LNP-Based Delivery of CRISPR/Cas12a for the Potential Treatment of Myocilin-Associated Glaucoma

Heather MacLeod

May 10, 2024

editas MEDICINE

Disclosure

The speaker and the co-authors are current or former employees and stockholders of Editas Medicine, Inc.

Disease Background on Primary Open Angle Glaucoma (POAG)

- Glaucoma is an optic neuropathy resulting in loss of peripheral vision followed by loss of central vison[1–3]
  - A leading cause of irreversible blindness in the developing world[1,2]
- Primary open-angle glaucoma (POAG) is a subset of glaucoma defined by an open, normal-appearing anterior chamber angle[1,3]
  - One major risk factors for POAG is elevated intraocular pressure (IOP)[1–3]
  - IOP lowering medications are the standard of care but begin to fail over time, resulting in the need for surgery[1–3]
  - Long-term prognosis is reduced vision and eventual vision loss[1,2]
  - 2%–5% of patients with POAG have mutations in the myocilin gene with an estimated 54,000–135,000 patients in the United States[1,2,4,5]

Normal vision

Early POAG

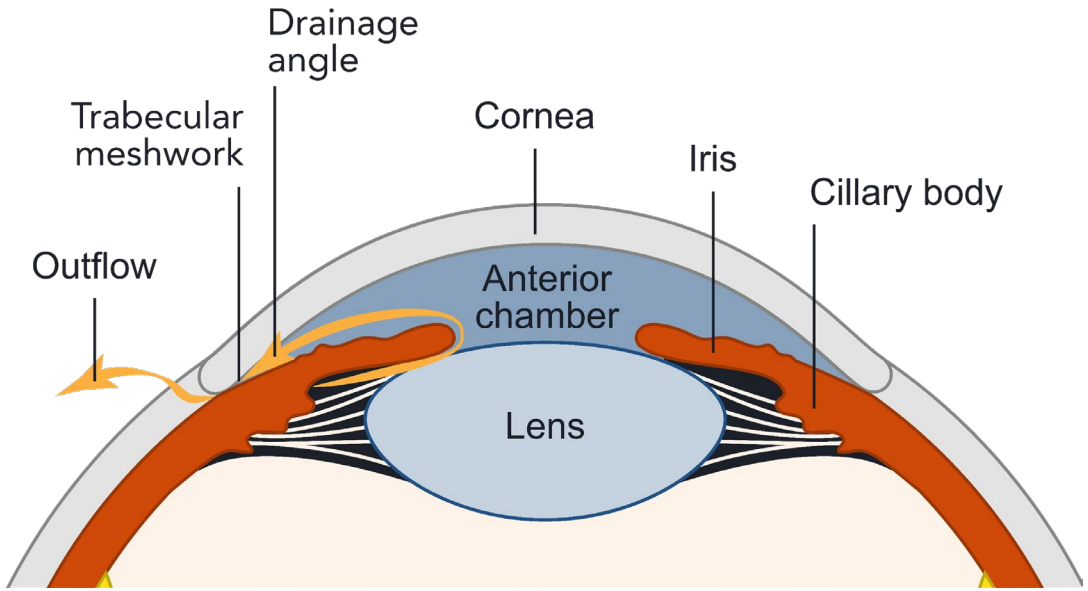
Advanced POAG

Extreme POAG

1. Fan and Wiggs. *J Clin Invest* 2010; 120 (9): 3064.
2. Weinreb *et al. JAMA* 2014; 311 (18): 1901.
3. Home and Work Optometry Care. Available at: https://homenworkoptometrycare.wordpress.com/2016/10/11/overview-of-primary-open-angle-glaucoma/. Accessed May 2024.
4. Fingert *et al. Human Molecular Genetics* 1999; 8: 899.
5. Glaucoma: Facts & Figures. Available at: https://www.brightfocus.org/glaucoma/article/glaucoma-facts-figures. Accessed May 2024.

IOP, intraocular pressure; POAG, primary open-angle glaucoma.

Rationale for *In Vivo* Gene Editing via CRISPR/Cas Knockout of Myocilin



Features of the anterior chamber of the eye

- The trabecular meshwork (TM) is responsible for regulating the outflow of aqueous humor and maintaining IOP at safe levels[1,3]
- Myocilin is a secreted protein of unknown function produced by the TM[2,3]
- Pathogenic gain-of-function mutations in myocilin result in buildup of the protein inside TM cells causing endoplasmic reticulum (ER) stress, dysfunction of the TM tissue, and elevated IOP[1,3]
- Gene editing via CRISPR/Cas knockout of myocilin is expected to restore function to the TM and subsequent lowering of IOP

Objective: To evaluate *in vivo* editing and efficacy of using lipid nanoparticles (LNP) to deliver engineered AsCas12a mRNA and gRNA in a mouse model of myocilin-associated glaucoma

AsCas12a, *Acidaminococcus sp.* CRISPR-associated protein 12a; CRISPR, clustered regularly interspaced short palindromic repeats; ER, endoplasmic reticulum; gRNA, guide RNA; IOP, intraocular pressure; LNP, lipid nanoparticle; mRNA, messenger RNA; RNA, ribonucleic acid; TM. trabecular meshwork.

