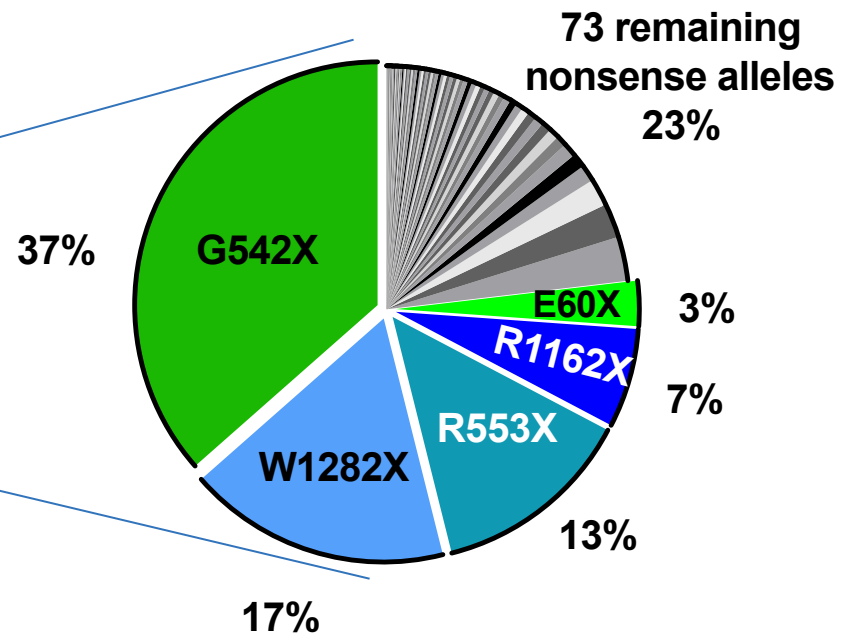
- Generally well tolerated in clinical studies to date supporting evaluation in Phase 2
- Consistent PK results across single and multiple dose studies, with no accumulation
- High bioavailability (98%) upon SC administration with highly reproducible PK over the dosage range studied (0.3-7.5 mg/kg)

Cystic Fibrosis Nonsense Mutation Patients Represent a Key Unmet Need Population

CF Nonsense Genotype Population

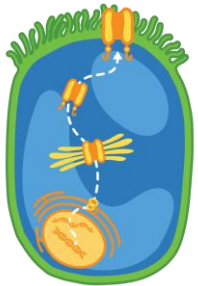


Nonsense Allele Frequency of Global CF Patient Population



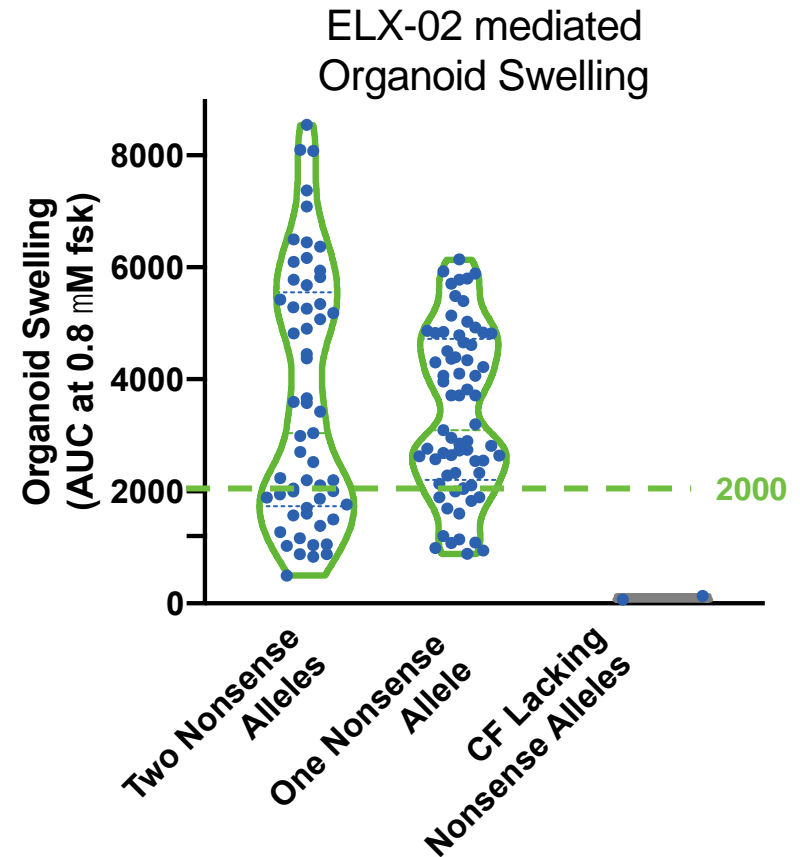
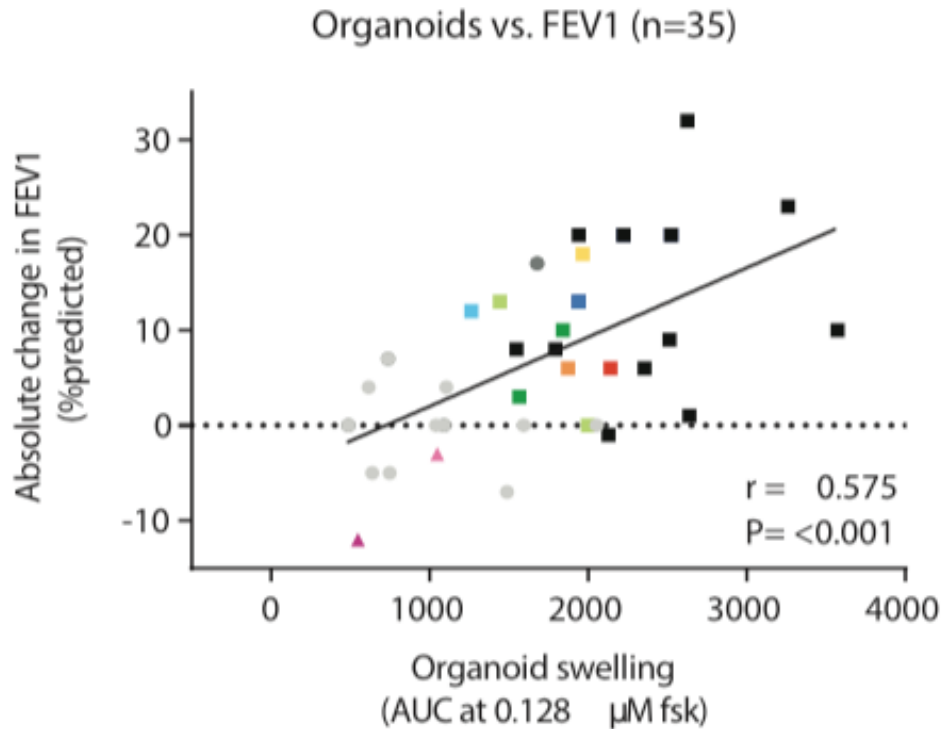
Sources: Allelic frequency based on CFTR2 database (July 2020);
CF population data based on 2019 Patient Registry Report.

