Research article

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Safety, pharmacodynamics, and antiviral activity of selgantolimod in viremic patients with chronic hepatitis B virus infection

Harry L. Janssen,[1,2,*] Young-Suk Lim,[3] Hyung Joon Kim,[4] Leonard Sowah,[5] Cheng-Hao Tseng,[6] Carla S. Coffin,[7] Magdy Elkhashab,[8] Sang Hoon Ahn,[9] Anh-Hoa Nguyen,[5] Diana Chen,[5] Jeffrey J. Wallin,[5] Simon P. Fletcher,[5] Circe McDonald,[5] Jenny C. Yang,[5] Anuj Gaggar,[5] Diana M. Brainard,[5] Scott Fung,[2] Yoon Jun Kim,[10] Jia-Horng Kao,[11] Wan-Long Chuang,[12] Anna E. Brooks,[13] P. Rod Dunbar[13]

[1]Department of Gastroenterology and Hepatology, Erasmus University Medical Center, Rotterdam, The Netherlands; [2]Toronto General Hospital, University of Toronto, Toronto, Ontario, Canada; [3]Asan Medical Centre, University of Ulsan College of Medicine, Seoul, South Korea; [4]Department of Internal Medicine, Chung-Ang University College of Medicine, Seoul, South Korea; [5]Gilead Sciences, Inc., Foster City, CA, USA; [6]Division of Gastroenterology and Hepatology, E-Da Cancer Hospital/I-Shou University, Kaohsiung, Taiwan; [7]Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada; [8]Toronto Liver Centre, Toronto, Ontario, Canada; [9]Department of Internal Medicine, Institute of Gastroenterology, Yonsei University College of Medicine, Seoul, South Korea; [10]Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul, South Korea; [11]Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine, Taipei City, Taiwan; [12]Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan; [13]School of Biological Sciences, and Maurice Wilkins Centre, University of Auckland, Auckland, New Zealand

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Background & Aims: Novel finite therapies for chronic hepatitis B (CHB) are needed, since lifelong treatment is usually required with current available oral antivirals. This phase II study (NCT03615066) evaluated the safety, pharmacodynamics, and antiviral activity of selgantolimod (a Toll-like receptor 8 agonist [TLR8]) with tenofovir alafenamide (TAF).
Methods: Viremic patients with CHB not receiving treatment were stratified by HBeAg status and randomized 2:2:1 to TAF 25 mg/day with selgantolimod 3 mg orally once weekly (QW), selgantolimod 1.5 mg QW, or placebo. Combination therapy continued until week (W)24, followed by TAF monotherapy until W48; patients then discontinued TAF and were followed until W96 (treatment-free follow-up [TFFU] period). The primary efficacy endpoint was the proportion with ≥1 $\log_{10}$ IU/ml HBsAg decline at W24.
Results: Sixty-seven patients received study drug; 27 were followed during TFFU. Nausea, headache, vomiting, fatigue, and dizziness were the most common adverse events. Most adverse events were grade 1. Alanine aminotransferase flares were not observed up to W48. Four patients experienced alanine aminotransferase and hepatitis flares during TFFU; all had HBV DNA increases. Selgantolimod increased serum cytokines and chemokines and redistributed several circulating immune cell subsets. No patients achieved the primary efficacy endpoint. Mean HBsAg changes were −0.12, −0.16, and −0.12 $\log_{10}$ IU/ml in the selgantolimod 3 mg, selgantolimod 1.5 mg, and placebo groups, respectively, at W48; HBV DNA declined in all groups by ≥2 $\log_{10}$ IU/ml as early as W2, with all groups rebounding to baseline during TFFU. No HBsAg or HBeAg loss or seroconversion was observed throughout TFFU.
Conclusions: Selgantolimod up to 3 mg was safe and well tolerated. Pharmacodynamics and antiviral activity in viremic patients support continued study of selgantolimod in combination CHB therapies.
Impact and implications: Novel therapeutics for chronic HBV infection are needed to achieve a functional cure. In this study, we confirmed the safety and tolerability of selgantolimod (formerly GS-9688, a TLR8) when administered with tenofovir alafenamide over 24 weeks in viremic patients with chronic HBV infection. Overall, declines in HBsAg levels with selgantolimod treatment were modest; subgroup analysis indicated that patients with alanine aminotransferase levels greater than the upper limit of normal had significantly greater declines compared to those with normal alanine aminotransferase levels (−0.20 *vs.* −0.03 $\log_{10}$ IU/ml; p <0.001). These findings suggest a potential differential response to selgantolimod based on patients' baseline HBV-specific immune response, which should be considered in future investigations characterizing the underlying mechanisms of selgantolimod treatment and in HBV cure studies using similar immunomodulatory pathways.
Clinical trial number: NCT03615066 be found at https://www.gileadclinicaltrials.com/transparency-policy/.

Keywords: Hepatitis B virus; oral antiviral; small molecule; immunotherapy; HBV cure; toll-like receptor; viremic.
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* Corresponding author. Address: Department of Gastroenterology and Hepatology, Erasmus University Medical Center, Rotterdam, The Netherlands; Tel.: +3110 703 5942.
E-mail address: h.janssen@erasmusmc.nl (H.L. Janssen).

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Introduction

The World Health Organization estimates that approximately 296 million people worldwide are chronically infected with HBV, and nearly 900,000 die annually from HBV-related causes, primarily from cirrhosis and/or hepatocellular carcinoma.[1–3] Clinical guidelines recommend using oral nucleos(t)ide analogues (NAs) or injectable interferons (IFN) to treat chronic hepatitis B (CHB).[1,4] Approved NAs target HBV polymerase and reverse transcriptase,[5,6] and injectable IFN exhibit non-specific immunomodulatory and antiviral effects.[7] Although current antiviral therapies are well tolerated and effective at suppressing HBV, leading to improved patient outcomes, a functional cure (*i.e.*, sustained loss of circulating HBsAg with undetectable HBV DNA) is rarely achieved, necessitating prolonged treatment.[6] Novel treatment candidates should be developed with the goal of inducing a functional cure for patients with CHB.[8] Current and investigational HBV treatments have diverse, potentially complementary, mechanisms of action (MoAs); thus, combination therapy likely represents the best path to a functional cure in the majority of patients living with CHB.

The host immune response to HBV infection plays a pivotal role in determining whether acute infection resolves or becomes chronic.[9] Individuals who are able to clear HBV spontaneously following an acute infection display a vigorous, polyclonal, HBV-specific $CD8^+$ and $CD4^+$ T-cell response, leading to resolution of active HBV infection (*i.e.*, presumptive "immune" control).[10–13] In contrast, CHB is associated with a limited and dysfunctional $CD8^+$ T-cell response, as well as the impaired antiviral function of natural killer (NK) cells and disruption of function of other immune cells, including HBsAg-specific B cells.[14,15]

Toll-like receptors (TLRs) are a family of membrane-bound pattern-recognition receptors that play a central role in both innate and adaptive immunity via the recognition of pathogen-associated molecular patterns from microorganisms.[16] Toll-like receptor 8 agonist (TLR8) is a transmembrane receptor located in the endosomal membrane in a subset of immune cells and recognizes single-stranded RNA.[17,18] TLR8 agonists can directly or indirectly activate innate and adaptive effector cell immune responses and may induce effective antiviral immunity in patients with CHB.[19–21] Activating TLRs in patients with CHB has been shown to be safe, with pharmacodynamic (PD) findings and serologic changes supporting the possibility of TLR agonist use in therapies to increase the immune response to HBV.[22–25]

Selgantolimod (formerly GS-9688) is a potent, selective, oral agonist of TLR8. In human peripheral blood mononuclear cells (PBMCs) *in vitro*, selgantolimod induced the production of the cellular immune mediator interleukin-12 (IL-12) and the antiviral cytokines tumor necrosis factor-α and interferon (IFN)-γ, while it had minimal effects on the levels of IFN-α, a TLR7-induced cytokine.[26] Selgantolimod also activated NK cells and stimulated $CD8^+$ T-cell proliferation.[26] A study in the woodchuck model of CHB showed that 8 weeks of treatment with selgantolimod was well tolerated and induced sustained antiviral efficacy and loss of woodchuck hepatitis virus surface antigen in a subset of animals.[27] A phase Ia study in healthy participants found that single doses of selgantolimod up to 5 mg were safe and induced a dose-dependent PD response;[28] a follow-up phase Ib study showed selgantolimod administration (up to 3 mg) was safe in patients with CHB.[29] In a recent phase II study in patients with CHB with suppressed viremia, selgantolimod up to 3 mg for 24 weeks was generally safe and well tolerated; treatment induced modest declines in HBsAg, while cytokine, chemokine, and T-cell responses suggested target engagement.[25]

The aim of this clinical study was to evaluate the safety, PD, and antiviral activity of 24 weeks of selgantolimod with tenofovir alafenamide (TAF) in patients with CHB not currently taking oral antivirals (OAVs). This study also evaluated the safety and durability of antiviral activity during a 48-week treatment-free follow-up (TFFU) phase.

Patients and methods

Study population and design

This was a phase II, randomized, double-blind, placebo-controlled, multicenter study (ClinicalTrials.gov: NCT03615066) designed to evaluate the safety, PDs, and antiviral activity of selgantolimod in patients with CHB not currently on treatment. The study included patients from 10 centers in Canada, South Korea, and Taiwan between August 2018 and April 2021.

Eligible patients were aged 18 to 65 years and had documented evidence of CHB (HBsAg positive for >6 months), with detectable HBsAg levels and HBV DNA ≥2,000 IU/ml at screening. Exclusion criteria included having extensive bridging fibrosis or cirrhosis, receiving a commercially available OAV for HBV, and receiving prolonged therapy with immunomodulators or biologics within 3 months of screening. A full list of inclusion and exclusion criteria (including histological assessment for bridging fibrosis or cirrhosis) are provided in the supplementary methods.

Enrolled patients were stratified into two cohorts by HBeAg status (Cohort 1, HBeAg positive; Cohort 2, HBeAg negative). Within each cohort, patients were randomly assigned 2:2:1 to either selgantolimod 3 mg orally once weekly (QW), selgantolimod 1.5 mg orally QW, or placebo. All patients were also treated with TAF 25 mg orally once daily. Patients received selgantolimod + TAF or placebo + TAF for 24 weeks, then continued TAF monotherapy until week 48. At week 48, patients discontinued TAF and were evaluated every 4 weeks for an additional 48 weeks or until the initiation of an alternative HBV therapy. Therefore, the total study duration for each patient was 48 total weeks of treatment with up to 48 weeks of TFFU. Study design and patient disposition are detailed in Fig. S1. Study drug was always administered in a fasted state (no food or drink except water for 8 h predose, with water also not allowed within 1 h predose). Patients were randomized using an Interactive Mobile Response System or Interactive Web Response System. For the duration of the study, patients and investigators were blinded to treatment group assignments.

This study was conducted in accordance with the principles of the 1975 Declaration of Helsinki, International Conference on Harmonisation guidelines, and good clinical practices. Study procedures were not conducted until written informed consent from patients was documented and approvals were confirmed by the Institutional Review Boards and Independent Ethics Committee.

Endpoints

The primary endpoints were the proportions of patients with treatment-emergent adverse events (TEAEs) and laboratory abnormalities at week 24 and the proportion of patients with an HBsAg decline ≥1 $\log_{10}$ IU/ml from baseline at week 24. Secondary endpoints were the proportion of patients with undetectable HBV DNA and HBsAg and HBeAg loss through week 48. Exploratory endpoints included changes from baseline in HBeAg, hepatitis B core-related antigen (HBcrAg), and HBV RNA; changes in PD markers (interleukin-12p40 [IL-12p40], IL-1 receptor antagonist, and IFN-γ) induction; changes in immune cell population; and changes in cell phenotype in peripheral blood. These endpoints were also assessed at the end of the TFFU period as applicable. Assays and evaluations used for efficacy and safety endpoints are described in the supplementary methods.

Statistical analysis

Baseline characteristics and demographics were reported descriptively. Efficacy analyses, including the primary endpoint, were conducted using the full analysis set (all randomized patients who took ≥1 dose of study drug). To compare treatment groups for HBeAg-positive and -negative patients separately, point estimates and the 2-sided 95% exact CIs of efficacy endpoints were provided based on the Clopper-Pearson method by HBeAg status and overall for each treatment group. CIs for the proportion differences were constructed based on the standardized statistic and inverting two one-sided tests. When treatment groups were compared in pooled patients, the proportion differences and corresponding 95% CIs were calculated using the stratum-adjusted Mantel-Haenszel method. *Post hoc* comparisons of HBV DNA and HBsAg changes from baseline, including subgroup analyses of alanine aminotransferase (ALT) and hepatitis flares, baseline ALT, or HBeAg values, were performed using Wilcoxon tests. Safety data were analyzed using the safety analysis set (all patients who took ≥1 dose of study drug). TFFU data were analyzed using the TFFU analysis set (all patients who were randomized into the study, received ≥1 dose of study drug, and entered the TFFU period). PD parameters were analyzed using the biomarker analysis set (all randomized patients who took ≥1 dose of study drug and had ≥1 biomarker value).

Due to the study's exploratory nature, the sample size was not determined by any formal power calculation. The number of patients in each treatment group was based on the feasibility of enrollment and prior HBV phase Ib studies. In general, missing data were not imputed. An initial analysis was conducted at week 24 after all patients completed or prematurely discontinued study treatment. Follow-up and final analysis occurred at week 48 with patients who entered the TFFU period. All statistical analyses were performed using the SAS Software version 9.4 (SAS Institute Inc., Cary, NC, USA) and R statistical package version 3.5.2 (Vienna, Austria).

The protocol is published online as supplementary material. For further details regarding the materials and methods used, please refer to the CTAT table and supplemental information.

Results

Baseline demographics and disease characteristics

Ninety-six patients were screened, 25 failed screening, and four were not randomized (withdrew consent, n = 2; outside of visit window, n = 1; other, n = 1), resulting in 67 patients randomized (between 25 September 2018 and 27 June 2019) and receiving ≥1 dose of study drug. Twenty-seven patients enrolled in the TFFU period (selgantolimod 3 mg, n = 13; selgantolimod 1.5 mg, n = 12; placebo, n = 2). The last patient visit was 12 April 2021. Sixty-seven important protocol deviations were reported during the study follow-up period, affecting 36 unique patients. Details of these deviations can be found in Table S1.

Overall, 39 of 67 patients were HBeAg positive. Most patients were Asian (99%), 58% were male, and mean BMI was 24.5 kg/m^2 (Table 1). The mean FibroTest score was numerically lower in HBeAg-positive *vs.* -negative patients at baseline, whereas mean HBV DNA, HBV RNA, HBsAg, and HBcrAg were numerically greater in HBeAg-positive *vs.* -negative patients at baseline. Mean ALT levels were lower in HBeAg-positive *vs.* -negative patients at baseline in patients assigned selgantolimod 3 mg and placebo but were numerically similar between HBeAg-positive and -negative patients receiving selgantolimod 1.5 mg. Most patients had HBV genotype B or C at baseline (63 of 67 [94%]; three patients had genotype D, and one had genotype I). For patients who previously used OAVs, the most common prior OAV was tenofovir disoproxil fumarate. Most patients did not have a history of prior IFN use, although data were missing for 15, 17, and 11 patients in the selgantolimod 3 mg, selgantolimod 1.5 mg, and placebo groups, respectively.

