No emergent core inhibitor resistance in patients with chronic hepatitis B virus infection treated with vebicorvir in combination with a nucleos(t)ide reverse transcriptase inhibitor

PO-1286

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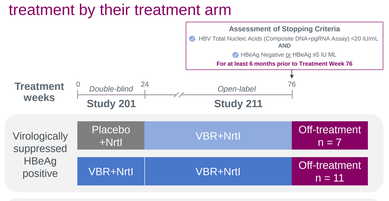
Introduction

- Chronic hepatitis B virus infection (cHBV) is a major cause of morbidity and mortality worldwide
- Long-term oral treatment with nucleos(t)ide reverse transcriptase inhibitors (NrtIs) is safe but results in a low rate of sustained response off-therapy
- Vebicorvir (VBR) is a first-generation hepatitis B virus (HBV) core inhibitor in development for the treatment of cHBV. Core inhibitors target multiple steps of the HBV replication cycle
- In Study 201 (NCT03576066), virologically suppressed patients with hepatitis B "e" antigen (HBeAg) positive or HBeAg negative cHBV were randomized in a blinded manner to VBR or placebo with an NrtI for 24 weeks, after which eligible patients entered the open-label extension (Study 211; NCT03780543) to receive ongoing treatment with VBR+NrtI
- 41 patients discontinued both VBR and NrtI after 76 weeks of treatment per protocol. All patients subsequently experienced virologic relapse 4–16 weeks after treatment discontinuation
- The aim of this post hoc analysis was to investigate whether any treatment-emergent core inhibitor substitutions or NrtI resistance mutations were observed during virologic relapse after treatment discontinuation

Methods

- Of the 69 patients enrolled in Studies 201 and 211, 43 met prospective criteria to discontinue both VBR and NrtI treatment after Week 76; however, only 2 decided to discontinue from the study. A total of 41 patients were analyzed in this study
- Patients discontinued VBR+NrtI if they had total HBV nucleic acids (DNA+pgRNA) <20 IU/mL and HBeAg levels ≤5 IU/mL for ≥6 months assessed at Week 52 or later[1]

Figure 1. Summary of 41 patients who discontinued treatment by their treatment arm



HBeAg, hepatitis B "e" antigen; HBV, hepatitis B virus; NrtI, nucleos(t)ide reverse transcriptase inhibitor; pg, pregenomic; VBR, vebicorvir.

- Viral nucleic acids were purified from patient plasma. Sanger sequencing of HBV core and polymerase/ reverse transcriptase (RT) regions was attempted on HBV RNA by RT-polymerase chain reaction (PCR) for baseline samples, since all patients enrolled in Study 201 were virologically suppressed (HBV DNA <20 IU/mL; COBAS TaqMan v2.0). HBV DNA was sequenced for the first 2 consecutive off-treatment visits with HBV DNA >20 IU/mL by PCR
- In vitro phenotyping assays were conducted for any novel core inhibitor substitution observed. The constructs were made by site-directed mutagenesis in the HBV wild-type (WT) glucosyltransferase domain backbone. Lunet cells were transiently transfected with WT plasmid or constructs with a mutation, then treated with dimethyl sulfoxide, VBR, or entecavir. HBV-replicative DNA in the cell lysate was extracted and used to evaluate the half maximal effective concentration

Sequence analysis results

Table 1. Proportions of patients with core inhibitor substitutions or NrtI resistance mutations during virologic relapse[a]

	HBeAg positive		HBeAg negative		
	Baseline sequencing positive[b]	Baseline sequencing negative[c]	Baseline sequencing positive[b]	Baseline sequencing negative[c]	Total
Core inhibitor substitutions detected	0/15	0/3	7/13	2/10	9/41 (22.0%)
NrtI resistance mutations detected	0/13	1/5	0/9	3/14	4/41 (9.8%)

[a]Sequence evaluations were conducted across core and the pol/RT region, focusing on core inhibitor substitutions (amino acids at positions 23, 24, 25, 29, 30, 33, 37, 38, 105, 106, 109, 110, 118, 124, 125, 127, 128, 132, 133, 134, 140, and 141) and NrtI resistance mutations in the pol/RT region (amino acids at positions 80, 169, 173, 180, 181, 184, 202, 204, 236, and 250).[2-4]
[b]Patients were successfully sequenced at baseline.
[c]Baseline sequence could not be amplified and analyzed successfully, and the HBV genotype A universal reference sequence (NCBI ID X02763) was used as a reference to define amino acid changes detected postbaseline.
HBeAg, hepatitis B "e" antigen; HBV, hepatitis B virus; NrtI, nucleos(t)ide reverse transcriptase inhibitor; pol/RT, polymerase/reverse transcriptase.

