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 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

Autosomal dominant cone-rod dystrophy type 6 (CORD6) is an early-onset retinal degeneration disease caused by mutations in *GUCY2D*, the gene encoding retinal guanylate cyclase-1 (retGC-1). It is characterized by infantile-onset of poor vision, abnormal color vision, photophobia, loss of visual field, and macular atrophy. There are currently no therapies available to treat CORD6. 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. To test this hypothesis, we investigated several different CRISPR/Cas9 systems to identify guides that selectively targeted the R838H mutation. Using “in vitro” guanylate cyclase functional assays, knock-in reporter cell lines, and therapeutically relevant human patient fibroblasts with the R838H mutation, we have identified gRNAs able to efficiently disrupt the GUCY2D R838H mutant allele without targeting the wild type allele. In order to reduce long-term in vivo expression of Cas9, we are currently evaluating self-inactivating AAV vectors carrying Cas9 and gRNA in rodent retina. Screening is ongoing for gRNAs able to efficiently target other CORD6-associated GUCY2D mutations.