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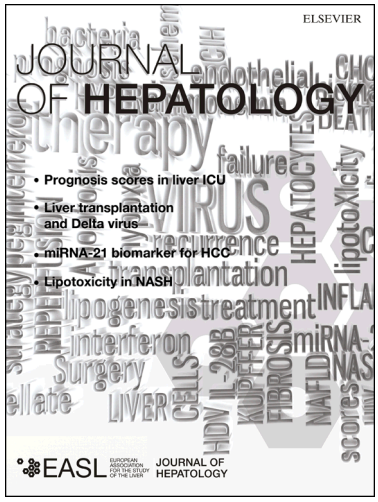

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The Oral Toll-Like Receptor-7 Agonist GS-9620 in Patients with Chronic Hepatitis B Virus Infection

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ABSTRACT [word limit: 250; currently 250]

Background: GS-9620 is an oral agonist of toll-like receptor 7, a pattern-recognition receptor whose activation results in innate and adaptive immune stimulation. We evaluated the safety, pharmacokinetics, and pharmacodynamics of GS-9620 in patients with chronic hepatitis B.

Methods: In two double-blind, phase 1b trials of identical design, 49 treatment-naïve and 51 virologically suppressed patients were randomized 5:1 to receive GS-9620 (at doses of 0.3 mg, 1 mg, 2 mg, 4 mg) or placebo as a single dose or as two doses 7 days apart. Pharmacodynamic assessment included evaluation of peripheral mRNA expression of interferon-stimulated gene 15 (ISG15), serum interferon gamma-induced protein 10 and serum interferon (IFN)-alpha.

Results: Overall, 74% of patients were male and 75% were HBeAg negative at Baseline. No subject discontinued treatment due to adverse events. Fifty-eight percent experienced ≥1 adverse event, all of which were mild to moderate in severity. Overall, the most common adverse event was headache. No clinically significant changes in HBsAg or HBV DNA levels were observed. Overall, a transient dose-dependent induction of peripheral ISG15 gene expression was observed peaking within 48 hours of dosing followed by return to baseline levels within 7 days. Higher GS-9620 dose, HBeAg positive status, and low HBsAg level at baseline were independently associated with greater probability of ISG15 response. Most patients (88%) did not show detectable levels of serum IFN-alpha at any time point.

Conclusions: Oral GS-9620 was safe, well tolerated, and associated with induction of peripheral ISG15 production in the absence of significant systemic IFN-alpha levels or related symptoms.

Keywords: Hepatitis B virus; HBsAg; immune response; TLR

INTRODUCTION

Chronic hepatitis B (CHB) is a major global health care challenge and one of the principal causes of chronic liver disease, cirrhosis, and hepatocellular carcinoma (HCC). Of the estimated 2 billion individuals worldwide who have been acutely infected with the hepatitis B virus (HBV), up to 350 million have developed CHB and approximately 600,000 people die annually from complications of CHB.[1] Treatment with peginterferon and nucleos(t)ide analogues can effectively suppress viral replication, but less than 10% of patients achieve loss of hepatitis B surface antigen (HBsAg) with HBsAg seroconversion.[2] These data underscore the need for new therapies that enhance rates of HBsAg loss with seroconversion thereby providing patients with a curative treatment option of finite duration.

Patients with CHB have compromised innate and adaptive immune responses, characterized by suboptimal antigen presentation, exhaustion of antigen-specific T cells, and insufficient antibody production.[3,4] One promising therapeutic strategy for CHB is stimulation of the immune system through targeted activation of toll-like receptor 7 (TLR7), a pattern recognition receptor expressed in the endo/lysosomal compartments of plasmacytoid dendritic cells (pDC) and B lymphocytes.[5] When activated by viral pathogen-associated molecular patterns, TLR7 initiates signaling that leads to production of type I IFN and other mediators which stimulate both innate and adaptive immune responses. Secreted type I IFN following TLR7 stimulation results in broad cellular activation and increased transcription of interferon-stimulated genes (ISGs), many of which have antiviral activity.[6] Type I IFNs can also inhibit HBV replication through epigenetic repression of the transcription of covalently closed circular DNA (cccDNA).[7]

TLR7 stimulation augments antigen presentation by pDCs with direct effects on subsequent T cell responses.[8] TLR7 activation in B cells, especially in combination with type I IFN, results in

polyclonal expansion and differentiation towards immunoglobulin (Ig)-producing plasma cells, providing an enhanced humoral component to the adaptive immune response.[9] Therefore, TLR7 agonists have the potential to augment anti-HBV immunity through both innate, pDC-dependent, as well as adaptive, B cell-dependent, effects.

GS-9620 is a TLR7 agonist that has been shown in preclinical animal studies to induce an immunological response marked by expression of ISGs in the liver in the absence of appreciable levels of serum interferon-alpha in the peripheral blood. This suggests that the predominant pharmacologic activity of GS-9620 occurs prior to systemic compound exposure, most likely by activation of gut-associated lymphoid tissue (GALT) and/or liver-resident lymphocytes, i.e., via a pre-systemic mechanism of action.[10] Consistent with its pre-systemic mechanism of action, a transient induction of ISG15 expression in the peripheral blood observed with low doses of GS-9620 (up to 6 mg) has not been accompanied by detectable serum IFN-alpha levels in healthy volunteers.[11] Oral dosing of GS-9620 demonstrated therapeutic efficacy in two preclinical models of chronic hepadnavirus infection. In HBV-infected chimpanzees, treatment with oral GS-9620 resulted in long-term suppression of serum and liver HBV DNA in all and persistent reduction of serum HBsAg levels in 2 of the 3 treated animals.[12] In the chronic infection model of woodchuck hepatitis virus (WHV), GS-9620 administered for up to 8 weeks resulted in marked reductions in serum levels of WHV DNA and WHV surface antigen (WHsAg) and dose related WHsAg loss in 13 of 26 animals. Treatment with GS-9620 dramatically reduced the incidence of hepatocellular carcinoma (3/18 animals treated with GS-9620 developed HCC). Importantly, induction of anti-WHsAg antibodies occurred and persisted in a subset of animals with WHsAg loss.[13]

Based on these data, we hypothesized that GS-9620 may facilitate an effective immune response against HBV infection in humans without producing the systemic adverse effects commonly associated with interferon-based therapy. We therefore conducted two clinical trials to evaluate the safety, pharmacokinetics, and pharmacodynamics of GS-9620 in treatment-naïve and virally suppressed CHB patients.

