CRISPR THERAPEUTICS

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Development of an *In Vivo* Non-Viral Delivery Platform for Ocular Editing and Application as a Potential Treatment for Glaucoma

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Disclosures

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Disclosure Information

I have the following relevant financial relationships to disclose:

Employee of: CRISPR Therapeutics

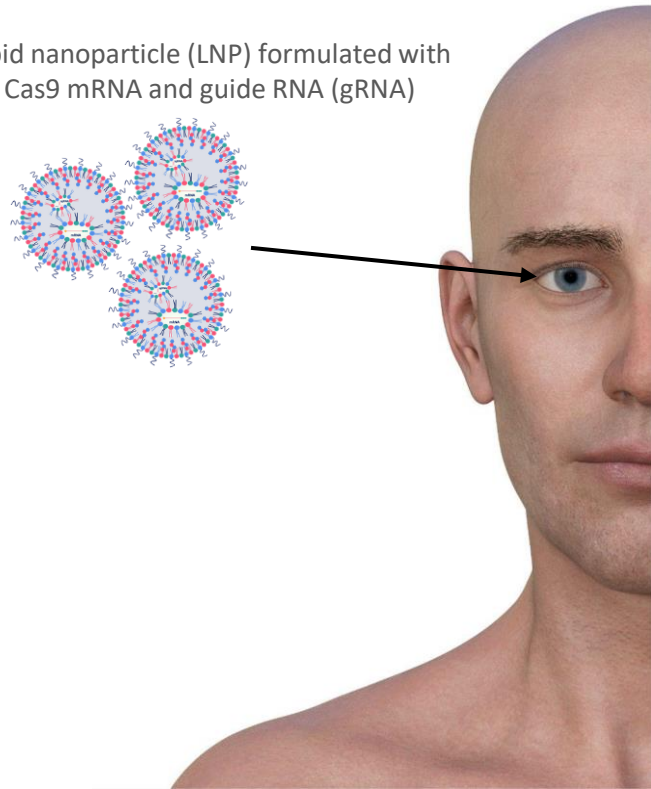
Stockholder in: CRISPR Therapeutics

Non-Viral Delivery – A Promising Approach for Ocular Gene Editing

- Potential to use CRISPR/Cas9 technology to address genetic diseases of the eye once considered untreatable

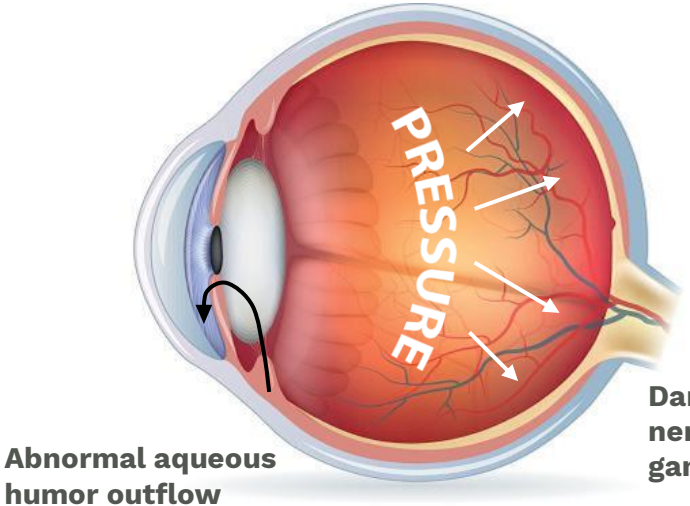
- Advantages of localized delivery to the eye:
  - Accessible for localized delivery
  - Small volume
  - Enclosed structure / limited systemic exposure
  - Immune-privileged organ

- Non-viral delivery of Cas9 mRNA offers several advantages:
  - Transient expression of Cas9 enzyme, no potential for vector integration
  - Ability to deliver larger cargoes
  - Simplified manufacturing
  - Repeat dosing feasible



