cccDNA inactivation using cytosine base editors

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2. Cancer Research Center of Lyon, INSERM, U1052, Lyon, France
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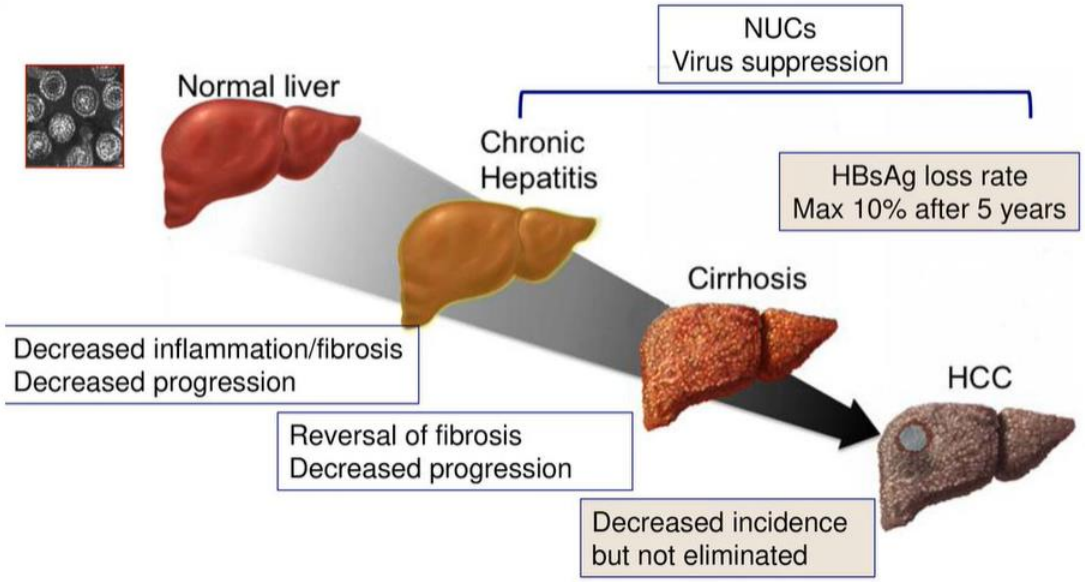
DISCLOSURE

- I am a Beam employee and shareholder

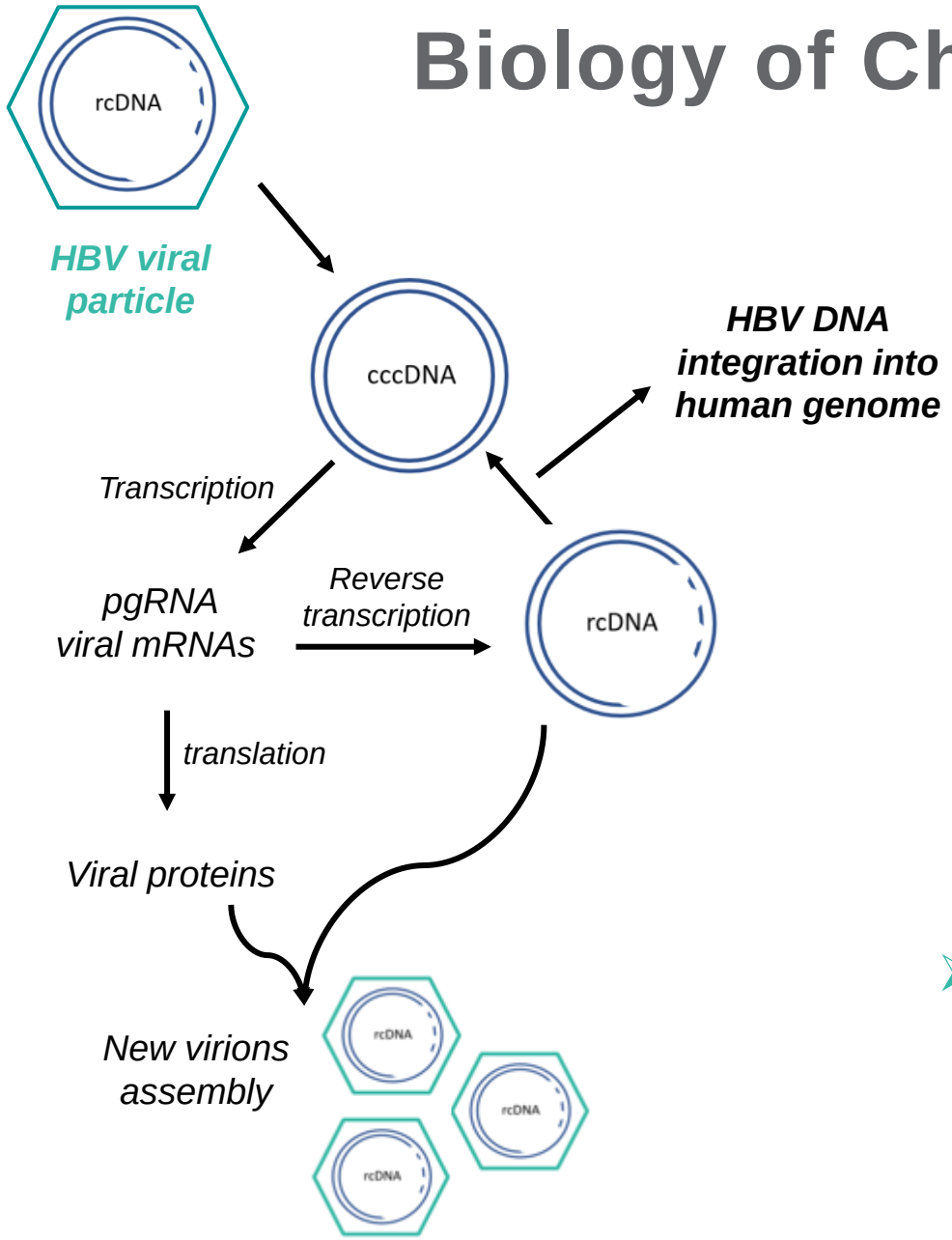
Unmet need in patients with chronic HBV

- Vaccination is 95% effective, and still not universally administered
- 257 million are chronically infected worldwide, 800,000 deaths/year
- Antiviral medications (NUC) manage HBV replication, but do not affect cccDNA – no cure
- 20-30% of adults with chronic infection develop HCC or cirrhosis (WHO)



Zoulim F., "Challenges towards the cure of HBV infection"

