Complete Blockage of HBV Virus Replication and Inhibition of cccDNA Formation by Core Protein Allosteric Modifiers

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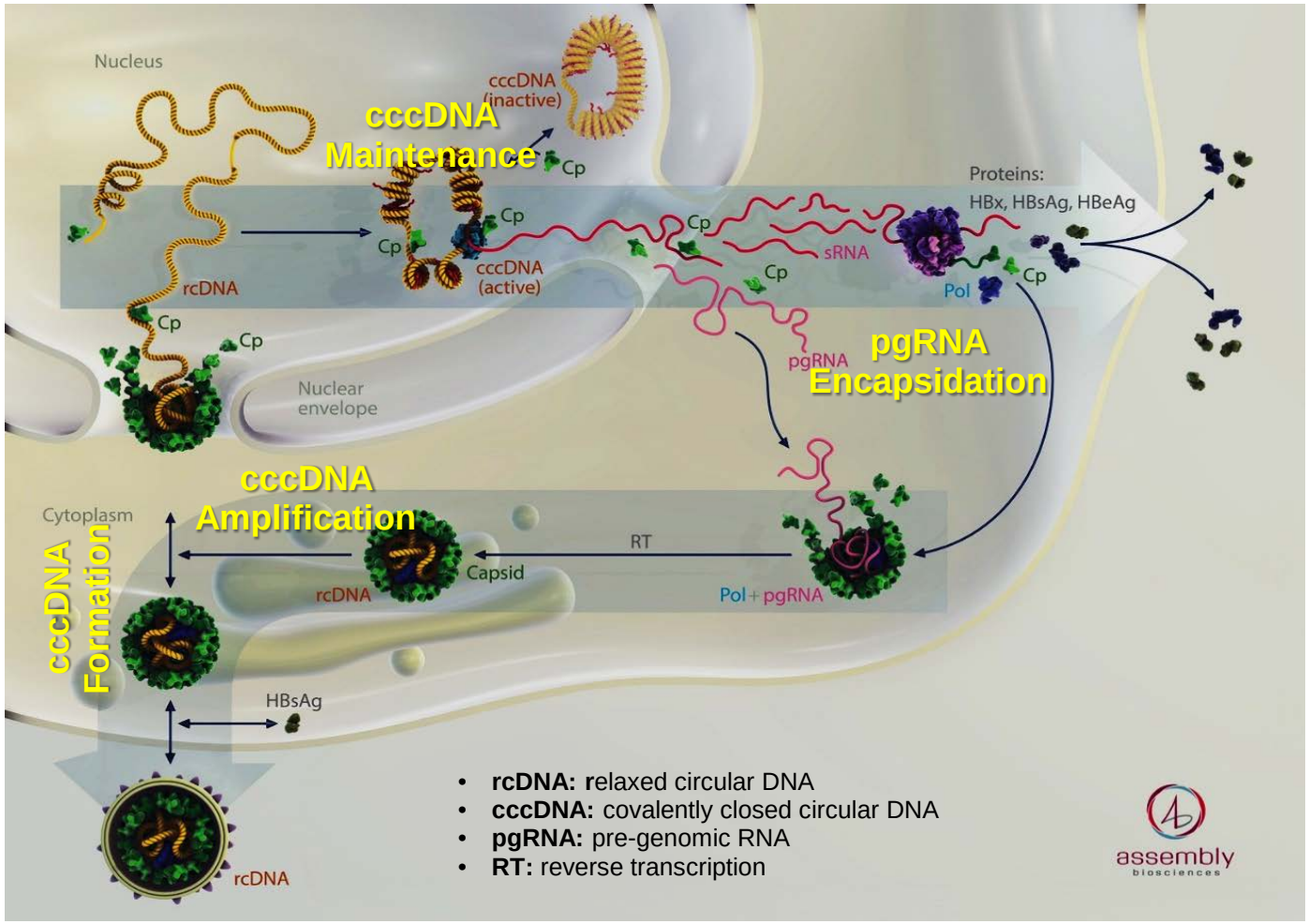
Assembly Biosciences