1. Fan and Wiggs. *J Clin Invest* 2010; 120 (9): 3064.
2. Fingert *et al. Genome Research* 1998; 8: 377.
3. Wang *et al. Int J of Molecular Med* 2019; 43: 671.

Development of a Lipid Nanoparticle for Delivery of CRISPR/Cas Editing Machinery to the Trabecular Meshwork

Lipid Nanoparticle (LNP)

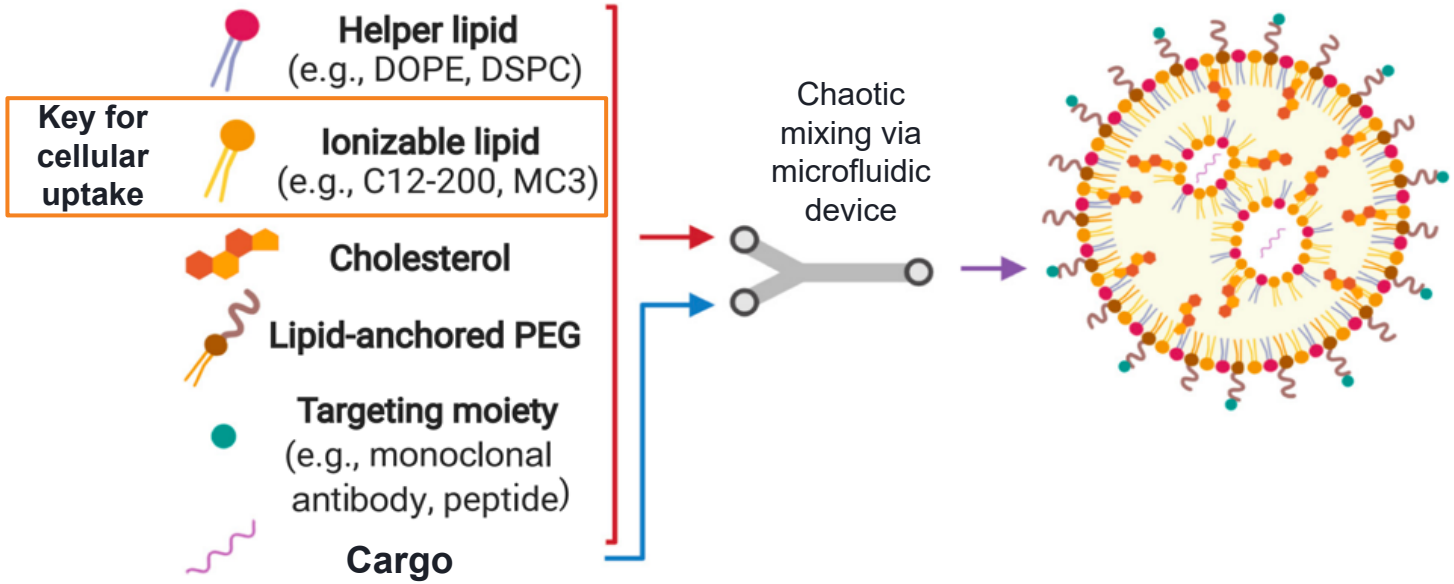
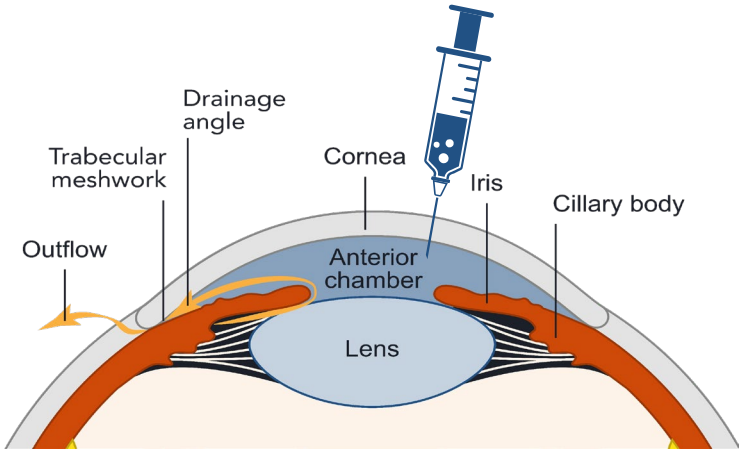


Figure adapted from Han X, 2023.[1]

- **Engineered AsCas12a mRNA**
- **gRNA with modifications to protect from endo- and exonucleases**

