Immunologic Biomarker Dynamics in Chronic Hepatitis B: Insights From a Phase 2a Open-Label Study on Combination Therapies With Small Interfering RNA, Selgantolimod, and Nivolumab

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Conclusions

- A comprehensive biomarker assessment of novel hepatitis B virus (HBV) cure combination strategies revealed:
  - Minimal immunologic changes during/after small interfering RNA lead-in
  - Limited increases in HBV-specific T-cell responses
  - Expected shifts in biomarkers associated with immune modulation
- These insights may guide the data-driven design of subsequent combination approaches

Plain Language Summary

- Multiple treatment options are approved for patients with chronic hepatitis B, but they rarely result in a cure
- Investigating drug combinations may uncover a successful cure in the future
- In this study, an assessment of investigational combination treatment strategies for hepatitis B virus cure showed some changes in the immune system, but very little impact on T cells specific for the virus

References: **1.** World Health Organization. Hepatitis B fact sheet. 2022. **2.** Seto WK, et al. *Lancet*. 2018;392:2313-24. **3.** Gane EJ, et al. *J Hepatol*. 2023;78:513-23. **4.** Janssen HL, et al. *JHEP Rep*. 2024;6:100975.

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Disclosures: **DP**, **NK**, **LS**, **BD**, **JJW**, **FA**, **AHL**, and **PMO** are employees of Gilead Sciences, Inc., and report owning stock in Gilead Sciences, Inc. **AA** is a stockholder of Vir Biotechnology. **DC** is a previous employee and shareholder of Vir Biotechnology and is an employee and shareholder of Revagenix. **PRD** reports consulting and conducting contract research for Arrowhead Pharmaceuticals, DrugFarm, and Gilead Sciences, Inc. **DJV** reports consulting and conducting contract research for Arrowhead Pharmaceuticals and Gilead Sciences, Inc.

Introduction

- Hepatitis B virus (HBV) infection impacts approximately 254 million individuals globally and is associated with cirrhosis, hepatic decompensation, and hepatocellular carcinoma if not properly treated[1,2]
- Novel therapeutic strategies for chronic hepatitis B (CHB) virus infection that aim to achieve HBV functional cure are currently being investigated
  - Current approved therapies for CHB rarely achieve this outcome
- Utilizing novel combinations of therapies that target HBV protein expression and boost the host immune response may offer added benefits
- A Phase 2a open-label study (NCT04891770) was conducted in patients with CHB to evaluate the safety and efficacy of combination therapies with VIR-2218 (a small interfering RNA targeting the HBx region of the HBV genome), selgantolimod (SLGN; an oral toll-like receptor 8 [TLR8] agonist), and nivolumab (NIVO; an anti–programmed cell death protein 1 monoclonal antibody)

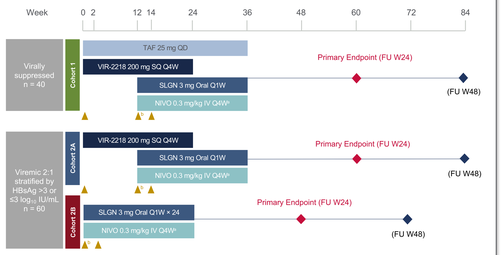
Objective

- To better understand the immunologic impact of dual immune modulation by SLGN and NIVO after reduction of hepatitis B surface antigen (HBsAg) with VIR-2218, we assessed longitudinal changes in peripheral cytokine levels, the whole blood transcriptome, and cellular immune responses

Methods

- Biomarker samples were collected longitudinally from patients
- Plasma cytokines were analyzed by singleplex enzyme-linked immunosorbent assay
- Whole blood was evaluated by RNA sequencing
- Peripheral blood mononuclear cells were immunophenotyped using a 37-parameter cytometry by time-of-flight panel
- HBV-specific T-cell responses were quantified using a 2-color Fluorospot (interferon gamma [IFNγ]/granzyme B)
- For all analyses, changes from baseline and differences between cohorts were evaluated using the nonparametric Wilcoxon rank-sum test

Study Design



Triangles indicate biomarker sample collection time points (predose).
‡NIVO was discontinued early in cohorts 1 and 2A, with the whole of cohort 2B (SLGN + NIVO) discontinued due to a low likelihood of efficacy with SLGN alone. †Denotes time points when biomarker samples were collected at predose, 4 h postdose, and 24 h postdose
FU, follow-up; h, hour; HBsAg, hepatitis B surface antigen; IV, intravenous; NIVO, nivolumab; Q1W, every 1 week; Q4W, every 4 weeks; QD, once daily; SLGN, selgantolimod; SQ, subcutaneous; TAF, tenofovir alafenamide; W, week.