METHODS

Patients and Study Design

In these two phase 1b, multicenter, randomized, double-blind, placebo-controlled single ascending dose (SAD) and multiple ascending dose (MAD) studies, we enrolled virally suppressed patients, who were required per protocol to have been on stable treatment with any approved anti-HBV antiviral agent for at least 3 months prior to screening, and treatment-naive patients aged 18-65 years with CHB in 20 centers in United States, New Zealand, and South Korea during the period from April 2012 to December 2013 and from June 2012 to October 2013, respectively (clinicaltrials.gov, numbers NCT01590654 and NCT01590641). At baseline, both treatment-naïve and virally suppressed patients were required to have HBsAg level ≥250 IU/mL, and virally suppressed patients were also required to have HBV DNA <LOQ (29 IU/mL). (See Supplement for amendments to inclusion criteria.) Major exclusion criteria included presence of extensive bridging fibrosis (Metavir ≥3) or cirrhosis as determined by liver biopsy within 3 years of screening, or by two non-invasive alternatives to liver biopsy (e.g. Fibroscan, Fibrotest, aspartate aminotransferase/platelet ratio index [APRI]) within 6 months of screening, and coinfection with hepatitis C virus, Delta virus or HIV. Based upon nonclinical data, GS-9620 is a substrate for CYP3A4 metabolism and, in the absence of a definitive clinical

evaluation, the use of CYP3A inhibitors (eg, clarithromycin) and inducers (eg, carbamazepine) was not permitted.

All patients provided informed consent. The studies were approved by the institutional review board at participating sites and conducted in compliance with the Declaration of Helsinki, Good Clinical Practice guidelines, and local regulatory requirements. Both studies were designed and conducted by the sponsor in collaboration with the principal investigators. The sponsor collected the data and monitored the study conduct. The investigators, participating institutions, and Sponsor agreed to maintain confidentiality of the data. All authors had access to the data and assumed responsibility for the integrity and completeness of the reported data. All authors approved the final manuscript.

Patients in both studies were enrolled into one of eight cohorts of six patients each and randomly assigned in a 5:1 ratio (active:placebo) to receive either active GS-9620 (0.3, 1, 2, or 4 mg) or placebo. Patients in the single-ascending dose (SAD) cohorts received single doses of GS-9620 (at doses of 0.3 mg, 1 mg, 2 mg, 4 mg) or placebo. Patients in the multiple-ascending dose (MAD) cohorts received 2 doses of GS-9620 (at doses of 0.3 mg, 1 mg, 2 mg, 4 mg) or placebo 7 days apart.

SAD cohorts were conducted in a sequential fashion. Before escalation to the next highest dose, SAD data through Day 8 was reviewed for all dose cohorts. Prior to MAD dose escalation or dose repetition decisions, safety data from the SAD cohorts at the same proposed dose were reviewed as well as MAD data through Day 15 of a prior (lower dose). An external data monitoring committee (DMC) provided external review of all safety and dose titration decisions.

Study assessments

Screening assessments included measurement of serum HBV DNA and HBsAg levels, and IL28B and TLR7 genotyping, in addition to standard laboratory and clinical tests.

Quantitative HBV DNA was determined by COBAS Taqman assay (Roche) (linear range: 29-110,000,000 IU/mL).

Quantification of HBsAg levels was determined by Quantitative Roche COBAS e601 Analyzer (linear range: 0.074 – 52000 IU/mL). HBV DNA and HBsAg assessments were conducted according the schedule provided in the Supplement.

HBV genotype was determined in the treatment-naïve patients by Inno-Lipa assay (minimum HBV DNA level required 5000 IU/mL). IL28B genotype was determined by means of polymerase-chain-reaction amplification and sequencing of the rs12979860 single-nucleotide polymorphism.

TLR7 genotype was determined on serum samples by means of polymerase-chain-reaction amplification and sequencing of rs179008 and rs3853839 single-nucleotide polymorphisms serum samples.[14] SNP rs179008 is associated with lower response to IFN-based HCV therapy.[15-17] SNP rs38538398 is associated with differential level of TLR7 expression and susceptibility to systemic lupus erythematosus and with spontaneous clearance of HCV infection.[18-19]

Whole blood samples and serum samples were collected for analysis of gene expression and for quantification of interferon-alpha (IFN-alpha) and interferon-γ-inducible protein (IP)-10 proteins, respectively, at the following time points: pre-dose and at 8, 24, 48, 96, and 168 hours

after a single dose (SAD cohorts Day 1) or after each of the two doses (MAD cohorts Day 1 and Day 8).

The magnitude and kinetics of the pharmacodynamic response were determined by measurement of ISG15 and TLR7 mRNA expression. Whole blood gene expression of ISG15 and TLR7 relative to GAPDH was analysed by quantitative reverse transcriptase PCR (qRT-PCR) (Covance Genomics Laboratories, Seattle, WA, USA). Fold change in ISG15 and TLR7 expression relative to baseline were calculated according to the ΔΔCt method,[20] and described as mean fold change.

