Intrahepatic Characterization of Virological and Immunological Markers in Two Distinct Populations of Chronic Hepatitis B: Baseline Assessment of Core Liver and Fine Needle Aspiration Biopsies From the Investigational INSIGHT Study

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Key Finding

- Contrasted intrahepatic distribution of liver viral markers was observed when comparing NCT HBeAg positive to VS HBeAg negative patients, which was reflected in peripheral viral markers

Conclusions

- Compared to patients with VS HBeAg negative CHB, NCT HBeAg positive patients had
  - A higher proportion of HBsAg positive hepatocytes
  - A higher proportion of HBcAg positive hepatocytes
  - A higher proportion of cccDNA/HBV RNA double-positive hepatocytes
  - In all VS HBeAg negative patients, the majority of hepatocytes were either cccDNA and HBV RNA negative (non-infected) or cccDNA positive and HBV RNA negative (silent)
  - In NCT HBeAg positive patients, a trend was observed for
    - A lower abundance of effector memory CD8+ T cells
    - An enriched expression of interferon-stimulated genes in CD8+ T cells

Introduction

- JNJ-73763989 (JNJ-3989) is a liver-targeted small interfering RNA (siRNA) that targets all hepatitis B virus (HBV) RNAs for degradation, thereby reducing all HBV proteins and pregenomic RNA[1]
- JNJ-56136379 (JNJ-6379; bersacapavir) is a capsid assembly modulator–empty (CAM-E) that interferes with HBV replication by causing the formation of structurally normal empty capsids that are devoid of HBV DNA and RNA[2]
- Recent studies investigating the combination treatment regimen of JNJ-3989 and nucleos(t)ide analogues (NAs), with or without JNJ-6379, have demonstrated profound reductions in HBV viral serum markers in patients with chronic hepatitis B (CHB)[3,4]

Objective

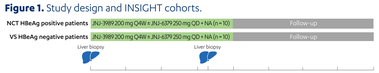
- The phase 2 INSIGHT study (ClinicalTrials.gov Identifier: NCT04585789) will assess the changes in intrahepatic viral and immune markers in response to JNJ-3989–containing combination regimens by comparing intrahepatic status between 2 distinct populations at baseline: virologically suppressed (VS) hepatitis B e antigen (HBeAg) negative and not currently treated (NCT) HBeAg positive patients with CHB

Methods

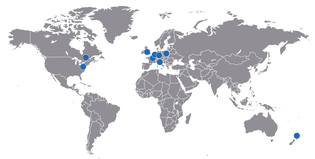
Study Design and Patients

- The phase 2, open-label, parallel-group, multicenter (across 9 countries in Europe, North America, and Oceania) INSIGHT study includes patients with CHB who are NCT HBeAg positive or VS by NA and HBeAg negative (**Figure 1**)
- Patients are receiving JNJ-3989 and NA, with or without JNJ-6379, for 48 weeks

Figure 1. Study design and INSIGHT cohorts.



Key inclusion criteria:
- Age 18–65 years
- Active CHB with HBsAg >100 IU/mL
- Non-cirrhotic (fibrosis stage F0–F2)
- Countries: Belgium, Canada, France, Germany, Italy, New Zealand, Poland, United Kingdom, United States
- NCT HBeAg positive patients (n = 10)
  - HBV DNA ≤20,000 IU/mL
  - ALT <10 × ULN
- VS HBeAg negative patients (n = 10)
  - HBV DNA <60 IU/mL
  - ALT <2 × ULN
  - NA* treatment for ≥6 months

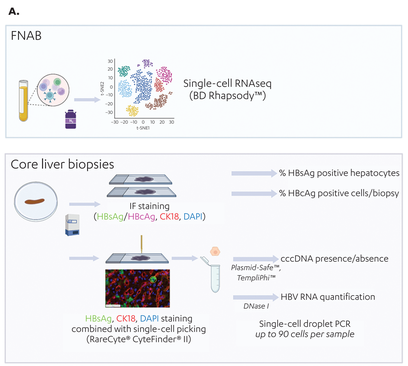


ALT, alanine transaminase; ETV, entecavir; HBsAg, hepatitis B surface antigen; Q4W, every 4 weeks; QD, daily; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; ULN, upper limit of normal.
*NA = ETV/TAF/TDF according to label.

