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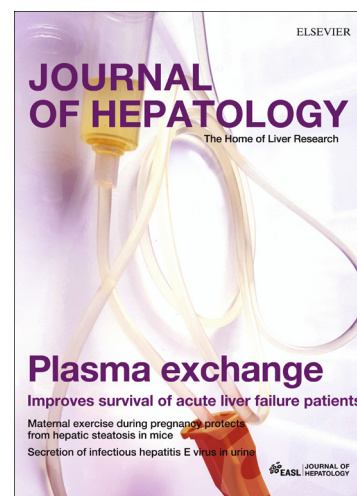
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# **Randomized phase II study of GS-4774 as a therapeutic vaccine in virally suppressed patients with chronic hepatitis B**

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**Abbreviations:** AE, adverse event; ALT, alanine aminotransferase; Anti-HBs, hepatitis B surface antibody; AST, aspartate aminotransferase; CI, confidence interval; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HBx, hepatitis B X, LLOQ, lower limit of quantification; OAV, oral antiviral; PBMC, peripheral blood mononuclear cell; SD, standard deviation; Tarmogen, Targeted Molecular Immunogen; ULN, upper limit of normal; YU, yeast unit.

**Keywords:** Hepatitis B virus; Hepatitis B surface antigen; Hepatitis B e antigen; GS-4774; Nucleoside/nucleotide analogue; Phase II

#### **Conflict of interest**

**AS Lok:** Research Grants: Bristol-Myers Squibb, Gilead Sciences, Merck; Advisor/Consultant: Gilead Sciences, GlaxoSmithKline, Merck.

**CQ Pan:** Grant/Research Support: Gilead Sciences, Merck; Advisor/Consultant: Gilead Sciences, Bristol-Myers Squibb, Johnson & Johnson; Speaker: Gilead Sciences, Bristol-Myers Squibb, AbbVie.

**S-HB Han:** Research Grants: Gilead Sciences; Speaker: Gilead Sciences.

**HN Trinh:** Funding: Gilead Sciences, Abbvie, Intercept; Advisor/Consultant: Gilead Sciences; Speaker: Gilead Sciences; Stock/Shares: Gilead Sciences.

**WJ Fessel:** Nothing to disclose.

**T Rodell:** Inventor of GS-4774; Employee of and holds stock and options in GlobeImmune, which owns an interest in GS-4774.

**B Massetto, L Lin, A Gaggar, GM Subramanian, and JG McHutchison:** Employees of and hold stock in Gilead Sciences.

**C Ferrari:** Research Grants: Gilead Sciences, Roche, Janssen; Advisor/Consultant: Gilead Sciences, Merck, AbbVie, Bristol-Myers Squibb, Arrowhead.

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**EJ Gane:** Scientific Advisory Boards: Gilead Sciences, Merck, AbbVie, Alnylam, Novira, Janssen; Speaker: Gilead Sciences, Merck, AbbVie.

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### **Author Contributions**

Conception/Design: AS Lok, A Gaggar, GM Subramanian, B Massetto, JG McHutchison; Data Acquisition: AS Lok, CQ Pan, S-HB Han, HN Trinh, WJ Fessel, C Ferrari, H Lee, SC Gordon, EJ Gane; Analysis/Interpretation: AS Lok, A Gaggar, L Lin; Manuscript Drafting/Review: All authors.

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**ABSTRACT**

**Background & Aims:** GS-4774 is a heat-inactivated, yeast-based, T-cell vaccine designed to elicit hepatitis B virus (HBV)-specific T-cell responses. We evaluated the safety, tolerability and efficacy of GS-4774 in patients with chronic HBV infection.

**Methods:** In this phase II study, 178 patients with chronic HBV infection and no cirrhosis who were virally suppressed on an oral antiviral (OAV) for  $\geq 1$  year were randomized (1:2:2:2) to continue OAV alone or receive OAV plus GS-4774 2, 10, or 40 yeast units (YU) subcutaneously every 4 weeks until week 20. OAV was continued for the remainder of the study. Efficacy was measured by decline in serum hepatitis B surface antigen (HBsAg) from baseline to week 24.

**Results:** Baseline characteristics were similar across groups (mean age, 45–50 years; male, 62–74%; Asian, 68–80%; hepatitis B e antigen (HBeAg)-positive, 24–26%; mean HBsAg, 2.5–3.1  $\log_{10}$  IU/ml). There were no significant differences between groups in mean HBsAg declines from baseline to week 24 or 48. Five HBeAg-positive patients receiving GS-4774 experienced HBeAg loss vs. none in the control group. Three GS-4774 40 YU-treated patients had HBsAg declines  $\geq 0.5 \log_{10}$  IU/ml, but no patient experienced loss of serum HBsAg. No virologic breakthrough occurred. Injection site reactions were the most frequent adverse event (AE), and there were no treatment discontinuations.

**Conclusions:** GS-4774 was well tolerated, but did not provide significant reductions in serum HBsAg in virally suppressed patients with chronic hepatitis B. Efficacy of GS-4774 in treatment naïve patients remains to be determined.

## LAY SUMMARY

GS-4774 is a therapeutic vaccine designed to improve the immune response against hepatitis B virus (HBV) in patients who already have chronic infection with HBV. In this study, GS-4774 was safe and well tolerated in patients with chronic HBV infection receiving oral antiviral therapy, but did not result in a clinical benefit. Combination approaches with other agents, and evaluation in other populations of patients with HBV are ongoing to determine if GS-4774 might have a therapeutic benefit.

## INTRODUCTION

Approximately 250 million people are chronically infected with the hepatitis B virus (HBV) [1]. Cirrhosis or hepatocellular carcinoma may develop in up to 25% of individuals infected with HBV as children [1,2]. The ultimate goal of treatment—the loss of detectable serum levels of hepatitis B surface antigen (HBsAg) with or without seroconversion— occurs in fewer than 10% of patients treated with nucleo(t)side analogues [3–6]. Several viral and host factors have been shown to influence responses to currently available treatments, including HBV genotype, hepatitis B e antigen (HBeAg) status, and levels of HBsAg, HBV DNA, and alanine aminotransferase (ALT) [7–14]. The low rate of HBsAg loss is attributed in part to decreased HBV-specific CD4-positive and CD8-positive T-cell responses in patients with chronic HBV infection. Diminished cell-mediated responses facilitate the development of reservoirs of infected hepatocytes, contributing to viral persistence [15–17].

