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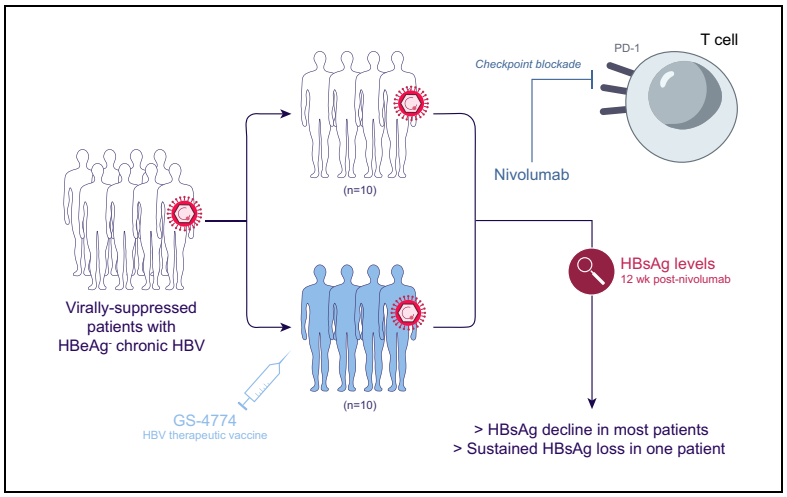
Graphical abstract



Highlights

- In patients with chronic HBV infection, T cell responses are inhibited, leading to an inability to control the virus.
- One of the most common inhibitors present on exhausted T cells is PD-1, which likely contributes to T cell dysfunction.
- A single dose of either 0.1 or 0.3 mg/kg of nivolumab, with or without GS-4774, was well tolerated and effective.

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Lay summary

Chronic hepatitis B virus infection (CHB) is characterized by a dysfunctional immune response. In patients with CHB, inhibitory receptors, such as programmed death receptor 1 (PD-1) are overexpressed on T cells, leading to an ineffective immune response in the liver. Herein, we show that the PD-1 inhibitor, nivolumab, is safe and effective for the treatment of virally suppressed patients with CHB.

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Anti-PD-1 blockade with nivolumab with and without therapeutic vaccination for virally suppressed chronic hepatitis B: A pilot study

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Background & Aims: To evaluate the hypothesis that increasing T cell frequency and activity may provide durable control of hepatitis B virus (HBV), we administered nivolumab, a programmed death receptor 1 (PD-1) inhibitor, with or without GS-4774, an HBV therapeutic vaccine, in virally suppressed patients with HBV e antigen (HBeAg)-negative chronic HBV.

Methods: In a phase Ib study, patients received either a single dose of nivolumab at 0.1 mg/kg (n = 2) or 0.3 mg/kg (n = 12), or 40 yeast units of GS-4774 at baseline and week 4 and 0.3 mg/kg of nivolumab at week 4 (n = 10). The primary efficacy endpoint was mean change in HBV surface antigen (HBsAg) 12 weeks after nivolumab. Safety and immunologic changes were assessed through week 24.

Results: There were no grade 3 or 4 adverse events or serious adverse events. All assessed patients retained T cell PD-1 receptor occupancy 6–12 weeks post-infusion, with a mean total across 0.1 and 0.3 mg/kg cohorts of 76% (95% CI 75–77), and no significant differences were observed between cohorts ($p = 0.839$). Patients receiving 0.3 mg/kg nivolumab without and with GS-4774 had mean declines of −0.30 (95% CI −0.46 to −0.14) and −0.16 (95% CI −0.33 to 0.01) $\log_{10}$ IU/ml, respectively. Patients showed significant HBsAg declines from baseline ($p = 0.035$) with 3 patients experiencing declines of >0.5 $\log_{10}$ by the end of study. One patient, whose HBsAg went from baseline 1,173 IU/ml to undetectable at week 20, experienced an alanine aminotransferase flare (grade 3) at week 4 that resolved by week 8 and was accompanied by a significant increase in peripheral HBsAg-specific T cells at week 24.

Conclusions: In virally suppressed HBeAg-negative patients, checkpoint blockade was well-tolerated and led to HBsAg decline in most patients and sustained HBsAg loss in 1 patient.

Lay summary: Chronic hepatitis B virus infection (CHB) is characterized by a dysfunctional immune response. In patients with CHB, inhibitory receptors, such as programmed death receptor 1 (PD-1) are overexpressed on T cells, leading to an ineffective immune response in the liver. Herein, we show that the PD-1 inhibitor, nivolumab, is safe and effective for the treatment of virally suppressed patients with CHB.

Australian New Zealand Clinical Trials Registry (http://www.anzctr.org.au/) number: ACTRN12615001133527.