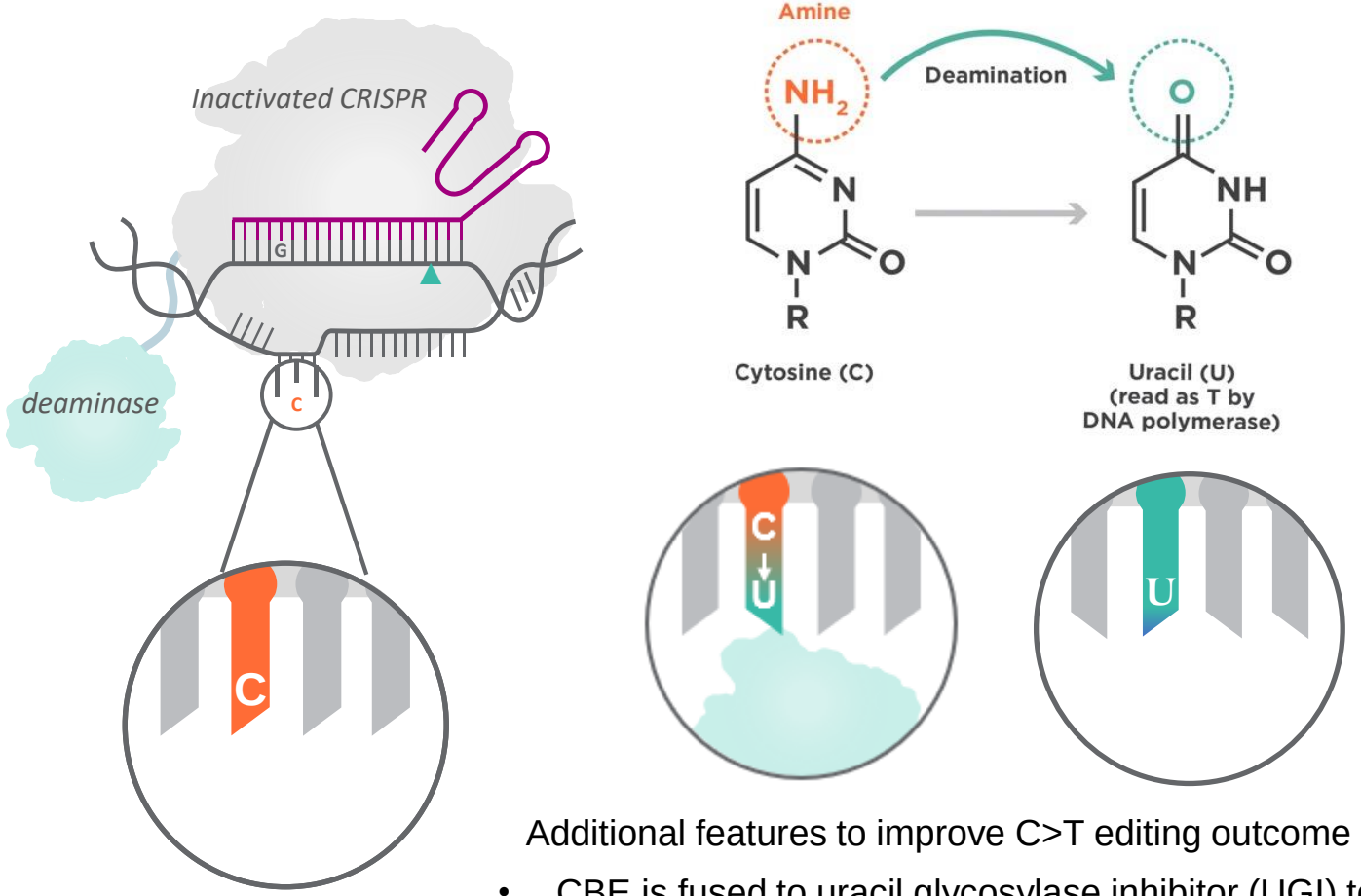
Biology of Chronic HBV infection



- HBV genome is maintained in the liver cell as 3.2kb nuclear episomal DNA (cccDNA)
- cccDNA – extremely stable, responsible for the persistence of chronic HBV infection
- HBV DNA integrates into the human genome and serves as a source of Hepatitis B Surface antigen (HBsAg) expression

➢ **Failure to prevent HBV infection rebound from cccDNA is the key challenge to cure HBV**

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



- Cytosine Base Editor (CBE) consists of a partially inactivated CRISPR protein fused to a deaminase enzyme
- Guide RNA (gRNA) directs the CBE to a target genomic DNA sequence and exposes the narrow editing window
- Deaminase chemically modifies target cytosine (C) to uracil (U) via deamination
- Uracil (U) is replaced into thymine (T) during DNA repair and/or replication.

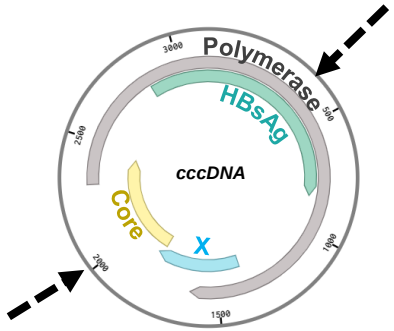
Additional features to improve C>T editing outcome

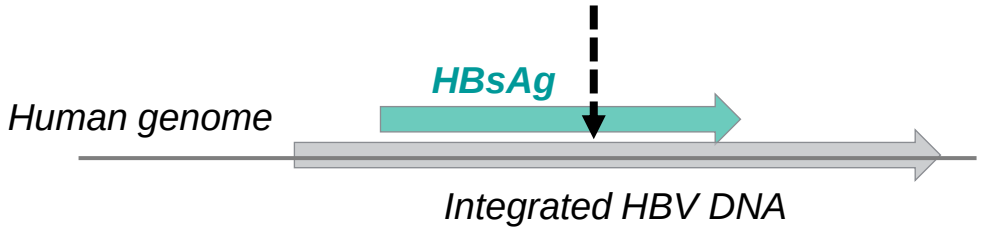
- CBE is fused to uracil glycosylase inhibitor (UGI) to block base excision repair system
- Cas9 retains nickase activity, which promotes mismatch repair system to resolve U-G into T-A

Base editing strategy: Targeting HBV genome with cytosine base editor (BE4)

- **Precise and permanent introduction of stop codons / missense mutations in viral genes without generating double-stranded breaks**
  - Viral gene silencing
  - Editing multiple sites simultaneously, without the risk of chromosome translocations

- **The goal is to achieve functional HBV cure using base editing:**
  - prevent HBV rebound by introducing permanent mutations in cccDNA
  - Irreversibly silence HBsAg expression from the integrated HBV DNA

