Greater sequence diversity during early hepatitis B virus decline on vebicorvir plus entecavir is associated with a lower level of virus rebound following switch to entecavir monotherapy

THU-346

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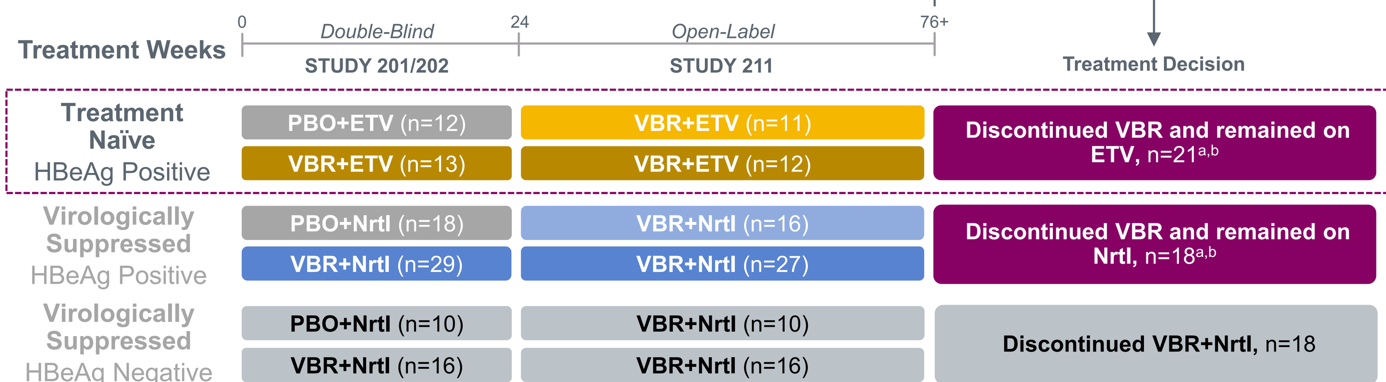
Introduction

- Core inhibitors are a novel class of hepatitis B virus (HBV) direct-acting antivirals, with multiple mechanisms of action and the potential to increase both on-treatment responses and cure rates
- Vebicorvir (VBR) is an investigational, first-generation core inhibitor being developed for the treatment of chronic HBV infection (cHBV)
  - VBR has demonstrated potent antiviral activity in Phase 1 clinical studies[1] and additive antiviral activity when combined with nucleos(t)ide reverse transcriptase inhibitors (NrtIs) compared to NrtIs alone in Phase 2 studies[2-5]
- In Study 202 (NCT03577171), treatment-naïve hepatitis B e antigen (HBeAg) positive cHBV patients were randomized to VBR + entecavir (ETV) or placebo (PBO) + ETV for 24 weeks
  - Eligible patients went on to receive open-label VBR+ETV in Study 211 (NCT03780543) for ≥52 weeks (**Figure 1**)
- In Study 211, 19 patients from Study 202 discontinued VBR and remained on ETV at end of study with sample data available. Following VBR discontinuation, a mean HBV DNA increase of 1.0 log_{10} IU/mL was observed, with 11/19 patients having a maximal increase of ≥1 log_{10} IU/mL, consistent with deeper suppression of viral replication when core inhibitors are combined with NrtIs[6]

Figure 1. Study design and treatment decisions



[a]Data available for 19 TN and 18 VS patients following VBR discontinuation. [b]Excluded patients who did not meet VBR stopping criteria or were terminated from the study.
ETV, entecavir; HBeAg, hepatitis B e antigen; NrtI, nucleos(t)ide reverse transcriptase inhibitor; PBO, placebo; TN, treatment naïve; VBR, vebicorvir; VS, virologically suppressed.