assembly biosciences

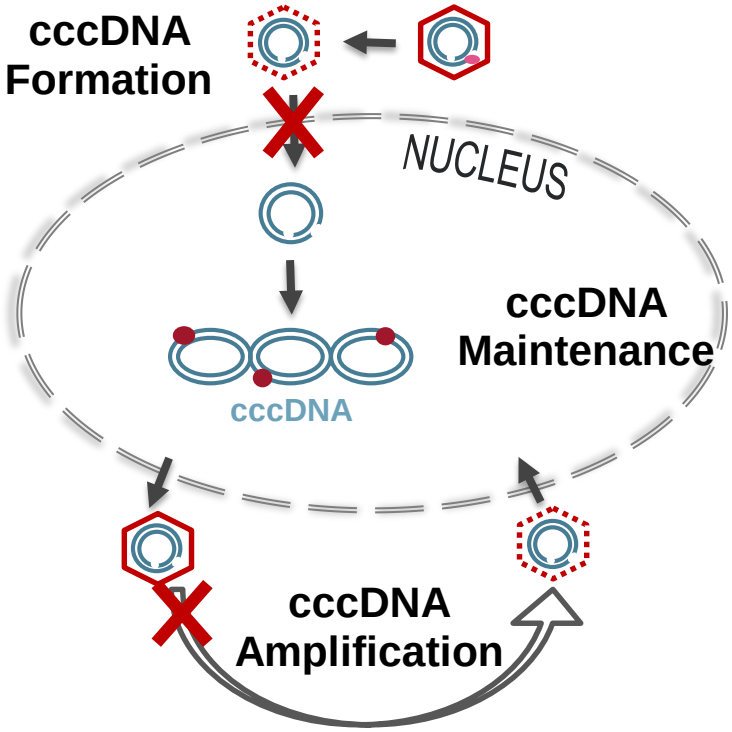
HBV Core Protein: Required Throughout Lifecycle

- Plays a critical role in the formation, amplification and maintenance of cccDNA
- Essential for creating new rcDNA from pgRNA



ASMB Goal: Curative Therapy for HBV

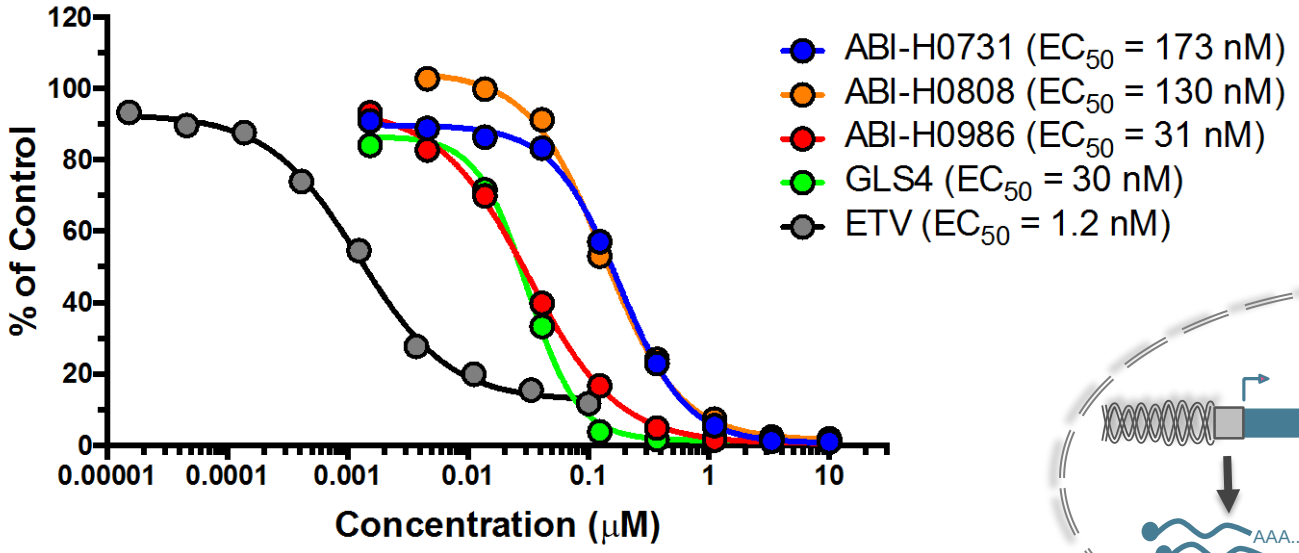
- **Current therapies are suboptimal**
  - IFNs are poorly tolerated, and often not effective
  - Nucs are highly effective in suppressing viral load to undetectable levels, but are not curative, and have little effect on cccDNA levels (key target to eliminate chronic infection)
- **To achieve clinical cure, new therapies must target cccDNA**