An intervention that can restore effective anti-HBV immune responses may lead to the elimination of infected hepatocytes and increased rates of HBsAg loss. Prior attempts at

therapeutic vaccines using recombinant HBV proteins or peptides with an adjuvant to induce a B-cell or T cell response have largely been unsuccessful [18–22].

GS-4774 is being developed and investigated as a therapeutic vaccine for patients with chronic HBV infection [24,25]. It was engineered using the Tarmogen (Targeted Molecular Immunogen) platform [26] to promote HBV-specific cell-mediated immune responses. GS-4774 consists of heat-inactivated, recombinant *Saccharomyces cerevisiae* yeast that express HBsAg, hepatitis B core antigen, and hepatitis B X (HBx) antigen. The yeast component is able to act as a natural adjuvant, potentially allowing for new T-cell responses to develop despite an overall high burden of HBV antigens in chronic HBV infection. In mouse models, GS-4774 can induce HBV Ag-specific CD4-positive and CD8-positive T cell responses, and can break immunological tolerance to tumor antigens [25]. In a phase I study, healthy volunteers were administered either weekly or monthly subcutaneous injections of GS-4774 10, 40, or 80 yeast units (YU; one YU is equivalent to  $10^7$  yeast cells). GS-4774 was found to elicit HBV-specific T-cell-mediated responses in 88% (52/60) of study participants, and two individuals developed low-level (<8.4 IU/ml) hepatitis B surface antibodies (anti-HBs). The most common adverse event (AE) was injection site reaction (38%, 23/60), all episodes of which were mild or moderate. No serious AE was reported [24].

We conducted an open-label, randomized, phase II study to assess the safety, tolerability, and efficacy of GS-4774 in patients with chronic HBV infection who were virally suppressed on nucleos(t)ide polymerase inhibitors (oral antiviral [OAV] therapy). This population was chosen for this proof-of-concept study because patients who are virally suppressed have lower levels of HBV antigen and decreased hepatic inflammation and fibrosis, which can enhance the safety profile of agents that are intended to stimulate immune responses to HBV [13,14].

## PATIENTS AND METHODS

### *Patients*

Study participants were adults (18–65 years) with a body mass index 18–33 kg/m<sup>2</sup> and chronic HBV infection (with documented evidence of HBsAg positivity for >6 months) that was virally suppressed through OAV therapy. A patient was considered to be virally suppressed if HBV DNA was <29 IU/ml at screening and both of the following conditions were met: (i) HBV DNA was persistently below the lower limit of quantification (LLOQ) by an approved assay for ≥1 year prior to screening and (ii) ≥2 HBV DNA values (measured ≥3 months apart) were below the LLOQ within 1 year of screening. All study participants had been using an approved HBV OAV therapy (adefovir dipivoxil, entecavir, lamivudine, telbivudine, or tenofovir disoproxil fumarate) either as a single agent or as part of combination therapy, with no change in regimen in the 3 months prior to screening. Patients with cirrhosis were excluded. Cirrhosis was defined as an Ishak fibrosis score ≥4 on liver biopsy within 5 years of screening, FibroTest score >0.48 plus aspartate aminotransferase (AST): platelet ratio index >1 at screening, or FibroScan® value >9 kPa within 6 months of screening [27]. Full eligibility criteria are provided in the Supplementary data. All patients provided written informed consent.

### *Study design and treatment*

In this parallel-group, multicenter (Supplementary Table 1) study (clinicaltrials.gov: NCT01943799), eligible patients were randomized (1:2:2:2) to receive OAV only, OAV plus GS-4774 2 YU, OAV plus GS-4774 10 YU, or OAV plus GS-4774 40 YU. Block randomization, with block size 14, was used. The randomization list was generated by SAS and was maintained by an outside vendor until the study was unblinded. An interactive web response



system was used for randomization allocation; patients were stratified by HBsAg levels ( $\leq 1000$  IU/ml or  $>1000$  IU/ml) and HBeAg status (positive or negative). GS-4774 was administered subcutaneously every 4 weeks until week 20. All patients continued with the OAV regimen that was ongoing at the time of screening until the end of the study at week 48. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

### *Endpoints*

The assessments performed at each visit are summarized in Supplementary Table 2. The primary efficacy endpoint was the mean change in  $\log_{10}$  IU/ml serum HBsAg from baseline to week 24. HBsAg levels were measured using the ARCHITECT® HBsAg Assay (Abbott Laboratories). Safety was a co-primary endpoint. All AEs were coded using the Medical Dictionary for Regulatory Activities, Version 17.1. The occurrence of liver-related laboratory abnormalities and liver toxicity was monitored. Liver-related laboratory abnormalities were defined as ALT or AST  $>3$  times upper limit of normal (ULN) or total bilirubin  $>2$  times ULN. Liver toxicity was defined as ALT  $\geq 10$  times ULN; confirmed elevations in ALT (grade shift or  $>2$  times previous value), with evidence of worsened hepatic function (e.g., total bilirubin  $>2$  mg/dL above baseline, elevated international normalized ratio  $\geq 1.7$  or  $>0.5$  over baseline, decrease serum albumin  $>1$  g/dl from baseline); or ALT  $\geq 3$  times to  $<10$  times ULN plus  $>2$  times nadir.

Secondary endpoints included the mean change in  $\log_{10}$  IU/ml serum HBsAg from baseline to weeks 12 and 48, proportion of patients with HBsAg loss and HBsAg seroconversion at weeks 24 and 48, and proportion of patients with HBeAg loss and HBeAg seroconversion at weeks 24 and 48. HBsAg loss and HBsAg seroconversion were determined based on quantitative assays for HBsAg (Abbott Architect) and anti-HBs (Siemens ADVIA Centaur XP), which have limits

of detection of 0.05 IU/ml and 5.0 mIU/ml, respectively. HBeAg loss and HBeAg seroconversion were determined by qualitative assays for HBeAg and anti-HBe (Diasorin Plus).

