Development of a Highly Potent Next-Generation Core Inhibitor for the Treatment of Chronic Hepatitis B Infection

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Science of HBV Cure
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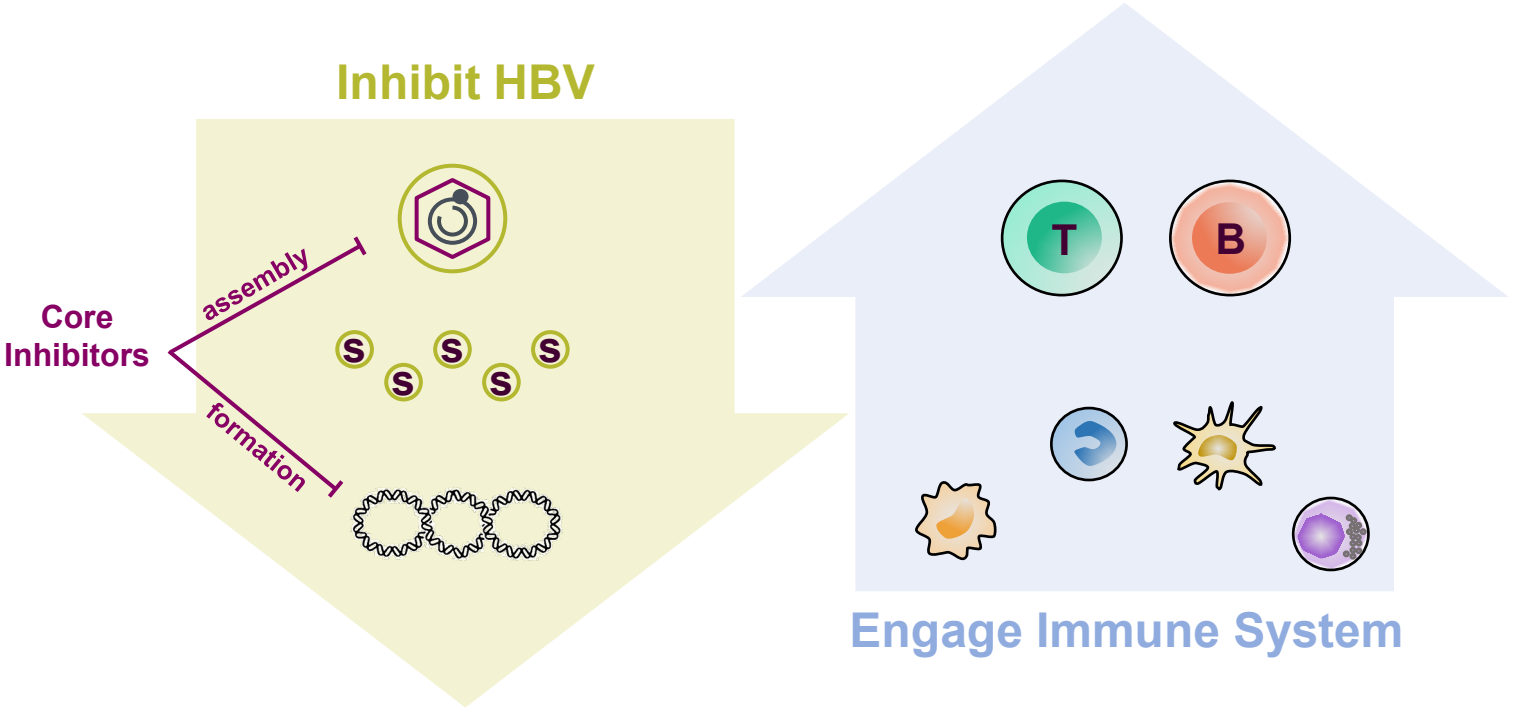
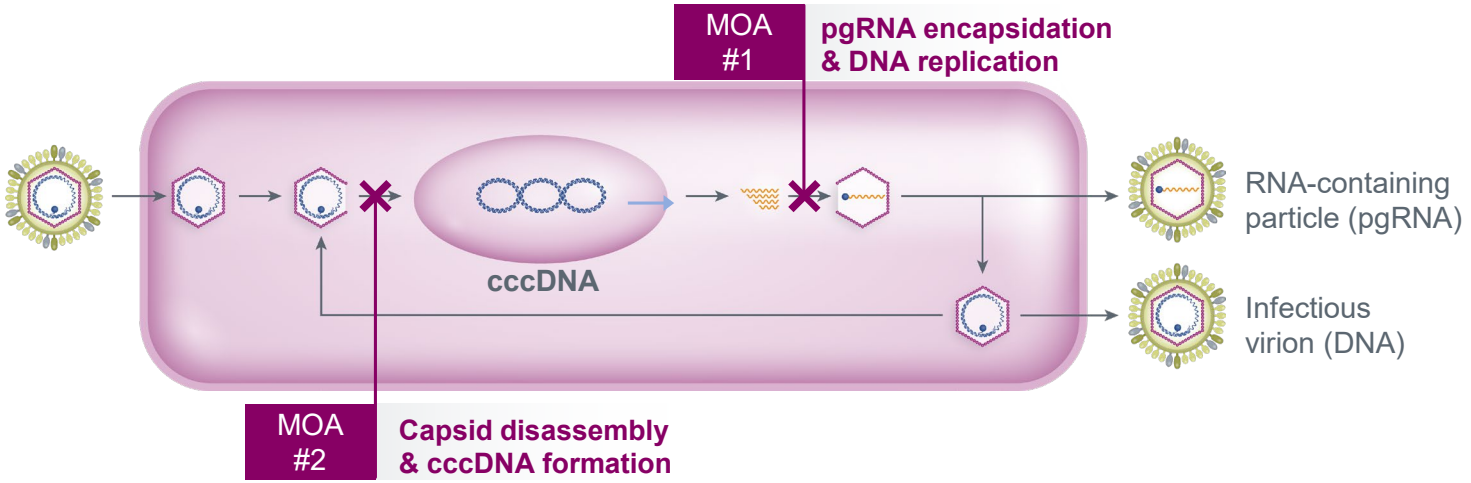
Stopping HBV Replication is a Key Component of HBV Cure Strategies



