Changes in Viral Antigens are More Strongly Associated with HBV pgRNA than HBV DNA in Studies of Vebicorvir and Nrtl in Treatment-naïve Patients with Chronic HBV Infection

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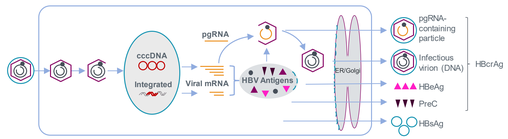
Background

- Worldwide, ~250 million people are chronically infected with hepatitis B virus (HBV), and 600,000 to 1 million die each year due to cirrhosis and hepatocellular carcinoma associated with chronic hepatitis B virus infection (cHBV)[1-4]; of the ~94 million patients eligible for treatment, only ~4.8 million (5%) receive antiviral therapy[5]
- Pregenomic RNA (pgRNA) plays an integral role in HBV replication (**Figure 1**) and provides an indirect assessment of the level of covalently closed circular (ccc)DNA transcriptional activity and number of productively infected cells
- The presence of pgRNA is associated with persistent viral infection and a higher risk of development of hepatocellular carcinoma and of relapse following cessation of nucleos(t)ide reverse transcriptase inhibitor (NrtI) therapy[6–8]
- To understand the correlations between changes in levels of HBV DNA and pgRNA with those of HBV antigens, post hoc statistical analyses were performed using clinical data from studies of vebicorvir (VBR; ABI-H0731), a novel first-generation inhibitor of HBV core protein
- Core inhibition leads to two-phase decline in pgRNA, an initial 2-$\log_{10}$ decline related to direct effect on encapsidation and a second phase believed to reflect reduction in cccDNA pools

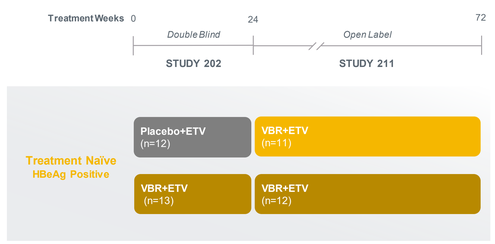
Figure 1. HBV Replication Cycle, pgRNA and HBV Antigens



cccDNA, covalently closed circular DNA; ER, endoplasmic reticulum; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B; mRNA, messenger RNA; pgRNA, pregenomic RNA; PreC, pre-core protein.

- Viral messenger RNA from cccDNA and integrated DNA are translated into viral proteins (including polymerase, core protein, hepatitis B e antigen [HBeAg], hepatitis B core-related antigen [HBcrAg], hepatitis B surface antigen [HBsAg])
- pgRNA, which is derived from cccDNA only, is incorporated into new nucleocapsids (along with polymerase and core), which can be secreted as pgRNA-containing particles

Figure 2. Design of Study 202 and Study 211



ETV, entecavir ; HBeAg, hepatitis e antigen; VBR, vebicorvir.

Methods

- Available clinical data through 72 weeks of treatment were evaluated from the Phase 2 Study 202 (NCT03577171) and Study 211 (NCT03780543) in treatment-naïve patients with HBeAg positive cHBV (**Figure 2**)[9]
- HBV virology assessments were based on the following assays:
  - HBV DNA: COBAS TaqMan (2.0), lower limit of quantification (LLOQ)=20 IU/mL
  - pgRNA: Assembly assay, LLOQ=135 U/mL
  - HBeAg: Abbott, LLOQ=0.11 IU/mL
  - HBcrAg: FujiRebio, LLOQ=1 kU/mL
  - HBsAg: Abbott, LLOQ=0.05 IU/mL
- Two approaches were taken to explore the correlations between HBV DNA, pgRNA, and HBV antigens:

1. Correlation Analysis with Pearson's Coefficient

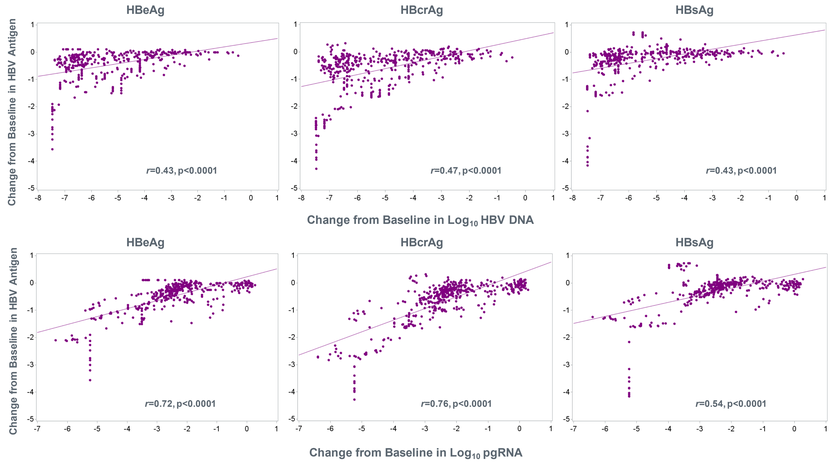
- Scatter plot analysis with Pearson's correlation coefficient
  - All patients who enrolled in Study 202/211 up to Week 72
  - All patients who received VBR+ETV in Study 202/211 up to Week 72 by magnitude of pgRNA decline (ie, ≤2 $\log_{10}$ vs >2 $\log_{10}$ pgRNA decline)
  - A correlation is considered high if the *r* value falls between 0.5 and 1, moderate if the *r* value is between 0.3 and 0.49, and low if the *r* value is less than 0.29

2. Correlation Analysis with Mixed-Effects Model for Repeated Measures (MMRM)

- A regression model to assess the linear relationship between the changes from baseline in $\log_{10}$ HBV DNA and pgRNA vs each HBV antigen (HBeAg, HBcrAg, HBsAg) accounting for other covariates and repeated measures including all patients (Model 1) and patients receiving VBR+ETV (Model 2) who enrolled in Study 202/211 up to Week 72
- Model 1 covariates: baseline $\log_{10}$ HBV antigen, change from baseline in $\log_{10}$ HBV DNA, change from baseline $\log_{10}$ pgRNA, and study visit
- Model 2: same covariates as Model 1 plus the magnitude of pgRNA decline (ie, ≤2 $\log_{10}$ vs >2 $\log_{10}$ pgRNA decline) and its interaction with the change from baseline in $\log_{10}$ pgRNA
  - Due to sample size limitations, a Toeplitz covariance structure was assumed for the model to converge

Results

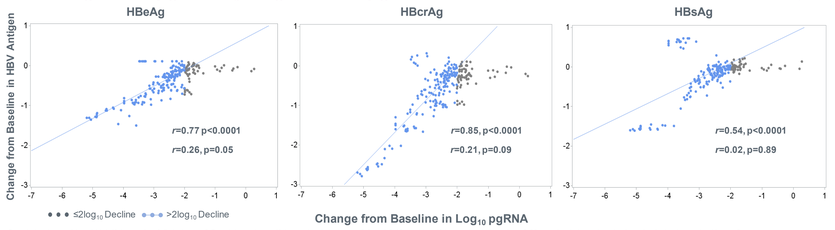
Figure 3. Correlation Analysis with Pearson's Coefficient
HBV Antigens vs HBV DNA or pgRNA (All Patients up to Week 72)



Correlation is considered high if the *r* value falls between 0.5 and 1, moderate if the r value is between 0.3 and 0.49, and low if the r value is less than 0.29. p-value is based on the hypothesis testing under H_0: ρ = 0.
HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; pgRNA, pregenomic RNA.

- Correlations between decline in HBV DNA and each HBV antigen were moderate
- Decline in pgRNA was highly correlated with decline in both HBeAg and HBcrAg, but less correlated with HBsAg

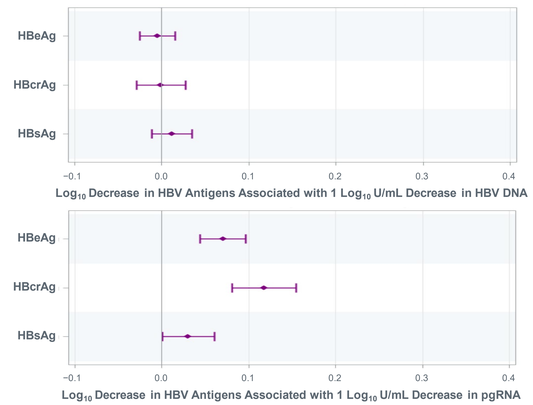
Figure 4. Correlation Analysis with Pearson's Coefficient
HBV Antigens vs pgRNA by Magnitude of pgRNA Decline (VBR+ETV Patients up to Week 72)