- Biomarker analyses were performed at the time points indicated in the Study Design
- Patients (n = 2) who did not receive the first dose of NIVO due to discontinuation were not included

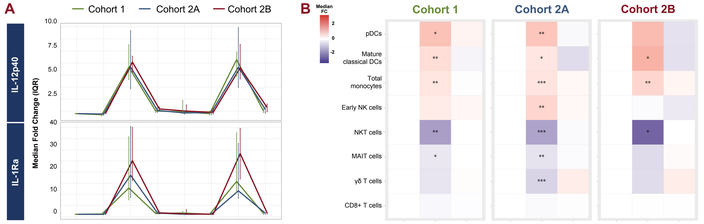
Results

Baseline Demographic and Disease Characteristics

Baseline Variables	Cohort 1 n = 42	Cohort 2A n = 38	Cohort 2B n = 20
Age, years	48 (41, 55)	41 (38, 46)	44 (42, 48)
Sex, male, n (%)	26 (62)	15 (39)	10 (50)
Race, Asian, n (%)	41 (98)	35 (92)	18 (90)
HBV DNA, $\log_{10}$ IU/mL	1.29 (1.28, 1.28)*	5.76 (4.10, 8.06)	4.80 (4.12, 8.14)
ALT, U/L	22 (18, 30)	28 (18, 39)	34 (23, 55)
HBsAg, $\log_{10}$ IU/mL	3.12 (2.70, 3.58)	3.80 (3.37, 4.17)	3.73 (3.22, 4.31)
HBeAg positive, n (%)	18 (43)	15 (39)	6 (30)

*Lower limit of quantification. Contnuous data shown as median (Q1, Q3).
ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; Q, quartile.

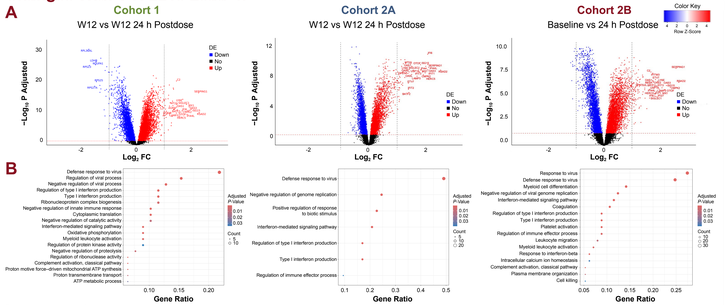
No Impact on SLGN Pharmacodynamic Responses With VIR-2218 Lead-In



Panel A: One subject excluded; cohort 2B adjusted by 12 weeks. Panel B: Two-sided Wilcoxon test: *P <.05; **P <.01; ***P <.001. pDCs: CD11c+CD123+; mature classical DCs: CD11c+CD38+; total monocytes: CD14+CD56−; early NK cells: CD56^{bright}CD57−; NKT cells: CD3+CD56+CD161+; MAIT cells: CD3+CD56−CD161+; γδ T cells: CD3+ TCRγδ+. Data may be incomplete for some patients due to missing samples or limited sample availability.
CD, cluster of differentiation; D, day; DC, dendritic cell; FC, fold change; IL, interleukin; IL-1Ra, interleukin receptor antagonist; MAIT, mucosal-associated invariant T; NK, natural killer; pDC, plasmacytoid dendritic cell; SLGN, selgantolimod; TCR, T-cell receptor; W, week.