Core Inhibitors: Multiple Mechanisms of Action

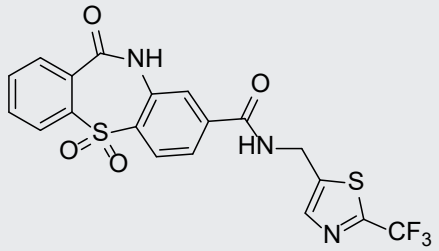


- Core inhibitors target multiple steps in the HBV replication cycle
- First-gen core inhibitors were optimized against viral DNA replication
- Assembly Bio's next-gen core inhibitor 4334 is optimized against **both** mechanisms: viral DNA replication and **cccDNA formation**

cccDNA, covalently closed circular DNA; MOA, mechanism of action; pgRNA, pregenomic RNA

Vebicorvir: A Novel Clinical Stage First-Generation HBV Core Inhibitor

- Disrupts HBV capsid by allosteric binding resulting in misassembly
  - Significantly more potent against MOA #1 compared to MOA #2
- Broad in vitro antiviral activity
- Administered orally, once daily at 300 mg without regard to food
- No drug interaction with NrtIs
- In 28-day Phase 1b studies HBV nucleic acid reductions at 300 mg
  - HBV DNA: 2.8 $\log_{10}$ IU/mL
  - HBV RNA: 2.0 $\log_{10}$ copies/mL
- Phase 2 combination studies of VBR with NrtIs
  - Safe and well tolerated
  - Enhanced suppression of both HBV DNA and RNA
  - Did not lead to cure



EC_{50} (MOA #1) = 173 nM

EC_{50} (MOA #2) = 5447 nM

CC_{50} = >10,000 nM

Huang et al. *Antimicrob Agents Chemother*. 2020; Yuen et al. *Lancet*. 2020; Yuen et al. *J Hep*. 2022; Sulkowski et al. *J Hep*. 2022.

