

ABI-4334, a novel hepatitis B core inhibitor, accelerates capsid assembly and inhibits cccDNA formation via multiple pathways

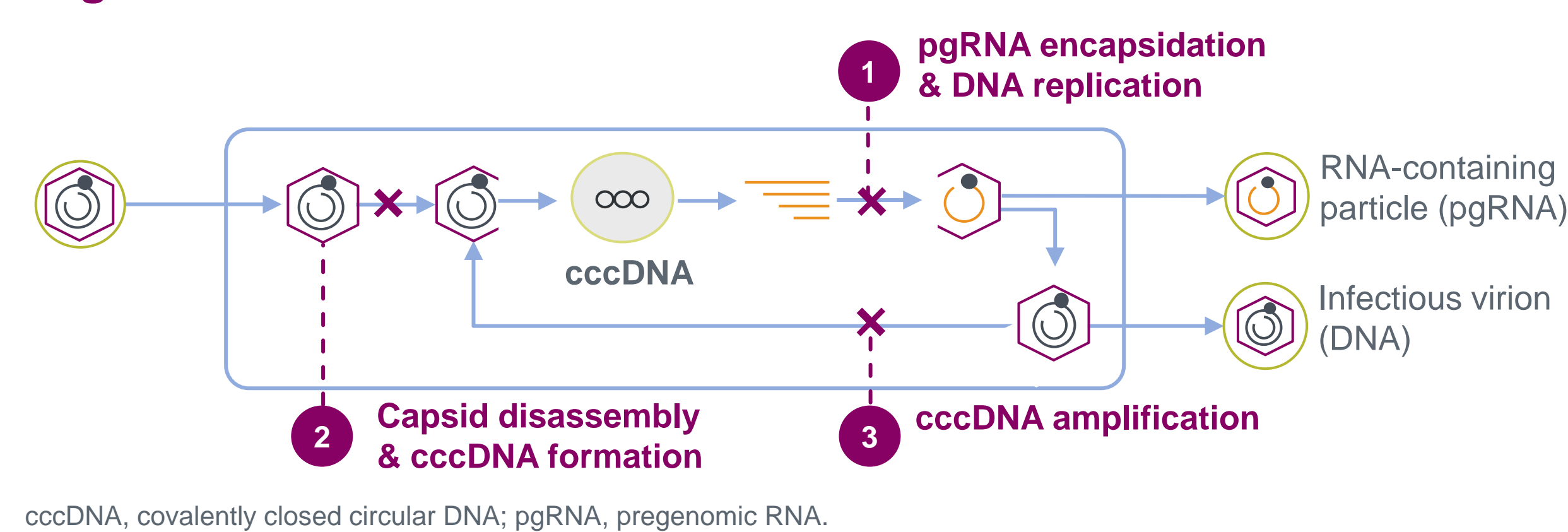
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BACKGROUND

- Chronic hepatitis B virus (cHBV) infection is a significant global health problem
 - Worldwide, an estimated 296 million people have cHBV infection, resulting in about 820,000 deaths each year, mostly due to cirrhosis and hepatocellular carcinoma¹⁻⁴
- Core inhibitors are a novel class of small molecules with the potential to improve cure rates in patients with cHBV. These agents:
 - Inhibit multiple steps in the HBV life cycle, including new capsid formation, pregenomic (pg)RNA encapsidation, formation of covalently closed circular (ccc)DNA from incoming HBV, and intracellular amplification of cccDNA (Figure 1)⁵
 - Demonstrated potent antiviral activity in Phase 1 studies^{6,7} and enhanced antiviral activity when combined with nucleos(t)ide reverse transcriptase inhibitors in Phase 2 studies^{8,9}
- ABI-4334 (4334) is a novel, next-generation core inhibitor with improved in vitro potency against pgRNA encapsidation and cccDNA formation compared to first-generation core inhibitors¹⁰