Serum was isolated and the concentrations of IP-10 and IFN-alpha were determined by Ciraplex assay (Aushon Biosystems, Billerca, MA, USA). Change in IP-10 levels, relative to baseline, were calculated as fold change; IFN-alpha levels are described as absolute values.

Plasma concentrations of GS-9620 were measured by a validated high-performance liquid chromatography-tandem mass spectroscopy method. The assay had a linear range of 50–10,000 pg/mL and was performed by QPS, Inc (Newark, DE, USA). Non-compartmental analysis of individual plasma concentration-time data was conducted using Phoenix WinNonlin (Version 6.3, Pharsight, Mountain View, California, USA). Dose proportionality was assessed using a power model. An alternate evaluation of dose proportionality was conducted using analysis of variance; the model included dose as a fixed effect.

Endpoints

The primary endpoint was safety and tolerability. Secondary endpoints included the pharmacodynamic response evaluated by change in peripheral gene expression and serum cytokine levels following administration of GS-9620. ISG15 response has been defined as fold

change from baseline >2 within 96 hours from Baseline. In addition, the HBsAg reduction and the HBV DNA decline after 1 or 2 doses were also evaluated.

Statistical Analysis

No formal statistical analysis was performed to determine sample size or to assess safety and pharmacodynamic outcomes. Multivariable logistic-regression analysis, including baseline demographic, disease characteristics and IL28/TLR7 SNPs was performed, and a stepwise procedure was used to identify independent predictors of ISG15 response.

All randomised patients who received at least one dose of study medication were included in the safety analysis. Patients who received study medication and for whom evaluable pharmacokinetic profiles were available were included in the pharmacokinetic analysis. Patients who completed the dosing period and had specific laboratory data available for calculating estimates of pharmacodynamics parameters were included in the pharmacodynamic analysis.

RESULTS

Baseline characteristics

Of the 232 patients who were initially screened across the 2 studies, 108 were randomized and 100 received one or two doses of GS-9620 (n=84) or placebo (n= 16) (See Supplementary Figure 1 and Supplementary Table 1). Of the 100 patients dosed, 49 were treatment-naïve and 51 were virologically suppressed. Eight patients were randomized, but not dosed; seven patients withdrew consent prior to 1st dose and one patient was excluded from participation for receiving a prohibited concomitant medication.

The median age of patients was 41 years, and most were male (74%), HBeAg negative (75%), and most IL28B CC genotype (62%). Forty-nine percent of patients were Asian. Median HBsAg

titer at baseline was 3.2 $\log_{10}$ IU/mL and 3.7 $\log_{10}$ IU/mL, respectively, in the virally suppressed and treatment-naive patients. Treatment-naive patients had a median baseline HBV DNA level of 3.95 $\log_{10}$ IU/mL (Table 1).

Safety

Treatment with GS-9620 was well tolerated in both patient populations. No patient discontinued study drug due to adverse events (Table 2). One virally suppressed patient experienced an SAE of forearm fracture during the post-treatment follow-up period, which was not considered drug related. Sixty percent of patients treated with GS-9620 and 50% of placebo patients experienced at least one adverse event, all of which were mild to moderate in severity. Of note, AEs were more frequently reported among treatment naïve patients (34/49, 69%) than virally suppressed patients (24/51, 47%). The same trend was observed when the treatment arm was considered: among patients treated with GS-9620, adverse events were reported in 71% and 49% of treatment naïve and virally suppressed patients, respectively; among patients who received placebo, adverse events were reported in 63% treatment naïve and in 38% of and virally suppressed patients. The most common adverse event in both treatment naïve and virally suppressed patients was headache with a frequency of 32% and 14%, respectively. Four patients experienced transient mild adverse events of flu-like symptoms. One virally suppressed patient experienced chills after receiving the second dose of GS-9620; this event resolved spontaneously a few hours later on the same day. Three treatment-naive patients experienced influenza-like symptoms: 2 patients had fever within 24 hours after second dose and one patient experienced influenza-like symptoms during the post-treatment follow-up. All these adverse events resolved within 48 hours of onset (Supplementary Table 2).

No clinically significant increase in serum levels of transaminases and γ- glutamal transpeptidase was observed; no patient experienced an ALT increase ≥5 × ULN (ULN was 43 IU/mL for males and 35 IU/mL for females) (Table 2). Overall, neither virally suppressed nor treatment-naive patients experienced a clinically significant change in white blood cells, neutrophils, or platelets; median values for these fluctuated within the normal range or slightly below (Supplementary Figures 3 and 4).

Pharmacokinetics

The pharmacokinetic parameters obtained following administration of single doses of GS-9620 (0.3, 1, 2, and 4 mg) are presented in Table 3. GS-9620 exposure, as described by area under the curve (AUC), plasma concentration over time, and the maximum observed plasma concentration (C_{max}), was generally dose-proportional up to 2 mg and greater-than-dose proportional at 4 mg. C_{max} was reached at approximately 1.0 to 5.0 hours (median T_{max}), and then concentrations decreased with a median half-life ($t_{1/2}$) of approximately 8 to 18 hours following administration of 1, 2, or 4 mg GS-9620. The median $t_{1/2}$ in subjects administered 0.3 mg could not be estimated accurately since GS-9620 concentrations approached the lower limit of quantitation (50 pg/mL) early in the concentration-time profile (data not shown); as such, $t_{1/2}$ values for 0.3 mg doses may represent distribution and not the elimination $t_{1/2}$. The pharmacokinetics of GS-9620 following single or 2 once-weekly doses were comparable, and, prior to administration of the second once-weekly dose of GS-9620 (on Day 8) in the MAD cohorts, all patients had concentrations of GS-9620 below the limit of quantitation, which is consistent with the half-life of GS-9620 and indicated that no dose accumulation occurred (data not shown).

