EPIGENETIC EDITORS TARGETING CHRONIC HEPATITIS B ACHIEVE HBV SURFACE ANTIGEN LOSS WITH A SINGLE COURSE OF TREATMENT IN MULTIPLE HBV MOUSE MODELS

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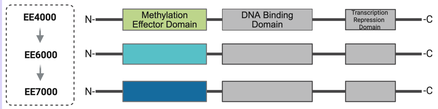
INTRODUCTION

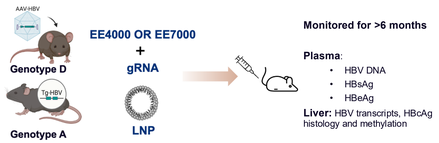
- Current standard-of-care therapies, including nucleos(t)ide analogs, effectively suppress HBV replication but rarely achieve functional cure, defined as sustained loss of HBsAg and undetectable HBV DNA for at least 24 weeks off therapy.
- Persistent viral expression from cccDNA and integrated HBV DNA (intDNA) remains a major hurdle.
- CRMA-1001is an optimized epigenetic silencer specifically designed to methylate both cccDNA and intDNA, resulting in durable silencing HBV expression at the transcriptional level.
- EE7000, the optimized effector component of CRMA-1001, and its precursor EE4000 were thoroughly characterized in multiple HBV mouse models, demonstrating reductions in HBsAg and HBV DNA following single or multi-dose treatment courses.

METHODS

- Construct optimization (Poster #1118).



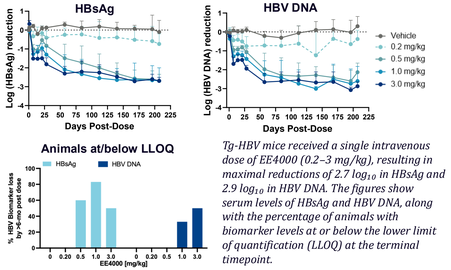
- Two HBV mouse models were used: AAV-HBV (AAV8-HBV1.2, ~4.5 copies/cell) and transgenic HBV (HBV1.28, ~2 copies/cell).



- EE7000, the lead candidate with enhanced potency and optimized pharmacological properties, is the epigenetic effector component used in CRMA-1001.

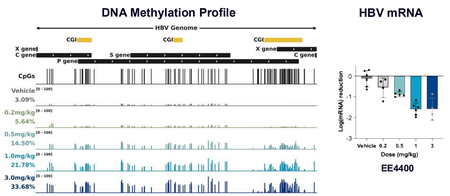
RESULTS

EE4000 induces dose-dependent HBV silencing to LLOQ >6 months in Tg-HBV mouse model



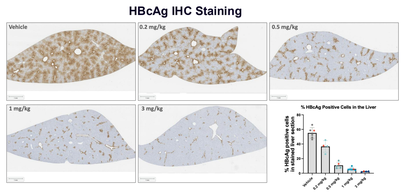
Tg-HBV mice received a single intravenous dose of EE4000 (0.2–3 mg/kg), resulting in maximal reductions of 2.7 log_{10} in HBsAg and 2.9 log_{10} in HBV DNA. The figures show serum levels of HBsAg and HBV DNA, along with the percentage of animals with biomarker levels at or below the lower limit of quantification (LLOQ) at the terminal timepoint.

EE4000 induces HBV CpG methylation linked to reduced hepatic HBV mRNA



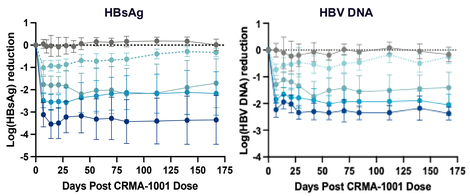
Mean methylation (%) per dose group is shown below each label. HBV genome features above: yellow bars = CpG islands, black arrows = HBV genes, vertical lines = CpG sites. Methylation increases dose-dependently and correlates with reduced hepatic HBV mRNA (log fold change vs. baseline or vehicle). Error bars = group.

Dose-dependent reduction of HBcAg⁺ cells in Tg-HBV livers following EE4000 treatment



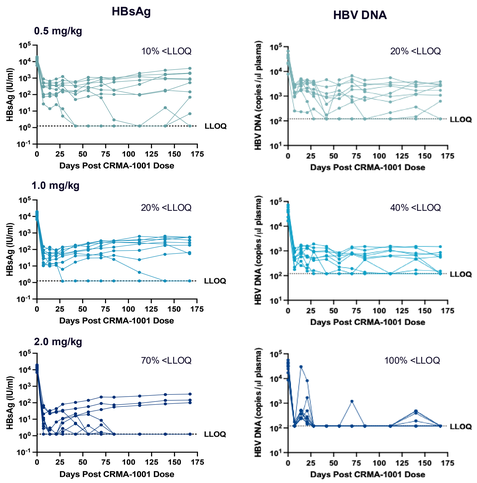
IHC staining in Tg-HBV livers at Day 207 post-dose (brown = HBcAg⁺ cells). Graph (bottom right) quantifies HBcAg⁺ cells: bars = mean ± SD, red dots = animals shown.