In an exploratory analysis, the effect of GS-4774 on changes in circulatory HBV-specific T-cell counts was assessed using ELISpot assay. Peripheral blood mononuclear cells (PBMCs) were to be isolated from blood samples that were shipped overnight and cryopreserved in liquid nitrogen until ELISpot analysis. However, the majority of samples collected at baseline and at the week 12 and week 24 time points were incorrectly stored at  $-80^{\circ}\text{C}$  for 9 months, which resulted in a loss of cell viability and functions. Once the error was detected, the sample handling procedure was revised, and fresh blood samples were shipped directly from clinical sites to a specialty laboratory, which isolated the PBMCs and performed the ELISpot analysis immediately. As this amendment was not initiated until the beginning of the week 48 sample collection, only ELISpot results at week 48 are reported. The following conditions were tested: recombinant HBsAg (ProSpec, Ness-Ziona, Israel), HBcAg (Fitzgerald Industries International, Acton, MA), and HBx antigen (ProSpec), as well as pools of 15-mer peptides that overlapped by 9 amino acids (Mimotopes, Clayton, Victoria, Australia) spanning the HBc, HBs, and HBx regions in GS-4774. A total of  $4 \times 10^5$  cells in triplicate were used for each testing condition. Assay medium and HIV peptides served as negative controls. Cytomegalovirus/Epstein-Barr virus/influenza virus peptides and phytohemagglutinin served as positive controls. Spot counts for each condition were quantified as the mean spot count of triplicates minus the mean spot count of medium-only controls. A positive response was required to meet all of the following criteria: (i) a spot count greater than the medium control mean spot count plus 3 times the standard deviation, (ii) a spot count  $>10$  spots/ $1\text{E}6$  cells, and (iii) a spot count  $>2$  times the mean of the medium control count.

### *Statistics*

The sample size calculation was based on the primary efficacy endpoint. A total of 25 patients in the OAV-only group and 50 patients in each of the OAV plus GS-4774 groups were determined to provide  $\geq 80\%$  power to detect a difference of  $0.15 \log_{10}$  IU/ml in HBsAg between the control arm and each of the OAV plus GS-4774 groups. The sample size calculation assumed a change from baseline to week 24 in the OAV-only group of  $-0.12 \log_{10}$  IU/ml, with a standard deviation of  $0.179 \log_{10}$  IU/ml and an alpha level of 0.016 (adjusting for three test groups). These assumptions were based on observations from two randomized phase III studies of tenofovir disoproxil fumarate [4].

The efficacy and safety analyses included all patients who had a baseline/day 1 visit (OAV-only group) or received  $\geq 1$  dose of GS-4774 (OAV plus GS-4774 groups). The primary efficacy endpoint was analyzed using a mixed-effects model for repeated measures. The model included treatment group, baseline HBsAg level ( $\leq 1000$  IU/ml or  $>1000$  IU/ml), baseline HBeAg status (positive or negative), study visit, and treatment-by-visit interaction as fixed effects and visit as a repeated measure. An unstructured, within-subject covariance matrix was used. Estimated least squares means of treatment effects and estimated differences in treatment effects between each of the GS-4774 groups and the OAV-only group at week 24 were calculated with 95% confidence intervals and unadjusted  $p$ -values. Only HBsAg data up to week 24 were included in the model for the primary analysis. For patients who prematurely discontinued GS-4774, off-treatment HBsAg data up to week 24 were included in the model. The secondary efficacy endpoints, changes in HBsAg from baseline to week 12 and from baseline to week 48, were analyzed using the same model, but only HBsAg data up to weeks 12 and 48, respectively, were included.

A stratified Cochran-Mantel-Haenszel test with baseline HBsAg level ( $\leq 1000$  IU/ml or  $> 1000$  IU/ml) and baseline HBeAg status (positive or negative) as stratification variables was used to compare the treatment effect between the OAV plus GS-4774 groups and the OAV-only group. Fisher's exact test was also used to compare the treatment effect between the OAV plus GS-4774 groups and the OAV-only group. The two-sided  $p$ -values generated from these analyses were unadjusted.

The exploratory HBV-specific T-cell analyses included patients who had a baseline/day 1 visit (OAV-only group) or received  $\geq 1$  dose of GS-4774 (OAV plus GS-4774 groups) and had T-cell data available. ELISpot results are summarized descriptively.

## RESULTS

### *Patients*

Of the 213 patients screened, 178 were enrolled between September 13, 2013 and March 25, 2014 (Figure 1). Of the 35 patients who failed screening, 26 did not satisfy the inclusion criteria, eight withdrew consent, and one was outside the screening window. In total, 27 patients were randomized to receive OAV-only, 51 to OAV plus GS-4774 2 YU, 50 to OAV plus GS-4774 10 YU, and 50 to OAV plus GS-4774 40 YU. One patient randomized to receive OAV plus GS-4774 2 YU actually received treatment with OAV plus GS-4774 10 YU; this patient was included in the OAV plus GS-4774 2 YU group for the efficacy analyses and the OAV plus GS-4774 10 YU for the baseline and safety analyses. Ten (6%) patients did not complete the study (withdrawal,  $n = 7$ ; loss to follow-up,  $n = 2$ ; protocol violation,  $n = 1$ ) (Figure 1).

Baseline demographics and disease characteristics were generally balanced across the four treatment groups (Table 1). The mean age of patients was 47 years (range, 23–65 years). Most

patients were men (69%), Asian (76%), HBeAg-negative (75%), and had a baseline HBsAg level >1000 IU/ml (59%). The most commonly used OAVs at baseline were tenofovir disoproxil fumarate (51%) and entecavir (43%).

### *Efficacy*

Declines in serum HBsAg from baseline to week 24 were seen for all groups. The mean declines in serum HBsAg between baseline and week 24 for the OAV, OAV plus GS-4774 2 YU, OAV plus GS-4774 10 YU, and OAV plus GS-4774 40 YU groups were  $-0.019$ ,  $-0.020$ ,  $-0.026$ , and  $-0.048$   $\log_{10}$  IU/ml, respectively (Table 2). The differences between treatment groups were not statistically significant. Statistically significant differences were also not found for the secondary endpoints, mean change in serum HBsAg from baseline to weeks 12 and 48 (Table 2). The largest mean decrease in serum HBsAg at weeks 12, 24, and 48 occurred in the OAV plus GS-4774 40 YU group. No patient exhibited HBsAg loss or seroconversion at either week 24 or 48. By week 48, three patients receiving GS-4774 40 YU, but none in the other groups, had reductions of  $\geq 0.5$   $\log_{10}$  IU/ml in serum HBsAg relative to baseline. All three patients were HBeAg negative with undetectable HBV DNA at baseline, two were Asian, two were men, ages ranged from 39-43 years, and duration of OAVs varied from 57-139 months.