ELX-02: Preclinical Data De-Risks CF Phase 2



- **ELX-02 is a small molecule that permits read-through of nonsense mutations**
 - ✓ High selectivity for the eukaryotic cytoplasmic ribosome relative to mitochondrial ribosome
 - ✓ Defined MOA: Demonstrated significant increases in *Cystinosin* & *CFTR mRNA*, protein and function
 - ✓ Demonstrated read-through in assays focusing on high prevalence Cystic Fibrosis & Cystinosis nonsense mutations
- **ELX-02 high activity in multiple cellular and animal models**
 - ✓ Pronounced *CFTR* read-through demonstrated in plasmid, HBE, FRT, transgenic mice and patient-derived organoids
 - ✓ Pronounced Cystinosin read-through demonstrated in plasmid, patient derived fibroblasts and transgenic mice
- **Phase 2 Studies enroll patients with defined genotypes**
 - Cystic Fibrosis trial focuses on *G542X* on one or both alleles

ELX-02 Response in Organoids Compares Favorably to Published Results

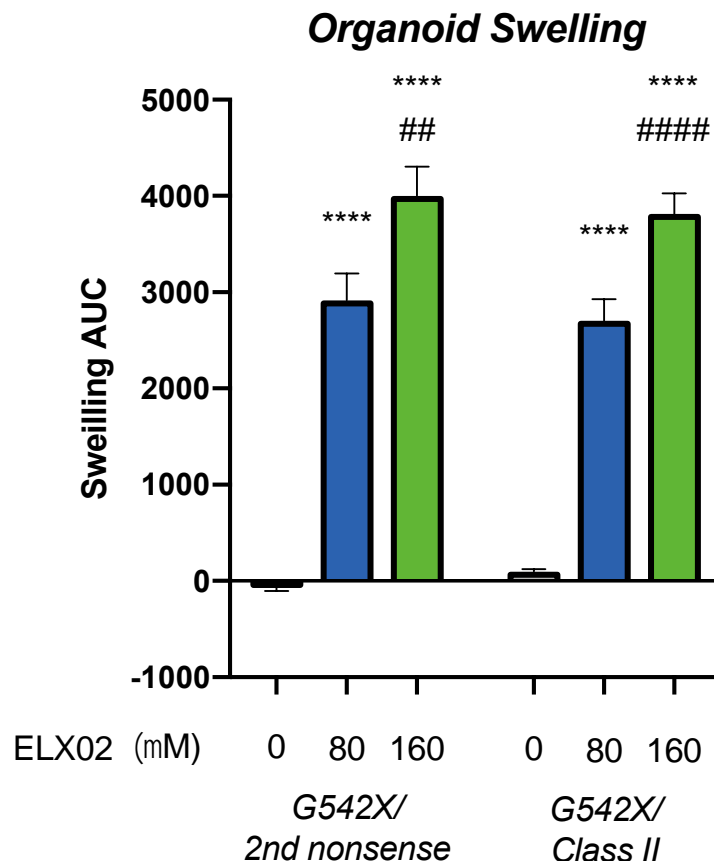


Berkers et al. Cell Reports (2019) Rectal Organoids Enable Personalized Treatment of Cystic Fibrosis.

Eloxx data on file.