- Transient increases in TLR8-associated cytokines and redistribution of innate and adaptive immune cells were observed 4 h after SLGN dosing in all cohorts, consistent with prior studies[3,4]

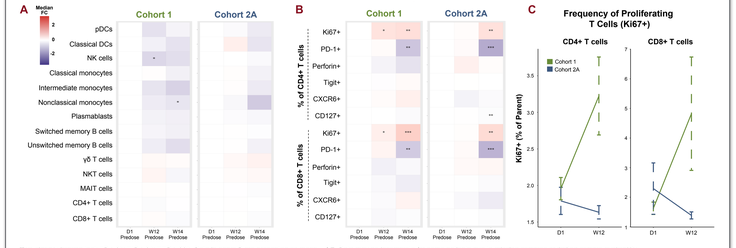
Whole Blood Transcriptomics Reveals Gene Signatures After Immune Modulation, but No Changes With VIR-2218 Lead-In



Data may be incomplete for some patients due to missing samples or limited sample availability.
ATP, adenosine triphosphate; DE, differential expression; FC, fold change; W, week.

- No changes were observed in whole blood transcriptional gene signatures during VIR-2218 lead-in (cohorts 1 and 2A; data not shown)
- Significant shifts in gene expression were observed at 24 h postdose with SLGN + NIVO in all cohorts, including upregulation of genes involved in interferon signaling and antiviral response pathways

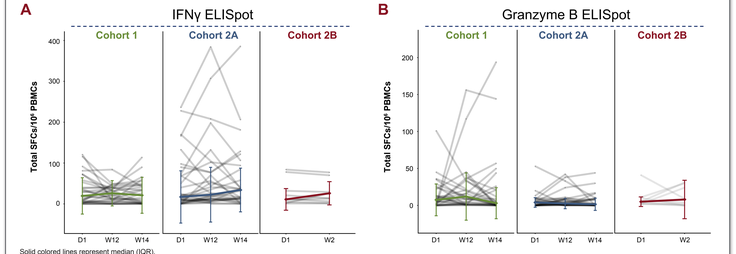
Impact of VIR-2218 Lead-In on Cellular Immune Responses



Two-sided Wilcoxon test; *P <.05; **P <.01; ***P <.001. Data in panel B are presented as mean ± SE. Data may be incomplete for some patients due to missing samples or limited sample availability.
CD, cluster of differentiation; CXCR, C-X-C motif chemokine receptor; D, day; DC, dendritic cell; FC, fold change; MAIT, mucosal-associated invariant T; NK, natural killer; PD-1, programmed cell death protein 1; pDC, plasmacytoid dendritic cell; W, week.

- The impact of VIR-2218 lead-in on the cellular immune response was minimal overall
- No significant changes in major innate or adaptive immune cell frequencies were observed during VIR-2218 lead-in
- In virally suppressed patients (cohort 1), proliferating T cells (CD4+ Ki67+ and CD8+ Ki67+) were significantly increased with VIR-2218 lead-in; no other changes in T-cell phenotype were noted

No Consistent Increases in HBV-Specific T-Cell Responses Were Observed in Any Cohort

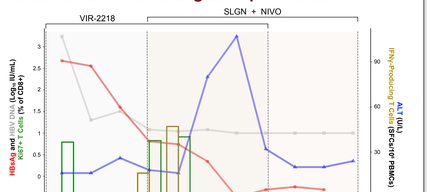


Solid colored lines represent median (IQR).
D, day; ELISpot, enzyme-linked immunospot; HBV, hepatitis B virus; IFNγ, interferon gamma; PBMC, peripheral blood mononuclear cell; SFC, spot-forming cell; W, week.

- Outliers observed in cohorts 1 and 2A showed increased HBV-specific T-cell responses by IFNγ or granzyme B

Biomarker Analysis of a Single Patient in Cohort 2A With HBsAg Loss per Protocol

- One patient in cohort 2A experienced HBsAg loss on study per protocol
- Alanine aminotransferase elevation prior to loss suggests intrahepatic immune activity
- An upward trend in peripheral HBV-specific T-cell responses was observed after the first dose of immune modulation
- Biomarker analyses at later time points are needed to provide additional insights into the immunology of HBsAg loss in this patient



Two other patients (cohort 1) lost HBsAg; however, BL HBsAg levels violated inclusion/exclusion criteria number 3 "HBsAg >1.5 $\log_{10}$ IU/mL".
ALT, alanine aminotransferase; BL, baseline; CD, cluster of differentiation; D, day; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IFNγ, interferon gamma; NIVO, nivolumab; PBMC, peripheral blood mononuclear cell; SFC, spot-forming cell; SLGN, selgantolimod; W, week.