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Safety, tolerability and immunogenicity of GS-4774, a hepatitis B virus-specific therapeutic vaccine, in healthy subjects: A randomized study

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ABSTRACT

Background: GS-4774 is a recombinant, heat-killed, yeast-based immunotherapy engineered to express hepatitis B virus (HBV)-specific antigens. GS-4774 is being developed as a therapeutic vaccine for chronic HBV infection. The aim of this study was to assess the safety, tolerability and immunogenicity of GS-4774 in healthy subjects.
Design: This was a randomized, open-label, dose-ascending study. Subjects were allocated to one of three dose groups ($n = 20$ per group) to receive 10, 40 or 80 yeast units (YU; 1 YU = 10^7 yeast) of GS-4774 in two immunization regimens (five subcutaneous injections at weekly intervals with one monthly booster or three subcutaneous injections at monthly intervals). T-cell-mediated responses were determined by interferon (IFN)-γ enzyme-linked immunospot (ELISpot) assay and lymphocyte-proliferation assay (LPA).
Results: Adverse events were reported by 39 of 60 (65%) subjects; all were mild or moderate and none was serious. Adverse events occurred most frequently in the highest dose group, 80 YU, and the number of individual events was higher after weekly immunization than monthly. The most common adverse events were injection-site reactions. Most (88%) subjects responded to GS-4774 by at least one of the T-cell assays. Following immunization with GS-4774, IFN-γ-producing T-cells specific for HBV antigens were detectable in 30 (51%) subjects. The ELISpot response was observed at all doses, with the highest frequency of responders occurring at the highest dose (10 YU: 45%; 40 YU: 35%; 80 YU: 74%). Proliferative responses to HBV recombinant antigens were observed in 90% subjects; responses were mainly independent of GS-4774 dose and immunization regimen.
Conclusions: GS-4774 was safe and well-tolerated in healthy subjects with injection-site reactions being the most frequently reported adverse events. With both weekly and monthly regimens, GS-4774 provided HBV-specific immune responses at all doses evaluated. Further evaluation of GS-4774 is ongoing in patients with chronic HBV infection.
Clinical trial registry: Clinicaltrials.gov (NCT01779505)

1. Introduction

Viral clearance of acute HBV infection depends on a rigorous $CD4^+$ and $CD8^+$ T-cell-mediated response directed against HBV-specific antigens that includes production of interferon (IFN)-γ [1–4]. In patients with chronic HBV infection, T-cell responses and IFN-γ production are both severely impaired, contributing to the persistence of their HBV infection [1,3,4]. Currently available drugs are capable of controlling viremia but rarely eradicate the virus [5]. Therefore, to achieve a cure (defined as hepatitis B surface antigen [HBsAg] seroconversion), new therapies targeting HBV replication and the immune system are needed [5].

GS-4774 (formerly GI-13020) is being developed to elicit an HBV-specific T-cell immune response in patients with chronic HBV infection. GS-4774 consists of heat-inactivated yeast cells that express well-conserved regions of HBV proteins, namely HBsAg, hepatitis B core antigen (HBcAg) and hepatitis B X protein (HBx) expressed as a single fusion protein. The recombinant heat-killed

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whole yeast platform has been previously shown to elicit a significant T-cell response upon subcutaneous administration [6]. Preclinical experiments in mice showed that GS-4774 elicited T-cell responses specific to HBsAg, HBcAg, and HBx and stimulated HBV-specific $CD8^+$ T-cells [7]. In cells from patients with chronic HBV infection, GS-4774 induced IFN-γ-producing $CD4^+$ and $CD8^+$ T cells that, in some cases, showed marked levels of expression of the Lamp-1/CD107a marker of cytotoxic function [8]. These experiments suggested that GS-4774 had potential to elicit an antiviral immune response. The present work was a first-time-in-human clinical trial of GS-4774 in healthy subjects.

2. Methods

2.1. Subjects

Healthy subjects aged ≥18 years were eligible. Subjects were recruited using a database of healthy volunteers elicited using advertisements in the community. Before enrolment, subjects had to demonstrate negative immunoglobulin (Ig) E-mediated hypersensitivity to *Saccharomyces cerevisiae*. Detailed exclusion criteria are provided in Supplementary File 1. All patients were negative for HBV DNA and anti-HBc antibodies. Four subjects had low-level antibodies to HBsAg below the threshold for positivity.

All subjects provided informed consent prior to screening. Local Ethics Review Committees approved the study, which was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki.