Length of exposure

Mean (SD) length of exposure to selgantolimod was similar between patients in the 3 mg (22.5 [5.43] weeks) and 1.5 mg (24.0 [0.11] weeks) groups. Mean (SD) exposure to TAF was 44.6 (11.96) weeks in the selgantolimod 3 mg group, 47.4 (3.78) weeks in the selgantolimod 1.5 mg group, and 44.9 (8.04) weeks in the placebo group.

Safety

Table 2 summarizes safety during the on-treatment period. Forty-nine of 67 patients (73%) had ≥1 TEAE by week 24, 52 of 67 (78%) had ≥1 adverse event (AE) by week 48, and 10 of 27 (37%) had ≥1 AE during the TFFU phase. Seventeen of 26 (65%) and 9 of 28 (32%) patients who received selgantolimod 3 mg or 1.5 mg, respectively, had TEAEs related to study drug, all of which were grade 2 or lower. Three of 13 (23%) patients who received placebo had TEAEs related to study drug, one of which was grade 3 (rhinorrhea). Through week 48, one patient (a 62-year-old Asian female) treated with selgantolimod 3 mg had study drug-related TEAEs of upper abdominal pain beginning at day 1 of dosing. On day 22, she reported grade 2 vomiting, which was determined to be related to the study drug, leading to study drug discontinuation. One person experienced a grade 1 serious AE (SAE) of limb injury on day 77 assessed as unrelated to study drug. No additional serious AEs or grade ≥3 TEAEs occurred during the TFFU phase, and no deaths occurred during this study. During the on-treatment period, the five most common TEAEs were nausea, headache, vomiting, fatigue, and dizziness (Table 2). No patients in the placebo group reported nausea, vomiting, fatigue, or dizziness.

A summary of laboratory abnormalities grade 2 or greater is presented in Table S2. Among patients treated with selgantolimod 1.5 mg, grade 4 elevated fasting triglycerides were reported in one (4%) patient during the 24- and 48-week periods, and grade 4 ALT increases were reported in two (17%) patients during

Table 1. Baseline characteristics (FAS and SAS).

	SLGN 3 mg[a]			SLGN 1.5 mg[a]			PBO[a]		
	HBeAg + (n = 14)	HBeAg – (n = 12)	Total (n = 26)	HBeAg + (n = 18)	HBeAg – (n = 10)	Total (n = 28)	HBeAg + (n = 7)	HBeAg – (n = 6)	Total (n = 13)
Age, years, mean (SD)	46 (10.7)	46 (10.2)	46 (10.3)	41 (13.5)	50 (6.0)	44 (12.1)	37 (7.1)	56 (6.6)	46 (12.0)
Sex, male, n (%)	7 (50.0)	8 (66.7)	15 (57.7)	10 (55.6)	5 (50.0)	15 (53.6)	4 (57.1)	5 (83.3)	9 (69.2)
Race, Asian, n (%)	14 (100)	11 (91.7)	25 (96.2)	18 (100)	10 (100)	28 (100)	7 (100)	6 (100)	13 (100)
BMI, kg/m^2, mean (SD)	24.5 (2.81)	25.2 (1.85)	24.8 (2.39)	24.0 (2.88)	24.3 (1.48)	24.1 (2.44)	23.8 (2.78)	25.3 (2.40)	24.5 (2.62)
FibroTest score, mean (SD)	0.2 (0.21)	0.4 (0.18)	0.3 (0.20)	0.2 (0.10)	0.3 (0.23)	0.2 (0.17)	0.1 (0.07)	0.4 (0.18)	0.2 (0.19)
HBV genotype, n (%)									
B	2 (14.3)	7 (58.3)	9 (34.6)	5 (27.8)	4 (40.0)	9 (32.1)	4 (57.1)	5 (83.3)	9 (69.2)
C	12 (85.7)	4 (33.3)	16 (61.5)	11 (61.1)	5 (50.0)	16 (57.1)	3 (42.9)	1 (16.7)	4 (30.8)
D	0	1 (8.3)	1 (3.8)	2 (11.1)	0	2 (7.1)	0	0	0
I	0	0	0	0	1 (10.0)	1 (3.6)	0	0	0
HBV DNA, $\log_{10}$ IU/ml, median (min, max)	8.2 (5.8, 8.9)	5.0 (3.5, 7.2)	6.6 (3.5, 8.9)	8.3 (7.4, 8.9)	4.7 (3.2, 7.2)	7.7 (3.2, 8.9)	8.2 (7.5, 8.6)	5.3 (3.7, 7.3)	7.5 (3.7, 8.6)
HBV RNA, $\log_{10}$ copies/ml, median (min, max)	6.56 (5.21, 7.90)	3.52 (2.48, 5.78)	5.57 (2.48, 7.90)	6.93 (5.65, 8.47)	3.35 (2.48, 5.09)	6.46 (2.48, 8.47)	6.78 (6.44, 7.39)	4.15 (2.48, 5.67)	6.44 (2.48, 7.39)
HBsAg, $\log_{10}$ IU/ml, median (min, max)	4.6 (2.4, 5.0)	3.5 (2.2, 4.3)	4.0 (2.2, 5.0)	4.6 (4.0, 5.1)	3.6 (2.1, 4.6)	4.6 (2.1, 5.1)	4.7 (3.6, 5.0)	3.5 (2.7, 3.8)	3.8 (2.7, 5.0)
HBeAg, $\log_{10}$ IU/ml, median (min, max)	3.00 (0.93, 3.15)	ND	ND	3.03 (2.01, 3.15)	ND	ND	2.96 (2.13, 3.15)	ND	ND
HBcrAg, $\log_{10}$ U/ml, median (min, max)	8.6 (6.8, 8.9)	4.7 (2.5, 6.4)	6.9 (2.5, 8.9)	8.6 (7.6, 9.0)	3.4 (2.8, 6.2)	8.3 (2.8, 9.0)	8.6 (6.9, 9.0)	4.7 (2.6, 6.6)	6.9 (2.6, 9.0)
ALT, U/L, mean (SD)	41 (30.8)	72 (91.9)	56 (66.7)	42 (28.6)	42 (46.6)	42 (35.2)	26 (10.3)	43 (18.2)	34 (16.5)
ALT ≤ULN, n (%)[b]	8 (57.1)	6 (50.0)	14 (53.8)	10 (55.6)	8 (80.0)	18 (64.3)	7 (100)	2 (33.3)	9 (69.2)
Prior NA treatment, n (%)									
Entecavir	0	3 (25.0)	3 (11.5)	2 (11.1)	0	2 (7.1)	0	0	0
Lamivudine	1 (7.1)	1 (8.3)	2 (7.7)	2 (11.1)	1 (10.0)	3 (10.7)	0	0	0
TDF	2 (14.3)	3 (25.0)	5 (19.2)	3 (16.7)	1 (10.0)	4 (14.3)	0	0	0
Prior IFN treatment for HBV, n (%)									
Yes	0	1 (14.3)	1 (9.1)	2 (25.0)	1 (33.3)	3 (27.3)	1 (100)	0	1 (50.0)
No	4 (100)	6 (85.7)	10 (90.9)	6 (75.0)	2 (66.7)	8 (72.7)	0	1 (100)	1 (50.0)
Missing	10	5	15	10	7	17	6	5	11

ALT, alanine aminotransferase; FAS, full analysis set; HBcrAg, hepatitis B core-related antigen; IFN, interferon; NA, nucleos(t)ide analogue; ND, not determined; PBO, placebo; SAS, safety analysis set; SLGN, selgantolimod; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; ULN, upper limit of normal.

[a] Also received TAF 25 mg once daily.

[b] ULN defined for ALT by the American Association for the Study of Liver Disease is 25 U/L for females and 35 U/L for males.

Table 2. Safety summary through week 48 and the most common TEAEs.

	24-week regimen			48-week regimen		
	SLGN 3 mg[a] (n = 26)	SLGN 1.5 mg[a] (n = 28)	PBO[a] (n = 13)	SLGN 3 mg[a] (n = 26)	SLGN 1.5 mg[a] (n = 28)	PBO[a] (n = 13)
Any TEAE	22 (84.6)	17 (60.7)	10 (76.9)	23 (88.5)	19 (67.9)	10 (76.9)
TEAE Grade ≥3	0	0	1 (7.7)	0	0	1 (7.7)
TEAE related to study drug	17 (65.4)	9 (32.1)	3 (23.1)	17 (65.4)	9 (32.1)	3 (23.1)
TEAE related to TAF	5 (19.2)	4 (14.3)	2 (15.4)	5 (19.2)	4 (14.3)	2 (15.4)
TE SAE	1 (3.8)	0	0	1 (3.8)	0	0
TEAE leading to premature discontinuation of study drug	1 (3.8)	0	0	1 (3.8)	0	0
TEAEs reported by ≥10% of patients in any treatment group						
Nausea	8 (30.8)	6 (21.4)	0	8 (30.8)	6 (21.4)	0
Headache	3 (11.5)	5 (17.9)	2 (15.4)	3 (11.5)	5 (17.9)	2 (15.4)
Vomiting	6 (23.1)	3 (10.7)	0	6 (23.1)	3 (10.7)	0
Fatigue	5 (19.2)	3 (10.7)	0	5 (19.2)	3 (10.7)	0
Dizziness	5 (19.2)	1 (3.6)	0	5 (19.2)	1 (3.6)	0
Diarrhea	3 (11.5)	1 (3.6)	2 (15.4)	3 (11.5)	1 (3.6)	2 (15.4)
Nasopharyngitis	1 (3.8)	2 (7.1)	1 (7.7)	1 (3.8)	2 (7.7)	1 (7.7)
Abdominal pain	1 (3.8)	3 (10.7)	0	1 (3.8)	3 (10.7)	0
Chills	4 (15.4)	0	0	4 (15.4)	0	0

PBO, placebo; SAE, serious adverse event; SLGN, selgantolimod; TAF, tenofovir alafenamide; TEAE, treatment-emergent adverse event.
Data shown as n (%).
[a] Also received TAF 25 mg once daily.

the TFFU period. No grade 4 laboratory abnormalities were reported with selgantolimod 3 mg.

ALT slightly decreased through week 48, with decreases from baseline in the selgantolimod groups being numerically greater than those in the placebo group (median changes of −6, −8, and +1 U/L in the selgantolimod 3 mg, selgantolimod 1.5 mg, and placebo groups, respectively, at week 48). ALT rebounded to near-baseline levels in all treatment groups during the TFFU period (Fig. S2). No patients experienced an ALT flare (serum ALT >2 × baseline and ≥5 × the upper limit of normal [ULN]) by week 24 and up to week 48. There were no confirmed treatment-emergent elevations in ALT meeting the American Association for the Study of Liver Diseases criteria for hepatitis flare (ALT ≥3 × baseline and >100 U/L)[4] by week 24 and up to week 48. Four patients had ALT elevations that met the criteria for both an ALT flare and hepatitis flare during the TFFU phase of the study, and two patients had ALT elevations that met the criteria for hepatitis flare only. No relationship was observed between ALT flares and cytokine changes.

Pharmacodynamics

In general, median IL-12p40, IL-1RA, and IFN-γ serum concentration ratios following dosing were higher in both selgantolimod treatment groups compared with placebo, with peak concentrations occurring approximately 4 h postdose for both selgantolimod doses and returning to near-baseline levels 24 h postdose (Fig. 1). The magnitude of increase in the IL-12p40, IL-1RA, and IFN-γ concentration ratios tended to be greater with selgantolimod 3 mg *vs.* 1.5 mg. Dose-dependent cytokine responses to selgantolimod are shown in Fig. 2. Many cytokines and chemokines (*e.g.* C–C motif chemokine ligands 8 and 20, IFN-γ, IL-12p70) increased on treatment in both selgantolimod dose groups. Acute phase proteins, serum amyloid A and C-reactive protein, had delayed kinetics with peak levels achieved at 24 h postdose. These also increased in a dose-dependent manner and returned to near-baseline levels before the next dose period.

Fold changes in HLA-DR$^+$ CD11c$^+$ peripheral blood myeloid cells (including monocytes and classical/conventional dendritic cells) of both selgantolimod-treated groups reached peak levels 4 h postdose (p <0.001 for selgantolimod 1.5 and 3 mg doses *vs.* placebo; Fig. 3A). A significant reduction in CD3$^+$ lymphocytes from circulation 4 h postdose in selgantolimod-treated patients was also apparent for both selgantolimod dose groups *vs.* placebo (p <0.001; Fig. 3B). For the myeloid- and T-cell subsets, the respective increases and decreases from circulation were near predose levels at 24 h postdose. No changes were detected in peripheral immune cell populations with selgantolimod postdose, with only minor alterations in HLA-DR$^+$ CD19$^+$ B- and CD56$^+$ CD3$^-$ NK-cell populations (Fig. 3C and D). The minor alterations in B- and NK-cell populations were generally comparable across treatment groups.

Antiviral activity

No patients achieved ≥1 $\log_{10}$ IU/ml decline from baseline in serum HBsAg at week 24 (primary efficacy endpoint). The greatest declines at weeks 24 and 48 in HBsAg occurred in patients who received selgantolimod (3 or 1.5 mg; Fig. 4A). At week 24, mean changes were −0.08, −0.11, and −0.03 $\log_{10}$ IU/ml in the selgantolimod 3 mg, selgantolimod 1.5 mg, and placebo groups, respectively; at week 48, respective changes were −0.12, −0.16, and −0.12 $\log_{10}$ IU/ml (p >0.05 for all treatment group comparisons at weeks 24 and 48; Table S3). In addition, at week 24, HBsAg declines of ≥0.5 $\log_{10}$ IU/ml were observed in 2 of 24 (8%), 1 of 28 (4%), and 0 patients receiving selgantolimod 3 mg, selgantolimod 1.5 mg, and placebo, respectively. At week 48, HBsAg declines of ≥0.5 $\log_{10}$ IU/ml were observed in 2 of 24 (8%), 2 of 27 (7%), and 0 patients receiving selgantolimod 3 mg, selgantolimod 1.5 mg, and placebo, respectively. In general, HBsAg decreased slightly through week 48, then returned to near-baseline levels during the TFFU phase (Fig. 4B).