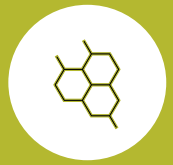
Improving the Therapeutic Potential of Next-Generation Core Inhibitors

Enhance Potency for MOA #1

- More potently block assembly and release of new viral particles

Enhance Potency for MOA #2

- Improved disruption of incoming capsids to block the establishment of new cccDNA
- Reduce the difference in activity between MOA #2 and MOA #1



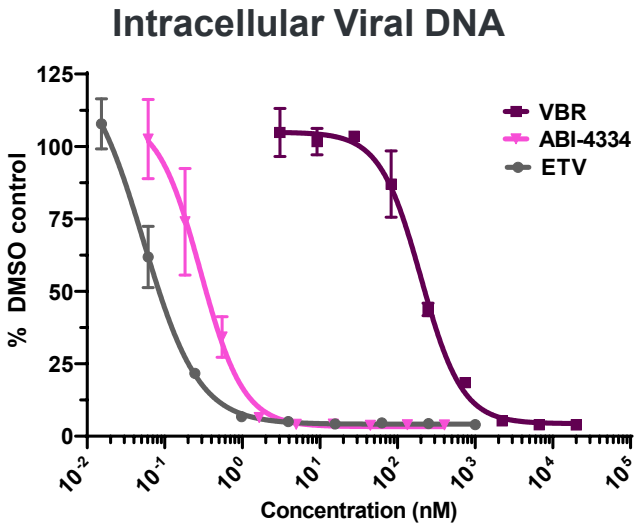
Improved Pharmacology

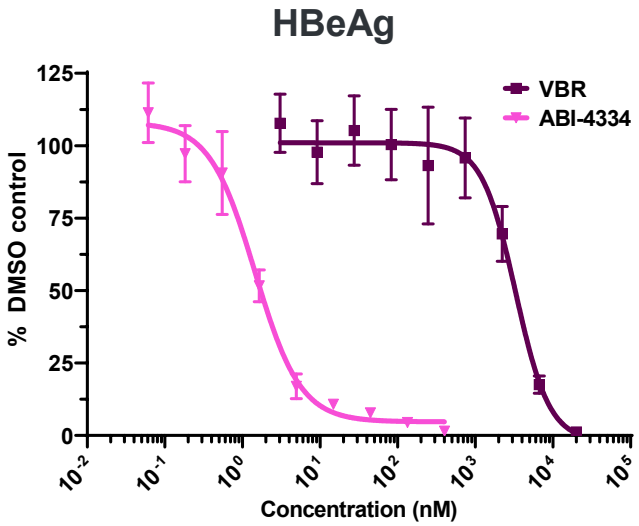
- Increase $t_{1/2}$ and C_{min} to provide enhanced target coverage over 24-hour dosing period
- Improve oral bioavailability
- Increase liver exposure

Safety

- Favorable clinical safety profile with chronic dosing
- No ALT elevations

4334 Potently Inhibits Intracellular Replication and cccDNA Formation





	VBR	4334	ETV
PHH DNA replication EC_{50} (nM)	200 ± 14	0.3 ± 0.1	0.05 ± 0.01
PHH cccDNA formation EC_{50} (nM)	3298 ± 578	1.5 ± 0.2	>1000
PHH cccDNA formation (direct quantification), EC_{50} (nM)	3443	3.1	-

PHH, primary human hepatocyte

Relative In Vitro Potency of Next-Generation Core Inhibitors

EC_{50} (nM)†	MOA	VBR	4334
Intracellular replication	1	200	0.3
cccDNA formation (direct quantification)	2	3443	3.1
C_{min} at 300 mg QD (known or predicted from preclinical studies; nM)		3080	600

