Intrahepatic changes in immunologic and virologic markers during siRNA JNJ-3989 (JNJ-3989) based treatment of chronic hepatitis B (CHB) patients: imaging mass cytometry (IMC) analyses from the INSIGHT study

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Background and Aims: The evaluation of patients with chronic hepatitis B (CHB) focuses primarily on the peripheral compartment due to difficulties obtaining samples directly from the liver, the site of viral infection. In the INSIGHT study, we analyzed intrahepatic changes in CHB patients under JNJ-3989 treatment.

Method: In INSIGHT, CHB patients who were hepatitis B e-antigen (HBeAg) positive and not currently treated (Group 1) or HBeAg-negative and virologically suppressed by nucleos(t)ide analogs (NA) (Group 2) received 48-weeks of siRNA JNJ-3989 + NA ± CAM-E JNJ-6379. Core liver biopsies (CLB) were collected at baseline and week 40 and assessed using Hyperion imaging mass cytometry. This method assessed non-parenchymal and liver immune cells for 30 proteins, including viral HBsAg, Ki67 as a proliferation marker, and several immune markers. All cells were classified into eight distinct phenotypes of lymphocytes/monocytes, hepatocytes, and structural cells using 11 markers: Collagen type1, Pan-keratin, cytokeratin18, CD45, CD20, CD3, CD4, CD8, CD57, CD68, and CD14. HBsAg was used to distinguish infected from non-infected hepatocytes.

Results: Nineteen baseline- and fifteen week 40 CLB samples were assessed. Differential protein abundance analysis showed higher proportions of immune cell markers in infected than non-infected hepatocytes. Nkp46, an activation marker of NK cells, was significantly enriched in infected (5%) vs non-infected hepatocytes (< 1%). Also, the proportion of PDL-2-positive cells was significantly higher (p = 0.003) among the infected hepatocytes. The proportion of Ki67-positive cells was higher (p = 0.022) among the non-infected than the infected hepatocytes. The proportion of HBsAg positive hepatocytes was significantly lower (p < 0.001) at week 40 compared to baseline in both cohorts, consistent with the effect of JNJ-3989. The proportion of CD8+ T-cells was significantly lower (p = 0.002) at week 40 compared to baseline in Group 1 but not in Group 2. Differential abundance analysis of proteins within the different cell types highlighted Ki67 as significantly reduced (p < 0.05) in both infected and non-infected hepatocytes at week 40 compared to baseline in Group 1. In addition, the proportion of PDL-1-positive cells among the infected hepatocytes showed a trend for
an increase in both groups during treatment (p = 0.058). This effect was absent in the non-infected hepatocytes.

**Conclusion:** Spatial single-cell profiling of immune and viral markers in the liver from CHB patients demonstrated a decrease in the numbers of infected hepatocytes with a trend towards higher proportions of PDL-1 positive hepatocytes after 40 weeks of treatment with JNJ-3989. Although there was a lower proportion of Ki67-positive cells among the infected hepatocytes, proportions were further reduced in all hepatocytes during treatment, indicating less proliferation.