Final LNP administered by injection into the anterior chamber of the eye

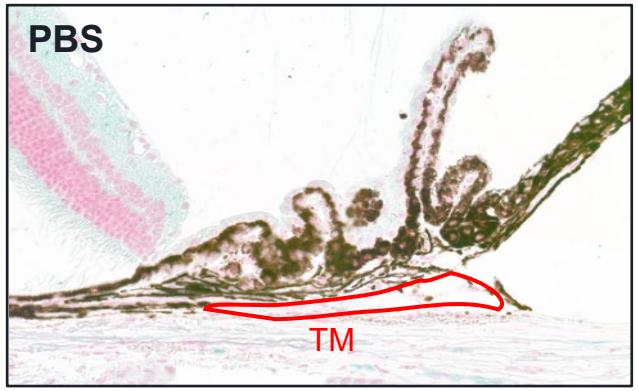


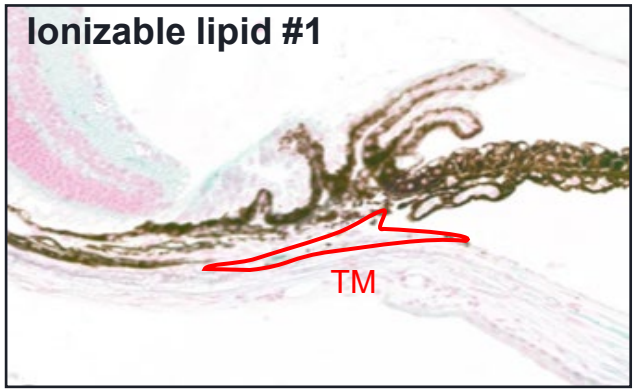
AsCas12a, *Acidaminococcus sp.* CRISPR-associated protein 12a; CRISPR, clustered regularly interspaced short palindromic repeats; DOPE, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine; DSPC, distearoylphosphatidylcholine; gRNA, guide RNA; LNP, lipid nanoparticle; mRNA, messenger RNA; PEG, polyethylene glycol; RNA, ribonucleic acid.

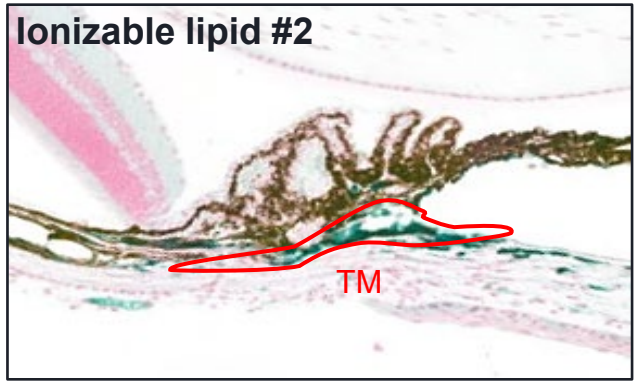
1. Han X *et al*. *Nat Commun* 2023; 14 (1): 75.

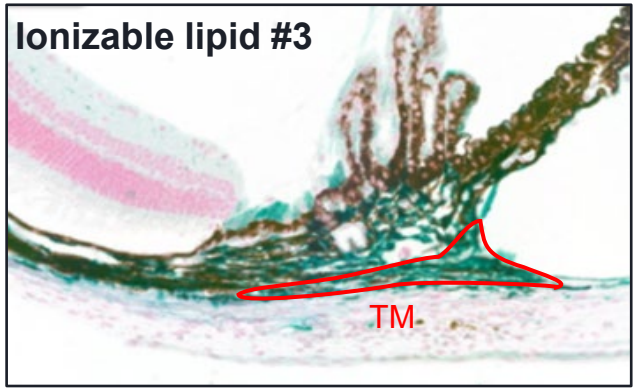
Selection of an Ionizable Lipid that Robustly and Specifically Targets the TM Tissue

LNPs encapsulating EGFP mRNA were evaluated in mice *in vivo* for transfection of the TM tissue









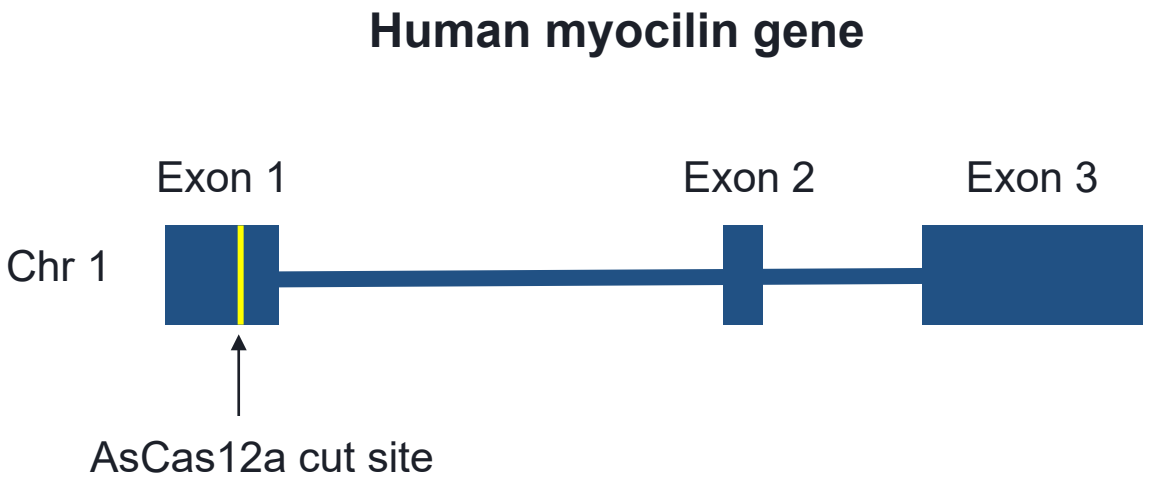
Trabecular meshwork (TM) area is outlined in red.
Representative images from five mice.