Antiviral activity

Effect on serum HBV DNA levels

Treatment-naive patients did not show clinically significant reductions in HBV DNA after 1 or 2 doses of GS-9620 (Supplementary Figure 5). In the treatment-naive patients, the median change from baseline to the end of treatment for HBV DNA was -0.142 $\log_{10}$ IU/mL after one dose (SAD cohorts) and -0.034 $\log_{10}$ IU/mL after 2 doses (MAD cohorts). Thirty-two percent of patients treated with GS-9620 (13/41) and 25% (2/8) of placebo patients showed a transient HBV DNA decline $\geq$0.5 $\log_{10}$ IU/mL.

All but one virally suppressed patient maintained HBV DNA <LLOQ (29 IU/mL) over time; one patient had an isolated single detectable value of HBV DNA of 220 IU/mL at the last evaluation on Day 8.

Effect on serum HBsAg levels

None of the treatment-naive or virally suppressed patients had clinically significant declines in HBsAg levels. No patient had an HBsAg decline $\geq$0.5 $\log_{10}$ IU/mL during the study; the median values ($\log_{10}$) at the end of treatment period were similar to the median values at baseline in all dose groups (the median change from Baseline to the end of treatment was 0.009 and 0.016 $\log_{10}$ IU/mL in the SAD cohorts, and -0.010 and 0.002 $\log_{10}$ IU/mL in the MAD cohorts, in treatment-naive and virally suppressed patients, respectively). Median change in HBsAg over time are shown in Supplementary Figure 6.

Pharmacodynamics

Both treatment-naive and virally suppressed patients showed a transient dose dependent induction of ISG15 mRNA expression after 1 or 2 doses of GS-9620. The highest induction was

observed in patients receiving 2- and 4- mg doses. The peak induction was observed within 24 hours in the virally suppressed patients and within 48 hours in the treatment-naive patients. In both patient populations, the peak fold change was followed by return to baseline level within 7 days (Fig 1). Consistent with the transient nature of the induction, the maximum ISG15 expression after second dose was comparable to the first dose, in both the virally suppressed and treatment-naive patients.

To understand the variability in ISG15 response, univariate and multivariate analyses were performed across the two study populations (Table 4 and Supplementary Table 3). Baseline factors found to be independently associated with higher probability of ISG15 response were higher study drug dose (odds ratio [OR] 211.62; 95% confidence interval [CI] 8.22 – >999.99, P=0.0012 for the 4-mg dose), high ISG15 mRNA expression at baseline (OR 2.00; 95% CI 1.18–3.37, P=0.0098), HBeAg positive status (OR 7.58; 95% CI 1.90–30.19, P=0.0041), and low HBsAg level at baseline (OR 3.17; 95% CI 1.18–8.52; P=0.0244) (Table 4). Different cut-off values of HBsAg levels were examined; significantly more HBeAg-positive patients with an HBsAg titer at baseline ≤5000 IU/mL had an ISG15 response than HBeAg negative patients with an HBsAg value >5000 IU/mL at baseline (83.3% vs 34.8%, p=0.0116) (Fig 2). The same pattern was observed when each study population was analyzed separately (Supplementary Figure 7 and 8).

ISG15 mRNA expression at baseline was associated with TLR7 expression at baseline (Pearson Correlation 0.5; p<0.0001) (Supplementary Figure 9). TLR7 expression at baseline was associated with race (white vs non white) (p= 0.0080) and with viral suppression during treatment with oral antiviral drugs compared to replicative status in absence of antiviral treatment (p=0.0260) (Supplementary Table 4).

Likewise, ISG15 response was associated with TLR7 induction post baseline (Pearson Correlation 0.7; $p<0.0001$) (Supplementary Figure 10). However, the fold change TLR7 mRNA expression post-dosing, was modest in both patient populations. Across the 2 studies, the median of the maximum fold change in ISG15 mRNA expression was 4.0 fold after single dose and 5.1 fold after two doses, as compared to a median of the maximum fold change in TLR7 expression of 1.7 and 1.8 fold after single dose and two doses of GS-9620, respectively.

No association was found between ISG15 maximum fold change and transient HBV DNA decline >0.5 $\log_{10}$ observed among 13 treatment naïve patients treated with GS-9620 (Supplementary Table 5).

A higher maximum ISG15 fold change was observed among patients treated with GS-9620 who experienced any type of AEs compared to patients for whom no AE was reported. An exact relationship between ISG15 fold change level and development of adverse events, however, is difficult to define given relatively small numbers of patients and variability in response (Supplementary Table 6).

Serum IP-10 was detectable in all patients at baseline. A transient peak induction in serum IP-10 was observed within 48 hours post-dose, with an induction in both treatment-naive and virally suppressed patients after one or two 4-mg doses of GS-9620 (Supplementary Figure 11). IP-10 and ISG15 maximum induction within 96 hours post Baseline were positively associated (Pearson Correlation 0.35, $p=0.0005$) (Supplementary Figure 12).

Serum IFN-alpha was not detected in 70 of the 80 patients (88%) who were dosed with GS-9620 and completed the study treatment period (8 days for the SAD cohorts and 15 days for the MAD cohorts). Minimal serum IFN-alpha levels were observed at one or more time points in 3/40

(7.5%) virally suppressed patients and in 7/40 (17.5%) treatment-naive patients. Of the 10 patients with detectable serum IFN-alpha, 8 (2 virally suppressed patients and 6 treatment-naive patients) had detectable serum IFN-alpha at baseline. Overall, the maximum level of serum IFN-alpha detected was 22.5 pg/mL, observed in a treatment-naive patient with a baseline value of 5.9 pg/mL. The 2 patients with undetectable levels at baseline showed a maximum value of 0.2 pg/mL and 1.8 pg/mL postdose. Overall, IFN-alpha levels were considerably lower than the levels seen with standard of care treatment with Peg-IFN (in which the average peak is ~300 pg/mL).[21] Of the 10 patients with detectable serum IFN-alpha at one or more time points, only two experienced systemic flu like symptoms: two treatment-naive subjects, one dosed with 0.3 mg and one with 1 mg GS-9620 had mild fever after second dose (Table 5). In both of these patients, the onset of fever coincided with peak serum IFN-alpha levels.