MYOC Mutations are the Most Common Genetic Cause of Glaucoma

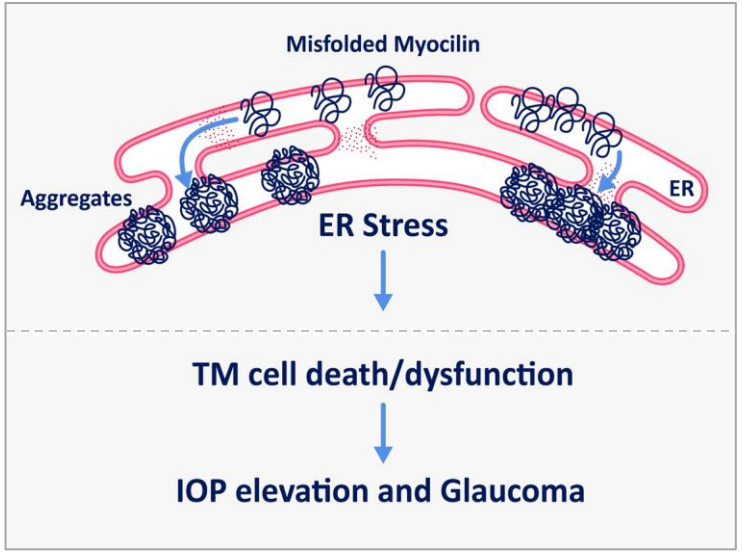
- Glaucoma is the second leading cause of blindness worldwide[1]
- Myocilin (MYOC) mutations are the most common genetic cause of glaucoma.[2] MYOC-associated glaucoma affects ~150k people in the United States alone[1,3]
- Myocilin mutations are autosomal dominant[4] and associated with earlier onset and more rapid progression of the disease[5]
- Mutations cause impaired outflow of aqueous humor through the trabecular meshwork (TM), leading to elevated intraocular pressure (IOP)[6]



[1] Allingham R.R. et al. 2009, [2] Fingert J.H. et al., 2011, [3] Zhang N. et al., 2021, [4] Sheffield V.C. et al. 1993, [5] Fingert J.H. et al., 1999, [6] Alward W.L.M. et al. 1998.

Myocilin Gene is a Promising Target for the Treatment of Glaucoma

- Myocilin is a glycoprotein highly expressed in the trabecular meshwork (TM)[1]
- Gain-of-function MYOC mutations cause misfolded protein aggregates in the endoplasmic reticulum (ER), inducing cellular stress that can lead to TM cell death/dysfunction[2]
- Knocking out MYOC expression in the TM using CRISPR/Cas9 was shown to reverse the ER stress, restore TM function, and reduce IOP[3,4]
- WT MYOC expression is not required for normal ocular function[5] and homozygous MYOC loss of function in humans is not associated with any disease[6]



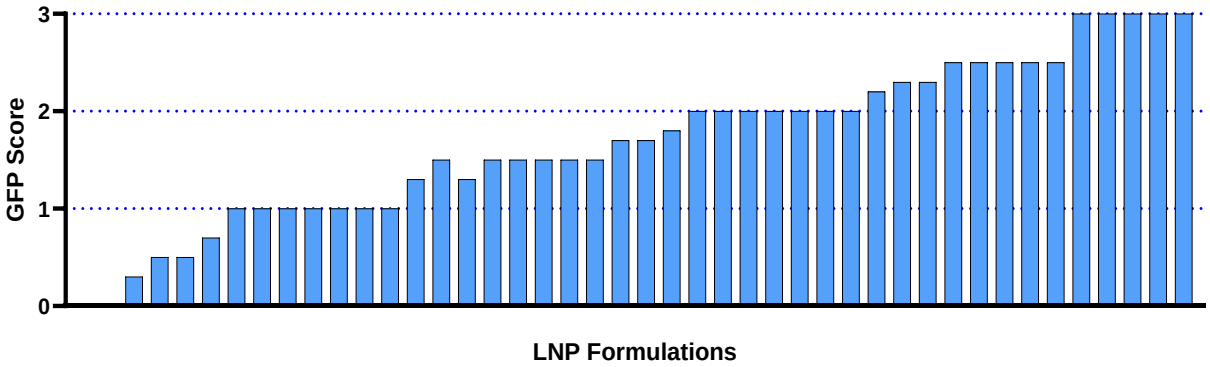
Adapted from Kasetti et al., 2021

[1] Pang C.P. et al. 2002, [2] Kasetti R.B. et al. 2016, [3] Jain A. et al., 2017, [4] Patil S.V. et al., 2024, [5] Kim B.S. 2001, [6] Lam D.S. 2000.

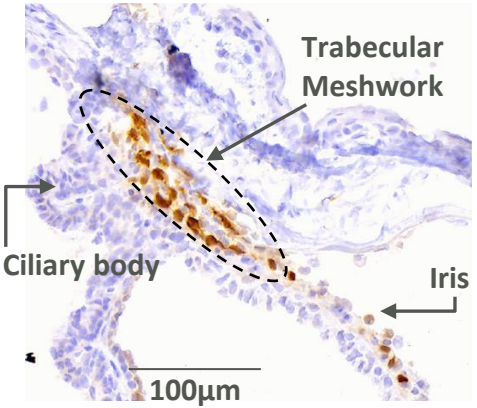
In Vivo Screen Identified LNPs that Deliver Efficiently to Mouse TM

- ~200 LNPs formulated with EGFP[1] mRNA were tested *in vitro* in primary TM cells
- ~40 LNP formulations were selected for *in vivo* testing