Figure 1. Core Inhibitor Mechanisms of Action



OBJECTIVE

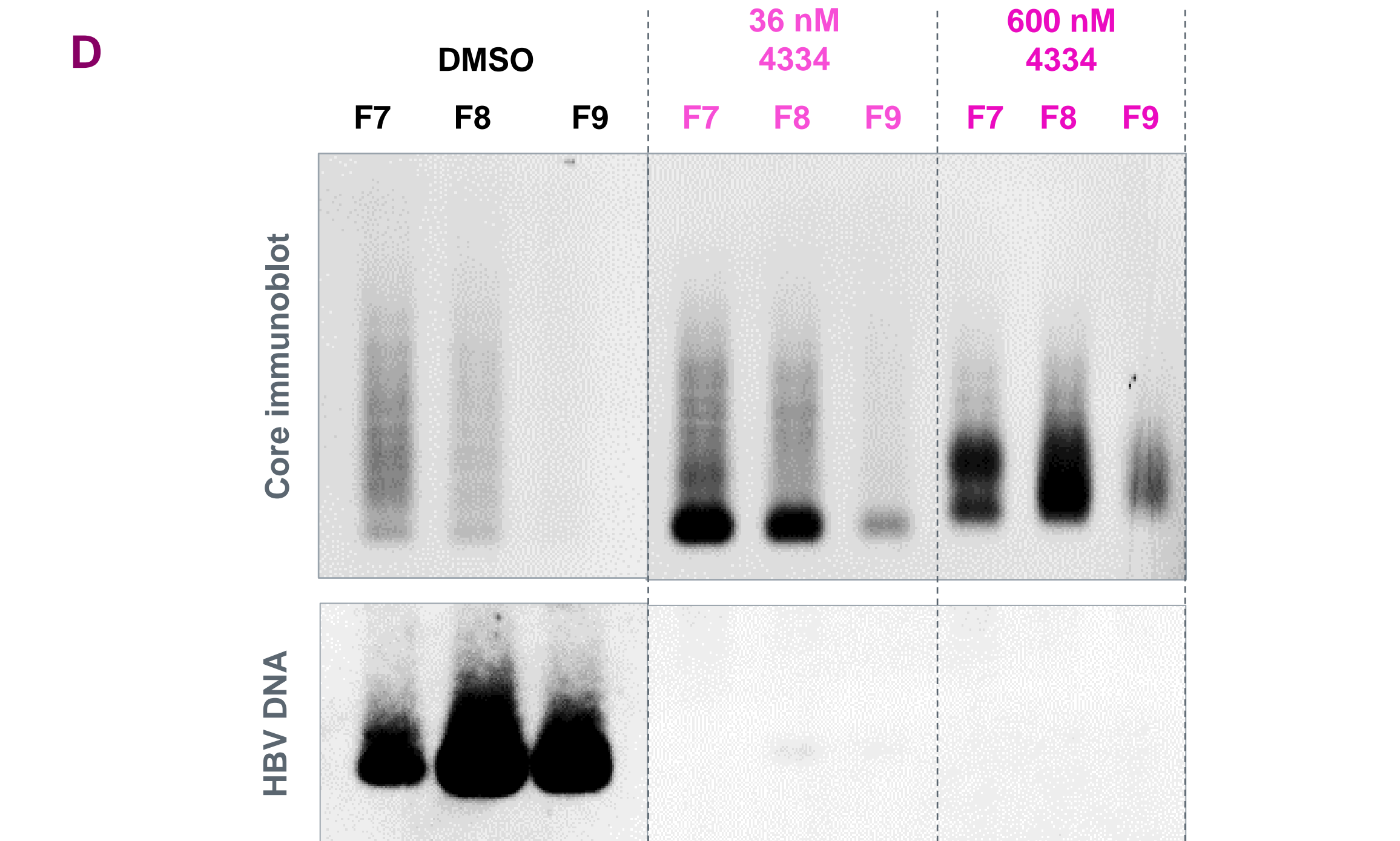
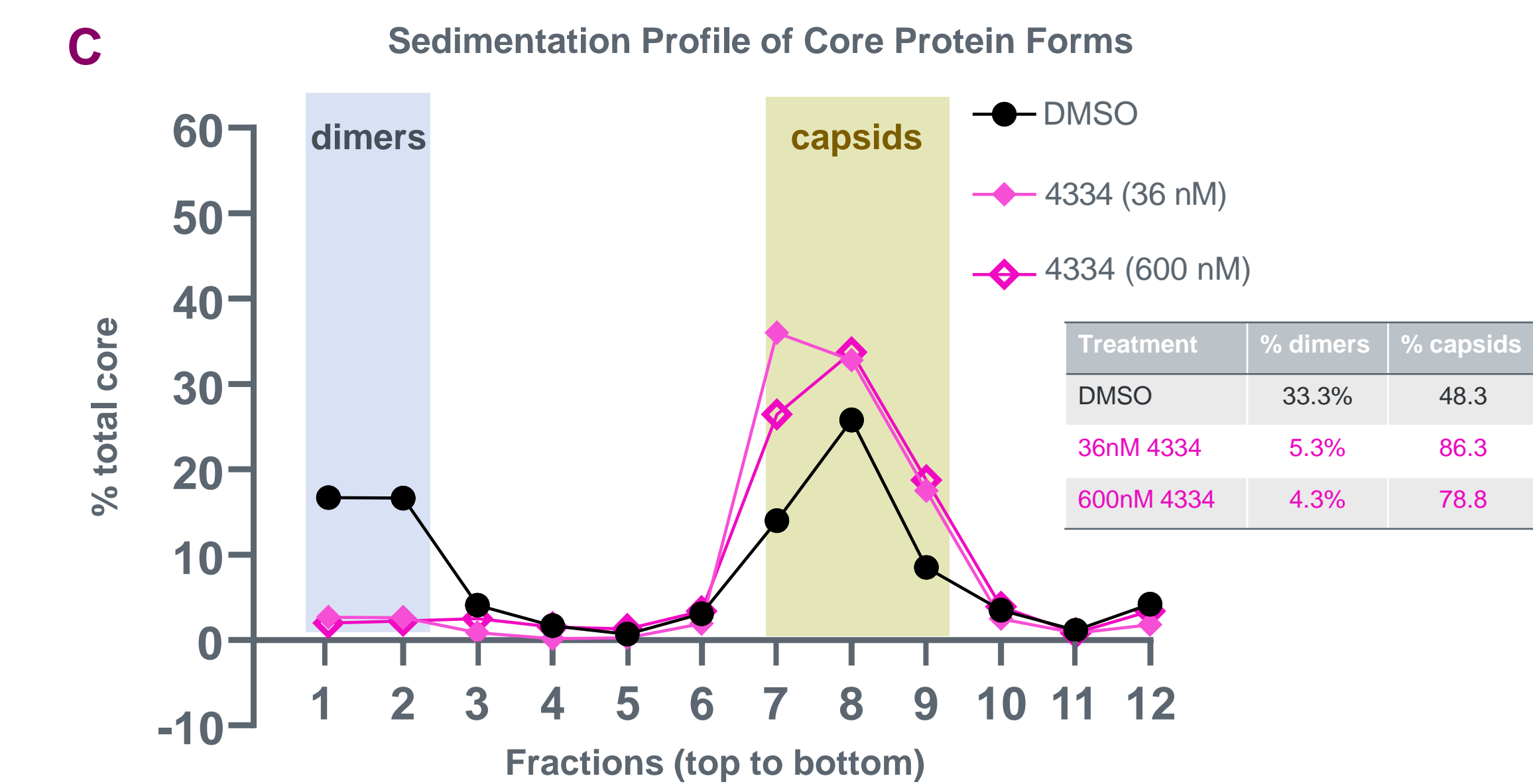