A total of 44 patients were HBeAg-positive at baseline (seven receiving OAV-only, 13 receiving OAV plus GS-4774 2 YU, and 12 each in the groups receiving OAV plus GS-4774 10 YU and GS-4774 40 YU). Three (7%) of these patients experienced HBeAg seroconversion at week 24; all three were treated with OAV plus GS-4774 10 YU. At week 48, five (11%) patients (OAV plus GS-4774 2 YU,  $n = 1$ ; OAV plus GS-4774 10 YU,  $n = 4$ ) had HBeAg loss, with four seroconverting to anti-HBe (OAV plus GS-4774 2 YU,  $n = 1$ ; OAV plus GS-4774 10 YU,  $n = 3$ ).

No patient randomized to OAV-only experienced HBeAg loss throughout the study. All five patients with HBeAg loss were Asian, ages ranged from 25-61 years, three were men, and duration of OAV varied from 41-203 months. Statistically significant differences were not observed in any efficacy measures between any of the OAV plus GS-4774 groups and the OAV-only group at any time point.

There were no apparent differences in HBsAg decline relative to baseline in subgroups defined by baseline HBsAg level or baseline HBeAg status. Most of the decline in HBsAg was seen in the subset of patients who were HBeAg-negative at baseline (Figure 2). Viral breakthrough was not observed in any patient.

### *Safety*

In total, 37%, 82%, 94%, and 92% of patients administered OAV-only, OAV plus GS-4774 2 YU, OAV plus GS-4774 10 YU, and OAV plus GS-4774 40 YU, respectively, experienced  $\geq 1$  treatment-emergent AE (Table 3). The proportion of AEs considered related to GS-4774 ranged from 58–80%, the most common of which were injection site reactions (erythema [34–64%], induration [36–41%], pruritus [24–46%], pain [24–66%], swelling [22–44%]). Upper respiratory tract infection (11%) was the most common AE in the OAV-only group. Following injection site reactions, the most common AEs in the OAV plus GS-4774 groups were fatigue (16–26%), headache (14–18%), and myalgia (8–26%).

Four grade 3 AEs were reported in three patients. One patient randomized to the OAV-only group experienced grade 3 influenza and grade 3 diarrhea, neither of which was considered related to study treatment. The other two grade 3 AEs—fatigue and injection site pain—were reported in the OAV plus GS-4774 10 YU group; both were deemed by the investigator to be

related to GS-4774. All grade 3 AEs resolved, and no grade 4 AE was reported. There were two serious AEs: a grade 2 suicide attempt in a patient administered OAV plus GS-4774 2 YU and a grade 2 colonic abscess in a patient administered OAV plus GS-4774 10 YU. Neither serious AE was considered related to GS-4774 (colonic abscess occurred after the patient discontinued GS-4774), and both resolved. No patient discontinued, modified, or interrupted treatment with GS-4774 due to AEs, and there were no deaths during the study. Seven patients experienced grade 3 laboratory abnormalities (Supplementary Table 3), none of which was considered clinically relevant. No grade 4 laboratory abnormality was reported.

Two patients met one of the criteria for a liver-related event (i.e., ALT >3 times ULN) and liver toxicity (i.e., ALT  $\geq$ 3 times to <10 times ULN plus >2 times nadir). The first patient received treatment with OAV plus GS-4774 2 YU and had grade 2 increased ALT at weeks 6 and 8, with concurrent grade 2 increases in AST. ALT and AST values peaked at 213 and 117 U/L, respectively and decreased to within normal ranges at a subsequent unscheduled visit and remained normal for the study duration. The second patient, who was treated with OAV plus GS-4774 10 YU, experienced grade 2 elevations in ALT at week 20. This was confirmed at a subsequent unscheduled visit, when grade 2 elevations in AST were also detected. ALT and AST peaked at 166 and 105 U/L, respectively, and both decreased to grade 1 at the following visit; the patient had grade 1 increased ALT and AST at screening. No patient had an ALT flare, which was defined as ALT >2 times baseline and >5 times ULN.

#### *HBV-specific T-cell response*

ELISpot responses to HBV core, surface, and X antigens were greater in GS-4774-treated than OAV-only-treated patients. Median ELISpot counts in response to all HBV antigens in the

OAV-only, OAV plus GS-4774 2 YU, OAV plus GS-4774 10 YU, and OAV plus GS-4774 40 YU groups were 19.2, 32.2, 31.3, and 23.7, respectively (Figure 3). Higher spot counts in the OAV plus GS-4774 groups were driven primarily by responses to HBV core (median spot counts: OAV plus GS-4774, 10.3–13.7; OAV-only, 6.2) and X antigens (median spot counts: OAV plus GS-4774, 7.7–12.2; OAV-only, 5.3). The proportions of patients who responded to HBcAg were 36% in the OAV-only group and 52%, 59%, and 58% in the OAV plus GS-4774 2 YU, 10 YU, and 40 YU groups, respectively (Table 4). Responses to HBcAg were greater in HBeAg-negative *vs.* HBeAg-positive patients (Table 4), with statistically significantly higher spot counts observed in GS-4774-treated *vs.* OAV-only-treated HBeAg-negative patients ( $p < 0.05$ ). There was no difference between the OAV-only and OAV plus GS-4774 groups in responses to HBsAg.

Compared with the spot counts in response to HBV antigens, median spot counts in response to HBV peptide pools were generally lower (OAV-only, 4.2; OAV plus GS-4774 2 YU, 7.0; OAV plus GS-4774 10 YU, 6.0; OAV plus GS-4774 40 YU, 6.8) (Figure 3). As with HBV core antigen, a trend of higher median spot counts was observed for the HBV core antigen peptide pool in the OAV plus GS-4774 groups *vs.* OAV-only group (median spot counts: OAV plus GS-4774, 2.7–5.0; OAV-only, 1.7). The proportions of patients who responded to HBcAg peptide pools were 7% in the OAV-only group and 28%, 24%, and 16% in the OAV plus GS-4774 2 YU, 10 YU, and 40 YU groups, respectively. Similar to HBcAg, responses to HBcAg peptide pools were greater in HBeAg-negative *vs.* HBeAg-positive patients (Table 4). There was no difference between the OAV-only and OAV plus GS-4774 groups in responses to the HBV surface or X antigen pools.

