

TREATMENT OF HERPETIC KERATITIS WITH CRISPR/CAS9 GENE EDITING IN A RABBIT DISEASE MODEL

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Introduction

• Recurrent Herpes Simplex Virus-1 (HSV-1) stromal keratitis is considered the leading cause of infectious corneal blindness worldwide^{1,2}, despite the current standard of care³.

• Recurrent HSV-1 ocular keratitis results from reactivation of latent virus within the trigeminal ganglia (TG), followed by an inflammatory response to viral infection within the stroma¹.

• To address this unmet medical need, we have developed a gene editing approach using Adeno-Associated Virus (AAV)-delivered CRISPR-associated protein-9 (Cas9) nuclease from *S.aureus* (SaCas9) and guide RNAs (gRNAs) to target latent HSV-1 genomes.

• The objective of this study was to evaluate the efficacy of the CRISPR/Cas9 gene editing system in a rabbit model of recurrent HSV-1 keratitis.

Methods

gRNA selection

• gRNAs (N=411) and SaCas9 assembled into ribonucleoproteins (RNPs) were screened against HSV-1-specific sequences in a high-throughput *in vitro* phenotypic assay (Figure 1).

Characterization of benchmark gRNAs

• Benchmark gRNAs were characterized in a viral replication assay using RNPs complexed with individual gRNAs. Briefly, Vero cells were transfected with RNPs, and 24 hours later, challenged with HSV-1 17Syn+ across a range of Multiplicities-Of-Infection (MOIs). At 24 hours post infection, levels of viral genomes in supernatants were quantified by qPCR. Percent inhibition of viral replication was calculated based on the AUC of the MOI dose response curves, relative to that of a non-targeting control gRNA (Figure 2).

Rabbit model of HSV-1 keratitis

• HSV-1 17Syn+ was applied topically to scarified corneas of New Zealand White rabbits to establish latency in the TG in 3.5 weeks. AAV expressing SaCas9 and gRNAs were dosed via corneal abrasion and allowed to express the transgenes for 4 weeks prior to HSV-1 reactivation by epinephrine iontophoresis on rabbit corneas.

• Slit lamp examinations (SLE) were conducted every other day to monitor symptoms of HSV-1 reactivation. HSV-1 viral production was determined by a plaque assay of daily tear swabs (Figure 4).

• TG and corneas were harvested 10 days post induction. HSV-1 viral genomes in TG and AAV viral genomes in both TG and corneas were quantified using qPCR. Statistical analyses utilized one-way ANOVA (Figures 7 - 9).

Study I: *in vitro* gRNA Selection

FIGURE 1. Design of the *in vitro* RNP screen.

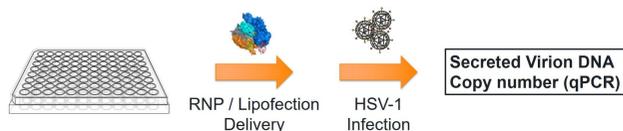


FIGURE 2. gRNAs targeting UL48 and RL2 showed the greatest efficacy in inhibiting viral replication.

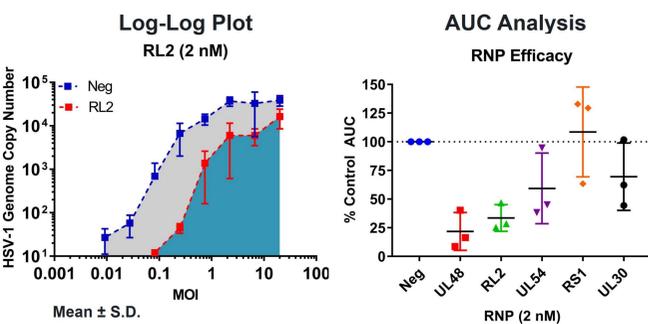
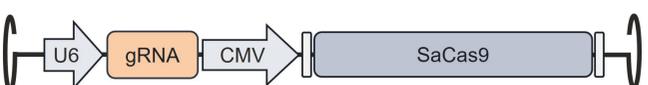


FIGURE 3. AAV vector map.



Reference: 1. Chapter 60, Fields Virology; 2. Herpes Simplex Virus Keratitis: A treatment Guideline – 2014, White et al. 2014, American Academy of Ophthalmology; 3. HEDS Study GROUP (1998) NEJM 339:300.

Study II: *in vivo* Efficacy Model

Table 1. Treatment groups for *in vivo* testing.

AAV-Guide Target	Dose (vg/eye)	Animal Group Size
AAV-GFP (Negative)	1.0 E+11	6
Acyclovir (Positive)	N.A.	5
AAV-UL48	1.0 E+11	6
AAV-RL2	1.0 E+11	6
AAV-UL48 + AAV-RL2	5.0 E+10 + 5.0 E+10	6

FIGURE 4. Study design of HSV-1 reactivation in a rabbit model.

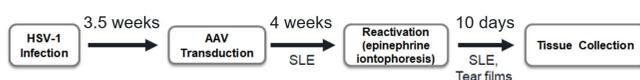
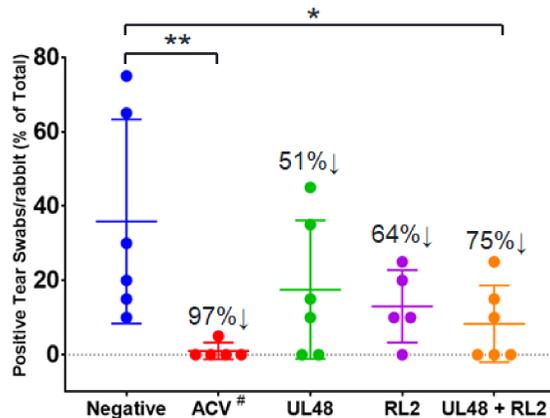


FIGURE 6. Infectious HSV-1 in tear films was inhibited by up to 75% with a dual-gRNAs approach.



