Editing of HBV DNA *in vitro* using a Gene Editor Approach

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Introduction

- It is estimated that 254 million people worldwide are living with chronic hepatitis B virus (HBV) infection[1]. Over 1 million people died from infection with HBV in 2022[1].
- Current therapies **do not cure** infection as they do not directly or efficiently target the HBV self-replenishing reservoir, the covalently closed circular DNA minichromosome (cccDNA)[2] or HBV integrated DNA.
- Novel therapeutics are needed to target both cccDNA and integrated DNA. The DNA editing tool CRISPR/Cas9 cytosine base editors (BEs) (Figure 1), which introduces specific DNA base edits[3], have potential to be a novel therapeutic.

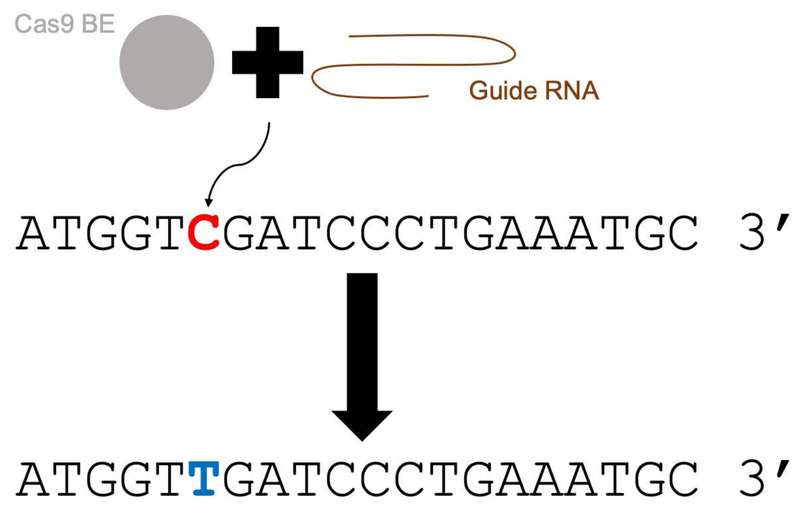

Figure 1:Cas9 BE guided by a guide RNA to edit target DNA. Cas9 BE introduces a specific cytosine to thymine DNA base edit at the target DNA site.

- Previous studies have shown success with reducing HBV replication and protein expression using the BE system to target HBV DNA[4-6].

AIM

To analyse guides targeting all HBV open reading frames and to test the efficacy of transient expression of BEs and guides in reducing HBV replication and protein expression *in vitro* and *in vivo*.

Methods

- All guides introduce premature stop codons across the HBV open reading frames (Figure 2). Impact of guides were compared to a non-targeting control (**NTC**) guide.

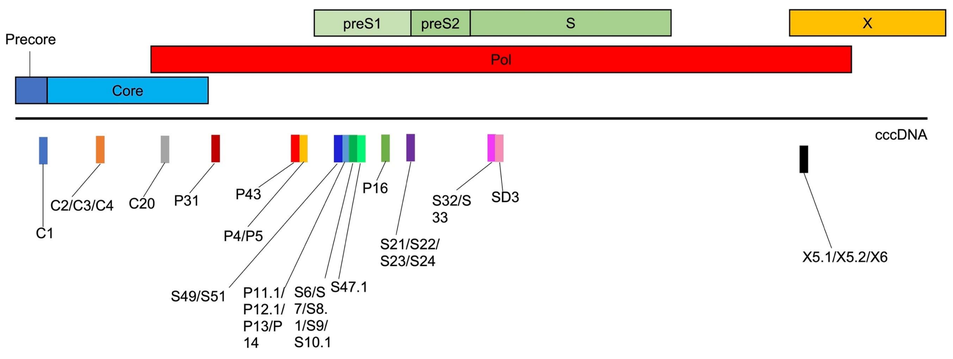

Figure 2: Linear map of the HBV DNA showing the 32 guides designed targeting all 4 HBV open reading frames.

- HBV genotype D3 targeting guides and Cas9 BEs were delivered into cells with HBV.
- HBV secreted protein HBV e antigen (HBeAg) was measured via quantitative serology 2 and 5 days post-delivery.
- Intracellular HBV DNA was investigated 5 days post-delivery.

Results

Core Open Reading Frame Guides Screen

- Guide C1 achieved significant knockdown of secreted HBeAg 2 and 5 days post-delivery (Figure 3).