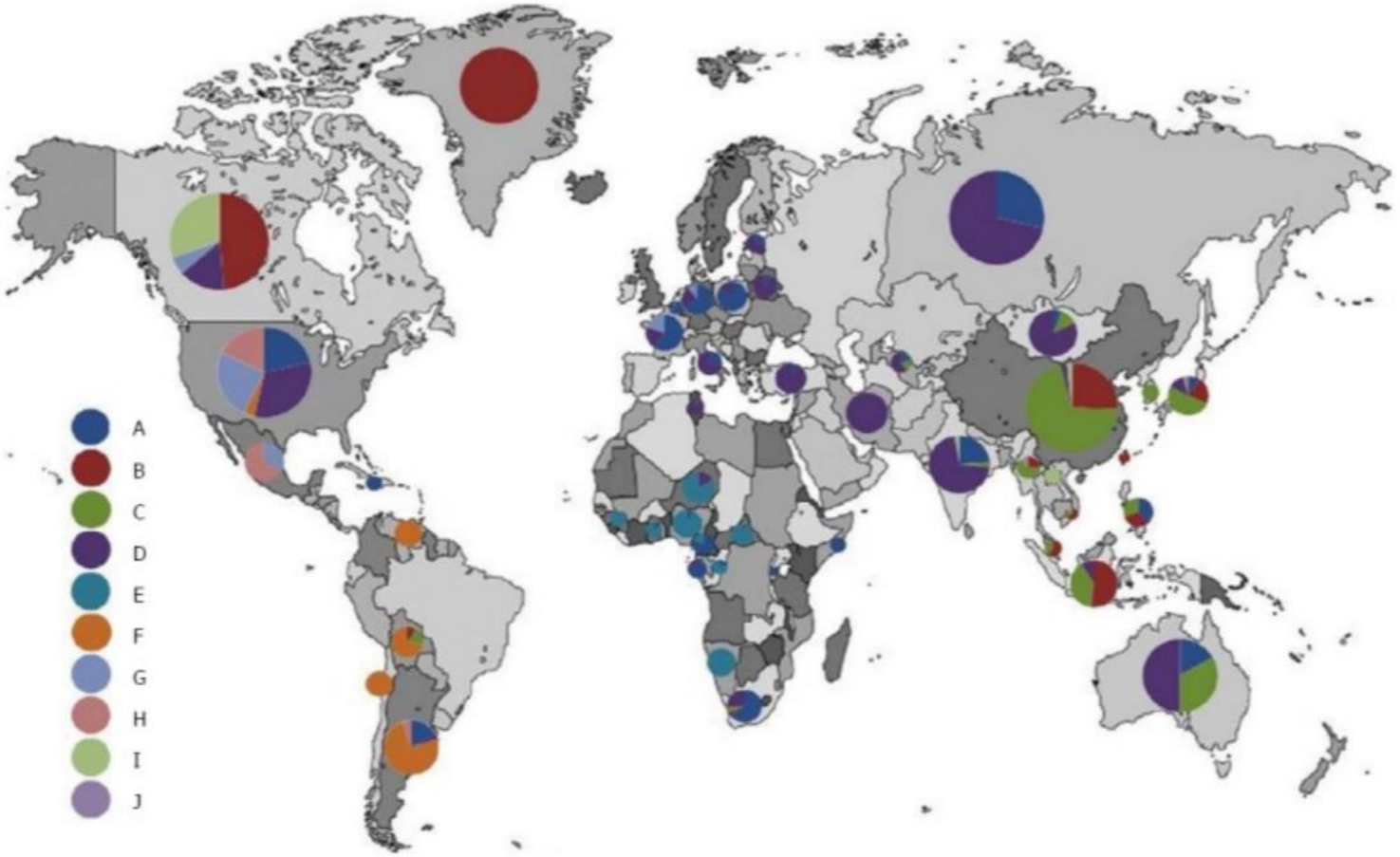




HBV genotype D chosen as a relevant viral genome sequence

Identifying conserved HBV regions with a focus on genotype D

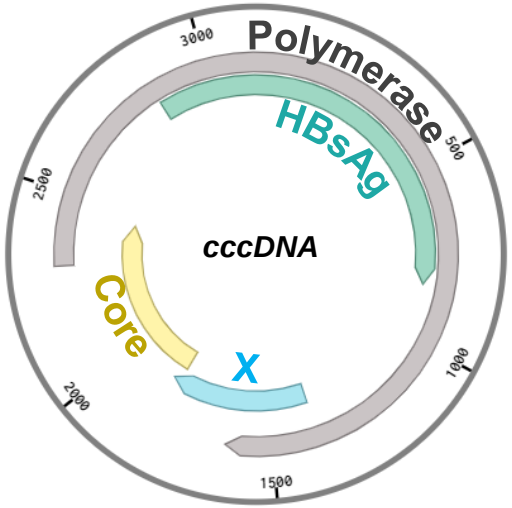
- Most abundant in US
- Existence of cellular and animal models



Geographic distribution of hepatitis B virus genotypes worldwide (World Gastroenterology Organisation Global Guideline, 2015)

Guide RNA selection and screen in Hek293-Lenti-HBV system

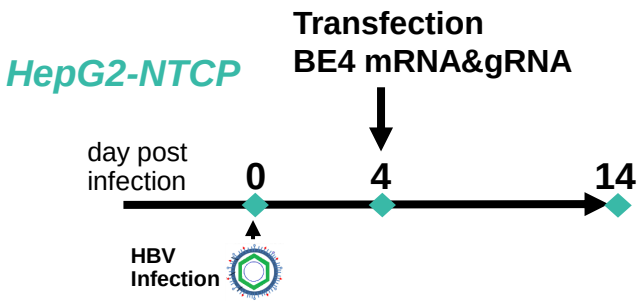
- **Targeting conserved HBV regions with a focus on *genotype D***
- ***In silico* gRNA selection based on their potential to silence HBV genes**
  - *100 gRNAs introducing Stop codons in viral genes (NGG, NGA, NNNRRT PAMs)*
  - *24 conserved gRNAs predicted to introduce missense mutations*
- **Final selection step:**
  editing in Hek293-Lenti-HBV cell line



Selected gRNAs

	ID	HBV gene	% Functional Edit	% Conservation across HBV genotypes
Stop strategy	C01	Stop-PreCore	42	95
	C02	Stop-PreCore	70	65
	P01	Stop-Pol	68	22
	P02	Stop-Pol	66	15
	X01	Stop-X	65	76
	X02	Stop-X	40	72
	S01	Stop-S	57	40
Conserved strategy	P/S1	Pol/S	47	96
	P/S2	Pol/S	25	92
	P/S3	Pol/S	65	95
	P/S4	Pol/S	49	92

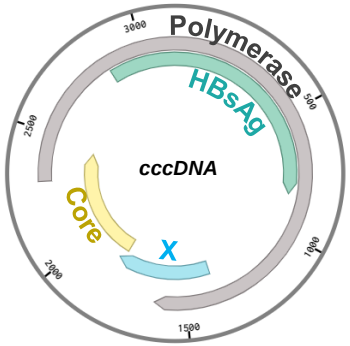
Functional gRNA screen in HBV-infected HepG2-NTCP

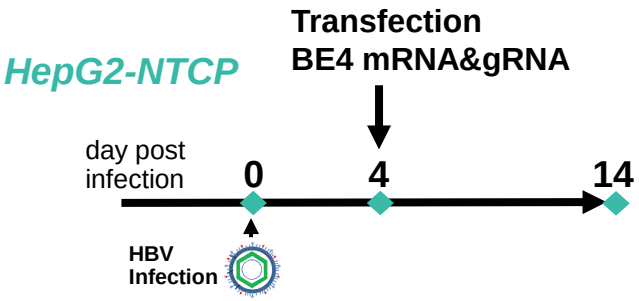


Assessment of HBV parameters:

Extracellular	Intracellular
• HBsAg	• Total HBV DNA
• HBeAg	• 3.5kb viral RNA

Functional gRNA screen in HBV-infected HepG2-NTCP identifies two lead gRNAs targeting HBsAg and Core genes





Assessment of HBV parameters:

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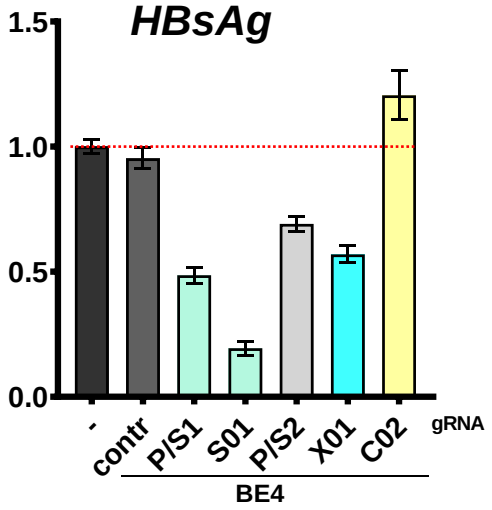
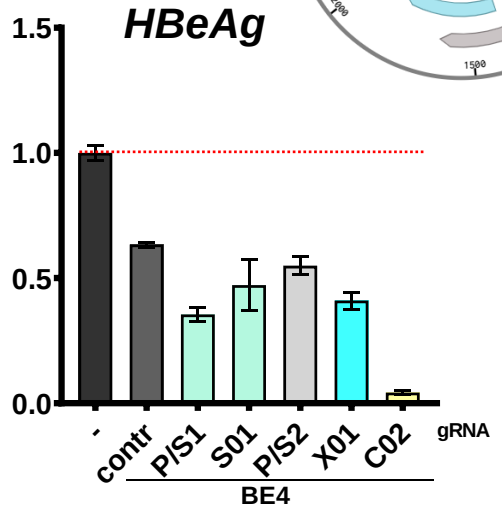
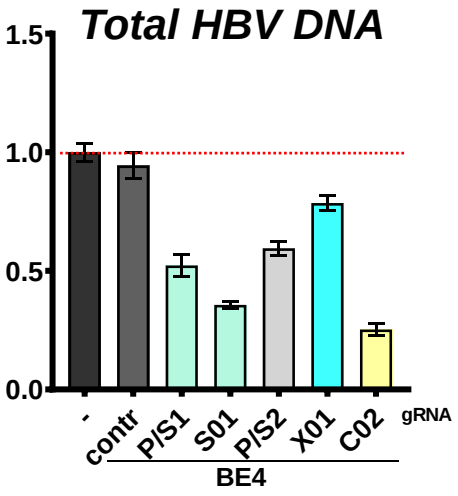
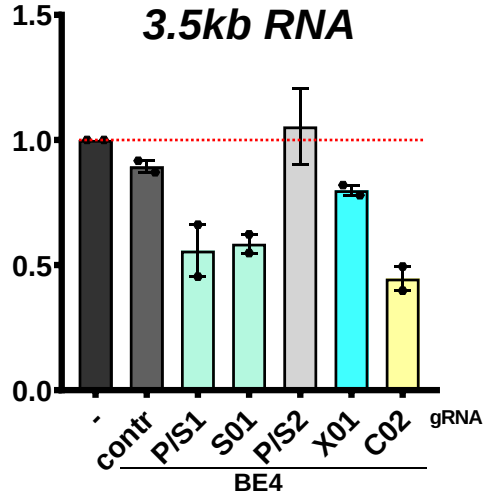