ELX-02 Mediated Organoid Swelling Is Equivalent in Organoids With One or Two Nonsense Mutations

- Significant increase in organoid swelling is observed in both *G542X* organoids with a second nonsense mutation and heterozygous organoids
- Experiments used 0.8 μ M Forskolin
- Scientific manuscript titled: Targeting *G542X CFTR* Nonsense Alleles with ELX-02 Restores CFTR Function in Human-Derived Intestinal Organoids” published in the *Journal of Cystic Fibrosis*



ordinary one-way ANOVA with Tukey's multiple comparison testing was used,, **** $p < 0.0001$ versus vehicle control, ## $p < 0.01$ versus next lower concentration, , ##### $p < 0.0001$ versus next lower concentration. Data represent >40 biological replicates per group from 2 or 4 independent patient organoids, respectively.

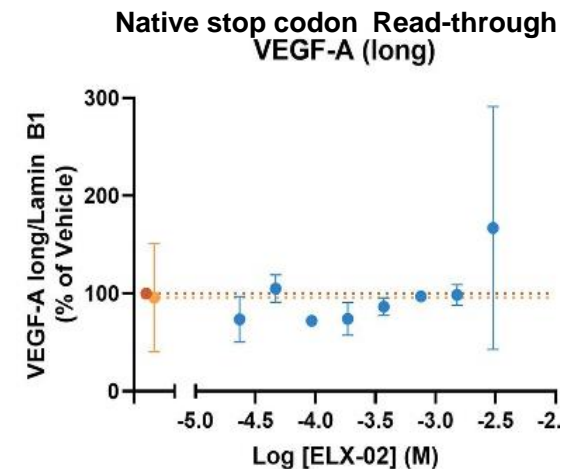
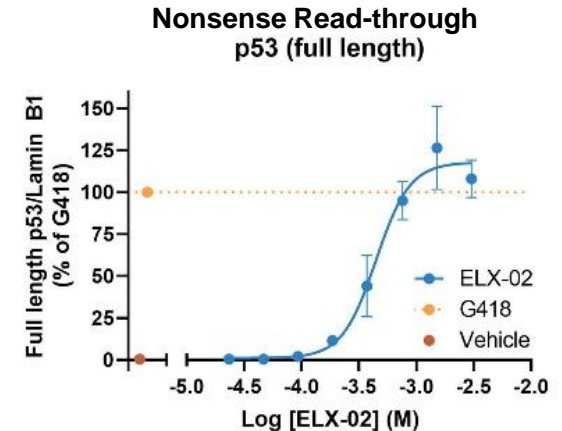
Eloxx data on file.

ELX-02 Mediates Read-through of Premature Stop Codons Without Read-through of Normal Stop Codons

- Recently published in JPET: “ELX-02 generates protein via premature stop codon read-through without inducing native stop codon read-through protein”

THE JOURNAL OF PHARMACOLOGY
AND EXPERIMENTAL THERAPEUTICS

- Manuscript addresses a common question: “*If ELX-02 promotes read-through of premature stop codons, what about the normal stop codons?*”
- Manuscript demonstrates:
 - premature stop codon read-through at protein level with ELX-02
 - three-complimentary techniques demonstrating ELX-02 does not promote read-through of normal stop codons at relevant concentrations tested



ELX-02 Phase 2 Cystic Fibrosis – Trial Design



ClinicalTrials.gov Identifier: **US Trial** NCT04135495 **EU/IL Trial** NCT04126473

Population

- Targeting up to 16 CF patients with a *G542X* mutation on one or both alleles
- Intra-patient dose escalation
- 4 increasing doses of ELX-02 ranging from 0.3 up to 3.0 mg/kg/day

Primary Outcome Measures

- Safety, tolerability, and pharmacokinetics

Secondary Outcome Measures

- PD changes from baseline in sweat chloride levels and FEV1
- Consistent with other Phase 2 trials for approved drugs

Locations

- Enrollment resumed in Europe, Israel & USA

Additionally

- Orphan drug designation granted in US and Europe
- Funding provided by CFF, sanctioned by CFF-TDN & ECFS-CTN (high priority ranking)



Eloxx is Developing New Indications for its ERSG Library to Expand the Portfolio

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 Nudelmann,³ Valery Belakhov,³ Timor Baasov,³ Tamar Ben-Yosef,² Uwe Wolfrum,^{1,4} and Kerstin Nagel-Wolfrum^{1,4}

PURPOSE. The human Usher syndrome (USH) is the most frequent cause of inherited combined deaf-blindness. USH is clinically and genetically heterogeneous, assigned to three clinical types. The most severe type is USH1, characterized by profound inner ear defects and retinitis pigmentosa. Thus far, no effective treatment for the ophthalmic component of USH exists. The p.R51X nonsense mutation in *USH1C* leads to a severe retinal degeneration.

CONCLUSIONS. Commercial aminoglycosides and NB30 induced significant read-through of the *USH1C* p.R51X nonsense mutation. However, the observed read-through efficiency, along with its significantly reduced toxicity and good biocompatibility, indicate that the novel derivative NB30 represents a better choice than commercial aminoglycosides to read-through the p.R51X mutation in other ocular diseases.