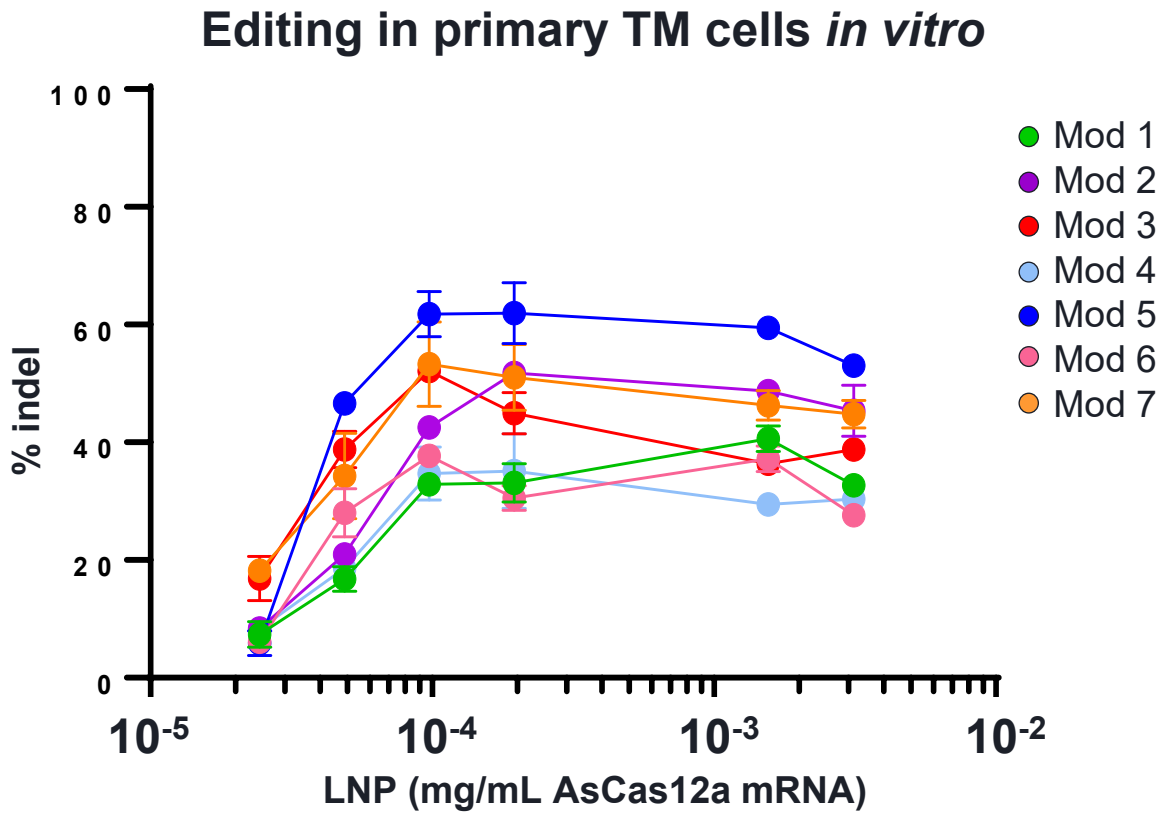
- Ionizable lipid #2 showed strongest GFP expression and specificity for the TM tissue
- Additional optimization was performed for the ratios of ionizable lipid #2, cholesterol, DSPC, and DMG-PEG

DMG-PEG, 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol; DSPC, distearoylphosphatidylcholine; EGFP, enhanced green fluorescence protein; GFP, green fluorescence protein; LNP, lipid nanoparticle; mRNA, messenger RNA; PBS, phosphate buffered saline; RNA, ribonucleic acid; TM, trabecular meshwork.
Additional LNP optimization data not shown.

editas MEDICINE

Development of an Optimized LNP Cargo: Selection of gRNA with Chemical Modifications to Improve Editing *In Vitro*





N=2. Data presented as mean ± SD.

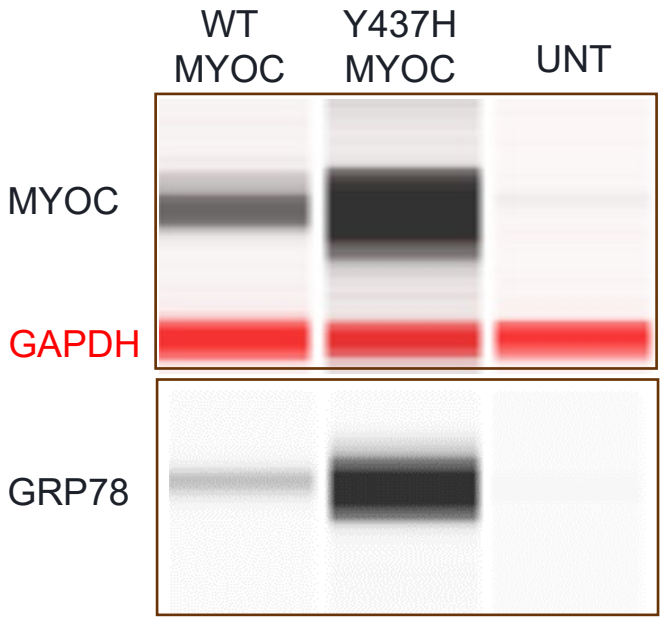
Modifications 3, 5, and 7 were selected to move forward for evaluation in mice