ACV-treated rabbits were maintained on acyclovir (oral gavage, 200 mg/kg) for 7 days post-reactivation: ACV blocks secretion of virus in tears. One positive swab identified Day 8. One death in the AAV-RL2 group (intestinal blockage) unrelated to study drug. Mean ± S.D. * = p<0.05, ** = p<0.01

FIGURE 5. Corneal lesions were suppressed by up to 91% with a dual-gRNA approach.

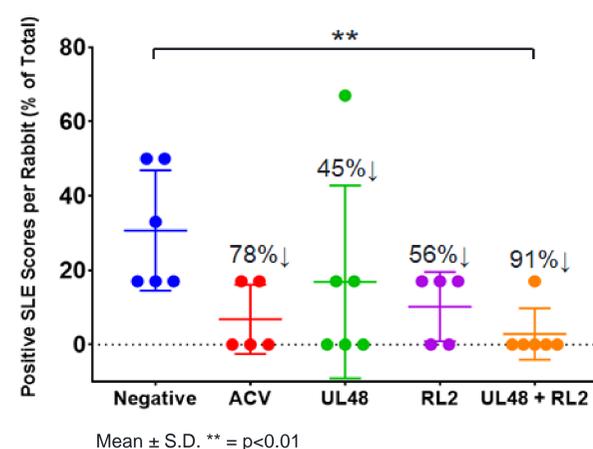


FIGURE 7. CRISPR/Cas9 or acyclovir had no significant impact on HSV-1 copy numbers in TG.

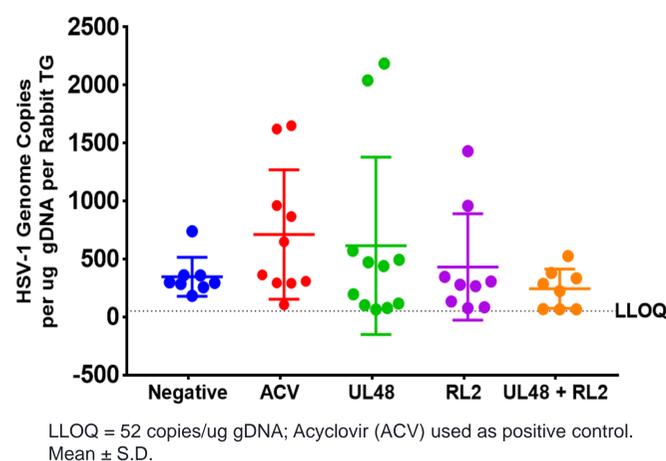


FIGURE 8. AAV copy numbers in TG were not above background.

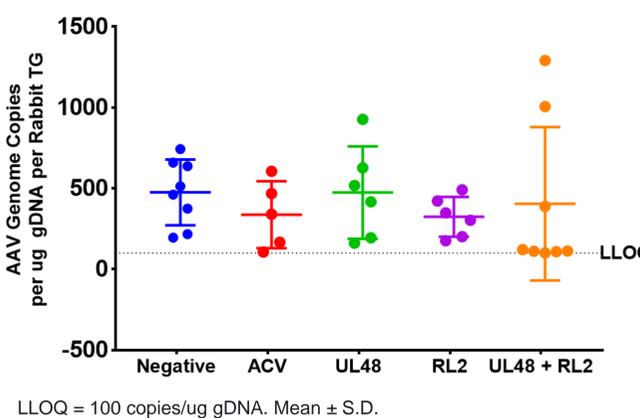
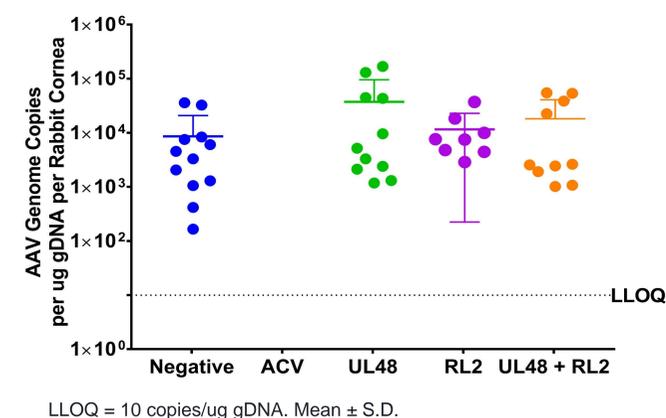


FIGURE 9. AAV vector genomes were detected in corneas.



Conclusions

1. An *in vitro* gRNA selection method was developed to identify gRNAs targeting HSV genes that are essential for viral replication.
2. *In vitro* characterization demonstrated that gRNAs targeting UL48 and RL2 had the greatest efficiency in inhibiting viral replication.
3. Evaluation of gRNAs targeting UL48 and RL2 in the rabbit reactivation model indicated that the combination of gRNAs resulted in the greatest efficacy.
 - 91% reduction in corneal lesions as measured by SLE;
 - 75% reduction of HSV-1 viral load in rabbit tear films as measured by plaque assays.
4. AAV vectors were detected in the corneas and not in the corresponding TG, implicating that CRISPR/Cas9 activity within the corneas contributed to the reduced viral load in tears and associated pathology.
5. In summary, we have identified gRNAs and demonstrated their ability to reduce viral shedding and corneal lesion development in a rabbit model of HSV-1 keratitis and reactivation. The impact of the gRNAs on viral load and pathology may reflect activity at the corneal surface.