Adenine Base Editing enables sustained reduction of HBsAg expression and inhibition of Hepatitis B viral replication in preclinical models

1. Unmet need in patients with chronic HBV

- Chronic Hepatitis B infection remains a global health problem (>296 million people infected worldwide, 800,000 deaths/year) (WHO, DHHS)
- HBV genome is maintained in human hepatocytes as episomal covalently closed circular DNA (cccDNA)
- cccDNA persistence in the liver is responsible for chronic HBV infection; failure to prevent HBV rebound from cccDNA is one of the key challenges to cure HBV
- HBV DNA integrates into the human genome and serves as a source of Hepatitis B Surface antigen (HBsAg) expression
- Standard antiviral medications (nucleoside analogue reverse transcriptase inhibitors) decrease viral replication, but do not provide a cure, and do not influence viral protein expression (HBsAg) from cccDNA and integrated HBV DNA

2. Adenine Base editors (ABE) convert A-T into G-C without double-stranded break (DSB)

3. Editing strategy

- Targeting HBV genome with ABE will allow precise introduction of missense mutations silencing viral genes without generating DSB, thus minimizing risk for chromosomal rearrangements
- Base editing will address two key aspects of Chronic Hepatitis B with the same reagent:
  1. Can prevent HBV rebound by introducing permanent mutations in cccDNA
  2. Irreversibly silences HBsAg expression from the integrated HBV DNA without DSBs

4. Adenine Base editing reduces HBV viral parameters in infected HepG2-NTCP hepatoma cell line

5. Base Editing reduces HBsAg expression in PLC/PRF/5 cells from naturally integrated HBV DNA

6. ABE reduces HBV viral parameters, including HBsAg level, in primary human hepatocytes (PHH)

7. In vivo PoC in HBV circle mouse model: ABE enables loss of HBsAg and sustained 98% reduction in HBV DNA

8. In vivo PoC in humanized mice: ABE enables sustained 90% reduction in serum HBsAg and HBV DNA

9. Conclusions

- Adenine Base Editing enables sustained reduction of the two major HBV markers, HBsAg and HBV DNA, in relevant in vitro and in vivo models
- ABE base editing reduces HBsAg, HBV DNA, and 35kDa RNA in HepG2-NTCP cells and primary human hepatocytes
- Combining base editing reagents with standard antiviral lamivudine does not compromise anti-HBV effect; Base editing strongly reduces HBsAg expression from integrated HBV sequences; Complete loss of HBsAg (3-4 log10) in HBV mimic mouse model; 10log10 HBsAg reduction in HBV humanized mice
- Potential to use single gRNA to inhibit HBsAg replication or multiplexing gRNAs to reduce other viral markers (HBcAg, 35kDa viral RNA)
- Potential to enable sustained effect with a single dose treatment