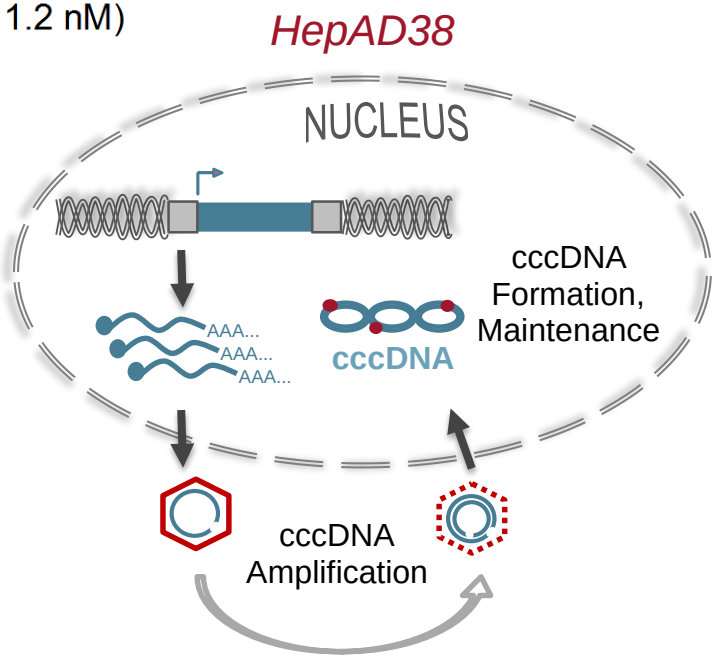


- **Identified and developed a series of potent Core Protein Allosteric Modifiers (CpAMs) which inhibit cccDNA**

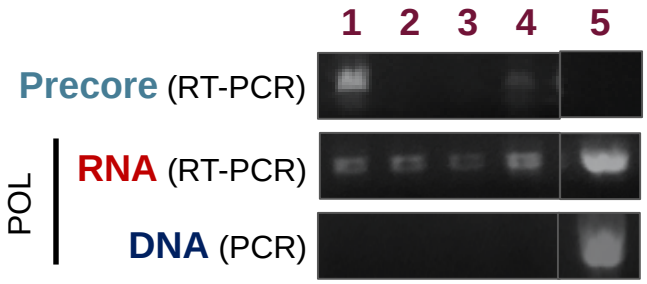
CpAMs Inhibit HBV Replication in HepAD38 cells

