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

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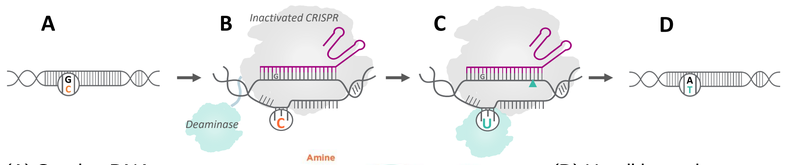
Unmet need in patients with chronic HBV

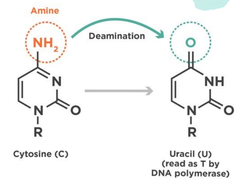
- Chronic Hepatitis B infection remains a global health problem (>250mln people infected worldwide, 800 000 deaths/year) (WHO, DHHS)
- HBV genome is maintained in hepatocytes as episomal covalently closed circular DNA (cccDNA)
- cccDNA persistence in the liver is responsible for chronic HBV infection; 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 (nucleotide 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 (Revill 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).



(D) Uracil is read as thymine by DNA polymerases. The nicked strand is repaired completing the conversion of C:G to T:A base pair (Komor et al., 2016).

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 nicks (▲) the opposite strand.

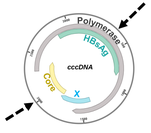
Base Editing strategy: potential functional HBV cure via introducing stop codons in HBV genes

- 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.

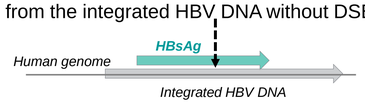
Glutamine	CAG → TAG	Stop codon
	CAA → TAA	
Arginine	CGA → TGA	
Tryptophan	TGG → TGA	
	TGG → TAG	
	TGG → TAA	

- 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 silence HBsAg expression from the integrated HBV DNA without DSBs



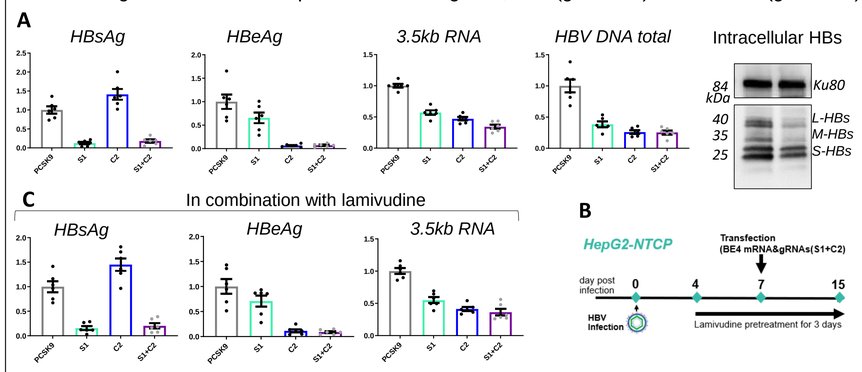
- A base editing strategy was devised to target conserved HBV regions with a focus on HBV genotype D (used to establish most *in vitro* and *in vivo* pre-clinical models of HBV infection)

References:

- Revill PA, Penicaud C, Brechot C, Zoulim F. Meeting the Challenge of Eliminating Chronic Hepatitis B Infection. Genes (Basel). 2019 Apr 1;10(4):260. doi: 10.3390/genes10040260. PMID: 30939846; PMCID: PMC6523454.
- Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature. 2016 May 19;533(7603):420-4. doi: 10.1038/nature17946. Epub 2016 Apr 20. PMID: 27096365; PMCID: PMC4873371.
- Ishii T, Tamura A, Shibata T, Kuroda K, Kanda T, Sugiyama M, Mizokami M, Moriyama M. Analysis of HBV Genomes Integrated into the Genomes of Human Hepatoma PLC/PRF/5 Cells by HBV Sequence Capture-Based Next-Generation Sequencing. Genes (Basel). 2020 Jun 18;11(6):661. doi: 10.3390/genes11060661. PMID: 32570699; PMCID: PMC7348787.
- Yan Z, Zeng J, Yu Y, Xiang K, Hu H, Zhou X, Gu L, Wang L, Zhao J, Young JAT, Gao L. HBVcircle: A novel tool to investigate hepatitis B virus covalently closed circular DNA. J Hepatol. 2017 Jun;66(6):1149-1157. doi: 10.1016/j.jhep.2017.02.004. Epub 2017 Feb 14. PMID: 28213165.

