CRISPR-Cas9 disruption of VEGF as a strategy to treat Exudative Age-Related Macular Degeneration

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INTRODUCTION
Age-related Macular Degeneration (AMD) is the number one cause of vision loss in people over the age of 50 in developed nations. Estimates suggest that currently over 1.2 million people in the United States have AMD, and this number is expected to only grow to 3 million in 2020. The neovascular “wet” form of AMD leads to the formation of abnormal blood vessels that can leak fluid into the subretinal space leading to vision loss (Figure C). Thus, there has been much work on the development of antiangiogenesis agents targeting vascular endothelial growth factor (VEGF). The two most popular current options include bevacizumab, ranibizumab, and aflibercept, which target secreted isoforms of VEGF, limiting its activity to form new blood vessels. However, these treatments often require monthly intravitreal injections indefinitely, which are invasive and can cost up to $16,114 per injection. A recent study from Dr. Yiu’s lab has successfully implemented CRISPR-Cas9 gene editing for VEGF in vivo with human retinal pigment epithelium (RPE) cells4. This study hopes to further our understanding by developing reproducible results in vivo with the use of small animals (mice) as evidence that this strategy may be one day translatable to humans. We expect to see a similar decrease in VEGF and neovascularization in mice. This will provide a more effective and efficient method for addressing many neovascular ocular diseases that would reduce the overall costs of treatment and may even possibly represent a cure for the millions affected.

OBJECTIVES
- In this study, we seek to: 1) Use CRISPR-Cas9 to reduce VEGF expression in vivo

These aims will help identify whether gene editing of VEGF through the use of CRISPR-Cas9 is a viable ocular antiangiogenesis therapy solution in vivo by testing it in a small animal model. These results will help show whether this strategy may ultimately be efficacious in humans.

MATERIALS AND METHODS
Design single guide RNA (gRNA) to target mouse VEGF gRNAs were designed against the protein coding regions of exon 1 of the mouse VEGF-A gene using computer software (Benchmark). gRNA with the best specificity as predicted by highest on-target probabilities and lowest off-target probabilities will be used. Clone gRNAs into adeno-associated virus (AAV) vectors expressing Staphylococcus aureus Cas9 and Campylobacter jejuni (CjCas9) We will clone gRNA into a single vector AAV system using SaCas9 or CjCas9 to express the CRISPR-Cas9 components targeting VEGF-A in mice. Vidal vectors expressing Cas9 without the VEGF-targeted gRNA sequences will be used as controls.

RESULTS
Check vitreous for VEGF levels using ELISA
After the mice are infected with the CRISPR-Cas9 system targeting VEGF-A, we will measure VEGF-A concentrations in the vitreous by ELISA compared to Cas9-only controls. We expect to see VEGF levels reduced in the eyes with gRNA expression compared to Cas9-only controls assessed statistically by ANOVA with Durman’s post-test.

CONCLUSIONS/FUTURE DIRECTIONS
- SaCas9 and CjCas9 constructs were both able to reduce VEGF levels in subretinally injected mice
- Laser-induced choroidal neovascularization will be assessed to show functional decrease
- Compare efficiency of designs in vitro

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REFERENCES

Figure 1. Fundus image of the eye. A) Normal eye. C) Eye with neovascularization (8).
Figure 2. Summary of CRISPR Cas9 gene editing strategy (7)
Figure 3. Vector designs for SaCas9 and CjCas9
Figure 4. Schematic of subretinal injection in mice. Mice were sacrificed 5 weeks postinjection.
Figure 5. Vegfa target sequence in CjCas9 with PAM sequence (red) and sgRNA (blue) (8).
Figure 6. Highest On-Target Score гRNA sequences for SaCas9 with PAM site NGP(R)R(N)