OCTOBER 11-12, 2024 MILAN, ITALY
DeltaCure 3rd International Meeting
P48
Poster for you

Adenine Base Editing of Hepatitis B surface antigen potently inhibits HDV

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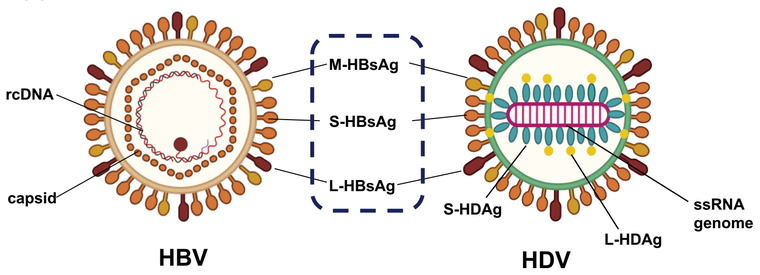
Introduction

Scientific Background

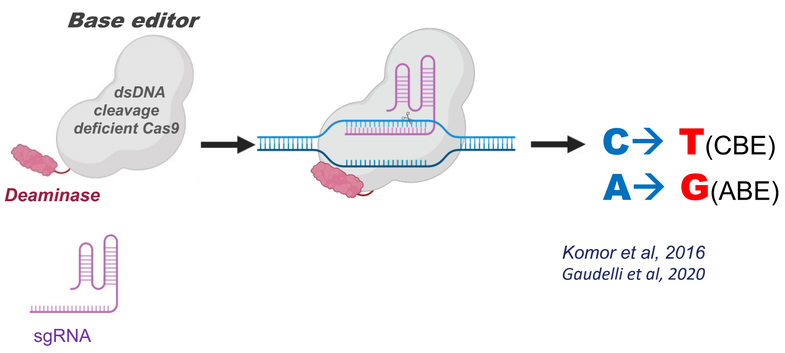
- Chronic Hepatits B virus (HBV) and Hepatitis D virus (HDV) infections represent serious health burden
- HBsAg is the common structural component of HBV and HDV
- HBsAg loss is considered as a desired endpoint of anti-HBV therapy, but is rarely achieved by current treatments
- Lowering HBsAg secretion is also being explored as an approach to inhibit HDV



Sausen et al, 2022

Base editing

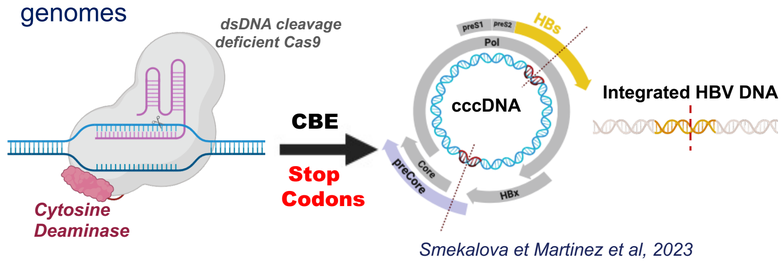
- Non dsDNA cleavage based technology
- Base editing leads to precise and permanent mutations in DNA



