A Gene Editing Approach to Eliminate Hepatitis B Virus Using ARCUS Meganucleases

Cassie Gorsuch[1], Mei Yu[2], Simin Xu[2], Janel Lape[1], Tyler Goodwin[1], Neil Leatherbury[1], Jeff Smith[1], Derek Jantz[1], Meghan Holdorf[2], Anuj Gaggar[2], Bill Delaney[2], Robert C. Muench[2], Amy Rhoden Smith[1]

[1]Precision BioSciences, Inc., Durham, NC, United States; [2]Gilead Sciences, Inc., Foster City, CA, United States

INTRODUCTION

- Hepatitis B virus (HBV) is a major, worldwide health concern with more than 250 million people chronically infected. Although treatments are available to control HBV infection, there is no cure for chronic Hepatitis B.
- 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 HBV-ARCUS nucleases that can target both covalently closed circular DNA (cccDNA) and integrated viral DNA.
- Here we evaluate HBV-ARCUS nucleases in immortalized cells containing integrated HBV DNA, primary human hepatocytes (PHH) that have been transduced with HBV, and a novel, noninfectious, episomal AAV mouse model.

OBJECTIVE

ARCUS Gene Editing Approach for HBV

- As a curative therapeutic approach, we have developed HBV genome-specific ARCUS meganucleases to target the viral polymerase gene (HBV-ARCUS-POL).
- Through subsequent rounds of nuclease engineering, we have generated highly specific and active HBV-ARCUS nucleases to cut cccDNA and integrated viral DNA to achieve durable viral antigen loss.

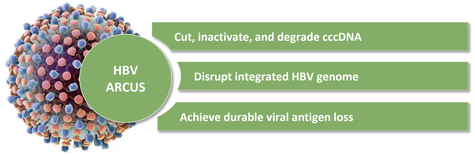
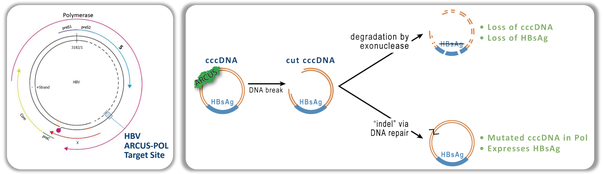


Figure 1: cccDNA Fate After ARCUS Cleavage

- HBV ARCUS-POL target site is conserved in >90% isolates.
- After ARCUS cleavage of cccDNA occurs, cccDNA can be degraded or repaired via DNA repair pathways.



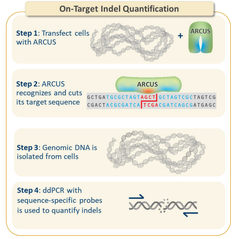
RESULTS

Figure 2: HBV-ARCUS-POL Nucleases Have Increased Activity Across Generations

- Hep3B cells, which contain integrated copies of HBV genomes, were transfected with various amounts of mRNA encoding HBV-ARCUS-POL nucleases. Indels at the genomic target sites were measured using ddPCR.
- ARCUS nucleases show a 2-log increase in on-target activity from earlier to later generations.

RESULTS

2A



2B. On-Target Indel Analysis of HBV-POL Nucleases

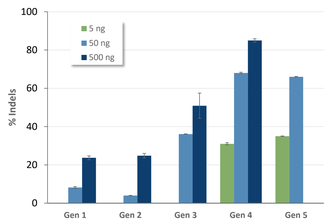
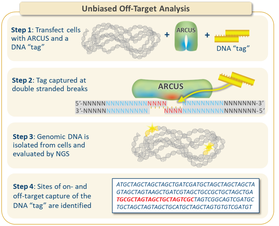


Figure 3: HBV-ARCUS-POL Nucleases Show Improved Specificity Across Generations

- HepG2 cells containing one, partial integrated HBV genome were transfected with high levels of HBV-ARCUS-POL nucleases along with a DNA "tag." gDNA was isolated and off-target editing was evaluated using Oligo Capture NGS.
- Each blue dot represents a potential cut site, with the X-axis indicating the number of reads recovered and the color of the dot indicating the number of mismatches each site has compared to the intended 22bp target site.
- Off target sites with the highest probability of being real are those with either a large number of reads or those with fewer mismatches. These are contained within the yellow outlined box. The orange circles indicate the intended target site which has been integrated into the genome of a HepG2 cell line.

