Prime Editors precisely correct pathogenic mutations in *RHO* and *USH2A* associated Retinitis Pigmentosa and prevent retinal degeneration

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Background & Introduction

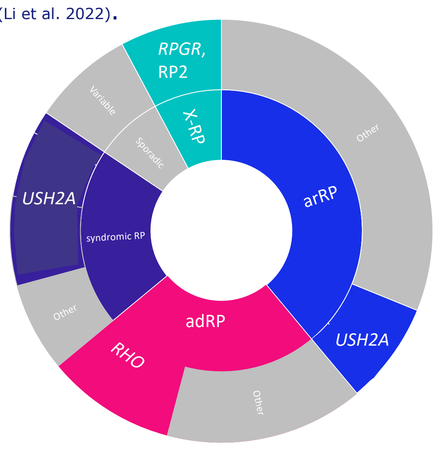
Retinitis pigmentosa (RP) is a group of rare inherited degenerative eye diseases affecting up to 1.5M individuals worldwide. RP is caused by mutations in multiple genes that affect the retina resulting in progressive vision loss leading to eventual blindness, with symptoms often manifesting in childhood and currently with no cure. RP is characterized by bilateral loss of rod photoreceptors followed by secondary loss of cone photoreceptors and degeneration of the retinal pigment epithelium (RPE).

RHO-mediated autosomal dominant RP is caused by mutations in the gene encoding rhodopsin, a light-sensitive G protein-coupled receptor that initiates the phototransduction cascade in rod photoreceptors (Zhen et al. 2023).

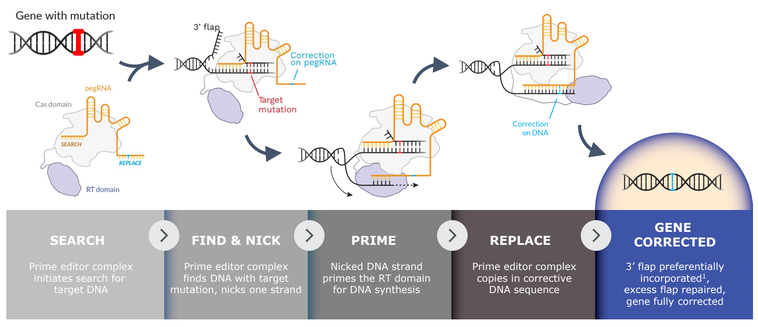
Mutations in the *USH2A* gene are a leading cause of autosomal recessive RP and Usher Syndrome. *USH2A* encodes usherin, a transmembrane protein mainly produced in the photosensitive layer of the retina, the hair cells of the cochlea, and the basement membrane of many tissues (Li et al. 2022).

Pathogenic variants associated with RP addressable by Prime Editing

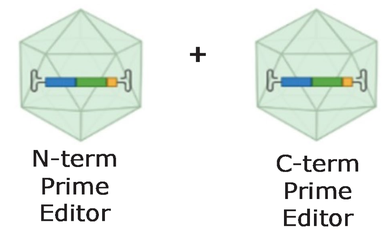
- **Syndromic RP (Usher Syndrome)**
- **adRP = autosomal dominant RP**
- **arRP = autosomal recessive RP**
- X-RP = X-linked RP



Prime Editing is a highly versatile gene editing technology that utilizes a modified CRISPR enzyme that can alter gene sequences with single nucleotide precision, including the correction of all twelve types of single base pair point mutations as well as multiple mutations spanning ~100 bp at genetic "hot spots". Collectively, the mutations that are addressable by Prime Editing account for approximately 90 percent of genetic variants associated with disease. Prime Editing creates permanent genetic corrections at the natural genomic location, thereby retaining native physiological gene control, and resulting in durable edits. Prime Editing can be programmed to produce a wide variety of precise, predictable, and efficient genetic outcomes at the targeted DNA sequence while minimizing unwanted bystander edits (such as insertions/deletions or indels) at the targeted genetic loci and minimizing off-target edits at non-targeted genetic loci.