Ben Zeev^{3,5}, Igor Nudelmann³, Yair Anikster³, Amos J. Simon³, Ninette Zeev^{3,5}, Timor Baasov³, Eva Gak^{1,2,4}

¹Center, Tel Hashomer, Israel; ²Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; ³Edmond and Lily Safra Center, Tel Hashomer, Israel; ⁴The Edith and Joseph Fischer Enzyme Inhibitors Laboratory, Schulich Faculty of Chemistry, Technion – Israel Institute of Technology, Haifa, Israel; ⁵Cancer Research Center, Sheba Medical Center, Tel Hashomer, Israel

ons in the X-linked methyl CpG-binding protein 2 (*MECP2*) comprise a significant proportion of Rett syndrome (RTT). Naturally occurring aminoglycosides, such as gentamicin, have been used to suppress the expression of mutant *MECP2* genes. However, the use of aminoglycosides is limited by their toxicity and the need for high doses.

NIH Public Access

Author Manuscript

Mol Genet Metab. Author manuscript; available in PMC 2015 March 01.

Published in final edited form as: *Genet Metab.* 2014 March; 111(3): 374–381. doi:10.1016/j.gymg.2013.12.007.

Long-Term Nonsense Suppression Therapy Moderates MPS I-H Disease Progression

Ben Zeev^{3,5}, Yanying Dai³, Ming Du³, Valery Belakhov³, Jayakumar Kandasamy², Trenton R. S. Hoeb³, Timor Baasov³, David M. Bedwell³, and Kim M. Keeling³

¹Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL, USA.

²The Edith and Joseph Enzyme Inhibitors Laboratory, Schulich Faculty of Chemistry, Technion-Israel Institute of Technology, Haifa, Israel

³Department of Genetics, University of Alabama at Birmingham, Birmingham, AL, USA

Abstract

Nonsense suppression therapy (NST) is a therapeutic approach to suppress the expression of mutant genes. In this study, we used NST to suppress the expression of mutant *PCDH15* genes in mice.

DOI: 10.1007/978-94-007-1210-7

RESEARCH REPORT

Aminoglycoside-Induced Premature Stop Codon Read-Through of Mucopolysaccharidosis Type I Patient Q70X and W402X Mutations in Cultured Cells

Makoto Kamei¹, Karina Kasperki¹, Maria Feller¹, Emma J. Perlman-Lawrence¹, Lisa Kangrgang¹, Valery Belakhov¹, Timor Baasov¹, John J. Hopwood¹, Doug A. Brooks¹

Received: 19 April 2013 / Revised: 11 August 2013 / Accepted: 25 September 2013 / Published online: 4 November 2013
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Abstract The premature stop codon mutations, Q70X and W402X, in the *IDS* gene, which cause mucopolysaccharidosis type I (MPS I), are the most common *exon-intron* gene mutations. Aminoglycosides promote read-through for the Q70X mutation. Inhibitors of the *W402X* mutation, which cause a severe form of MPS I, are not known.

Aminoglycoside antibiotics restore dystrophin function to skeletal muscles of *mdx* mice

Elisabeth R. Barton-Davis,¹ Laurence Cordier,¹ Daria I. Shoturma,¹ Stuart E. Leland,² and H. Lee Sweeney¹

¹Department of Physiology, and ²Institute for Human Gene Therapy, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania
Address correspondence to: H. Lee Sweeney, A700 Richards Building, Department of Physiology, 3700 Hamlo University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-6085, USA. Phone: (215) 898-8988; Fax: (215) 898-0475; E-mail: lsweeney@mail.med.upenn.edu.

Received for publication July 14, 1999, and accepted July 16, 1999.

Duchenne muscular dystrophy (DMD) is caused by mutations in the dystrophin gene that result in the absence of the dystrophin protein in skeletal muscle. A significant number of these mutations are premature stop codons. On the basis of the observation that aminoglycoside antibiotics induce read-through of premature stop codons in cultured cells, we tested the effect of these antibiotics on dystrophin expression in *mdx* mice.

ORIGINAL RESEARCH

Synthetic Aminoglycosides Efficiently Suppress Cystic Fibrosis Transmembrane Conductance Regulator Nonsense Mutations and Are Enhanced by Ivacaftor

Xiaojiao Xue^{1,2}, Venkateshwar Mutyama², Liping Tang², Silpak Biswas², Ming Du^{3,4}, Laura A. Jackson², Yanying Dai³, Valery Belakhov³, Moran Shalev³, Fuqian Chen³, Jochen Schacht³, Robert J. Bridges³, Timor Baasov³, Jeong H. David M. Bedwell^{3,4,5,6}, and Steven M. Rowe^{2,3,4,6}

Departments of ¹Genetics, ²Medicine, ³Pediatrics, ⁴Cell Developmental and Integrative Biology, and ⁵Microbiology, and ⁶Gregory Fleming James Cystic Fibrosis Research Center, University of Alabama at Birmingham, Birmingham, Alabama; ⁷The Edith and Joseph Fischer Enzyme Inhibitors Laboratory, Schulich Faculty of Chemistry, Technion-Israel Institute of Technology, Haifa, Israel; ⁸Kresge Hearing Research Institute, Department of Otolaryngology, University of Michigan Medical School, Ann Arbor, Michigan; and ⁹Department of Physiology and Biophysics, Rosalind Franklin University, North Chicago, Illinois

Abstract

New drugs are needed to enhance premature termination codon (PTC) suppression in the setting of cystic fibrosis (CF). We have developed a library of synthetic aminoglycosides that efficiently suppress PTCs in CF cells. We have also developed a library of synthetic aminoglycosides that efficiently suppress PTCs in CF cells.