2.2. Study design

Single-site, randomized, open-label, dose-ascending, multi-arm study conducted in the USA between January and July 2013. Subjects were allocated to one of three dose groups ($n = 20$ per group) to receive 10, 40 or 80 yeast units (YU) (1 YU = 10^7 yeast cells) of study treatment. Within each group, subjects were randomized into two dosing cohorts (each $n = 10$): Cohort A received five injections of study treatment at weekly intervals plus another dose on day 57; Cohort B received three injections of study treatment at monthly intervals; the post-treatment period was defined as the 4-week period following the last dose. Further details of the protocol are given in Supplementary File 1.

At the start of the study, the exclusion threshold for anti-HBsAg antibody levels was 8.4 IU/L. However, in February 2013, the threshold levels were reduced to <3.5 IU/L to exclude any subjects with even low levels of HBV immunity. Four subjects enrolled and dosed who had screening levels ≥3.5 but ≤8.4 IU/L were permitted to continue the study. These subjects all had values for anti-HBsAg that were below the threshold of having a positive anti-HBsAg test and were negative for anti-HBcAg and for HBV DNA.

2.3. Study treatment

GS-4774 (Supplementary Figure 1; Globeimmune, Louisville, CO, and Integrity Bio, Camarillo, CA) was administered by 25 Gauge 5/8′ needle.

2.4. Safety and tolerability assessments

Primary endpoints were: frequency of serious adverse events, discontinuations from treatment due to adverse events, abnormal common laboratory parameters, dose-limiting toxicities, and frequency and intensity of common adverse events. Safety was assessed by physical examination, vital signs measurements, electrocardiogram (ECG), clinical laboratory tests and adverse event and concomitant medications monitoring.

2.5. Immunogenicity assessments

Secondary endpoint was immunogenicity of different dosing regimens of GS-4774.

2.6. Samples for immune function assays

Blood samples were collected before study treatment administration at baseline (day 1 or screening), on days 15, 29, 36, and 57 of treatment and on day 28 of the post-treatment period. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll density gradient centrifugation and frozen in liquid nitrogen until analysis.

2.7. Interferon-γ enzyme-linked immunospot assay

Sterile 96-well plates (PVDF membranes, Millipore, Bedford, MA) were coated overnight at 4 °C with anti-human IFN-γ antibody (Thermo Scientific, Rockford, IL), then stimulants and PBMCs were added each in a volume of 100 μL. Thawed PBMCs (4×10^5 cells/well) were stimulated with: assay medium alone (serum-free medium, CTL-Test™ PLUS medium, Cellular Technology Ltd. [CTL], Shaker Heights, OH); HBV recombinant antigens namely HBsAg (Prospec-Tany Technogene, Ness Ziona, Israel), HBcAg (Fitzgerald Industries International, Acton, MA), and HBx (Prospec-Tany) (10 μg/mL each); pools of overlapping 15-mer HBV peptides (overlapping by nine amino acids) spanning the entire GS–4774 insert sequence (12.5 μg/mL each); pools of discrete peptides (8–17 amino acids in length) known to be HBV-specific T-cell epitopes (25 μg/mL); and single peptides also known to be HBV-specific T-cell epitopes (25 μg/mL) (Supplementary Tables 1 and 2). All HBV peptides were based on HBV Genotype D and produced by Mimotopes (Clayton, Australia) except for single peptides FLLTRILTI and FLPSDFFPSV (Peptide 2.0, Chantilly, VA).

Positive controls were phytohemagglutinin (PHA; Sigma–Aldrich, St. Louis, MO; 5 μg/mL) and a pool of known $CD8^+$ T-cell epitopes (2 μg/mL) from cytomegalovirus, Epstein–Barr virus, and influenza virus ([9]; EZBioLab, Carmel, IN). A pool of HIV peptides (Mimotopes; 25 μg/mL) was used as negative control (Supplementary Table 3). Cells were incubated with stimulants at 37 °C and 5% CO_2 for 24 h. Plates were washed and biotinylated anti-human IFN-γ antibody (Thermo Scientific) was added to each well. Plates were refrigerated overnight. Thereafter, plates were washed and streptavidin-HRP (BD Biosciences, San Jose, CA) was added to each well and incubated for 2 h. Plates were washed and air-dried, and the substrate 3-amino-9-ethyl carbazole was added. Numbers of IFN-γ-secreting cells ("spots") were measured by anti-IFN-γ capture antibody and adjusted for background (medium alone) and baseline response. Spots were counted by CTL ImmunoSpot® Analyzer (CTL); data were processed by SpotMap® software. An immune response was pre-specified by algorithms that evaluated T-cell IFN-γ responses in terms of breadth, duration, and magnitude. In addition, a response to any pool or antigen was required to be ≥2-fold over assay background and display at least a 2-fold increase from baseline (Supplementary Table 4).