Komor et al, 2016
Gaudelli et al, 2020

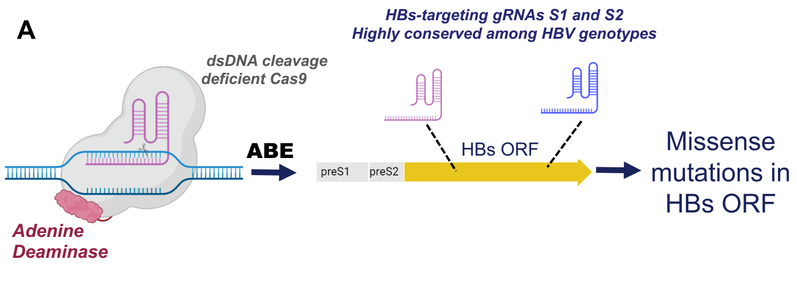
Base editing & HBV

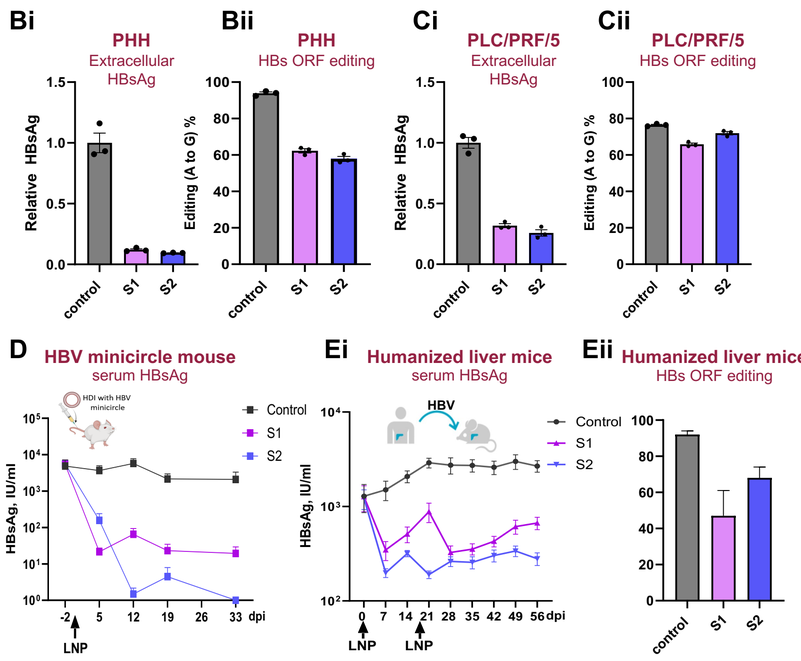
- Cytosine Base Editing (CBE) to functionally inactivate HBV genomes



Smekalova et Martinez et al, 2023

- Adenine Base Editing (ABE) for sustained HBsAg reduction



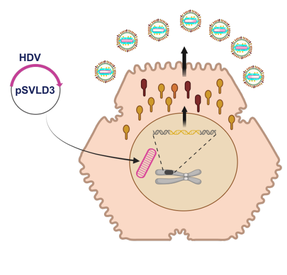


(A) Schematic of the ABE strategy to incorporate missense mutations in HBs ORF utilizing two gRNAs S1 and S2. (B-E) Effect of gRNA S1 and S2 on HBsAg levels in different *in vitro* and *in vivo* models. The editing efficiency on HBs ORF was determined by amplicon sequencing/NGS.

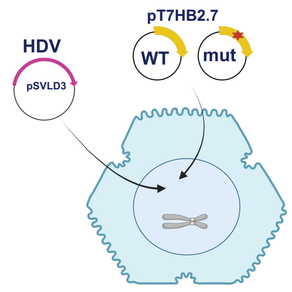
Aim of the study

To explore the potential of adenine base editing of HBs ORF in inhibiting HDV

Methods



Freitas et al, 2014



Base editing

- PLC/PRF/5 cells harboring replication incompetent integrated HBV DNA sequences capable of producing HBsAg. These cells support assembly and release of infectious HDV particles.
- HDV replication initiation was performed by transfecting PLC/PRF/5 cells with an HDV-encoding plasmid, pSVLD3.
- ABE was performed by transfecting PLC/PRF/5 cells with an ABE encoding mRNA and gRNA S1 or S2.
- **Base Editing followed by HDV replication (Results Panel I)**
- **HDV replication followed by Base Editing (Results Panel II)**