Introduction

More than 240 million people worldwide are chronically infected with the hepatitis B virus (HBV)[1,2]. In the Asia-Pacific region, where it is most prevalent, chronic HBV infection is the leading cause of cirrhosis, liver failure, and hepatocellular carcinoma.[2–6] While currently approved oral nucleos(t)ide analogs effectively suppress viral replication, providing important clinical benefits, there are several disadvantages of this therapeutic approach. First, since antiviral therapy is rarely curative, most patients must receive life-long treatment with the attendant cost, cumulative toxicity, and risk of breakthrough through either non-adherence or the emergence of antiviral resistance. Moreover, viral suppression with nucleos(t)ide analogs does not eliminate the risk of hepatocellular carcinoma.[7] A finite course of treatment that can provide sustained off-treatment HBV suppression and clinical response is a clear unmet medical need. Current guidelines recognize clearance of the hepatitis B surface antigen (HBsAg) from the patient's serum as a so-called "functional cure" and can be distinguished from a "sterilizing cure" which requires the elimination of the covalently closed circular DNA (cccDNA) nuclear reservoir of the virus.[5,8,9] Functional cure has been associated with improved outcomes, however, and is a current goal of curative approaches for HBV.[10]

A major barrier to achieving a cure of chronic HBV infection is the presence of a dysfunctional immune response to the virus.[11] Whereas in acute self-limited infection, the CD8 T cell response is diverse and vigorous, the T cell response in patients with chronic infection is characterized by a depleted population of antigen-specific T cells and an inability to control or eliminate the virus. During the course of chronic HBV infection, inhibitory receptors on T cells are overexpressed, limiting T cell effector function.[12] Of the many inhibitory receptors present on exhausted T cells in patients with HBV, programmed death receptor 1 (PD-1) is the most highly expressed, especially on HBV-specific T cells within the liver.[13,14] One of the ligands

Keywords: Chronic hepatitis B; GS-4774; Nivolumab; Receptor occupancy; T cell response; HBV; Immune-checkpoint inhibitors; Immunology.
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for PD-1, programmed cell death ligand 1 (PD-L1), is normally found on cells within the liver, but its expression is increased in the setting of chronic infection.[15] Together, this increased expression of PD-L1 in HBV-infected hepatocytes and increased PD-1 on HBV-specific T cells likely contribute to T cell effector dysfunction. Reversal of "T cell exhaustion" through blockade of the PD-1:PD-L1 axis, has improved specific anti-HBV T cell responses in human intrahepatic T cells and in models of HBV, including mouse and woodchuck models.[16–18] In woodchucks with chronic woodchuck hepatitis virus infection, blockade of PD-L1 combined with DNA vaccination effectively controlled viremia, while antiviral treatment alone or antiviral treatment plus vaccination had no effect.[19]

To date, checkpoint blockade inhibitors have been tested mainly in the treatment of cancer and have shown efficacy in relieving *in situ* T cell dysfunction in patients with advanced malignancy. PD-1 and PD-L1 inhibitors disrupt PD-1 immune checkpoint signaling and restore antitumor activity of otherwise suppressed effector T cells. In clinical studies, nivolumab (Bristol-Myers Squibb, Princeton, NJ, United States), a fully human immunoglobulin G4, increased time to progression and improved survival in patients with advanced melanoma, non-small cell lung cancer, and non-Hodgkin's lymphoma. Nivolumab is now approved treatment for these and other cancers.[20] In the first clinical study of nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040 Study), 3 of 51 patients (6%) with chronic HBV infection who received 3.0 mg/kg every 2 weeks demonstrated a 1 log decline in HBsAg during nivolumab therapy.[21] These studies suggest that the new PD-1 and PD-L1 inhibitors have potential in the treatment of patients with chronic HBV infection.

Doses of nivolumab selected for this pilot study (0.1 mg/kg and 0.3 mg/kg) were based on receptor occupancy (a surrogate marker of efficacy) and safety data reported from phase I dose-finding studies of nivolumab in patients with malignancies.[22,23] In these studies, peripheral receptor occupancy was similar across doses ranging from 0.1 mg/kg to 10 mg/kg. Importantly, doses below 1 mg/kg were safe with no serious adverse events (SAEs) and fewer treatment-related grade 3–4 adverse events (AEs) and no autoimmune disorders have been reported in patients who received single or multiple doses below 1 mg/kg.

One cohort of patients received GS-4774, a yeast-based therapeutic HBV vaccine along with nivolumab. At the time of study initiation, GS-4774 was being evaluated in phase II studies for the treatment of chronic HBV infection. GS-4774, which contains a fusion antigen including major antigenic regions of the HBx, HBc, and HBs antigens, had been shown to stimulate modest T cell responses in preclinical and phase I studies. However, in phase II evaluations, GS-4774 did not demonstrate antiviral efficacy against HBV.[24–26]

Patients and methods

Patient inclusion criteria

Eligible patients were 18 to 65 years of age (inclusive), with documented evidence of chronic HBV infection (*i.e.*, positive for HBsAg for more than 6 months), and negative for HBV e antigen (HBeAg) at screening. Pregnant or lactating women were excluded. Patients were also required to have been receiving approved HBV oral antiviral treatment for ≥1 year prior to screening, with HBV DNA <69 IU/ml (measured at least once) for ≥6 months prior to screening, and HBV DNA <20 IU/ml at screening. Extensive bridging fibrosis or cirrhosis (defined as Fibroscan >9 kPa within 1 year of screening) were exclusionary. Full inclusion and exclusion criteria are provided in the supplementary information.