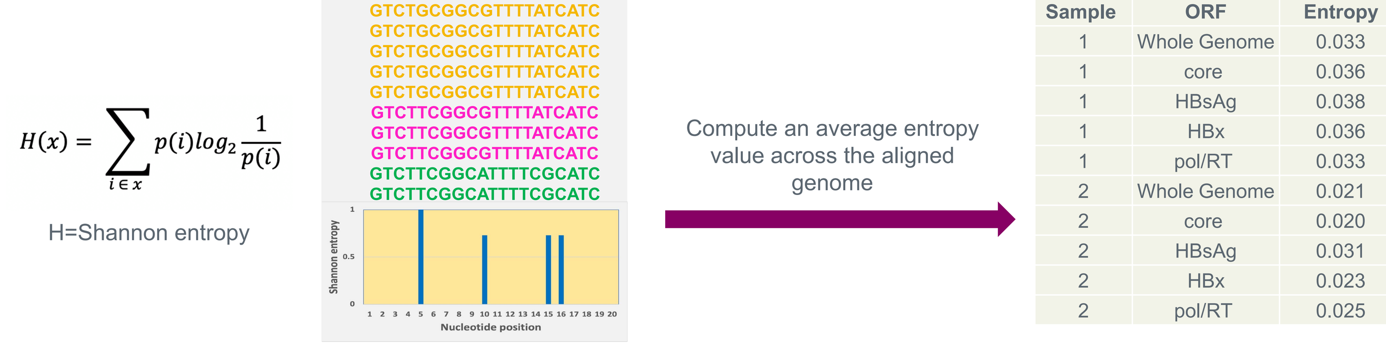
Objective

- To investigate the role of sequence diversity in viral rebound, during HBV decline in patients who discontinued VBR and remained on ETV in Studies 202/211, by ad hoc next-generation sequencing (NGS)-based analysis

Methods

- Viral nucleic acids were purified from Study 202 patient plasma samples collected at Baseline, Week 4, and Study 211 rebound (if possible), and whole genome NGS (Illumina MiSeq) was conducted
- 18/19 patients had NGS data available: 11 with ≥1 log_{10} IU/mL HBV DNA maximal increase and 7 with <1 log_{10} IU/mL HBV DNA maximal increase after VBR discontinuation

Figure 2. Shannon entropy calculation



HBsAg, hepatitis B surface antigen; HBx, hepatitis B virus X protein; pol/RT, polymerase reverse transcriptase; ORF, open reading frame.

- To determine sequence diversity, Shannon entropy[7] was calculated from demultiplexed FASTQ read files using an in-house pipeline with genotype-specific reference sequences (**Figure 2**)
- HBV whole genome nucleotide sequences for reference genotypes were obtained from the National Center for Biotechnology Information with the following accessions: genotype A (X02763), B (AB219428), and C (GQ358158)
- The pipeline includes data quality control, read trimming and alignment, variant calling, and variant annotation in a workflow orchestrated by Nextflow running on Amazon Cloud Service

Results

Table 1. Study 202 patients who had NGS data available and discontinued VBR+ETV by maximum DNA increase following VBR discontinuation