2.8. Lymphocyte proliferation assay

Thawed PBMCs (2×10^5 cells/well) were incubated with HBsAg, HBcAg, and HBx (1 and 10 μg/mL each). *Candida albicans* extract (Greer Labs., Lenoir, NC; 20 μg/mL), tetanus toxoid (Colorado Serum Company, Denver, CO; 0.25 limes flocculation units/mL), and PHA (Roche Diagnostics, Indianapolis, IN, 5 or 12.5 μg/mL)

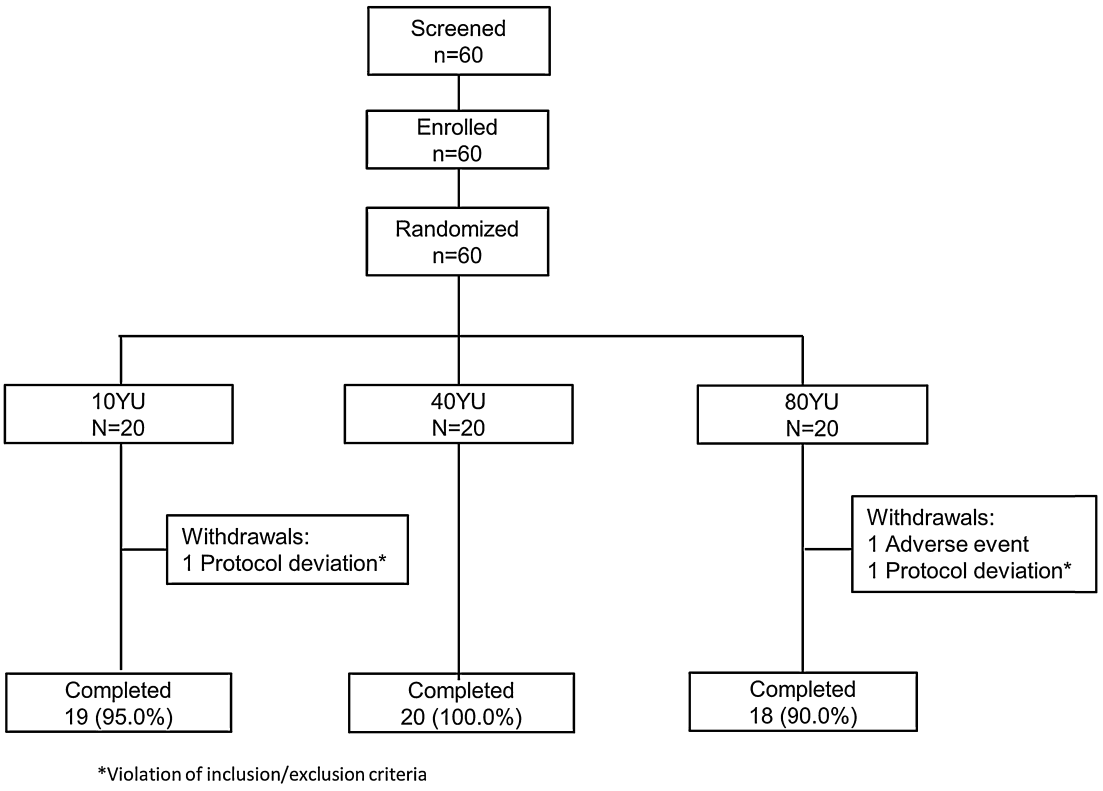

Fig. 1. CONSORT diagram describing subject flow through the study.

were used as positive controls. Assay medium was used as negative control. Cells were incubated with test antigens in a humidified incubator at 37 °C and 5% CO_2 for 6 days. Proliferation was measured by uptake of ^{3}H-thymidine (Packard Topcount NXT, Downers Grove, IL), which was added for the final 6 h of incubation, using a beta scintillation counter. PHA stimulation was measured after 3 days. The stimulation index (SI) for each antigen was calculated as the ratio of the median response in the presence and absence of antigen. A response was defined as SI ≥2 over baseline.

2.9. Anti-S. cerevisiae antibody detection

Serum was harvested from blood samples collected before study treatment administration on days 1 and 29, and on day 28 of the post-treatment period. Anti-*S. cerevisiae* antibody (ASCA) IgA and IgG levels were measured by Quanta Lite™ ELISA kits (INOVA Diagnostics, San Diego, CA). Both ASCA IgA and IgG are known to bind to a specific epitope present in the cell wall of *S. cerevisiae* [10,11]. An ASCA value ≥25 U on treatment after subtraction of baseline unit value was considered to be a positive response.