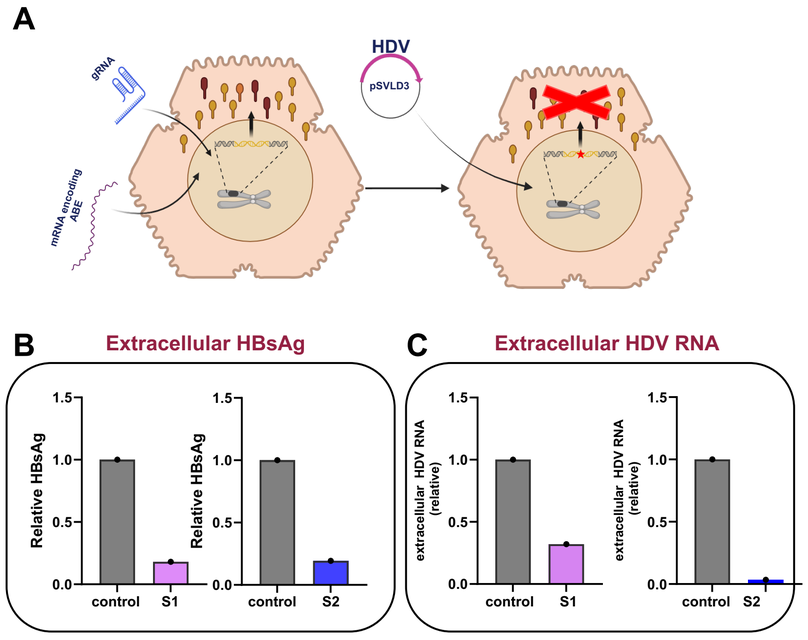
Transfection

- Huh7 cells were used to perform plasmid transfection.
- gRNA S2 corresponding mutations were incorporated in HBs-encoding plasmid, pT7HB2.7.
- Huh7 cells were co-transfected with pSVLD3 and either WT or mutant pT7HB2.7.
- **Cotransfection: pSVLD3 and HBs-encoding WT/mutant (Results Panel III)**

- Readout : **Assessement of HBsAg and extracellular HDV RNA**

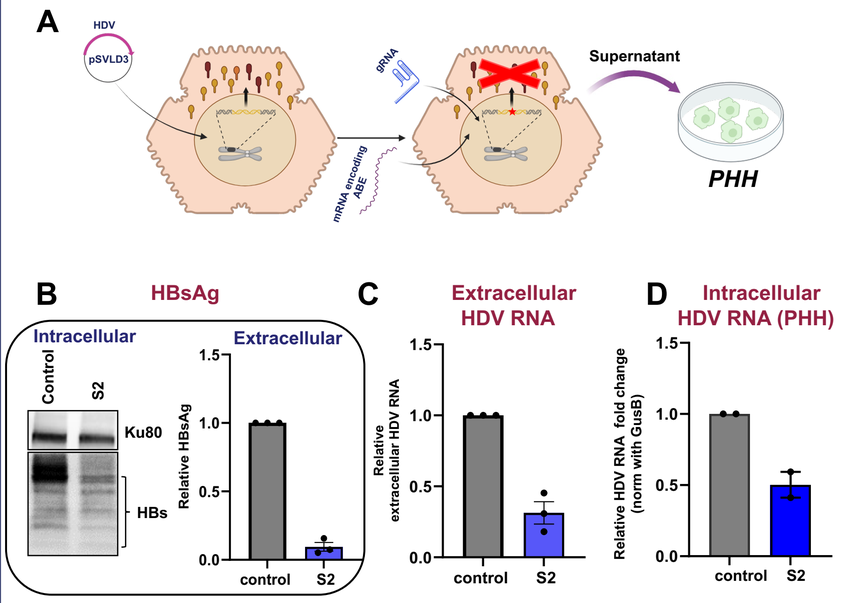
Results

I - Base editing of HBs ORF inhibits HDV release



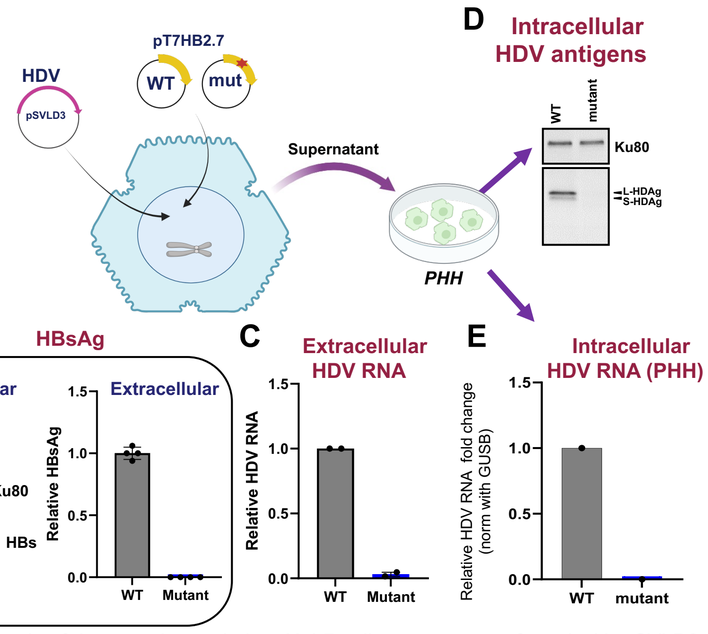
(A) Schematic of the experimental plan. PLC/PRF/5 cells were first transfected with ABE-encoding mRNA and gRNA (S1/S2) to perform base editing, followed by transfection with pSVLD3 5 days later. (B) At 15th day post-pSVLD3 transfection, levels of extracellular HBsAg were determined by ELISA and (C) extracellular HDV RNA was quantified by one-step qRT-PCR. Control represents non HBs-targeting gRNA.