Clinical trial design

In this open-label, single-center phase I study, 2 patients were to receive a single intravenous dose of 0.1 mg/kg of nivolumab (Sentinel A) (Fig. 1). Provided the initial dose was deemed safe during a 28-day follow-up, an additional 2 patients were to receive a single intravenous dose of 0.3 mg/kg of nivolumab (Sentinel B). If no safety signal was detected in the 28-day follow-up, 10 additional patients were given a single intravenous dose of nivolumab at 0.3 mg/kg (Cohort A). Following completion of enrollment of this cohort, an additional group of 10 patients received 40 yeast units (YU) of GS-4774 subcutaneously on Day 1 of treatment, and 40 YU of GS-4774 along with a single intravenous dose of nivolumab at 0.3 mg/kg on Day 28 (Cohort B). All patients were followed for 24 weeks after receiving nivolumab.

Clinical study assessments

Efficacy: To determine HBsAg and HBV DNA levels, blood samples were collected at screening, baseline/Day 1, Weeks 1, 2, 3, 4, 6, 8, 12, 16, 20, and 24 from patients who received nivolumab only, and at screening, baseline/Day 1, Weeks 2, 4, 5, 6, 7, 8, 10, 12, 16, 20, 24, and 28 in patients who received GS-4774+ nivolumab. The Elecsys HBsAg II Quant Assay (Roche Diagnostics, Indianapolis, IN, United States) with a lower detection limit of 0.05 IU/ml (linear range 0.05 to 52,000 IU/ml) was used to quantify HBsAg levels.

Safety: Safety assessments included monitoring of AEs and concomitant medications, measurement of vital signs, clinical laboratory tests, and physical examinations. AEs of special interest in this study were those previously reported with PD-1 blockade in oncology patients, including onset of any autoimmune disorder and hepatitis flare whether HBV-related or autoimmune.

Immunology methods

The pharmacodynamics of anti-PD-1 antibody were assessed by the receptor occupancy assay which quantifies the binding of PD-1 onto circulating CD3+ T cells.[22,24] In brief, the presence of nivolumab bound to the surface of T cells in peripheral blood mononuclear cells (PBMCs) was detected with fluorophore-labelled anti-IgG4. Pre-incubation with either excess nivolumab or an IgG4 isotype control allowed for determination of the percentage of total cell surface PD-1 occupied by nivolumab on circulating T cells.[22] Co-staining with anti-CD3; -CD4; -CD8;

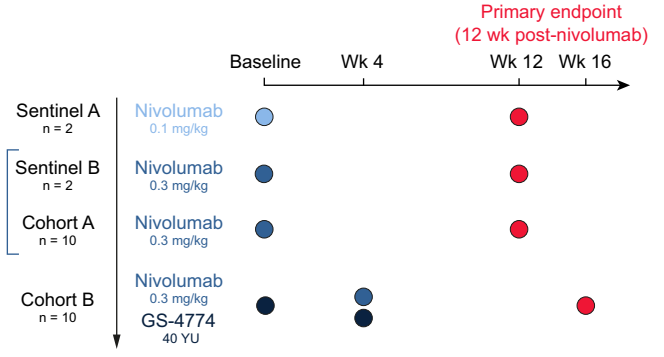

Fig. 1. Study design. (This figure appears in colour on the web.)

-CD45RA; -CCR7 was performed to evaluate occupancy within T cell lineages and naïve/memory subsets.

Two-color FluoroSpot (Mabtech/AiD iSpot, Mabtech AB, Stockholm, Sweden) was performed for interferon (IFN)-γ and tumor necrosis factor (TNF)-α production directly *ex vivo*, following overnight rest, without peptide-mediated pre-expansion, using iSPOT reader (Autoimmun Diagnostika GmbH, Strassberg, Germany) in response to HBV surface and core peptides. A total of 108 surface antigen peptides covered amino acids 1–270 and 270–389, genotypes A-D, while 76 core antigen peptides covered amino acids 1–183, genotypes A-D.

Flow Cytometry was performed on BD SORP FACSAria II (BD Biosciences, San Jose CA, United States) and analyzed using FlowJo Software vX (Tree Star, Inc. Ashland, OR, United States).

Statistics

All patients who received at least 1 dose of study drug were included in all analyses.

Statistical comparisons of *p* values between treatment groups used the nivolumab 0.3 mg/kg group as the reference group. The primary efficacy endpoint was the mean change in serum HBsAg at week 12 compared to baseline (Day 1) following administration of nivolumab. The primary efficacy endpoint was analyzed using a mixed-effect model for repeated measures. Estimated least square means of treatment effects and estimated differences in treatment effects between the treatment groups at week 12 were presented with the 95% CIs and unadjusted *p* values. Secondary efficacy endpoints included the change in HBsAg (log_{10} IU/ml) at week 4 compared to baseline (Day 1) following administration of nivolumab was analyzed as described above for the primary efficacy endpoint.

HBsAg loss was defined as a change from positive at baseline to negative at any postbaseline visit within the targeted time window. Seroconversion of HBsAg was defined as HBs antibody changing from negative at baseline to positive at any postbaseline visit with HBsAg loss occurring within the targeted time window.

Study oversight

All patients provided informed consent. The study was approved by the Institutional Review Board at both participating sites and was conducted in compliance with the Declaration of Helsinki, Good Clinical Practice guidelines, and local regulatory requirements. The study was 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 study data and reviewed and approved the final manuscript.

Results

Patients were enrolled and treated at 1 site in New Zealand from November 29, 2015 through November 28, 2016. Of the 27 patients with HBeAg-negative chronic hepatitis B on oral antiviral therapy who were screened for this study 24 were enrolled (Fig. S1). Three patients withdrew consent before the initiation of dosing. All 24 participants completed the study and are included in the final analysis.

