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

A joint venture between The University of Melbourne and The Royal Melbourne Hospital

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Introduction

- New finite therapeutic approaches are required that target multiple aspects of the HBV replication cycle[1].
- HBV is a DNA virus that replicates through an RNA intermediate. The HBV RNAs are potential targets for new therapies.
- Bacterial CRISPR-Cas13b targets RNA and can be used to target viral RNAs[2,3] to reduce viral replication and protein expression.
- Cas13b has high specificity due to the 30-nucleotide CRISPR RNA (crRNA)[4] that is complementary to the target RNA.
- We have shown that Cas13b can suppress HBV replication and protein expression in pre-clinical models when using plasmids to deliver Cas13b[5].
- We are now testing Cas13b in additional pre-clinical models using mRNA to deliver Cas13b, to further develop Cas13b as a potential therapy for chronic HBV infection.

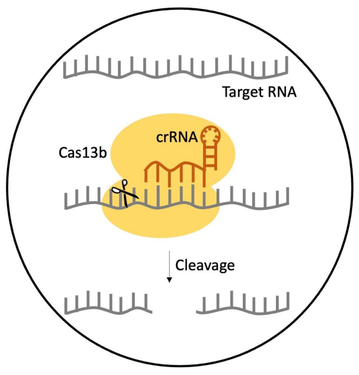

Fig. 1. Cas13b mechanism. Cas13b binds to the crRNA which directs Cas13b to the target RNA. Cas13b then cleaves the target RNA.

Aim

To target the HBV RNAs using CRISPR-Cas13b to reduce HBV replication and protein expression *in vitro* and *in vivo*

Methods

- Cas13b crRNAs were designed to target either the 5′ or 3′ regions of the HBV RNAs (Fig. 2) (Patent application no. 2023901165).

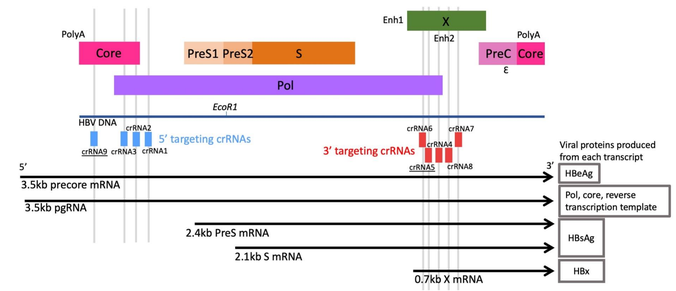

Fig. 2. Map of HBV-targeting Cas13b crRNAs designed in this study.

- HBV, Cas13b and crRNA plasmids were delivered to cells to determine the best crRNAs (Fig. 3).

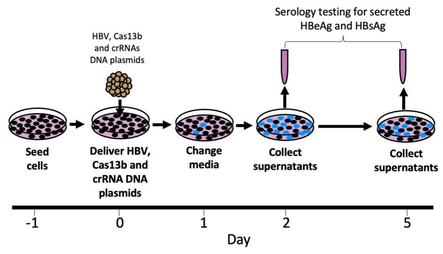

Fig. 3. Method for delivery of Cas13b plasmid into cells.

- Cas13b mRNA and crRNA were delivered in HBV-infected primary human hepatocytes and secreted HBeAg and HBsAg were measured (Fig. 4).

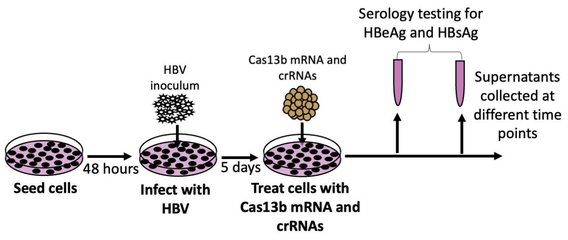

Fig. 4. Method for delivery of Cas13b mRNA and crRNA in HBV-infected primary human hepatocytes.

- Mice were injected with HBV, and then eight weeks later, treated with lipid nanoparticles containing Cas13b mRNA. Serum HBsAg and HBV DNA was monitored over time after treatment (Fig. 5).

