In Vivo Proof of Concept for EDIT-102: A CRISPR/Cas9-Based Experimental Medicine for *USH2A*-Related Inherited Retinal Degeneration Caused by Mutations in Exon 13

P# 719

Swati Mukherjee, Anila Mehta, Dawn Ciulla, James Bochicchio, Georgia Giannoukos, Eugenio Marco, George Bounoutas, Shengfang Jin, Andrea de Erkenez, Cecilia A. Fernandez, Charles F Albright, Kate Zhang, Carrie M. Margulies

Editas Medicine, Inc. | 11Hurley Street, Cambridge, MA 02141

Usher Syndrome Type 2A and EDIT-102

- Mutations in the *USH2A* gene are a major cause of autosomal recessive retinitis pigmentosa (RP) and Usher syndrome Type II (USH2A). Patients with USH2A mutation have early hearing loss, progressive peripheral vision loss, and eventual legal blindness.
- *USH2A* gene encodes the transmembrane protein Usherin which is localized in connecting cilia of photoreceptors and believed to play a role in stabilizing the photoreceptor outer segment
- c.2299delG mutation in exon 13 is the most common mutation in *USH2A* gene, causing a frameshift and truncated protein, which leads to ciliary defect. It is a common cause of inherited retinal degeneration (IRD).
- There are currently no approved disease-modifying therapies for RP caused by USH2A mutations
- We have developed EDIT-102, an experimental medicine for *USH2A*-related IRD through CRISPR/Cas9-mediated excision of USH2A exon 13.
- EDIT-102 is an AAV5 vector expressing S. aureus CRISPR/Cas9 driven by the photoreceptor-specific G protein-coupled receptor kinase 1 (GRK1) promoter and two U6 promoter-driven gRNAs that flank hUSH2A exon 13 (Figure 1).

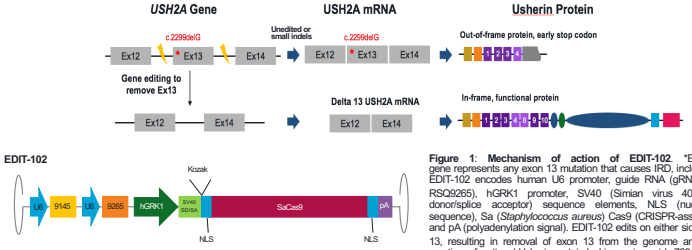

Figure 1: Mechanism of action of EDIT-102. *Ex13 in *USH2A* gene represents any exon 13 mutation that causes IRD, including c.2299delG. EDIT-102 encodes human U6 promoter, guide RNA (gRNA; RSQ9145 and RSQ9265), hGRK1 promoter, SV40 (Simian virus 40) SD/SA (splice donor/splice acceptor) sequence elements, NLS (nuclear localization sequence), Sa (*Staphylococcus aureus*) Cas9 (CRISPR-associated protein 9) and pA (polyadenylation signal). EDIT-102 edits on either side of *USH2A* exon 13, resulting in removal of exon 13 from the genome and mRNA thereby creating a functional Usherin protein lacking amino acids 723-936.

USH2A Humanized Knock-In (KI) Mouse Models

- A humanized *USH2A* KI mouse model (hUSH2A^{2299delG}) was generated, where mouse *Ush2a* exon 12 was replaced with the human *USH2A* exon 13 containing the 2299delG mutation and a portion of the flanking introns (Figure 2A).
- Around 17% of USH2A transcripts in heterozygous hUSH2A^{2299delG} mouse retinal tissue contain human exon 13, as measured by Nanostring.

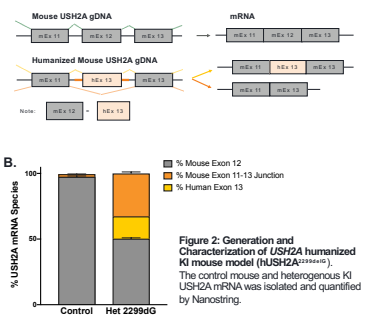

Figure 2: Generation and Characterization of *USH2A* humanized KI mouse model (hUSH2A^{2299delG}). The control mouse and heterogenous KI USH2A mRNA was isolated and quantified by Nanostring.

Methods

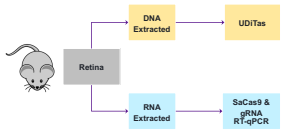

Figure 3: Experimental Schematic for EDIT-102 Pharmacology Studies. Heterozygous hUSH2A^{2299delG} KI mice were subretinally injected with EDIT-102 or vehicle. At different time points, retinas were harvested and split equally for DNA & RNA assays. On-target *USH2A* gene editing was determined using the retinal DNA in a unidirectional targeted sequencing (UDiTaS) method. Expression levels of SaCas9 and guide RNA (gRNA) were evaluated using the retinal RNA in a real time quantitative polymerase chain reaction (RT-qPCR) assay.