2.10. Anti-HBsAg and anti-HBcAg antibody detection

Serum was harvested from blood samples collected before study treatment administration at screening and on days 1, 15, 29, 57, and on day 28 of the post-treatment period; for subjects in Cohort A of each group, further samples were collected on days 8 and 22. Anti-HBsAg antibodies were measured by electrochemiluminescence assays (anti-HBS kits, Roche) and anti-HBcAg antibodies by qualitative chemiluminescent immunoassays (ADVIA Centaur HBc Total kits [Siemens Healthcare Diagnostics, Malvern, PA]).

2.11. Human leukocyte antigen typing

HLA typing was performed by DNA sequence-based methodology (Abbott Molecular, Abbott Park, IL) using buccal swabs obtained from subjects prior to dosing on day 1. The following exons were routinely sequenced: HLA-A, B, C: Exons 2, 3, 4; HLA-DRB1: Exon 2; HLA-DQB1: Exons 2, 3. Remaining ambiguities were resolved by application of "heterozygosity ambiguity resolution primers" (Abbott) or by PCR-SSP (Life Technologies, Carlsbad, CA).

2.12. Statistical analysis

No formal analysis was performed to determine sample size or to assess safety data. The IFN-γ ELISpot and LPA algorithms and response criteria together with ASCA response criteria were predefined. All randomized subjects who received at least one dose of study treatment were included in the safety analysis.

3. Results

3.1. Subjects

Sixty subjects were randomized of whom 57 completed the study (Fig. 1). Three subjects were discontinued because of an adverse event ($n = 1$) and protocol violation ($n = 2$). Demographic and baseline subject characteristics were similar for Cohorts A and B (Table 1).

3.2. Safety and tolerability

Thirty-nine (65%) subjects reported adverse events (Table 2); all were graded mild or moderate and none was serious. A full listing of moderate adverse events is shown in Supplementary Table 5.

Table 1
Demographic and baseline subject characteristics.

	GS-4774 dose					
	10 YU		40 YU		80 YU	
	Cohort A (Weekly) $N=10$	Cohort B (Monthly) $N=10$	Cohort A (Weekly) $N=10$	Cohort B (Monthly) $N=10$	Cohort A (Weekly) $N=10$	Cohort B (Monthly) $N=10$
Age in years, mean [range]	48.8 [31–69]	42.9 [27–65]	37.7 [30–50]	33.2 [22–54]	38.9 [28–57]	39.3 [22–50]
Male, *n* (%)	2 (20)	4 (40)	4 (40)	7 (70)	5 (50)	3 (30)
Weight in lbs, mean (SD)	172.6 (40.94)	164.9 (26.15)	181.2 (41.70)	165.6 (29.76)	178.9 (56.35)	170.2 (43.22)
Race, *n* (%)						
White	9 (90)	10 (100)	10 (100)	10 (100)	8 (80)	9 (90)
Black	1 (10)	0	0	0	0	1 (10)
Native American	0	0	0	0	2 (20)	0
Ethnicity, *n* (%)						
Hispanic/Latino	5 (50)	6 (60)	8 (80)	8 (80)	7 (70)	8 (80)
Other	5 (50)	4 (40)	2 (20)	2 (20)	3 (30)	2 (20)

SD, standard deviation.

One subject who received monthly injections of 80 YU GS-4774 was discontinued due to mild paresthesia, which resolved and was judged by the Investigator to be related to study treatment. The number of individual adverse events increased with dose and more adverse events were reported following weekly than monthly dosing. Most adverse events reported were judged related to study treatment by the Investigator; all of these were injection-site reactions except for one transient episode of headache in the 40 YU group and another of myalgia in the 80 YU dose group. Adverse events experienced by more than one subject in a single cohort are shown in Supplementary Table 6.

The most frequent adverse events were injection-site reactions, reported by 23 (38%) subjects (Table 2). Injection-site reactions were reported more frequently after weekly ($n=15$ subjects) than monthly dosing ($n=8$). All reactions resolved and were mild with the exception of two episodes of moderate injection-site pain reported by one subject in Cohort A 80 YU. Both episodes resolved without treatment and were judged to be related to study treatment. Two of the mild injection-site reactions (induration and pain) required treatment (acetaminophen and ice).

Four patients had Grade 3 decreases in hemoglobin (two in Cohort A 10 YU, one in Cohort B 40 YU, and one in Cohort B 80 YU). There were no other Grade ≥2 laboratory abnormalities. Only two laboratory abnormalities were reported as adverse events: decrease in absolute neutrophils and white blood cell counts by one subject in Cohort A 40 YU. Both events were mild and considered not related to study treatment. No clinically relevant changes were reported for vital signs or ECG.