3A



3B. Off-Target Analysis of HBV-POL Nucleases

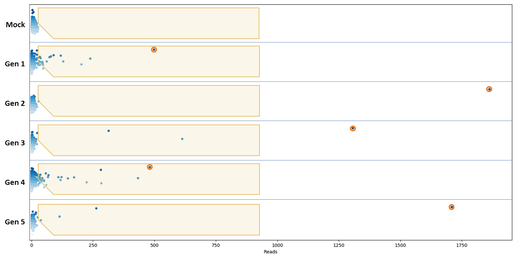
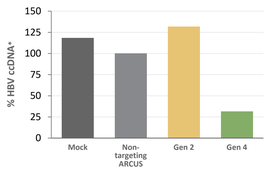


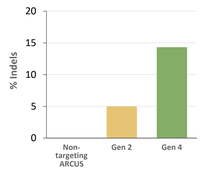
Figure 4: HBV-ARCUS-POL Nucleases Show Antiviral Activity in HBV-Infected PHH

- Primary human hepatocytes (PHH) were de novo transduced with HBV. HBV-ARCUS-POL mRNA was transfected into cells and multiple viral endpoints were assessed.
- The 2-log activity increase between Gen 2 and Gen 4 observed in Hep3B cells (Fig 2B) correlates with increased efficacy in HBV-infected PHH.
- Generation 4 nuclease shows 70% reduction in cccDNA with the remaining cccDNA containing 15% indels. This level of cccDNA editing results in ~70% reduction in extracellular viral surface antigen (HBsAg) with no observed toxicity, as indicated by steady levels of hAlbumin.

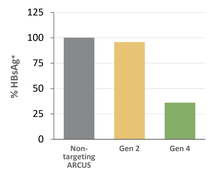
4A. cccDNA Levels



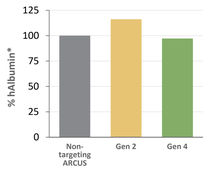
4B. cccDNA Indel Frequency in Remaining cccDNA



4C. Extracellular HBsAg Levels



4D. hAlbumin Levels

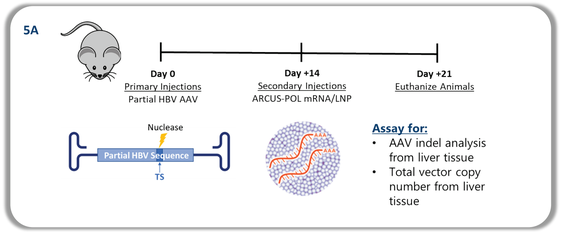


*Normalized to control

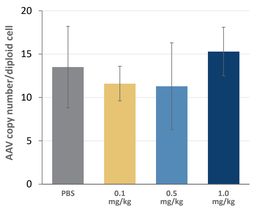
Figure 5: HBV-ARCUS-POL Generation 4 Nuclease Edit Episomal DNA *In Vivo*

- To evaluate HBV-ARCUS-POL nucleases in vivo, we developed a AAV Episomal Mouse model. NSG mice receive an AAV carrying a partial HBV genome. Two weeks later, LNPs containing Generation 4 nuclease mRNA are administered. One week post LNP, mice are euthanized and livers are collected. AAV copy number and indel frequency are measured by ddPCR.
- Generation 4 HBV-ARCUS POL nuclease shows robust editing at all doses tested.

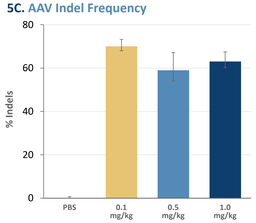
5A



5B. HBV-AAV Copy Number



5C. AAV Indel Frequency



CONCLUSIONS

- ARCUS-POL nucleases show robust cutting efficiency of integrated HBV DNA and cccDNA, with increased activity shown across nuclease generations.
- In HBV-infected PHH cells transiently exposed to HBV-POL nucleases, we observe a ~70% reduction in cccDNA as well as ~15% indel formation in remaining cccDNA. This results in a ~70% reduction in secreted HBsAg with no observed cellular toxicity.
- Furthermore, we demonstrate >60% editing *in vivo* using an AAV episomal target sequence and LNP/mRNA-delivery of an ARCUS-POL nuclease.
- These data support an ARCUS gene editing approach for elimination of cccDNA and an HBV cure.

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

For more information, please contact Cassie Gorsuch (cassie.gorsuch@precisionbiosciences.com).

PRECISION BIOSCIENCES