HBV Viral Load Inhibition in HepAD38 cells





Precore Transcript Levels

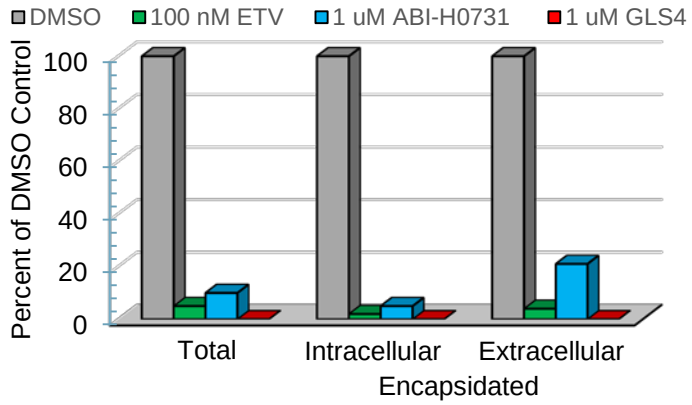


1 = DMSO
2 = ETV *0.1 μM*
3 = ABI-H0731 *1 μM*
4 = GLS4 *1 μM*
5 = virion DNA

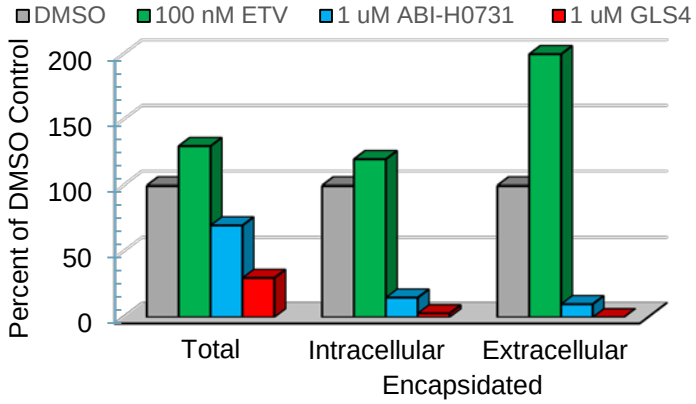
N≥3

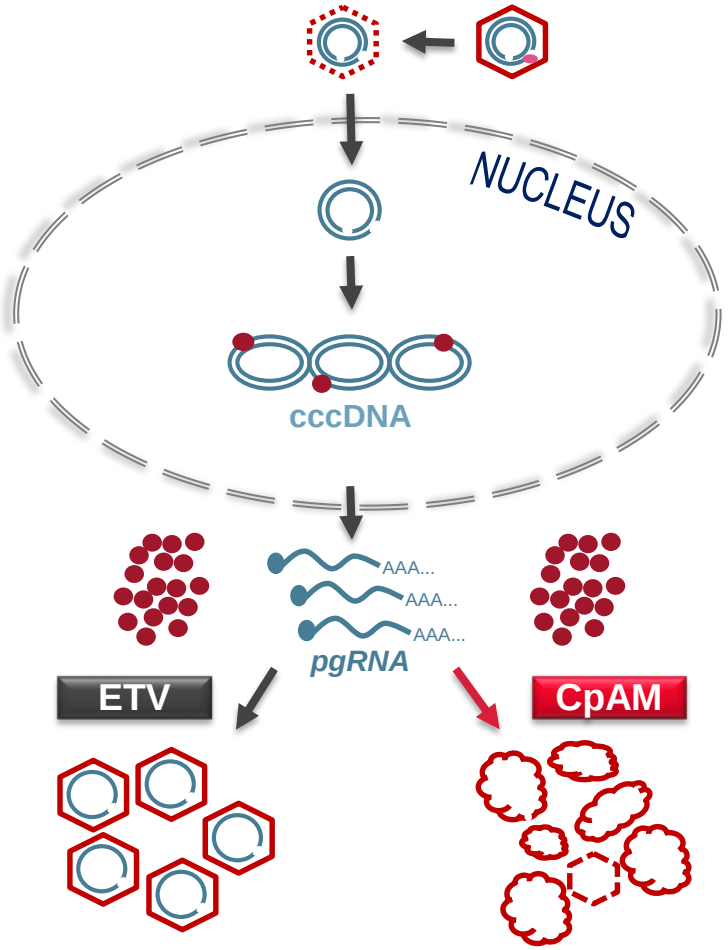
CpAMs Inhibit HBV DNA Replication by Blocking pgRNA Encapsidation