†In primary human hepatocytes (PHH) assays; no protein adjustment

MOA

1 pgRNA encapsidation & DNA replication

2 Capsid disassembly & cccDNA formation

Improved Target Coverage with 4334 for MOA #1 and #2

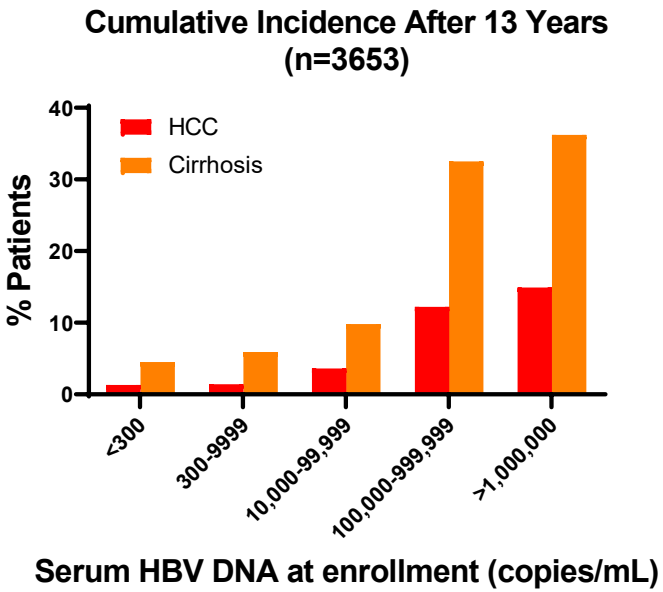
$C_{min}/paEC_{50}$†	MOA	VBR	4334
Intracellular replication	1	1.9	364
cccDNA formation (direct quantification)	2	0.1	35

†C_{min} at 300 mg QD (known or predicted from preclinical studies)

MOA

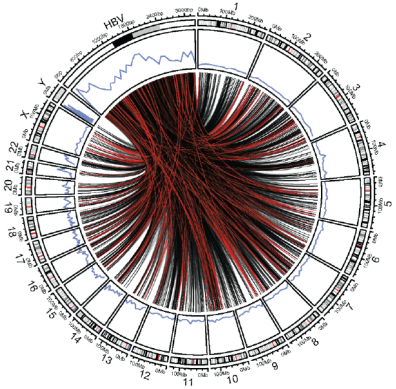
1. pgRNA encapsidation & DNA replication
2. Capsid disassembly & cccDNA formation

HBV Replication Drives Pathogenesis

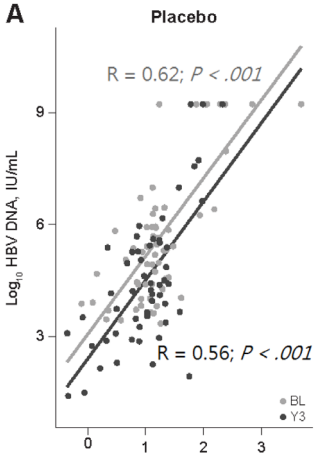


Chen et al. *JAMA.* 2006.

HBV integrates throughout genome



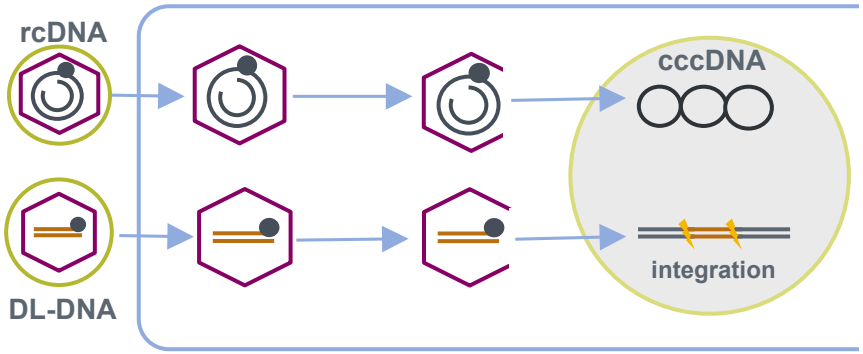
More replication →
More integration →
More cirrhosis/HCC



Hsu et al. *Gastroenterology.* 2022.