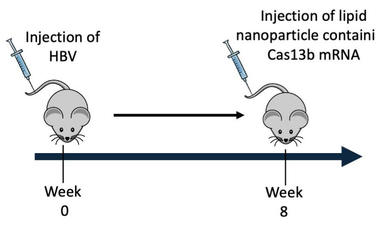

Fig. 5. Method for delivery of Cas13b mRNA and crRNA in HBV-infected mice.

References

1. Revill, P. A. *et al.* Lancet Gastroenterology Hepatology (2019)
2. Freije, C. A. *et al.* Molecular Cell (2019)
3. Fareh, M. *et al.* Nature Comms (2021)
4. Abudayyeh, O. O. *et al.* Nature (2017)
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Results

Cas13b plasmid strongly reduced HBV replication and protein expression

- Secreted HBeAg and HBsAg were reduced by 95% and 96% respectively (Fig. 6). Both crRNAs strongly reduced HBV DNA (Fig. 7), HBV RNAs and HBV protein expression (not shown).

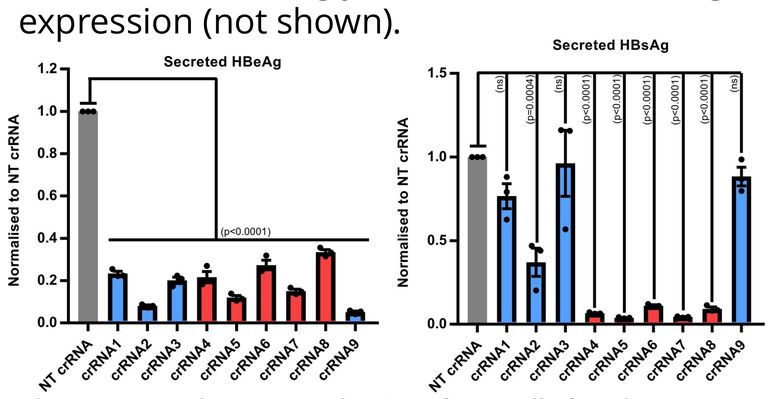

Fig. 6. Secreted HBeAg and HBsAg from cells five days post-delivery of HBV, Cas13b and crRNA plasmids. N=3. NT crRNA: Non-targeting crRNA control.

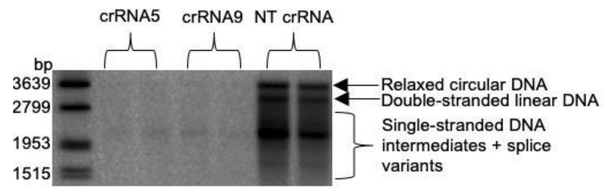

Fig. 7. Intracellular HBV DNA from cells five days post-delivery of HBV, Cas13b and crRNA plasmids. NT crRNA: Non-targeting crRNA control.

Cas13b mRNA reduced HBV proteins in HBV-infected primary human hepatocytes

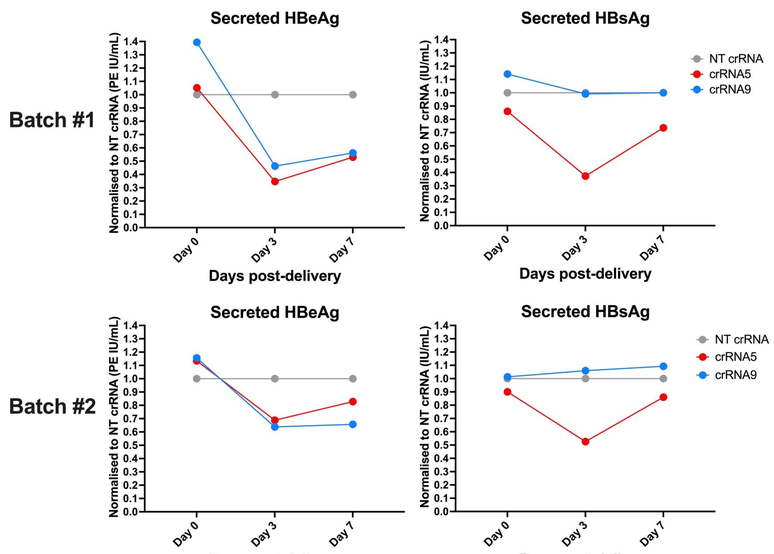

Fig. 8. Secreted HBeAg and HBsAg from HBV-infected primary human hepatocytes after treatment with Cas13b mRNA. Two different batches of primary human hepatocytes were used. NT crRNA: Non-targeting crRNA control.

