Bepirovirsen immune mechanism of action may potentiate infected hepatocyte killing: Indirect evidence from B-Together peripheral longitudinal biomarker analysis

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Conclusions

Two key findings support the hypothesis that bepirovirsen treatment leads to the modulation of the immune response and subsequent killing of infected hepatocytes in chronic HBV infection:

- Bepirovirsen appears to induce immune responses, as evidenced by increased cytokines and activation/proliferation of immune cells
- ALT increases on bepirovirsen appear to be the outcome of infected hepatocyte cell death, as evidenced by concurrent transient increase in HBV DNA, and several liver and apoptosis-related proteins in serum

Background

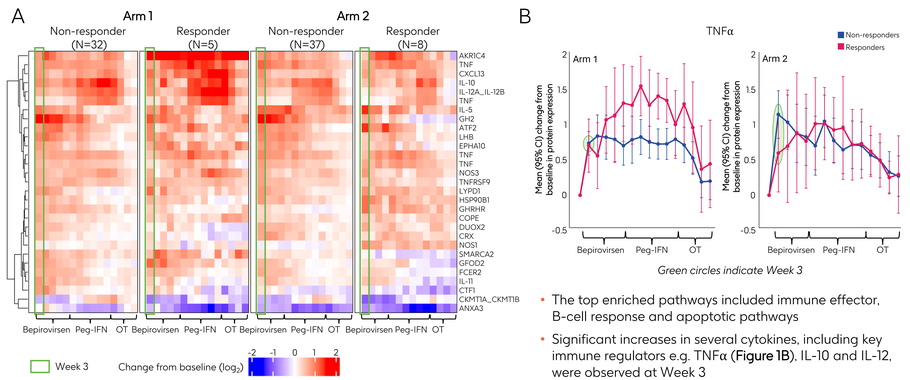
- Transient ALT increases, which were associated with concurrent HBsAg decline,[1,2] have been observed in some participants treated with bepirovirsen, an HBV-targeting unconjugated antisense oligonucleotide[3]
- Using data from the B-Together study (NCT04676724), we investigated bepirovirsen's immune mechanism of action, its role with respect to virological response, surrogate markers associated with hepatocyte cell death and ALT increases

Methods

- B-Together was a Phase 2b trial; 108 participants with chronic HBV infection on stable NA received bepirovirsen for 24 (Arm 1) or 12 (Arm 2) weeks, then up to 24 weeks of Peg-IFN[4]
  - Responders: achieved the primary endpoint: HBsAg <0.05 IU/mL and HBV DNA <20 IU/mL sustained for 24 weeks after the end of sequential bepirovirsen + Peg-IFN treatment (OT period) in the absence of rescue medication
  - Non-responders: did not meet the primary endpoint or had missing data in the OT period
- In this longitudinal peripheral biomarker exploratory analysis, which was performed post hoc on 82 participants (excluding China), flow cytometry of peripheral blood mononuclear cells, serum proteomics and whole blood transcriptomics were performed
- Relative expression of biomarkers was measured at baseline and post-baseline at multiple timepoints during the bepirovirsen, Peg-IFN and OT periods
- To determine differential expression, multivariate models were fit that included treatment arms and response subgroups