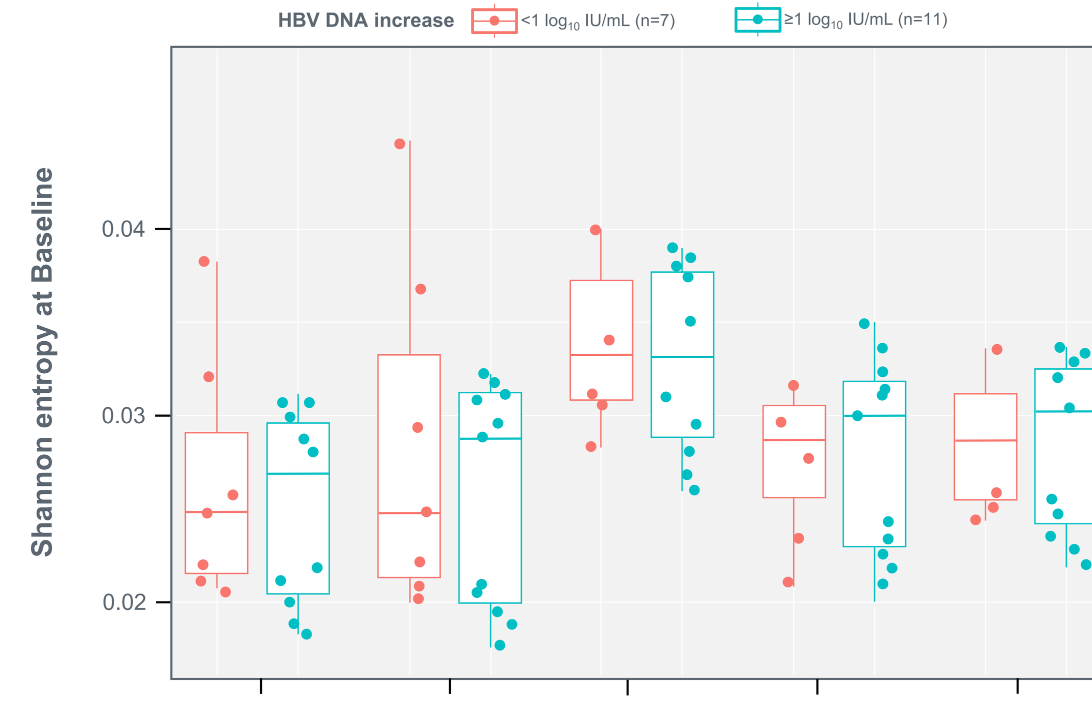
Parameter[a]	Max DNA increase ≥1 log_{10} IU/mL (n=11)	Max DNA increase <1 log_{10} IU/mL (n=7)	*P*-value[b]
HBV DNA (BL), log_{10} IU/mL	8.3	7.6	0.42
HBV DNA (Week 24), log_{10} IU/mL	3.4	1.8	0.06
Maximum HBV DNA increase, log_{10} IU/mL	1.8	0.3	**<0.01**
HBV pgRNA (BL), log_{10} U/mL	7.6	6.2	0.15
HBeAg (BL), log_{10} IU/mL	3.0	1.6	**0.01**
HBcrAg (BL), log_{10} kU/mL	5.8	5.2	**0.01**
HBsAg (BL), log_{10} IU/mL	4.8	4.3	0.07
HBsAg (change from BL to Week 24), log_{10} IU/mL	−0.1	−0.2	0.79
ALT >ULN (BL), n (%)	3 (27)	5 (71)	0.14
Genotype A/B/C, n	0/6/5	1/1/5	—
Treatment in Study 202: VBR+ETV/PBO+ETV	6/5	4/3	—

[a]All parameters are median values unless otherwise specified. [b]As determined by parametric t-tests, except for ALT >ULN, where Fisher's test was used.
ALT, alanine aminotransferase; BL, Baseline; ETV, entecavir; HBeAg, hepatitis B e antigen; HBcrAg, hepatitis B core-related antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NGS, next-generation sequencing; PBO, placebo; pgRNA, pregenomic RNA; ULN, upper limit of normal; VBR, vebicorvir.

Results

- The subgroup of patients with maximal <1 log_{10} IU/mL HBV DNA increase following VBR discontinuation
  - Had lower Baseline HBeAg and hepatitis B core-related antigen (HBcrAg) (*P*<0.05)
  - Trended lower for HBV DNA, pregenomic RNA (pgRNA), and hepatitis B surface antigen (HBsAg)
  - The majority had ALT >upper limit of normal (ULN) at Baseline
  - Had a 0.2 log decrease in HBsAg at Week 24 in contrast to a 0.1 log decrease in the subgroup of patients with maximal ≥1 log_{10} IU/mL HBV DNA increase following VBR discontinuation (**Table 1**)