Stratifying patients by HBV genotype (types B and C; Fig. S3) or HBsAg levels at baseline (<1,000 IU/ml *vs.* ≥1,000 IU/ml; Fig. S4) did not reveal differences in HBsAg levels through week 48. Levels of viremia (stratifying patients with baseline HBV DNA >20,000 IU/ml) did not influence HBsAg levels through week 48 (Fig. S5). A further subgroup analysis comparing HBsAg changes from baseline among those with and without ALT flares

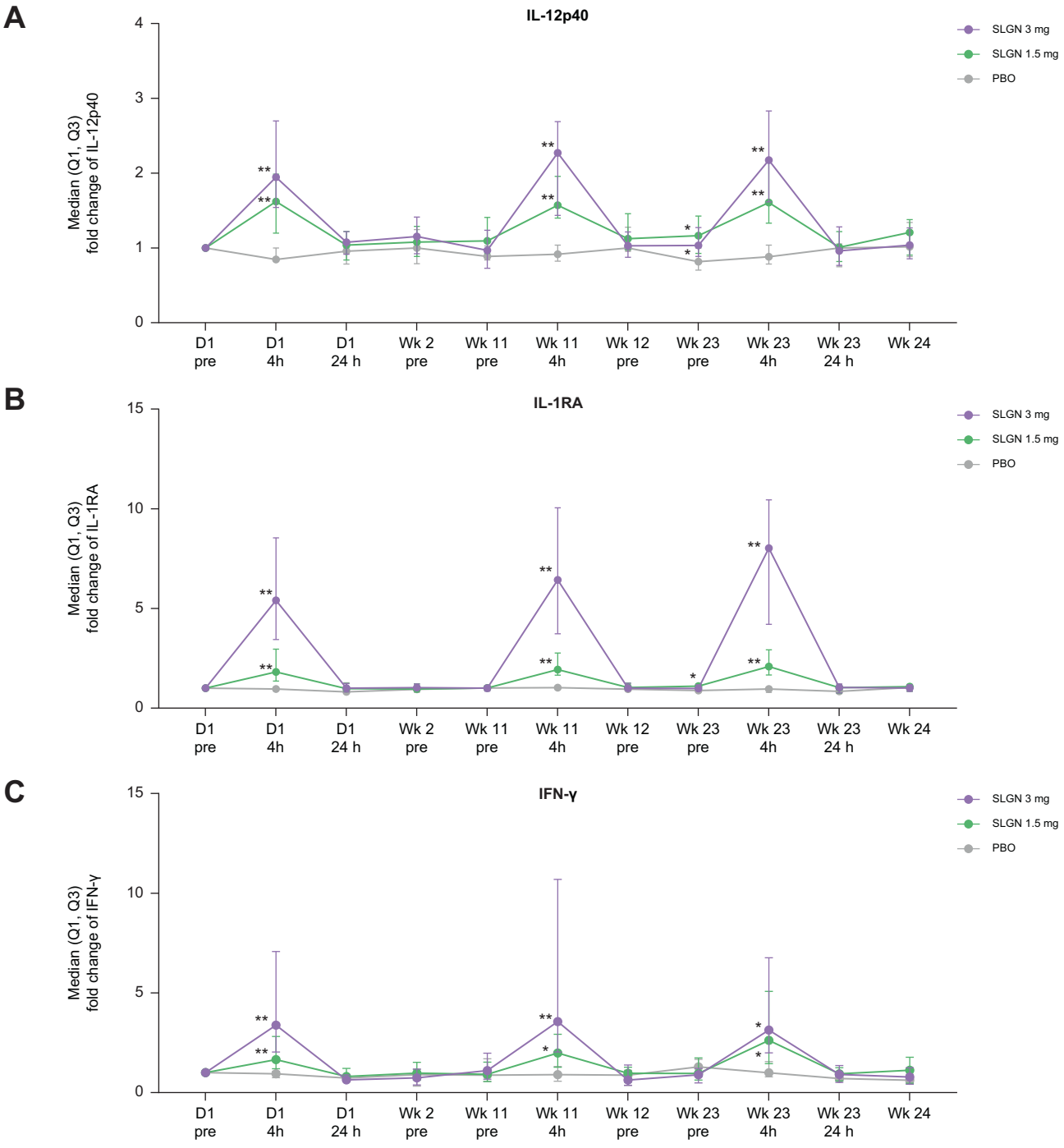

Fig. 1. Median IL-12p40, IL-1RA, and IFN-γ concentration ratios following multiple-dose administrations of SLGN (biomarker analysis set). Median (IQR) fold change of (A) IL-12p40, (B) IL-1RA, and (C) IFN-γ *vs.* time by visit following dosing with SLGN (1.5 mg or 3 mg) or PBO in viremic patients. *p <0.05 and **p <0.001 based on the Wilcoxon test. IFN, interferon; IL-, interleukin-; PBO, placebo; pre, predose; Q, quartile; RA, receptor agonist; SLGN, selgantolimod.

demonstrated similar changes in HBsAg at the end of selgantolimod treatment (week 24; −0.06 and −0.09 $\log_{10}$ IU/ml, respectively; p = 0.26; Table S4). HBsAg changes up to week 48 were numerically less in those with flares *vs.* those without flares (−0.07 *vs.* −0.14, respectively; p = 0.21).

Overall, selgantolimod-treated patients with a baseline ALT >ULN had greater HBsAg change at week 24 (−0.20 *vs.* −0.03 $\log_{10}$ IU/ml; p <0.001) and week 48 (−0.27 *vs.* −0.05 $\log_{10}$ IU/ml; p = 0.001) than those with ALT ≤ULN. In patients with a baseline ALT >ULN, changes in $\log_{10}$ IU/ml HBsAg were −0.20 and −0.11 at week 24 among patients who received selgantolimod *vs.* placebo, respectively (p = 0.54); the respective changes in $\log_{10}$ IU/ml HBsAg were −0.27 and −0.18 at week 48 (p = 0.79). In patients with a baseline ALT ≤ULN, HBsAg changes were similar between selgantolimod and placebo groups at weeks 24 and 48. Change in HBsAg was greater in patients receiving selgantolimod

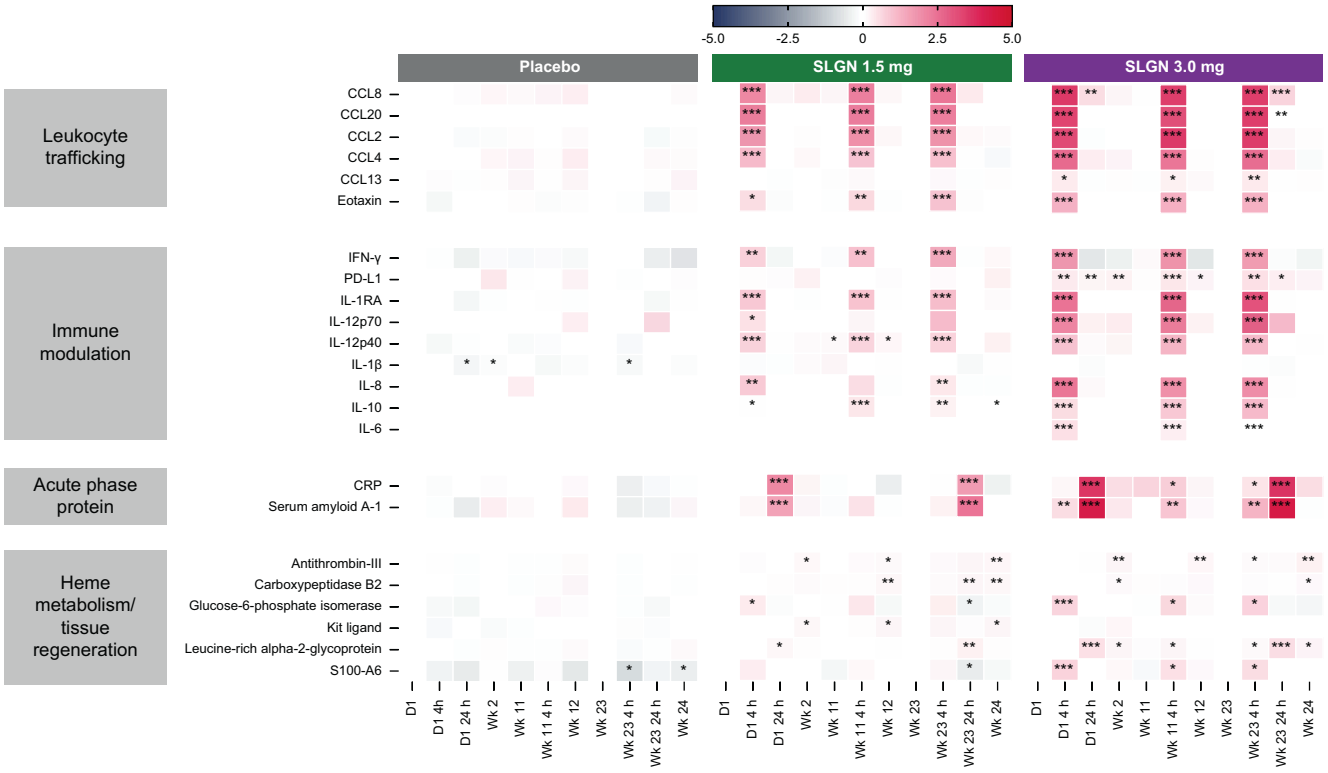

Fig. 2. Dose-dependent cytokine responses to SLGN. *p <0.05; **p <0.01; ***p <0.001; two-sided Wilcoxon test comparison to baseline; unadjusted p value reported. CCL, C–C motif chemokine; CRP, C-reactive protein; IFN, interferon; IL-, interleukin-; PD-L1, programmed cell death ligand 1; RA, receptor antagonist; SLGN, selgantolimod.

who were HBeAg positive *vs.* negative at baseline: –0.14 and –0.03 $\log_{10}$ IU/ml, respectively, at week 24 (p <0.001) and –0.22 and –0.02, respectively, at week 48 (p <0.001).

No patients achieved HBsAg or HBeAg loss (defined as a negative qualitative antigen result) or seroconversion. Decreases in HBeAg (in HBeAg-positive patients only) and HBcrAg were numerically greater in patients receiving selgantolimod *vs.* placebo through week 48, with both HBeAg and HBcrAg returning to near-baseline levels during the TFFU period (Fig. S6).

At week 24, 9 of 24 (38%), 9 of 28 (32%), and 4 of 12 (33%) patients receiving selgantolimod 3 mg, selgantolimod 1.5 mg, and placebo, respectively, had HBV DNA below the lower limit of quantification. Of these patients, one in the selgantolimod 3 mg group was HBeAg positive at baseline. At week 48, 12 of 24 (50%), 12 of 27 (44%), and 5 of 11 (46%) patients receiving selgantolimod 3 mg, selgantolimod 1.5 mg, and placebo, respectively, had HBV DNA below the lower limit of quantification. Of these patients, three in the selgantolimod 3 mg group and three in the selgantolimod 1.5 mg group were HBeAg positive at baseline. A 2 $\log_{10}$ IU/ml decline in HBV DNA was observed in all treatment groups as early as week 2, with HBV DNA continuing to decline until week 24, then remaining numerically stable through week 48. HBV DNA levels returned to near-baseline levels during the TFFU period (Fig. 5A). Patients with or without ALT and hepatitis flares had similar changes in HBV DNA (Table S5). All six patients with an ALT flare had an increase in HBV DNA during the TFFU period. The rate of rebound in HBV DNA was numerically greater in HBeAg-positive *vs.* -negative patients, most likely due to greater baseline levels of HBV DNA.

Over the course of 96 weeks, median total HBV RNA did not significantly change from baseline, regardless of treatment (Fig. 5B). Stratifying patients by HBV genotype did not reveal any significant changes in HBV RNA throughout the study (Fig. S7). Overall, the rate of HBV RNA rebound during TFFU was equivalent between HBeAg-positive and -negative patients. No patients developed treatment-emergent resistance to any study drug.

Discussion

This phase II study evaluated the safety, PD, and antiviral activity of selgantolimod with TAF in viremic patients with CHB not currently on treatment. Selgantolimod at doses of 1.5 mg or 3 mg administered QW for 24 weeks was generally well tolerated. Selgantolimod administered with TAF resulted in dose-dependent increases in immunomodulatory cytokines and led to numerically greater declines in HBsAg levels compared with TAF monotherapy during the treatment phase.

The safety profile of selgantolimod in this study is similar to that observed in prior phase II and phase Ia/b studies.[25,28,29] Most TEAEs associated with selgantolimod during the treatment period were grade 1. Gastrointestinal-related TEAEs were the most common during treatment (Table 2). TEAEs considered related to study drug were dose proportional, particularly nausea, fatigue, and vomiting. Only one patient (1.5 mg group) had a TEAE (upper abdominal pain and vomiting) related to selgantolimod that led to treatment discontinuation; this TEAE resolved the same day it occurred. Overall, most TEAEs reported with selgantolimod likely represented drug-related signals and

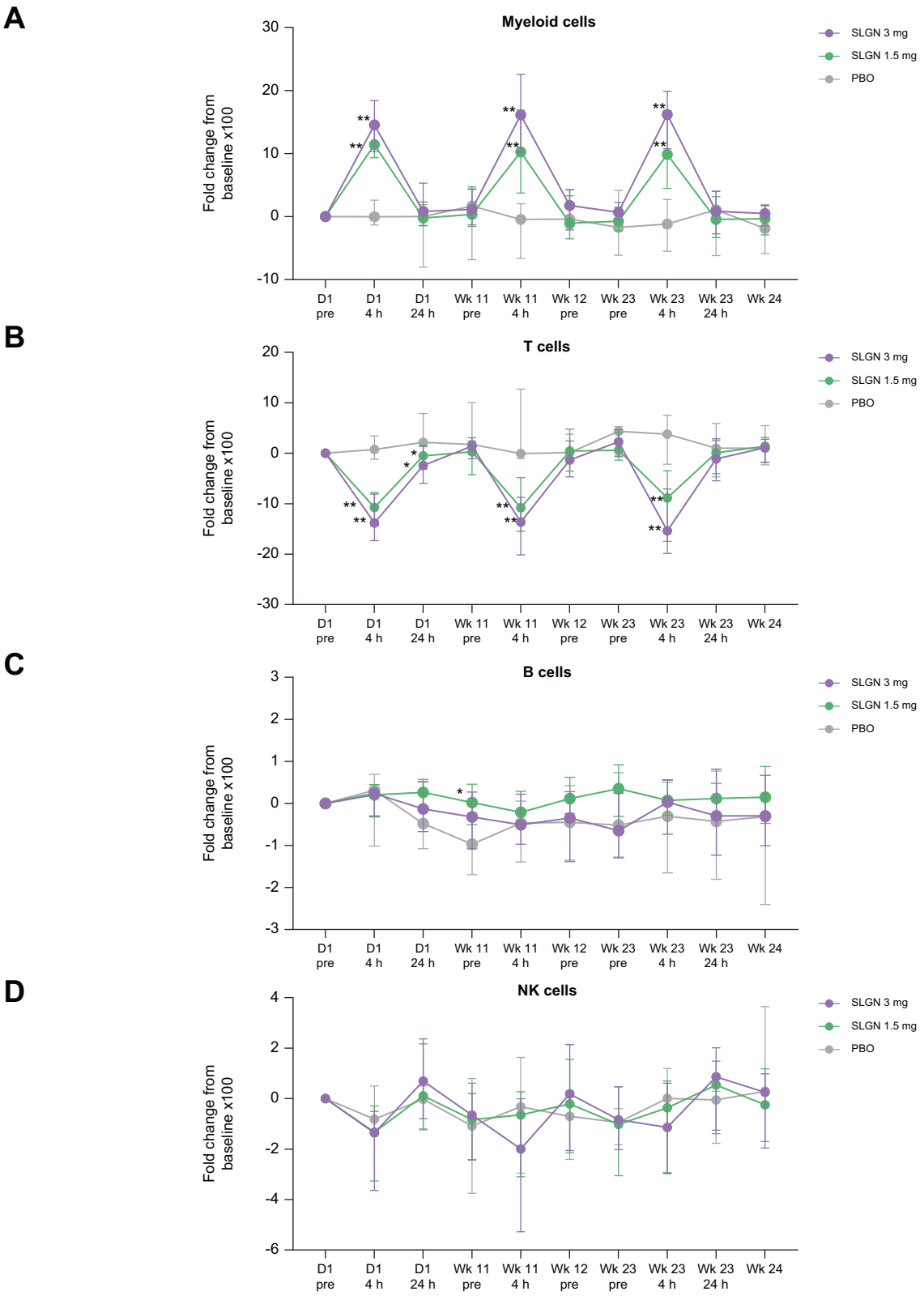

Fig. 3. Immune cell shifts in peripheral blood with SLGN. Redistribution of (A) myeloid cells (HLA-DR$^+$ CD11c$^+$, including monocytes and classical/conventional dendritic cells), (B) T cells (CD3$^+$), (C) B cells (HLA-DR$^+$ CD19$^+$), and (D) NK cells (CD56$^+$ CD3$^-$) in the peripheral blood of patients. *p <0.05; **p <0.001; two-sided Wilcoxon test comparison to baseline value; unadjusted p value reported. NK, natural killer; PBO, placebo; Pre, predose; SLGN, selgantolimod.