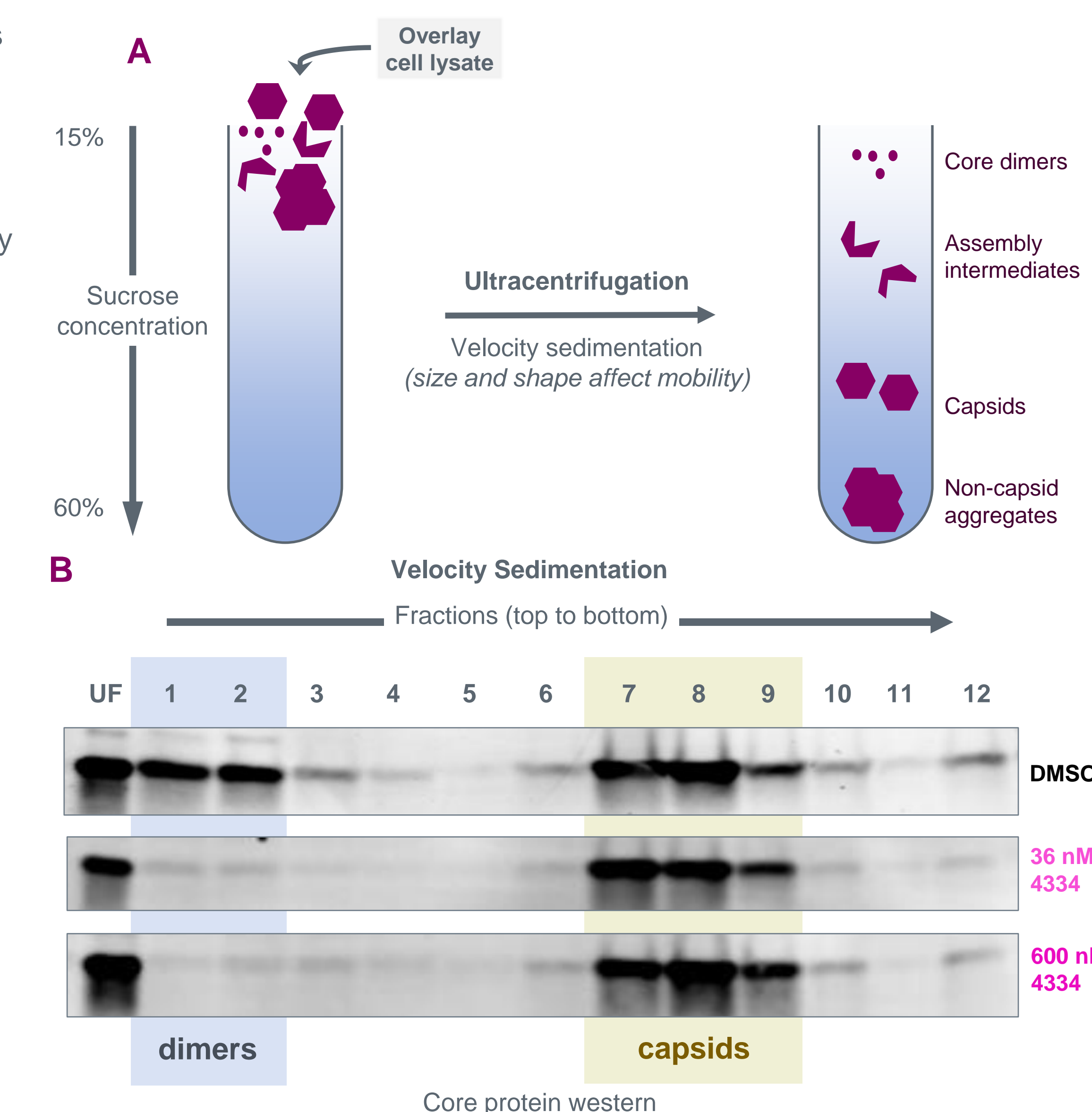
- To further characterize the mechanisms of action (MOA) of 4334, a novel, investigational next-generation core inhibitor