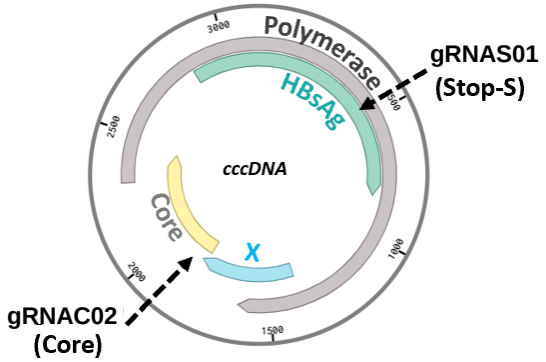


- gRNAS01 (Stop-S) reduces HBsAg
- gRNAC02 (Stop-PreCore) efficiently reduces HBeAg
- Each also lowers the total HBV DNA and 3.5kb RNA levels



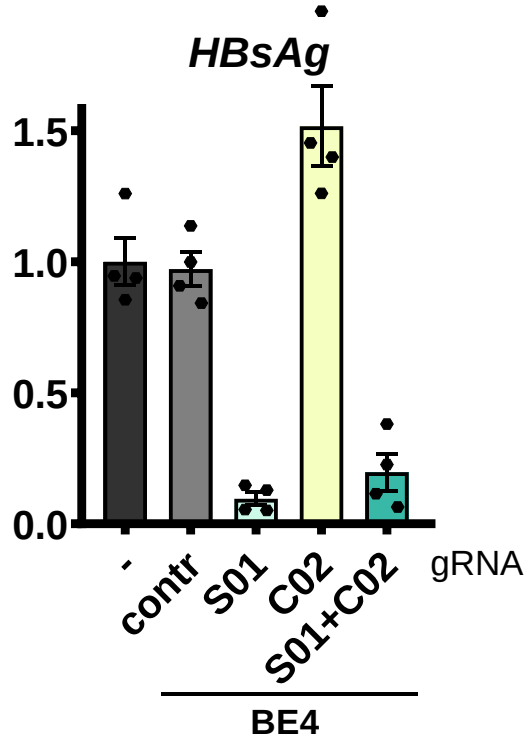


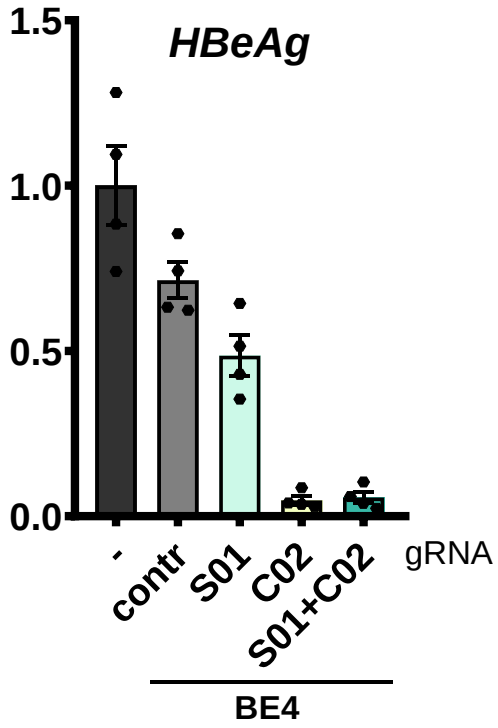
Multiplexing gRNAs simultaneously reduces respective HBV viral parameters in HepG2-NTCP

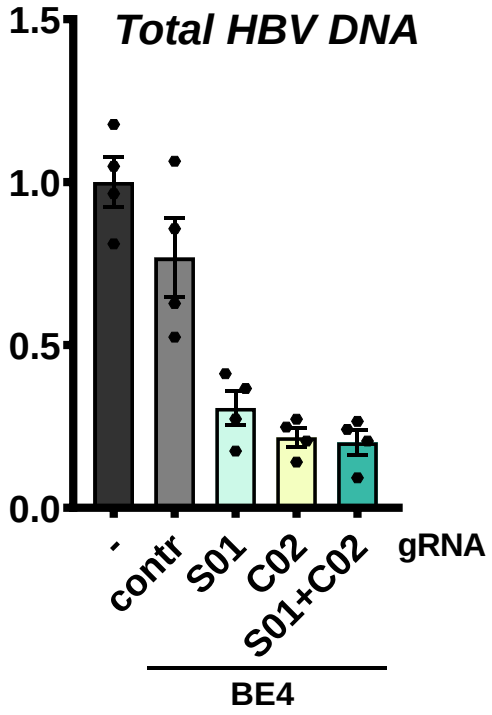


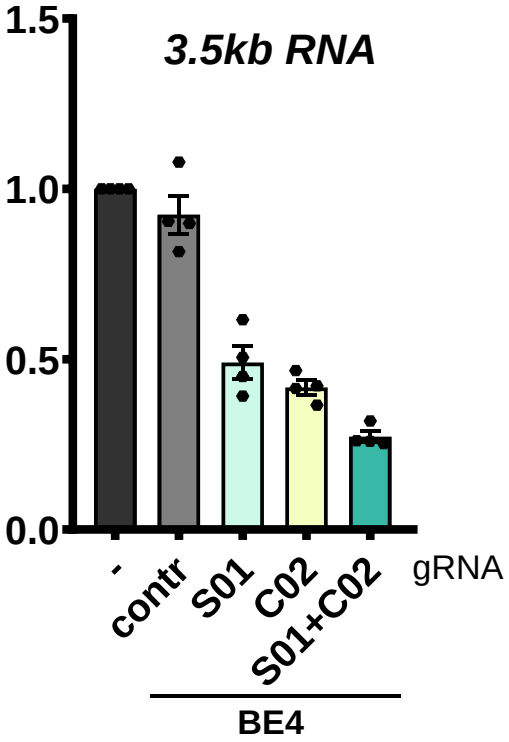
BE4 & gRNAS01(Stop-S) + gRNAC02(Stop-PreCore)

- Target same cccDNA strand; > 1kb distance in between
- **Multiplexing gRNAS01+gRNAC02 reduces HBsAg <u>and</u> HBeAg, as well as total HBV DNA and 3.5kb RNA**



