# Suppression of hepatitis B virus replication and surface antigen using CRISPR-Cas13b – pre-clinical investigations of a new therapeutic approach

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**Background:** Low hepatitis B surface antigen (HBsAg) levels are associated with better clinical outcomes. As current treatments do not directly target HBsAg, new treatments targeting multiple stages of the hepatitis B virus (HBV) replication cycle, including HBsAg, are urgently required to improve rates of HBV functional cure.

CRISPR-Cas13b endonuclease, used by bacteria to target and suppress bacteriophage RNAs, has been repurposed to target RNA in mammalian cells by designing highly specific 30 nucleotide guide RNAs (gRNAs) complementary to the target RNAs of interest. Here, in a world first study, we targeted the HBV RNAs using CRISPR-Cas13b to reduce HBV replication and HBsAg *in vitro* and *in vivo*.

**Methods:** gRNAs were designed to target the HBV RNAs. HepG2 cells were transfected with plasmids expressing wildtype HBV of multiple genotypes, Cas13b and gRNA. A HBV stable cell line and HBV infection model were transfected with Cas13b and gRNA plasmids. The impact on HBV replication and HBsAg was determined. Wildtype HBV, Cas13b and gRNA plasmids were simultaneously hydrodynamically injected into CBA mice and sera HBsAg was measured. Cas13b mRNA and gRNA were delivered by lipid nanoparticles (LNPs) in a HBsAg-expressing stable cell line and secreted HBsAg was measured.

**Results:** Cas13b strongly suppressed HBV replication and reduced secreted HBsAg by 96% ($p<0.0001$) in HepG2 cells. The effect was pan-genotypic. HBV replication and secreted HBsAg was reduced in an HBV stable cell line and HBV infection model. Sera HBsAg was reduced by ~50% ($p<0.0001$) *in vivo* when Cas13b was co-injected with wildtype HBV. Finally, LNP-encapsulated Cas13b mRNA reduced secreted HBsAg by 87% ($p=0.0168$) in a HBsAg-expressing stable cell line.

**Conclusion:** The HBV RNAs were successfully targeted and degraded using CRISPR-Cas13b to significantly reduce HBV replication and HBsAg in cell culture and *in vivo*, demonstrating its potential as a novel treatment option for chronic HBV infection.

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