Targeting the Hepatitis B cccDNA with a Sequence-Specific ARCUS Nuclease to Eliminate Hepatitis B Virus In Vivo

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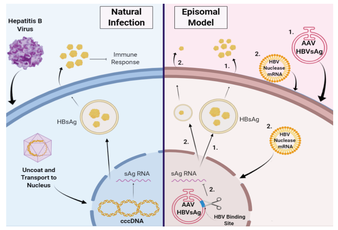
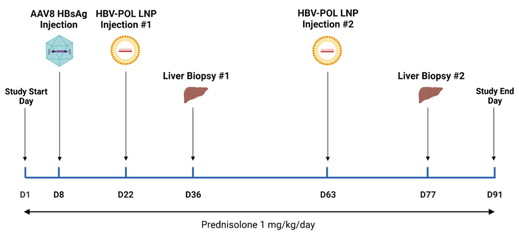
INTRODUCTION

- Hepatitis B virus (HBV) is a major health concern, affecting millions of people worldwide, with no curative treatments currently available.
- HBV infects hepatocytes and persists as covalently closed circular DNA (cccDNA), producing an abundance of surface antigen (HBsAg) subsequently suppressing the host immune system (schematic, right).
- A functional cure for Hepatitis B has been defined as undetectable HBV DNA and a sustained loss of hepatitis B surface antigen (HBsAg), with or without HBsAg seroconversion.
- We have developed an HBV-targeting nuclease (HBV-POL) that can specifically target both covalently closed circular DNA (cccDNA) and integrated viral DNA.
- Here we evaluate HBV-targeting ARCUS nucleases in immortalized cells containing integrated HBV DNA, HBV-infected primary human hepatocytes (PHH), and novel, noninfectious, episomal AAV mouse and NHP models (NHP study schematic, below).
- Our data support an in vivo gene editing approach for elimination of cccDNA and a potential HBV cure.



NHP Episomal AAV Study Outline



THERAPEUTIC STRATEGY

- We hypothesized that a nuclease-mediated double-stranded break could lead to degradation of the cccDNA or generate mutated, replication-incompetent cccDNA, with both outcomes potentially leading to reductions in HBsAg.
- In an effort towards achieving an HBV cure, we engineered and optimized a gene-editing ARCUS nuclease (ARCUS-HBV) capable of specifically cleaving a 22 base pair sequence in the HBV DNA polymerase open reading frame (ORF).

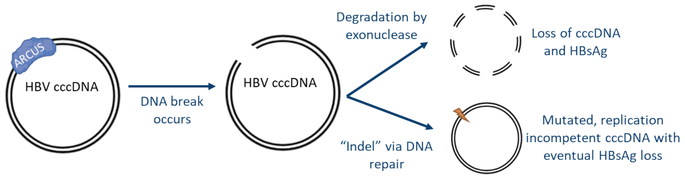


Figure 1. Outcomes for cccDNA after ARCUS editing

RESULTS

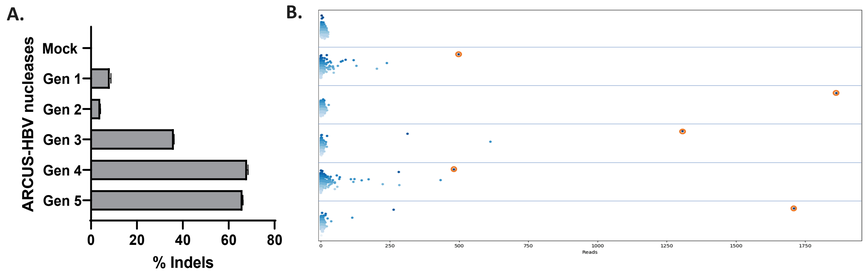


Figure 2. Optimization of nucleases targeting the HBV polymerase

- Successive generations of ARCUS-HBV nucleases were shown to exhibit enhanced on-target activity in Hep3B cells (**2A**) and improved specificity in HEK293 cells using oligo capture (**2B**).