- No core inhibitor substitutions or NrtI resistance mutations were detected in the majority of patients (68%) at any timepoint investigated
- No patient had both core inhibitor substitutions and NrtI resistance mutations detected

Table 2. Comparison of baseline demographics and disease characteristics between patients with or without substitutions

	Core inhibitor substitution (n = 9)	NrtI resistance mutation (n = 4)	Without any mutation (n = 28)	Overall (N = 41)
Baseline demographics				
Age, years, mean (SD)	50.4 (6.69)	46.0 (12.11)	44.5 (10.73)	45.9 (10.18)
Male	6 (67)	3 (75)	20 (71)	29 (71)
Asian	6 (67)	4 (100)	22 (79)	32 (78)
BMI, kg/m^2, mean (SD)	25.2 (4.37)	22.9 (1.46)	24.1 (2.80)	24.2 (3.11)
Genotype[a] A	3 (33)	0	5 (18)	8 (20)
B	1 (11)	0	5 (18)	6 (15)
C	1 (11)	1 (25)	4 (14)	6 (15)
D	1 (11)	0	0	1 (2)
B/C	1 (11)	3 (75)	9 (32)	13 (32)
B/C/D	1 (11)	0	0	1 (2)
C/D	1 (11)	0	0	1 (2)
E	0	0	0	0
F	0	0	0	0
G	0	0	1 (4)	1 (2)
Unknown	0	0	-	4 (10)
Duration of NrtI at randomization, years, mean (SD)	5.5 (4.63)	4.0 (7.05)	4.0 (3.87)	4.4 (4.31)
Baseline disease characteristics				
HBV DNA (COBAS) <LLOQ, Target Detected[b]	2 (22)	2 (50)	12 (43)	16 (39)
HBV DNA (Assembly Lab), Target Detected[a]	4 (44)	0	16 (57)	20 (49)
HBV RNA (log_{10} U/mL), mean (SD)[a]	1.7 (0.43)	1.5 (0.00)	2.1 (0.88)	2.0 (0.77)
HBeAg (log_{10} IU/mL), mean (SD)[b]	–1.0 (0.0)	–0.8 (0.25)	–0.4 (0.65)	–0.6 (0.59)
HBsAg (log_{10} IU/mL), mean (SD)[b]	3.2 (0.61)	3.1 (0.66)	3.4 (0.60)	3.3 (0.60)
HBcrAg (log_{10} IU/mL), mean (SD)[c]	0.4 (0.69)	1.1 (0.72)	1.7 (1.10)	1.4 (1.13)
ALT (U/L), mean (SD)[b]	22.8 (16.92)	25.0 (12.57)	28.9 (21.32)	27.2 (19.57)

Data is shown as n (%) unless otherwise specified.
[a]Reported tests are based on Assembly Lab: genotype, HBV pgRNA LLOQ = 35 U/mL.
[b]Reported tests are based on central lab: HBV DNA LLOQ = 20 IU/mL and LOD = 10 IU/mL; HBeAg LLOQ = 0.11 IU/mL; HBsAg LLOQ = 0.05 IU/mL, ALT ULN (Covance) of 34 U/L for female and 43 U/L for male and ULN (AASLD) of 25 U/L for female and 33 U/L for male.
[c]Reported test is based on University of Hong Kong lab: HBcrAg LLOQ = 1 kU/mL.
AASLD, American Association for the Study of Liver Disease; ALT, alanine aminotransferase; BMI, body mass index; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B "e" antigen, HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; LLOQ, lower limit of quantification; LOD, limit of detection; NrtI, nucleos(t)ide reverse transcriptase inhibitor; pg, pregenomic; SD, standard deviation; ULN, upper limit of normal.