The other two patients who experienced influenza-like symptoms did not have concomitant detectable serum IFN-alpha levels.

DISCUSSION

In these two studies, treatment with GS-9620 was safe and well-tolerated, with no evidence of clinically significant AEs or laboratory abnormalities. Interestingly the frequency of reported AES was higher among treatment naïve patients than in virally suppressed, and this may reflect a role of the HBV DNA level in the safety and tolerability of the treatment with GS-9620 or specific patients related factors affecting tolerability of any treatment. Of note, no patient experienced clinically significant increase in liver transaminases, GGT, or anaemia or thrombocytopenia previously described in the preclinical animal studies with chimpanzees and woodchucks.[22]

Dose-dependent ISG15 induction of similar magnitude after either 1 or 2 doses and a return to baseline levels of ISG15 within 7 days post dosing was observed in both treatment-naïve and virally suppressed patients. This suggests (i) that an amplification of the response did not occur after the repeat dose and (ii) that the molecular networks controlling the kinetics of the response elicit similar activity after single and repeat dosing. These results support once-weekly administration of GS-9620 from a safety point of view.

The induction of peripheral ISG15 in absence of serum detectable levels of IFN-alpha in most of the patients dosed with GS-9620 is consistent with similar findings in preclinical studies and in the healthy volunteer study suggesting a pre-systemic mechanism of action for GS-9620: oral administration of GS-9620 likely results in local activation of TLR7-expressing cells, such as pDCs, particularly in gut-associated lymphatic tissue and/or in the liver. This, in turn, stimulates localised production of interferon-alpha and other mediators, including IP-10. Intra-hepatic IFN-alpha has the potential to protect HBV specific CTL from NK cell-mediated cytolysis[23,24]; in contrast the exposure to systemic IFN was associated with decline of total CTL.[25] Induction of ISG15 expression in the blood in the absence of concomitant detectable serum interferon-alpha may result from peripheral lymphocyte trafficking through the gut and liver vasculature and exposure of these cells to the locally produced interferon-alpha.

Alternative explanations for the lack of serum IFN-alpha detection may be the transient nature of the elevation that may have occurred at a timing different from the sample collections, or the assay limitation to detect IFN subtypes.

Interestingly, in the multivariate analysis of possible baseline factors associated with ISG15 response to GS-9620, ISG induction was only associated with viral factors, such as HBeAg

status and HBsAg levels, but not with host factors, such as IL28B or TLR7 polymorphisms. The association of ISG15 response with HBeAg positive status is an intriguing observation as a relatively higher immune response resulting in higher rates of HBsAg loss during antiviral therapy for CHB has been observed in HBeAg positive patients compared to HBeAg negative patients.[26] Although HBeAg status was the baseline factor most highly associated with ISG15 response (OR 7.58, p=0041), the probability of response varied by the HBsAg level at baseline. These findings suggest that HBsAg levels may be a contributing factor to regulation of TLR7-dependent anti-HBV responses; the mechanism of action for this is not clear. It is worth mentioning that a role of HBsAg in maintenance of immunological tolerance has been previously proposed.[27, 28]

The observation of IP-10 induction after dosing with GS-9620 and its association with ISG15 induction are consistent with the TLR7 dependant activation of pDC and type I IFN induction.[29] We anticipate that, similar to the mechanisms indicated for type I IFN production described above, the majority of the peripherally detected IP-10 was produced and secreted in the liver by pDC cells.[30] As IP-10 has potent chemokine activity through CXCR3, we hypothesize that GS-9620 administration may increase intra-hepatic frequency of CXCR3-positive cells, including CD4+ T helper type 1 cells, CD8+ cytotoxic T cells, and pDC.[31,32] Interestingly, higher pre-treatment IP-10 levels and on-treatment decline have been reported to be associated with HBsAg loss during antiviral treatment , and with HBsAg decline during CHB treatment with peg-IFN-alpha.[33,34]

A limitation of the two studies discussed here is that neither ISG expression nor IFN-alpha production could be evaluated at the intra-hepatic level. These studies are also limited by reliance on a single representative ISG—(ISG15)—for the assessment of the biological activity

of GS-9620 at the gene expression level. However, ISG15 is one of the best described ISGs to be induced upon exposure to endogenous or exogenous interferon (IFN)[35] and it was chosen based on previous data in healthy volunteers showing that it had the most robust inducible expression compared to other ISGs tested.[9] The studies described here did not show evidence of clinical efficacy of GS-9620 in terms of HBV DNA decline or HBsAg reduction. However the lack of antiviral efficacy may not be unexpected given the short duration of dosing explored in these studies.

In conclusion, in these two phase 1b studies, one or two low doses of TLR7 agonist GS-9620 administered once a week was safe, well tolerated, and associated with transient peripheral induction of interferon stimulated genes in absence of detectable serum levels of IFN-alpha in the majority of patients with chronic hepatitis B, including both untreated patients and patients on oral antiviral therapy. These findings support further clinical development of GS-9620 in patients with chronic hepatitis B.

