Research Article

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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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**Background & Aims**: 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 seven 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 $\geqslant$1 adverse event, all of which were mild to moderate in severity. 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 seven 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.



## 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

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*Abbreviations:* CHB, Chronic hepatitis B; AUC, Area under the curve; DMC, Data monitoring committee; GALT, Gut-associated lymphoid tissue; HBV, Hepatitis B virus; HCV, Hepatitis C virus; HCC, Hepatocellular carcinoma; ISG, Interferon-stimulated genes; MAD, Multiple-ascending dose; SAD, Single ascending dose; WHV, Woodchuck hepatitis virus; IFN-alpha, serum interferon (IFN)-alpha.

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 two of the three 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.

## Patients and 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 $\geqslant$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 $\geqslant$3) or cirrhosis as determined by liver biopsy within three years of screening, or by two non-invasive alternatives to liver biopsy (e.g. Fibroscan, Fibrotest, aspartate aminotransferase/platelet ratio index [APRI]) within six 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 (e.g. clarithromycin) and inducers (e.g. 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 two doses of GS-9620 (at doses of 0.3 mg, 1 mg, 2 mg, 4 mg) or placebo seven 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: 0.074–52,000 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-$\gamma$-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 analyzed 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 $\Delta\Delta$Ct 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, Billerica, MA, USA). Change in IP-10 levels, relative to baseline, were calculated as fold change; IFN-alpha levels are described as absolute values.