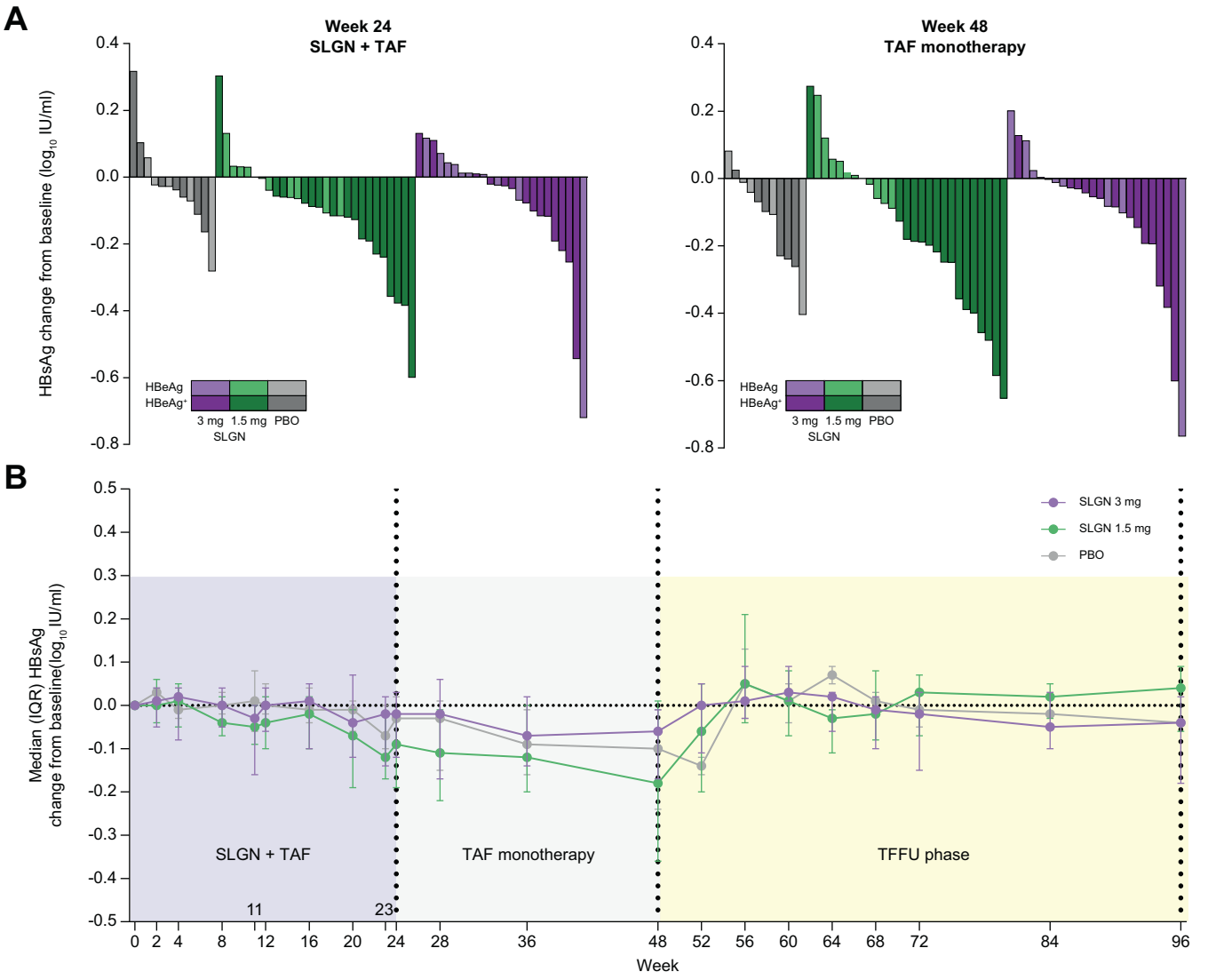

Fig. 4. HBsAg (log_{10} IU/ml) changes from baseline (FAS). (A) Individual patient HBsAg change from baseline (log_{10} IU/ml) at weeks 24 and 48. (B) Median (IQR) change from baseline in HBsAg through week 96. All treatment groups also received TAF 25 mg through week 48. FAS, full analysis set; PBO, placebo; SLGN, selgantolimod; TAF, tenofovir alafenamide; TFFU, treatment-free follow-up.

were manageable. An ongoing phase IIa trial (NCT04891770) is evaluating selgatolimod and the immune-checkpoint inhibitor nivolumab in combination with the small interfering ribonucleic acid VIR-2218 in NA-treated patients with viremic HBV infection; the results of this trial will help to characterize selgantolimod's safety as part of combination regimens.

Increased ALT and hepatic inflammation in the natural course of CHB infection are often due to host immune-mediated antiviral response.[30] During the treatment period, ALT levels declined from baseline in all treatment groups then rebounded to near-baseline levels during the TFFU phase. Six patients exhibited ALT flares during the TFFU period. This was most likely related to TAF discontinuation.[31] After discontinuation of OAVs, viral rebound may occur and trigger greater responsiveness of T cells; less exhausted T cells may be more likely to achieve antigen suppression and show a higher proliferative capacity.[32] These flares did not appear to be associated with any statistically significant changes in HBV DNA or HBsAg levels based on comparison with patients who did not experience such flares (up to week 48).

Selgantolimod-induced cytokines may be important for the expansion and activity of multiple T-cell subsets as well as some innate immune subsets. Median IL-12p40, IL-1RA, and IFN-γ levels tended to peak approximately 4 h after selgantolimod dosing. The magnitude of IL-12p40, IL-1RA, and IFN-γ PD responses postdose was similar at each study time point, indicating a lack of tachyphylaxis, which supports weekly dosing in future studies. These changes were accompanied by apparent shifts in the balance of circulating myeloid and lymphoid cell populations, with some T-cell populations depleted from circulation, while B- and NK-cell populations were unaffected. These data suggest selgantolimod induces rapid redistribution of some immune cell subsets from circulation, possibly into the liver or other tissues. Flow cytometry data at these time points did not indicate signals of lymphocyte activation (data not shown), which have been observed in selgantolimod studies with PBMCs

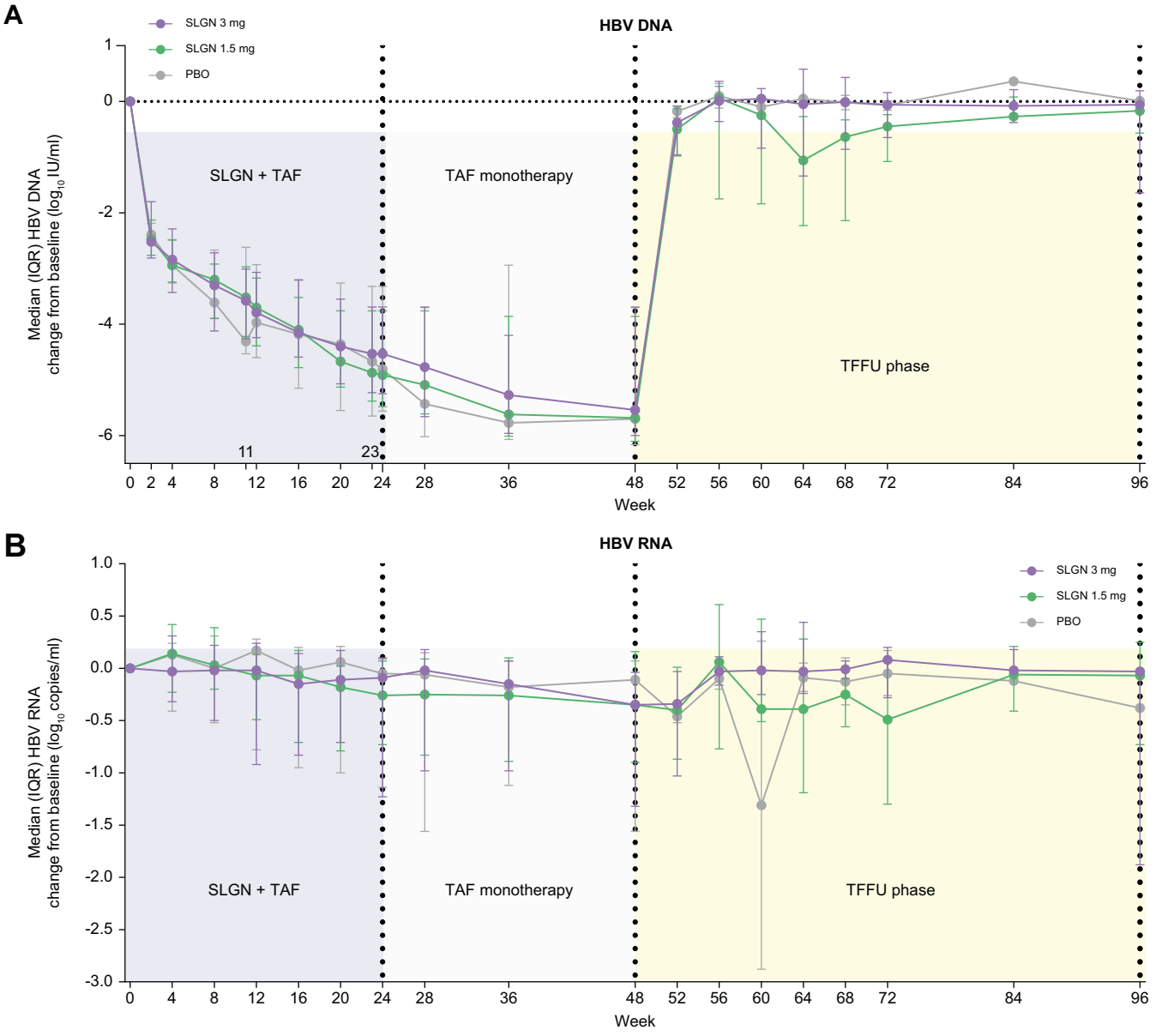

Fig. 5. HBV DNA (log_{10} IU/ml) and HBV RNA (log_{10} copies/ml) changes from baseline (FAS). Median (IQR) change from baseline in (A) HBV DNA and (B) HBV RNA through week 96. All treatment groups also received TAF 25 mg through week 48. FAS, full analysis set; PBO, placebo; SLGN, selgantolimod; TAF, tenofovir alafenamide; TFFU, treatment-free follow-up.

in vitro.[26] This could be explained by the greatest exposure of selgantolimod occurring in the gut and liver, whereas blood cells may have limited exposure to the study drug.

The primary efficacy endpoint (≥1 log_{10} IU/ml decline from baseline in HBsAg at week 24) was not met by any study patient. No patients achieved HBsAg or HBeAg seroconversion. During the treatment period, the greatest changes from baseline in HBsAg were in the selgantolimod-treated groups, including HBsAg declines ≥0.5 log_{10} IU/ml observed only in selgantolimod-treated patients. Among patients who received selgantolimod, changes were greater in those with a baseline ALT >ULN or who were HBeAg positive. These observed differences in HBsAg by HBeAg status and baseline ALT are reported in other studies.[33,34] Following the decline in HBV DNA during the treatment period (which was numerically greater in the selgantolimod 3 mg group *vs.* the placebo group among HBeAg-positive patients), HBV DNA returned to near-pretreatment levels during TFFU. Small declines during treatment in HBV RNA, HBeAg, and HBcrAg were nonsignificant and returned to near-baseline levels during TFFU. These findings are similar to those observed in virologically suppressed patients.[25] No patients experienced treatment-emergent resistance in this study.

The current goal of HBV cure strategies is to achieve durable off-treatment HBsAg loss with or without seroconversion to anti-HBsAg antibodies. NAs do not directly target covalently closed circular DNA, the template for HBV replication.[35] Novel combination-treatment approaches using distinct, but complementary, MoAs likely represent the best path to achieve durable

HBV responses off treatment. As proof of concept for this approach, combining pegylated-IFN-α with NAs resulted in increased antiviral activity and HBsAg loss compared with NA monotherapy.[36] Given the poor tolerability of pegylated-IFN-α, new immune-modifying agents are needed for combination therapies (*e.g.*, with antigen-reducing agents) to achieve HBV cure in the majority of individuals living with CHB.

This study has several limitations, including a small sample population that was predominantly Asian. Selgantolimod was administered for only 24 weeks, which may not be long enough to reveal any long-term treatment-related AEs or further changes in antiviral activity. For example, HBcrAg, a surrogate marker for the transcriptional activity of covalently closed circular DNA, may not show changes from baseline within a 24-week treatment period. Previous reports of agents with different MoAs suggest this may take an extended period of time.[37–39]

In conclusion, selgantolimod was safe and well tolerated at doses up to 3 mg when administered with TAF to patients with CHB. This study demonstrated limited activity for selgantolimod as a monotherapy. The observed PDs and antiviral activity suggest that future studies should focus on combining varying MoAs, which is likely the best path to functional cure of HBV.