METHODS

- Primary human hepatocyte (PHH) assays**
 - Capsid disruption:** PHHs were infected with HBV at a multiplicity of infection (MOI) of 2500 viral genome equivalents (vge)/cell for 1 hour, followed by drug treatment. Three hours posttreatment, cells were harvested and lysed with 1% NP-40 lysis buffer; cytoplasmic fractions were electrophoresed on a native agarose gel and then blotted onto a nylon membrane for probing against HBV DNA
 - cccDNA formation:** PHHs were infected with HBV at an MOI of 500 vge/cell for 3 hours, followed by drug treatment. At Day 4, cells were harvested, and total DNA was extracted using a modified Hirt DNA extraction procedure. Total DNA was treated with T5 exonuclease to digest linear and nicked double-stranded DNA, followed by EcoRI digestion to linearize cccDNA. Extracted cccDNA was analyzed by Southern blotting
- HepAD38 cell assays**
 - Velocity sedimentation:** Induced and treated HepAD38 cells were lysed at Day 4 with 1% NP-40 lysis buffer. Cytoplasmic lysate was loaded onto a step sucrose gradient (15%–60% sucrose), and the gradient was centrifuged for 2.5 hours at 277,000 g and 10°C. Twelve fractions were separated from top to bottom. Each fraction was run on a sodium dodecyl sulfate-polyacrylamide gel, followed by western blotting for core protein
 - Capsid analysis:** Cytoplasmic fractions subjected to velocity sedimentation were electrophoresed on native agarose gels, blotted, and either immunostained for core using a custom rabbit polyclonal core antibody or probed against HBV minus-strand DNA
 - Intracellular amplification:** HepAD38 cells were induced and treated with 2 mM foscarnet. At Day 4, the culture medium was changed, and cells were treated with core inhibitor or entecavir (ETV) and 3 µg/mL tetracycline treatment for an additional 3 days. At Day 7, extracted cccDNA was analyzed via Southern blotting, as described for PHH
- HepG2-sodium taurocholate cotransporting polypeptide (NTCP) assays**
 - Capsid disruption:** Cells were infected with HBV at an MOI of 3000 vge/cell for 1 hour, followed by drug treatment. Three hours posttreatment, cells were harvested and lysed with 1% NP-40 lysis buffer; cytoplasmic fractions were treated with micrococcal nuclease followed by extraction of encapsidated nucleic acids. Nucleic acids were electrophoresed and analyzed via Southern blotting

