Amino Acid Substitutions in the Inhibitor Binding Pocket of HBV Core Protein Confer Differential Changes in Susceptibility to Three Generations of HBV Core Inhibitors

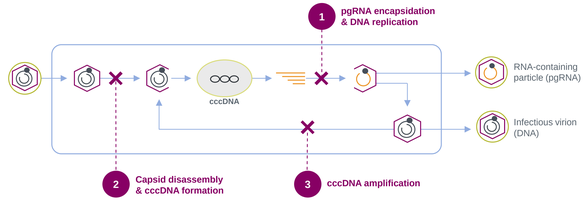
Dawei Cai, Emily Connelly, Renuka Kumar, Yuhua Zong, Ran Yan, Yi Zhou, Alexandre Mercier, Richard Colonno, Qi Huang, and William Delaney

Assembly Biosciences, Inc., South San Francisco, USA

Background

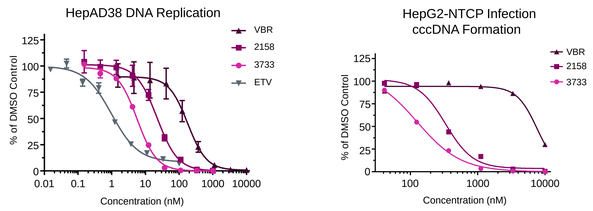
- Core inhibitors are a novel class of hepatitis B virus (HBV) direct-acting antivirals with the potential to increase on-treatment responses and cure rates after finite treatment
  - Core inhibitors prevent the assembly and release of new viral particles containing pregenomic RNA (pgRNA) or HBV DNA
  - Core inhibitors block the establishment of new covalently closed circular DNA (cccDNA) during de novo infection (**Figure 1**)
- All known core inhibitors bind to the same highly conserved pocket on the core protein[1]
- Amino acid substitutions in the binding pocket can confer reduced sensitivity to core inhibitors[2–4]
- Vebicorvir (VBR; ABI-H0731), ABI-H2158 (2158), and ABI-H3733 (3733) represent three generations of structurally distinct core inhibitors selected for increasing potency against cccDNA formation (**Figure 2**)[5–7]
- VBR, 2158, and 3733 are currently in clinical development
  - VBR and 2158 are being investigated in Phase 2 studies in combination with nucleos(t)ide reverse transcriptase inhibitors (NrtIs)
  - 3733 is being investigated in a Phase 1a study

Figure 1. Core Inhibitor Mechanisms of Action



cccDNA, covalently closed circular DNA; pgRNA, pregenomic RNA.

Figure 2. Potency of VBR, 2158, 3773, and ETV[7]



	VBR (1st Gen)	2158 (2nd Gen)	3733 (3rd Gen)	ETV
HepAD38 DNA replication, EC_{50} (nM)	173±40	22±2	5.7±0.2	0.98±0.06
HepG2-NTCP infection cccDNA formation, EC_{50} (nM)	5447	334	125	>>1000

cccDNA, covalently closed circular DNA; DMSO, dimethyl sulfoxide; EC_{50}, concentration of a drug that gives half-maximal response; ETV, entecavir; Gen, generation; VBR, vebicorvir.

Objectives

- To evaluate the antiviral activity of VBR, 2158, and 3733 against known substitutions in the core inhibitor binding pocket
- To determine if substitutions that confer reduced susceptibility to core inhibitors affect assembly of new viral particles (antiviral activity) and prevention of new cccDNA formation (capsid melting activity)

Methods

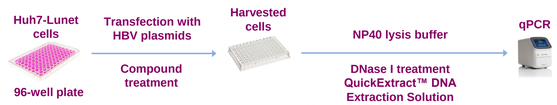
- The antiviral activity of VBR, 2158, 3733, and entecavir (ETV) was tested against HBV encoding 11 individual substitutions in HBV core protein (**Table 1**)
- The transient transfection-based quantitative polymerase chain reaction (qPCR) assay and Southern blot assay used to profile antiviral activity and capsid melting activity are illustrated in **Figure 3** and **Figure 4**, respectively

Table 1. Amino Acid Substitutions in the Inhibitor Binding Pocket of HBV Core Protein

Substitution	Prevalence (INSERM database)[8]	Association with Resistance (references)
P25A	<0.1%	References[9,10]
D29G	0.1%	References[4,10]
T33N	<0.1%	References[4,9–12]
Y38C	0.1%	Observed in an Assembly clinical trial
Y38F	3.2%	References[4,10,13]
I105L	0.7%	References[2,3,10,13]
I105T	0.6%	References[2–4,10–14]
T109I	0.2%	References[2,3,10,13]
T109M	0.8%	Observed in an Assembly clinical trial[15] References[2,3,10,13]
I116L	[a]	Observed in an Assembly clinical trial
Y118F	0.4%	References[2–4,10,13]

[a]Genotype variant, genotype I116 (20.5%), L116 (77.4%). HBV, hepatitis B virus.

