Preclinical efficacy and safety of ARCUS-POL nucleases for chronic hepatitis B: a potentially curative strategy

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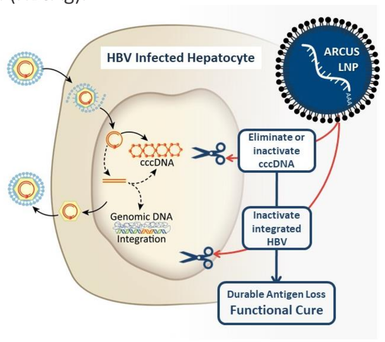
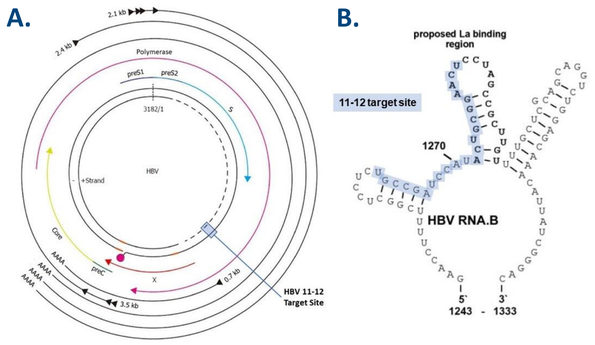
INTRODUCTION

- 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).
- Previous POC studies indicate that ARCUS nucleases engineered to recognize a highly conserved target sequence in the HBV polymerase gene (HBV 11-12) durably eliminate cccDNA and reduce HBV surface antigen (HBsAg).
- The previously described ARCUS-POL nuclease, construct, and formulation were each optimized for increased activity and safety (ARCUS-POL v.2), then further engineered to enhance specificity for the HBV target site (ARCUS-POL v.3).



THERAPEUTIC STRATEGY

- Cleavage of the highly conserved HBV 11-12 target site (A) leads to cccDNA elimination and reduction of all viral products. Indel formation in the viral polymerase ORF and the HBV RNA.B regulatory element (B) within cccDNA or integrated HBV sequences in the human genome leads to decreased levels of HBsAg.



RESULTS

Episomal AAV *in vivo* Studies

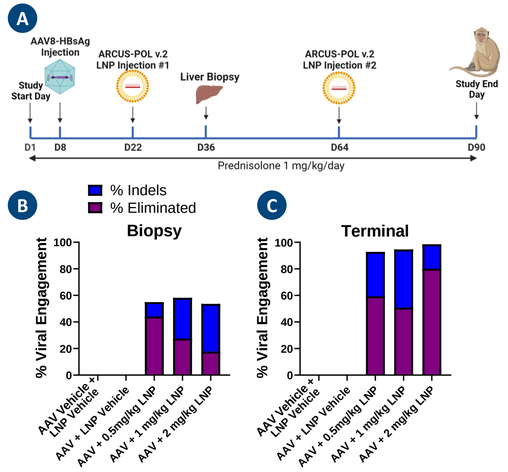


Figure 1. ARCUS-POL v.2 nuclease edits up to 99% in an episomal AAV NHP model

- ARCUS-POL v.2 resulted in >53% and >92% viral engagement at all doses at biopsy (1B) and necropsy (1C), respectively.
- ARCUS-POL v.2 achieved 99% editing by terminal timepoint.
- ARCUS-POL v.2 was well tolerated at all doses.

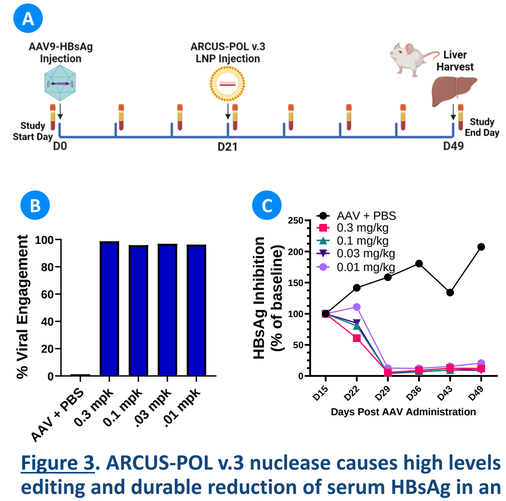


Figure 3. ARCUS-POL v.3 nuclease causes high levels editing and durable reduction of serum HBsAg in an episomal AAV mouse model

- ARCUS-POL v.3 achieved >95% viral engagement at all dose levels (3B).
- ARCUS-POL v.3 sustainably reduced HBsAg by >95% at D49 compared to PBS-treated mice (3C).
- ARCUS-POL v.3 was well tolerated at all doses.