NIH-PA Author Manuscript

NIH Public Access

Author Manuscript

Mol Genet Metab. Author manuscript; available in PMC 2013 January 1.

Published in final edited form as: *Mol Genet Metab.* 2012 January; 105(1): 116–125. doi:10.1016/j.gymg.2011.10.005.

The Designer Aminoglycoside NB84 Significantly Reduces Glycosaminoglycan Accumulation Associated with MPS I-H in the *Idua-W392X* Mouse

Dan Wang¹, Valery Belakhov², Jayakumar Kandasamy², Timor Baasov², Su-Chen Li³, Yu-Teh Li³, David M. Bedwell^{1,4}, and Kim M. Keeling^{1,4}

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³Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294, USA

⁴Department of Pediatrics, University of Alabama at Birmingham, Birmingham, AL 35294, USA

ADPKD (*Autosomal Dominant Polycystic Kidney Disease*)

- 200,000-600,000 people with ADPKD in the US
 - 12 million people worldwide
- ~141,000 diagnosed cases of ADPKD in the US (2 main genes are *PKD1* and 2)

***PKD1* Mutation**

- 85% of all ADPKD cases
- Cysts may appear when patients are young adults
- Disease can progress rapidly
- Median age of ESRD onset is 54 yrs.
- Gene location: short arm on chromosome 16 (16p13.3)

***PKD2* Mutation**

- 15% of all ADPKD cases
- Disease progresses more slowly vs. PKD1
- Median age of ESRD onset is 74 years
- Gene location: long arm on chromosome 4 (4q21)

- ~6,000 new cases diagnosed each year in the US
- ~***6-10% of individuals receiving dialysis and renal transplant treatment in the US have ADPKD***

Blanchette, C. et al; Burden of Autosomal Dominant Polycystic Kidney Disease: Systematic Literature Review, Am J Pharm Benefits, 2015; 7(2): e27-e36
Uncoverpkd.com (Otsuka HCP site)

NORD, <https://rarediseases.org/rare-diseases/autosomal-dominant-polycystic-kidney-disease/>

PKD International, <https://pkdinternational.org/what-is-pkd/adpkd>



ADPKD Nonsense Mutation Prevalence

- Genetic disorder characterized by cysts localized within the kidney. Majority of patients progress to ESRD.
- Mutations in *PKD1* or *PKD2* cause a disruption in the production of functional polycystin, which through signaling process leads to excess vasopressin, leading to cyst growth.