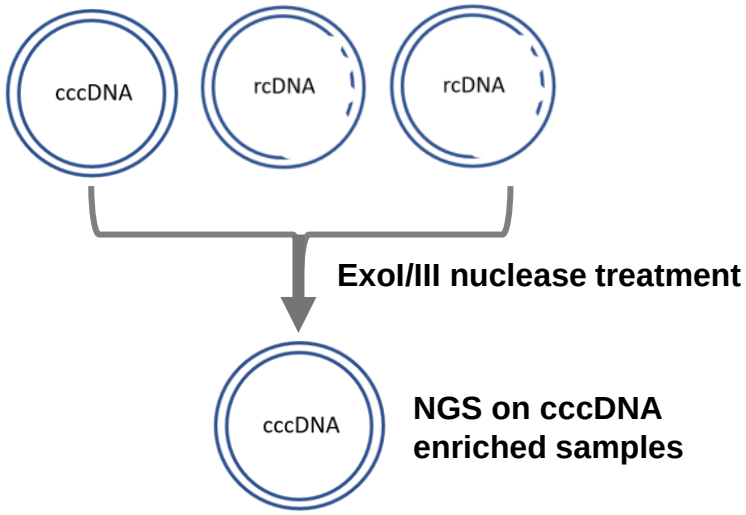


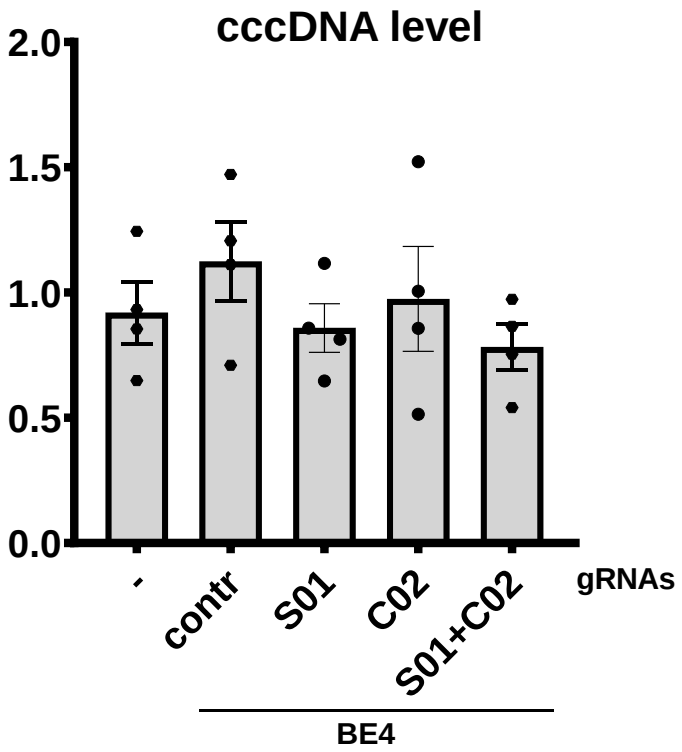




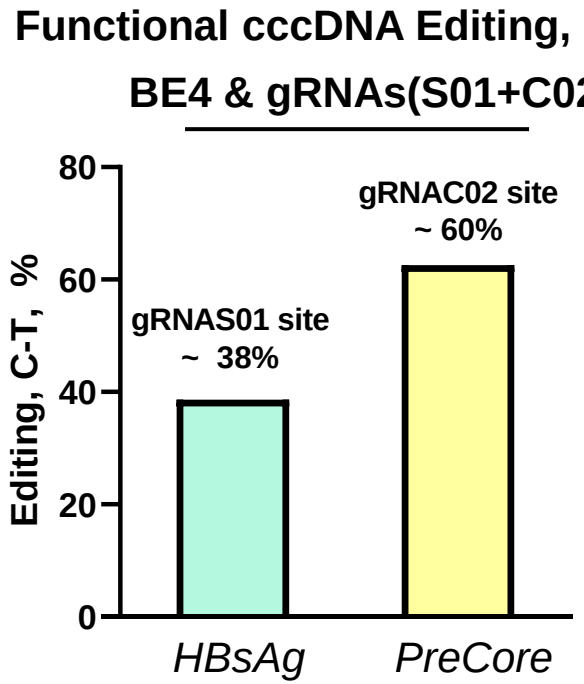
Base editors function through cccDNA editing, without reducing cccDNA level

- HepG2-NTCP
- cccDNA enriched through ExoI/ExoIII nuclease treatment





➢ **Base editing does not affect cccDNA level**

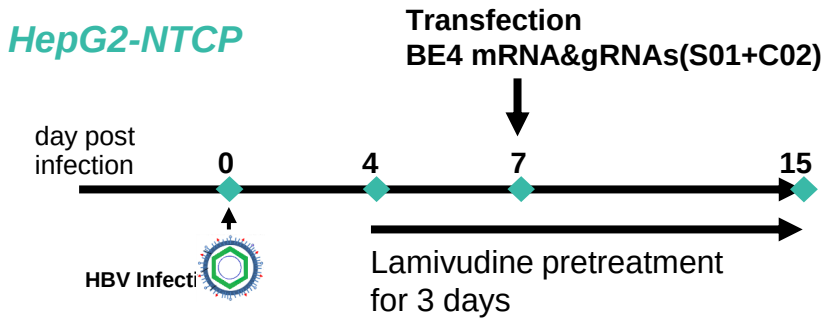


➢ **Robust cccDNA editing**

~ 38% for gRNAS01 (Stop-S)

~ 60% for gRNAC02 (Stop-PreCore)

Efficacy of base editing + lamivudine in HepG2-NTCP



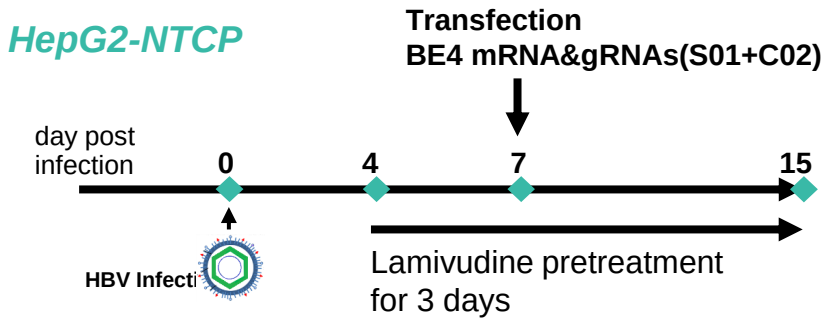
Lamivudine – standard of care antiviral, nucleoside reverse transcriptase inhibitor, which blocks HBV viral replication

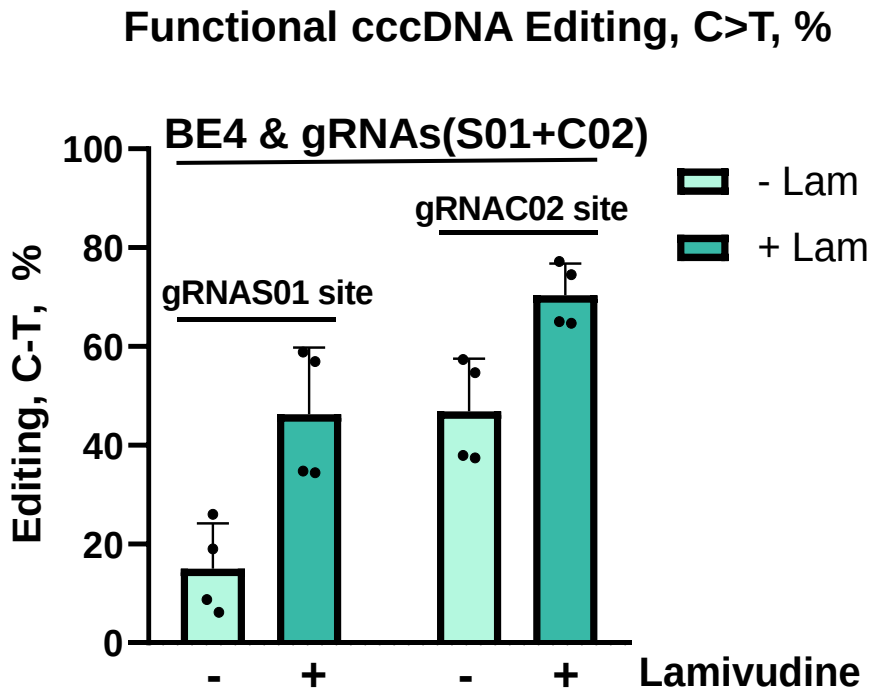
Objectives:

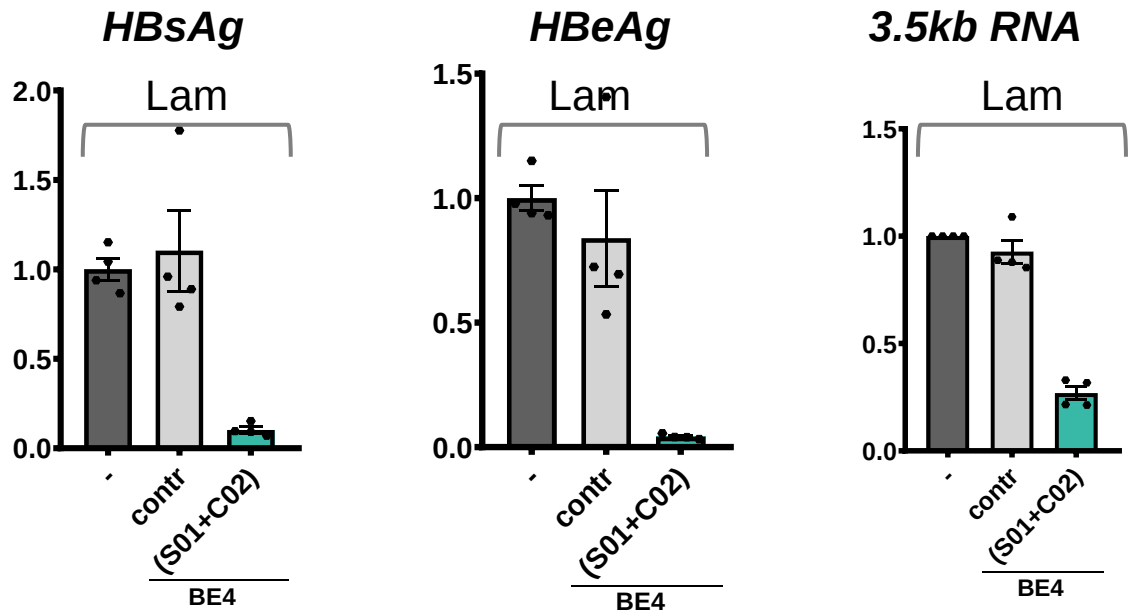
1) Pretreatment with lamivudine will remove intermediate HBV DNA species, leaving only cccDNA – efficacy in this system would confirm MoA

2) Assessing the efficacy of the combinatorial treatment

Pretreatment with lamivudine improves editing, which results in high antiviral efficacy in HepG2-NTCP



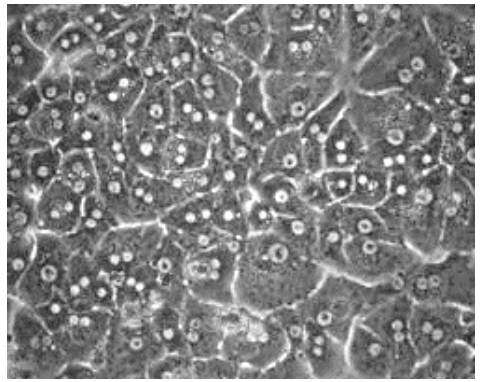




- Pretreatment with Lamivudine improves base editing by 20% in HepG2-NTCP
- High cccDNA editing in Lam pretreated conditions suggests that CBE directly targets cccDNA
- Combinatorial treatment leads to robust reduction of HBV viral markers

Primary human hepatocyte (PHH) co-culture system for longitudinal studies of HBV infection

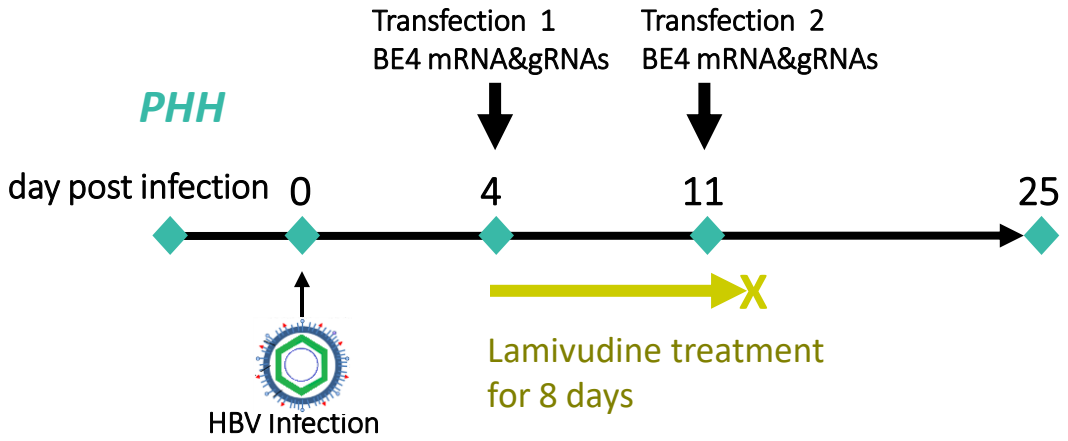
- Primary hepatocyte co-culture system (PhoenixBio) maintains hepatocyte differentiation and metabolic activity for over 30 days
- Persistent HBV infection, maintains cccDNA level



Assessment of antiviral activity of base editing relative to lamivudine

Conditions:

1) *HBV, non treated control*
2) *Lamivudine, 8 days, then discontinued*
3) *BE4 / control gRNA*
4) *BE4 / HBV_gRNAs(37+40)*