Overall, patients were 75% male, 38% Asian, 50% Polynesian, with median baseline HBsAg levels ranging from 2.6–3.1 log_{10} IU/ml across treatment arms (Table 1). All patients were HBeAg negative and were receiving antiviral treatment with either tenofovir disoproxil fumarate or entecavir. The overall median duration of oral HBV therapy was 7.0 (range 2–16) years.

Receptor occupancy

In PBMCs from the 24 patients who were treated with 1 cycle of anti-PD-1 antibody at a dose of 0.1 or 0.3 mg/kg, the maximum PD-1-receptor occupancy by anti-PD-1 antibody was 68.9–88.2%, similar to levels seen in prior evaluations of nivolumab[23] (Fig. 2). Receptor occupancy was detected in all patients at week 6 and in some patients receiving 0.3 mg/kg, persisted up to week 12 following administration of nivolumab.

Safety

AEs were reported in 15 out of the 24 patients: 50% (1 of 2) of those receiving nivolumab 0.1 mg/kg, 58% (7 of 12) of those receiving nivolumab 0.3 mg/kg, and 70% (7 of 10) of those receiving GS-4774 + nivolumab 0.3 mg/kg (Table 2). Most AEs were grade 1 in severity and were considered not related to

Table 1. Demographics and baseline characteristics.

	Nivolumab 0.1 mg/kg (n = 2)	Nivolumab 0.3 mg/kg (n = 12)	Nivolumab 0.3 mg/kg + 40 YU GS-4774 (n = 10)
Median age, yrs (range)	50 (44–55)	51 (41–63)	54 (38–64)
Male, n (%)	1 (50)	9 (75)	8 (80)
Race, n (%)			
Asian	1 (50)	6 (50)	2 (20)
Polynesian	1 (50)	5 (42)	6 (60)
Median BMI, kg/m^2 (range)	26 (22–29)	28 (22–38)	33 (25–62)
Median HBsAg, log_{10}IU/ml (range)	3.1 (3.0–3.2)	2.7 (1.0–4.0)	2.6 (1.9–3.6)
HBsAg >1,000 IU/ml, n (%)	2 (100)	4 (33)	3 (30)
HBeAg negative, n (%)	2 (100)	12 (100)	10 (100)
Median ALT, U/L (range)	23 (16–29)	26 (18–34)	23 (12–58)
ALT >ULN, n (%)	0	5 (42)	3 (30)
Oral HBV therapy, n (%)			
Entecavir	2 (100)	7 (58)	6 (60)
Tenofovir disoproxil fumarate	0	5 (42)	4 (40)
Median duration of oral HBV therapy (range)	3.5 (3–4)	6.0 (2–14)*	11.5 (3–16)

*Due to a data issue, one patient was not included in the median duration of oral HBV therapy calculation.

ALT, alanine aminotransferase; BMI, body mass index; HBeAg, HBV e antigen; HBsAg, HBV surface antigen; HBV, hepatitis B virus; ULN, upper limit of normal; YU, yeast unit.

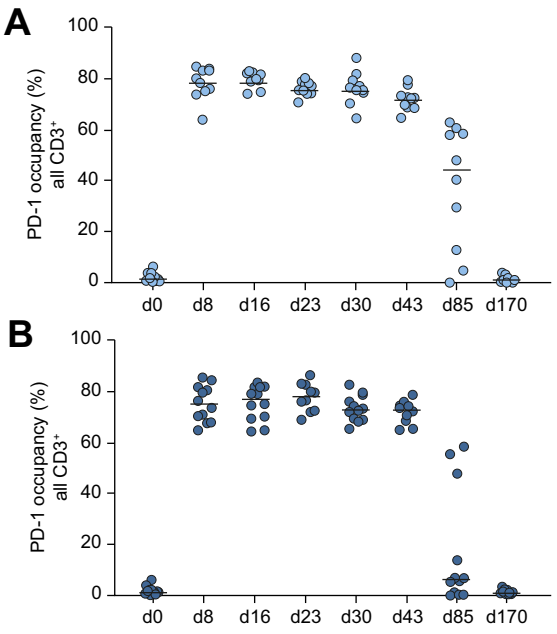

Fig. 2. Receptor occupancy. (A) Nivolumab 0.1 mg/kg or 0.3 mg/kg, and (B) nivolumab 0.3 mg/kg + GS-4774.

the study drugs. One patient receiving GS 4774 + nivolumab 0.3 mg/kg experienced an AE (injection site pain) considered by the investigator as related to GS-4774, and 3 patients receiving nivolumab 0.3 mg/kg experienced an AE (fatigue, headache, cough [1 patient each]) considered by the investigator as related to nivolumab. The most common AEs were fatigue (3 patients [nivolumab 0.3 mg/kg group]), upper respiratory tract infection (2 patients [GS-4774 + nivolumab 0.3 mg/kg group]), and nasal congestion (2 patients [GS-4774 + nivolumab 0.3 mg/kg group]). There were no grade 3 or 4 AEs or SAEs. Only 3 AEs were attributed to nivolumab treatment: mild fatigue, mild headache, and mild cough each of which occurred in a single individual. There were no AEs of autoimmune disorders such as pneumonitis, colitis, rash, or endocrinopathies.