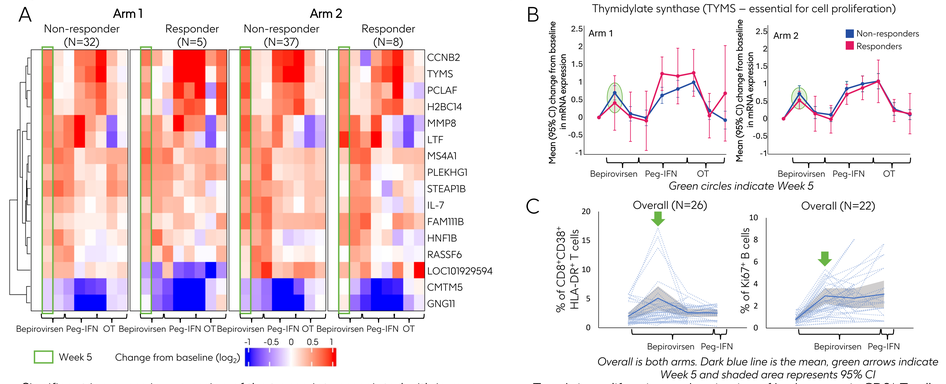
Results

Figure 1: **Bepirovirsen induces key immune response regulators at Week 3**



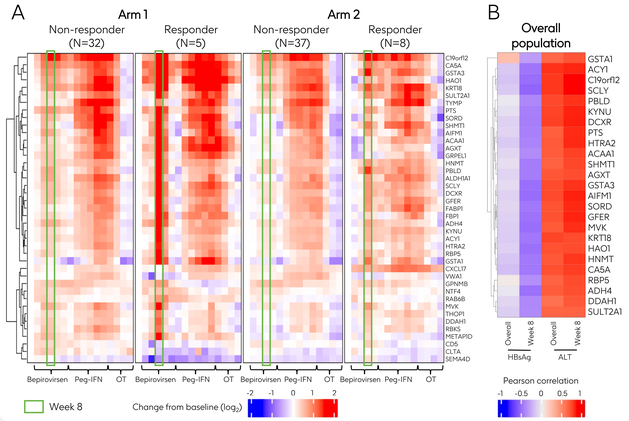
- The top enriched pathways included immune effector, B-cell response and apoptotic pathways
- Significant increases in several cytokines, including key immune regulators e.g. TNFα (**Figure 1B**), IL-10 and IL-12, were observed at Week 3

Figure 2: **Bepirovirsen increases immune cell activation and proliferation at Week 5**



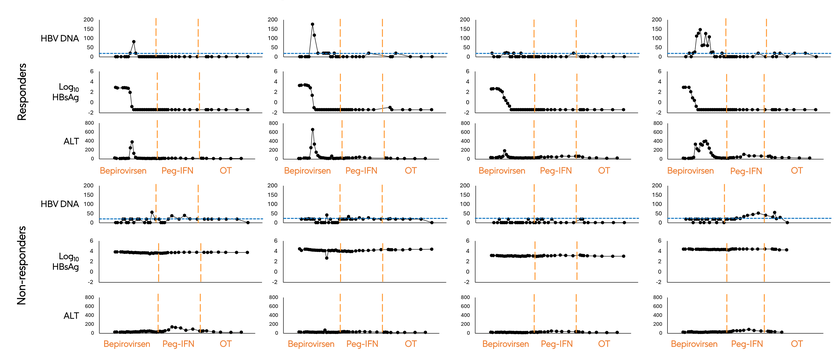
- Significant increases in expression of the transcripts associated with immune cell activation and proliferation e.g. TYMS (**Figure 2B**) was observed by Week 5
- Trends in proliferation and activation of both cytotoxic CD8⁺ T cells (CD38⁺; HLA-DR⁺) and B cells (Ki67⁺) were also observed (**Figure 2C**)

Figure 3: **Bepirovirsen may mediate hepatocyte cell death by Week 8**



- Liver and apoptosis-specific proteins were more abundant in the serum of responders than non-responders at Week 8 (**Figure 3A**). The top enriched pathways included metabolic pathways
- The top 25 of these proteins showed high association with ALT and a trend of inverse correlation with HBsAg at Week 8 (**Figure 3B**)
- Significant direct association between expression of the apoptosis marker AIFM1 and ALT levels (**Figure 3C**)

Figure 4: **Bepirovirsen potentiates killing of infected hepatocytes**



Figures show representative individual participant-level profiles for responders and non-responders in Arm 1. The blue line indicates the LLOQ for HBV DNA (20 IU/mL)

- During the bepirovirsen treatment period, concurrent transient increases in HBV DNA alongside ALT elevations, indicative of infected hepatocyte death and release of HBV DNA into the periphery, appeared to be more prominent in responders than non-responders (**Figure 4**)

Abbreviations

AIFM1, apoptosis-inducing factor mitochondria-associated 1; ALT, alanine aminotransferase; CI, confidence interval; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IL, interleukin; LLOQ, lower limit of quantification; NA, nucleos(t)ide analogue; OT, off treatment; Peg-IFN, pegylated interferon-α-2a; TNF, tumour necrosis factor; TYMS, thymidylate synthase

References

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