Abbreviations

AE, adverse event; ALT, alanine aminotransferase; CHB, chronic hepatitis B; HBcrAg, hepatitis B core-related antigen; IFN, interferon; IL-, interleukin-; MoAs, mechanisms of action; NA, nucleos(t)ide analogue; NK, natural killer; OAV, oral antiviral; PBMC, peripheral blood mononuclear cell; PD, pharmacodynamic; QW, once weekly; TAF, tenofovir alafenamide; TEAE, treatment-emergent adverse event; TFFU, treatment-free follow-up; TLR, Toll-like receptor.

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Conflicts of interest

Harry L. Janssen reports receiving grants from AbbVie; Arbutus Biopharma; Gilead Sciences, Inc.; Janssen; Merck; and Roche; and serving as a consultant for Arbutus Biopharma; Arena Pharmaceuticals; Enyo Pharma; Gilead Sciences, Inc.; GSK; Janssen; Merck; Roche; Vir Biotechnology; and Viroclinics Biosciences. Young-Suk Lim reports serving on the advisory board for Gilead Sciences, Inc., and receiving research funding from Bayer Healthcare and Gilead Sciences, Inc. Hyung Joon Kim reports no conflicts of interest. Leonard Sowah reports being an employee of and owning stock in Gilead Sciences, Inc. Cheng-Hao Tseng reports serving as a speaker for Abbvie; Bayer Healthcare; Bristol Myers Squibb; Gilead Sciences, Inc.; and Merck Sharp & Dohme. Carla S. Coffin reports investigator-initiated research grants/research materials from Arbutus Biopharma; Gilead Sciences, Inc.; GSK; and Janssen; having served as a consultant for Gilead Sciences, Inc., and Roche; and serving on an advisory board for Altimmune Pharmaceuticals with funds paid to the Canadian HBV Network, University of Calgary. Magdy Elkhashab reports receiving grants from AbbVie; Bristol Myers Squibb; Eisai; Gilead Sciences, Inc.; and Roche; and serving on advisory boards for AbbVie; Bristol Myers Squibb; Gilead Sciences, Inc.; and Merck. Sang Hoon Ahn has acted as an advisor and investigator for AbbVie; Aligos; Arbutus; Assembly Biosciences, Inc.; Brii; GeneOne Life Science; Gilead Sciences, Inc.; GSK; GreenCross; Ildong; Inovio; Janssen; Roche; Samil; SL Vaxigen; Vaccitech; Vir Biotechnology; and Yuhan. Anh-Hoa Nguyen reports being an employee of Gilead Sciences, Inc., and owning stock. Diana Chen reports being a former employee of Gilead Sciences, Inc., and owning stock. Jeffrey J. Wallin reports being an employee of Gilead Sciences, Inc., and owning stock. Simon P. Fletcher reports being an employee of Gilead Sciences, Inc., and owning stock. Circe McDonald reports being an employee of Gilead Sciences, Inc., and owning stock. Jenny C. Yang reports being a former employee of Gilead Sciences, Inc., and owning stock. Anuj Gaggar reports being a former employee of Gilead Sciences, Inc., and owning stock. Diana M. Brainard reports being a former employee of Gilead Sciences, Inc., and owning stock. Scott Fung reports receiving fees for speaking, teaching, and/or serving on advisory committees for AbbVie; Gilead Sciences, Inc.; Janssen; Lupin; Novo Nordisk; and Pfizer. Yoon Jun Kim reports no conflicts of interest. Jia-Horng Kao reports no conflicts of interest. Wan-Long Chuang reports serving as a consultant for AbbVie; Bristol Myers Squibb; Gilead Sciences, Inc.; Merck Sharp & Dohme; and PharmaEssentia; and has served as a speaker for AbbVie; Bristol Myers Squibb; Gilead Sciences, Inc.; Merck Sharp & Dohme; and PharmaEssentia. Anna E. Brooks reports consulting and conducting contract research for Gilead Sciences, Inc. P. Rod Dunbar reports consulting and conducting contract research for Arrowhead Pharmaceuticals, DrugFarm, and Gilead Sciences, Inc.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

All authors approved the final manuscript prior to submission. Conceptualization: Harry L. Janssen, Simon Fletcher, Anuj Gaggar, Diana M. Brainard. Data curation: Circe McDonald. Data interpretation: Anh-Hoa Nguyen, Diana Chen, Jeffrey J. Wallin. Formal analysis: Anh-Hoa Nguyen, Diana Chen, Jeffrey J. Wallin, Circe McDonald, Diana M. Brainard, Leonard Sowah, P. Rod Dunbar. Investigation: Harry L. Janssen, Young-Suk Lim, Hyung Joon Kim, Cheng-Hao Tseng, Carla S. Coffin, Magdy Elkhashab, Sang Hoon Ahn, Scott Fung, Yoon Jun Kim, Jia-Horng Kao, Wan-Long Chuang, Anna E. Brooks. Methodology: Anuj Gaggar, Diana M. Brainard, P. Rod Dunbar. Resources: Carla S. Coffin. Supervision: Carla S. Coffin, Jenny C. Yang, Anuj Gaggar, Diana M. Brainard. Visualization: Circe McDonald, P. Rod Dunbar. Writing – Review and Editing: Harry L. Janssen, Carla S. Coffin, Anh-Hoa Nguyen, Diana Chen, Jeffrey J. Wallin, Simon Fletcher, Anuj Gaggar, Diana M. Brainard, Leonard Sowah, P. Rod Dunbar.

Data availability statement

Anonymized individual patient data will be shared upon request for research purposes dependent upon the nature of the request, the merit of the proposed research, the availability of the data, and its intended use. The full data-sharing policy for Gilead Sciences, Inc., can be found at https://www.gileadclinicaltrials.com/transparency-policy/

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jhepr.2023.100975.

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Author names in bold designate shared co-first authorship

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Supplemental information

Safety, pharmacodynamics, and antiviral activity of selgantolimod in viremic patients with chronic hepatitis B virus infection

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Table of contents

Supplementary methods

Inclusion criteria

Patients were required to meet all of the following inclusion criteria to be eligible for participation in this study:

1. Must have the ability to understand and sign a written informed consent form, which must be obtained prior to initiation of study procedures
2. Adult male or nonpregnant, non-lactating female patients aged 18 to 65 years on the date of the screening visit
3. Documented evidence of chronic HBV infection (eg, HBsAg positive for more than 6 months) with detectable HBsAg levels at screening
4. Females of childbearing potential must have a negative serum pregnancy test at screening and a negative urine pregnancy test at baseline prior to enrollment
5. Male and female patients of childbearing potential who engage in heterosexual intercourse must agree to use protocol-specified method(s) of contraception
6. Screening HBV DNA ≥2000 IU/mL
7. Screening electrocardiogram without clinically significant abnormalities and with QTcF interval (QT corrected using Fridericia's formula) ≤450 msec for males and ≤470 msec for females
8. Must be willing and able to comply with all study requirements

Exclusion criteria

Patients who met any of the following exclusion criteria were not enrolled in this study:

1. Extensive bridging fibrosis or cirrhosis as defined clinically, by imaging, or by the following:
    a. Metavir ≥3 or Ishak fibrosis score ≥4 by a liver biopsy within 3 years of screening, or, in the absence of an appropriate liver biopsy, either:
    b. Screening FibroTest score of >0.48 and aspartate aminotransferase to platelet ratio index >1, or
    c. Historic FibroScan with a result >9 kPa within ≤6 months of screening (if available)
        i. If an appropriate liver biopsy is available, the liver biopsy result supersedes (b) and/or (c, if available)
        ii. If an appropriate liver biopsy is not available, fibrosis will be evaluated by (b) and/or (c, if available). In the event of discordance between (b) and (c), the FibroScan results will take precedence
2. Received 1 or more commercially available HBV oral antiviral treatments (tenofovir alafenamide, tenofovir disoproxil fumarate, entecavir, adefovir dipivoxil, lamivudine, telbivudine, either as single agents or in combination) within the 3 months prior to screening
3. Received prolonged therapy with immunomodulators (eg, corticosteroids) or biologics (eg, monoclonal antibody, interferon) within 3 months of screening
4. Patients meeting any of the following laboratory parameters at screening:
    a. Hemoglobin <12 g/dL (for males) or <11 g/dL (for females)
    b. White blood cell count <2500 cells/mm^3

c. Neutrophil count <1500 cells/mm^3 (or <1000 cells/mm^3 if considered a physiological variant in a patient of African descent)
d. Alanine aminotransferase (ALT) >5× upper limit of normal (ULN)
e. International normalized ratio (INR) >ULN unless the patient is stable on an anticoagulant regimen affecting INR
f. Albumin <3.5 g/dL
g. Direct bilirubin >1.5× ULN
h. Platelet count <100,000/μL
i. Estimated creatinine clearance <60 mL/min (using the Cockcroft-Gault method) based on serum creatinine and actual body weight as measured at the screening evaluation

5. Coinfection with HIV, HCV, or HDV
    a. Patients who are HCV antibody positive, but have a documented negative HCV RNA, are eligible
6. Prior history of HCC (eg, as evidenced by prior imaging) or screening alpha-fetoprotein ≥50 ng/mL without imaging to rule out HCC
7. Malignancy within 5 years prior to screening, with the exception of specific cancers that are cured by surgical resection (eg, basal cell skin cancer). Patients under evaluation for possible malignancy are not eligible
8. Significant cardiovascular, pulmonary, or neurological disease in the opinion of the investigator
9. Diagnosis of an autoimmune disease (eg, systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, autoimmune hepatitis,

sarcoidosis, psoriasis of greater than mild severity, autoimmune uveitis), poorly controlled diabetes mellitus, significant psychiatric illness, severe chronic obstructive pulmonary disease, hemoglobinopathy, or retinal disease, or are immunosuppressed

10. Chronic liver disease of a non-HBV etiology (eg, Wilson's disease, hemochromatosis, alpha-1-antitrypsin deficiency, cholangitis, nonalcoholic steatohepatitis), except for nonalcoholic fatty liver disease
11. Received a solid organ or bone marrow transplant
12. Use of another investigational agent within 90 days of screening, unless allowed by the sponsor
13. Current alcohol or substance abuse judged by the investigator to potentially interfere with patient compliance
14. Known hypersensitivity to study drugs or formulation excipients
15. Women who are breastfeeding, pregnant, or who wish to become pregnant during the course of the study
16. Female patients unwilling to refrain from egg donation and in vitro fertilization during and until at least 30 days after the last study drug dose
17. Male patients unwilling to refrain from sperm donation during and until at least 90 days after the last study drug dose
18. Use of any prohibited concomitant medications
19. Believed by the study investigator to be inappropriate for study participation for any reason not otherwise listed

Assays and evaluations

Serum HBV DNA, viral resistance, and HBsAg were assessed on day 1 and weeks 2, 4, 8, 11, 12, 16, 20, and 23. HBV DNA was quantified using the COBAS Ampliprep/COBAS TaqMan version 2.0 (Roche, Basel, Switzerland; lower limit of quantification [LLOQ], 20 IU/mL). HBsAg and HBeAg were quantified using the Architect i2000SR HBsAg assay (Abbott Laboratories, Abbott Park, IL, USA), with an LLOQ of 0.05 IU/mL for HBsAg and 0.11 IU/mL for HBeAg. HBeAg, HBV RNA, and hepatitis B core-related antigen (HBcrAg) were assessed on day 1 and weeks 4, 8, 12, 16, and 20. Total HBV RNA was quantified using previously validated methods involving total nucleic acid isolation from plasma using the NucleiSENS easyMAG system (Biomerieux, Marcy-I-Etolie, France), followed by quantitative reverse transcriptase-PCR by the LC480 II real-time PCR instrument according to manufacturer instructions (Roche, Basel, Switzerland).[1] HBcrAg was quantified using the Lumipulse G HBcrAg assay (Fujirebio Europe, Gent, Belgium; LLOQ, 2.6 $\log_{10}$ U/mL). ALT was assessed according to standard methods with normal ranges of ≤25 U/L for females and ≤35 U/L for males, as defined by the American Association for the Study of Liver Diseases. Covance Central Laboratory Services, Inc., (Indianapolis, IN, USA) processed all the routine clinical laboratory data. For resistance testing, Illumina-MiSeq deep sequencing of the polymerase and reverse transcriptase region was performed for qualified patients. Sequence analysis of the HBV full genome was performed by DDL Diagnostic Laboratory (Rijswijk, Netherlands) for the characterization of resistance mutations for any patients for whom DNA was available and who were being treated with oral

antivirals and experienced virologic breakthrough, as well as for patients who were not suppressed by week 48.

Safety laboratory tests were conducted on day 1 and weeks 2, 4, 8, 11, 12, 16, 20, and 23. Clinical and laboratory adverse events (AEs) were coded using the Medical Dictionary for Regulatory Activities (version 23.0). A treatment-emergent AE for the primary analysis was defined as any AE with an onset date on or after the study drug date and no later than 30 days after permanent discontinuation of the study drug or any AE leading to premature discontinuation of the study drug. Serious AEs were defined as an event that, at any dose, resulted in death, a life-threatening situation, inpatient hospitalization, persistent or significant disability, a congenital abnormality, or a medically significant event that, based on medical judgment, may have jeopardized the patient or have required surgical intervention. Covance Central Laboratory Services, Inc., (Indianapolis, IN, USA) processed all the routine clinical laboratory data.

Serum and plasma pharmacodynamic (PD) biomarkers were assessed predose at day 1 and at weeks 2, 8, 11, 12, 16, 20, 23, 24, and 28. Postdose assessments were also made on day 1 and weeks 11 and 23. Serum levels of cytokines and chemokines were assessed using Luminex xMAP (Myriad, Austin, TX, USA). For assessment of IL-12p70 expression levels, the single molecule array Simoa (Quanterix, Billerica, MA, USA) ultrasensitive platform was used on plasma samples. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation with Lymphoprep (Axis-shield, Dundee, UK) and Leucosep tubes (Greiner, Kremsmünster, Austria). Cryopreserved PBMCs were thawed and rested in Roswell Park Memorial Institute Medium 1640 containing 10% fetal bovine serum (FBS) and DNAse-1 (20

µg/mL; Sigma-Aldrich, St. Louis, MO, USA) for 1 hour at 37 °C/5% CO_2. After incubation, cells were spun at 800 *g* for 10 minutes and resuspended in PBS supplemented with Trustain FcX Fc Receptor Blocking Solution (5 µL per tube; Biolegend, San Diego, CA, USA), True Stain Monocyte Blocker (5 µL per tube; Biolegend, San Diego, CA, USA), Zombie NIR Fixable Viability dye (1:6400 final dilution; Biolegend, San Diego, CA, USA), and EDTA (2 mM final concentration; Thermofisher, Waltham, MA, USA). After incubating for 20 minutes at room temperature, cells were stained with antibodies and incubated for another 20 minutes at room temperature. After staining of cell surface markers, cells were washed (Dulbecco's PBS + 1% FBS, 2 mM EDTA) and fixed with Fluorofix (Biolegend, San Diego, CA, USA) prior to acquisition. All data were acquired on a 4-laser Cytek Aurora (Cytek Biosciences, Freemont, CA, USA). Data analysis was performed using flow cytometry software express version 7 (De Novo Software, Pasadena, CA, USA). Covance Central Laboratory Services, Inc., (Indianapolis, IN, USA) processed all the PD biomarker serum samples for IL-12p40 and IL-1 receptor antagonist.