HCC, hepatocellular carcinoma

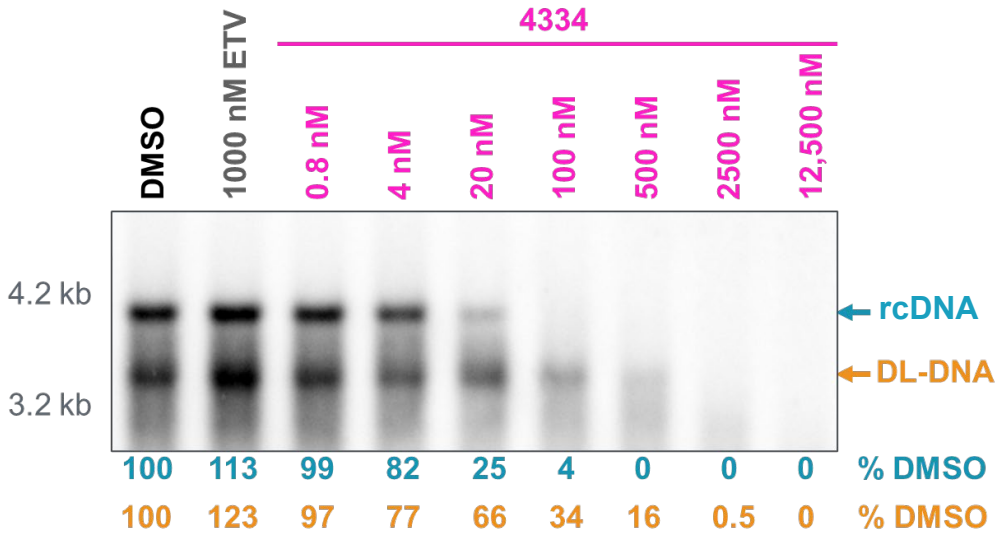
Two Forms of HBV DNA Can Enter the Nucleus Upon Infection



- 4334 disrupts capsids containing rcDNA and prevents formation of cccDNA
- Does 4334 disrupt capsids containing DL-DNA and prevent integration?

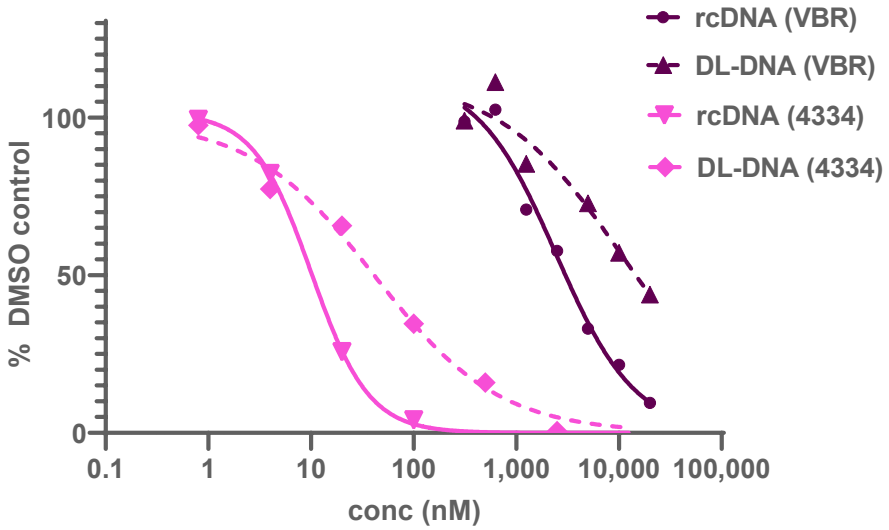
DL-DNA, duplex linear DNA; rcDNA, relaxed circular DNA