ADPKD: Cyst formation

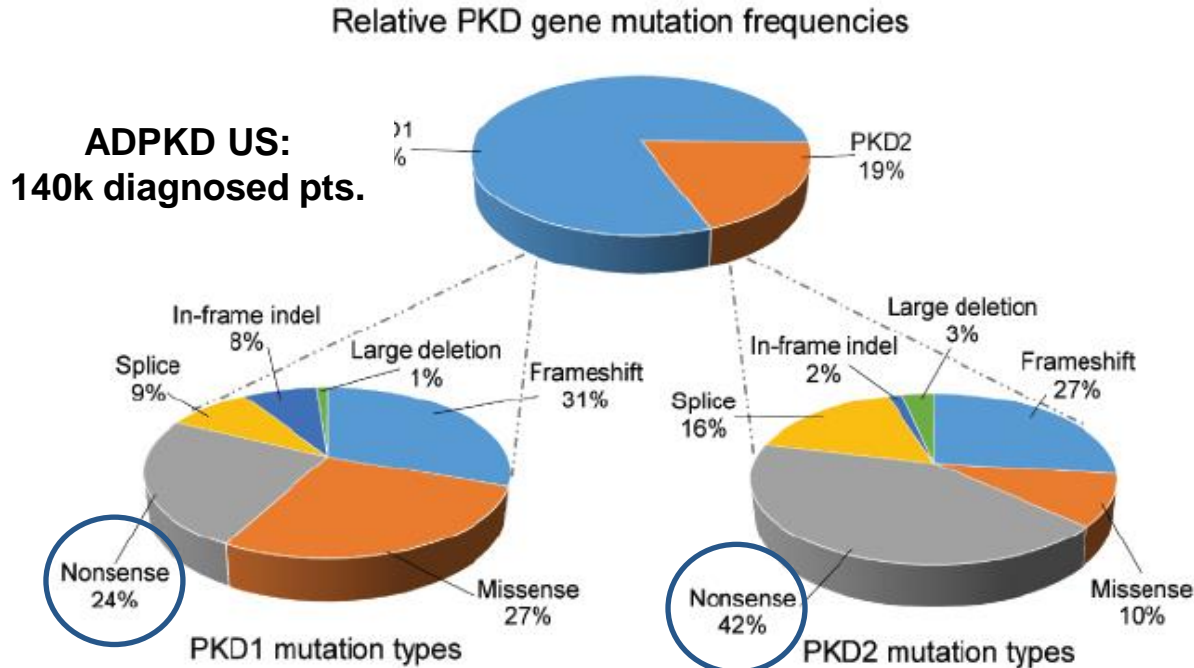
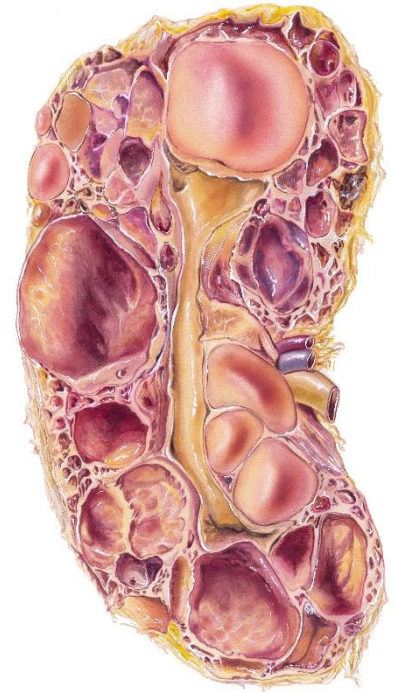
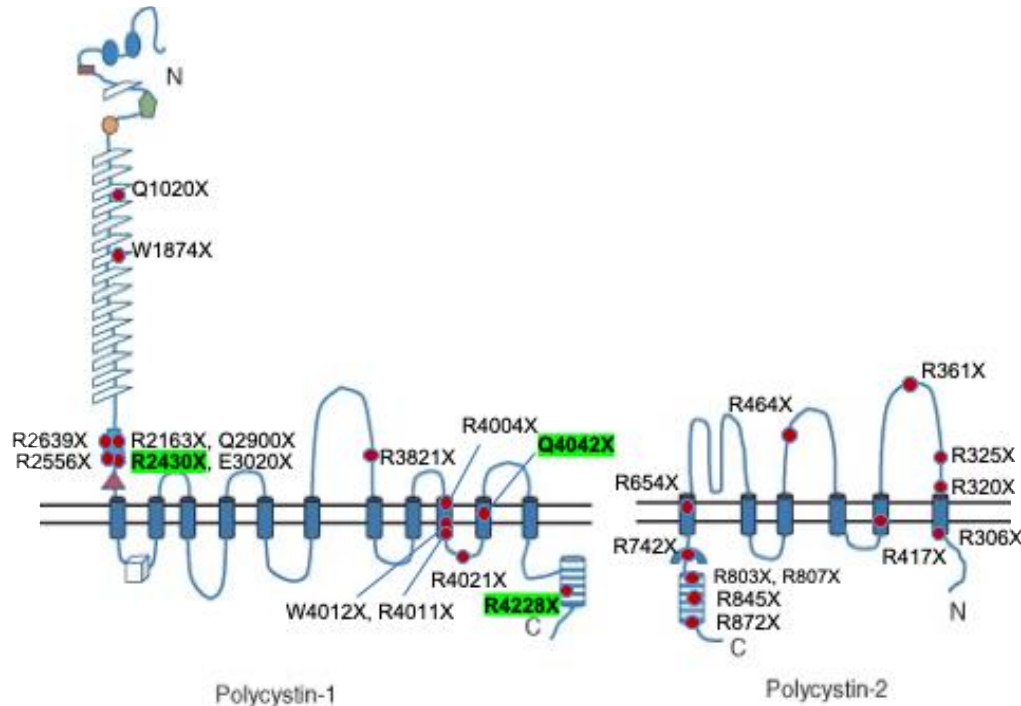


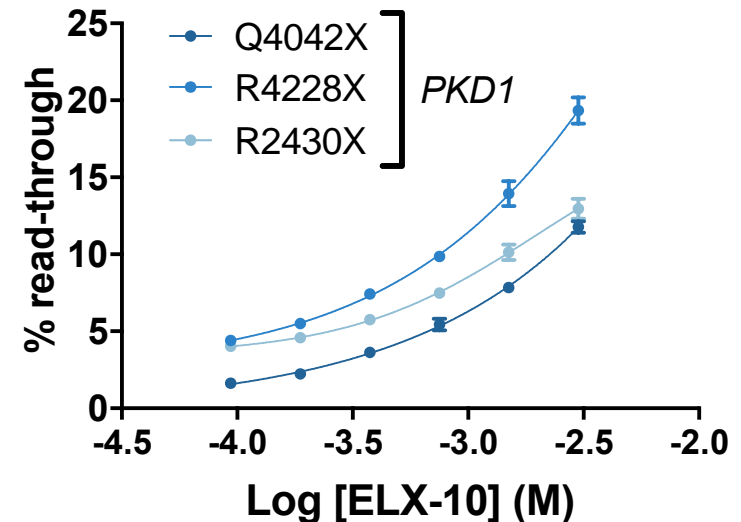
Figure 2. Frequency and type of *PKD1* and *PKD2* mutations from the PKD mutation database. All mutation types have been reported for both genes. The relative infrequency of missense mutations and in-frame insertions or deletions for *PKD2* could reflect the under-diagnosis of these patients present in the general population. The PKD mutation database is available at <http://pkdb.mayo.edu/index.html> (accessed 22 April 2016).