## DISCUSSION



This is the first study to examine the efficacy and safety of GS-4774 in patients with chronic HBV infection. No clinically or statistically significant differences were seen between the OAV-only group and any of the GS-4774 groups for the primary efficacy endpoint (decline in serum HBsAg from baseline to week 24), and no patient experienced HBsAg loss by week 48. Declines in serum HBsAg levels  $\geq 0.5 \log_{10}$  IU/ml have been shown to be predictive of a sustained response to interferon treatment [28,29]. In the present study, three patients—all of whom received GS-4774 40 YU—had declines in HBsAg serum levels  $\geq 0.5 \log_{10}$  IU/ml between baseline and week 48. Demographics of these patients did not identify clear predictors of response and with only 3 patients having significant declines, the association with GS-4774 treatment cannot be determined. Only patients randomized to treatment with OAV plus GS-4774 experienced HBeAg loss by week 48 (11%). Of the five GS-4774-treated patients who lost HBeAg by week 48, four seroconverted, but no significant differences in HBeAg loss were seen upon comparison of each of the OAV plus GS-4774 groups with the OAV-only group.

Treatment with OAV plus GS-4774 2, 10, or 40 YU every 4 weeks for 20 weeks was well-tolerated in virally suppressed patients with chronic HBV infection and no cirrhosis: no patient discontinued treatment with GS-4774 due to AEs, and no serious AE related to GS-4774 was reported. The majority of treatment-emergent AEs were grade 1–2, with only four grade 3 AEs reported in three patients. As expected with its subcutaneous route of administration, the most common AEs in GS-4774-treated patients were injection site reactions, all but one instance of which was grade 1–2. The occurrence of injection site-related AEs appeared to be dose-related, with a numerically greater proportion of patients randomized to OAV plus GS-4774 40 YU experiencing injection site reactions relative to patients treated with OAV plus GS-4774 2 YU. Seven patients had transient grade 3 laboratory abnormalities. Transient occult blood and/or

blood in urine was the only grade 3 laboratory abnormality reported in more than one patient ( $n = 3$ , one woman in each of the OAV plus GS-4774 groups). No patient had grade 3–4 increases in ALT, but two patients had transient grade 2 ALT increases. The significance of these ALT elevations is not known as they were not associated with symptoms or increase in bilirubin and not accompanied by HBsAg declines or HBeAg loss nor were they related to the dose of GS-4774. Overall, the safety profile of GS-4774 in patients with chronic HBV was consistent with that observed in a phase I study of healthy volunteers [24].

Exploratory ELISpot assays at week 48 demonstrated that HBV-specific T-cell responses were generated in all patients, irrespective of treatment regimen. However, there was a trend for higher median spot counts at week 48 in GS-4774-treated vs. OAV-only-treated patients. Further analysis indicated that these responses were primarily directed against the HBV core and X antigens. The reasons for weaker responses to HBsAg are unclear. One possibility is that these patients had been exposed to high levels of HBsAg for many years or decades. As the HBV-specific immune response elicited in GS-4774-treated patients was weak, more potent stimulation of the immune response and/or the activation of pathways to overcome T-cell exhaustion may be required to restore HBV-specific immune responses in patients with chronic HBV infection. In this study, the median duration of continuous OAV use prior to study entry was 70.6 months and the maximum was 211.7 months. Chronic HBV infection is an immunologically complex disease and it remains to be determined whether GS-4774 can elicit a stronger immune response in other patient populations, such as treatment-naïve patients or patients in the immune tolerant phase, which are the focus of ongoing studies. It is also unclear if a stronger immune response may be observed in patients with shorter duration of OAV therapy. Although the results of this study are disappointing, other approaches to stimulate

immune response to HBV in combination with novel antiviral agents that target other steps in the HBV life cycle should be explored.

In conclusion, in this randomized phase II study, OAV plus GS-4774 in virally suppressed, non-cirrhotic patients with chronic HBV infection was well-tolerated, but of limited efficacy. The efficacy and safety of GS-4774 in treatment-naïve patients with chronic HBV is being examined in an ongoing phase II study (NCT02174276).

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**TABLES**

**Table 1. Baseline demographics and disease characteristics.**

**Table 2. Change from baseline in HBsAg ( $\log_{10}$  IU/ml).**

**Table 3. Treatment-emergent AE frequency and severity.**

**Table 4. ELISpot responder status.\***

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**FIGURE LEGENDS**

**Figure 1. Patient disposition.** OAV, oral antiviral.

**Figure 2. Change from baseline to Week 48 in HBsAg ( $\log_{10}$  IU/ml) in patient subgroups stratified by baseline HBsAg level ( $\leq 1000$  or  $>1000$  IU/ml) and HBeAg status (positive or negative).** HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; OAV, oral antiviral; YU, yeast units.

**Figure 3. ELISpot responses to hepatitis B antigens and peptide pools at week 48.** Data are median spot counts  $\pm$  interquartile range. IFN- $\gamma$ , interferon- $\gamma$ ; OAV, oral antiviral; YU, yeast units.