Intrahepatic Assessments

- Paired percutaneous core liver biopsies and fine needle aspiration biopsies (FNABs) are being collected pre-baseline and at Week 40 of treatment using standardized collection procedures and storage protocols across sites (**Figure 2A**)
  - The quality of the samples is being assessed during collection (capturing the size and volume of the sample and potential blood contamination) and with profiling at a central laboratory
- Viral intrahepatic markers, including HBsAg, hepatitis B core antigen (HBcAg), and HBV RNA, are being assessed by immunofluorescent (IF) core biopsy staining combined with single-cell digital droplet polymerase chain reaction (PCR) from fresh frozen tissue sections (**Figure 2A**)
- Immune cells are characterized by single-cell RNA sequencing (RNAseq) from FNABs (**Figure 2B**)

Figure 2. (A) Liver sample collection* and (B) intrahepatic viral and immune profiling.†



cccDNA, covalently closed circular DNA; LOD, limit of detection.
*Liver sample collection was standardized across sites. Samples were cryopreserved at the sites and shipped to a central laboratory for analysis. FNAB profiling was performed by single-cell RNAseq using BD Rhapsody™ whole transcriptome analysis to assess variation in intrahepatic immune cells; core biopsies were analyzed by IF staining to assess the fraction of HBsAg positive hepatocytes and HBcAg positive cells per sample. In a subset of core biopsy samples (n = 9), up to 90 hepatocytes per sample were individually picked (RareCyte® CyteFinder® II) for deep viral characterization (HBsAg positivity, cccDNA presence/absence, and HBV RNA quantification for each individual hepatocyte). The LOD of the HBV RNA assay was 1.6 copies/cell.
†Primers were designed for single-cell droplet PCR at the 3′ end of HBV genome to enrich for cccDNA-derived HBV RNAs.

Results

Demographic and Disease Characteristics

- Baseline liver samples were collected from 20 patients (10 per group; **Table 1**)
- For NCT HBeAg positive and VS HBeAg negative patients, the mean age was 33.4 and 43.4 years, respectively, and 50% of patients in each group were female; the NCT HBeAg positive group had a higher number of patients who were Asian versus the VS HBeAg negative group (80% vs 30%; **Table 1**)

Table 1. Baseline Demographic and Disease Characteristics of NCT HBeAg Positive and VS HBeAg Negative Patients Enrolled in the INSIGHT Study

N (%) or mean (SD)	NCT HBeAg positive patients	VS HBeAg negative patients
Analysis set: ITT	n = 10	n = 10
Demographic characteristics		
Age, years	33.4 (14.73)	43.4 (12.63)
Female	5 (50%)	5 (50%)
Asian	8 (80%)	3 (30%)
Disease characteristics		
Virologically suppressed	0	10 (100%)
HBeAg positive	10 (100%)	0
HBeAg, $\log_{10}$ IU/mL*	2.80 (0.52)	–
HBsAg, $\log_{10}$ IU/mL	4.47 (0.64)	3.40 (0.59)
HBsAg ≤1,000 IU/mL	10 (100%)	8 (80%)
HBV DNA, $\log_{10}$ IU/mL	8.01 (0.66)	0.84 (0.23)
HBV DNA <LLOQ*	0	10 (100%)
HBV RNA <LOD*	0	5 (50%)
HBcrAg, $\log_{10}$ U/mL	8.41 (0.80)	3.53 (0.99)
HBcrAg <LLOQ§	0	4 (40%)
ALT ≤ULN	4 (40%)	10 (100%)
ALT ≤ULN and DNA >7 $\log_{10}$ IU/mL	3 (30.0%)	0
FibroScan® score, kPa	5.49 (1.51)	4.84 (1.30)
Type of NA at study entry: TDF/TAF/ETV¶	–	5 (50%)/2 (20%)/3 (30%)
Duration of NA at baseline, years‡	–	6.2 (3.12)
HBV genotype	2 (20%) GT-B, 6 (60%) GT-C, 2 (20%) GT-E	2 (20%) GT-D¶

HBcrAg, hepatitis B core-related antigen; ITT, intention-to-treat; LLOQ, lower limit of quantification; SD, standard deviation. The viral markers and ALT reported in this table are serum based (ie, peripheral).
*Among NCT HBeAg positive patients.
†For HBV DNA, LLOQ = 1.3 $\log_{10}$ IU/mL.
‡For HBV RNA, LOD = 1.4 $\log_{10}$ cp/mL.
§For HBcrAg, LLOQ = 3.0 $\log_{10}$ U/mL.
¶Among VS HBeAg negative patients.
‡2 of 10 patients with available HBV genotype data based on historical information.