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

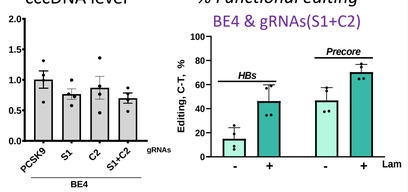
- Two lead gRNAs introduce stop codons in HBV genes, HBs (gRNA S1) and Precore (gRNA C2)



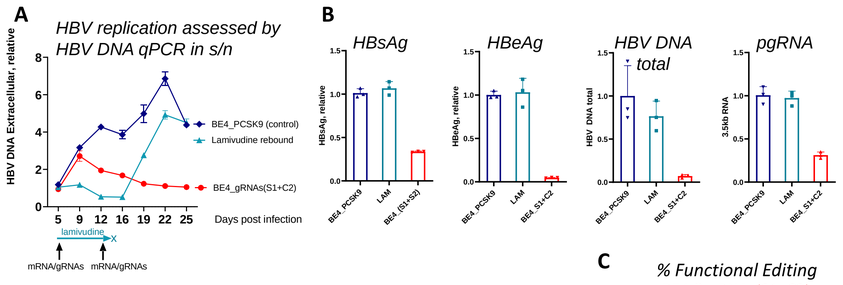
(A) Base editing leads to the efficient reduction of viral extracellular (HBsAg, HBeAg) as well as intracellular (3.5kbRNA, and HBV DNA) parameters relative to a control sample treated with base editing reagents targeting the unrelated PCSK9 gene; BE4/gRNAs(S1+C2) treatment inhibited all HBs isoforms, 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 Lam pretreated conditions suggests that CBE directly targets cccDNA.

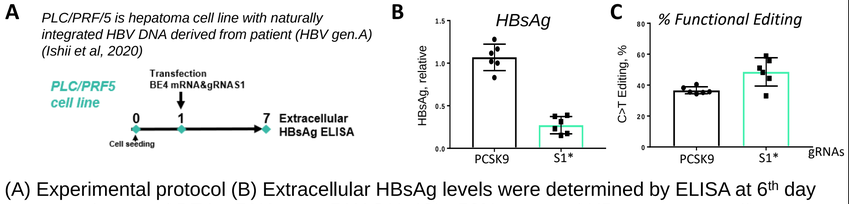


Base editing prevents viral rebound in PHH



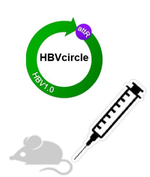
A) HBV replication assessed by HBV DNA qPCR in primary hepatocyte (PHH) supernatant. Discontinuation of lamivudine leads to HBV rebound, while base editing prevents viral rebound. B) Base editing leads to the efficient reduction of HBsAg, HBeAg, 3.5kbRNA, and HBV DNA. (C) Editing was assessed by NGS on cccDNA enriched samples: ~55% Editing HBs and ~80% Editing PreCore genes

Base editing reduces HBsAg from naturally integrated HBV DNA

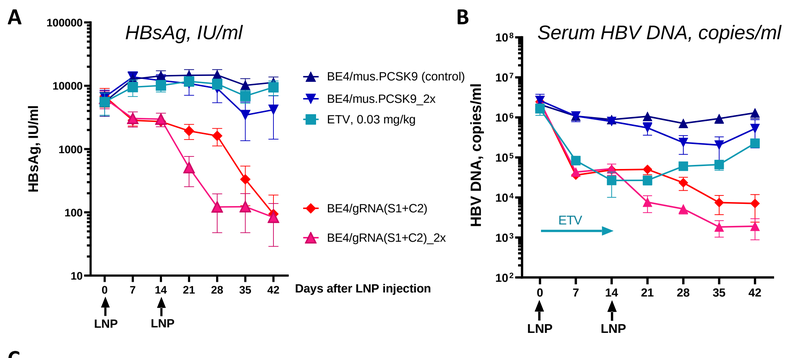


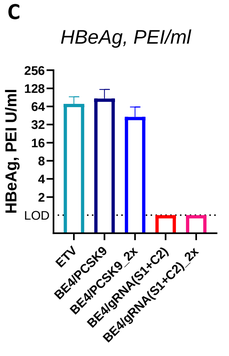
(A) Experimental protocol (B) Extracellular HBsAg levels were determined by ELISA at 6th day post-transfection of BE4 mRNA and gRNAS1*. (C) ~50% editing of HBs gene was sufficient to enable robust reduction of HBsAg. *gRNAS1 adapted to match integrated HBV DNA in PLC cells

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



- HBV minicircle mouse model supports durable production of HBV-like viral particles and HBV 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 antiviral 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(S1+C2):

(A) > 2log mean HBsAg reduction; 6/9 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 (positive control);
>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
(C) Loss of HBeAg 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

CONCLUSIONS

- Multiplexing two gRNAs introducing Stop codons in HBV genes HBs and Precore with CBE leads to a simultaneous reduction of HBsAg, HBeAg, HBV DNA, and 3.5kb RNA in HepG2-NTCP and human primary hepatocytes
- MoA: Reduction in viral markers appears to be driven by base editing of cccDNA, without reduction in cccDNA levels
- Base editing strongly reduces HBsAg produced from naturally integrated HBV sequences
- In vivo PoC in HBV minicircle mouse model: IV injection(s) with LNP formulated with HBV targeting base editors leads to sustained reduction in HBsAg, with several mice showing loss in HBsAg, as well as reduction of HBeAg and serum HBV DNA.
- Combined, the data indicate that base editing can inactivate cccDNA and integrated HBV DNA by introducing mutations abrogating HBV replication and silencing viral protein expression.

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