AsCas12a, *Acidaminococcus sp.* CRISPR-associated protein 12a; Chr 1, chromosome 1; CRISPR, clustered regularly interspaced short palindromic repeats; gRNA, guide RNA; LNP, lipid nanoparticle; Mod, gRNA modification; mRNA, messenger RNA; RNA, ribonucleic acid; SD, standard deviation.

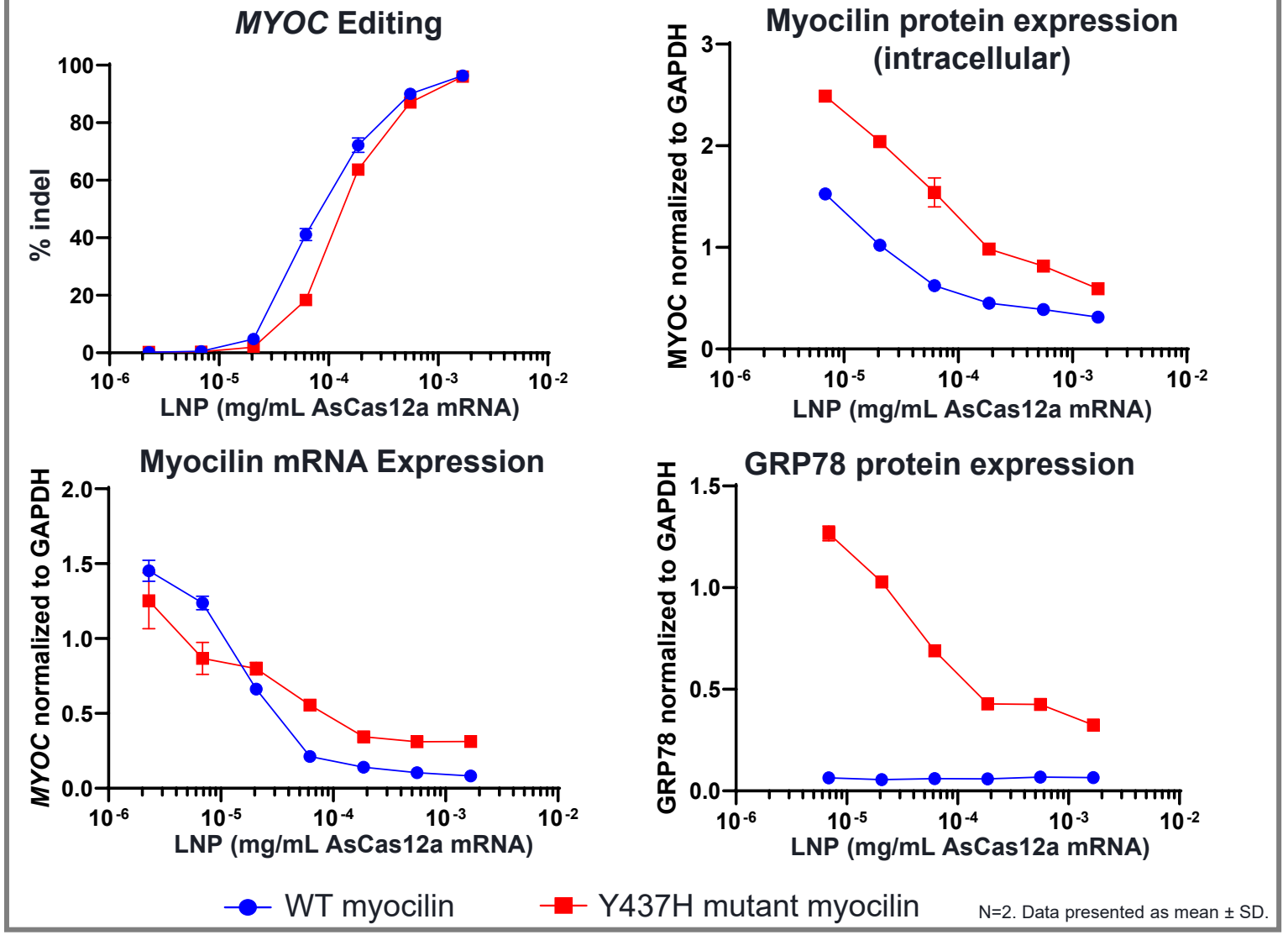
Wrighton PJ *et al.* Poster 1182 presented at ASGCT 2024; Baltimore, MD, USA, 7–11 May 2024.