Figure 1

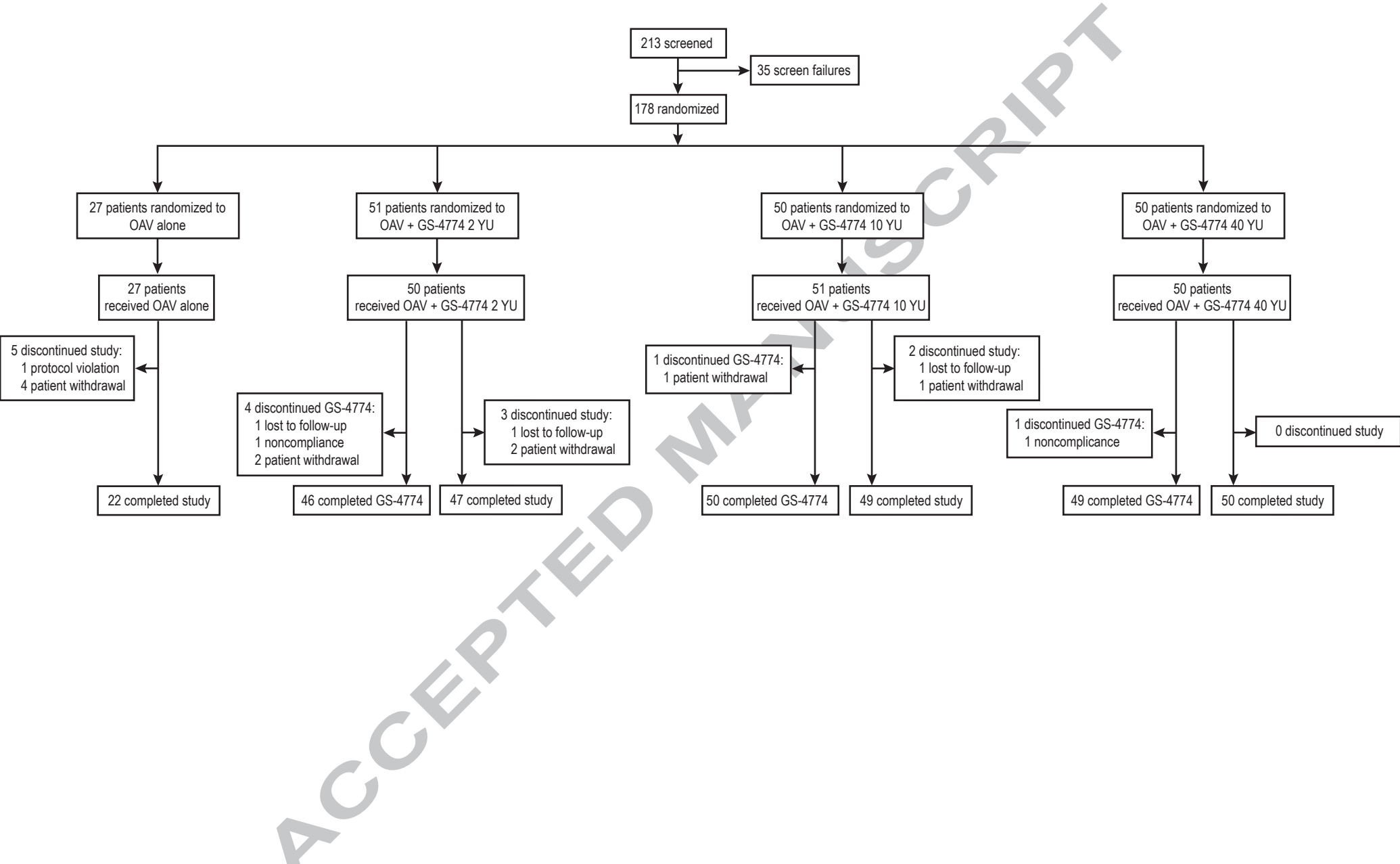


Figure 2

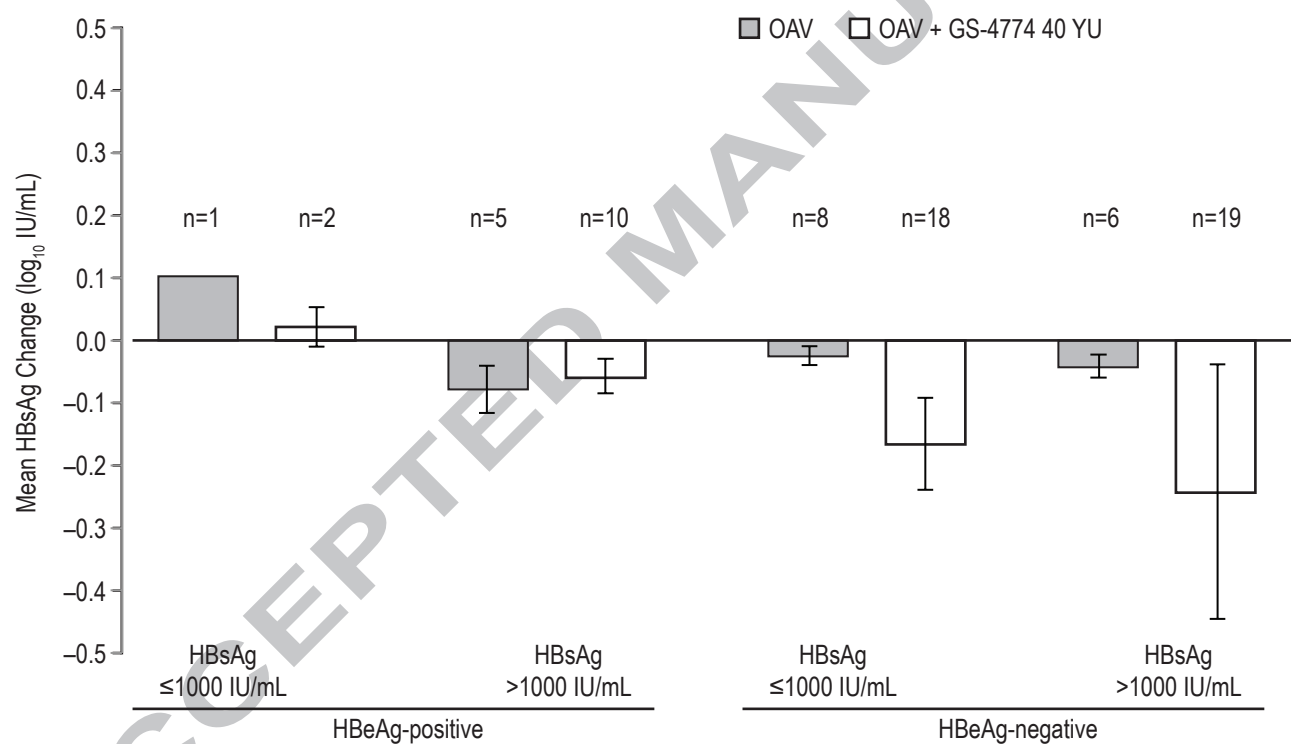
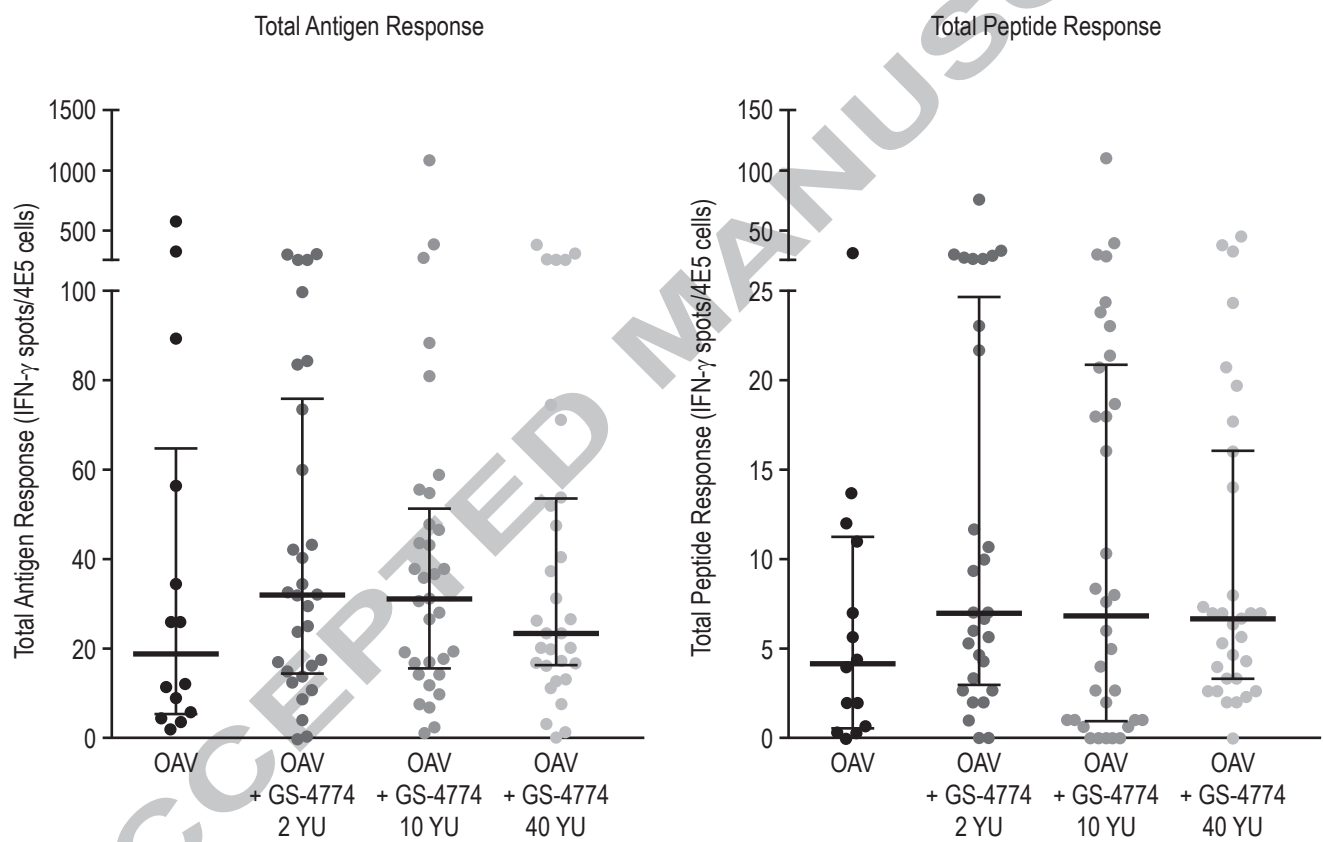


