CRISPR Cas9/gRNAs Selective Targeting of the GUCY2D Mutant Allele for Autosomal Dominant Cone-Rod Retinal Dystrophy

Cindy Park-Windhol[1], Paul Weingarden[1] , Ryo Takeuchi[1] , Akiko Noma[1], Maria L. Muraca[1], Mariacarmela Allocca[1], Julian Esteve-Rudd[2], Mike Lukason[1], Jonas Neubauer[3] , Daniyar Dauletbekov[3], Andrew Scharenberg[1], Dominik Fischer[3] and Abraham Scaria[1]

[1]Casebia Therapeutics LLC, Cambridge, MA; [2]Bayer AG, Wuppertal, Germany; [3]University of Tübingen, Tübingen, Germany

Introduction and Objective

Autosomal dominant cone-rod dystrophy type 6 (CORD6) is a retinal degenerative disease accounting for ~35% of all cone-rod dystrophy cases. The onset of disease occurs between childhood and early adulthood and is characterized clinically by a progressive loss of visual acuity, visual field, color vision, increased photophobia, and macular atrophy. There are currently no therapies available to treat CORD6.

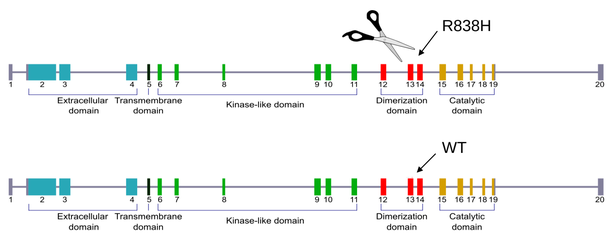
CORD6 is caused by mutations in *GUCY2D*, the gene encoding retinal guanylate cyclase-1 (retGC-1). RetGC-1 is a key enzyme in the recovery phase of phototransduction in both cone and rod photoreceptor cells. Upon light stimulation, an enzymatic cascade of events occurs which leads to the hydrolysis of cGMP and the closure of the cGMP gated cation channels. This results in hyperpolarization of the plasma membrane and a reduction in intracellular Ca^{2+} levels. Lowered Ca^{2+} levels via the Ca^{2+}-ligand guanylyl cyclase activating protein 1 (GCAP-1) stimulates retGC-1 leading to cGMP-gated channels restoring photosensitivity to the photoreceptor cell.

Mutations clustered at, or adjacent to, residue R838 in the dimerization domain of retGC1 have been linked to the CORD6 locus. Codon 838 seems particularly prone to mutational change and is believed to be responsible for the severity of the disease, depending on the precise amino acid replacement and the presence of additional substitutions in adjacent codons. One of the common mutations found in CORD6 patients is c.2513G>A in codon 838 (R838H).

Selective disruption of such a pathogenic GUCY2D mutant could potentially be an effective treatment of CORD6. This study investigates several different CRISPR/Cas9 systems to identify guides that selectively target the R838H mutation.

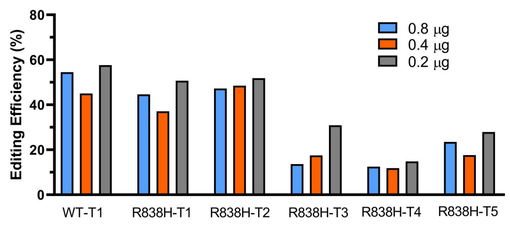
Therapeutic Strategy

- Develop guide(s) to overlap the GUCY2D R838H mutation.
- Non-homologous end-joining (NHEJ) introduces insertions or deletions (indels) at the target site and creates frameshift mutations.
- To design gRNA(s) containing the R838H mutation to cut the mutant allele while maintaining expression of the wild-type (WT) allele of GUCY2D.

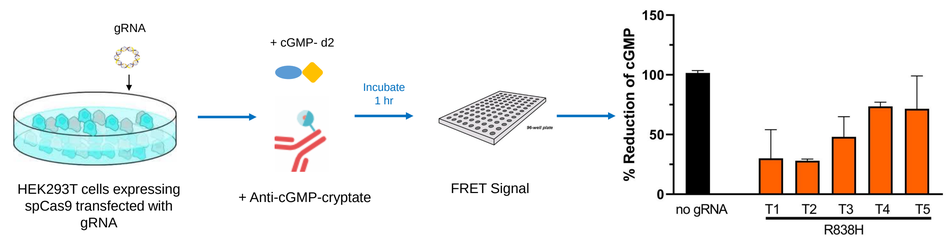


On-Target Editing Using TIDE Analysis and cGMP Functional Assay

- Indels were analyzed by TIDE using HEK293T-SpCas9 cells transfected with plasmids encoding gRNAs targeting WT or the R838H mutation within the GUCY2D gene.