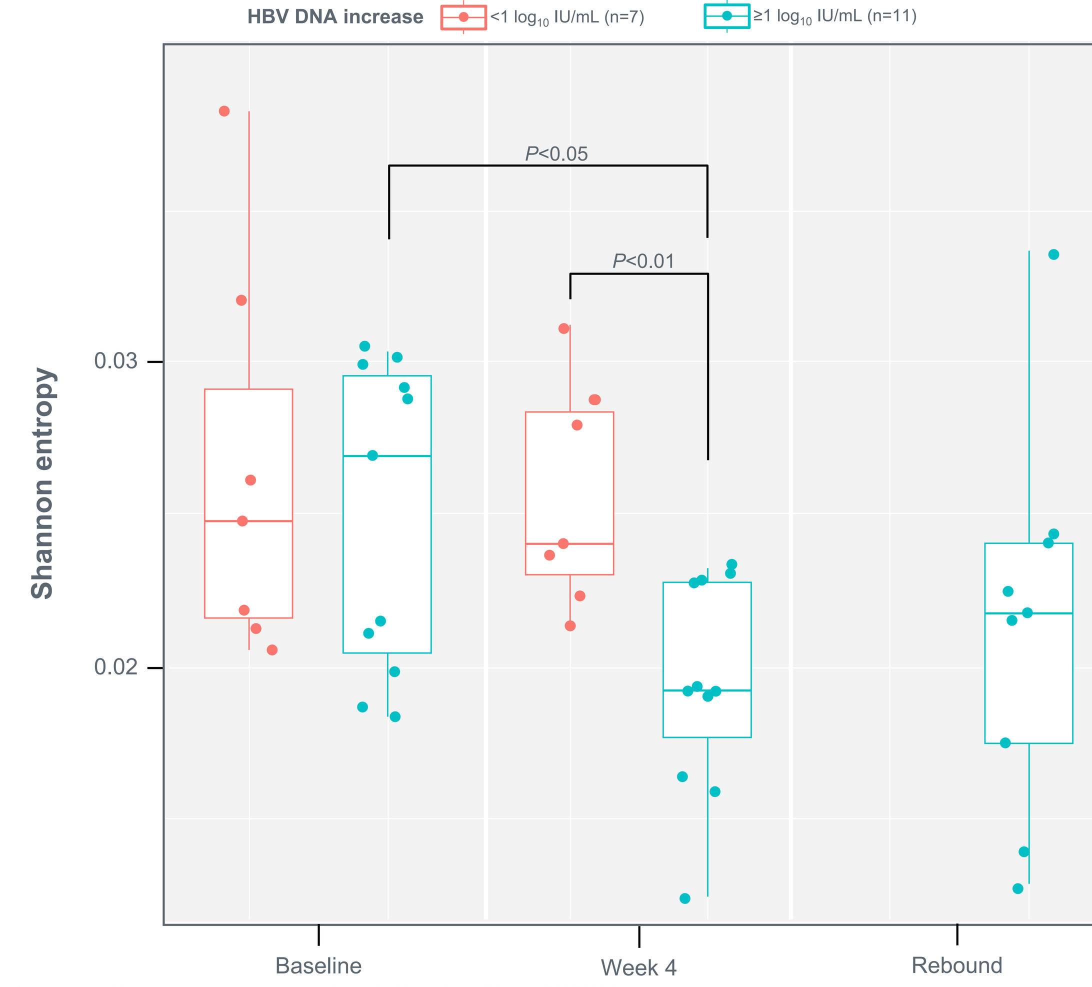
Figure 3. HBV nucleotide sequence diversity at Baseline by maximum HBV DNA increase after VBR discontinuation



HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBx, hepatitis B virus X protein; pol/RT, polymerase reverse transcriptase; VBR, vebicorvir.

- At Baseline, similar diversity levels were observed across the viral genome in both subgroups, with the highest and lowest diversity observed in the open reading frame (ORF) corresponding to the surface antigen and core protein, respectively (**Figure 3**)