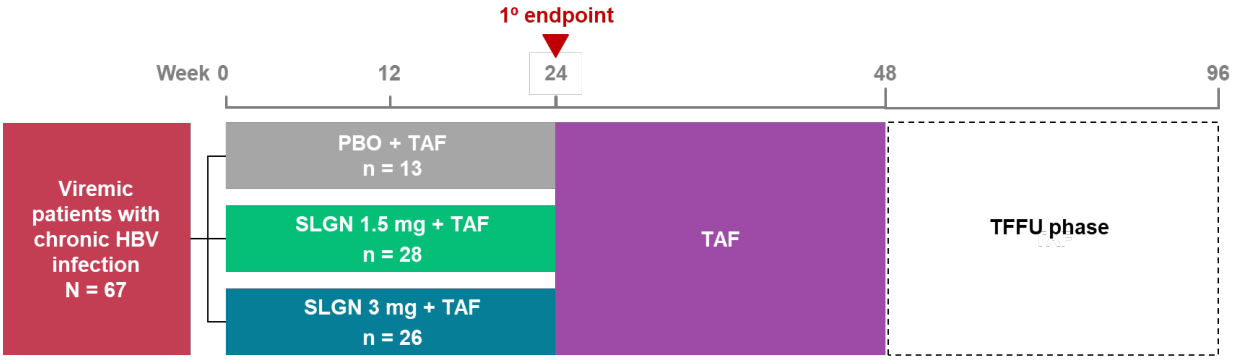
During the treatment-free follow-up phase, safety assessments, HBV serology, HBV virology, and exploratory biomarkers were assessed at weeks 52, 56, 60, 64, 68, 72, 84, and 96.

Supplementary figures

Fig. S1. Patient disposition and study design.

(A) The primary endpoint was assessed at week 24. After week 24, patients continued to receive TAF open label until week 48 and then were treatment free until week 96. **(B)** Of the 67 patients who enrolled and were randomized to receive study drug, 64 (96%) completed study treatment and 27 completed the TFFU. PBO, placebo; SLGN, selgantolimod; TAF, tenofovir alafenamide; TFFU, treatment-free follow-up; W, week.

A



B

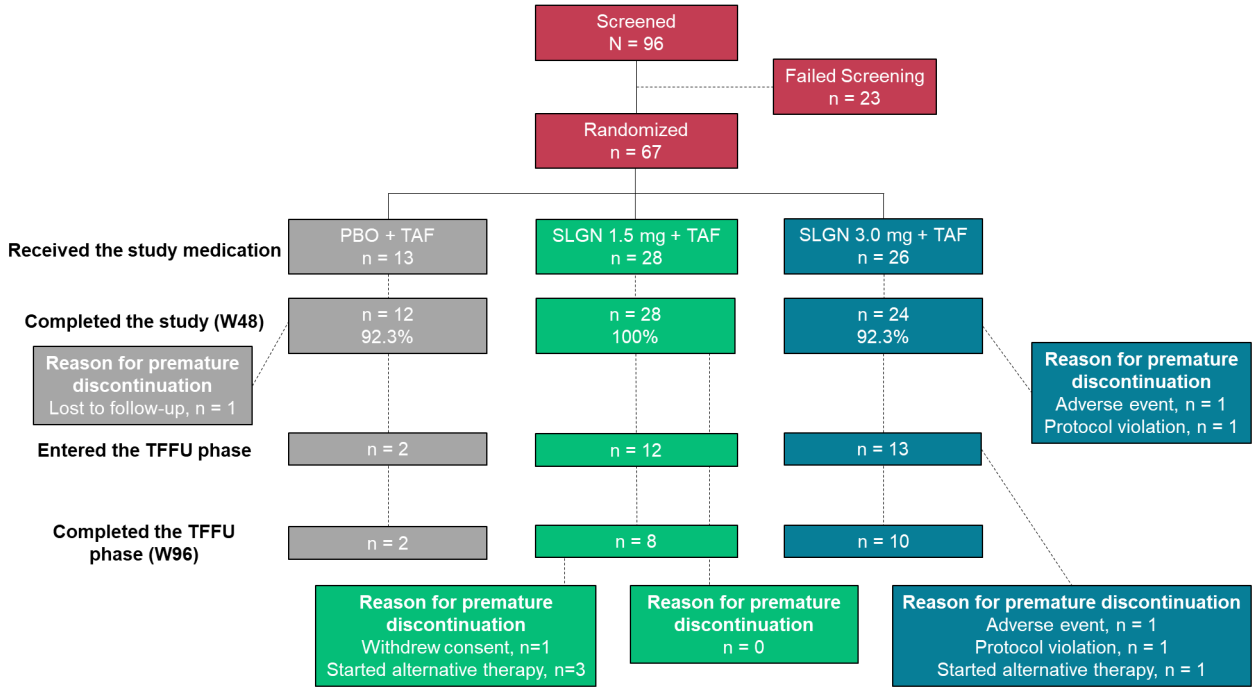


Fig. S2. Change from baseline in ALT (U/L) through week 96 (SAS).

ALT, alanine aminotransferase; IQR, interquartile range; PBO, placebo; SAS, safety analysis set; SLGN, selgantolimod; TAF, tenofovir alafenamide; TFFU, treatment-free follow-up.

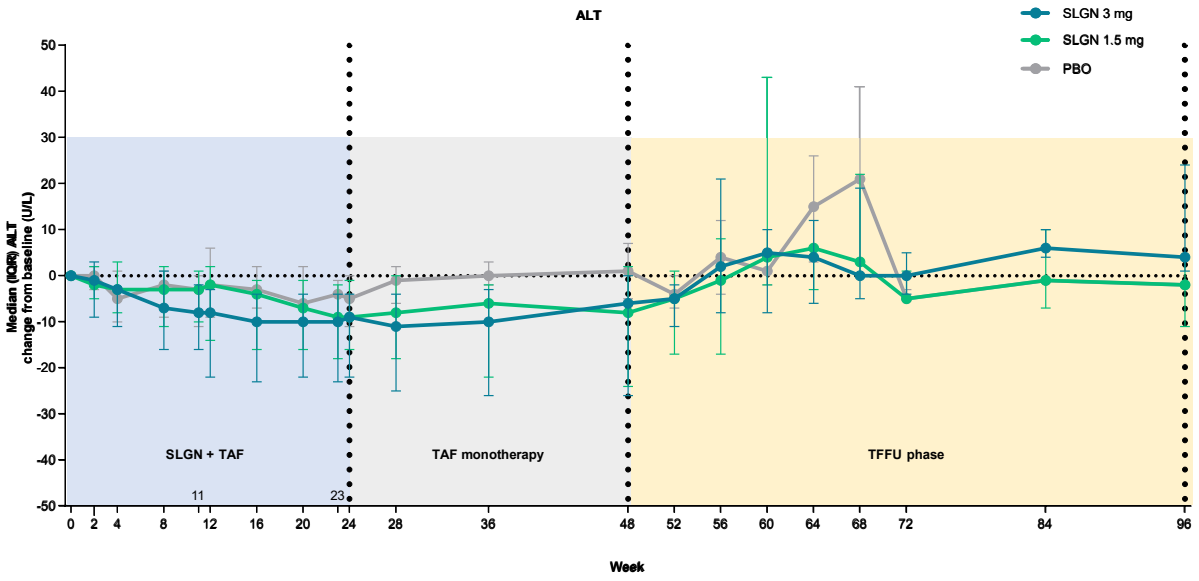


Fig. S3. Change from baseline in HBsAg (log_{10} IU/mL) in patients (FAS) with (A) HBV genotype B and in patients with (B) HBV genotype C.

FAS, full analysis set; IQR, interquartile range; PBO, placebo; SLGN, selgantolimod; TAF, tenofovir alafenamide.

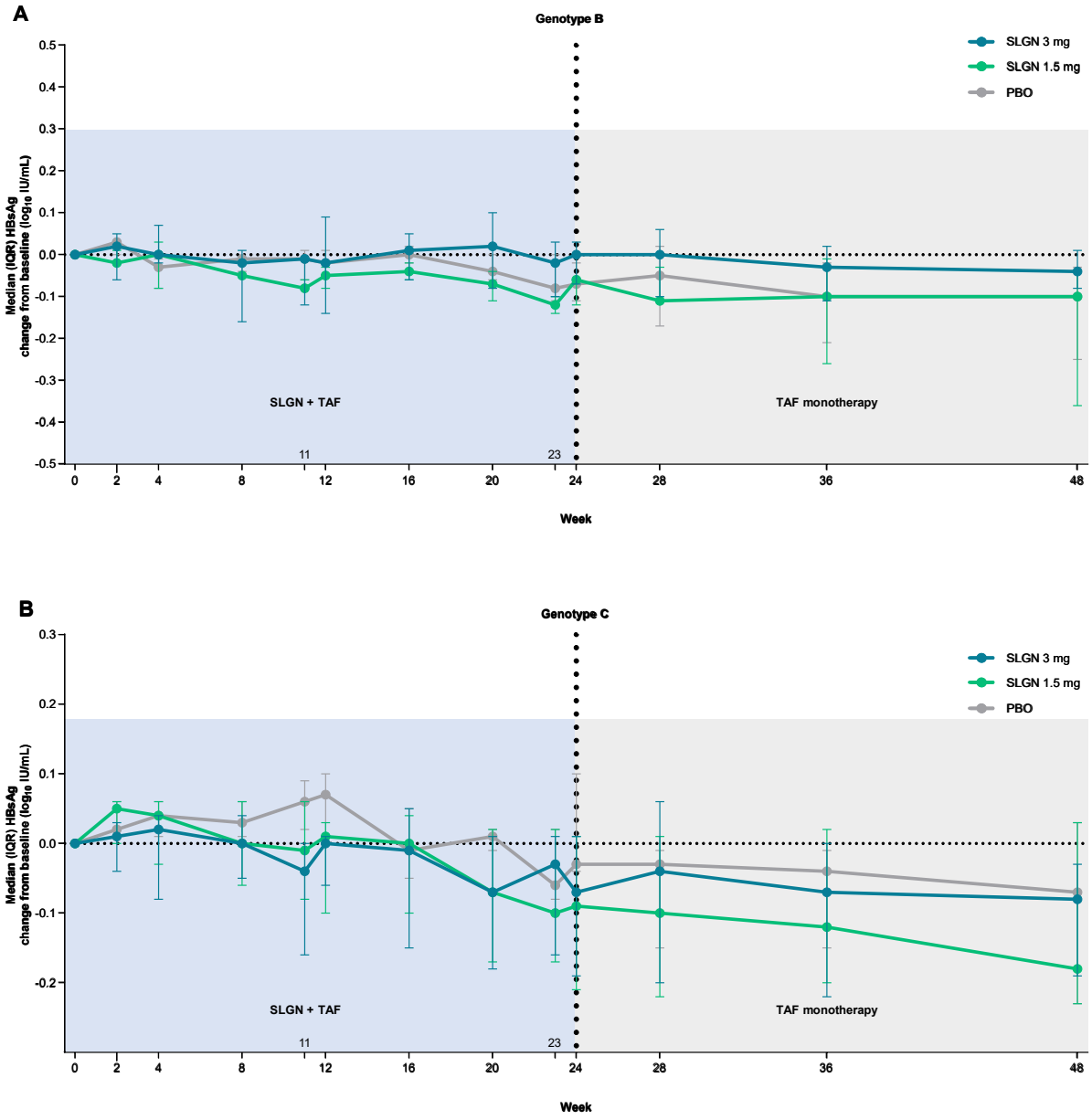


Fig. S4. Change from baseline in HBsAg (log_{10} IU/mL) by HBsAg level (FAS) in patients with (A) HBsAg <1000 IU/mL and in patients with (B) HBsAg ≥1000 IU/mL.

FAS, full analysis set; IQR, interquartile range; PBO, placebo; SLGN, selgantolimod; TAF, tenofovir alafenamide.

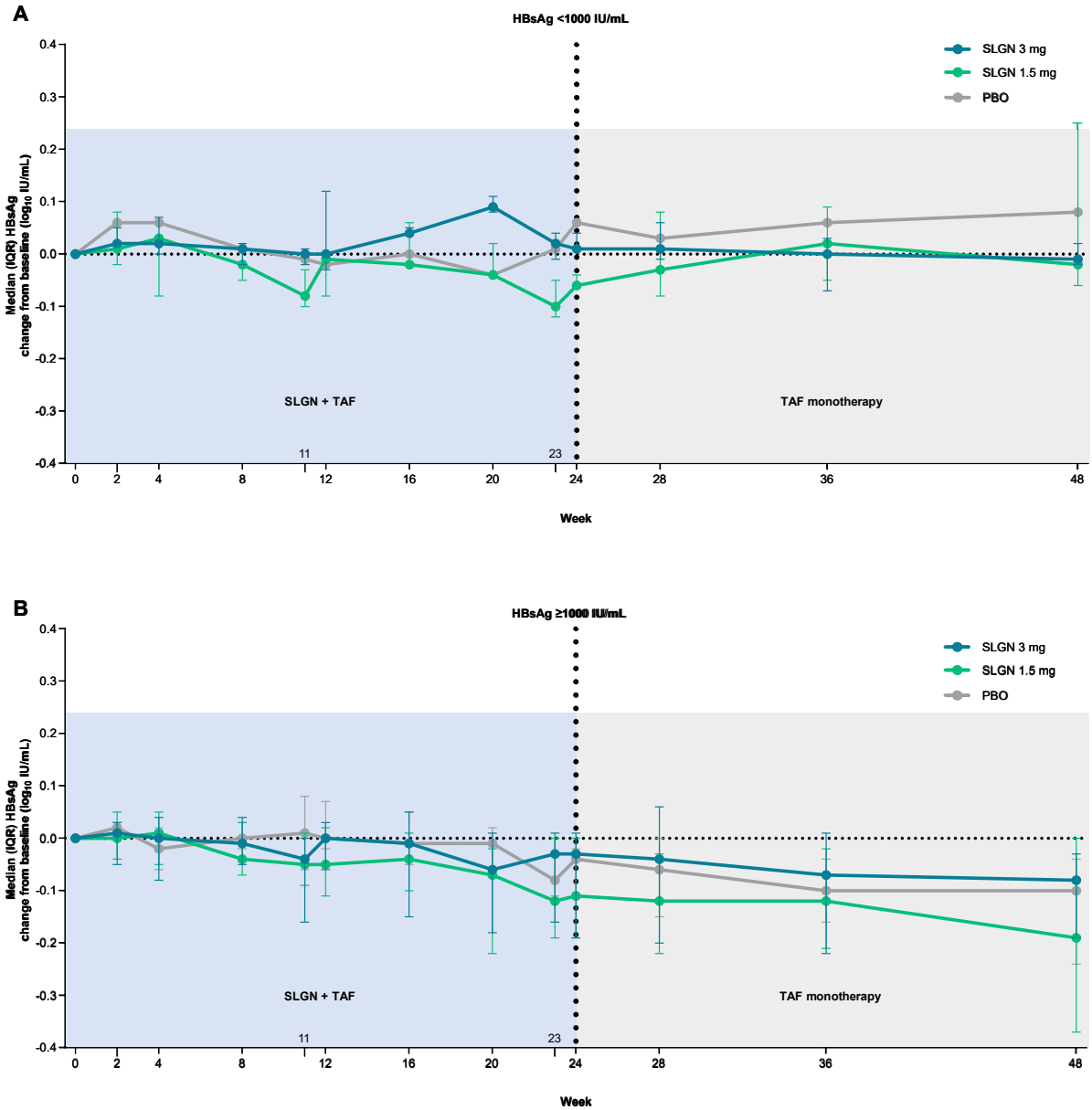


Fig. S5. Change from baseline in HBsAg (log_{10} IU/mL; FAS), in patients with (A) HBV DNA >20,000 IU/mL and in patients with (B) HBV DNA ≤20,000 IU/mL.