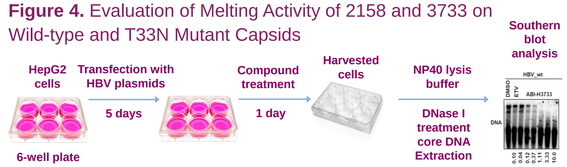
Figure 3. Evaluation of Antiviral Activity of VBR, 2158, and 3733 against Known Substitutions in the Core Inhibitor Binding Pocket



HBV, hepatitis B virus; qPCR, quantitative polymerase chain reaction; VBR, vebicorvir.

- Huh7-Lunet cells were transiently transfected with plasmids encoding wild-type HBV or HBV encoding core protein substitutions previously reported in the literature **(Figure 3, Table 1)**
- Transfected cells were treated with VBR, 2158, 3733, or ETV for 1 week, after which the amount of replicating HBV DNA in the cells was evaluated by qPCR on lysates extracted from cells **(Figure 3)**

Figure 4. Evaluation of Melting Activity of 2158 and 3733 on Wild-type and T33N Mutant Capsids



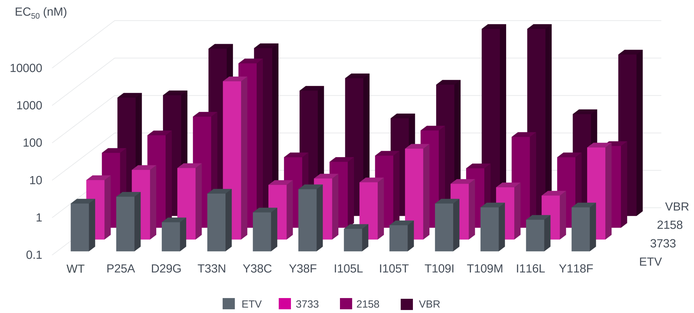
HBV, hepatitis B virus.

- HepG2 cells were transiently transfected with plasmids encoding wild-type or T33N mutant HBV
- Transfected cells were free of compound treatment for 5 days to allow wild-type and T33N capsid accumulation, then treated with 2158, 3733, or ETV for 1 day, after which the intact relaxed circular DNA-containing capsids were evaluated by Southern blot analysis **(Figure 4)**

Results

- VBR had a >10-fold loss of activity against the D29G, T33N, T109I, T109M, and Y118F substitutions; these substitutions had reduced replicative capacity and are present in <1% of sequences in public databases
- 2158 and 3733 had improved resistance profiles with >10-fold resistance to only the T33N
- For all three inhibitors, there were substitutions that conferred <10-fold shifts in EC_{50}; importantly, these substitutions differed between inhibitors
- Compared with wild-type, the T33N mutant had reduced sensitivity in capsid melting (**Figure 6**)

Figure 5. EC_{50} Values for VBR, 2158, 3733 and ETV Against HBV encoding Amino Acid Substitutions in the Inhibitor Binding Pocket of Core Protein



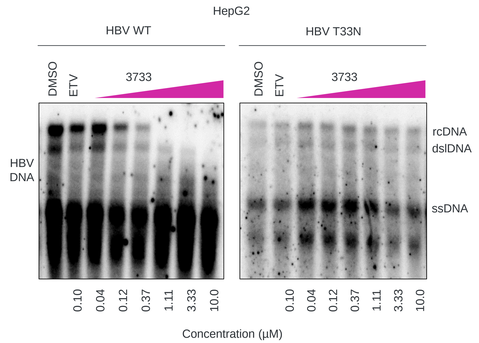
EC_{50}, concentration of a drug that gives half-maximal response; ETV, entecavir; VBR, vebicorvir; WT, wild-type.

