Nonclinical phenotypic evaluation of clinically identified baseline and treatment-emergent hepatitis B Virus variants that contain single nucleotide polymorphisms in the bepirovirsen binding site

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Background and Aims: Bepirovirsen (GSK3228836, BPV) is an antisense oligonucleotide under Phase 3 investigation for treatment of Chronic Hepatitis B. BPV targets a highly conserved 20 nucleotide sequence common among HBV pgRNA and mRNA transcripts, where HBx and HBV pol open reading frames overlap. SNPs in the BPV binding site of HBV genomes from B-Clear Phase 2b (NCT04449029) samples were previously reported among a minority of pre- and post-treatment isolates. Clinically identified baseline (BL) HBV variants were previously evaluated nonclinically for their susceptibility to the GalNAc-conjugated form of BPV, GSK3389404, in primary human hepatocytes (PHH) and to BPV in the case of HBV variant C9A using the RNA Launch model. In this study, we evaluated the susceptibility of BL variants (C9A, T10A, T10G, C20T) and treatment emergent (TE) variants (C9A, G8A, C15T) to BPV in PHH. HBV variant fitness and susceptibility to BPV compared with WT HBV were assessed by the levels of secreted HBsAg and HBeAg.

Method: HBV variant stocks containing clinically identified SNPs in the BPV binding site were generated by transfection of HepG2 cells with 1.3X HBV variant genome plasmids. PHH were infected with WT or HBV variant stocks for 7 days, followed by BPV treatment every 3-4 days totaling 4 sequential doses. After 15 days of BPV treatment, culture supernatant was collected for HBsAg and HBeAg quantification. The susceptibility of HBV variants to BPV compared with WT HBV was determined using dose-response analysis. Mid and high fitness variants were defined as those displaying HBsAg and HBeAg levels of 20-60% or >60% relative to WT HBV, respectively.

Results: HBV variant fitness and susceptibility to BPV were largely consistent between HBsAg and HBeAg readouts. HBV variants exhibited varying levels of fitness, with C9A, T10A, T10G, C20T being high fitness variants, and G8A and C15T being low fitness variants compared with WT HBV. C9A exhibited the lowest susceptibility to BPV compared to WT HBV (about 4.1-fold and 3.6-fold reduction in EC50 as indicated by secreted HBsAg and HBeAg levels, respectively). T10G exhibited approximately 2-3-fold reduction in susceptibility across both readouts. C15T and G8A exhibited approximately 2-3-fold reduction as determined by HBsAg levels and nearly 2-fold reduction as determined by HBeAg levels. T10A and C20T exhibited less than 2-fold reduction across HBsAg and HBeAg readouts.
**Conclusion:** Using our two-step system of HepG2 and PHH, we evaluated the susceptibility of clinically identified HBV BL and TE variants containing SNPs in the BPV site to BPV. The biological basis for varying levels of fitness of each variant, through potential impact of SNPs on HBx and HBV DNA polymerase function, is under evaluation. While the frequency of these HBV variants is very low, the relevance of the *in vitro* phenotypic data to clinical outcome is subject to further investigation.