Targeting HBV RNAs using CRISPR-Cas13b to reduce hepatitis B virus replication and antigen expression *in vitro* and *in vivo*

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Background: New treatments targeting multiple stages of the hepatitis B virus (HBV) replication cycle are urgently required to improve the rates of HBV functional cure. CRISPR-Cas13b, naturally used by bacteria, targets RNA and can be re-purposed to target mammalian and viral RNAs. We have previously designed CRISPR RNAs (crRNAs) complementary to the HBV RNAs and have shown that CRISPR-Cas13b can target the HBV RNAs to reduce HBV replication and protein expression in cell culture. Here, we expand on these studies to test Cas13b in HBV-infected primary human hepatocytes and in a mouse model with persistent HBV replication.

Methods: Primary human hepatocytes were infected with HBV and then treated with Cas13b mRNA and crRNA five days post-infection. Secreted HBeAg and HBsAg were measured as markers of viral replication. Mice were hydrodynamically injected (HDI) with HBV and then intravenously injected (IV) with lipid nanoparticle (LNP)-encapsulated Cas13b mRNA and crRNA eight weeks post-HDI. Sera HBeAg and HBsAg were measured at several time points.

Results: Cas13b strongly reduced secreted HBeAg and HBsAg from primary human hepatocytes compared to the non-targeting crRNA control. Mice studies have commenced with results yet to be analysed.

Conclusion: These studies expand on previous findings that the HBV RNAs can be successfully targeted and degraded using CRISPR-Cas13b to significantly reduce HBV replication and antigen expression. This further demonstrates the potential of using CRISPR-Cas13b as a novel treatment option for chronic HBV infection.

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