RESULTS

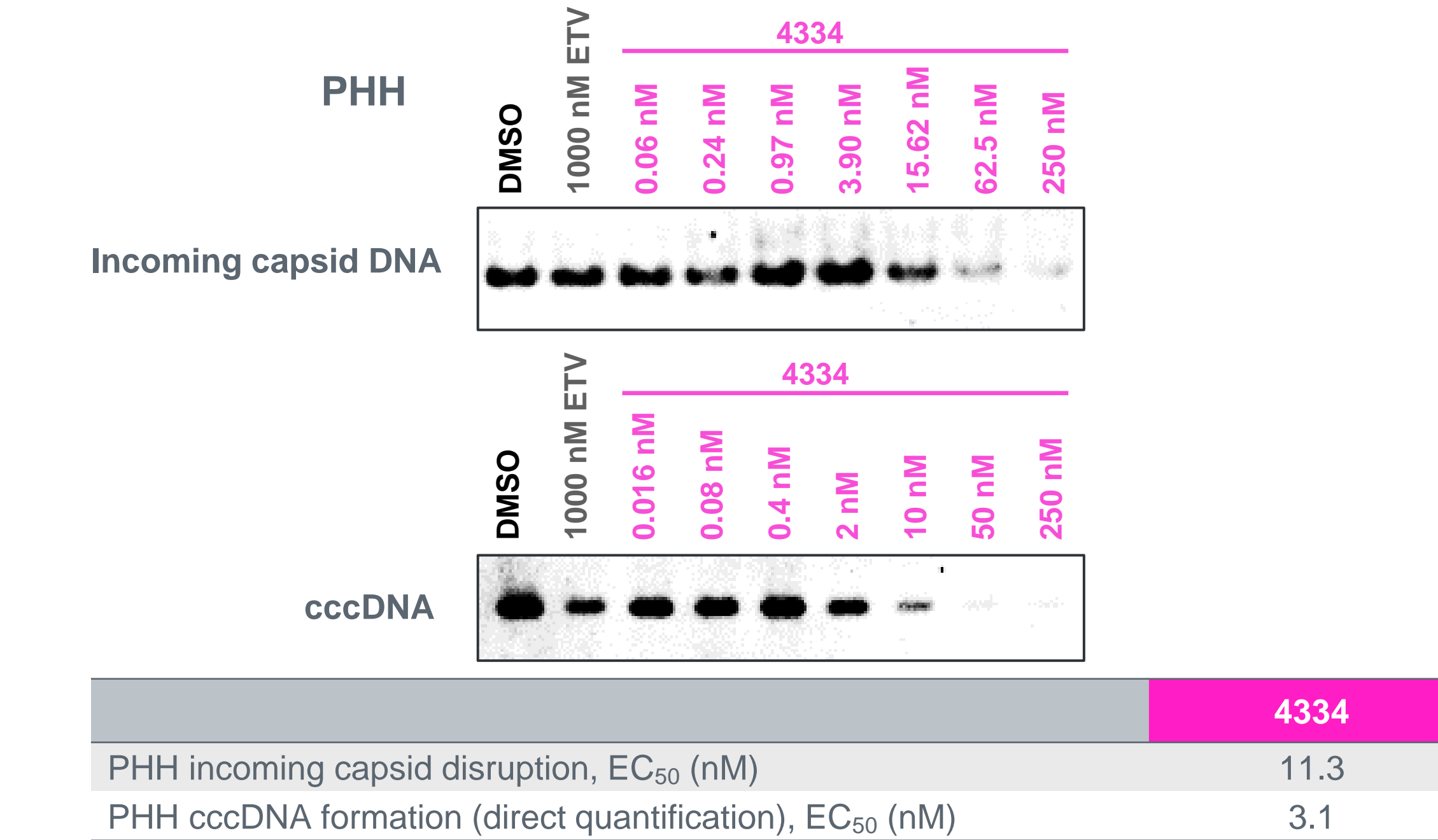
Figure 2. 4334 Accelerates the Formation of Empty Capsids Devoid of HBV DNA



