Transcriptional analysis of HBV infected liver after treatment with Selgantolimod reveals longitudinal changes in both inflammation-related pathways and B cell receptor repertoire

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Key Findings

- SLGN elicits an inflammatory response in the liver microenvironment of chronic HBV patients shortly after dosing.
- Gene pathways associated with the regulation of macrophage chemotaxis (*CSF1*), inflammatory cytokines (*STAT1, IL6, TNFAIP3, MYD88, TLR7*), activation and proliferation of cytotoxic T cells (*CD69, CD44, TAP1*), and mature B cell differentiation (*LYN*) were upregulated after treatment with SLGN.
- Cell deconvolution analyses showed an increase in the averages of M1 Macrophages (1.7-fold), regulatory T cells (4.6-fold), and B cells (1.9-fold) within the liver.
- Analysis of B cell receptor repertoires in liver revealed an increase in clonotypic diversity after treatment. Increases in sequences containing VH3-49, VH3-7, VH3-21 were observed in 2 of 3 subjects characterized.

Conclusions

SLGN treatment in CHB patients induced upregulation of inflammation-associated genes within the liver microenvironment, promoting activation of pro-inflammatory myeloid, B and T lymphocytes.

Changes to the intrahepatic B cell repertoire were observed suggesting that subsequent weekly treatment with SLGN may increase diversity of the BCR repertoire and enrich for antibody families potentially specific to HBV. These findings support the hypothesis that SLGN is an effective immunomodulator that promotes activation of hepatic immune microenvironment and suggests SLGN will be a valuable addition to combination therapeutic approaches for HBV Cure.

References: 1. Ayithan N, et al. *Viruses* 2021;13(12):2400. **2.** Gane EJ, et al. *Hepatology* 2021;74(4):1737-1749.

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Introduction

- Selgantolimod (SLGN; GS-9688) is a potent, selective, oral, small-molecule agonist of toll-like receptor 8 (TLR8) in clinical development for the treatment of Chronic Hepatitis B (CHB).
- SLGN has the potential to induce intrahepatic HBV immunity through the activation of intrahepatic CD8$^+$ T, B, natural killer, and mucosal-associated invariant T cells.[1]
- Previous clinical studies have characterized the peripheral PD response to SLGN in CHB subjects demonstrating that SLGN elicits increases in pro-inflammatory cytokines (IL-12p40, INFγ, IL-1RA) in the periphery shortly after administration.[2]

Objective

- To evaluate the effect of oral treatment with SLGN on the liver microenvironment via transcriptional analyses.

Methods

- Paired core needle liver biopsies were collected from 3 Inactive Carrier (IC) CHB participants during screening and week 23, 2-6 hours post-dosing. PBMCs and fixed whole blood samples were collected at the same timepoints.

Figure 1. GS-US-389-5458 Clinical Study Design

Immunotolerant (IT) CHB	Inactive Carrier (IC) CHB	HBV/HDV
- High viral load (HBV DNA ≥1 × 10^6 IU/mL) - HBeAg positive - ALT ≤ULN - Not on oral antiviral therapy	- Low viral load (HBV DNA ≤2000 IU/mL) - HBeAg negative - ALT ≤ULN - Not on oral antiviral therapy	- Detectable HDV RNA at screening - HBV DNA <69 IU mL - On oral antiviral therapy

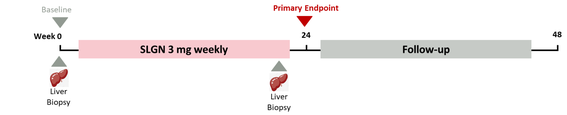
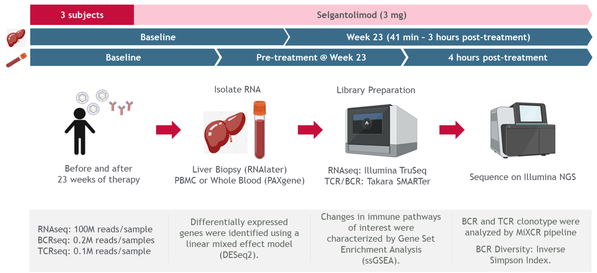
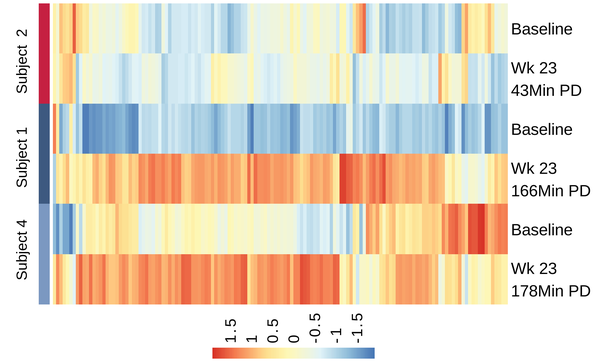