Leveraging a highly-modular Dual-AAV platform for delivery of Prime Editing components to the retina



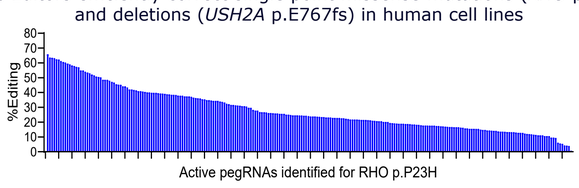
Leveraging the knowledge and experience previously established in the ophthalmology field for effective gene delivery and good safety profile of AAV vectors, Prime Medicine has developed a Dual-AAV platform to deliver Prime Editing components to photoreceptors in the retina via subretinal injection. Our therapeutic approach utilizes Dual-AAV Prime Editors (PEs) to precisely correct prevalent disease-causing mutations in *RHO* and *USH2A*, which are the most recurrently mutated genes causing RP.

A new Prime Editor Dual-AAV can be created by swapping guide RNAs in otherwise constant AAV genomes (promoters, coding sequences, regulatory elements) and capsids.

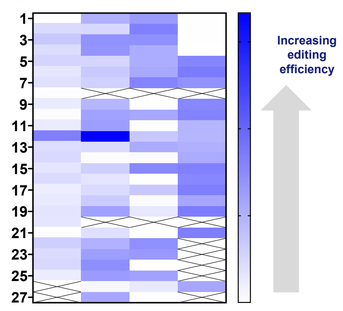
Results

Prime Editing can correct prevalent pathogenic mutations for RP

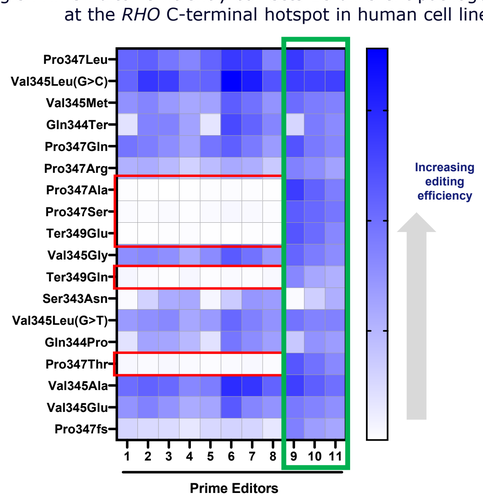
A Prime Editors efficiently correct single point missense mutations (*RHO* p.P23H) and deletions (*USH2A* p.E767fs) in human cell lines



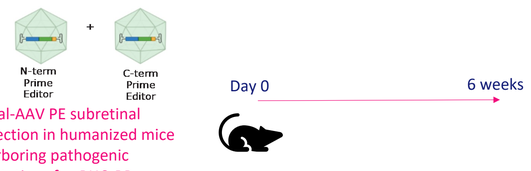
Active pegRNAs identified for *USH2A* p.E767fs (c.2299delG)



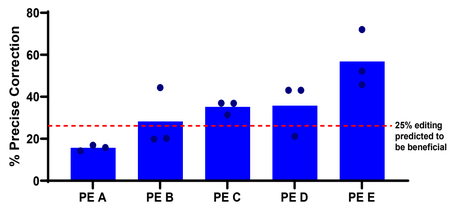
B A single Prime Editor efficiently corrects 18 different pathogenic mutations at the *RHO* C-terminal hotspot in human cell lines



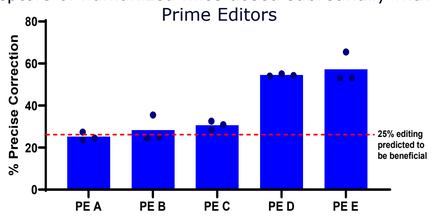
Robust *in vivo* editing of pathogenic mutations for Retinitis Pigmentosa observed in photoreceptors of humanized mice