Figure 3



**Table 1. Baseline demographics and disease characteristics.**

	OAV	OAV + GS-	OAV + GS-	OAV + GS-	Total
	(n = 27)	2 YU (n = 50*)	10 YU (n = 51*)	40 YU (n = 50)	(N = 178)
Mean age (median, range), years	45 (48, 28–65)	50 (51, 25–64)	47 (47, 25–63)	47 (48, 23–65)	47 (48, 23–65)
Male, n (%)	20 (74)	34 (68)	37 (73)	31 (62)	122 (69)
Race, n (%)					
Asian	21 (78)	40 (80)	41 (80)	34 (68)	136 (76)
White	5 (19)	6 (12)	3 (6)	7 (14)	21 (12)
Black or African American	0 (0)	1 (2)	2 (4)	5 (10)	8 (4)
Other	1 (4)	3 (6)	5 (10)	4 (8)	13 (7)
Mean BMI (median, range), kg/m <sup>2</sup>	24.9 (24.4, 18.6– 32.9)	24.7 (24.8, 18.4– 32.7)	24.2 (24.3, 18.2– 33.3)	25.0 (24.2, 16.5– 35.9)	24.7 (24.6, 16.5– 35.9)
Mean HBV DNA (median, range), log <sub>10</sub> IU/ml	1.4 (1.4, 1.4–1.4)	1.5 (1.4, 1.4–1.6)	1.4 (1.4, 1.4–1.4)	1.5 (1.4, 1.4–2.0)	1.5 (1.4, 1.4–2.0)
Mean HBsAg (median, range), log <sub>10</sub> IU/ml	2.5 (3.0, –1.4 to 4.0)	3.1 (3.2, 1.6 to 4.5)	3.0 (3.2, –1.4 to 4.3)	3.0 (3.2, 0.1 to 4.4)	2.9 (3.1, –1.4 to 4.5)

HBsAg level $\leq 1000$	13 (48)	18 (36)	21 (41)	21 (42)	73 (41)
IU/ml, n (%)					
HBeAg-positive, n	7 (26)	13 (26)	12 (24)	12 (24)	44 (25)
(%)					
Mean ALT (median,	29.4	25.3	28.0	26.7	27.1
range), U/L	(24.0, 15.0–	(23.0, 8.0–	(25.0, 11.0–	(22.0, 10.0–	(24.0, 8.0–
	59.0)	57.0)	86.0)	64.0)	86.0)
Baseline OAV, n (%)					
Tenofovir	17 (63)	27 (54)	29 (57)	17 (34)	90 (51)
disoproxil					
fumarate					
Entecavir	9 (33)	22 (44)	18 (35)	28 (56)	77 (43)
Adefovir	0 (0)	0 (0)	1 (2)	1 (2)	2 (1)
dipivoxil					
Lamivudine	1 (4)	0 (0)	0 (0)	0 (0)	1 (1)
Telbivudine	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Combination	0 (0)	1 (2)	3 (6)	4 (8)	8 (4)
therapy					
Mean duration of	77.3	71.6	65.8	70.8	70.6
continuous	(72.2, 19.5–	(66.0, 17.0–	(61.6, 15.4–	(67.6, 12.8–	(66.4, 12.8–
OAV prior to	138.5)	211.7)	176.1)	152.2)	211.7)
randomization					
(median, range),					



months

Prior use of	5 (19)	5 (10)	9 (18)	6 (12)	25 (14)
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interferons to treat

HBV, n (%)

Mean duration of	18	16	16	14	16
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HBV-positivity	(14, 6–43)	(15, 2–53)	(11, 4–48)	(10, 2–65)	(12, 2–65)
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(median, range),

years

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\*One patient randomized to receive OAV plus GS-4774 2 YU actually received treatment with OAV plus GS-4774 10 YU. This patient was included in the OAV plus GS-4774 2 YU group for the efficacy analyses and the OAV plus GS-4774 10 YU for the baseline and safety analyses.