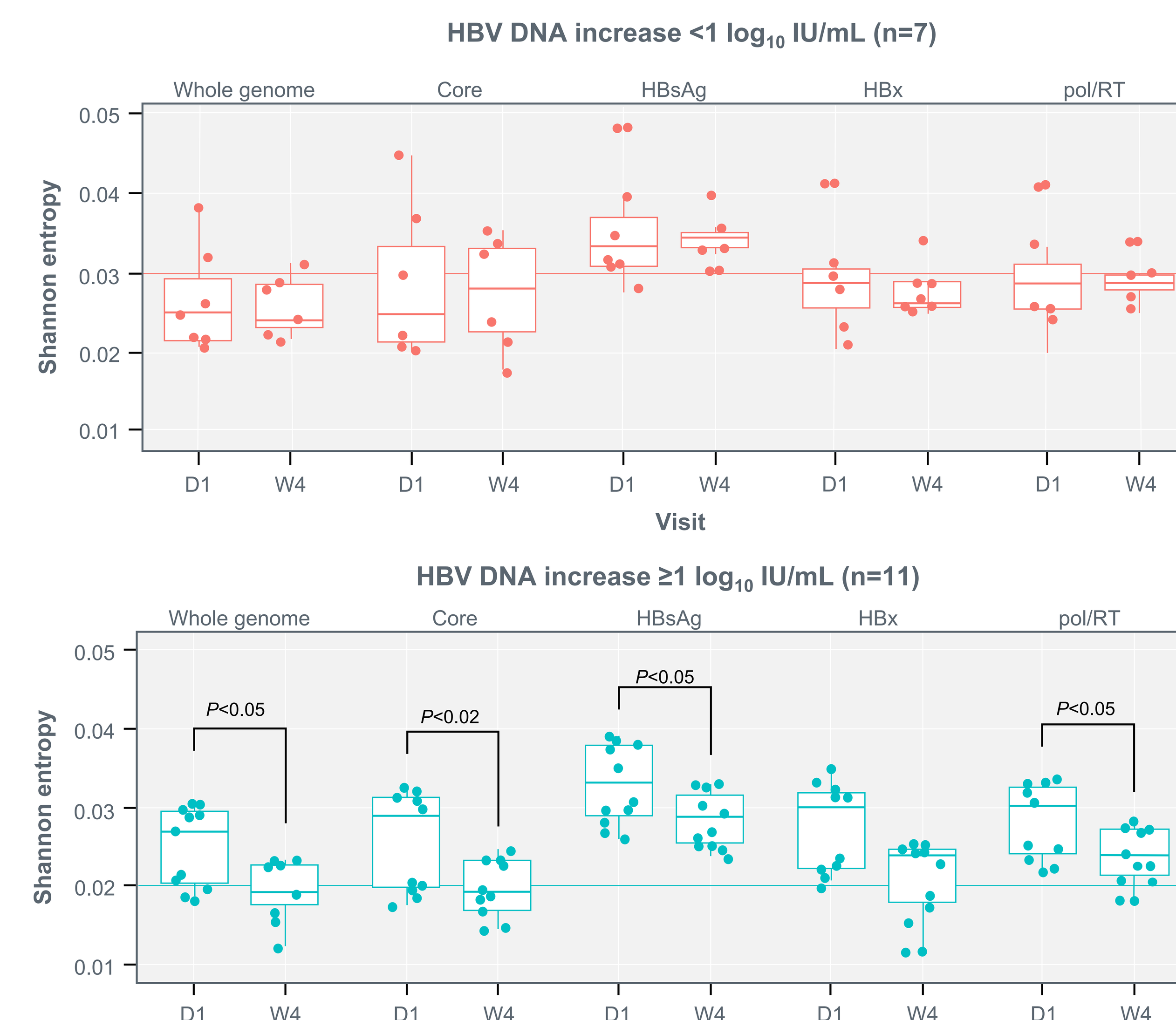
Figure 4. HBV whole genome nucleotide sequence diversity during initial HBV decline and after VBR discontinuation by maximum HBV DNA increase after VBR discontinuation



We were unable to sequence patients at rebound with <1 log_{10} IU/mL HBV DNA increases.
HBV, hepatitis B virus; VBR, vebicorvir.

- At Week 4, diversity remained stable for patients with <1 log_{10} IU/mL HBV DNA increases following VBR discontinuation, while diversity declined at Week 4 for patients with ≥1 log_{10} IU/mL HBV DNA increases following VBR discontinuation (*P*<0.05) (**Figure 4**)
- For the 9 of 11 patients with available NGS data and ≥1 log_{10} IU/mL HBV DNA increase after VBR discontinuation, diversity returned in the direction toward Baseline levels at rebound, which occurred 4 to 12 weeks after VBR discontinuation (**Figure 4**)