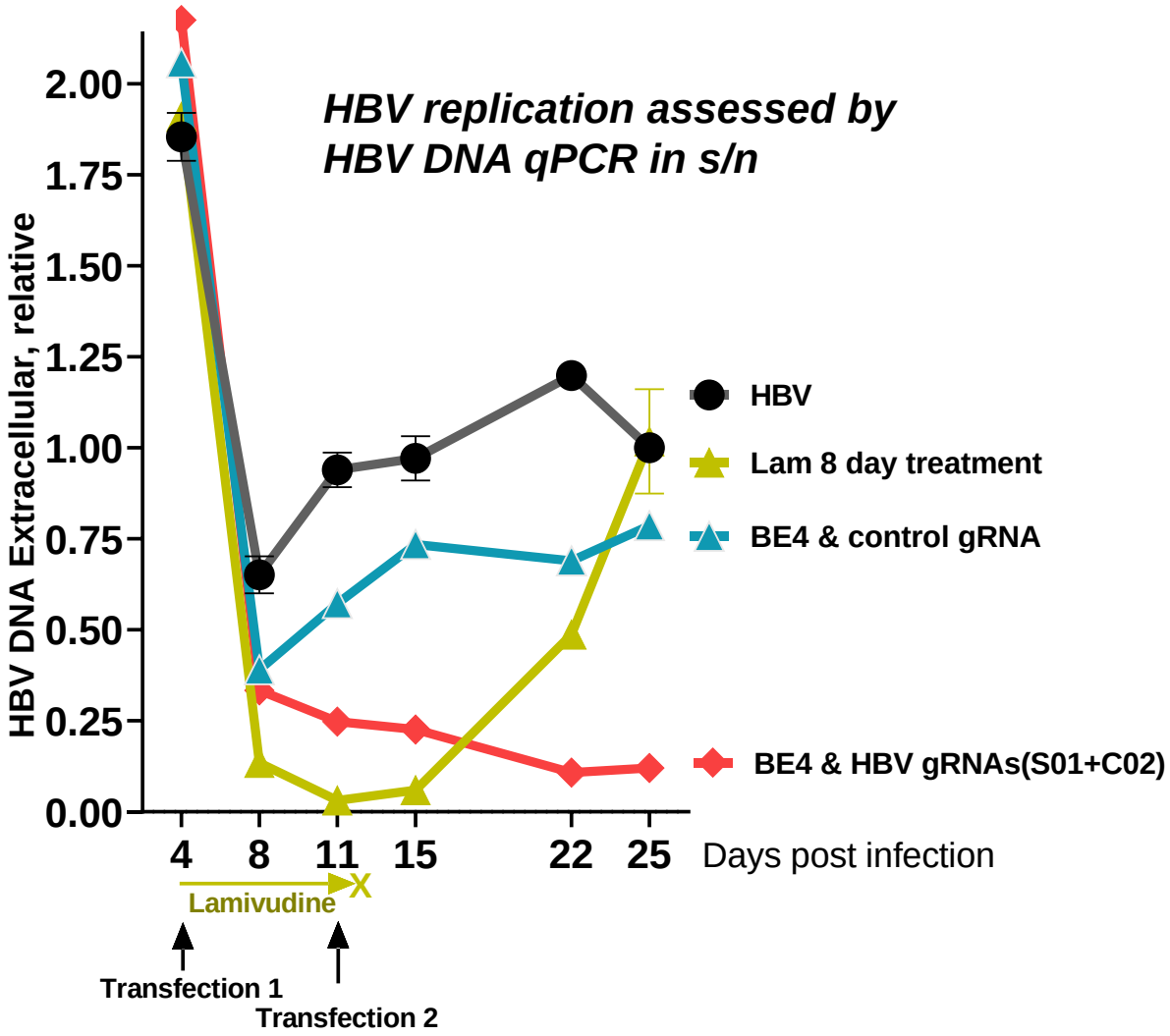


HBV parameters assessment:

Extracellular
- HBV DNA (time course)
- HBsAg
- HBeAg

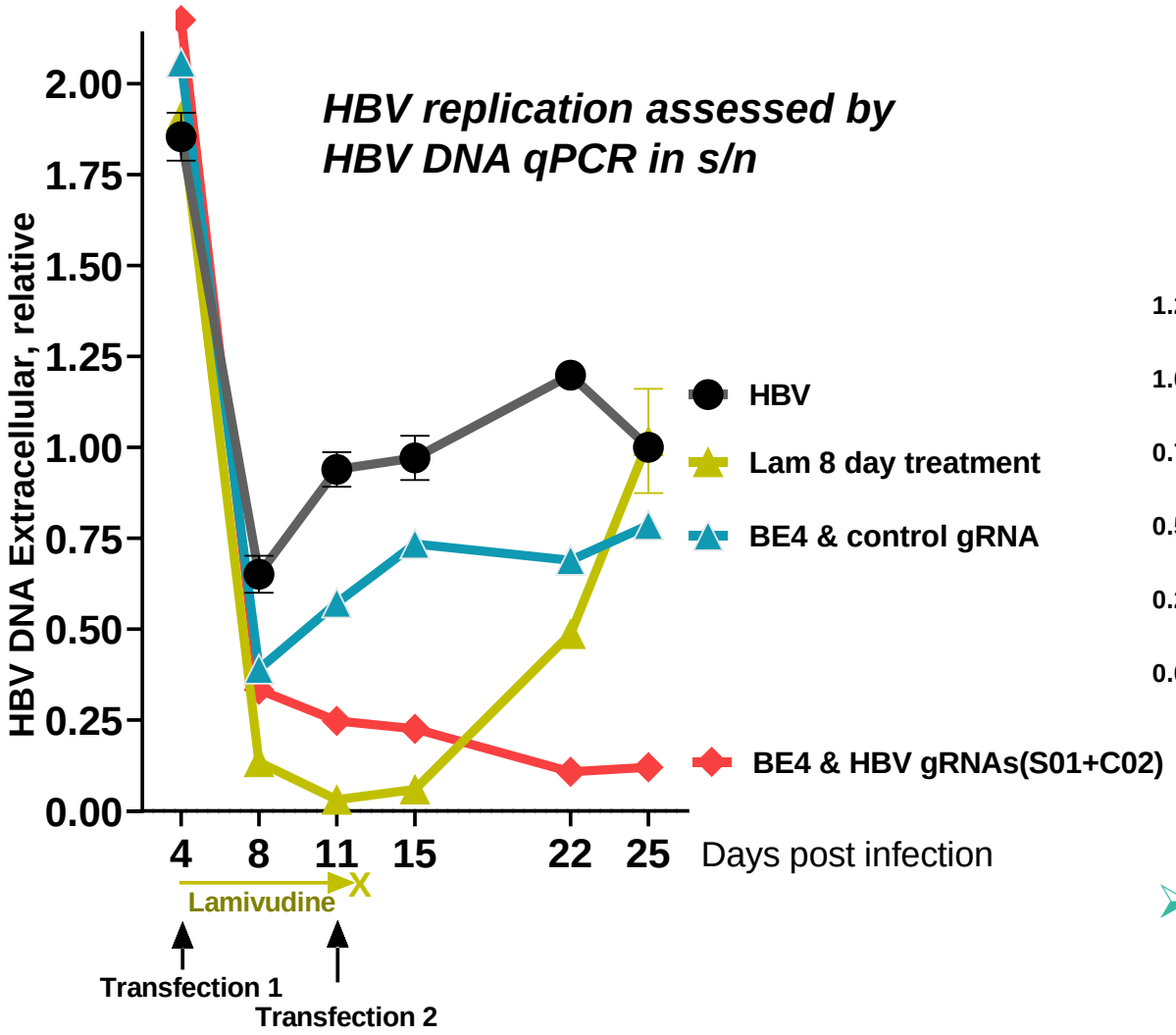
Intracellular
- HBV DNA total
- 3.5kb RNA
- cccDNA editing

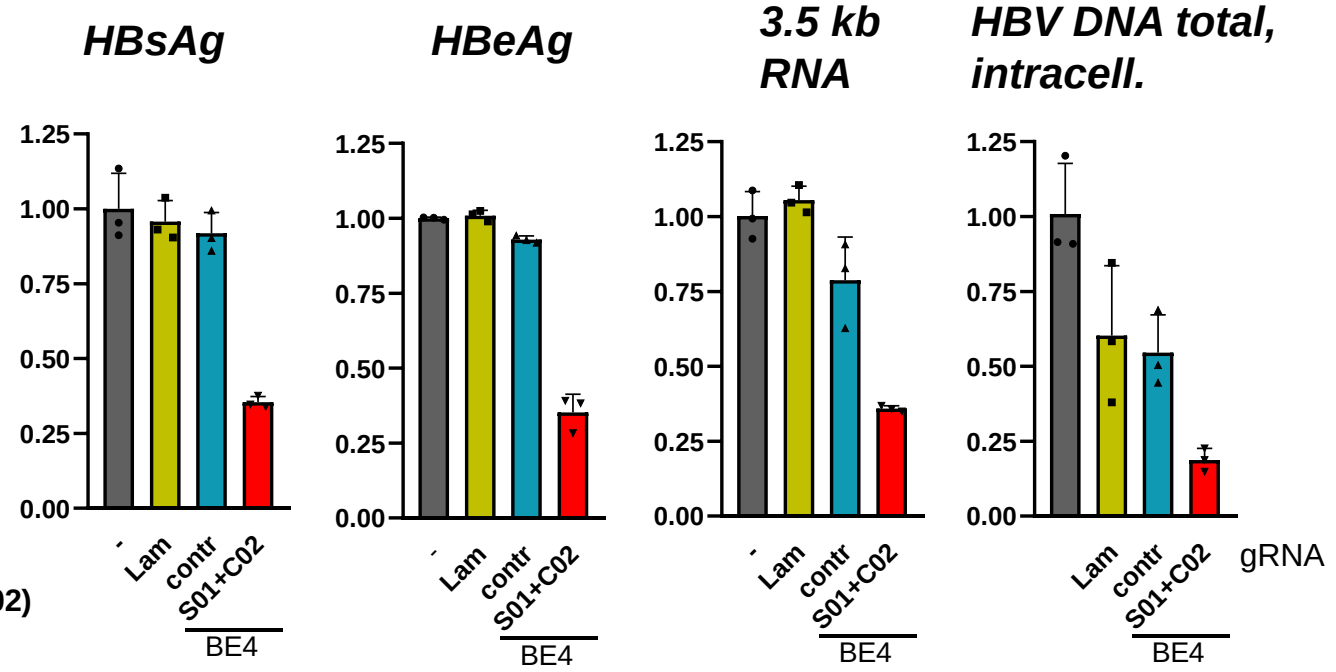
Base editing prevents HBV rebound in primary hepatocyte co-cultures



