Research Article

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Viral Hepatitis

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

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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 $\geqslant$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 $\geqslant$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.

Clinical trial registration number: NCT01943799.

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].

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

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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.

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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 [18–23].

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 cervisiae 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^2 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 $\geqslant$1 year prior to screening and (ii) $\geqslant$2 HBV DNA values (measured $\geqslant$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 $\geqslant$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 material. 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 ($\leqslant$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 $\geqslant$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 $\geqslant$1.7 or >0.5 over baseline, decrease serum albumin >1 g/dl from baseline); or ALT $\geqslant$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 °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 4×10^5 -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/1E6 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 $\geqslant$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 fumurate [4].

The efficacy and safety analyses included all patients who had a baseline/day 1 visit (OAV-only group) or received $\geqslant$1 dose of GS-4774 (OAV plus GS-4774 groups). The primary efficacy endpoint was analyzed using a mixed-effects model