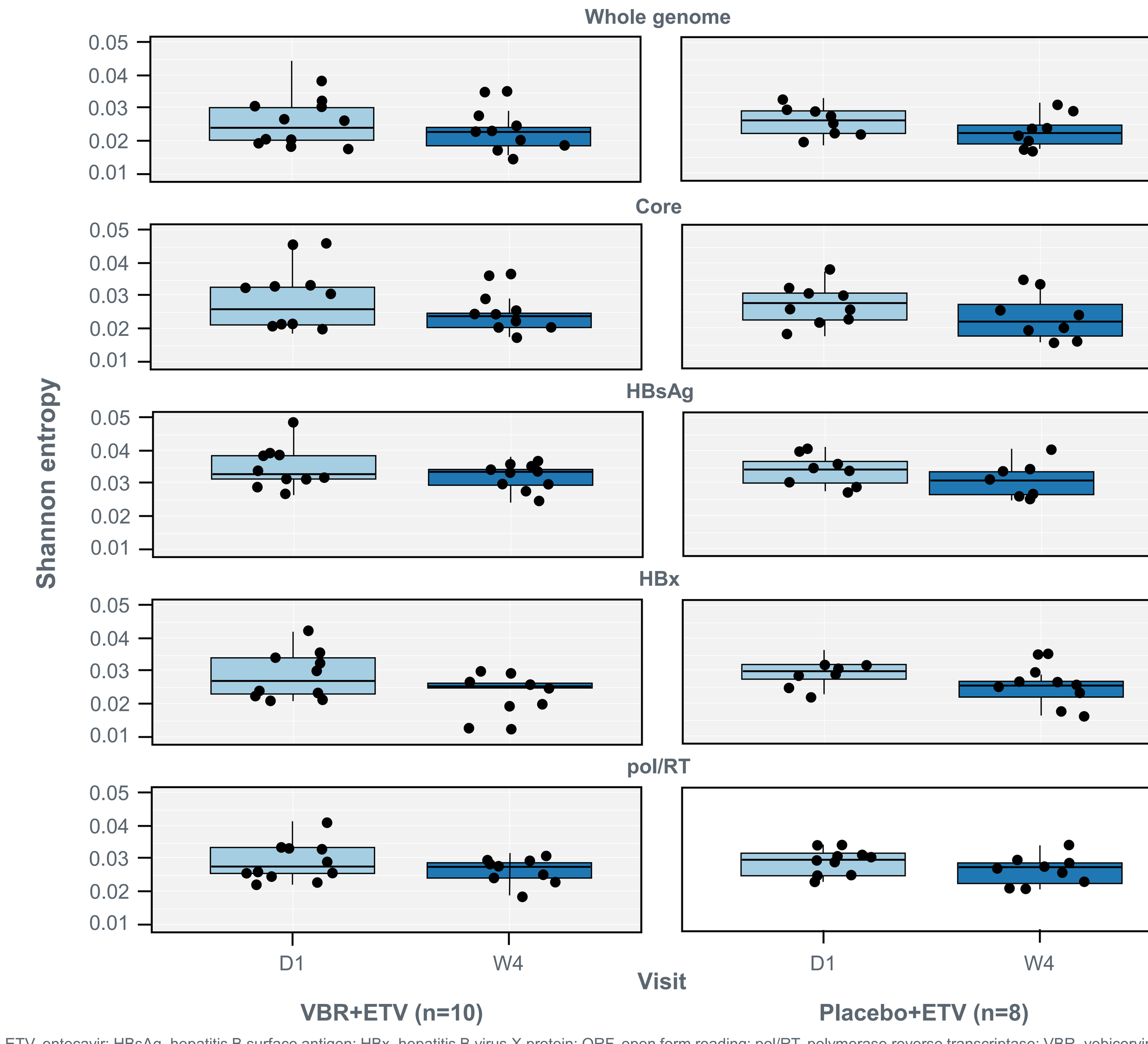
Figure 5. HBV genome nucleotide sequence diversity across the different ORFs along with HBV DNA increase after VBR discontinuation



Statistical significance was estimated by the Wilcoxon non-parametric test.
HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBx, hepatitis B virus X protein; pol/RT, polymerase reverse transcriptase; VBR, vebicorvir.

- Decreases in sequence diversity from Baseline to Week 4 were observed in patients with ≥1 log_{10} IU/mL HBV DNA increases following VBR discontinuation, achieving statistical significance (*P*<0.05) in all ORFs except hepatitis B virus X protein (HBx)
- Diversity remained stable across all ORFs in patients with <1 log_{10} IU/mL HBV DNA increases following VBR discontinuation (**Figure 5**)