Editing Myocilin *In Vitro* Reduces ER Stress Associated with Mutant Myocilin

HEK293T cells stably expressed either wildtype or Y437H mutant myocilin



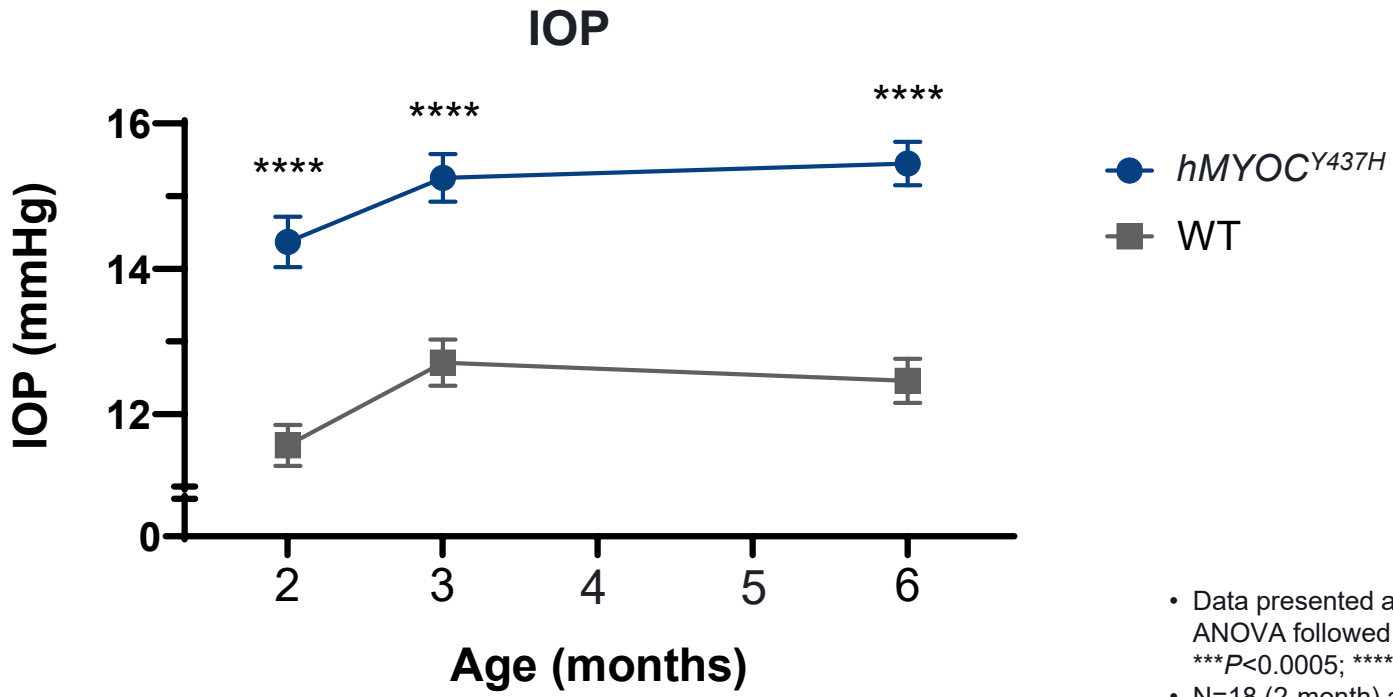
- Mutant myocilin protein is not secreted and builds up inside the cell resulting in elevated GRP78, a marker of ER stress



AsCas12a, *Acidaminococcus sp.* CRISPR-associated protein 12a; CRISPR, clustered regularly interspaced short palindromic repeats; ER, endoplasmic reticulum; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GRP78, Glucose-Regulated Protein 78; LNP, lipid nanoparticle; mRNA; messenger RNA; *MYOC*; myocilin gene; RNA, ribonucleic acid; SD, standard deviation; UNT, Untreated; WT, wildtype.

Mice Expressing Human Myocilin with the Pathogenic Y437H Mutation Exhibit Elevated IOP

Full length human myocilin with the Y437H mutation replaced the mouse myocilin gene