Correlation is considered high if the *r* value falls between 0.5 and 1, moderate if the r value is between 0.3 and 0.49, and low if the r value is less than 0.29. p-value is based on the hypothesis testing under H_0: ρ = 0.
ETV, entecavir; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; pgRNA, pregenomic RNA; VBR, vebicorvir.

- Greater correlations were observed with the >2 $\log_{10}$ pgRNA decline compared with those with the ≤2 $\log_{10}$ pgRNA decline
- All the correlations with >2$\log_{10}$ pgRNA decline were high (*r*>0.5, p-value<0.0001)
- All the correlations with ≤2$\log_{10}$ pgRNA decline were low (*r*<0.3, p-value≥0.05)

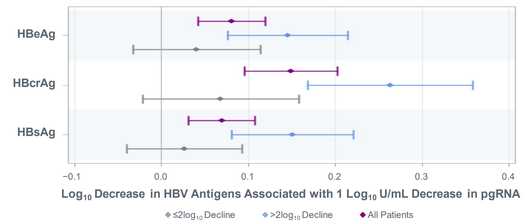
Figure 5. Correlation Analysis with MMRM
Model 1 (All Patients up to Week 72)



Reported parameter estimates and CIs are based on MMRM model.
CI, confidence interval; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; MMRM, mixed-effects model for repeated measures; pgRNA, pregenomic RNA.

- Reductions in HBV DNA do not predict statistically significant reductions in HBV antigens
- Reductions in pgRNA predict statistically significant reductions in HBV antigens (p<0.0001 for HBeAg and HBcrAg; p=0.045 for HBsAg, lower bounds of the 95% confidence interval exceed 0)

Figure 6. Correlation Analysis with MMRM
Model 2 (Adjusting for Magnitude of pgRNA Decline: VBR+ETV Patients up to Week 72)



Reported parameter estimates and CIs are based on MMRM model.
CI, confidence interval; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; MMRM, mixed-effects model for repeated measures; pgRNA, pregenomic RNA.
Due to the convergence issue, the covariate, change in $\log_{10}$ HBV DNA, was not included in the MMRM model for all patients.

- Magnitude of pgRNA decline is a significant factor of the prediction; reductions >2 $\log_{10}$ in pgRNA were a stronger predictor for decline in HBV antigens than reductions ≤2 $\log_{10}$

Conclusions

- Data from ongoing Phase 2 clinical studies with VBR analyzed by two distinct statistical approaches show that the changes in HBV antigens are more strongly associated with the change in pgRNA compared with the change in HBV DNA
- The correlations between pgRNA and HBeAg and HBcrAg were greater relative to the correlations with HBsAg, likely due to the substantial contribution of HBV integrants to HBsAg levels (whereas pgRNA, HBcAg, and HBcrAg are known to arise only from cccDNA)
- A >2 $\log_{10}$ decline in pgRNA in patients receiving VBR+ETV more significantly predicted the decline in the HBeAg and HBcrAg consistent with the second phase decline with core inhibitor treatment reflecting reduction in cccDNA pools
- These results demonstrate the importance of pgRNA as a key biomarker for cHBV

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1) European Association for the Study of the Liver. *J Hepatol*. 2017;67:370-98; **2)** World Health Organization. Global Hepatitis Report. 2017; **3)** El-Serag HB et al. *Gastroenterology*. 2012;142(6):1264-73; **4)** Colvin, HM & Mitchell, AE. National Academies Press. 2010; **5)** The Polaris Observatory Collaborators. *Lancet Gastroenterol*. 2018;3:383-403; **6)** Halgand B et al. *Hepatology*. 2018;67(1):86-96. **7)** Mak et al. AASLD 2020 Poster. **8)** Wang J et al. *J Hepatol*. 2016;65(4):700-10. **9)** Yuen M-F et al. EASL 2020 Poster.

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