- There were no significant demographic and disease characteristic differences between patients with and without core inhibitor substitutions or NrtI resistance mutations

Core inhibitor substitutions

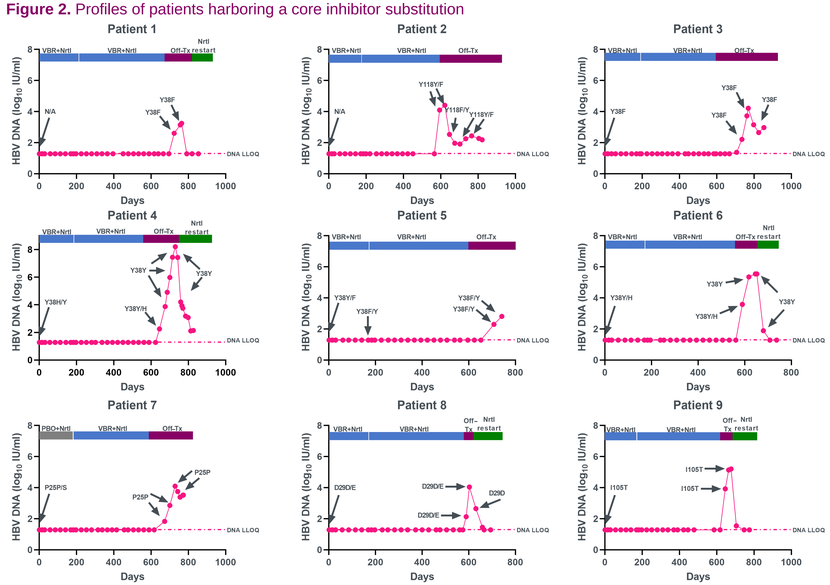
Table 3. Core inhibitor substitutions observed in 9 patients during virologic relapse

Patient	Baseline	Off-treatment 1[a]		Off-treatment 2[b]	
	RNA sequences	DNA sequences	RNA sequences	DNA sequences	RNA sequences
1	No baseline	Y38F	Y38F	Y38F	Y38F
2	No baseline	Y118Y/F	Y118Y/F	Y118Y/F	Y118Y/F
3	Y38F	Y38F	ND[c]	Y38F	ND[c]
4	Y38H/Y	Y38Y/H	Y38H/Y	Y38 WT	Y38Y/H
5	Y38Y/F	Y38F/Y	ND	Y38F/Y	Y38F
6	Y38Y/H	Y38Y/H	Y38Y/H	Y38 WT	Y38 WT
7	P25P/S	P25 WT	ND	P25 WT	P25 WT
8	D29D/E	D29D/E	D29D/E	D29D/E	D29D/E
9	I105T	I105T	I105T	I105T	I105T

[a]First off-treatment visit with HBV DNA >20 IU/mL. [b]The sequential visit after off-treatment 1. [c]RNA sequencing was successful at later timepoints, and the Y38F substitution was detected.
ND, not determinable; WT, wild-type.

- For the 9 patients with a core inhibitor substitution, no enrichment compared to baseline was observed after treatment was discontinued
- Sequencing results using DNA or RNA were consistent

Figure 2. Profiles of patients harboring a core inhibitor substitution



Initial treatment use was in the context of Study 201. Eligible patients enrolled in the open-label extension (Study 211) receiving VBR+NrtI. Patients could be eligible to stop treatment at Week 76. Some off-treatment patients restarted their standard-of-care NrtIs. Arrows point to core inhibitor substitutions in individual patients.
HBV, hepatitis B virus; LLOQ, lower limit of quantification; NrtI, nucleos(t)ide reverse transcriptase inhibitor; Tx, treatment; VBR, vebicorvir.