HBV DNA Levels



HBV pgRNA Levels

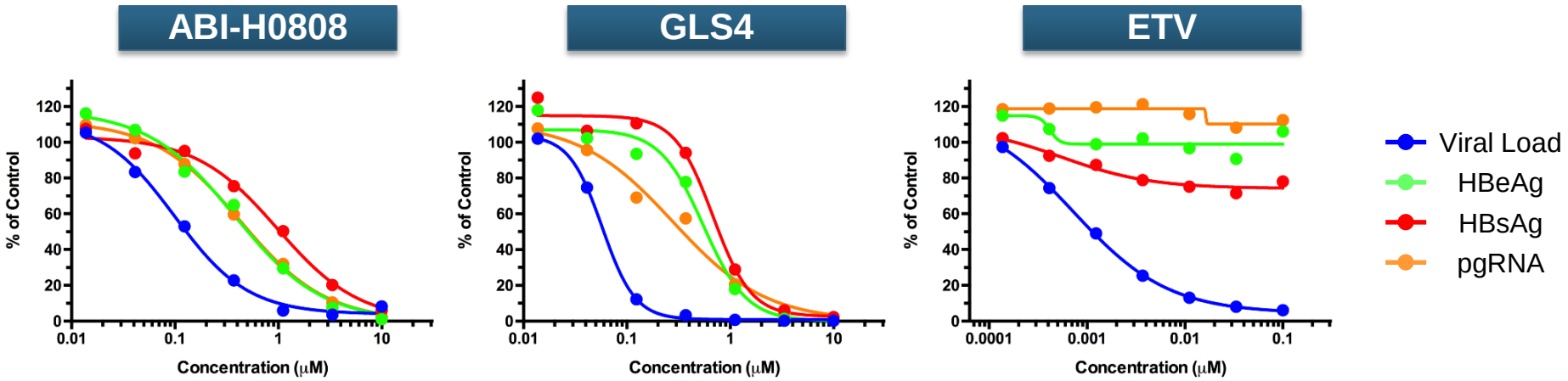




- ABI-H0731 & GLS4 inhibited both viral RNA and DNA in HBV capsids
- ETV reduced quantity of HBV DNA, but increased (~50%) the amount of pgRNA packaged in intracellular capsids and secreted virions

CpAMs Inhibit cccDNA Formation in NTCP-HepG2 Cells

Viral DNA, pgRNA, HBeAg and HBsAg levels in infected NTCP-HepG2 cells

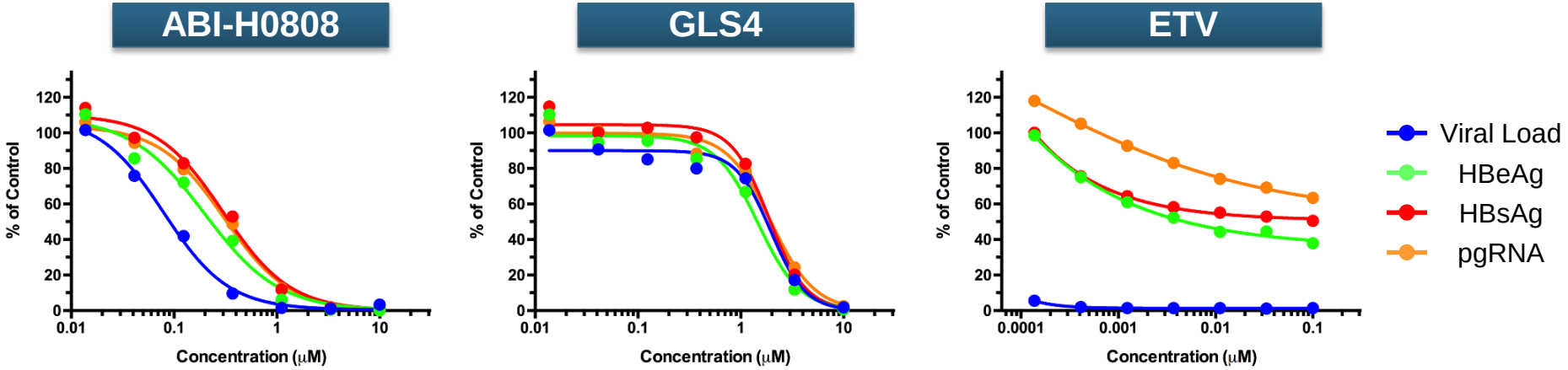


	EC_{50} ± SD (nM)			
Compound	**Viral DNA**	**HBeAg**	**HBsAg**	**pgRNA**
ABI-H0808	95	372	945	419
GLS4	58	549	673	279
ETV	0.8	>>100	>>100	>>100