4334, ABI-4334; DMSO, dimethyl sulfoxide; F, fraction; HBV, hepatitis B virus; UF, unfractionated.

- Velocity sedimentation was conducted to determine if 4334 treatment resulted in formation of capsid-like particles (CLPs) or non-capsid aggregates (Figure 2A)
- Treatment with 4334 accelerated dimer-dimer interactions to predominantly form CLPs (fractions 7–9) as compared to dimethyl sulfoxide-treated cells (Figure 2B and 2C). The acceleration of dimer-dimer interactions was observed in cells treated with low and high concentrations of 4334
- Capsid fractions from velocity sedimentation retained gel migration properties (Figure 2D) that were identical to the pattern observed with cell lysates,¹⁰ indicating that subtle structural differences occur in CLPs formed at high concentrations of 4334 (600 nM) that likely account for the striking gel migration pattern difference when compared to low concentrations of 4334 (36 nM)
- All CLPs formed under 4334 treatment were predominantly devoid of HBV DNA (Figure 2D)

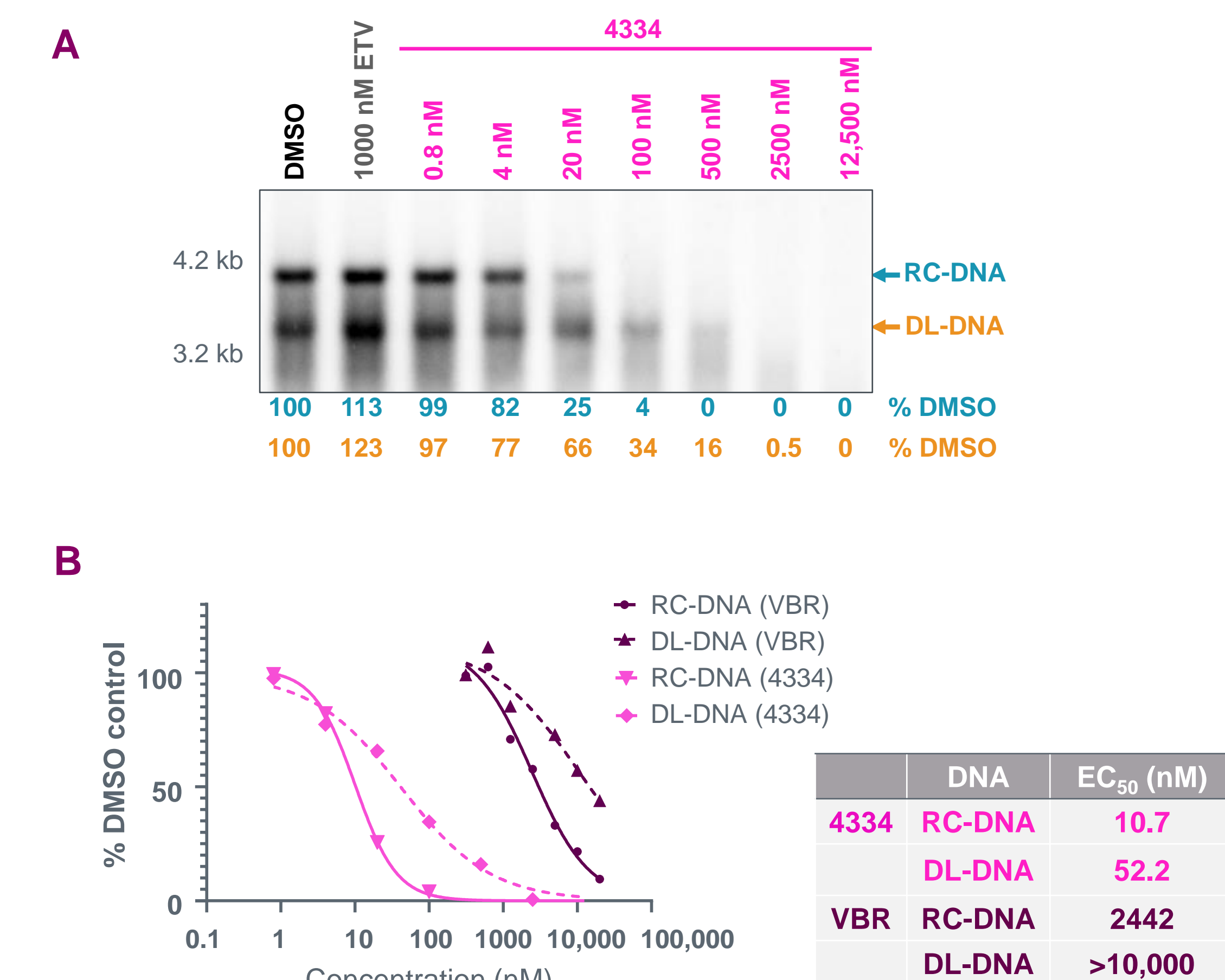
Figure 3. 4334 Prematurely Disrupts Capsids and Inhibits the Formation of cccDNA