FAS, full analysis set; IQR, interquartile range; PBO, placebo; SLGN, selgantolimod; TAF, tenofovir alafenamide.

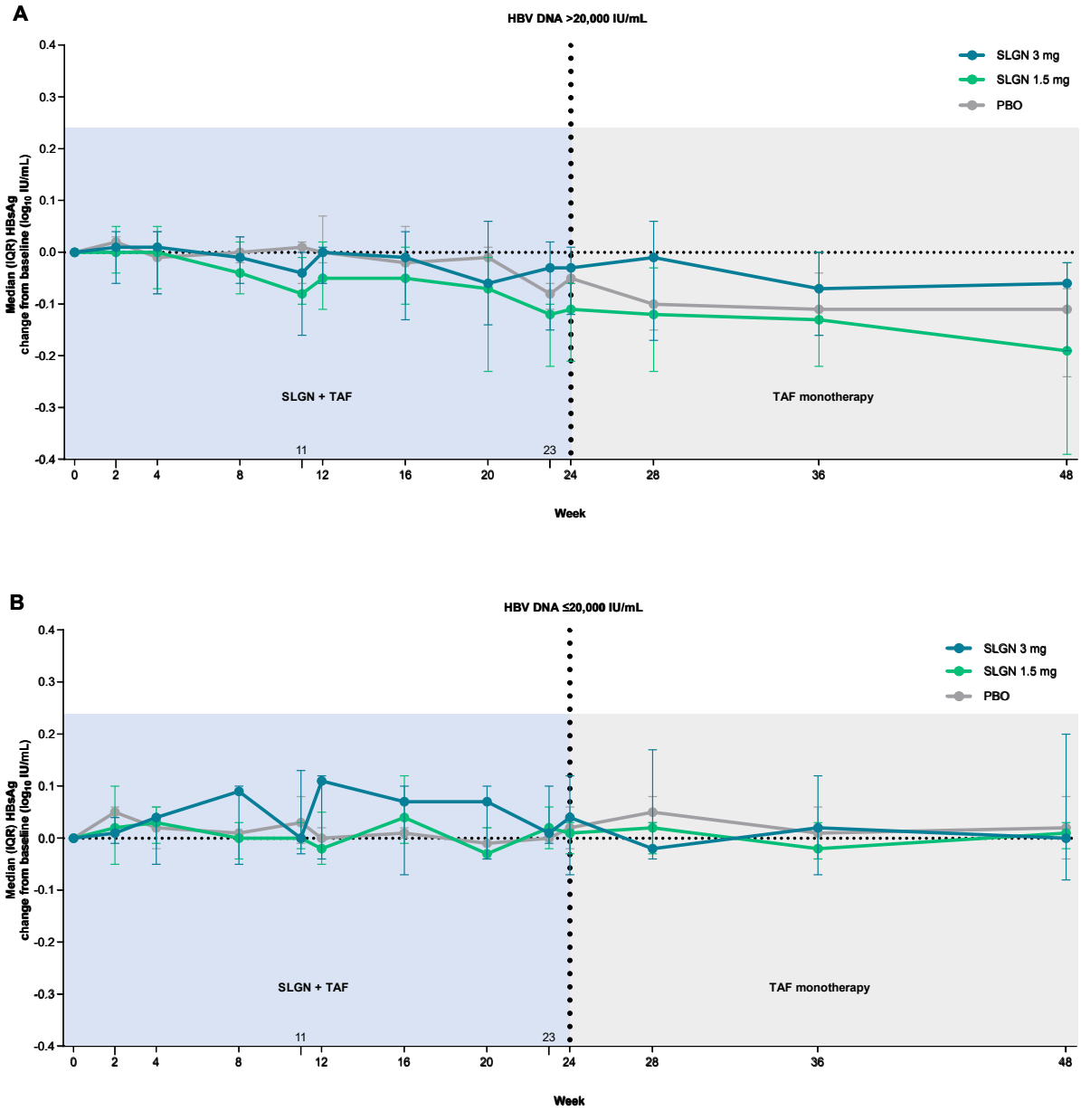
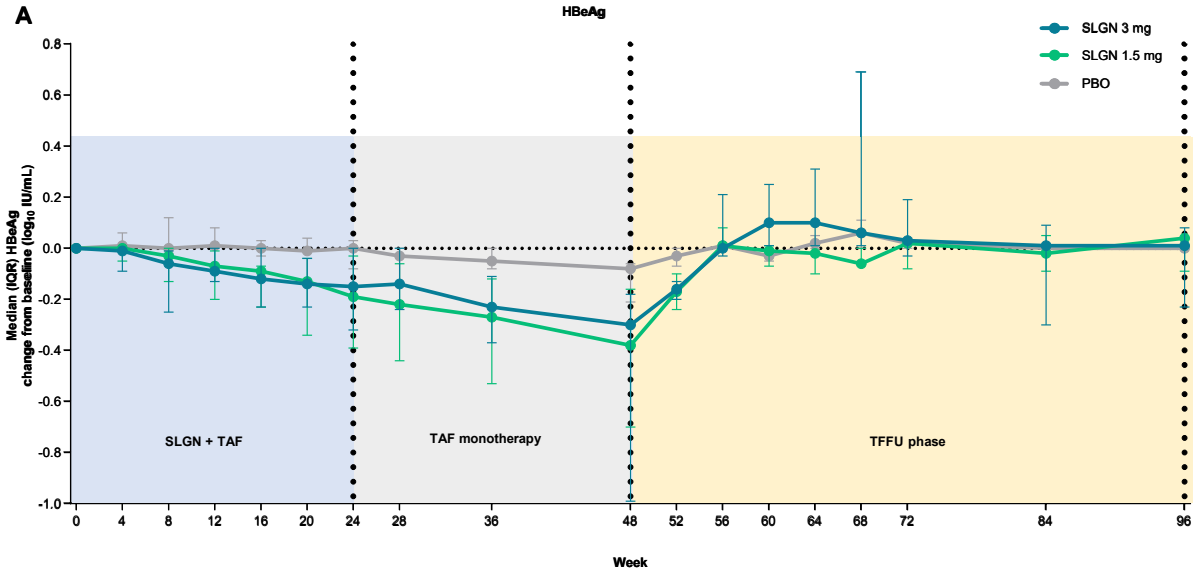


Fig. S6. Change from baseline in (A) HBeAg (log_{10} IU/mL; HBeAg-positive patients only) and (B) HBcrAg (log_{10} U/mL), FAS, throughout the study.

Both antigens experienced a numerically greater decrease from baseline in patients treated with SLGN+TAF compared with TAF monotherapy. Both antigens returned to near-baseline levels during TFFU.

FAS, full analysis set; HBcrAg, hepatitis B virus core-related antigen; IQR, interquartile range; PBO, placebo; SLGN, selgantolimod; TAF, tenofovir alafenamide; TFFU, treatment-free follow-up.



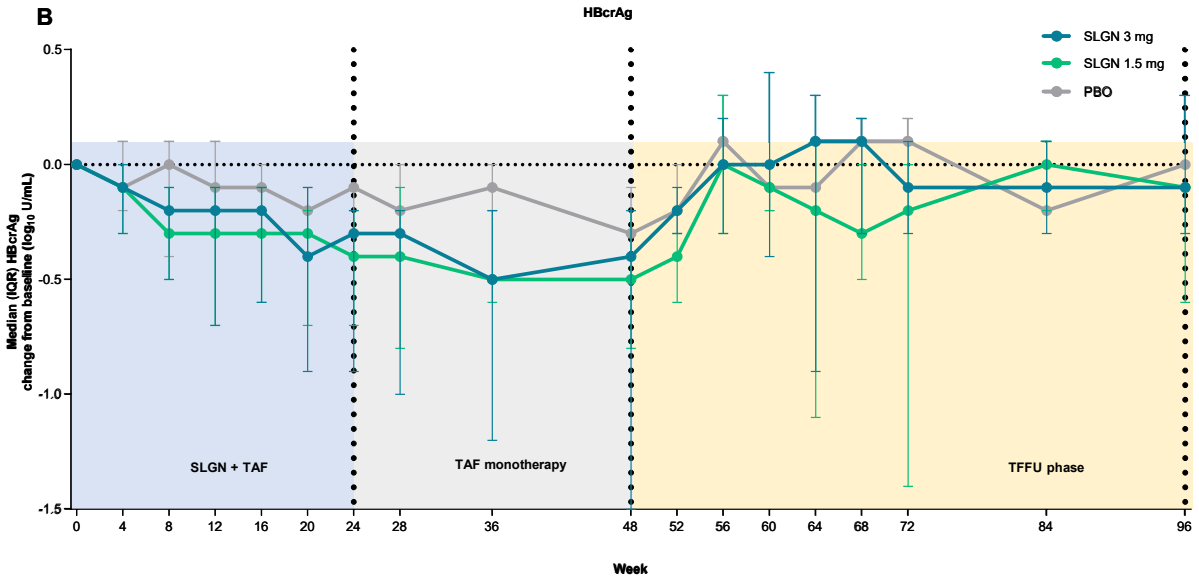
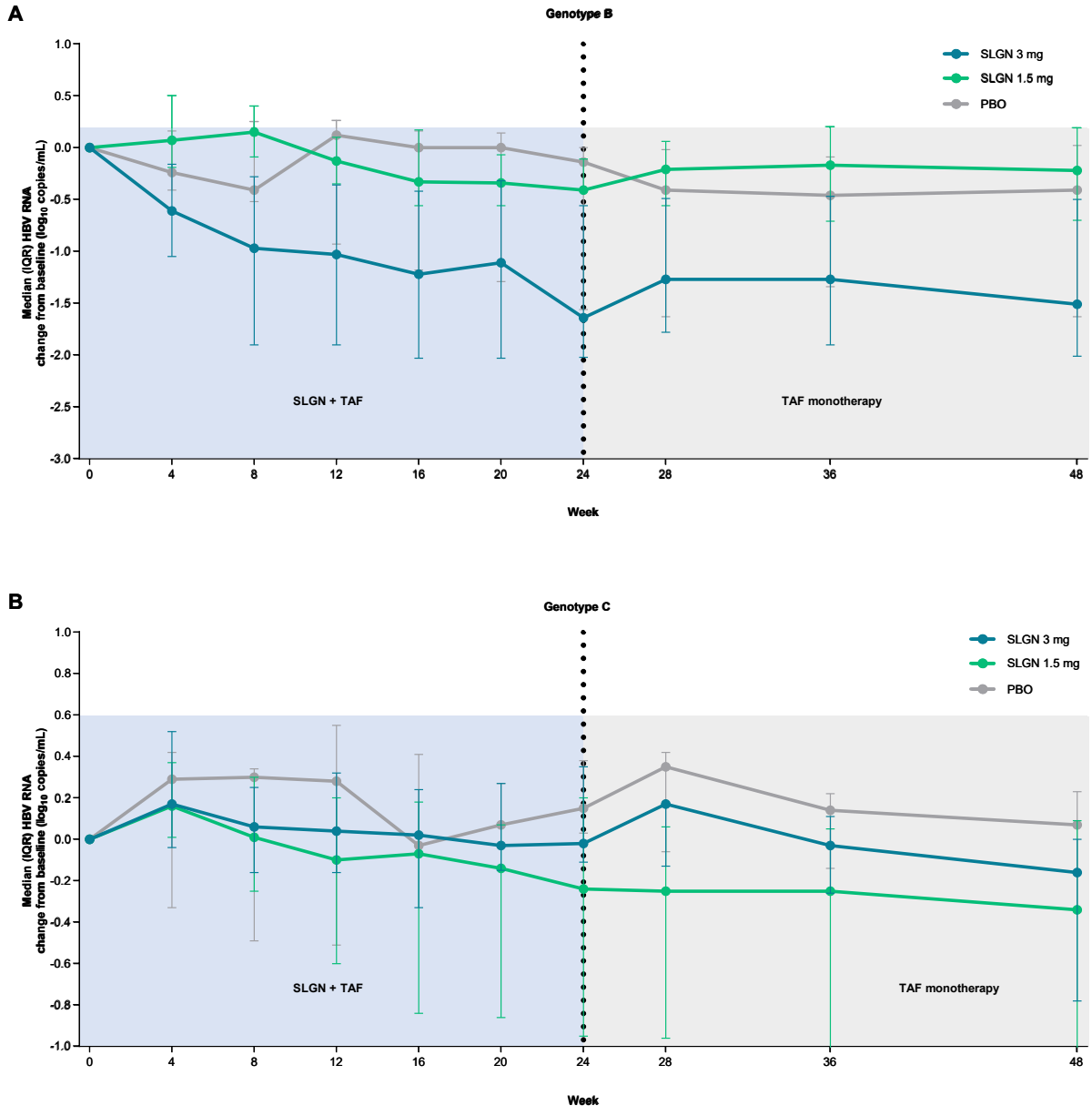

B
HBcrAg
SLGN 3 mg
SLGN 1.5 mg
PBO
Median (IQR) HBcrAg change from baseline (log10 U/mL)
0.5
0.0
-0.5
-1.0
-1.5
SLGN + TAF
TAF monotherapy
TFFU phase
0
4
8
12
16
20
24
28
36
48
52
56
60
64
68
72
84
96
Week

Fig. S7. Change from baseline in HBV RNA (log_{10} copies/mL) by genotype (FAS) in patients with (A) HBV genotype B and in patients with (B) HBV genotype C.

FAS, full analysis set; IQR, interquartile range; PBO, placebo; SLGN, selgantolimod; TAF, tenofovir alafenamide.



Supplementary tables

Table S1. Important protocol deviations (FAS).

