Preclinical safety data for PBGENE-HBV gene editing program supports advancement to clinical trials as a potentially curative treatment for chronic hepatitis B

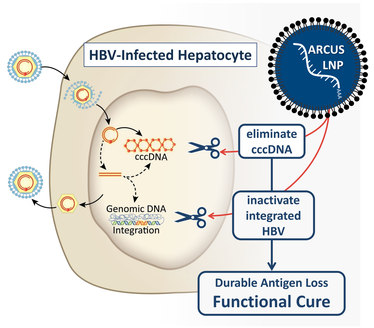
LBP-022

Emily B Harrison. [1], Keely Dulmage[1], Katie Evans[1], Haley Grimason[1], Nicole Heard[1], Adepeju Aseyiga[1], Whitney E. Lewis[1], Ben Morris[1], Arpan Mukherjee[1], Traci Reddick[1], Andrew Van Cott[1], Dan Nazarenko[1], Ying Tam[2], Paulo Lin[2], Sean Semple[2], Neil Leatherbury[1], Janel Lape[1], Jeff Smith[1], Cassandra L. Gorsuch[1]

[1]Precision BioSciences Inc., Durham, NC 27701, USA, [2]Acuitas Therapeutics, Vancouver, British Columbia

INTRODUCTION

- Persistence of chronic Hepatitis B (CHB) is attributed to maintenance of the intrahepatic pool of the hepatitis B virus (HBV) covalently closed circular DNA (cccDNA).
- PBGENE-HBV is a lipid nanoparticle encapsulating ARCUS mRNA that encodes an ARCUS nuclease. Once taken up into hepatocytes the mRNA is translated to an ARCUS protein that cuts a highly conserved target sequence in cccDNA and integrated HBV DNA.
- Cleavage of the target site leads to cccDNA elimination and inactivation of integrated HBV DNA leading to a reduction of all viral products.
- **AIM: preclinical safety of PBGENE-HBV was evaluated to support advancement to clinical trials.**



Summary of PBGENE-HBV Pharmacology[1,2]

- Proof of concept for elimination of cccDNA:
  - 99% viral engagement in AAV non-human primate (NHP) model.
  - 95% durable reduction in HBsAg in AAV mouse model.
  - Reduction of cccDNA in HBV-infected primary human hepatocytes.
- Proof of concept for inactivation of integrated HBV DNA:
  - Multi-log durable reductions in HBV DNA in HBV transgenic mouse.

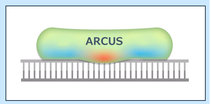
ARCUS: Unique gene editing platform

- ARCUS is derived from I-CreI, a homing endonuclease from algae.
- Iterative protein engineering optimizes for efficacy and safety.

Size:

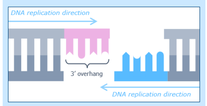
ARCUS is the smallest gene editor allowing for clinically relevant delivery and better access to cccDNA[3]

Simplicity:



ARCUS recognizes target site through a protein-DNA interface making it a single component editor

Cut:



3′ overhang "sticky-ends" allows for higher sensitivity off-target identification than blunt cuts

RESULTS

PBGENE-HBV Generates No Off-Target Editing at Therapeutically Relevant Doses

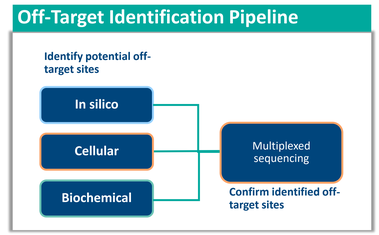
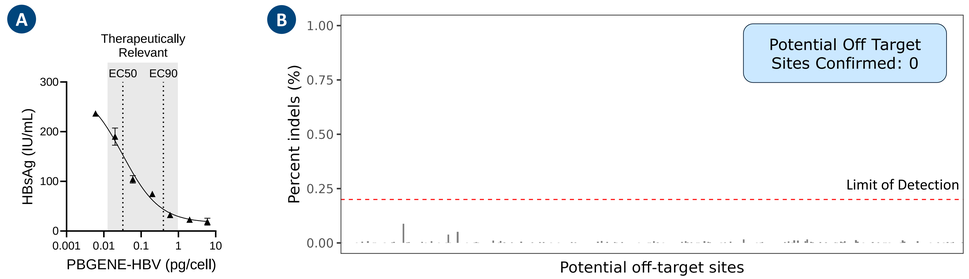


Figure 1. Genome-wide screening identifies potential off-target sites

- In silico search used to identify similar sites in human genome.
- Cellular assays used to detect potential "sticky end" ARCUS cuts with high sensitivity in 2 cell lines with integrated HBV.
- Biochemical assays performed using gDNA from cells lines representing tissues with known LNP biodistribution.