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Conflict of interest: Edward J. Gane: Grant/Research – Gilead; Advisory Board – AbbVie, Boehringer Ingelheim, Gilead, Janssen, Novartis, Roche, Tibotec; Speaker – Gilead, Novartis, Roche, Tibotec; Patents – Gilead. Young-Suk Lim: Grant/Research – Bayer Healthcare, Gilead Science, Novartis; Advisory Board – Bayer Healthcare, Gilead Science. Stuart C. Gordon: Grant/Research – Gilead, GlaxoSmithKline, Intercept, Kadmon, Abbvie, Bristol-Myers Squibb, Merck, Vertex. Kumar Visvanathan: Grant/Research – Gilead; Speaker – AbbVie. Stuart Roberts: Consultant – Gilead Sciences. Bradley Freilich: Grant/Research – Gilead. Wendy

Cheng: Advisory Board – AbbVie, Boehringer Ingelheim, BMS, MSD, Janssen. Benedetta Massetto, Zhishen Ye, Stefan Pflanz, Kimberly L. Garrison, Anuj Gaggar, G. Mani Subramanian, and John G. McHutchison are current employees of Gilead Sciences. No other disclosures were reported.

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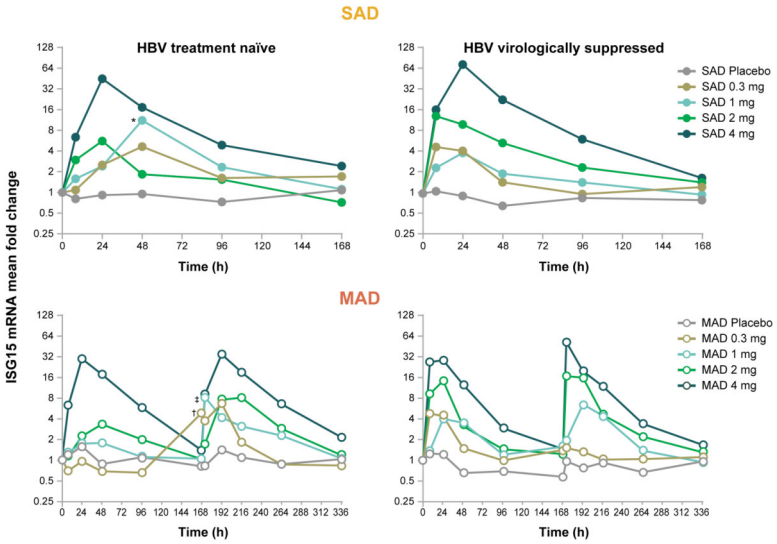
FIGURE LEGENDS

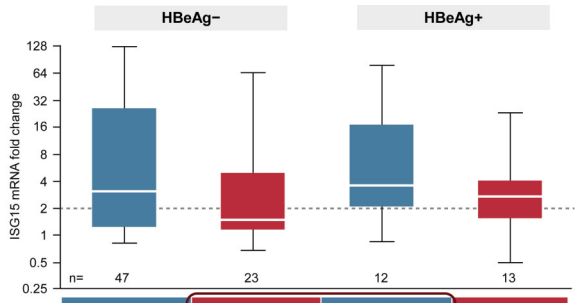
Fig 1: ISG15 (mRNA) fold change

The figure shows the ISG15 mRNA mean fold change over time in the SAD and MAD cohorts in each patient population

Figure 2: Association of HBeAg positivity and low HBsAg level with ISG15 induction

The figure shows the association between HBsAg level and HBeAg status. The boxes represent interquartile range (Q1 and Q3), the central bar in the boxes represents the median value, and the whiskers minimum and maximum values. Responder is defined as a patient with >2 fold change of ISG induction from baseline within 96 h after first dose in the SAD and MAD cohorts. P=0.0116 (Fisher exact test) for the comparison between HBeAg negative patients with HBsAg >5000 IU/mL and HBeAg positive and HBsAg ≤5000 IU/mL.





n (%)	HBsAg ≤5000 IU/mL	HBsAg >5000 IU/mL	HBsAg ≤5000 IU/mL	HBsAg >5000 IU/mL
Responders (ISG15 >2-fold change)	28 (60)	8 (35)	10 (83)	8 (62)
Nonresponders (ISG15 ≤2-fold change)	19 (40)	15 (65)	2 (17)	5 (38)