4334 Disrupts/Destabilizes rcDNA and DL-DNA–Containing Capsids



- 4334 prematurely disrupted incoming HBV capsids containing rcDNA and DL-DNA

4334 Disrupts HBV Capsids Containing DL-DNA

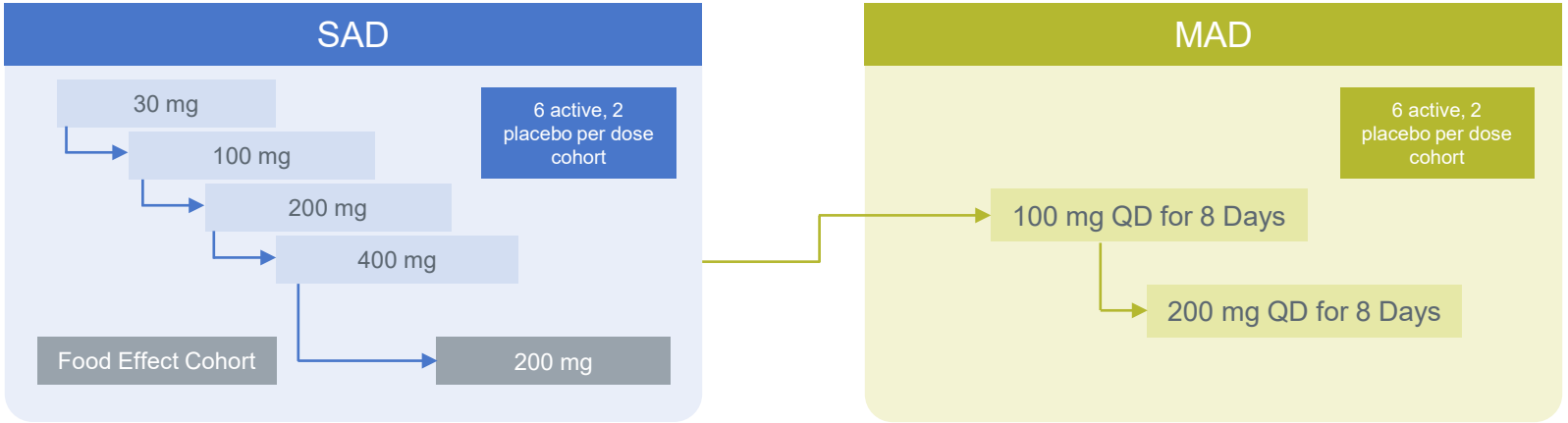


	DNA	EC_{50} (nM)
4334	rcDNA	10.7
	DL-DNA	52.2
VBR	rcDNA	2442
	DL-DNA	>10,000

- ~5x higher 4334 concentrations needed for DL-DNA disruption vs rcDNA disruption
- Studies ongoing to evaluate impact of 4334 on HBV DNA integration

Study ABI-4334-101: Phase 1a Study in Healthy Volunteers
Overall Design (NCT05569941)

- Healthy volunteers enrolled in 4 SAD cohorts and 2 MAD cohorts
- 4334 concentrations assessed by validated liquid chromatography mass spectrometry
- Safety assessed by physical exams, adverse events, and laboratory abnormalities



Summary

- 4334 has significantly improved potency against both HBV DNA replication and cccDNA formation compared to first-generation core inhibitors
  - 4334 is predicted to have ~30x coverage over cccDNA formation
- 4334 prematurely disrupts capsids containing DL-DNA
  - Studies ongoing to determine if 4334 directly impacts HBV integration
- Phase 1a study has recently completed and will be presented at EASL 2023

Acknowledgement

To our study participants and their families

To our study participating investigators and staff