One patient in the 0.3 mg/kg nivolumab arm experienced a grade 3 elevation in alanine aminotransferase (ALT). This patient had a baseline ALT of 49 IU/ml which increased to 275 IU/ml at week 4 after nivolumab administration before returning to near baseline levels of 54 IU/ml by the end of the study. The ALT elevation was accompanied by a 3-log reduction in serum HBsAg level and subsequent HBsAg seroconversion. Two other patients (1 in the nivolumab 0.3 mg/kg arm and 1 in the GS-4774 + nivolumab arm) experienced grade 1 ALT elevations with peak ALT values of 59 IU/ml and 61 IU/ml, respectively, both accompanied by >0.5 log reduction in serum HBsAg levels at 24 weeks. The association of these transient ALT elevations with HBsAg responses in all 3 patients suggested that these were immune-mediated HBV flares rather than *de novo* autoimmune hepatitis.

There were no clinically significant abnormal findings for electrocardiograms or vital signs.

Efficacy

Twelve weeks after administration of nivolumab, no significant difference in levels of serum HBsAg were observed in the 0.1 mg/kg group (Fig. 3A). Of the 22 patients who received 0.3 mg/kg nivolumab with or without GS-4774, 20 (91%) had a reduction in HBsAg from baseline during the study (90% [9/10] with GS-4774, and 92% [11/12] without GS-4774). The mean decline in levels of HBsAg from baseline at week 12 in the nivolumab 0.3 mg/kg group was −0.30 $\log_{10}$ IU/ml (95% CI −0.46 to −0.14), which was similar to the GS-4774 + nivolumab 0.3 mg/kg group, −0.16 $\log_{10}$ IU/ml (95% CI −0.33 to 0.01). At week 24, the mean decline in the nivolumab 0.3 mg/kg group was −0.48 $\log_{10}$ IU/ml (95% CI −0.83 to −0.13) (Fig. 3B). Proportions of patients with HBsAg decline >0.1 log10 IU/ml at week 12 and week 24 are shown in Table S1.

Using an arbitrary cut-off of patients with ≥0.5 $\log_{10}$ IU/ml decrease in HBsAg, 1 of 10 patients (10%) receiving GS-4774 + nivolumab 0.3 mg/kg and 1 of 12 patients (8%) receiving nivolumab 0.3 mg/kg was found to meet this criteria at week 12, while no patient in the nivolumab 0.1 mg/kg group did (Fig. 3C). At week 24, the proportion of patients with a decrease in serum HBsAg ≥0.5 log10 IU/ml increased to 2 of 10 patients (20%) in the GS-4774 + nivolumab 0.3 mg/kg group but stayed the same (8.3%) in the nivolumab 0.3 mg/kg group (Fig. 3D, Fig. S2A,B).

The single patient with a ≥0.5 log10 IU/ml decrease in HBsAg at week 12 in the nivolumab only arm had an associated ALT flare from week 3 to week 8 (described in AE section above) (Fig. 4). This patient's HBsAg titer continued to decline and became undetectable at week 16, and the patient remained HBsAg negative thereafter. He developed an anti-HBs response 10 weeks after the end of study, upon which antiviral treatment was discontinued. He remained HBsAg negative with anti-HBs titers above 500 IU/L at 12 months post-treatment.

Table 2. Safety.

n (%)	Nivolumab 0.1 mg/kg (n = 2)	Nivolumab 0.3 mg/kg (n = 12)	Nivolumab 0.3 mg/kg + 40 YU GS-4774 (n = 10)
Overall safety			
AE	1 (50)	7 (58)	7 (70)
Any grade 3/4	0	0	0
Nivolumab-related AE	0	3* (25)	0
SAE	0	0	0
Death, n (%)	0	0	0
Laboratory abnormalities			
Any grade 3–4	0	1 (8)	0
ALT grade 3	0	1 (8)†	0
ALT grade 2	0	0	0
ALT grade 1	0	1 (8)	1 (10)

*The 3 nivolumab-related AEs of fatigue, headache, and cough occurred in one patient and all were mild in severity.
AE, adverse event; ALT, alanine aminotransferase; SAE, serious adverse event.

