Background and Aims: B-Together assessed efficacy and safety of sequential bepirovirsen (BPV), an unconjugated antisense oligonucleotide, followed by pegylated interferon (PegIFN) in participants (pts) with chronic hepatitis B virus (HBV) infection. Previously, we demonstrated that pts treated with BPV may experience transient alanine transaminase (ALT) increases. We investigated the role of BPV’s immune mechanism of action (MoA) with respect to virological response (VR) and surrogate markers associated with hepatocyte cell death using peripheral longitudinal biomarker exploratory analysis.

Method: B-Together was a Phase 2b trial in 108 pts on stable nucleos(t)ide analogues. Pts were randomised (1:1) to receive BPV for 12 or 24 weeks followed by up to 24 weeks of PegIFN. The primary endpoint was the proportion of pts with hepatitis B surface antigen and HBV DNA below the limit of detection for 24 weeks after end of treatment (tx). Here, peripheral blood mononuclear cells, blood, and serum samples were subjected to flow cytometry, whole blood transcriptomics (WBT) and proteomics analyses, respectively. Relative expression was measured at baseline (BL) and post-BL at multiple timepoints during the BPV, PegIFN and off-tx phases. To determine differential expression, multivariate models were fit that included tx arms and VR subgroups.

Results: By Week 3, BPV led to a significant increase from BL in mean expression of serum proteins, including several cytokines, independent of arms or VR subgroups. These proteins showed enrichment in immune effector response and apoptotic pathways. After 4 weeks of BPV, upward trends in proliferating activated CD8+ T-cells and B-cells were observed with a significant increase in mean expression from BL of some genes associated with proliferation in WBT independent of arms or response subgroups. After 7 weeks of BPV tx, a subset of proteins including liver and apoptosis-specific proteins showed increased abundance in serum and were more pronounced in BPV responders. Abundance of these proteins was highly correlated with ALT levels, which in turn was sometimes associated with transient low level HBV DNA elevations in serum; indirectly linking the observation to infected hepatocyte death.

Conclusion: BPV led to activation of the immune response in treated pts, providing support for an immune MoA. Three observations provide indirect evidence of a role for BPV in infected hepatocyte
death: 1) presence of proliferating, activated adaptive immune cells in all response subgroups before rise in ALT; 2) increases in several liver and apoptosis-specific proteins in the serum concomitant with ALT elevations; 3) intermittent increase in HBV DNA during ALT elevations occurring more frequently in BPV responders than non-responders. Work is ongoing to further elucidate the multiple MoA of BPV and their roles in HBV functional cure.

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