Figure 2. Multiplexed sequencing identifies no detectable off-target editing in HBV-infected Primary Human Hepatocytes (PHH) at therapeutically relevant doses

HBsAg reduction was used to determine the EC90 of PBGENE-HBV in PHH (2A). Multiplexed sequencing was performed to quantitate editing at identified potential off-target sites after 2 administrations of PBGENE-HBV at a dose 1.5x EC90 in HBV-infected PHH (2B).



No Editing-Associated Translocations in HBV-infected PHH

A

Condition	% HBV Integrations	% HBV Translocations
No Treatment	0.18	0.002
PBGENE-HBV EC50	0.22	0.002
PBGENE-HBV EC90	0.19	0.002

Treatment with PBGENE-HBV does not show additional translocation risk vs. no treatment

B

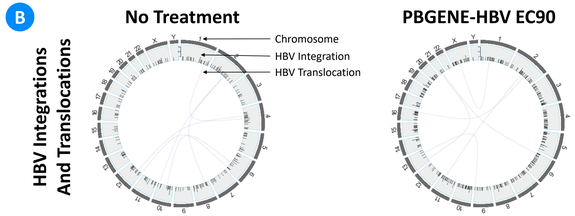


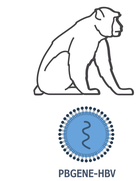
Figure 3. Assessment of genomic alterations in HBV-infected PHH

Hybrid capture followed by long read sequencing was used to detect insertions and translocations involving HBV DNA (3A+B)

PBGENE-HBV is Well-Tolerated in Non-Human Primates

Figure 4. PBGENE-HBV was rapidly cleared after administrations

Preliminary data from a GLP study in which cynomolgus monkeys were administered 2 doses of 1.5 mg/kg PBGENE-HBV. Lipids and ARCUS mRNA in the plasma were measured using HPLC-MS/MS and RT-qPCR, respectively.



No altered clearance after multiple administrations

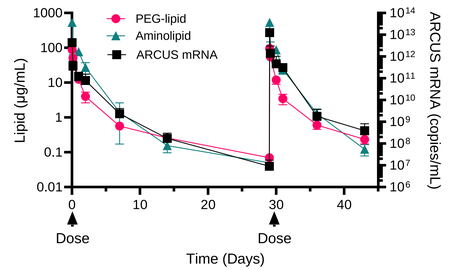
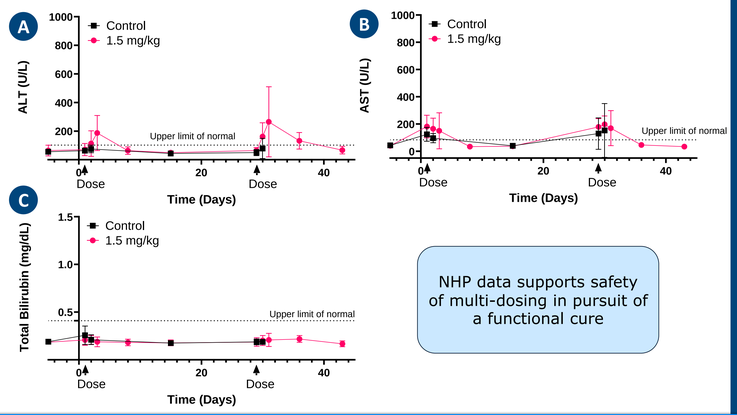


Figure 5. PBGENE-HBV produced minor and transient elevations in liver transaminases with no total bilirubin elevations

- Preliminary alanine transaminase (ALT) and aspartate aminotransferase (AST) and total bilirubin data from a GLP study (5A-C). Cynomolgus monkeys were administered PBGENE-HBV on Day 1 and 29.
- Transaminase elevations and non-adverse changes in blood parameters normalized within 2 weeks after each dose administration and did not increase in magnitude after subsequent administrations.



NHP data supports safety of multi-dosing in pursuit of a functional cure

CONCLUSIONS

- PBGENE-HBV specifically cuts HBV DNA leading to elimination of cccDNA and inactivation of integrated HBV DNA without impacting any sites in the human genome.
- PBGENE-HBV was well tolerated in non-human primates over multiple administrations.
- Preclinical safety data supports the advancement of PBGENE-HBV to clinical trials as a potentially curative, finite treatment for HBV.

References:

[1]Gorsuch, C.L. et al. Targeting the hepatitis B cccDNA with a sequence-specific ARCUS nuclease to eliminate hepatitis B virus in vivo. Mol. Ther. 2022, 30,2909–2922.
[2]Harrison, E.B. et al. Preclinical efficacy and safety of ARCUS-POL Nucleases for Chronic Hepatitis B: A Potentially Curative Strategy. American Association for the Study of the Liver. Boston, MA. 2023, 5040-C
[3]Martinez, M.G. et al. Gene Editing Technologies to Target HBV cccDNA. Viruses. 2022, 14(12), 2654

PRECISION BIOSCIENCES

European Association for the Study of the Liver ● June 5-8th, 2024 ● Milan, Italy