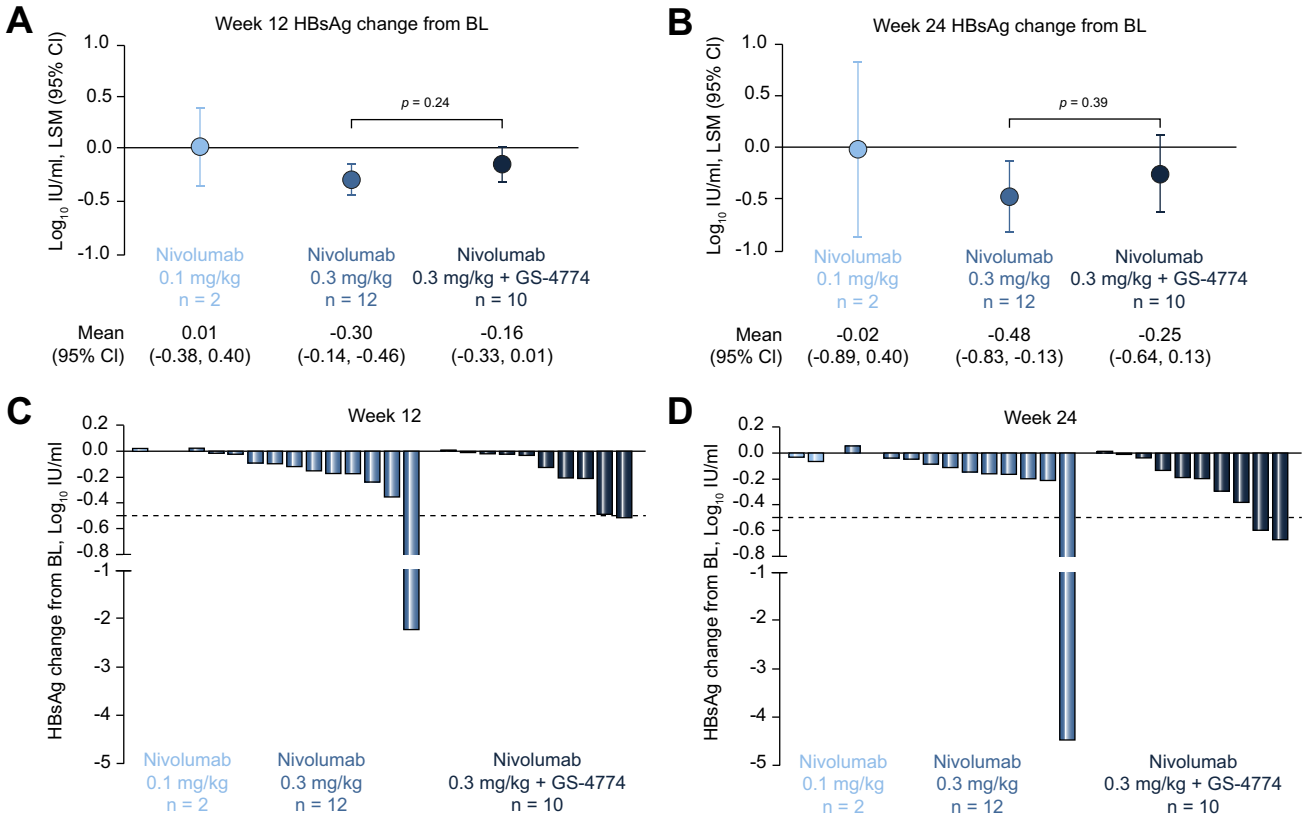

Fig. 3. HBsAg decline. Mean changes in serum HBsAg (A) 12 or (B) 24 weeks after dosing with nivolumab. Individual patient changes in HBsAg (C) 12 or (D) 24 weeks after nivolumab treatment. BL, baseline; HBsAg, HBV surface antigen; LSM, liver stiffness measurement.

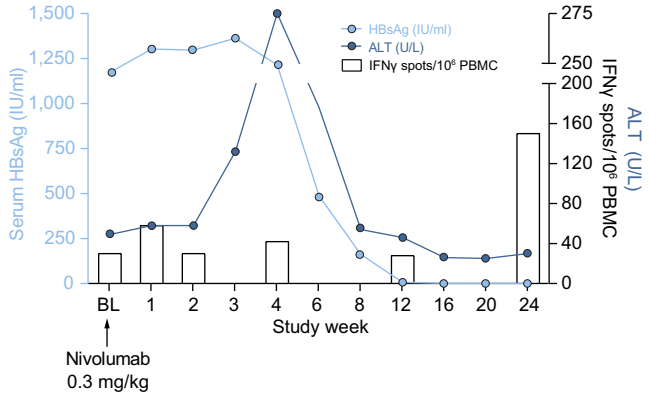

Fig. 4. Clinical measures of patient who achieved HBsAg loss. ALT alanine aminotransferase; HBsAg, HBV surface antigen; IFN, interferon; PBMC, peripheral blood mononuclear cell.

Immunology

Core- and surface- antigen-specific T cells could be detected directly in PBMCs in 18 of 24 patients, but did not consistently increase in frequency following nivolumab treatment. T cell responses to surface antigen amino acids 1–270 were detectable in 7 of 24 patients; to surface antigen amino acids 270–389 in 13/24 patients and to core antigen amino acids 1–183 in 16/24 patients (Fig. 5 and Table S2).

Maximal T cell responses were observed in the patient who achieved HBsAg loss. These IFN-γ and TNF-α FluoroSpot responses were maximal at treatment week 24 (Fig. 6).

Flow cytometry was performed in all patients throughout the study. At baseline, PD-1 levels were found mainly on T cells with a higher frequency of expression on effector memory cells compared with central memory cells (Fig. S3A). Similar levels of PD-1 occupancy with nivolumab were observed among these T cell subsets throughout the dosing period (Fig. S3B). In general, no significant changes were seen in global cell counts, in specific subsets of natural killer (NK) cells, B-cells, T cells, or monocytes, or in activation state of peripheral T cells and NK-cells across dosing groups. For the patient who developed ALT elevation and HBsAg loss, no significant changes were observed in peripheral cells during the ALT elevation, however, by the end of the study (week 24), an increase in CCR7-CD45RA- effector memory CD4+ T cell frequency with a compensatory reduction in naïve CD4+ T cell frequency was observed (Fig. 5). Although no change in the frequency of circulating CD4+ FoxP3+ CD25HI CD127- regulatory T cells was seen across the study, within this subset a notable decrease in the frequency of activated regulatory T cells expressing the ectonucleotidase CD39 was observed at week 24 (Fig. 5). In addition, CD8+ T cells also demonstrated a reduction in naïve T cell frequency with concomitant increases in the frequencies of circulating CCR7-CD45RA- effector memory, CCR7-CD45RA+ effector memory RA, and CD56+ and CD57 + T cells at week 24 (Fig. 6).