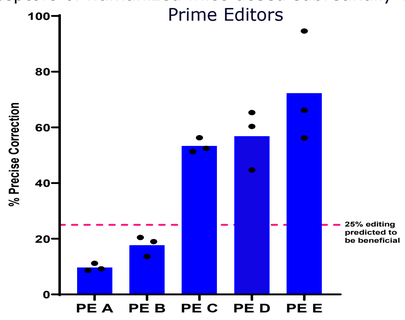
A Up to 70% precise correction at *RHO* p.P23H in photoreceptors of humanized mice dosed subretinally with dual-AAV Prime Editors



B Up to 65% precise correction at *RHO* C-terminal mutation hotspot in photoreceptors of humanized mice dosed subretinally with Dual-AAV Prime Editors

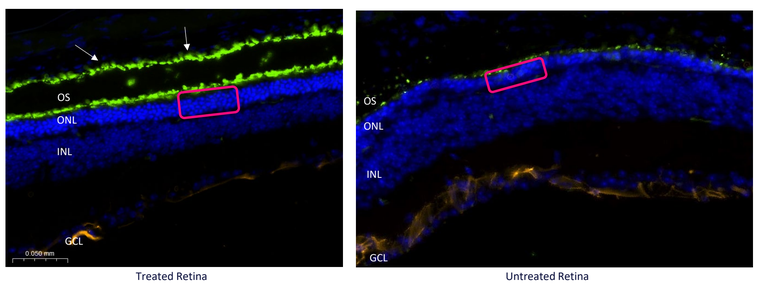


C Up to 95% precise correction at *USH2A* p.E767fs (c.2299delG) in photoreceptors of humanized mice dosed subretinally with Dual-AAV Prime Editors



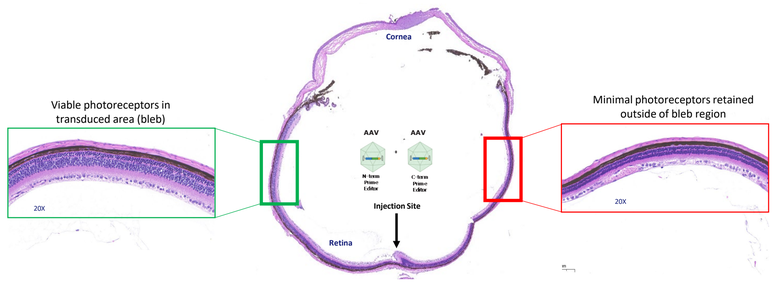
Prime Editor correction *in vivo* results in restoration of RHO expression and preservation of photoreceptors

A Prime Editor correction results in restoration of rhodopsin expression in photoreceptors of mice dosed subretinally with Dual-AAV Prime Editors

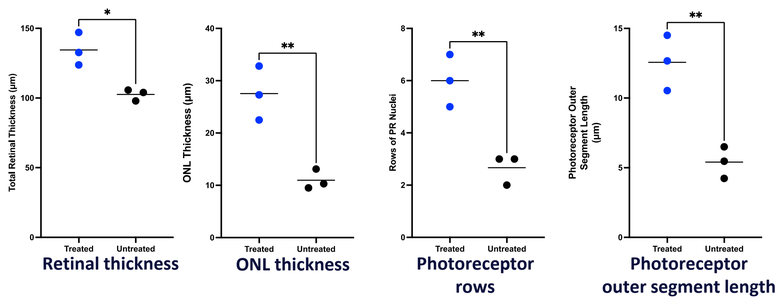


Rhodopsin, Nuclei , Glial cells (GFAP), Pink box demonstration of PR preservation.
OS = outer segment; ONL = outer nuclear layer; INL = inner nuclear layer; GCL = ganglion cell layer

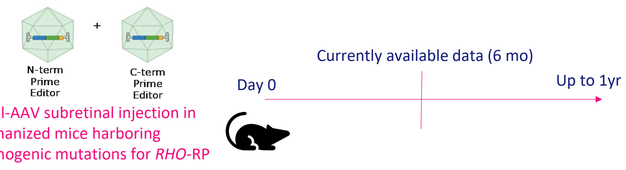
B Prime Editor correction results in preservation of photoreceptors in treated retinal areas