Table 1: Baseline Demographic and Clinical Characteristics of the CHB Patients

	Virally Suppressed			Treatment-naive			
	Placebo (N=8)	**GS-9620 (N=43)**	**Total (N=51)**	**Placebo (N=8)**	**GS-9620 (N=41)**	**Total (N=49)**	**Total (N=100)**
Age at Baseline (years)							
Median, (range)	54 (30, 60)	42 (23, 65)	43 (23, 65)	42 (26, 60)	37 (19, 61)	37 (19, 61)	41 (19, 65)
Sex, n (%)							
Male	7 (88%)	33 (77%)	40 (78%)	5 (63%)	29 (71%)	34 (69%)	74 (74%)
Race, n (%)							
Asian	5 (63%)	23 (54%)	28 (55%)	4 (50%)	17 (42%)	21 (43%)	49 (49%)
White	3 (38%)	9 (21%)	12 (24%)	1 (13%)	5 (12%)	6 (12%)	18 (18%)
Black	0	4 (9%)	4 (8%)	2 (25%)	13 (32%)	15 (31%)	19 (19%)
Other*	0	7 (16%)	7 (14%)	1 (13%)	6 (10%)	7 (14%)	13 (13%)
Ethnicity, n (%)							
Non-Hispanic/Latino	8 (100%)	41 (95%)	49 (96%)	8 (100%)	41 (100%)	49 (100%)	98 (98%)
IL28B Genotype, n (%)							
C/C	4 (50%)	29 (67%)	33 (65%)	4 (50%)	25 (61%)	29 (59%)	62 (62%)
C/T	4 (50%)	10 (23%)	14 (28%)	2 (25%)	14 (34%)	16 (33%)	30 (30%)
T/T	0	4 (9.3)	4 (8%)	2 (25%)	2 (5%)	4 (8%)	8 (8%)
TLR7 SNP rs179008, n (%)							
A/AA	6 (75%)	38 (88%)	44 (86%)	5 (63%)	33 (81%)	38 (78%)	82 (82%)
AT	0	0	0	1 (13%)	0	1 (2%)	1 (1%)
T/TT	2 (25%)	1 (2%)	3 (6%)	0	2 (5%)	2 (4%)	5 (5%)
Unknown	0	4 (9%)	4 (8%)	2 (25%)	6 (15%)	8 (16%)	12 (12%)
TLR7 SNP rs3853839, n (%)							
C/CC	3 (38%)	18 (42%)	21 (41%)	1 (13%)	12 (29%)	13 (27%)	34 (34%)
CG	0	2 (5%)	2 (4%)	1 (13%)	4 (10%)	5 (10%)	7 (7%)
G/GG	5 (63%)	19 (44%)	24 (47%)	3 (38%)	18 (44%)	21 (43%)	45 (45%)
Unknown	0	4 (9%)	4 (8%)	3 (38%)	7 (17%)	10 (20%)	14 (14%)
HBeAg Status, n (%)							
Negative	7 (88%)	31 (72%)	38 (75%)	7 (88%)	30 (73%)	37 (76%)	75 (75%)
HBV DNA (log_{10} IU/mL)							
Median (range)	1.5 (1.5, 1.5)	1.5 (1. 5, 1. 5)	1. 5 (1. 5, 1. 5)	2.9 (1.9, 8.2)	4.1 (1. 5, 9.2)	4.0 (1.5, 9.2)	NA
HBV Genotype, n (%)							
A	NA	NA	NA	0	5 (17%)	5 (10%)	NA
B	NA	NA	NA	0	4 (13%)	4 (8%)	NA
C	NA	NA	NA	1 (33%)	13 (43%)	14 (29%)	NA
D	NA	NA	NA	2 (67%)	5 (17%)	7 (14%)	NA
E	NA	NA	NA	0	3 (10%)	3 (6%)	NA
Unable to genotype	NA	NA	NA	7 (29%)	9 (36%)	16 (33%)	16 (33%)
HBsAg (log_{10} IU/mL)							
Median (range)	2.8 (2.6, 3.4)	3.3 (2.4, 4.5)	3.2 (2.4, 4.5)	3.3 (2.7, 4.0)	3. 8 (2.3, 4.7)	3.7 (2.3, 4.7)	3.4 (2.3, 4.7)
Oral antiviral treatment				NA	NA	NA	NA
Tenofovir	5	21	26	NA	NA	NA	NA
Entecavir	2	16	18	NA	NA	NA	NA
Telbivudine	0	1	1	NA	NA	NA	NA

	Virally Suppressed			Treatment-naive			
	Placebo (N=8)	**GS-9620 (N=43)**	**Total (N=51)**	**Placebo (N=8)**	**GS-9620 (N=41)**	**Total (N=49)**	**Total (N=100)**
Tenofovir+Entecvair	1	0	1	NA	NA	NA	NA
Tenofovir + lamivudine	0	3	3	NA	NA	NA	NA
Adefovir +Telbivudine	0	1	1	NA	NA	NA	NA
Adefovir +Lamivudine	0	1	1	NA	NA	NA	NA

* Other include American Indian, Alaska Native, Native Hawaiian or Pacific Islander

** Race and ethnic group were self reported

Table 2: Treatment Discontinuation, Adverse Events, Hematological Abnormalities and Serum Transaminase Elevations

	Virally Suppressed		Treatment Naïve	
	Placebo (N=8)	GS-9620 (N=43)	Placebo (N=8)	GS-9620 (N=41)
Discontinuation of GS-9620 due to an AE, n (%)	0	0	0	0
Treatment-Emergent Serious AE, n (%)	0	1 (2%)	0	0
Any Treatment-Emergent AE, n (%)	3 (38%)	21 (49%)	5 (63%)	29 (71%)
Grade 1	3 (38%)	16 (37%)	5 (63%)	22 (54%)
Grade 2	0	5 (12%)	0	7 (17%)
Grade 3	0	0	0	0
Grade 4	0	0	0	0
AEs in >2 patient in any patient population, n (%)				
Headache	1 (13%)	6 (14%)	1 (13%)	13 (32%)
Myalgia	0	3 (7%)	1 (13%)	4 (10%)
Fatigue	1 (13%)	1 (2%)	1 (13%)	4 (10%)
Contusion	0	2 (5%)	0	4 (10%)
Nausea	0	1 (2%)	0	3 (7%)
Dizziness	0	1 (2%)	0	3 (7%)
Diarrhea	0	0	0	3 (7%)
Somnolence	0	0	1 (13%)	2 (5%)
Cough	0	0	0	3 (7%)
Oropharyngeal Pain	0	0	0	3 (7%)
Treatment-emergent hematologic abnormalities, n (%)				
Lymphocytes ($\times 10^3/\mu L$) Toxicity ≥Grade 3	0	0	0	0
Neutrophil ($\times 10^3/\mu L$) Toxicity ≥Grade 3	0	2 (5%)	0	0
Platelet ($\times 10^3/\mu L$) Toxicity ≥Grade 3	0	0	0	0
WBC ($\times 10^3/\mu L$) Toxicity ≥Grade 3	0	0	0	0
Treatment Emergent ALT Elevation				
Grade 2 (>2.50 to 5 x ULN*)	0	0	0	2 (5%)**
Grade 3 (> 5 to 10 x ULN*)	0	0	0	0
Grade 4 (>10 x ULN*)	0	0	0	0
ALT Elevation from Predose				
ALT ≥2 × Baseline	0	0	0	2 (5%)**
ALT ≥5 × ULN*	0	0	0	0
ALT ≥2 × Baseline and ALT ≥10 × ULN*	0	0	0	0
Treatment emergent AST elevation				
Grade 2 (>2.50 to 5 x ULN*)	0	1	0	1
Grade 3 (> 5 to 10 x ULN*)	0	1	0	1
Grade 4 (> 10 x ULN*)	0	0	0	0
Treatment emergent GGT elevation				
Grade 2 (>2.50 to 5 x ULN*)	0	0	0	0
Grade 3 (> 5 to 10 x ULN*)	0	0	0	0
Grade 4 (> 10 x ULN*)	0	0	0	0

*Upper Limit of Normal = 43 IU/mL for males and 35 IU/mL for females.