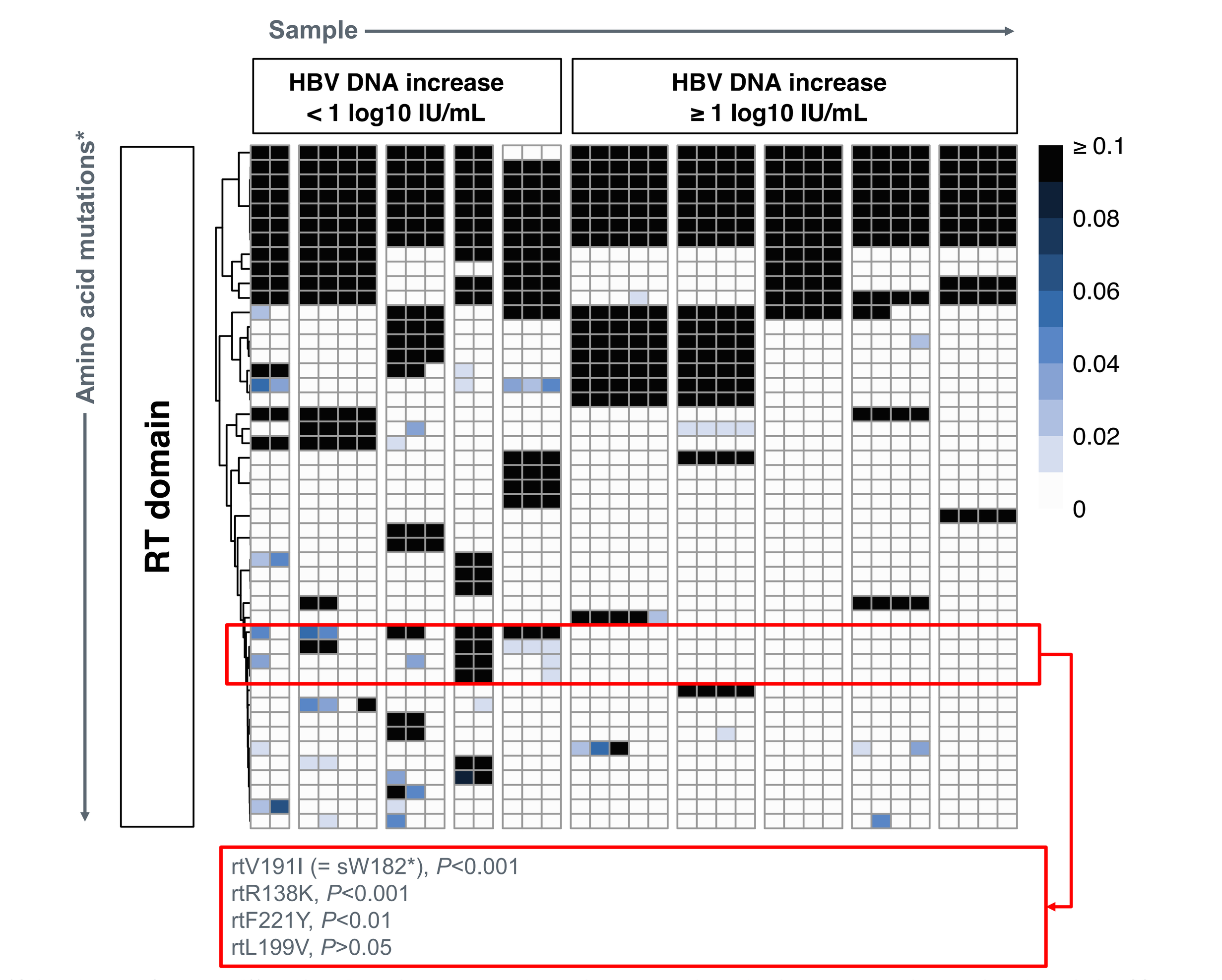
Figure 6. HBV nucleotide sequence diversity across the different ORFs of the HBV genome along with VBR+ETV or PBO+ETV treatment



ETV, entecavir; HBsAg, hepatitis B surface antigen; HBx, hepatitis B virus X protein; ORF, open form reading; pol/RT, polymerase reverse transcriptase; VBR, vebicorvir.