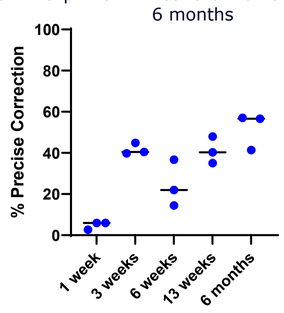
C Prime Editing correction results in improved photoreceptor cell health in treated retinal areas



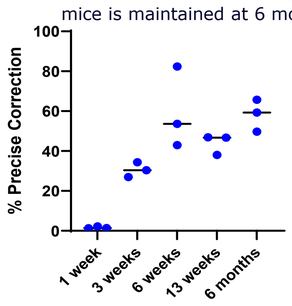
Durable robust gene correction observed in humanized mice dosed with Dual-AAV Prime Editors for Retinitis Pigmentosa



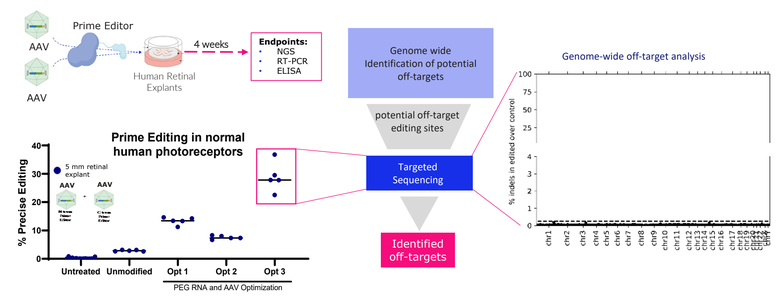
A Robust editing of *RHO* p.P23H in retina of humanized mice is maintained at 6 months



B Robust editing of *RHO* C-terminal mutation hotspot in retina of humanized mice is maintained at 6 months



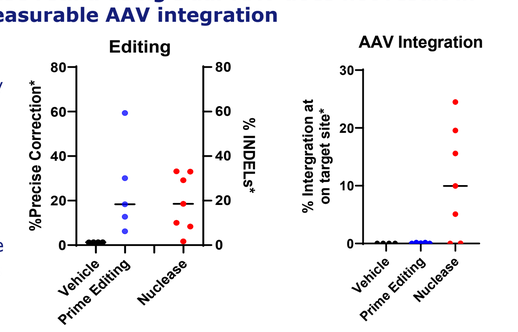
Preliminary analysis of potential off-target sites does not identify off-target editing in human photoreceptors



Prime Editing of mouse retina using Dual-AAV does not result in measurable AAV integration

Prime Editing of mouse retinas via subretinal delivery of AAV results in editing at a non-*RHO* control site at similar rates to nuclease editors

Prime Editing avoids measurable integration of the AAV vector at the edit site as measured by one-sided PCR



Conclusions

- Prime Editors correct the prevalent pathogenic mutations causing Retinitis Pigmentosa, including single base pair point mutations and multiple mutations within *RHO* C-terminal hotspot
- Our highly-modular Dual-AAV Prime Editor platform effectively delivers Prime Editor components to photoreceptors with high efficiency, enabling the precise correction of pathogenic mutations that cause RP with no evidence of unintended edits
- Correction of the *RHO* p.P23H RP mutation in humanized mouse model with rapid photoreceptor degeneration results in restoration of rhodopsin expression and preservation of photoreceptors in the retina
- Dual-AAV Prime Editor platform for Retinitis Pigmentosa enables one-time treatment for sustained precise correction of pathogenic mutations in humanized mice out to 6 months
- Correction of most prevalent mutations in the *RHO* and *USH2A* genes using Prime Editing is expected to restore photoreceptor cell function and/or preserve cell number and expected to halt Retinitis Pigmentosa disease progression to impact the overall functional vision of individuals with *RHO*-RP and *USH2A*-RP