EGFP Expression in Mouse TM 24h After IVT[2] Injection



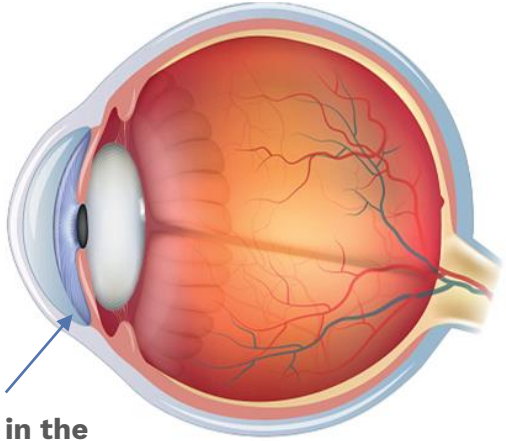
EGFP Detected in TM by IHC[3] 24h After LNP Dosing



[1] EGFP – Enhanced Green Fluorescent Protein; [2] IVT – Intravitreal; [3] IHC - Immunohistochemistry

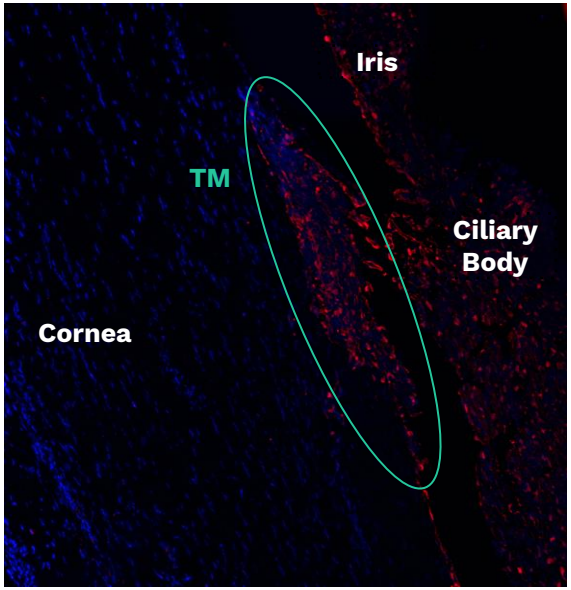
LNPs Selected for Targeted Delivery to Non-Human Primate TM

Intracameral Injection in Cynomolgus Monkey



Injection in the anterior segment of LNP formulated with EGFP mRNA

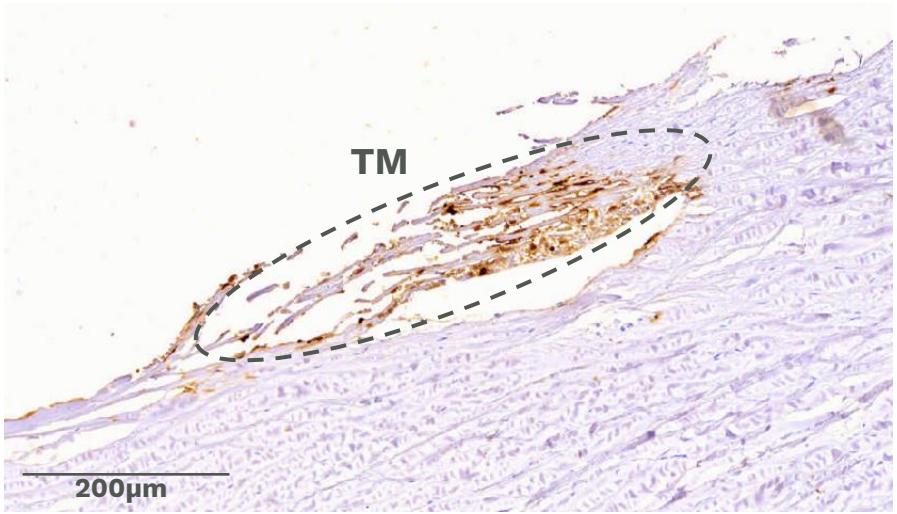
EGFP Detected in TM by IHC 24h After Dosing with LNP



LNP Formulation Also Successfully Delivered to Human TM in *Ex Vivo* Model

Human Eye Anterior Segment Organ Culture (ASOC)[1]

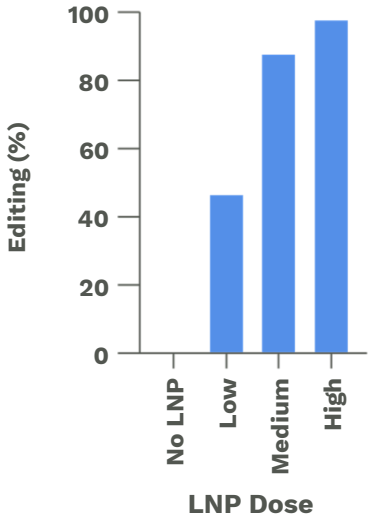
EGFP Detected in Human TM by IHC 24h After LNP Infusion



