239

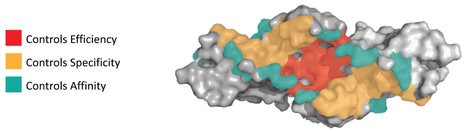
Optimization of Hydroxyacid Oxidase 1 (HAO1) Targeting ARCUS Nucleases for the Treatment of Primary Hyperoxaluria Type 1 (PH1)

Gary Owens, Whitney Lewis, Nicole Heard, Rachel Polak, Matt Jordan-Steele, Janel Lape, Jeff Smith, Jason Holt, Greg Falls, Derek Jantz

Precision BioSciences, Durham, NC

INTRODUCTION

PH1 is a rare, autosomal recessive disorder affecting an estimated 1 to 4 individuals per million, with most patients diagnosed as children or young adults. PH1 is caused by a mutation in the alanine glyoxylate aminotransferase (AGXT) gene, which encodes a key metabolic enzyme responsible for converting glyoxylate to glycine in the liver. The inability to metabolize glyoxylate to glycine leads to an overproduction of systemic oxalate, resulting in the formation of insoluble calcium oxalate crystals in the kidneys. These calcium oxalate crystals lead to kidney stone formation, kidney failure, and further effects in the liver, heart, and other organs. ARCUS nucleases possess several attractive attributes for therapeutic application, including a single-component protein containing both a site-specific DNA recognition interface and endonuclease activity. The combination of the substrate-recognition and catalytic motifs into a single protein allows for both viral and non-viral delivery modalities and iterative improvements in both activity and specificity through protein engineering. To determine if ARCUS gene editing could be utilized to reduce systemic oxalate levels in PH1 patients, ARCUS nucleases were designed to target and disrupt the HAO1 gene that encodes hydroxyacid oxidase 1 (HAO1) also known as glycolate oxidase (GO), an upstream enzyme in the metabolic pathway responsible for the conversion of glycolate to glyoxylate. By inhibiting the formation of glyoxylate, oxalate production should be minimized.

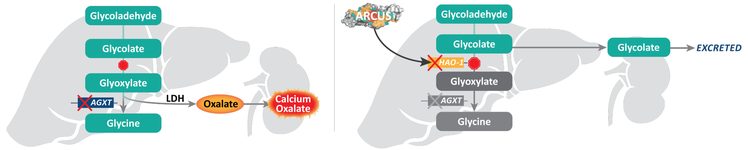


- ARCUS is derived from I-CreI, a homodimeric protein that recognizes and cleaves a semi-palindromic 22 base pair target sequence in the chloroplast genome of *Chlamydomonas reinhardtii*.
- ARCUS is a small (~350 amino acids), monomeric protein that does not require a guide RNA to edit DNA and can be optimized to improve specificity

Figure 1. Metabolic Pathway in PH1 Patients and ARCUS Approach

Mutations in AGXT prevent glyoxylate metabolism to glycine resulting in accumulation of oxalate

Targeting HAO1 prevents the conversion of glycolate to glyoxylate, allowing glycolate to be safely excreted



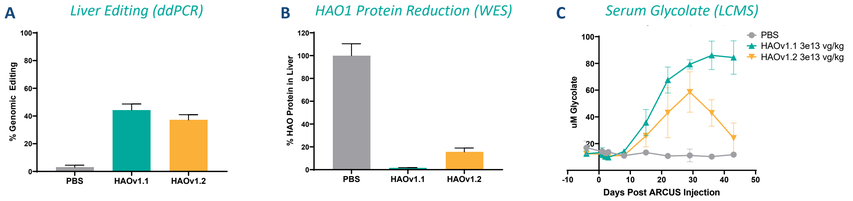
METHODS

- As a proof of concept, first generation HAO1-targeting ARCUS nucleases delivered via AAV were characterized in non-human primates (NHPs) at the genomic level by measuring editing by digital droplet PCR (ddPCR), at the protein level by measuring HAO1 protein reduction in the liver using the WES system by Protein Simple, and at a functional level by measuring changes in serum glycolate using LCMS.
- Candidate HAO nucleases were subjected to multiple rounds of optimization to increase efficacy while minimizing off-target effects.
- Efficacy of optimized HAO nucleases were tested in NHPs and characterized for specificity using super-saturating doses of nuclease in a highly sensitive oligo capture assay.