- At Baseline and Week 4, no significant difference in sequence diversity was observed between VBR+ETV- and PBO+ETV-treated patients across the HBV genome (**Figure 6**)

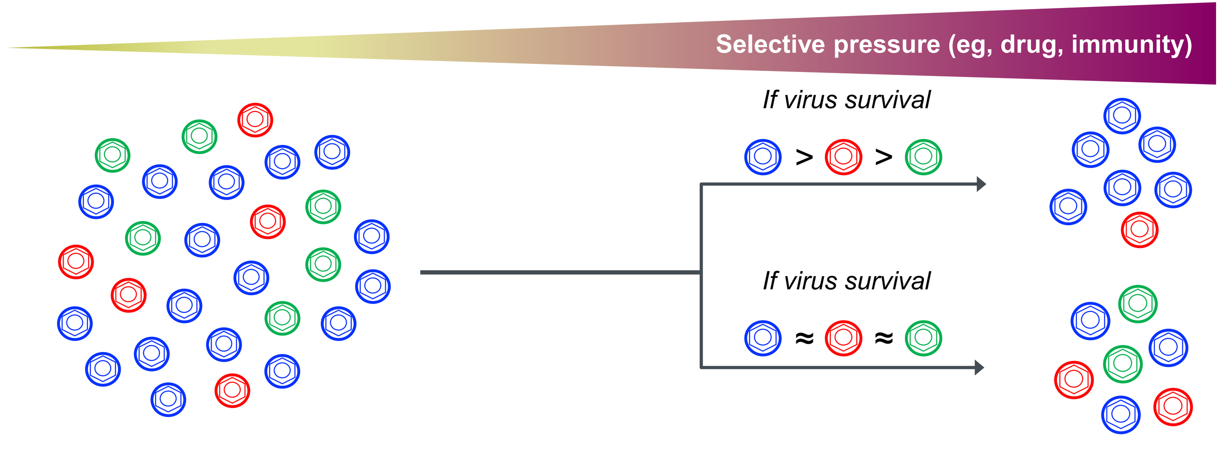
Figure 7. Allele frequency of amino acid mutations in pol/RT found among patients infected with HBV genotype C (n=10) by maximum HBV DNA increase after VBR discontinuation



*Only mutations at frequency ≥2% in at least 2 samples were used in this analysis, which included Baseline and postbaseline samples with available NGS data.
HBV, hepatitis B virus; pol/RT, polymerase reverse transcriptase; RT, reverse transcriptase; VBR, vebicorvir.

- In genotype C, which was the only genotype well-represented across both groups (**Table 1**), a selected set of amino acid mutations in the polymerase-reverse transcriptase domain was consistently observed in the subgroup with <1 log_{10} IU/mL HBV DNA increases but was not detected in the subgroup with ≥1 log_{10} IU/mL HBV DNA increases after VBR discontinuation (**Figure 7**)
  - The detailed biological roles played by these mutations in how HBV may respond to various drugs, including NrtIs, remain to be elucidated
- No core inhibitor binding pocket substitutions were identified by NGS at any time point sequenced, including time points after VBR discontinuation
- One NrtI resistance mutation (rtM204I) associated with ETV resistance was observed at 12 weeks post-treatment follow-up in a single patient who had exposure to ETV

Figure 8. Quasispecies in response to selective pressure



- Taken together, our results were consistent with the hypothesis that HBV survival against selective pressure was influenced by multiple factors, including viral load, replication rate, genetic heterogeneity, and viral fitness, emphasizing the relevance of quasispecies for viral pathogenesis and possible host/viral responses to drug treatments (**Figure 8**)

Conclusions

- Decreases in sequence diversity were observed during initial HBV DNA decline during HBV treatment among patients with higher rebound when VBR was discontinued and ETV continued
- The early differences in sequence diversity may reflect differences in HBV-specific immune responses (also reflected in ALT levels) and viral load set points between these 2 groups, although the mechanism remains to be further defined
- NGS is a useful tool to study the quasispecies change in cHBV patients undergoing antiviral therapy

References: **1.** Yuen M-F, et al. *Lancet Gastroent Hep.* 2020;5:152–166. **2.** Fung SK, et al. Oral presentation at EASL: Aug 27–29, 2020. **3.** Yuen M-F, et al. Poster presentation at EASL: Aug 27–29, 2020. **4.** Ma X, et al. Oral presentation at EASL: April 10–14, 2019. **5.** Jacobson I, et al. Poster presentation at the Liver Meeting: Nov 13–16, 2020. **6**. Yuen M-F et al. Oral presentation at the Liver Meeting 2021; Nov 12–15, 2021. **7.** Shannon CE. *MD Comput*. 1997;14:306–17.

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