In vitro Integrated HBV DNA

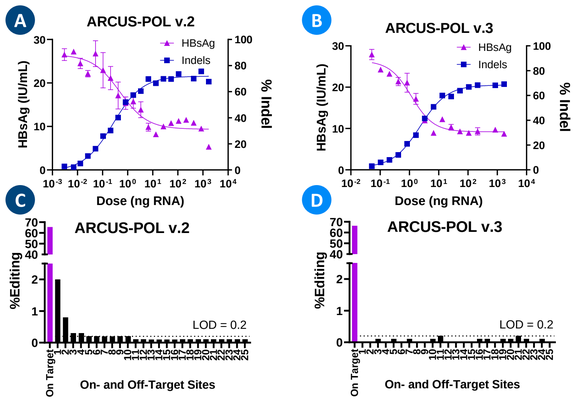


Figure 2. ARCUS-POL v.3 nuclease maintains on-target potency and demonstrates no off-target editing in an engineered HepG2 cell line with integrated HBV DNA

- Both variants achieved similar editing levels and reductions in secreted HBsAg (2A, B).
- The v.3 variant showed enhanced specificity compared to v.2 by eliminating all off-target editing above limit of detection (LOD, 0.2%) (2C, D) at a saturating dose when examining 384 potential off-target sites.

HBV Disease Models

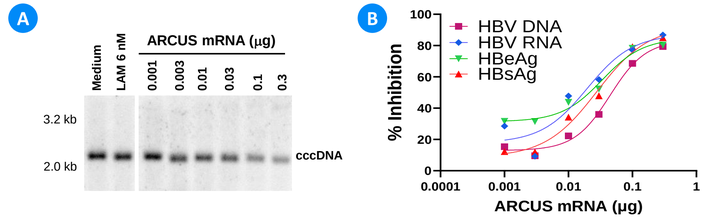


Figure 4. ARCUS-POL v.3 nuclease eliminates cccDNA and inhibits viral endpoints in HBV-infected primary human hepatocytes

- At 15 days post HBV infection, ARCUS-POL v.3 transfection showed dose-dependent elimination of cccDNA (4A) and reduced HBsAg, HBeAg, HBV DNA, and HBV RNA by ~80% (4B). Lamivudine (LAM) did not eliminate cccDNA.

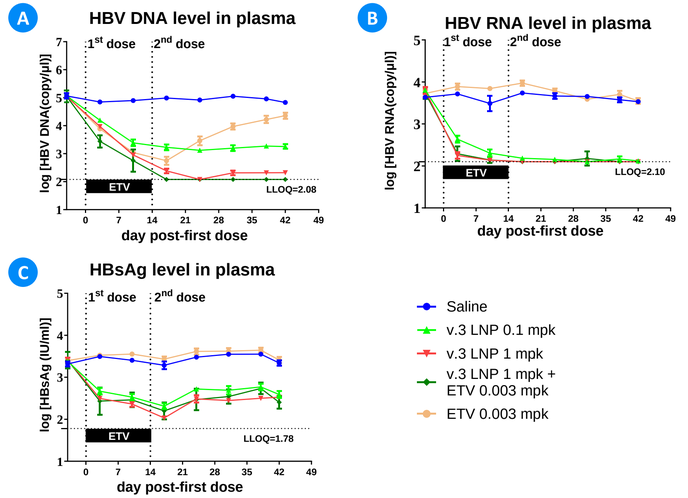


Figure 5. ARCUS-POL v.3 nuclease durably reduces HBV DNA, HBV RNA, and HBsAg in a transgenic HBV mouse model

- Mice were administered two doses of ARCUS-POL v.3 alone or in combination with a limited course of entecavir (ETV). Treatment with ARCUS-POL v.3 with and without ETV reduced HBV DNA (5A), HBV RNA (5B) and HBsAg (5C).

CONCLUSIONS

- The fully optimized ARCUS-POL v.3 eliminates cccDNA and inactivates integrated HBV DNA *in vitro* and *in vivo* resulting in durable reductions in HBsAg, HBV DNA, and HBV RNA.
- ARCUS-POL v.3 achieved 99% viral engagement in NHPs.
- No off-target editing with ARCUS-POL v.3 at 384 sites examined.

PRECISION BIOSCIENCES

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