Protocol deviation category	SLGN 3 mg (n = 26)	SLGN 1.5 mg (n = 28)	PBO (n = 13)	Total (N = 67)
Patients with at least 1 important protocol deviation	14 (53.8)	16 (57.1)	6 (46.2)	36 (53.7)
Missing data	6 (23.1)	6 (21.4)	4 (30.8)	16 (23.9)
Off-schedule procedure	4 (15.4)	9 (32.1)	2 (15.4)	15 (22.4)
Other treatment compliance issue	4 (15.4)	1 (3.6)	3 (23.1)	8 (11.9)
Eligibility criteria	3 (11.5)	3 (10.7)	0	6 (9.0)
Informed consent	1 (3.8)	4 (14.3)	0	5 (7.5)
Excluded concomitant medication	2 (7.7)	1 (3.6)	0	3 (4.5)
Other	0	1 (3.6)	0	1 (1.5)
Wrong treatment or incorrect dose	0	0	1 (7.7)	1 (1.5)

Data shown as n (%).

FAS, full analysis set; PBO, placebo; SLGN, selgantolimod.

Table S2. Laboratory abnormalities of Grade ≥2.

	24-week regimen			48-week regimen			TFFU phase		
	SLGN 3 mg[a] (n = 26)	SLGN 1.5 mg[a] (n = 28)	PBO[a] (n = 13)	SLGN 3 mg[a] (n = 26)	SLGN 1.5 mg[a] (n = 28)	PBO[a] (n = 13)	SLGN 3 mg (n = 13)	SLGN 1.5 mg (n = 12)	PBO (n = 2)
Any laboratory abnormality	22 (84.6)	26 (92.9)	12 (92.3)	24 (92.3)	27 (96.4)	12 (92.3)	12 (92.3)	12 (100)	2 (100)
Grade 2	3 (11.5)	16 (57.1)	3 (23.1)	3 (11.5)	16 (57.1)	4 (30.8)	5 (38.5)	4 (33.3)	1 (50.0)
Grade 3	7 (26.9)	2 (7.1)	3 (23.1)	9 (34.6)	4 (14.3)	3 (23.1)	3 (23.1)	2 (16.7)	1 (50.0)
Grade 4	0	1 (3.6)	1 (7.7)	0	1 (3.6)	1 (7.7)	0	2 (16.7)	0
ALT increased									
Grade 2	0	0	0	0	1 (3.6)	0	3 (23.1)	0	0
Grade 3	0	0	0	0	0	0	1 (7.7)	0	0
Grade 4	0	0	0	0	0	0	0	2 (16.7)	0
AST increased									
Grade 2	1 (3.8)	0	0	1 (3.8)	0	0	2 (15.4)	0	0
Grade 3	0	0	0	0	0	0	0	2 (16.7)	0
Amylase increased									
Grade 2	0	0	0	0	1[b]	0	0	0	0
Bicarbonate decreased									
Grade 2	1 (3.8)	0	0	1 (3.8)	0	0	0	0	0
Creatine kinase increased									
Grade 3	2 (7.7)	0	0	2 (7.7)	0	0	0	1 (8.3)	0
GGT increased									
Grade 2	0	0	0	0	0	1 (8.3)[c]	0	0	0
LDL, fasting, increased									
Grade 2	0	6 (21.4)	2 (15.4)	0	7 (25.0)	3 (23.1)	0	2 (16.7)	0
Grade 3	1 (3.8)	1 (3.6)	2 (15.4)	3 (11.5)	1 (3.6)	2 (15.4)	0	1 (8.3)	0
Lipase increased									
Grade 3	0	0	0	0	1 (3.6)[d]	0	0	0	0
Serum glucose, fasting, hyperglycaemia									
Grade 2	1 (3.8)	4 (14.3)	2 (15.4)	2 (7.7)	5 (17.9)	2 (15.4)	1 (7.7)	1 (8.3)	0
Serum glucose, nonfasting, hyperglycemia									

Grade 2	0	1 (33.3)[e]	1 (50.0)[f]	0	1 (20.0)[g]	1 (33.3)[e]	0	0	0
Serum sodium, hyponatremia									
Grade 2	0	0	1 (7.7)[h]	0	0	1 (7.7)[i]	0	0	0
Total cholesterol, fasting, hypercholesterolemia									
Grade 2	2 (7.7)	6 (21.4)	3 (23.1)	3 (11.5)	6 (21.4)	4 (30.8)	0	2 (16.7)	1 (50.0)
Grade 3	0	1 (3.6)	0	0	1 (3.6)	0	0	0	0
Triglycerides, fasting, increased									
Grade 2	0	0	0	0	0	0	0	1 (8.3)	0
Grade 4	0	1 (3.6)	1 (7.7)	0	1 (3.6)	1 (7.7)	0	0	0
Urinalysis, occult blood									
Grade 2	1 (3.8)	3 (10.7)	1 (7.7)	1 (3.8)	3 (10.7)	1 (7.7)	1 (7.7)	1 (8.3)	1 (50.0)
Grade 3	1 (3.8)	0	0	1 (3.8)	0	0	2 (15.4)	0	0
Urine glucose, glycosuria									
Grade 2	0	2 (7.1)	0	0	2 (7.1)	1 (7.7)	0	0	0
Grade 3	1 (3.8)	0	0	1 (3.8)	0	0	0	0	0
Urine protein, proteinuria									
Grade 2	0	1 (3.6)	0	0	1 (3.6)	0	1 (7.7)	0	0
Urine RBC, hematuria, quantitative									
Grade 2	0	2 (7.1)	0	0	2 (7.1)	0	1 (7.7)	1 (8.3)	0
Grade 3	3 (11.5)	0	1 (7.7)	3 (11.5)	1 (3.6)	1 (7.7)	2 (15.4)	0	1 (50.0)
Hemoglobin, decreased									
Grade 2	0	0	0	0	0	0	0	1 (8.3)	0
Neutrophils, decreased									
Grade 3	0	0	0	0	0	0	0	1 (8.3)	0

Data are n (%).

[a]Also received TAF 25 mg once daily. [b]n = 1. [c]n = 12. [d]n = 28. [e]n = 3. [f]n = 2. [g]n = 5. [h]n = 0. [i]n = 13.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; PBO, placebo; RBC, red blood cell; SLGN, selgantolimod; TAF, tenofovir alafenamide; TFFU, treatment-free follow-up.

Table S3. Mean HBsAg (log_{10} IU/mL) and change from baseline in patients on selgantolimod treatment or placebo.

	SLGN 3 mg (n = 26)	SLGN 1.5 mg (n = 28)	PBO (n = 13)
Baseline			
Mean (SD)	3.84 (0.855)	4.16 (0.831)	4.05 (0.735)
Week 2			
Mean (SD)	3.82 (0.848)	4.16 (0.819)	4.07 (0.734)
Mean (SD) change from BL	–0.02 (0.101)	0.00 (0.075)	0.02 (0.040)
p-value	0.96	0.83	0.57
Week 4			
Mean (SD)	3.82 (0.862)	4.14 (0.835)	4.03 (0.742)
Mean (SD) change from BL	–0.04 (0.155)	–0.01 (0.081)	–0.02 (0.065)
p-value	0.98	0.87	0.94
Week 8			
Mean (SD)	3.84 (0.904)	4.11 (0.815)	4.10 (0.750)
Mean (SD) change from BL	–0.04 (0.161)	–0.05 (0.121)	0.00 (0.081)
p-value	0.59	0.32	0.86
Week 11			
Mean (SD)	3.80 (0.868)	4.09 (0.835)	4.01 (0.779)
Mean (SD) change from BL	–0.08 (0.170)	–0.05 (0.094)	–0.01 (0.102)
p-value	0.98	0.26	0.45
Week 12			
Mean (SD)	3.84 (0.879)	4.12 (0.823)	4.08 (0.765)
Mean (SD) change from BL	–0.04 (0.163)	–0.05 (0.108)	0.01 (0.099)
p-value	0.61	0.30	0.89
Week 16			
Mean (SD)	3.83 (0.850)	4.11 (0.800)	4.06 (0.751)
Mean (SD) change from BL	–0.05 (0.172)	–0.06 (0.156)	–0.01 (0.120)
p-value	0.78	0.63	1.0
Week 20			
Mean (SD)	3.80 (0.831)	4.06 (0.795)	4.05 (0.755)

Mean (SD) change from BL	–0.08 (0.200)	–0.11 (0.163)	–0.02 (0.133)
p-value	0.45	0.25	0.92
Week 23			
Mean (SD)	3.80 (0.844)	4.04 (0.811)	4.01 (0.745)
Mean (SD) change from BL	–0.08 (0.201)	–0.13 (0.166)	–0.06 (0.136)
p-value	0.22	0.30	0.82
Week 24			
Mean (SD)	3.80 (0.846)	4.05 (0.805)	4.04 (0.744)
Mean (SD) change from BL	–0.08 (0.198)	–0.11 (0.174)	–0.03 (0.146)
p-value	0.32	0.24	1.0
Week 28			
Mean (SD)	3.80 (0.854)	4.04 (0.793)	4.04 (0.757)
Mean (SD) change from BL	–0.08 (0.212)	–0.13 (0.182)	–0.07 (0.110)
p-value	0.31	0.70	0.82
Week 36			
Mean (SD)	3.76 (0.853)	4.02 (0.784)	4.00 (0.760)
Mean (SD) change from BL	–0.11 (0.218)	–0.13 (0.177)	–0.11 (0.139)
p-value	0.64	0.96	0.78
Week 48			
Mean (SD)	3.76 (0.835)	3.99 (0.753)	3.99 (0.740)
Mean (SD) change from BL	–0.12 (0.218)	–0.16 (0.235)	–0.12 (0.145)
p-value	0.61	0.94	0.84

p-values were determined from Wilcoxon rank sum tests and are presented for SLGN 3 vs 1.5 mg, SLGN 1.5 mg vs PBO, and PBO vs SLGN 3 mg from left to right, respectively.

BL, baseline; PBO, placebo; SLGN, selgantolimod.

Table S4. Mean HBsAg (log_{10} IU/mL) and change from baseline in patients with or without ALT hepatitis flare.

	With ALT hepatitis flare (n = 6)	Without ALT hepatitis flare (n = 61)	*p*-value
Baseline			
Mean (SD)	3.54 (0.702)	4.06 (0.826)	
Week 2			
Mean (SD)	3.48 (0.674)	4.06 (0.817)	
Mean (SD) change from BL	–0.06 (0.173)	0.00 (0.066)	0.49
Week 4			
Mean (SD)	3.44 (0.652)	4.05 (0.828)	
Mean (SD) change from BL	–0.10 (0.280)	–0.01 (0.081)	0.96
Week 8			
Mean (SD)	3.47 (0.651)	4.07 (0.836)	
Mean (SD) change from BL	–0.07 (0.292)	–0.03 (0.106)	0.36
Week 11			
Mean (SD)	3.46 (0.661)	4.02 (0.839)	
Mean (SD) change from BL	–0.08 (0.323)	–0.05 (0.095)	0.25
Week 12			
Mean (SD)	3.48 (0.675)	4.06 (0.833)	
Mean (SD) change from BL	–0.06 (0.313)	–0.03 (0.099)	0.57
Week 16			
Mean (SD)	3.47 (0.696)	4.05 (0.805)	
Mean (SD) change from BL	–0.07 (0.305)	–0.04 (0.136)	0.60
Week 20			
Mean (SD)	3.44 (0.668)	4.01 (0.797)	
Mean (SD) change from BL	–0.10 (0.342)	–0.08 (0.152)	0.41
Week 23			
Mean (SD)	3.44 (0.705)	3.99 (0.805)	
Mean (SD) change from BL	–0.10 (0.341)	–0.10 (0.153)	0.40
Week 24			

Mean (SD)	3.48 (0.718)	4.00 (0.805)	
Mean (SD) change from BL	–0.06 (0.354)	–0.09 (0.156)	0.26
Week 28			
Mean (SD)	3.44 (0.699)	4.00 (0.804)	
Mean (SD) change from BL	–0.10 (0.348)	–0.10 (0.163)	0.41
Week 36			
Mean (SD)	3.41 (0.665)	3.97 (0.804)	
Mean (SD) change from BL	–0.13 (0.339)	–0.12 (0.167)	0.29
Week 48			
Mean (SD)	3.47 (0.697)	3.95 (0.778)	
Mean (SD) change from BL	–0.07 (0.367)	–0.14 (0.194)	0.21

p-values were determined from Wilcoxon rank sum test.

ALT, alanine transaminase; BL, baseline.

Table S5. Mean HBV DNA (log_{10} IU/mL) and change from baseline in patients with or without ALT hepatitis flare.

	With ALT hepatitis flare (n = 6)	Without ALT hepatitis flare (n = 61)	*p*-value
Baseline			
Mean (SD)	6.31 (1.040)	6.84 (1.818)	
Week 2			
Mean (SD)	4.20 (1.215)	4.37 (1.830)	
Mean (SD) change from BL	–2.11 (0.519)	–2.45 (0.495)	0.14
Week 4			
Mean (SD)	3.63 (1.312)	4.00 (1.840)	
Mean (SD) change from BL	–2.68 (0.718)	–2.86 (0.730)	0.38
Week 8			
Mean (SD)	3.14 (1.578)	3.54 (1.756)	
Mean (SD) change from BL	–3.17 (1.046)	–3.36 (0.881)	0.58
Week 11			
Mean (SD)	3.00 (1.782)	3.23 (1.618)	
Mean (SD) change from BL	–3.31 (1.329)	–3.63 (0.925)	0.62
Week 12			
Mean (SD)	2.89 (1.797)	3.20 (1.526)	
Mean (SD) change from BL	–3.42 (1.281)	–3.73 (0.890)	0.62
Week 16			
Mean (SD)	2.38 (1.502)	2.90 (1.431)	
Mean (SD) change from BL	–3.94 (0.973)	–4.02 (1.049)	0.77
Week 20			
Mean (SD)	2.14 (1.153)	2.67 (1.452)	
Mean (SD) change from BL	–4.17 (0.720)	–4.26 (1.318)	0.54
Week 23			
Mean (SD)	2.32 (1.678)	2.48 (1.272)	
Mean (SD) change from BL	–4.00 (1.468)	–4.45 (1.330)	0.36
Week 24			

Mean (SD)	2.31 (1.562)	2.48 (1.342)	
Mean (SD) change from BL	–4.00 (1.294)	–4.45 (1.410)	0.22
Week 28			
Mean (SD)	2.01 (1.129)	2.23 (1.057)	
Mean (SD) change from BL	–4.31 (1.017)	–4.68 (1.348)	0.29
Week 36			
Mean (SD)	1.57 (0.364)	2.00 (0.803)	
Mean (SD) change from BL	–4.74 (0.767)	–4.89 (1.513)	0.30
Week 48			
Mean (SD)	2.15 (1.443)	1.86 (0.674)	
Mean (SD) change from BL	–4.16 (1.280)	–5.04 (1.427)	0.11

p-values were determined from Wilcoxon rank sum test.

ALT, alanine transaminase; BL, baseline.

Supplementary reference

[1] van Bömmel F, Bartens A, Mysickova A, Hofmann J, Krüger DH, Berg T, et al. Serum hepatitis B virus RNA levels as an early predictor of hepatitis B envelope antigen seroconversion during treatment with polymerase inhibitors. Hepatology 2015;61:66-76. doi:10.1002/hep.27381.