ACKNOWLEDGEMENTS: *Precision's Core group for Nuclease Generation & NGS Support, OpAns (LCMS), Sinclair Research Center LLC (NHP POC Study), and Labcorp Early Development Laboratories Inc (NHP Optimization Study)*

RESULTS

Figure 2. NHP POC: Genomic Editing, HAO1 Protein Knockdown in NHP Liver, and Serum Glycolate levels at Day 43 using first generation HAO nucleases



- A single infusion of AAV to deliver HAO nucleases resulted in greater than 35% editing at the *HAO1* locus, greater than 95% knock-down of HAO1 protein in the liver and up to 80 µM of glycolate in the serum at Day 43

Figure 3. Optimization of ARCUS Nucleases

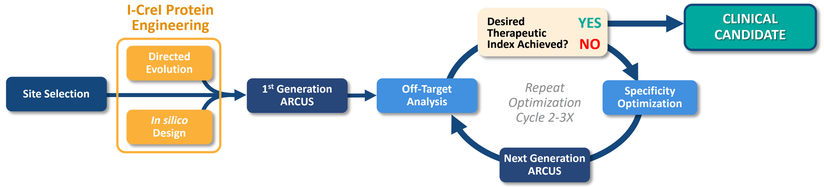
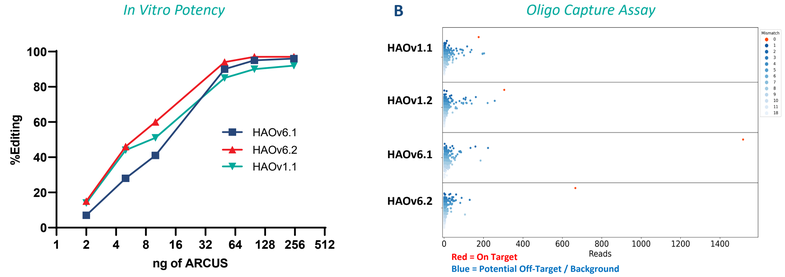
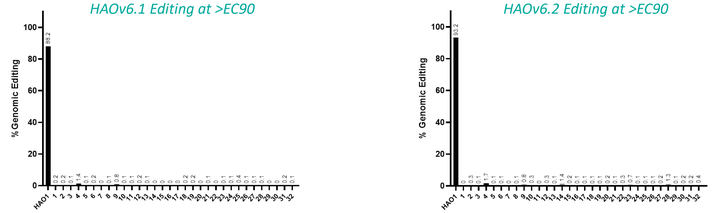


Figure 4. Optimized HAO Nucleases Retain Potency with Increased Specificity In Vitro



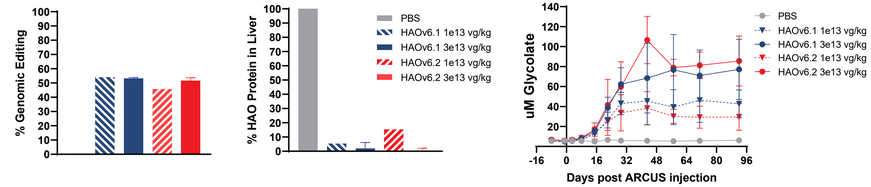
- (**A**) Optimized nucleases have equal or greater potency than HAOv1.1 as measured by genomic editing (ddPCR)
- (**B**) A highly sensitive oligo capture assay was completed with super-saturating mRNA doses of v1 and v6 HAO nucleases to qualitatively identify potential off-target sites.

Figure 5. Multi-target Amplification (MTA) and NGS to Quantify Editing



- Optimized nuclease mRNAs were characterized in vitro at a high concentration (>EC90) in liver cells, and indels were quantitated by amplicon NGS sequencing at the on-target site, as well as 32 potential off target sites detected in oligo capture.

Figure 6. Optimized HAO Nucleases in NHP: Genomic Editing, HAO1 Protein Knockdown in NHP Liver & Serum Glycolate levels at Day 92



- A single infusion of AAV delivering optimized HAO nucleases resulted in greater than 50% genomic indels in liver at the high dose (A), resulting in greater than 98% HAO1 protein knockdown in the liver (**B**) and increased serum glycolate levels consistent with previously achieved levels in the POC study (**C**)
- HAO nucleases were well tolerated through in life portion

CONCLUSIONS

1. Optimization through the ARCUS platform resulted in improvements in both potency and specificity of HAO ARCUS nucleases
2. A single infusion of AAVs carrying first generation HAO1 nucleases resulted in >30% editing at the *HAO1* locus, >95% knock-down of HAO1 protein in NHP liver, and therapeutically meaningful increases in serum glycolate up to 80µM
3. Optimized HAO1 nucleases achieved >50% editing at the *HAO1* locus, 98% knock-down of HAO1 protein in NHP liver, with serum glycolate levels greater than 80µM out to 92 days
4. Using a highly sensitive oligo capture assay along with super-saturating levels of HAO nuclease mRNA, we demonstrate the ability to reduce off-target editing through iterative rounds of protein engineering
5. These data demonstrate the ability to optimize HAO ARCUS nucleases to specifically edit the *HAO1* gene and impact the glyoxylate metabolic pathway at therapeutically meaningful levels after a single administration in NHPs