**One patient in the SAD 4 mg cohort had an ALT value of 119 U/L at Day 5, a level that was ≥ twice the baseline value of 44 U/L. This level progressively declined to a normal value (29 U/L) at Follow-Up Visit 2.

One patient in the MAD 2 mg cohort had a Grade 2 ALT value of 109 U/L at Day 15. At all other time points the ALT values were of Grade 1 (from Baseline to Follow up visit 1) or Grade 2 (Follow up visit 2).
One patient in the MAD 4 mg cohort had a Grade 1 ALT value of 93 U/L at Follow up visit 1, a level that was ≥ twice the baseline value of 43 U/L. This value decreased to ungraded level of 51 U/L at the following visit Follow up 2.

Table 3: Pharmacokinetic Parameters following a Single Dose of GS-9620 in Virally Suppressed or Treatment Naive Patients

PK Parameter mean (%CV)[a]	0.3 mg (N =5)[b]	1 mg (N = 5)	2 mg (N = 5)	4 mg (N = 5)
Virally Suppressed				
AUC_{inf} (pg•h/mL)	1488.7 (77.7)	9008.2 (81.8)	13420.3 (76.2)	31716.5 (59.8)
C_{max} (pg/mL)	176.1 (52.7)	1146.6 (124.5)	1344.9 (68.6)	3548.5 (56.0)
T_{max} (h)	4.50 (4.00, 5.00)	5.00 (3.50, 5.00)	2.50 (1.50, 4.50)	1.50 (1.47, 2.50)
Treatment Naive				
AUC_{inf} (pg•h/mL)	1763.1 (39.5)	6234.9 (50.5)	10945.2 (58.0)	51564.6 (38.8)
C_{max} (pg/mL)	161.4 (67.7)	518.5 (34.2)	560.1 (34.6)	5663.5 (85.1)
T_{max} (h)	3.57 (2.07, 5.00)	2.00 (2.00, 2.52)	4.50 (1.50, 5.00)	5.00 (5.00, 6.00)

a Median (Q1, Q3) for T_{max}
b N = 4 for Treatment Naive

Table 4: Baseline Factors Associated with ISG15 Induction* (Multivariate Analysis)

	Odds Ratio	95% CI	P value
Dose of GS-9620, vs placebo			
0.3 mg	2.28	0.38–13.68	0.3669
1 mg	6.30	1.02–38.88	0.0475
2 mg	15.89	2.34–107.97	0.0047
4 mg	211.62	8.22–>999.99	0.0012
ISG15-GAPDH at baseline	2.00	1.18–3.37	0.0098
–**HBsAg, log_{10} IU/mL	3.17	1.18–8.52	0.0224
HBeAg positive vs negative	7.58	1.90–30.19	0.0041

CI = confidence interval
*Induction is defined as >2 fold change in ISG15 from baseline within 96 hours after first dose.
** minus: lower level of HBsAg at baseline was associated with higher probability of ISG15 induction

Table 5: Patients with detectable serum IFN-alpha levels

Patient population	Cohort	IFN alpha (pg/mL)						AEs within 48 hours from detectable serum IFN-alpha	ISG15 Max fold change any time
		BL	D1 8h	D8	D8 8hr	D15	max		
suppressed	SAD 0.3 mg	1.6	2	2	na	na	3.6 (D3)	Phlebitis	2.9
suppressed	MAD 0.3 mg	<0.1	<0.1	<0.1	<0.1	<0.1	0.2	-	1.4
suppressed	MAD 1 mg	0.3	<0.1	<0.1	<0.1	<0.1	1.1 (D12)	-	9.6
suppressed	PBO	<0.1	< 0.1	<0.1	na	na	0.3	-	1.2
suppressed	PBO	<0.1	<0.1	<0.1	<0.1	<0.1	0.2	-	2.1
suppressed	PBO	6.3	5.6	4.9	3.7	2.3	6.3 (BL)	-	2.4
suppressed	PBO	<0.1	<0.1	<0.1	<0.1	<0.1	0.2	-	1
naive	SAD 2 mg	4.7	6.2	5.8	na	na	6.3 (D5)	Bruise, headache	16.2
naive	MAD 0.3 mg	6.7	9.6	11	<0.1	9.2	16.3 (D3)	-	1.2
naive	MAD 0.3 mg	<0.1	<0.1	0.4	0.3	<0.1	1.8 (D9)	Pyrexia (D9)	29
naive	MAD 1 mg	5.9	22.5	12	16.3	8.5	22.5 (D1 8hr)	Chills, Pyrexia (Day 8 post dose), dizziness, vomiting, pharyngeal erythema	33
naive	MAD 2mg	17.1	0.9	2.6	1.6	4.6	22 (D5)	Fatigue, bruising, Sore throat, nausea, headache	5.4
naive	MAD 2 mg	5.5	2.8	5.3	3.6	6.5	6.5 (D15)	Fatigue	15.4
naive	MAD 4 mg	10.8	8.6	5.4	3	2.4	10.8 (BL)	Mouth ulcers, headache, vomiting	3.2

SAD=single ascending dose; MAD=multiple ascending dose; PBO=placebo; BL=baseline; D=day; ISG=interferon-stimulated gene; AE=adverse event.