ALT, alanine aminotransferase; BMI, body mass index; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; OAV, oral antiviral.

**Table 2. Change from baseline in HBsAg ( $\log_{10}$  IU/ml).**

HBsAg, $\log_{10}$ IU/ml (95% CI)	OAV	OAV + GS-4774 2 YU	OAV + GS-4774 10 YU	OAV + GS-4774 40 YU
	(n = 27)	(n = 51*)	(n = 50*)	(n = 50)
Week 12	n = 21	n = 48	n = 49	n = 49
Mean change from baseline	-0.004 (-0.041 to 0.033)	-0.016 (-0.041 to 0.010)	-0.017 (-0.043 to 0.008)	-0.028 (-0.054 to -0.003)
Mean treatment difference <sup>†</sup>		-0.012 (-0.056 to 0.033)	-0.013 (-0.058 to 0.031)	-0.025 (-0.069 to 0.020)
<i>p</i> -value		0.61	0.56	0.28
Week 24	n = 21	n = 47	n = 48	n = 49
Mean change from baseline	-0.019 (-0.070 to 0.031)	-0.020 (-0.055 to 0.014)	-0.026 (-0.060 to 0.008)	-0.048 (-0.082 to -0.014)
Mean treatment difference <sup>†</sup>		-0.001 (-0.061 to	-0.007 (-0.067 to	-0.029 (-0.089 to 0.031)

		0.059)	0.053)	
<i>p</i> -value		0.98	0.83	0.34
Week 48	n=20	n=48	n=48	n=49
Mean change from baseline	-0.043 (-0.179 to 0.094)	-0.055 (-0.148 to 0.038)	-0.051 (-0.143 to 0.041)	-0.166 (-0.257 to -0.075)
Mean treatment difference <sup>†</sup>		-0.012 (-0.177 to 0.153)	-0.008 (-0.172 to 0.156)	-0.123 (-0.287 to 0.041)
<i>p</i> -value		0.89	0.92	0.14

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\*One patient randomized to receive OAV plus GS-4774 2 YU actually received treatment with OAV plus GS-4774 10 YU. This patient was included in the OAV plus GS-4774 2 YU group for the efficacy analyses and the OAV plus GS-4774 10 YU for the baseline and safety analyses.

<sup>†</sup>Treatment difference=GS-4774-containing group – OAV-only group.

CI, confidence interval; HBsAg, hepatitis B surface antigen; OAV, oral antiviral.

**Table 3. Treatment-emergent AE frequency and severity.**

		OAV + GS-	OAV + GS-	OAV + GS-
	OAV	4774	4774	4774
Patients, n (%)	(n = 27)	2 YU (n = 50*)	10 YU (n = 51*)	40 YU (n = 50)
Any treatment-emergent AE	10 (37)	41 (82)	48 (94)	46 (92)
AE related to GS-4774	N/A	29 (58)	41 (80)	38 (76)
Any-grade AE reported in ≥10% of patients in any group				
Injection site pain	0 (0)	19 (38)	34 (67)	39 (78)
Injection site erythema	0 (0)	18 (36)	29 (57)	36 (72)
Injection site induration	0 (0)	18 (36)	21 (41)	23 (46)
Injection site pruritus	0 (0)	13 (26)	16 (31)	26 (52)
Injection site swelling	0 (0)	12 (24)	17 (33)	25 (50)
Fatigue	0 (0)	8 (16)	13 (25)	13 (26)
Headache	1 (4)	9 (18)	7 (14)	8 (16)
Myalgia	0 (0)	4 (8)	7 (14)	13 (26)

Chills	0 (0)	2 (4)	5 (10)	7 (14)
Cough	0 (0)	5 (10)	6 (12)	3 (6)
Nasopharyngitis	0 (0)	5 (10)	8 (16)	1 (2)
Nausea	0 (0)	3 (6)	4 (8)	6 (12)
Upper respiratory tract infection	3 (11)	2 (4)	2 (4)	3 (6)
Grade 3 or 4 AE <sup>†</sup>	1 (4)	0 (0)	2 (4)	0 (0)
Serious AE	0 (0)	1 (2)	1 (2)	0 (0)
AE leading to permanent discontinuation, modification, or interruption of GS-4774	N/A	0 (0)	0 (0)	0 (0)
Death	0 (0)	0 (0)	0 (0)	0 (0)

\*One patient randomized to receive OAV plus GS-4774 2 YU actually received treatment with OAV plus GS-4774 10 YU. This patient was included in the OAV plus GS-4774 2 YU group for the efficacy analyses and the OAV plus GS-4774 10 YU for the baseline and safety analyses.

<sup>†</sup>Three patients reported four grade 3 AEs (influenza, diarrhea, fatigue, injection site pain). No grade 4 AE was reported.

AE, adverse event; N/A, not applicable.

**Table 4. ELISpot responder status.\***

		OAV + GS-	OAV + GS-	OAV + GS-
Patients, n/N (%)	OAV	4774	4774	4774
		2 YU	10 YU	40 YU
All patients				
HBcAg	5/14 (36)	15/29 (52)	20/34 (59)	18/31 (58)
HBcAg peptide pool	1/14 (7)	8/29 (28)	8/34 (24)	5/31 (16)
HBeAg-negative patients				
HBcAg	3/9 (33)	13/22 (59)	17/25 (68)	15/24 (63)
HBcAg peptide pool	1/9 (11)	8/22 (36)	6/25 (24)	5/24 (21)
HBeAg-positive patients				
HBcAg	2/5 (40)	2/7 (29)	3/9 (33)	3/7 (43)
HBcAg peptide pool	0/5 (0)	0/7 (0)	2/9 (22)	0/7 (0)

\*A positive response was defined as a spot count greater than the mean (plus 3 times the standard deviation) of the medium alone wells plus a spot count >10 spots/1E6 cells plus a spot count >2 times the mean of the medium control count.

HBcAg, hepatitis B core antigen; HBeAg, hepatitis B e antigen; OAV, oral antiviral; YU, yeast units.