Figure 2. Experimental Design



Results

Figure 3. GSVA of immune pathways from liver biopsies collected before and after SLGN treatment



- Changes to pathways were evaluated using gene set variation analysis (GSVA) for selected immune related gene sets. Legend indicates GSVA score
- Magnitude of gene set changes from baseline correlates with the amount of time between SLGN administration and liver biopsy collection.

Figure 4. Change in gene expression associated with inflammation, TLR, and immune cell activation after treatment with SLGN treatment

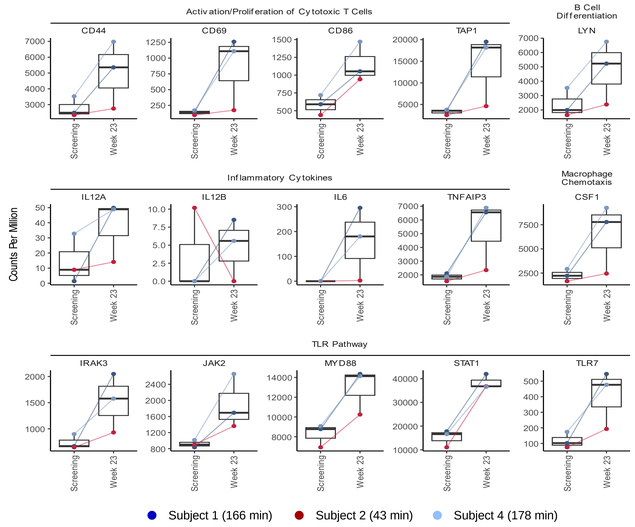
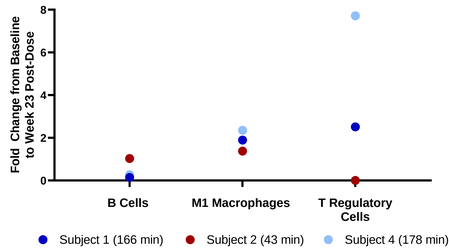
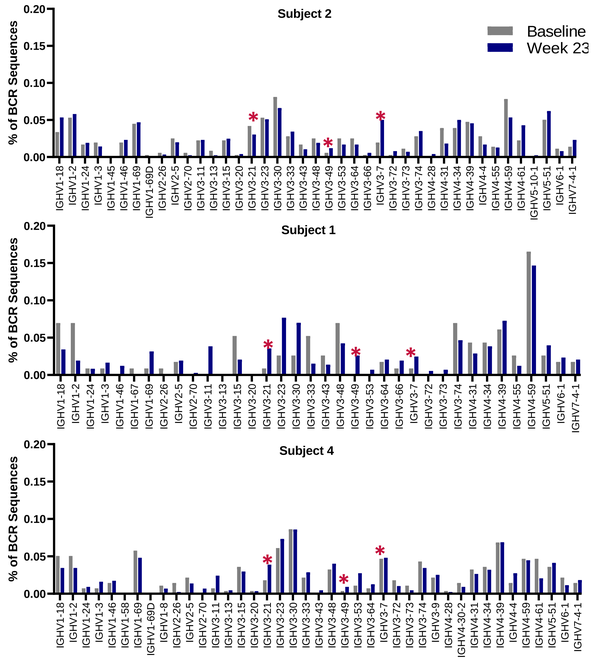


Figure 5. Cell deconvolution suggests increased proportion of liver macrophages and B cells after SLGN treatment



Deconvolution of cell populations by quantTIseq is based on constrained least squares regression from normalized expression of signature genes.

Figure 6. BCR sequencing of liver biopsies indicate modifications in heavy chain gene usage after treatment with SLGN



*Indicate VH families that were expanded or reduced upon treatment with SLGN; however, these data are not statistically significant due to reduced n number.

*Clinical data generated from this clinical study will be presented by Agarwal et.al – Poster ID SAT-182