Intrahepatic Viral Markers

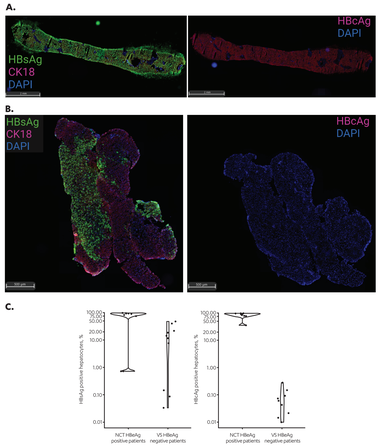
- Higher expression of intrahepatic viral markers was observed in biopsies from NCT HBeAg positive compared to VS HBeAg negative patients (**Table 2**; **Figure 3** and **4**)
- A significantly lower fraction of HBsAg positive hepatocytes and HBcAg positive cells was observed in VS HBeAg negative patients compared to NCT HBeAg positive patients (beta binomial model; **Figure 3**)

Table 2. Baseline Intrahepatic HBV Viral Markers in the 2 Populations of Patients Enrolled in INSIGHT

Mean (SD)	NCT HBeAg positive patients	VS HBeAg negative patients
Analysis set: ITT	n = 9	n = 10
HBsAg positive hepatocytes, %	69.4 (39.6)	16.1 (16.5)
HBcAg positive cells, %	84.3 (19.9)	6.6 (8.5)
HBV RNA positive hepatocytes, %	96.6 (5.3)	19.5 (9.1)
cccDNA positive hepatocytes, %	46.9 (33.3)	46.3 (15.2)
cccDNA positive HBV RNA negative (silent) hepatocytes, %	1.9 (3.7)	36.4 (10.1)

The viral markers reported were measured in core liver biopsies. Percentages of positive HBsAg (median = 11,252; range: 640-31,051) and HBcAg (median = 13,095; range: 1,648-33,998) cells were based on IF staining; percentages of cccDNA and HBV RNA positive cells were based on single-cell digital droplet PCR from up to 90 hepatocytes picked from a section (LOD = 1.6 copies/cell).

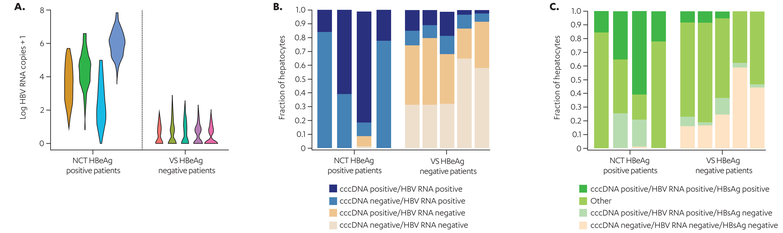
Figure 3. Baseline HBsAg and HBcAg IF staining.



Representative examples of multiplex IF staining of a core biopsy collected in (A) NCT HBeAg positive and (B) VS HBeAg negative patients showing contrasted fraction and distribution of infected cells (with clusters of HBsAg positive cells in samples collected in VS HBeAg negative patients). (C) Overall proportion of detected HBsAg positive hepatocytes and HBcAg positive cells comparing NCT HBeAg positive and VS HBeAg negative patients (P <0.01 in both comparisons, applying a beta binomial model). A median of 11,251 (range: 1,309-22,430) hepatocytes were analyzed per biopsy.