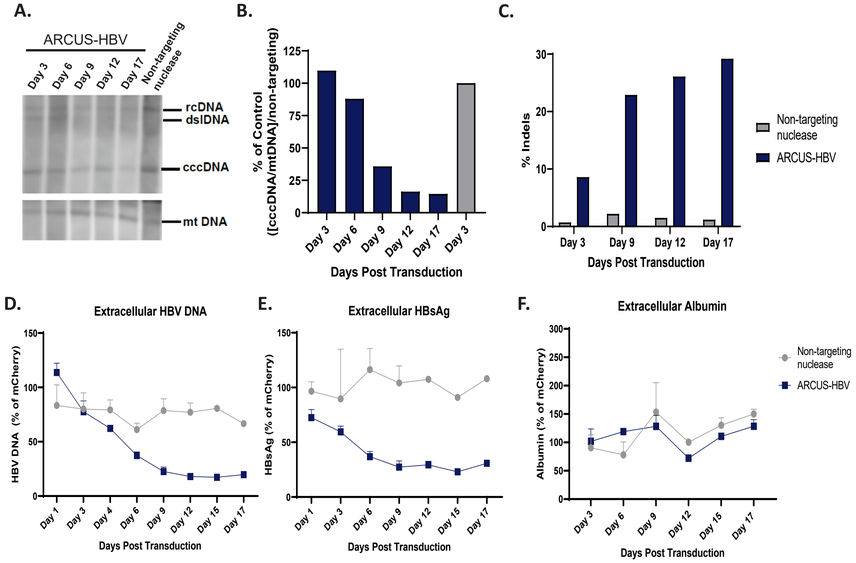


Figure 3. Antiviral effect of Gen 5 ARCUS-HBV nuclease in HBV-infected PHH

- Primary human hepatocytes (PHH) were de novo transduced with HBV and transfected with either Gen 5 ARCUS-HBV or a non-targeting nuclease.
- ARCUS-HBV nuclease treatment results in ~85% reduction in cccDNA (**3A**, **3B**) and 29% indels in the remaining cccDNA (**3C**) at day 17 post transduction. ARCUS-HBV-treated cells showed an 80% reduction in extracellular HBV DNA (**3D**), a 77% reduction in secreted HBsAg (**3E**), and no change in secreted albumin (**3F**).

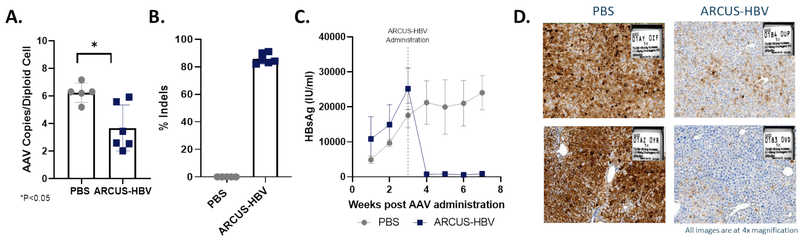


Figure 4. Gen 5 ARCUS-HBV nuclease evaluation in an episomal AAV mouse model

- NSG mice were administered an AAV9-HBsAg vector, and three weeks later received LNPs containing Gen 5 ARCUS-HBV mRNA. Mice were bled weekly throughout the study and euthanized and necropsied at seven weeks post AAV administration.
- ARCUS-HBV nuclease treatment resulted in significant loss of AAV copies in the liver compared to PBS controls (**4A**) with 80% indels in the remaining AAV (**4B**). Editing resulted in 96% reduction in serum HBsAg levels (**4C**) and substantial loss of HBsAg expression in liver (**4D**).

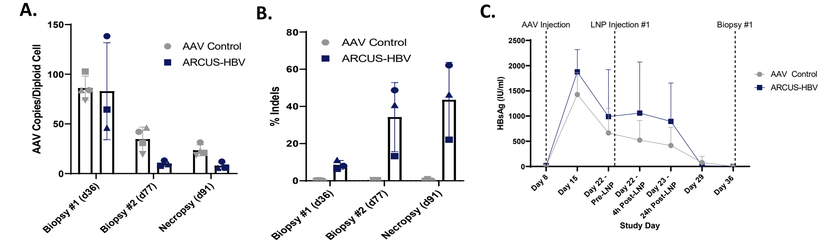


Figure 5. Gen 5 ARCUS-HBV nuclease evaluation in an episomal NHP model

- NHPs were given prednisolone daily starting one week prior to AAV administration. Two and eight weeks after AAV administration, NHPs received LNPs containing Gen 5 ARCUS-HBV mRNA. Liver biopsies were collected two weeks after each LNP administration and animals were euthanized twelve weeks post AAV administration.
- After a single LNP administration, AAV copies were not significantly reduced, but nuclease-treated animals showed 9% indels. After a second LNP dose and at necropsy, nuclease-treated animals showed a significant reduction in AAV copies in the liver and an average of 34% and 44% indels, respectively (**5A**, **5B**). Despite immunosuppression, NHPs were unable to maintain secreted HBsAg to use as a biomarker (**5C**).

CONCLUSIONS

- Our studies show that Gen 5 ARCUS-HBV is a highly specific and effective nuclease, showing large reductions in cccDNA in HBV-infected PHH with subsequent HBsAg knockdown.
- We've developed a novel *in vivo* model to test ARCUS-HBV nucleases and demonstrated high levels of editing and HBsAg reductions in mice and NHPs.

Acknowledgments: We thank our colleagues at Precision BioSciences who have participated in this project, as well as our collaborators at Gilead Sciences and Acuitas Therapeutics.

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