Productive USH2A Gene Editing in EDIT-102 treated Mice

- Subretinal injection of 1 µl of AAV5 vector AAV5-GKR1-GFP show 30% transduction of mouse neural retina in previous study (Maeder 2019).
- The total on-target edits is calculated by including large deletions, inversions, AAV/plasmid integrations and small indels.
- Inversions and deletions were previously defined as productive edits that generate USH2A$^{\Delta 13}$ mRNA. Normalized productive editing is obtained by multiplying the percentage of productive editing with the transduction multiplier, as shown below:

Transduction area of retina: 30%
Transduction multiplier: 100/30 = 3.3
Normalized Productive Editing = Productive Editing * 3.3

Results

Normalized Productive Editing of *USH2A* Gene and Cas9 mRNA/gRNA Expression Increased with the Increase of EDIT-102 dose

Dose Response Study Design

Groups	Dosing Regimen	Dose (vg/ml)	Number of Retinas
1	Vehicle	0	10
2	EDIT-102	1E+11	30
3	EDIT-102	3E+11	30
4	EDIT-102	1E+12	31
5	EDIT-102	3E+12	30
6	EDIT-102	1E+13	31

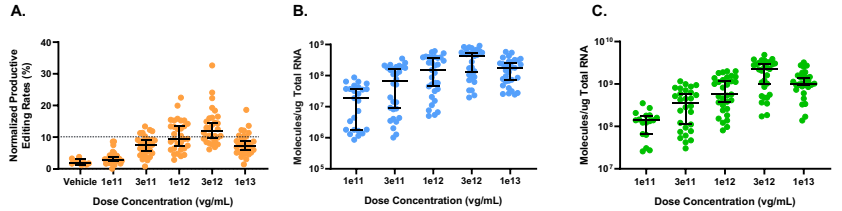

Figure 4: Dose Response Study For EDIT-102. The figure shows the normalized productive editing rates (Median ± 95% confidence interval) (A), Cas9 mRNA (B) and gRNA (C) expression levels in hUSH2A^{2299delG} KI mice. Each dot represents a single retinal sample and the dotted line represents 10% normalized productive editing of photoreceptors.

The level of normalized productive editing in the mouse retina increases with the dose of EDIT-102 from 1e11 to 3e12 vg/mL and decreases at 1e13 vg/ml. We expect a therapeutic benefit when 10% foveal cone photoreceptors are productively edited (Geller 1992, Geller 1993). The dose response of EDIT-102 from 3e11 to 3e12 vg/ml achieve 10% or greater productive editing in the treated mouse retinas.

Normalized Productive *USH2A* Gene Editing and Cas9 mRNA/gRNA Increased with Time

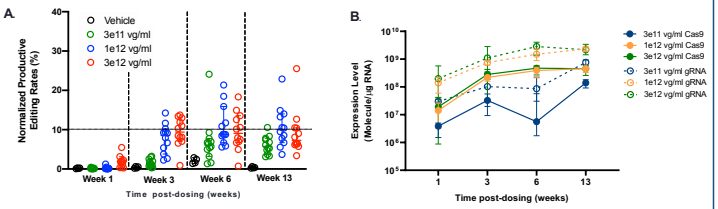

Figure 5. Time course study for EDIT-102. The figure indicates the normalized productive editing of *USH2A* gene (A) and Cas9 mRNA and gRNA expression (B) over time. The Vehicle group includes 16 retinas and the dose 3e11, 1e12 and 3e12 vg/ml of EDIT-102 groups have 47, 48 and 48 retinas. Each point represents an individual mouse eye harvested at various timepoints from 1 to 13 weeks post-dosing.

The median level of normalized productive editing and expression level of Cas9 mRNA and gRNA, at the doses of 1e12 and 3e12 vg/ml, plateaued at 6 weeks and was maintained at a stable level over 13 weeks post dose.

Total Editing Correlates with gRNA and Cas9 mRNA Expression

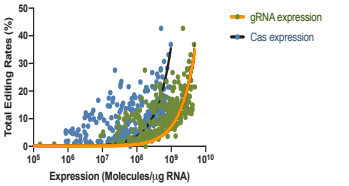

Figure 6: Total *USH2A* Gene Editing Rates and Cas9 mRNA and gRNA Levels in EDIT-102 Treated hUSH2A^{2299delG} KI Mice. Each data point indicates a single mouse retina processed in EDIT-102 dose-response and time-course studies, 1, 3, 6, or 13-week post subretinal injection. Curve fitting between total non-normalized editing and Cas or gRNA expression was fitted using a non-linear regression model using GraphPad Prism. Spearman rank correlation coefficient with P-value <0.0001.

Conclusions

- Pharmacokinetic and pharmacodynamic studies indicated Cas9 and gRNA expression correlated with total editing. Dose response of EDIT-102 from 1e12 to 3e12 vg/ml achieved close to 10% or greater normalized productive editing in the mouse retinas.
- All animals used in the pharmacological studies demonstrated tolerability as determined by clinical observations and body weight measurement.
- These data support the continued development of EDIT-102 for the potential treatment of USH2A-related IRD caused by mutations in exon 13.

References

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Acknowledgements

We would like to thank the following Editas teams for supporting this project: Sequencing, Screening, Sample Management, Bioinformatics, Computational Biology, Regulators, and Scientific Communications. Additionally, we would like to thank Clifford Yudoff, Binit Kumar and Mrudula Donepudi for valuable scientific insight. Graphic design support was provided by Robert Brown.

Disclosures:
Employees and shareholders of Editas Medicine: S.M., A.M., D.C., J.B., G.G., E.M., S.J., A.D.E., C.F.A., K.Z., and C.M.M.