- HBV RNA expression was significantly higher in biopsies collected from NCT HBeAg positive versus VS HBeAg negative patients (**Table 2**; **Figure 4**), and the fraction of cccDNA positive, HBV RNA positive, and HBsAg positive cells (infected cells with active replication and HBsAg expression) was higher in NCT HBeAg positive patients
- Core biopsies from VS HBeAg negative patients were associated with a higher frequency of cccDNA negative, HBV RNA negative, and HBsAg negative cells (a proxy for non-infected hepatocytes) compared to NCT HBeAg positive patients

Figure 4. Differences in baseline HBV RNA expression and cccDNA/HBV RNA positivity in up to 90 individual hepatocytes per section picked from a subset of 4 core biopsies of NCT HBeAg positive patients and 5 biopsies of VS HBeAg negative patients.

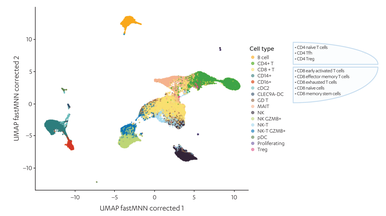


(A) Violin plot representing the distribution of HBV RNA copies. (B) Joint distribution of cccDNA positive and HBV RNA positive (HBV infected and actively replicating) hepatocytes, cccDNA negative and HBV RNA positive hepatocytes, cccDNA positive and HBV RNA negative (silent) hepatocytes, and cccDNA negative and HBV RNA negative (non-infected) hepatocytes. (C) Joint distribution of cccDNA positive, HBV RNA positive, and HBsAg positive hepatocytes (infected and actively expressed HBV RNA and HBsAg) and cccDNA negative, HBV RNA negative, and HBsAg negative hepatocytes. The LOD for cccDNA positivity and HBV RNA positivity is 1.6 copies/cell. Other includes any combination of cccDNA, HBV RNA, and HBsAg not listed in the other 3 categories.

Intrahepatic Immune Composition

- Limited differences in intrahepatic cell composition were observed when comparing FNABs from NCT HBeAg positive patients and VS HBeAg negative patients (**Figure 5**)
  - A trend for higher CD8+ effector memory T cells in NCT HBeAg positive patients was observed
- No significant change was detected in the normalized cell abundance of each cell population in FNABs collected from NCT HBeAg positive and VS HBeAg negative patients
- A trend for enrichment was observed in the expression of interferon-stimulated genes in CD8+ effector memory T cells of samples collected in NCT HBeAg positive compared to VS HBeAg negative patients (associated with a false discovery rate [FDR] <10%; **Figure 6** and **Table 3**)
- Tumor necrosis factor (TNF) expression in mucosal-associated invariant T cells (MAIT) is associated with peripheral HBsAg categories (1,000 IU/mL and 10,000 IU/mL; **Figure 7**)

Figure 5. Projection of single-cell RNAseq FNAB profiling (UMAP[5] fastMNN[6] corrected) at baseline.



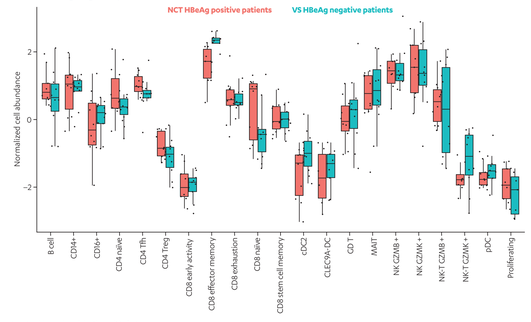
cDC2, type-2 conventional dendritic cell; DC, dendritic cell; GZMB+, granzyme B positive; NK, natural killer; pDC, plasmacytoid dendritic cell; TFh, T follicular helper.
19 FNAB samples collected at baseline were successfully profiled, with approximately 2,000 genes/cell quantified (NCT HBeAg positive patients, n = 9; 15,828 liver resident cells; VS HBeAg negative patients, n = 10; 21,925 liver resident cells). On average, 1,988 cells per sample were profiled, varying from 282 to 5,200 cells. 24 major immune cell populations could be identified based on their transcriptome.