Mao et al., F1000 Research 2016

ERSGs Promote Read-through of Most Common *PKD1* and *PKD2* Nonsense Mutations



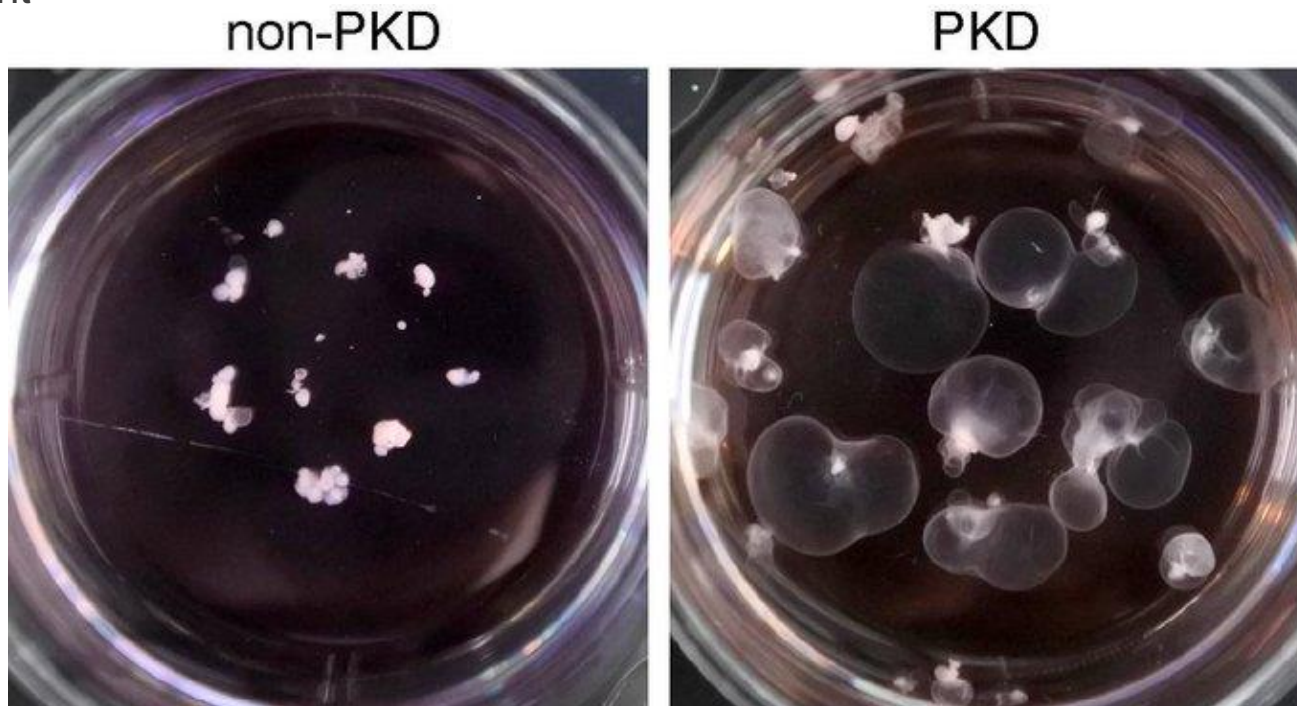
***PKD1* Dual luciferase
% Read-through, ELX-10**



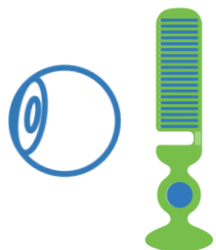
- Nonsense (X) mutations can be found across both *PKD1* (PC1) and *PKD2* (PC2) genes
- Read-through of **most common nonsense alleles** shown in dual luciferase assay
- Dose-dependent read-through of the top three *PKD1* nonsense alleles (according to the Mayo ADPKD database) is observed with multiple ERSGs

Encouraging ERSG Results in ADPKD Organoids

- Nonsense mutation kidney organoids model ADPKD cyst formation
 - Model system flexible enough to evaluate genetically heterogenous ADPKD population
 - Ongoing collaboration with Benjamin Freedman, University of Washington
- ERSG compounds are under evaluation in iPSC-derived (induced pluripotent stem cell-derived) and primary organoids for impact on cyst formation and reduction
 - Encouraging preliminary results show reduced cystogenesis and cyst size with ERSG treatment



Ocular Program Development



- High unmet medical need and prevalence of nonsense mutations across inherited retinal diseases (IRDs)
- Screened multiple compounds from ERSG library for read-through and tolerability
- IND-enabling studies are progressing



- Emerging Profile Across Models
 - Compounds are appropriate for intravitreal administration
 - Compounds show retinal tolerability at doses 10-fold greater than anticipated efficacy range in sensitive species
 - No adverse ELX compound-related retinal anatomic or functional changes observed to date by histopathology and ERG
 - Dose-dependent read-through of Usher mutations greater than gentamicin reference
 - Encouraging PK demonstrating retina exposure
- In vitro sustained release rates are consistent with the target range of one to three months