- HBV rebounds after discontinuation of lamivudine
- No HBV rebound for 2 weeks after the 2nd transfection with the base editing reagents

Base editing prevents HBV rebound in primary hepatocyte co-cultures

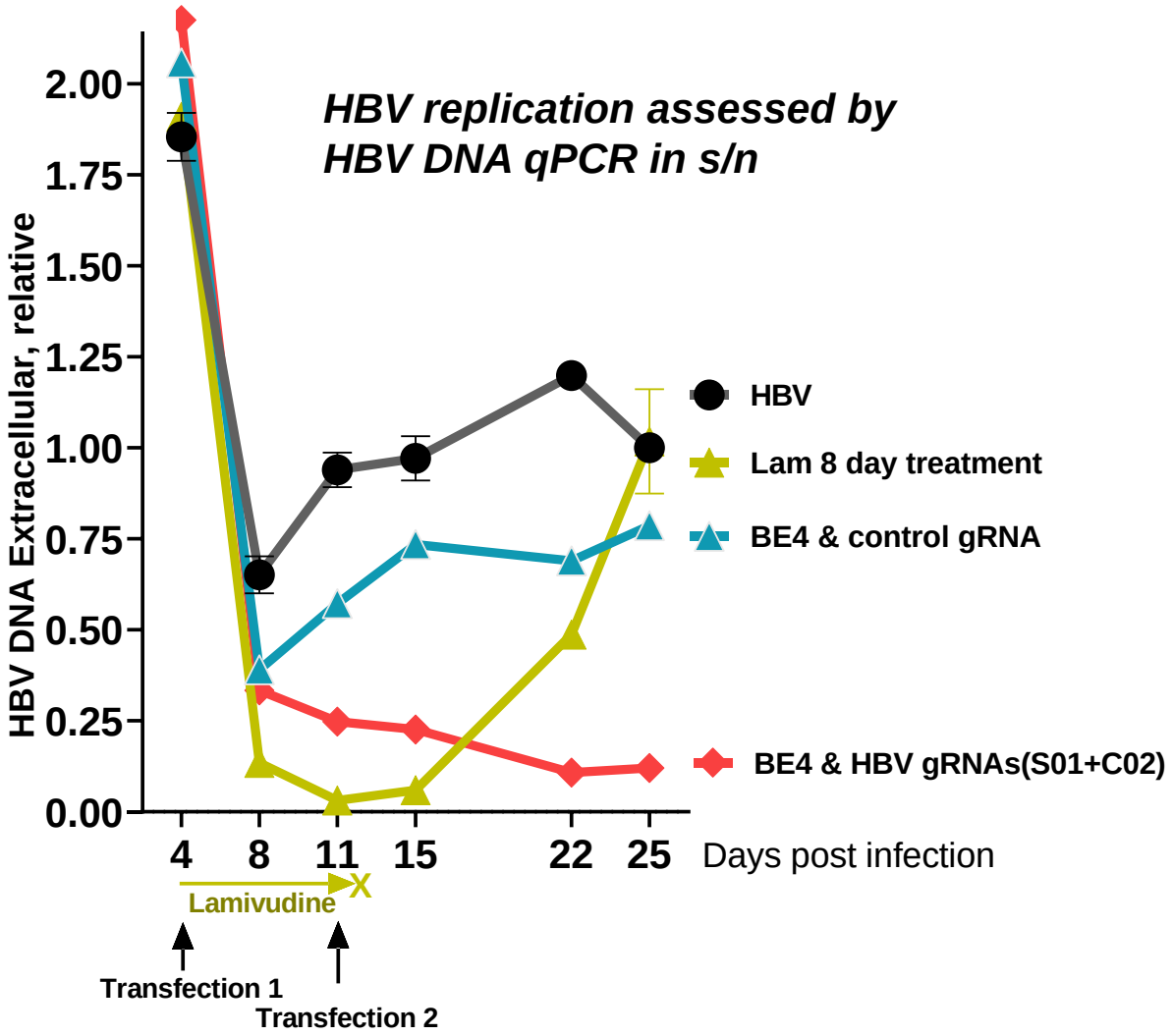


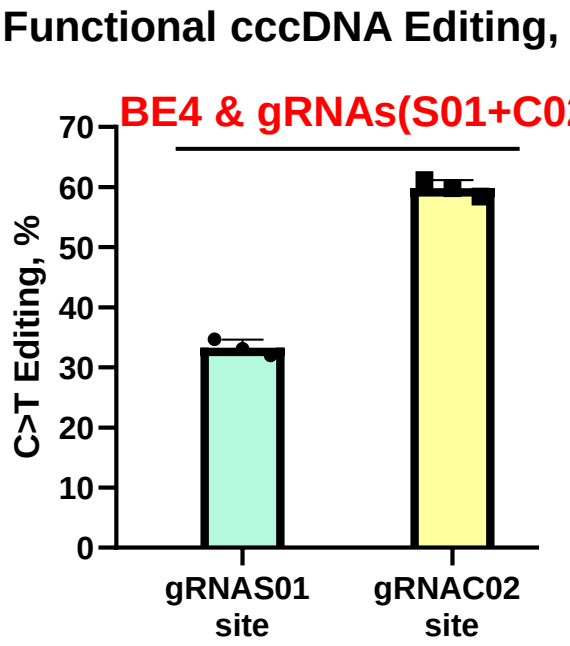


- Reduction of all HBV viral markers by 70-80% at the end of the experiment

- HBV rebounds after discontinuation of lamivudine
- No HBV rebound for 2 weeks after the 2nd transfection with the base editing reagents

Base editing prevents HBV rebound in primary hepatocyte co-cultures





- ~30% Editing S antigen and ~60% Editing PreCore gene sufficient to enable high antiviral efficacy and prevent rebound in PHH
- HBV rebounds after discontinuation of lamivudine
- No HBV rebound for 2 weeks after the 2nd transfection with base editing reagents

Conclusions

- **Cytosine base editing results in gRNA-specific reduction of HBV viral markers in relevant in vitro systems**
- **Multiplexing two gRNAs introducing Stop codons with CBE leads to a simultaneous reduction of HBsAg, HBeAg, HBV DNA, and 3.5kb RNA in HepG2-NTCP and primary hepatocytes**
- **Reduction in viral markers appear to be driven by base editing of cccDNA, but not a reduction in cccDNA levels**
- **Combinatorial treatment of the base editing reagents with standard antiviral lamivudine results in higher base editing efficiency**
- **Base editing prevents HBV rebound in infected primary hepatocytes**

➢ **Cytosine base editing introduces permanent mutations in cccDNA preventing HBV rebound in relevant in vitro models**

Thank You

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