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

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Introduction: Current hepatitis B virus (HBV) treatments do not target the HBV covalently closed circular DNA (cccDNA) minichromosome reservoir, nor do they target HBV integrated DNA. There is a desperate need to develop novel therapeutics that target both cccDNA and integrated DNA; to improve HBV cure rates.

CRISPR/Cas9 base editors (BEs) are a promising approach as they utilise the CRISPR/Cas9 guiding system to introduce specific C:G to T:A edits into target DNA. Edits can be predicted and there is less chance of genome instability when targeting integrated HBV DNA. The aim of this project is to design new single guide RNA (sgRNAs) targeting all HBV open reading frames (ORFs) and to test the efficacy of transient expression of BEs and sgRNAs in reducing HBV replication and protein expression *in vitro* and *in vivo*.

Methods: All sgRNAs introduced premature stop codons to reduce HBV protein expression. The efficacy of sgRNAs were tested via plasmid transfection with HBV DNA and Cas9 BEs in HepG2 cells. HBV replication markers and proteins were measured and compared to a non-targeting control sgRNA.

Results: sgRNAs with different BEs targeting HBV ORFs achieved knockdown of HBV proteins and intracellular core-associated HBV DNA with varying efficacy. Furthur analysis of the impact on HBV RNA, DNA and protein expression *in vitro* will be performed. A combination of sgRNAs will also be tested. Different models will be used including HBV stable cell lines, an HBV infection system, and a murine model. BEs will also be delivered as mRNA packaged in lipid nanoparticles *in vitro* and *in vivo*, with and without current therapeutics.

Conclusion: These studies will determine the utility of Cas9 BEs for introducing specific base changes into the HBV cccDNA and integrated DNA, as an important first step towards developing this approach as a novel HBV therapeutic.

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