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

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INTRODUCTION

- Persistence of chronic Hepatitis B (CHB) is attributed to maintenance of the intrahepatic pool of the viral covalently closed circular DNA (cccDNA).
- While current therapeutics aim to achieve a functional cure, our approach has the potential to achieve a sterilizing cure.
- We have developed a gene-editing meganuclease, ARCUS-POL, that has demonstrated the ability to cut cccDNA and integrated HBV DNA, leading to reduction in both sAg and cccDNA.
- Transient ARCUS-POL expression in HBV-infected primary human hepatocytes produced substantial reductions in both cccDNA and Hepatitis B surface antigen (HBsAg).
- To evaluate ARCUS-POL in vivo, we developed episomal adeno-associated virus (AAV) mouse and non-human primate (NHP) models containing a portion of the HBV genome serving as a surrogate for cccDNA.
- Together, these data support a gene editing approach for elimination of cccDNA and an HBV cure.

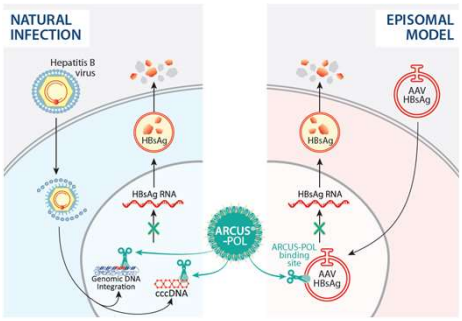

Figure 1. Transient expression of an ARCUS nuclease targeting the HBV polymerase (ARCUS-POL)

WHAT IS ARCUS?

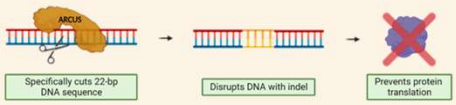

Figure 2. Mechanism of ARCUS gene disruption

- ARCUS is a single-component protein derived from I-CreI containing both a 22 bp site-specific DNA recognition interface and endonuclease activity.
- The small size of ARCUS (364 aa) makes it easy to package into AAV or a lipid nanoparticle (LNP) for efficient delivery to the nucleus.
- Multiple rounds of optimization are performed to increase both cutting efficiency and specificity with safety as the top priority.

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-POL) capable of specifically cleaving a specific 22 base pair sequence in the HBV DNA polymerase open reading frame (ORF).

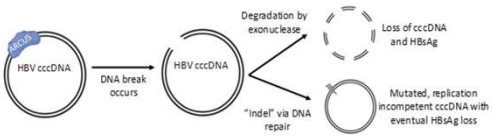

Figure 3. Outcomes for cccDNA after ARCUS editing

RESULTS

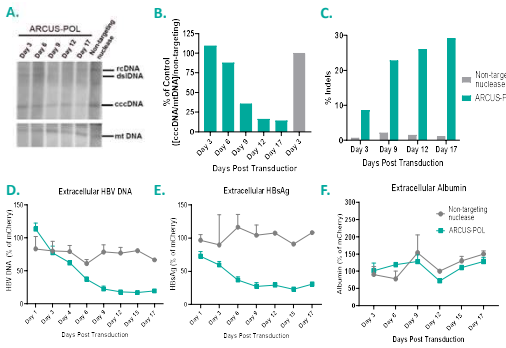

Figure 4. Antiviral effect of Gen 5 ARCUS-POL nuclease in HBV-infected PHH

- Primary human hepatocytes (PHH) were de novo transduced with HBV and transfected with either Gen 5 ARCUS-POL or a non-targeting nuclease.
- ARCUS-POL nuclease treatment results in ~**85%** reduction in cccDNA (**3A**, **3B**) and 29% indels in the remaining cccDNA (3C) at day 17 post transduction. ARCUS-POL-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**).

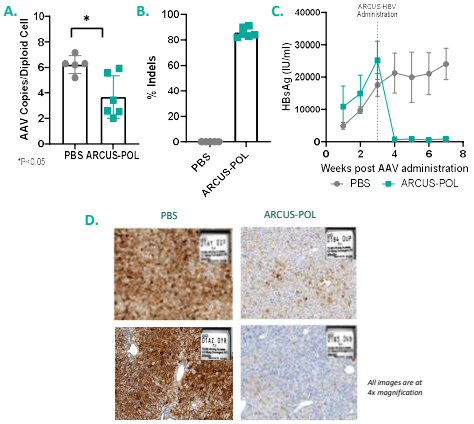

Figure 5. Gen 5 ARCUS-POL nuclease evaluation in an episomal AAV mouse model

- NSG mice were administered an AAV9-HBsAg vector, and three weeks later received LNPs containing Gen 5 ARCUS-POL mRNA. Mice were bled weekly throughout the study and euthanized and necropsied at seven weeks post AAV administration.
- ARCUS-POL 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**).

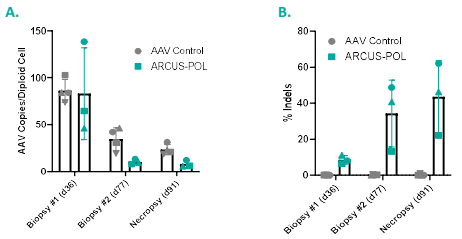

Figure 6. Gen 5 ARCUS-POL nuclease evaluation in an episomal NHP model

- Two and eight weeks after AAV administration, NHPs received LNPs containing Gen 5 ARCUS-POL mRNA. Liver biopsies were collected two weeks after each LNP administration and animals were euthanized twelve weeks post AAV administration.
- Animals receiving ARCUS-POL LNP had an average of 9% indels at the first liver biopsy, 34% indels at the second liver biopsy, and 44% indels at necropsy, as well as a significant reduction in overall AAV copy number (**5A**, **5B**). Overall nuclease engagement resulting in either editing or degradation of the AAV-HBsAg cccDNA surrogate was estimated to be 83%.
- Despite immunosuppression, NHPs were unable to maintain secreted HBsAg to use as a biomarker.
- Together these data demonstrate the ability of the ARCUS-POL nuclease to cut episomal AAV8-HBsAg in NHPs, leading to reduced AAV genome copies and indels at the intended target site.

CONCLUSIONS

1. **We have developed both mouse and NHP models to assess in vivo HBV gene editing with ARCUS using clinically translatable LNP/mRNA delivery.**
2. **Our data demonstrate the viability of a gene editing approach using the ARCUS-POL nuclease to decrease cccDNA and secreted HBsAg with the goal of achieving a cure for HBV patients.**

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PRECISION BIOSCIENCES