Discussion

In this first clinical study of checkpoint inhibition in chronic HBV infection, a single dose of nivolumab with or without GS-4774 was safe and well-tolerated. No symptoms or signs of autoimmune disorders were observed, and the single grade 3 ALT flare occurred in the patient who underwent HBsAg

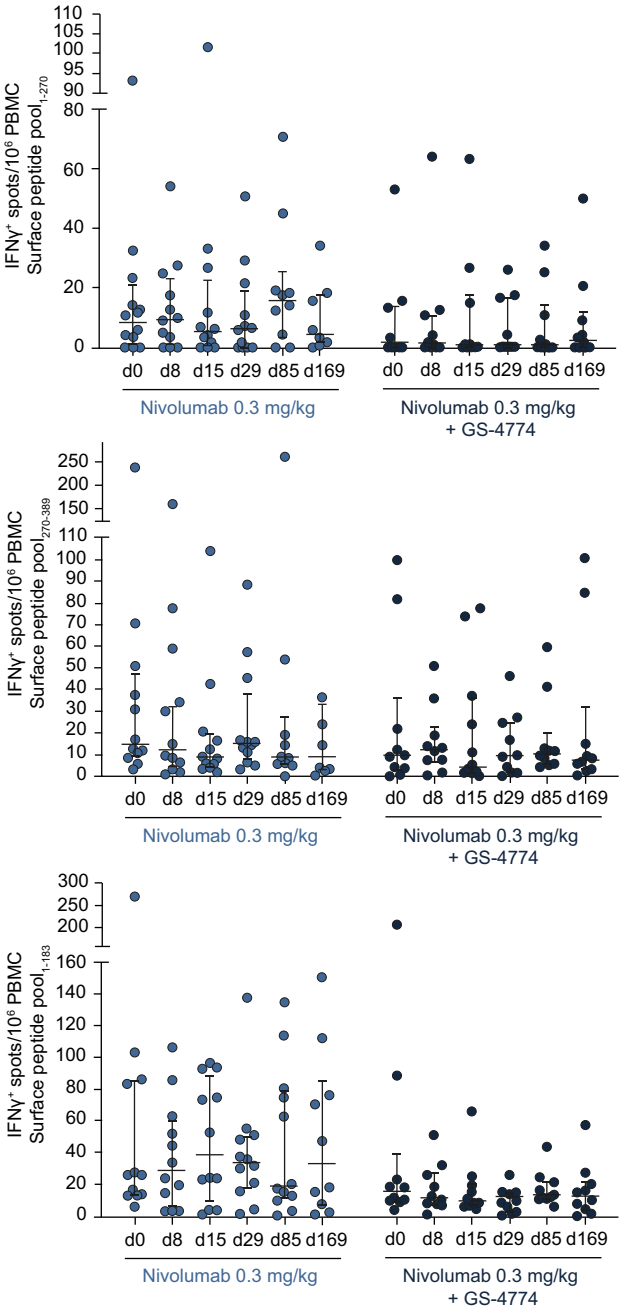

Fig. 5. Fluorospot responses by peptide pool. IFN, interferon; PBMC, peripheral blood mononuclear cell.

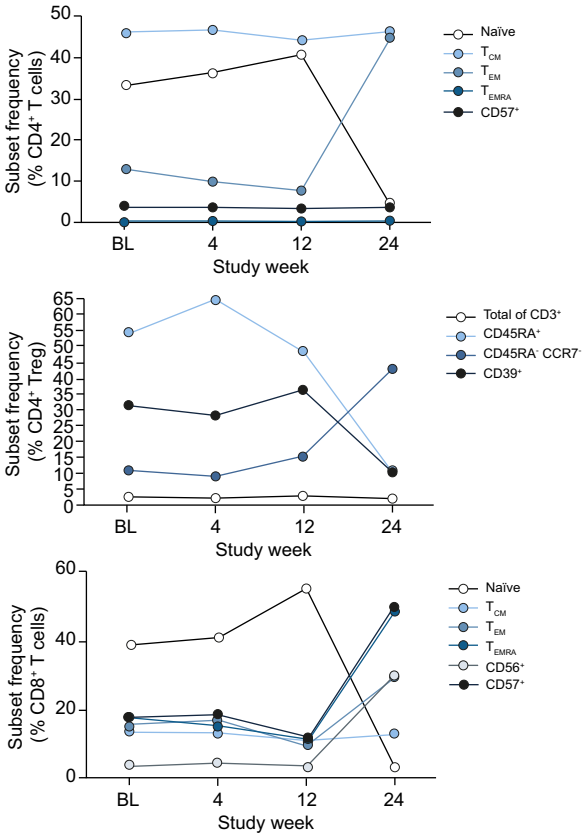

Fig. 6. T cell subset frequency changes observed in patient who achieved HBsAg loss.

seroconversion. Nivolumab treatment was associated with a decrease in HBsAg titers in 20/22 (91%) patients receiving 0.3 mg/kg. HBsAg titers decreased by 0.5 log reduction in 3 patients at week 24 post nivolumab dosing and a single patient achieved sustained HBsAg loss and seroconversion.