Table 3. Differentially Expressed Genes at Baseline in Detected Cell Populations in Samples From NCT HBeAg Positive and VS HBeAg Negative Patients

Cell population	Gene	Direction	FDR-adjusted *P* value
CD8+ T cells	*EPSTI1*	NCT HBeAg+	0.027
MAIT	*GBP1*	NCT HBeAg+	0.047
MAIT	*GBP4*	NCT HBeAg+	0.047
NK cells GzmB+	*STAT1*	NCT HBeAg+	0.060
NK cells GzmB+	*SPON2*	NCT HBeAg+	0.060
CD8+ T cells	*TAP1*	NCT HBeAg+	0.069
CD8+ T cells	*ITM2C*	NCT HBeAg+	0.069
CD8+ T cells	*JAK3*	NCT HBeAg+	0.069
CD8+ T cells	*JAML*	NCT HBeAg+	0.069
CD8+ T cells	*MPP6*	VS HBeAg−	0.076
CD8+ T cells	*STAT1*	NCT HBeAg+	0.076
CD8+ T cells	*ARHGAP9*	NCT HBeAg+	0.085
CD8+ T cells	*MYO6*	VS HBeAg−	0.085

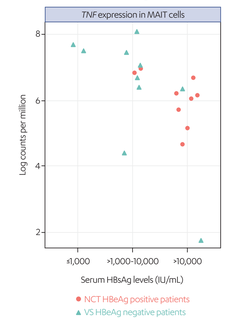
HBeAg−, HBeAg negative; HBeAg+, HBeAg positive.
An FDR of 10% was used as the cutoff within each population. Pseudobulk analysis was performed with edgeR.[7] The group associated with enrichment in a specific gene is listed under "Direction."

Figure 6. Normalized cell abundance of each cell population in FNABs collected at baseline from NCT HBeAg positive and VS HBeAg negative patients.



Negative binomial model with an FDR of 5%.

Figure 7. Relationship between baseline *TNF* expression in MAIT cells and peripheral HBsAg levels.



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PL serves on the advisory board/speakers bureau for Bristol Myers Squibb, Roche, Gilead, GSK, AbbVie, MSD, Arrowhead, Alnylam, Janssen, Spring Bank, MYR, Eiger BioPharmaceuticals, Antios Therapeutics, Aligos, and Vir Biotechnology. TA is a speaker and investigator for Antios Therapeutics, AbbVie, Eiger BioPharmaceuticals, ENYO, Gilead, Janssen, Roche, and Vir Biotechnology. EG is a member of scientific advisory boards for AbbVie, Abbott Diagnostics, Aligos, Arbutus, Arrowhead, Assembly Biosciences, Avalia, CkeriX Therapeutics, Discerna, Gilead, GSK, Intellia, Janssen, Merck, Novartis, Genentech-Roche, Vaccitech, Vir Biotechnology, and Virion Therapeutics; and is a speaker for AbbVie, Abbott Diagnostics, Gilead, and Intellia. SF received investigator-initiated research funding from Gilead; received advisory board/consulting fees from Gilead, AbbVie, Janssen, Pfizer, and Novo Nordisk; and received speaker fees from Gilead, AbbVie, and Lupin. PTK reports relationships with Abbott, Aligos, Antios Therapeutics, Assembly Biosciences, Gilead, Janssen, GSK, Immunocore, and Drug Farm. T Vanwolleghem receives grants from Gilead and Bristol Myers Squibb; and serves as a consultant for Janssen Pharmaceuticals, Gilead, and AbbVie. EJ receives fees for clinical trials from Janssen-Cilag, GSK, Immunocore, Bristol Myers Squibb, Novo Nordisk, Inventiva, CymaBay, Dr. Falk, Cirifols, Cellaion, MSD, Exelixis, Callidtas, Sagimet, and Axcella; and serves on advisory boards for Novo Nordisk and Cellaion. JSchW serves as a consultant for Gilead. MS serves as a consultant for AbbVie, Atea, Antios Therapeutics, Aligos, Gilead, F2G, Virion Therapeutics, and Precision Bio; receives grants from Janssen, Vir Biotechnology, and GSK (directed to Johns Hopkins University); and reports relationships with DSMB Gilead (HIV) and Immunocore (HBV). HW, DFC, NC-N, EdT, KVdB, HWHG, JA, JJ, ZA, OL, TV Verbinnen, TNK, CG-A, MT, and MB are employees of Janssen and may hold stock in Johnson & Johnson.

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