4334, ABI-4334; cccDNA, covalently closed circular DNA; DMSO, dimethyl sulfoxide; EC₅₀, half-maximal effective concentration; ETV, entecavir; PHH, primary human hepatocyte.

- ABI-4334 treatment resulted in the premature disruption of incoming HBV capsids (half-maximal effective concentration [EC₅₀] = 11.3 nM), which inhibits cccDNA formation (EC₅₀ = 3.1 nM; Figure 3)
- The results observed in PHH are consistent with what was previously observed in HepG2-NTCP cells¹¹

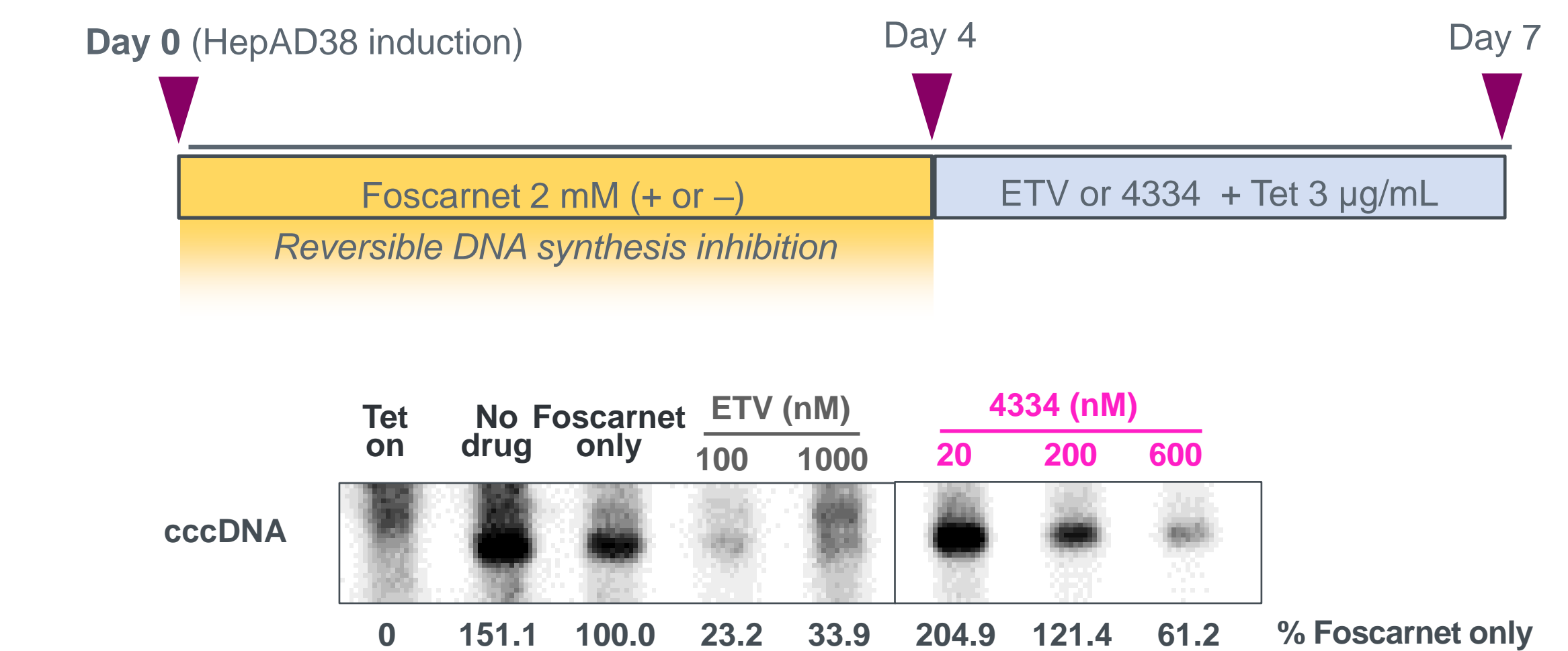
Figure 4. 4334 Disrupts HBV Capsids Containing DL-DNA