Nivolumab treatment was associated with changes in T cell and NK subsets in some patients. The highest core- and surface-antigen specific T cell responses were observed in the single patient who achieved functional cure. These HBV-specific immune responses were maximal 24 weeks after nivolumab dosing, and almost 12 weeks after resolution of the ALT flare. This delay may reflect the site of the anti-PD1 effect within the liver, where trapping of the activated T cells may occur. The measurable T cell response in the peripheral circulation may be delayed until expansion of these intrahepatic T cells and clearance of their cognate antigen within the liver is complete. In future studies, the evolution of antigen-specific T cell responses within the liver could be better evaluated by sampling liver tissue through core biopsies or fine needle aspirates rather than PBMC sampling. These data possibly highlight the limitations of using peripheral immune evaluation to monitor in real time specific anti-HBV activity within the liver, though more mechanistic evaluations are required.

Although the single ALT flare observed in the patient who underwent HBsAg loss could represent a “bad” flare due to immune checkpoint inhibitor-related hepatotoxicity, this would seem most unlikely. Immune-related hepatitis has not been observed after a single low dose of an immune checkpoint inhibitor and would not be associated with a reduction in HBsAg levels or restoration of HBV-specific T cell responses.

The hypothesis for combining nivolumab with GS-4774 was that the therapeutic vaccine would increase the number of HBV-specific effector T cells and nivolumab would reverse immune exhaustion associated with chronic HBV infection and thereby restore the antiviral activity of these T cells. However, in this small pilot study, the addition of GS-4774 had no significant benefit. This may reflect the poor immunogenicity of this vaccine or possibly the need for checkpoint blockade prior to therapeutic vaccination to prime HBV-specific T cells.

A single 0.1 mg/kg or 0.3 mg/kg dose of nivolumab was administered in this study, one-tenth of the approved dose in oncology patients. These very low doses were selected because they were safe and well-tolerated and associated with high PD-1 receptor occupancy in the early oncology dose-finding studies.

However, in those same studies, objective cancer response rates were significantly higher in patients who received 3–10 mg/kg. This dose-response relationship in the oncology studies suggests that repeated and higher doses of nivolumab could also increase efficacy in patients with chronic HBV infection. However, any improvement in HBsAg response must be countered by a real, albeit small, risk of adverse effects, activation of a severe, potentially life-threatening chronic autoimmune disorder in a patient who does not have advanced malignancy. In addition, case reports have emerged describing HBV reactivation in patients receiving checkpoint inhibitors.[27] Though the addition of antivirals to patients receiving checkpoint inhibitors, as was done in this study, likely mitigates this risk.

High peripheral PD-1 receptor occupancy was maintained in patients with chronic hepatitis B for up to 85 days following a single 0.3 mg/kg IV dose of nivolumab, reflecting the life span of the receptors. This prolonged receptor occupancy suggests that dosing intervals could be increased to 12 weeks in future studies, though the peripheral occupancy may not completely reflect occupancy on T cells within the liver. Further, this prolonged receptor occupancy could also delay resolution of adverse effects after treatment discontinuation. The future replacement of monoclonal antibodies with small molecules that reversibly inhibit the PD-1/PD-L1 axis should alleviate this risk and allow oral administration with more predictable pharmacology.

In summary, this pilot study provides the first evidence that immune checkpoint inhibition can restore HBV-specific immune responses in patients with chronic HBV infection and should support the inclusion of PD-1/PD-L1 blockade in future combination strategies towards functional cure of chronic HBV infection. Planned studies will also evaluate higher and repeated doses of anti-PD1/PD-L1. If these studies confirm the safety and efficacy results from this current study, then the targeted populations could be expanded to include patients with HBeAg positive infection (previously termed "immunotolerant") and those with HBeAg-negative infection (previously termed "inactive"). The development of functional cure to a wider HBV population would dramatically reduce the disease burden associated with chronic HBV infection.[28]

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Conflicts of interest
Edward Gane has served on advisory boards for Gilead Sciences, Janssen, Roche, and AbbVie, and as a speaker for Gilead Sciences and AbbVie. Daniel Verdon, Anna Brooks, and Rod Dunbar have served as consultants and have conducted contract research for Gilead Sciences. Anuj Gaggar, Anh Hoa Nguyen, and G. Mani Subramanian, are employees of and hold stock interest in Gilead Sciences. Christian Schwabe declares no conflicts of interest.

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

Role of study sponsor
The sponsor (Gilead Sciences, Inc.) was involved with the study design, statistical analysis, interpretation of data, and drafting of this manuscript.

Authors' contributions
Edward Gane, Anuj Gaggar, G. Mani Subramanian, and P. Rod Dunbar designed the study. Edward Gane and Christian Schwabe collected study data. Daniel J. Verdon, Anna E. Brooks, and P. Rod Dunbar analyzed the data. Anh Hoa Nguyen provided statistical analysis. All authors analyzed the study data, and reviewed the manuscript prior to submission.

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Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2019.06.028.

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