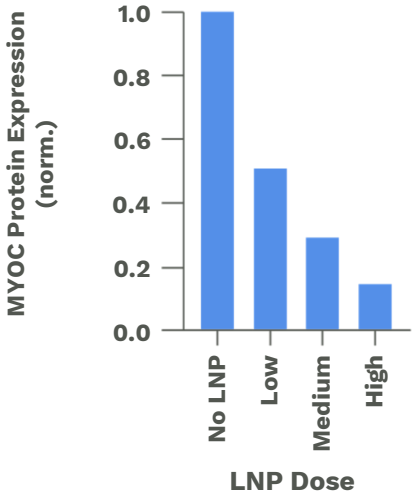
[1] *LNP formulated with EGFP mRNA was infused through syringe/pump. Flow: 2.5 µl/min (method adapted from Boice and Snider, JOVE, 2021)*

Prioritized MYOC gRNA Shows Efficient Editing in Primary Human TM cells

>90% MYOC Gene Editing *In Vitro* with Prioritized gRNA[1]



MYOC Protein Reduction Up to 85% with Prioritized gRNA

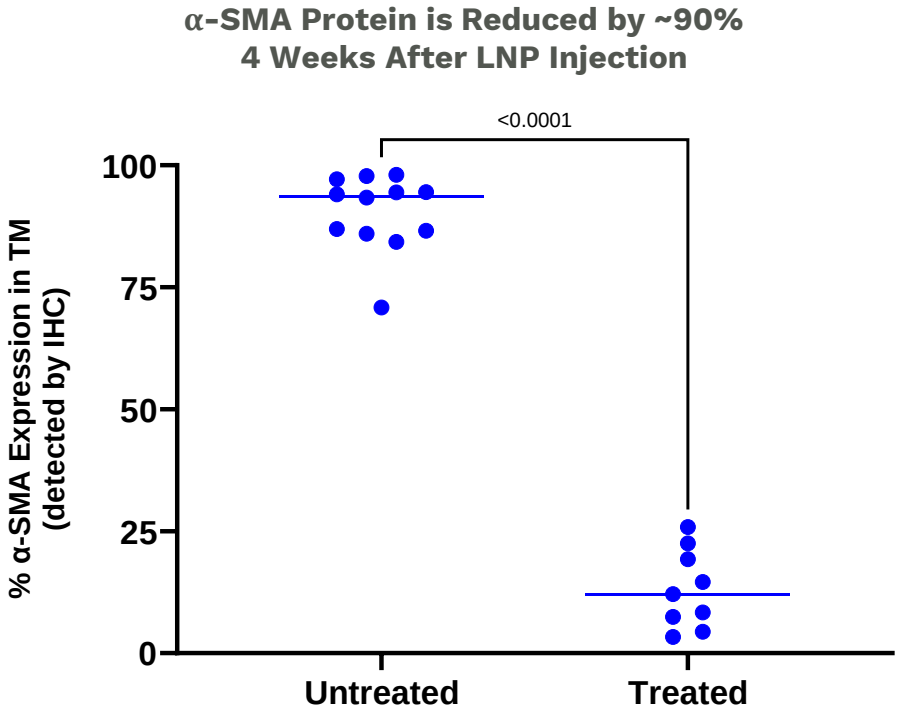


- Cross-reactive human/NHP MYOC gRNA selected based on *in vitro* screen
- Comprehensive off-target assessment completed with no off-target editing detected for prioritized gRNAs

[1] *gRNA – guide RNAs*

Efficient Reduction of Protein Expression Achieved in Mouse TM using LNP Delivery

- α-smooth actin (α-SMA), encoded by *Acta2* gene, was selected as a surrogate target in a mouse model
- LNP-mediated delivery of Cas9 mRNA & gRNA by intravitreal injection
- α-SMA expression was analyzed by immunohistochemistry (IHC)



Conclusions

- Identified LNPs to deliver efficiently and selectively to the Trabecular Meshwork (TM), a therapeutically relevant target tissue for the treatment of glaucoma
- Demonstrated ~90% surrogate protein reduction in mouse TM using *in vivo* LNP-mediated delivery of Cas9 mRNA and gRNA
- Identified highly-efficient gRNA to edit MYOC in human TM cells, showing >90% editing of the gene locus and up to 85% reduction in myocilin protein expression
- Ongoing: *in vivo* proof of concept study in mouse model of MYOC-associated glaucoma

Acknowledgments

CRISPR Therapeutics

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Thank you!

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