Cytosine base editing inhibits Hepatitis B Virus replication and reduces HBsAg expression in vitro and in vivo

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Unmet need in patients with chronic HBV
- Chronic Hepatitis B infection remains a global health problem (>250 million people infected worldwide, 800,000 deaths/year) (WHO, DHHS)
- HBV genome is maintained in 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 analogs 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 (Reville et al, 2019)

Cytosine Base Editors (CBE) convert C-G into T-A without double-stranded breaks

(A) Starting DNA sequence with the target base pair (C-G).
(B) Cytosine Base Editor (CBE) consists of a partially inactivated CRISPR protein (grey) fused to a deaminase enzyme (green).
Guide RNA (gRNA) directs the CBE to a target genomic DNA sequence and exposes the narrow editing window. (C) Deaminase chemically modifies target cytosine (C) to uracil (U) via deamination and the Cas enzyme nick (A) the opposite strand.

Base Editing strategy: potential functional HBV cure via introducing stop codons in HBV genes
- Targeting HBV genome with CBE will allow precise and permanent introduction of stop codons/missense mutations in viral genes without generating DSB, thus minimizing risk for chromosomal rearrangements / deletions.
- Base editing will address two key aspects of Chronic Hepatitis B with the same reagents:
  1) Can prevent HBV rebound by introducing permanent mutations in cccDNA
  2) Irreversibly silences HBsAg expression from the integrated HBV DNA without DSBs in hepatocytes
- A base editing strategy was devised to target conserved HBV regions with a focus on HBV genotype D (used to establish most in vitro and in vivo pre-clinical models of HBV infection)

Multiplexing two gRNAs with BE4 base editor simultaneously reduces HBV viral parameters in HepG2-NTCP

(A) Base editing leads to the efficient reduction of viral extracellular HBsAg (HBsAg, BE4/gRNA) as well as intracellular (3.5 kb RNA, and HBV DNA) parameters relative to a control sample treated with base editing reagents targeting the unrelated PCSK9 gene: BE4/gRNAs (C1–S2) treatment inhibited all HBs isoforms, as observed in Western blotting. (B) Experiment schedule in case of pretreatment with lamivudine. (C) Combinatorial treatment with lamivudine leads to the robust reduction of HBV viral markers, similarly to the panel A.

Base editors function through cccDNA editing, without reducing cccDNA level.

(D) Base editing does not reduce cccDNA level in HepG2-NTCP. (E) Editing assessed by NGS on the cccDNA enriched samples. Pretreatment with Lamivudine increases base editing rates by 20% in HepG2-NTCP. High cccDNA editing in Lamb pretreated conditions suggests that CBE directly targets cccDNA.

LNP-mediated delivery of BE4 mRNA and gRNAs S1/C2 leads to sustained reduction of viral markers in HBV mouse model

(A) HBV minicircle mouse model supports durable production of HBV-like viral particles and HBsAg antigen expression in immunocompetent mice (Yan et al., 2017)
- 4 weeks after hydrodynamic injection with cccDNA-like minicircle plasmid, mice received one or two doses (2x) of the base editing reagents (mRNA & gRNA formulated into a lipid nanoparticle (LNP), at 2mg/kg)
- Entecavir (ETV) treated mice received entacavir at 0.03mg/kg orally for two weeks, then the treatment was discontinued

Antiviral efficacy of the base editing reagents in HBV minicircle mouse model. At day 35 (6 weeks) after the 1st injection with the base editing reagents BE4/gRNAs (C1-S2): (A) >3 log mean HBsAg reduction; 6/8 mice showed HBsAg reduction below the limit of detection (B) HBV replication is reduced in entecavir treated mice, and then rebounds as soon as the treatment is discontinued (C) 3 log sustained reduction in serum HBV DNA in base editing treated groups; no HBV rebound; 2 injections with LNP lead to a better reduction in serum HBV DNA (D) Loss of HBsAg expression in all mice below the limit of detection two weeks after the 1st LNP injection

Data represented as mean ± SEM, n=4 or 5 per group

References:

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