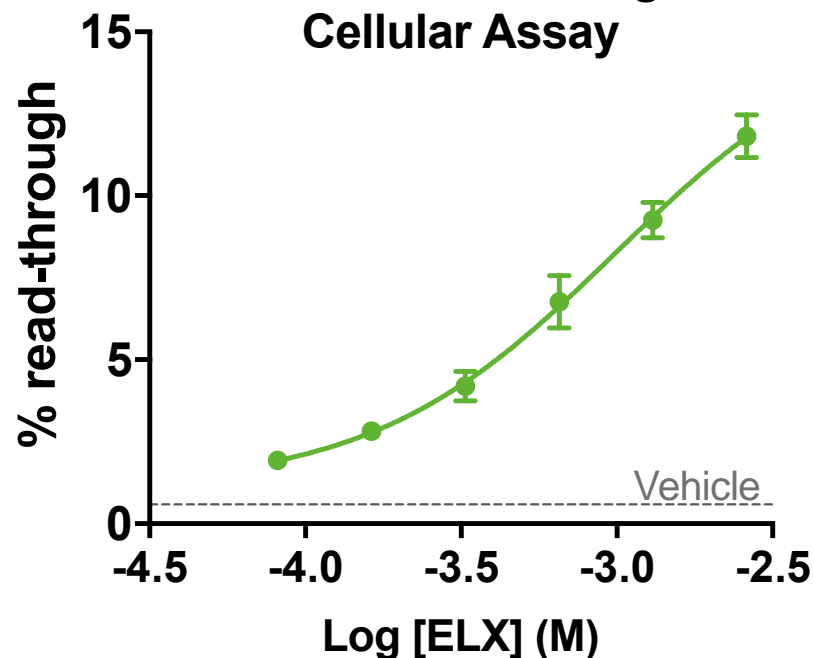


Intravitreal Administration Modeling



- SJL/J mice have a *R262X* mutation (UGA) in the *OCA2* gene¹
- *OCA2* is a channel involved in establishing organelle (melanocyte) pH in the RPE (retinal pigment epithelium)
- Cellular read-through testing demonstrates significant read-through potential across ELX compounds
- Model is being used to screen Eloxx compounds for *in vivo* read-through activity at the back of the eye

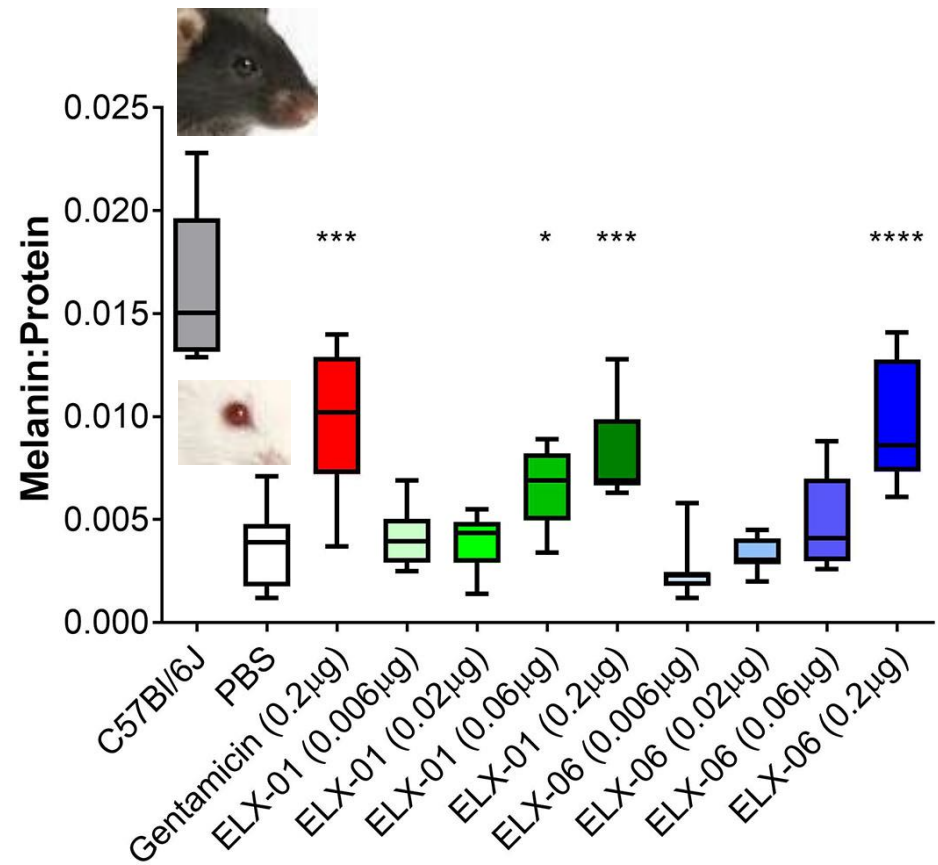
In Vitro Dual Luciferase Assay *R262X* Read-through Cellular Assay



1. Shoji et al, Exp. Anim 2015; 64(2)
2. Bellono et al., eLife 2014
3. Eloxx data on file.

ERSGs Promote Functional Read-through in the Eye by Intravitreal Administration (*In Vivo*)

- Intravitreal dosing of ELX compounds demonstrate a dose-dependent increase in melanin production in the eye
 - Single dose on Study Day 1
 - Melanin measured on Study Day 3
- Multiple ERSG compounds demonstrate increased OCA2 function after single intravitreal injection
- Data support that ERSG compounds can reach cells deep in the neurosensory retina, including the retinal pigment epithelium and choroid
- New data presented at the **Association for Research in Vision and Ophthalmology (ARVO) Virtual Annual Meeting** May 6, 2020



Eloxx Pharmaceutical Highlights

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- Cash runway extends through the end of 2021
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 - Independent Safety Review Committees have allowed dose escalation to the top dose
 - Expect to report top line data in the first half of 2021, barring any COVID disruptions
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 - Preclinical studies demonstrate dose-dependent read-through across most prevalent *PKD1* and *PKD2* alleles
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Thank you

February 2021