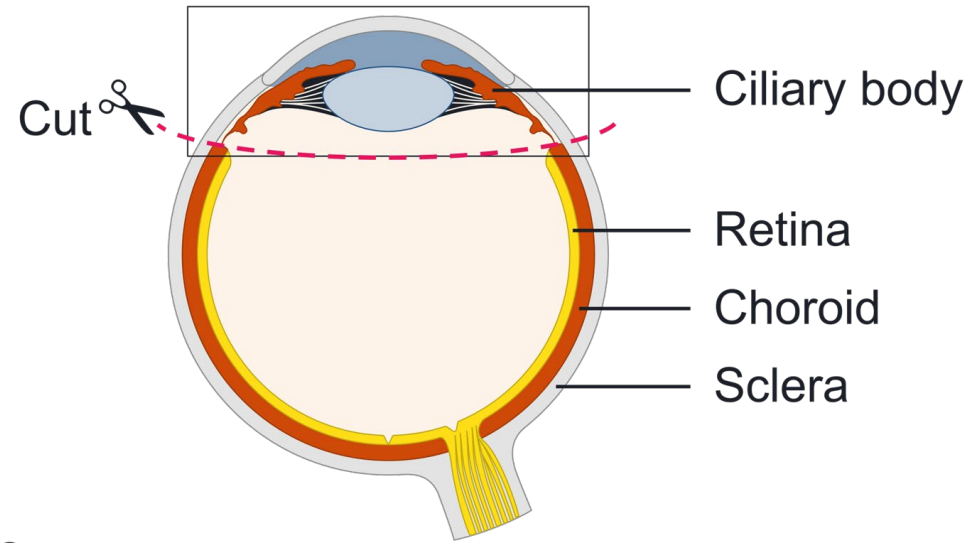


- Data presented as mean ± SEM and analyzed with 2-way ANOVA followed by Tukey's test. **P*<0.05; ***P*<0.005; ****P*<0.0005; *****P*<0.0001 compared with age-matched WT.
- N=18 (2-month) and 32 (3- and 6-month) for WT; N=28 (2-month), 40 (3-month), and 37 (6-month) for *hMYOC*^{Y437H}.

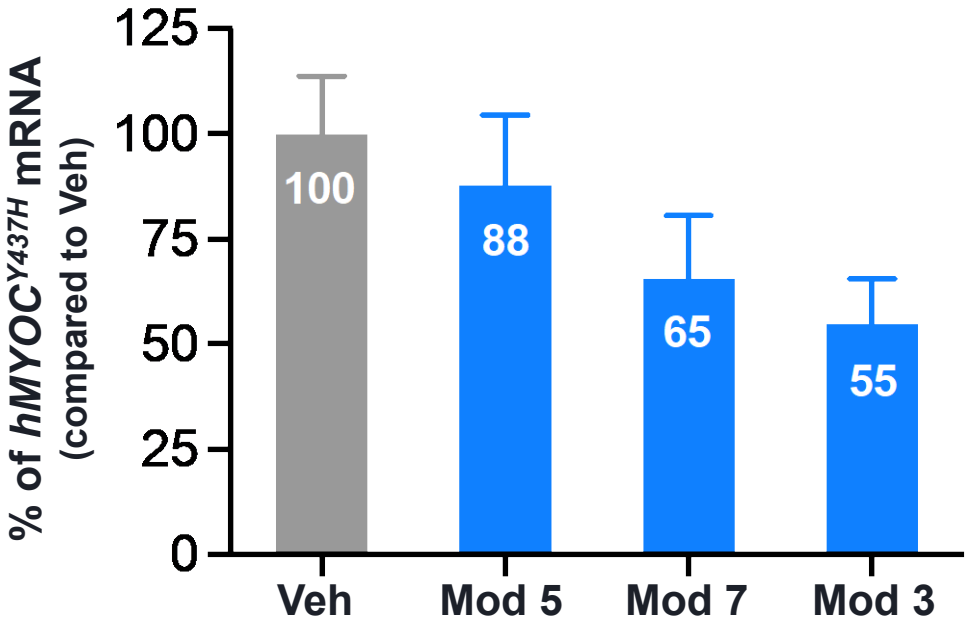
ANOVA, analysis of variance; AsCas12a, *Acidaminococcus sp.* CRISPR-associated protein 12a; Chr 1, chromosome 1; CRISPR, clustered regularly interspaced short palindromic repeats; IOP, intraocular pressure; *hMYOC*^{Y437H}, human myocilin with the Y437H mutation; WT, wildtype; HOM, homozygous mutants; SEM, standard error of the mean.

LNP Delivery of AsCas12a mRNA and Modified gRNA Reduces Expression of *hMYOC* mRNA *In Vivo*

