Downregulation of soluble FASLG as a potential mechanism of enhanced immune-related clearance of infected hepatocytes induced by JNJ-73763989 in HBeAg-negative virologically suppressed chronic hepatitis B patients

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Background and Aims: Persistent exposure to hepatitis B surface antigen (HBsAg) in chronically infected hepatitis B patients can interfere with the host immune response to clear infected hepatocytes. JNJ-73763989 (JNJ-3989), a small interfering RNA (siRNA) targeting all HBV ribonucleic acid (RNA) transcripts resulting in profound HBV surface antigen (HBsAg) reductions, is currently under development for treatment of chronic hepatitis B. Here, we evaluated serum proteome changes during JNJ-3989 treatment across two clinical studies, to evaluate the effect of JNJ-3989 on host responses.

Method: Serum was collected from HBeAg-negative, virologically suppressed (VS) patients from two phase 2 studies with 48 weeks of JNJ-3989 based treatment: REEF-1 (n=44) and REEF-2 (n=35). Soluble serum proteins were evaluated with Olink Explore®, a high-throughput protein biomarker discovery platform used before in chronic hepatitis B studies. A mixed effects model was applied to assess serum protein changes during JNJ-3989 based treatment. Significantly affected proteins were explored by evaluating overlap between both studies and association with HBsAg levels.

Results: 84/1460 (5.8%) proteins (REEF-1) and 669/2943 (22.7%) proteins (REEF-2) were differentially expressed comparing baseline with on-treatment. 62 proteins were consistently changed under JNJ-3989 based treatment across both studies. Network analysis showed apoptosis (FAS, FASLG, TNFRSF 10B, CTSF, CTS) and glyoxylate/dicarboxylate, sphingolipid and carbohydrate metabolism (AGXT, SHMT1, HAO1, SMPD1, ASAH2, ENPP7) as the main pathways affected during JNJ-3989 treatment. Most of the upregulated proteins (56/62; 90.3%) were liver-enriched cytoplasmic proteins involved in hepatocyte metabolism, suggesting increased hepatocyte death. Soluble Fas Ligand (FASLG), a key protein involved in cell death induced by cytotoxic T/NK cells was downregulated in both studies and at multiple timepoints. Strikingly, we did not observe any association with FASLG levels and HBsAg levels at baseline or on-treatment. Only 1/62 protein (PON2) was associated with HBsAg levels at baseline, whereas 4/62 proteins (AGXT, C19orf12, CTSF, SEZ6L) were negatively associated with HBsAg levels at W48 of treatment, but not at baseline.

Conclusion: Serum proteomic analyses in VS HBeAg negative patients across two clinical studies showed that JNJ-3989 treatment reduces soluble FASLG and increases serum levels of intracellular hepatocyte proteins, suggestive of increased hepatocyte cell death. As soluble FASLG can interfere with the pro-apoptotic action of cytotoxic T/NK cells through steric hinderance of the membrane bound FAS/FASLG interaction, these data suggest JNJ-3989 treatment might improve clearance of infected hepatocytes by cytotoxic immune cells through restoration of FAS/FASLG mediated apoptotic signaling.