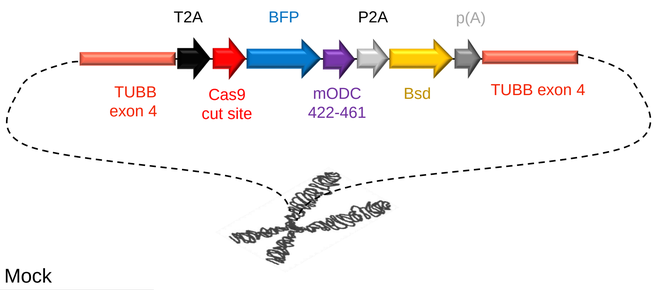


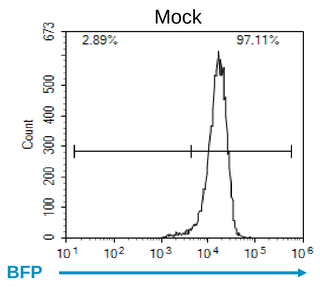
- Function of GUCY2D, as observed by a reduction in cGMP production, was used to assess on-target editing of plasmids encoding gRNA(s) targeting the R838H mutation within GUCY2D.

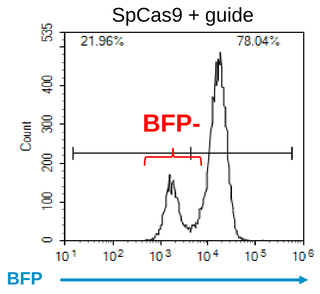


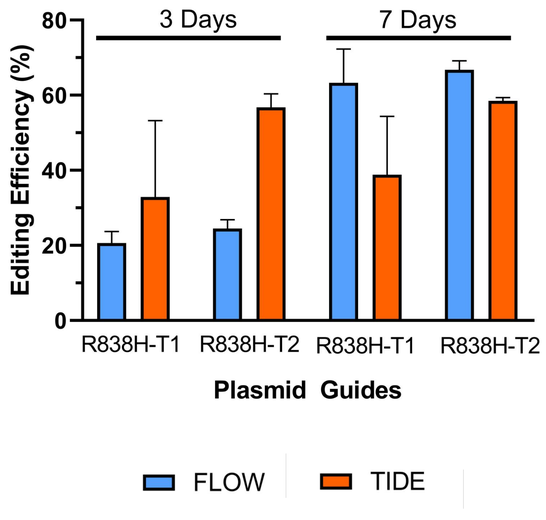
Characterization of R838H Guides using a BFP-Reporter Cell Line

- On-target editing of gRNAs targeting the WT GUCY2D and R838H mutation within the GUCY2D gene of a plasmid. Cas9-mediated double-strand breaks promotes frame-shift mutations, leading to loss of BFP expression correlating to gene editing.



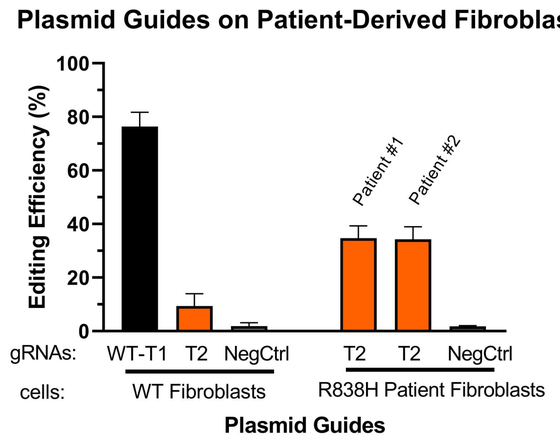






Characterization of R838H Guide using Patient-derived R838H Fibroblasts

- Patients with a R838H mutant allele provided skin biopsies to create an immortalized primary fibroblast cell line.



Conclusions

A cGMP functional assay can be used as an on-target screening approach for R838H gRNA screening.

On-target activity in human patient-derived fibroblasts that have a R838H mutant allele shows >33 % editing.

R838H-T2 shows low editing (6%) in WT fibroblasts indicating specificity against WT allele with R838H gRNA.

Hamel CP. *Orphanet J Rare Dis*. 2: 7 (2007); Zobor D, et al., *Retina*. 34: 8 (2014); Mukherjee R., et al., *Eye.* 28: 4 (2014); Schmid-Burgk JL et al., *Nat. Communications*. 7: 12338 (2016)