4334, ABI-4334; DL-DNA, duplex linear DNA; DMSO, dimethyl sulfoxide; EC₅₀, half-maximal effective concentration; ETV, entecavir; HBV, hepatitis B virus; RC-DNA, relaxed circular DNA; VBR, vebicovir.

- 4334 treatment resulted in the premature disruption of incoming HBV capsids containing relaxed circular DNA (RC-DNA) and duplex linear DNA (DL-DNA; Figure 4A)
 - In contrast, ETV treatment, even at high concentrations, did not disrupt incoming RC-DNA- and DL-DNA-containing capsids
- The potency of 4334 against DL-DNA-containing capsids was ~5 times lower (EC₅₀ = 52.2 nM) than RC-DNA-containing capsids (EC₅₀ = 10.7 nM; Figure 4B)

Figure 5. Impact of 4334 on cccDNA Formation Via Intracellular Amplification



4334, ABI-4334; cccDNA, covalently closed circular DNA; ETV, entecavir; Tet, tetracycline.

- 4334 prevented cccDNA formation via intracellular amplification, albeit at higher concentrations than needed to inhibit cccDNA formation via incoming capsids (Figure 5)

Table 1. Summary of in Vitro Potency

EC ₅₀ (nM)	Cell Type	MOA	VBR	4334	ETV	
Intracellular replication	PHH	1	154 ⁵	0.29 ¹⁰	0.05 ¹⁰	
cccDNA formation (HBeAg)		2	2210 ⁵	1.5 ¹⁰	Inactive	
cccDNA formation (direct quantification)		2	3443 ⁵	3.1	Inactive	
Capsid disruption		2	1360 ⁵	11.3	Inactive	
RC-DNA disruption		HepG2-NTCP	2	2442	10.2	Inactive
DL-DNA disruption			2	>10,000	52.2	Inactive

4334, ABI-4334; cccDNA, covalently closed circular DNA; DL-DNA, duplex linear DNA; EC₅₀, half-maximal effective concentration; ETV, entecavir; HBeAg, hepatitis B e antigen; MOA, mechanism of action; ND, not determined; NTCP, sodium taurocholate cotransporting polypeptide; PHH, primary human hepatocyte; RC-DNA, relaxed circular DNA; VBR, vebicovir.

- 4334 demonstrated low or subnanomolar potency against the majority of core inhibitor MOA, with EC₅₀ concentrations well below the predicted minimum plasma concentration of 600 nM (Table 1)¹² In contrast, vebicovir EC₅₀ concentrations for all cccDNA formation MOAs are either at or above the minimum plasma concentrations observed in patients with cHBV⁶
- ETV potentially inhibits intracellular replication, but does not impact incoming capsids or prevent cccDNA formation

CONCLUSIONS

- ABI-4334 is a potent, next-generation core inhibitor with activity against cccDNA formation via incoming capsids as well as intracellular amplification
- ABI-4334 prematurely disrupts capsids containing DL-DNA, which has the potential to impact HBV integration
- A Phase 1a study with ABI-4334 is planned to be initiated in the second half of 2022

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