- CpAMs reduced HBeAg, HBsAg and pgRNA levels (surrogates for establishment of cccDNA), while ETV was only effective at inhibiting viral load (HBV DNA)

CpAMs Inhibit cccDNA Formation in PHH Cells

Viral DNA, pgRNA, HBeAg and HBsAg in Primary Human Hepatocytes



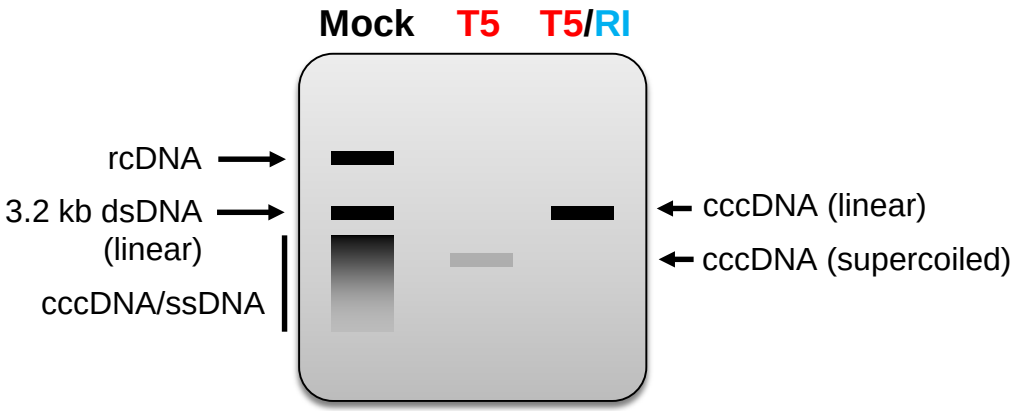
	EC_{50} ± SD (nM)			
Compound	**Viral load**	**HBeAg**	**HBsAg**	**pgRNA**
ABI-H0808	80	196	310	305
GLS4	1,940	1,480	1,860	1,930
ETV	<0.1	Incomplete	Incomplete	Incomplete

- CpAMs reduced HBeAg, HBsAg and pgRNA levels (surrogates for establishment of cccDNA), while ETV was only effective at inhibiting viral load (HBV DNA)
- GLS4 lost potency in PHH, likely due to metabolic instability

CpAMs Inhibit cccDNA Amplification in HepAD38 cells

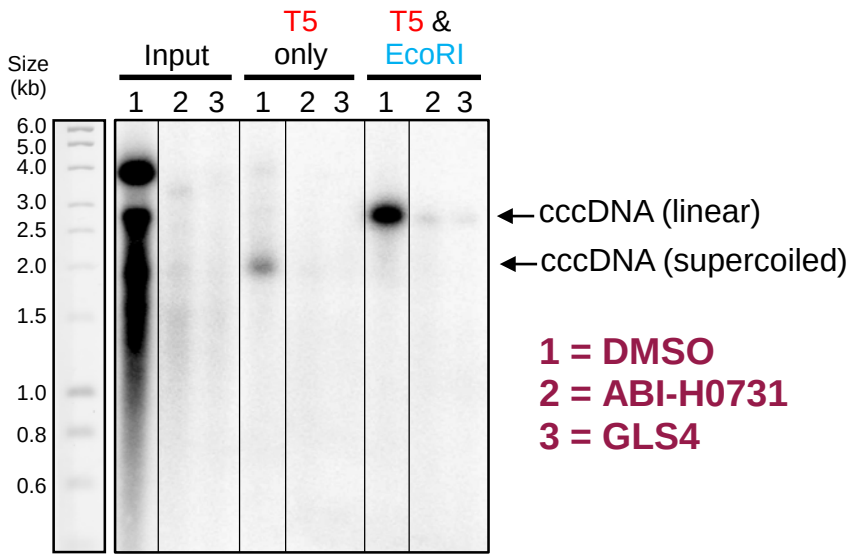
Detection of HBV cccDNA

Extraction of extrachromosomal DNA
*Sequential digestion with **T5 exonuclease***
*and **EcoRI endonuclease***
Southern Blot