II - Base editing of HBs ORF inhibits HDV release & spread



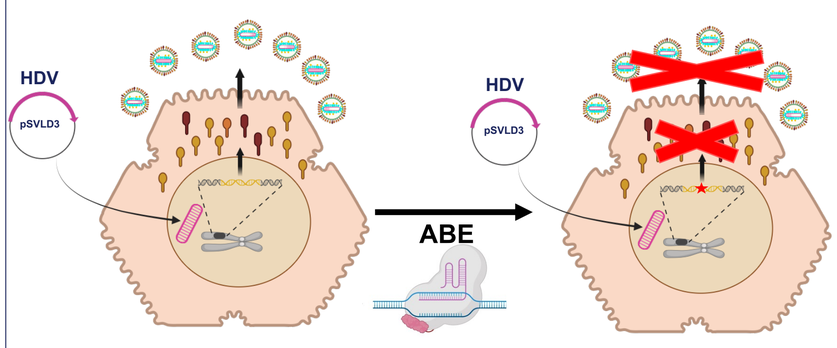
(A) Schematic of the experimental plan. PLC/PRF/5 cells were first transfected with pSVLD3 followed by ABE 5 days later (B) At 11 th day post pSVLD3 transfection, levels of intracellular and extracellular HBsAg were determined by Western blot and ELISA, respectively. Ku80 served as internal control of loading. (C) Extracellular HDV RNA was quantified by one-step qRT-PCR. (D) PHH were infected with equal volume of HDV released from control gRNA or gRNA S2-edited PLC/PRF/5 cells. At 6th day post infection, levels of intracellular HDV RNA were quantified by one-step qRT-PCR.

III - HBs ORF mutation abrogates HDV release and PHH infectivity



(A) Schematic of the experimental plan. Huh7 cells were co-transfected with pSVLD3 and wildtype (WT) or gRNA S2 corresponding mutant. (B) At 11th day post transfection, intracellular and extracellular HBsAg levels were determined by Western blot and ELISA, respectively. (C) Extracellular HDV RNA was quantified by one-step qRT-PCR. (D-E) PHH were infected with equal volume of HDV released from Wildtype (WT) or mutant conditions. At 6th day post infection, intracellular HDAg levels and intracellular HDV RNA levels were determined by Western blot and one-step qRT-PCR, respectively.

Conclusions



Adenine base editing is a promising approach to mutate HBs ORF to :

- **inhibit HBsAg expression from cccDNA and integrated HBV DNA**
- **potently reduce HDV release and spread**

Acknowledgements

Inserm Institut national de la santé et de la recherche médicale
Beam THERAPEUTICS
LYON HEPATOLOGY INSTITUTE COMPREHENSIVE LIVER CENTER
cnrs
CRCL CENTRE DE RECHERCHE EN CANCÉROLOGIE DE LYON
HCL HOSPICES CIVILS DE LYON
CENTRE DE LUTTE CONTRE LE CANCER LEON BERARD
Lyon 1

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Disclosure

This work was funded by Beam Therapeutics, a public company developing base editing technology for human therapeutics. E. Smekalova, S. Dejene, M. Packer, C-Y Chen, G. Ciaramella, are/were employees/shareholders of Beam Therapeutics.