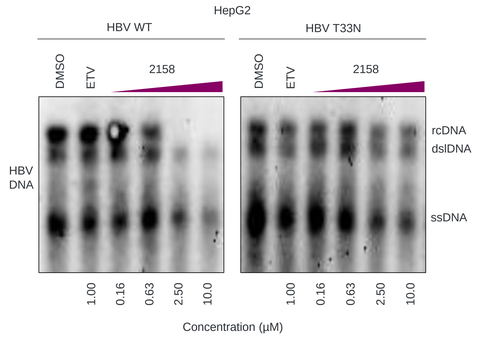
Table 2. EC_{50} Fold Shift of Amino Acid Substitutions in the Inhibitor Binding Pocket of HBV Core Protein

Fold Change in EC_{50} vs Wild-type					
Core Protein Substitutions	Replication Capacity (%WT)	VBR WT EC_{50}=146 nM	2158 WT EC_{50}=10 nM	3733 WT EC_{50}=4 nM	ETV WT EC_{50}=2 nM
P25A	122%	1.2	2.9	1.9	1.5
D29G	19%	20.2	9.3	2.1	0.3
T33N	61%	20.9	245	442	1.8
Y38C	92%	1.5	0.8	0.7	0.6
Y38F	55%	3.3	0.6	1.1	2.4
I105L	73%	0.3	0.8	0.9	0.2
I105T	52%	2.2	4.0	7.0	0.4
T109I	52%	>68.4	0.4	0.8	1.0
T109M	29%	>68.4	2.7	0.6	0.8
I116L	88%	0.4	0.8	0.4	0.4
Y118F	9%	14.2	1.5	7.6	0.8

EC_{50}, concentration of a drug that gives half-maximal response; ETV, entecavir; VBR, vebicorvir; WT, wild-type.

Figure 6. Capsid Melting Activity of 2158 and 3733 on Wild-Type and T33N Mutant Capsids





DMSO, dimethyl sulfoxide; dslDNA, double stranded linear; ETV, entecavir; HBV, hepatitis B virus; rcDNA, relaxed circular DNA; ssDNA, single-stranded DNA; WT, wild-type.

Conclusions

- Although all HBV core inhibitors bind in the same pocket, specific amino acid substitutions confer differential changes to drug susceptibility based on inhibitor structure
- T33N affected encapsidation of pgRNA and viral replication and also affected the ability of core inhibitors to disrupt DNA-containing nucleocapsids and block cccDNA formation
- Overall, 2158 and 3733 showed greater potency compared with VBR and had more favorable resistance profiles against a panel of substitutions
- ETV retains activity against all tested core protein substitutions; combination therapy with NrtIs may prevent viral breakthrough due to pre-existence or potential emergence of core protein substitutions

References

1) Viswanathan U, et al. *Antiviral Res*. 2020;182:104917. 2)Klumpp K, et al. *Proc Natl Acad Sci U.S.A*. 2015;112:15196-201. 3) Zhou Z, et al. *Sci Rep*. 2017;7:1-12. 4) Berke JM, et al. *Antivir Res*. 2017;144:205-15. 5) Huang Q, et al. *Antimicrob Agents Chemother*. 2020;AAC.01463-20. 6) Huang Q, et al. *Hepatol*. 2017;66(1 Suppl): 493A. 7) Huang Q, et al. EASL 2019 Oral Presentation. 8) Genotype A-H, RF (11145 sequences), March 29, 2020; https://hbvdb.lyon.inserm.fr/data/proteins/repertoires/all_HBc.num. 9) Lam AM, et al. *Antimicrob Agents Chemother*. 2019;63:e01734-18. 10) Verbinnen T, et al. *J Antimicrob Chemother*. 2020;75:2526-34. 11) Janssen, HLA et al. EASL 2020 Poster. 12) Mani N, et al. AASLD 2019 Poster #0701. 13) Verbinnen T, et al. *J Viral Hepat*. 2020;27(11):1127-37. 14) Yuen M-F, et al. AASLD 2019 Poster #LP7. 15) Yuen M-F, et al. *Lancet Gastroenterol Hepatol*. 2019;S2468-1253:30346-2.

Acknowledgments

- Writing and editorial support was provided by Lauren Hanlon, PhD, of AlphaBioCom, LLC, and funded by Assembly Biosciences
- This study was sponsored by Assembly Biosciences

Presented at American Association for the Study of Liver Diseases (AASLD) The Liver Meeting Digital Experience 2020, November 13-16, 2020