Cas13b-nanoluciferase mRNA was expressed in the liver of mice after lipid nanoparticle delivery

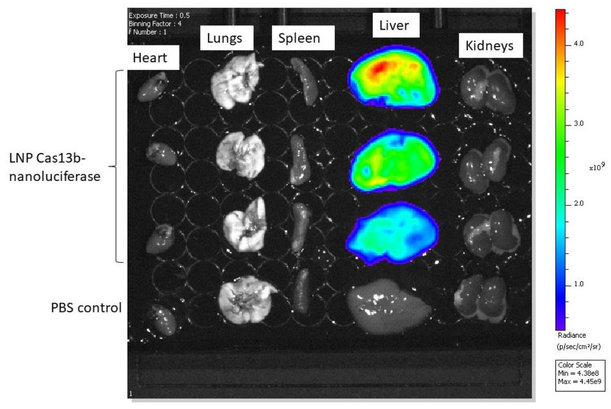

Fig. 9. Nanoluciferase expression in different mice organs 24 hours post-intravenous injection of lipid nanoparticles containing Cas13b-nanoluciferase mRNA.

Cas13b reduced HBV replication and proteins in one out of three mice in a pilot study

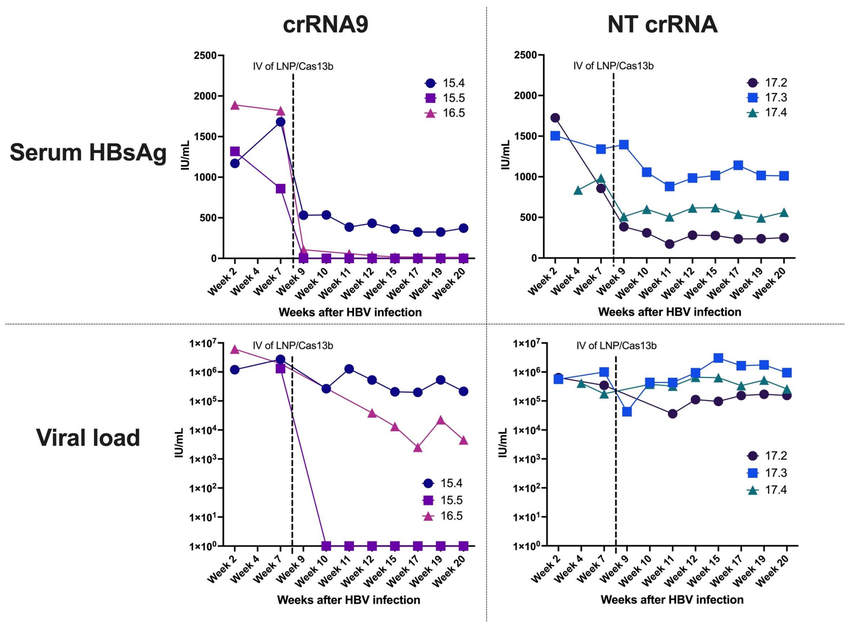

Fig. 10. Serum HBsAg and HBV DNA viral load over time in mice with persistent HBV infection that were treated with lipid nanoparticles containing Cas13b mRNA. NT crRNA: Non-targeting crRNA control.

Conclusions

- Cas13b mRNA reduced secreted HBV proteins in HBV-infected primary human hepatocytes.
- Cas13b mRNA was delivered to the liver of mice and reduced HBV replication and protein expression in one out of three mice with HBV.
- CRISPR-Cas13b may be a potential novel treatment option for chronic hepatitis B.