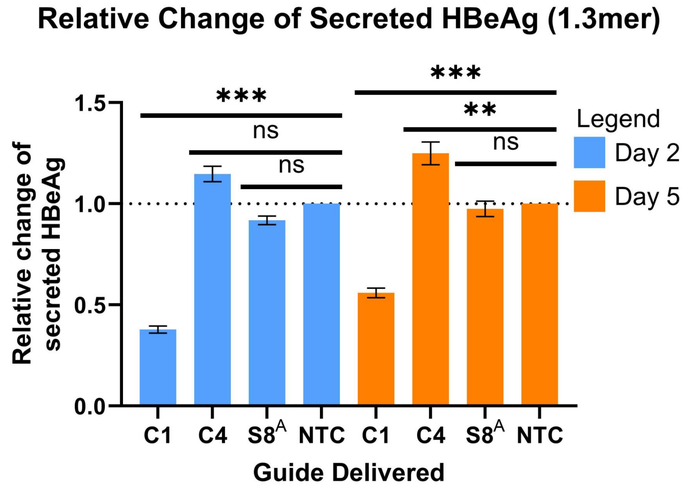

Figure 3: Relative change of secreted HBeAg at different time points post-delivery using core open reading frame targeting guides. [a]Previously published guide[4]. Error bars indicate the standard error of the mean of the 3 independent experiments. ***=p<0.001, **=p<0.01, ns=not significant.

Different Base Editors

- Guide C1 achieved significant decrease of secreted HBeAg compared to the NTC guide when using different BEs (Figure 4).

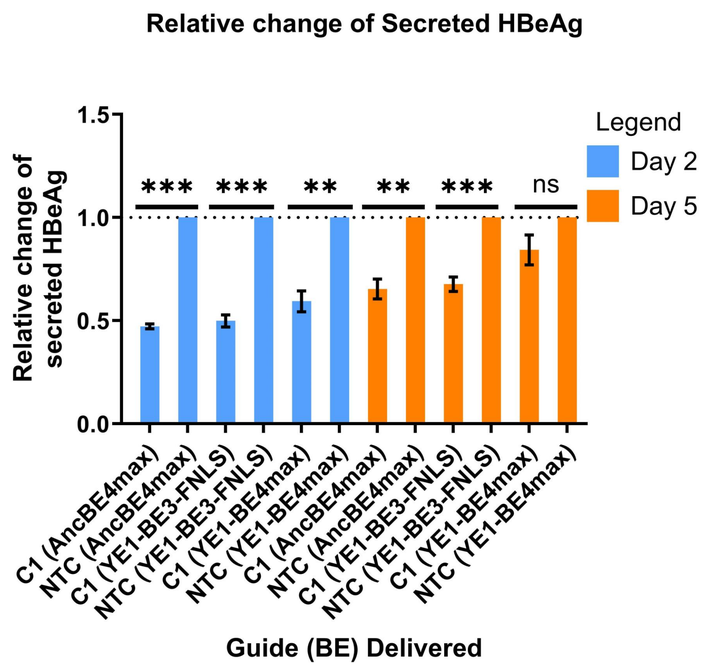

Figure 4: Relative change of secreted HBeAg at different time points post-delivery using different BEs. All results were compared to the NTC guide with the corresponding BE. Error bars indicate the standard error of the mean. **=p<0.01, ***=p<0.001 ns=not significant.

- Guide C1 also negatively impacted HBV replication when using different BEs (Figure 5).

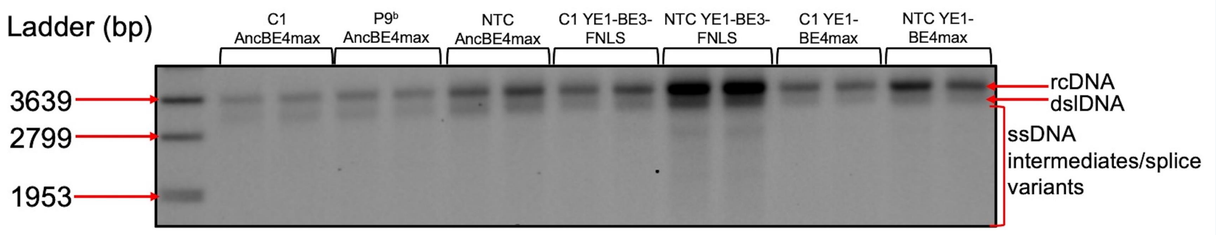

Figure 5: Southern blot of intracellular core associated HBV DNA 5 days post-delivery with different BEs and guides. [b]Previously published guide[4]. rcDNA=HBV relaxed circular DNA, dslDNA=HBV double stranded linear DNA and ssDNA=HBV single stranded DNA, bp=base pair.

Conclusion

- Guide C1 achieved significant reduction of secreted HBeAg as well as intracellular HBV DNA compared to the NTC guide.
- Future experiments include delivering the system as RNA in lipid nanoparticles and testing guides in an infection system and in murine models. Additionally, sequencing of the target editing site as well off-target editing will be investigated.
- Overall, these preliminary studies are an important first step towards developing this approach as a novel HBV therapeutic.

References

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