Model Illustration of Expected Pattern

Inhibitory effect of CpAMs on cccDNA

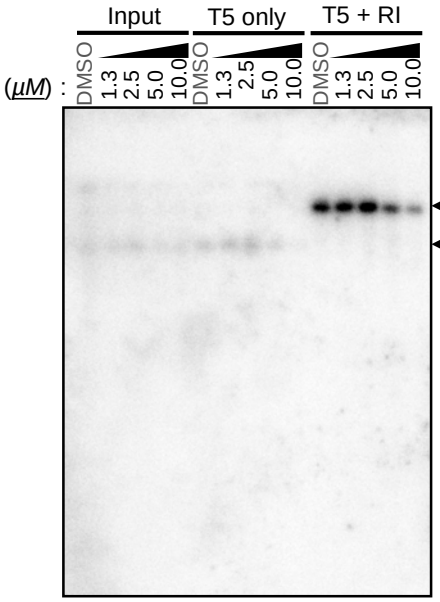


Southern Blot on total extrachromosomal DNA from induced HepAD38 cells treated with compounds at 10x their EC_{50} for 11 days

CpAMs Block cccDNA Formation in NTCP-HepG2

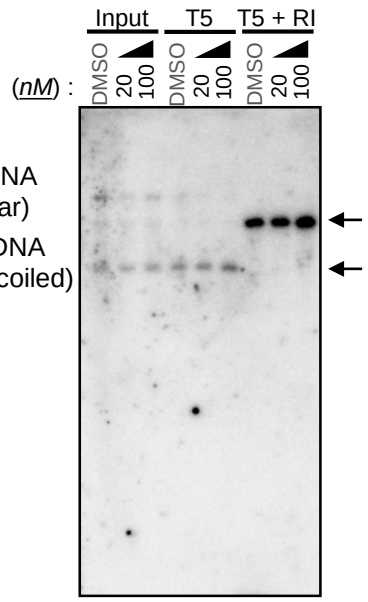
cccDNA Southern Blot

ABI-H0731



ABI-H0731 up to ~50x EC_{50}

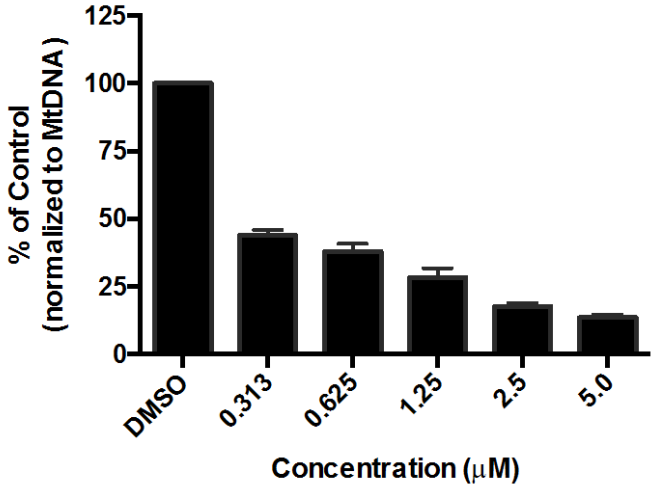
ETV



ETV up to ~125x EC_{50}

cccDNA qPCR

ABI-H0808



ABI-H0808 T5+EcoRI samples qPCR with cccDNA specific primers normalized to MtDNA levels

Summary

- Important to identify treatments/strategies that result in significantly higher cure rates
- HBV cccDNA appears to be obvious target, as this moiety is believed to be responsible for sustaining a chronic infection and is not impacted by “standard of care” therapy
- HBV Core protein plays multiple roles throughout the HBV lifecycle and represents an excellent target by which to impact cccDNA levels
- CpAMs represent a new class of direct acting antivirals that are selective for HBV and inhibit *de novo* cccDNA formation
- Assembly Biosciences has established assays to specifically measure cccDNA levels and is currently progressing the first candidate into clinical development this year

Acknowledgements

Assembly Biosciences HBV Team

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