- For patients with sequence results at baseline, core inhibitor substitutions observed were pre-existing before treatment with VBR
- Additional relapse timepoints were assessed for the 9 patients with core inhibitor substitutions. Following treatment discontinuation, all substitutions were considered as no proportion change or were replaced by WT
- The presence of observed core inhibitor substitutions did not impact HBV DNA resuppression with NrtI restart
- No emergent resistance to VBR was observed

Table 4. Assessment of association between core inhibitor substitutions and DNA relapse

Presence of core inhibitor substitution	Lower viral load[a] (n = 17)	Higher viral load[a] (n = 24)	Total
No	12 (37.5)	20 (62.5)	32
Yes	5 (55.6)	4 (44.4)	9
Total	17	24	41
2-sided *p*-value	Chi-square test		0.331

Data is shown as n (%) unless otherwise specified. [a]Patients were categorized as lower viral load, those who maintained HBV DNA <80,000 (4.9 log_{10}) IU/mL for ≥8 weeks off-treatment, or as higher viral load (maximum HBV DNA ≥80,000 (4.9 log_{10}) IU/mL or restarted NrtI before 8 Weeks off-treatment).
HBV, hepatitis B virus; NrtI, nucleos(t)ide reverse transcriptase inhibitor.

- The presence of core inhibitor substitutions did not impact the ability to control viremia off-treatment

In vitro phenotyping assay

Table 5. Activity of VBR against HBV core inhibitor substitutions

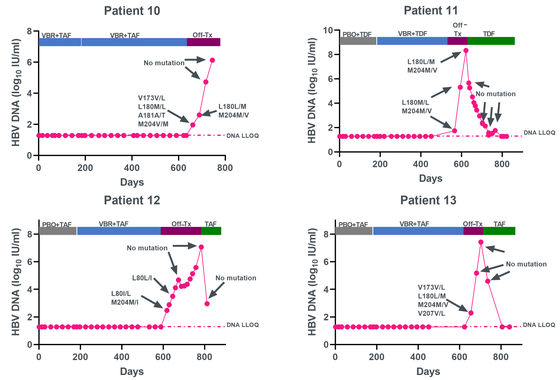
Core inhibitor substitution	Prevalence[a]	Fitness	VBR EC_{50} (nM)[b]	VBR Fold shift	ETV EC_{50} (nM)	ETV Fold shift
WT			146 ± 21		1.9 ± 0.9	
P25S	0.1%	38%	253 ± 137	1.7	2.7 ± 0.9	1.4
D29E	0.5%	22%	82 ± 17	0.6	1.7 ± 0.5	0.9
Y38F	3.0%	55%	481 ± 34	3.3	4.6 ± 0.6	2.4
Y38H	1.1%	12%	492 ± 80	3.4	1.9 ± 0.2	1.0
I105T	0.6%	52%	324 ± 20	2.2	0.5 ± 0.3	0.4
Y118F	0.3%	9%	2070 ± 264	14.2	1.5 ± 0.9	0.8

[a]12294 HBV genome sequences archived as of April 19, 2021, at https://hbvdb.lyon.inserm.fr/HBVdb/.
[b]Mean EC_{50} values for intracellular rcDNA inhibition (n ≥2 ± SD).
EC_{50}, half maximal effective concentration; ETV, entecavir; HBV, hepatitis B; rc, relaxed circular; SD, standard deviation; VBR, vebicorvir; WT, wild-type.

- In vitro phenotyping assay demonstrated that most of the core inhibitor substitutions observed had minimal (<3.5× fold) changes in sensitivity to VBR compared with WT

NrtI resistance mutations

Figure 3. Profiles of patients with NrtI resistance mutations



Initial treatment use was in the context of study 201. Eligible patients enrolled in the extension study receiving VBR+NRTI. Patients could be eligible to stop treatment at Week 76. Some off-treatment patients restarted their standard of care NrtIs. Arrows point to core inhibitor substitutions in individual patients.
HBV, hepatitis B virus; LLOQ, lower limit of quantification; NrtI, nucleos(t)ide reverse transcriptase inhibitor; PBO, placebo; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; Tx, treatment; VBR, vebicorvir.

- NrtI resistance mutations were detected off-treatment in 4 patients (1 HBeAg positive and 3 HBeAg negative patients); all patients were missing baseline sequences
- Detected NrtI resistance mutations were known to be associated with lamivudine, adefovir, or telbivudine treatment but not with the NrtI the patient was taking in this study
- Observed mutations did not impact HBV DNA resuppression with NrtI restart
- After treatment discontinuation, all NrtI resistance mutations reverted toward WT

Conclusions

- The majority of patients had no core inhibitor substitutions
- No emergence or enrichment of core inhibitor substitutions was observed

References

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