Mouse anterior chamber dissection



Reduction of *hMYOC*^{Y437H} mRNA

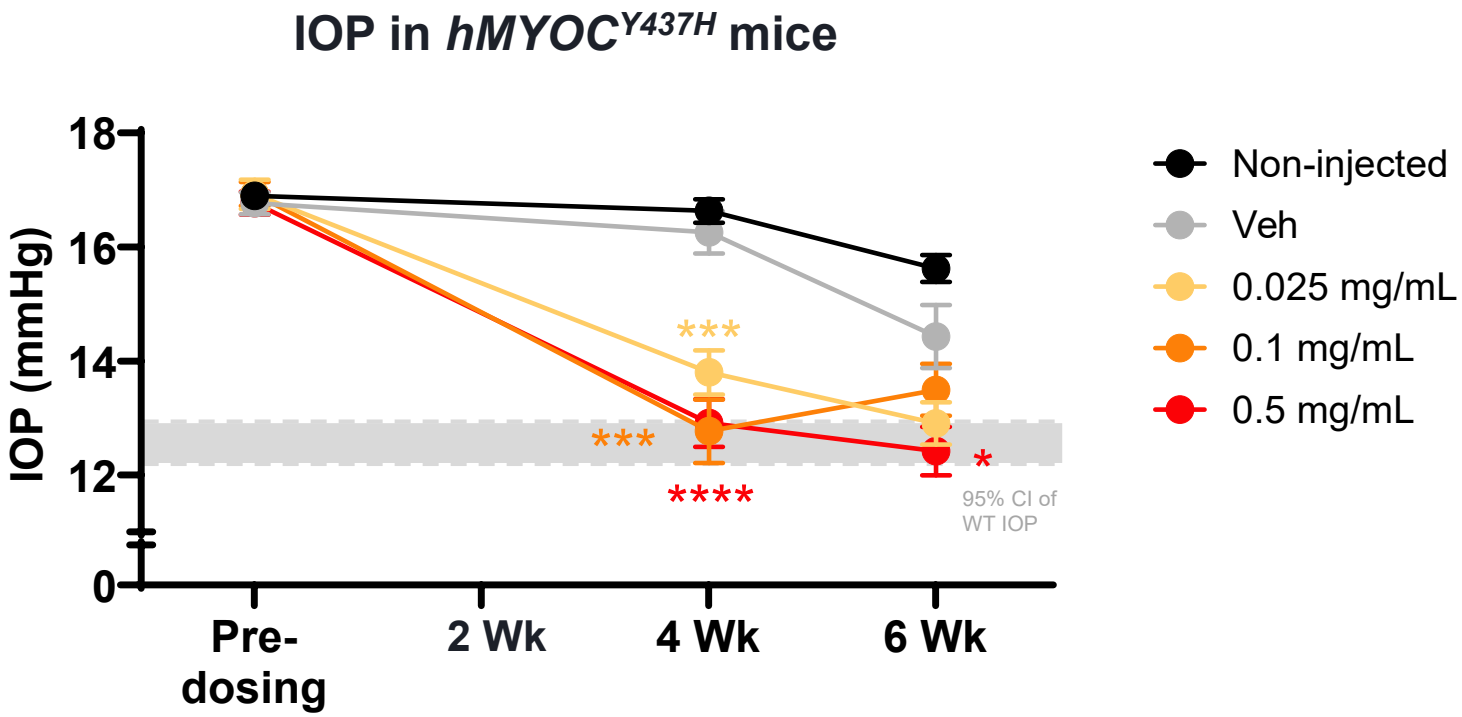


- Data presented as mean ± SEM and analyzed by one-way ANOVA with Tukey's multiple comparisons test compared to vehicle group (Veh)
- N=3 samples/group; two anterior chambers/sample

gRNA modification #3 showed highest reduction in *hMYOC* mRNA and was moved forward for evaluation in phenotypic assays

ANOVA, analysis of variance; *hMYOC*^{Y437H}, human myocilin with the Y437H mutation; gRNA, guide RNA; Mod, gRNA modification; mRNA, messenger RNA; RNA, ribonucleic acid; SEM, standard error of the mean; Veh, vehicle.

Editing the Myocilin Gene *In Vivo* Significantly Reduces IOP in a Mouse Model of Myocilin-Associated POAG



MYOC gene editing resulted in a reduction of IOP at all doses tested

AsCas12a, *Acidaminococcus sp.* CRISPR-associated protein 12a; CI, confidence interval; CRISPR, clustered regularly interspaced short palindromic repeats; *hMYOC*^{Y437H}, human myocilin with the Y437H mutation; IOP, intraocular pressure; LNP, lipid nanoparticle; *MYOC*, myocilin gene; POAG, primary open-angle glaucoma; SEM, standard error of the mean; Veh, vehicle; Wk, week; WT, wildtype.

- Data presented as mean ± SEM and analyzed by mixed-effect model with Tukey's multiple comparisons test against vehicle group (Veh). *P<0.05; **P<0.005 ; ***P<0.0005; ****P<0.0001.
- N=75–80 eyes for non-injected group; N=19–20 eyes for Veh; N=17–20 eyes for LNP-treated groups.
- The gray shading on the graph indicates 95% CI for the mean IOP of age-matched WT mice.

Summary and Conclusions

Successful development and optimization of an LNP for *in vivo* gene editing in myocilin-associated POAG

- Identified an ionizable lipid and lipid ratios that robustly and specifically transfects the TM
- Established modifications to gRNA that improve *in vivo* editing
- Developed a mouse model where expression of human, mutant myocilin results in elevated IOP

LNP-mediated delivery of an optimized gRNA and engineered AsCas12a mRNA results in:

- Reduced ER stress in HEK293T cells expressing mutant myocilin *in vitro*
- Rescued IOP *in vivo* in a mouse model of myocilin-associated POAG

MYOC gene knockout by LNP-based delivery of CRISPR/Cas12a is a potential therapeutic approach for treating myocilin-associated POAG

AsCas12a, *Acidaminococcus sp.* CRISPR-associated protein 12a; CRISPR, clustered regularly interspaced short palindromic repeats; ER, endoplasmic reticulum; gRNA, guide RNA; IOP, intraocular pressure; LNP, lipid nanoparticle; mRNA, messenger RNA; MYOC, myocilin gene; POAG; primary open-angle glaucoma; RNA, ribonucleic acid; TM, trabecular meshwork.

Thank you