Single-dose CRMA-1001 achieves durable HBV suppression in AAV-HBV mouse model



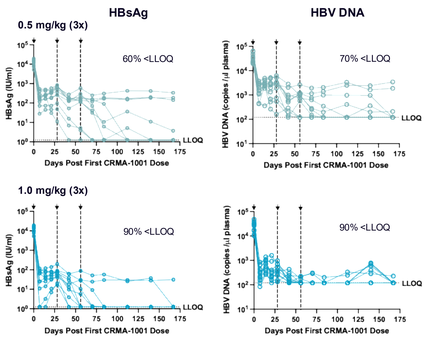
A single administration of CRMA-1001 led to a dose-dependent and sustained reduction in circulating HBsAg (up to ~3.4 log_{10}) and HBV DNA (up to ~2.3 log_{10}) for at least 6 months.

Single-dose CRMA-1001 lowers HBsAg and HBV DNA to LLOQ in most animals



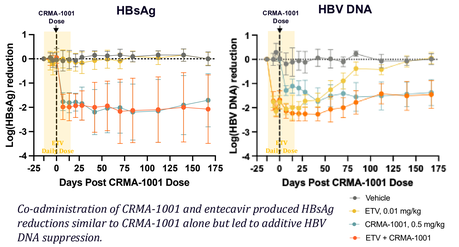
At ≥0.5 mg/kg, a single dose leads to undetectable HBsAg in up to 70% and HBV DNA in up to 90% in the AAV-HBV mice (n=10/group).

Three-dose CRMA-1001 regimen improves rate of HBsAg and HBV DNA loss in AAV-HBV mice



Three monthly doses of CRMA-1001 at 0.5 mg/kg reduced HBsAg to undetectable levels in up to 60% of animals and lowered HBV DNA in up to 70%. At the higher dose of 1.0 mg/kg, both HBsAg and HBV DNA were eliminated in up to 90% of animals (n=10/group).

Co-administration of CRMA-1001 and entecavir enhances and sustains HBV DNA suppression



Co-administration of CRMA-1001 and entecavir produced HBsAg reductions similar to CRMA-1001 alone but led to additive HBV DNA suppression.

REFERENCE

1. Wong, G. L. H., *et al.* How to achieve functional cure of HBV: Stopping NUCs, adding interferon or new drug development? *J Hepatol* **76**, 1249–1262 (2022).
2. Guidotti, J. G. *et al.* High-level hepatitis B virus replication in transgenic mice. *J Vir.* **69,** 6158–6169 (1995).
3. Ko, C. *et al.* Intramolecular recombination enables the formation of hepatitis B virus (HBV) cccDNA in mice after HBV genome transfer using recombinant AAV vectors. Antiviral Res. **194**, 105140 (2021).

CONCLUSIONS

- **Epigenetic silencing without cutting or nicking**: CRMA-1001 durably silences cccDNA and HBV intDNA via DNA methylation, without introducing DNA breaks.
- **Finite dosing, lasting efficacy:** A single dose or short-course treatment achieves multi-log HBsAg and HBV DNA reductions sustained for ≥6 months.
- **Broad and robust antiviral activity:** Demonstrated efficacy in Tg-HBV and AAV-HBV preclinical mouse models.
- **Synergistic with standard therapies:** Additive antiviral effects with entecavir support incorporation into existing treatment regimens.

ACKNOWLEDGEMENTS

- Thank you to the nChroma Bio team, collaborators and partners.

ADDITIONAL INFORMATION

- For additional information on this abstract, please contact: info@nchromabio.com.
- For additional details on **CRMA-1001**, please see: *Preclinical pharmacology and safety of CRMA-1001, a novel epigenetic editor for chronic hepatitis B that demonstrates HBV surface antigen loss in animal models via precise HBV DNA methylation* (**Poster #1118**).
- For additional details on **CRMA-1001 Specificity Assessment**, please see: *Establishment of assay limits for differential gene expression and DNA methylation to characterize specificity of epigenetic editing* **(Poster #4304).**