3.3. Immunogenicity

Fifty-two (88%) subjects showed an immune response by at least one measure of T-cell immune function (Table 3).

3.4. IFN-γ ELISpot assay

The highest frequency of IFN-γ ELISpot responders occurred in the highest dose group: 9/20 (45%) subjects in the 10 YU group, 7/20 (35%) subjects in the 40 YU group, and 14/19 (74%) subjects in the 80 YU group (Table 3). The frequency of responders was similar between immunization cohorts across all dose groups, including the two highest dose groups. A total of 14/29 (48%) subjects responded in Cohort A (weekly dosing) and 16/30 (53%) subjects in Cohort B (monthly) (Table 3). At the lowest dose, however, the monthly immunization regimen generated a 2-fold higher frequency of ELISpot responders than the weekly regimen.

The kinetics of the emergence of the IFN-γ ELISpot response was dependent on dose and immunization regimen. While responses were seen as early as day 15, generally the highest responses occurred at later time-points (Fig. 2). For 80 YU, 7/14 (50%) of eventual responders exhibited IFN-γ responses by day 15 whereas for 10 and 40 YU there was a slower kinetics with only 1/9 (11%) and

Table 2
Adverse events including injection-site reactions reported by the subjects in each cohort.

	GS-4774 dose					
	10 YU		40 YU		80 YU	
	Cohort A (Weekly) $N=10$	Cohort B (Monthly) $N=10$	Cohort A (Weekly) $N=10$	Cohort B (Monthly) $N=10$	Cohort A (Weekly) $N=10$	Cohort B (Monthly) $N=10$
Patients with ≥1 AE, *n* (%)	7 (70)	4 (40)	9 (90)	6 (60)	7 (70)	6 (60)
Mild	5 (50)	4 (40)	8 (80)	5 (50)	6 (60)	5 (50)
Moderate	2 (20)	0	1 (10)	1 (10)	1 (10)	1 (10)
Patients with ≥1 SAE, *n* (%)	0	0	0	0	0	0
Number of AEs	19	10	70	19	101	93
Mild, *n* (% total)	17 (89)	10 (100)	69 (99)	18 (95)	99 (98)	91 (98)
Moderate, *n* (% total)	2 (11)	0	1 (1)	1 (5)	2 (2)	2 (2)
Severe, *n* (% total)	0	0	0	0	0	0
Injection-site reactions						
Subjects with reactions, *n* (% total)	1 (1)	1 (10)	8 (80)	2 (20)	6 (60)	5 (50)
Injection-site reactions, *n* (% total AEs)	4 (21)	1 (10)	51 (73)	3 (16)	76 (77)	67 (74)
Mild, *n* (% reactions)	4 (100)	1 (100)	51 (100)	3 (100)	74 (97)	67 (100)
Moderate, *n* (% reactions)	0	0	0	0	2 (3)	0

AE, adverse event; SAE, serious adverse event.

Table 3
Immunogenicity summary.

	GS-4774 dose					
	10 YU		40 YU		80 YU	
	Cohort A (Weekly) $N=10$	Cohort B (Monthly) $N=10$	Cohort A (Weekly) $N=10$	Cohort B (Monthly) $N=10$	Cohort A (Weekly) $N=10$	Cohort B (Monthly) $N=10$
ELISpot, n/N (%)	3/10 (30.0)	6/10 (60.0)	4/10 (40.0)	3/10 (30.0)	7/9 (77.8)[a]	7/10 (70.0)
LPA, n/N (%)[b]	6/8 (75.0)	4/5 (80.0)	9/9 (100.0)	9/9 (100.0)	9/9 (100.0)[a]	8/10 (80.0)
Total immune responders, n/N (%)[c]	8/10 (80.0)	7/10 (70.0)	10/10 (100.0)	9/10 (90.0)	9/9 (100.0)	9/10 (90.0)

n, number of responders; *N*, number of subjects analyzed.
[a] One subject terminated early and only received Day 1 dosing. This subject was excluded from ELISpot and LPA analysis.
[b] Some subjects had insufficient recovery of peripheral blood mononuclear cells for LPA analysis.
[c] Response in either ELISpot or LPA or both assays.

1/7 (14%), respectively, eventual responders exhibiting responses at this early timepoint. On day 15, there were also almost twice as many responders in Cohort B than in Cohort A across dose groups: 3/14 (21%) eventual responders in Cohort A versus 6/16 (38%) in Cohort B. The majority of ELISpot responders (18/30 [60%]) demonstrated responsiveness by the end of the study, 28 days following the final immunization.

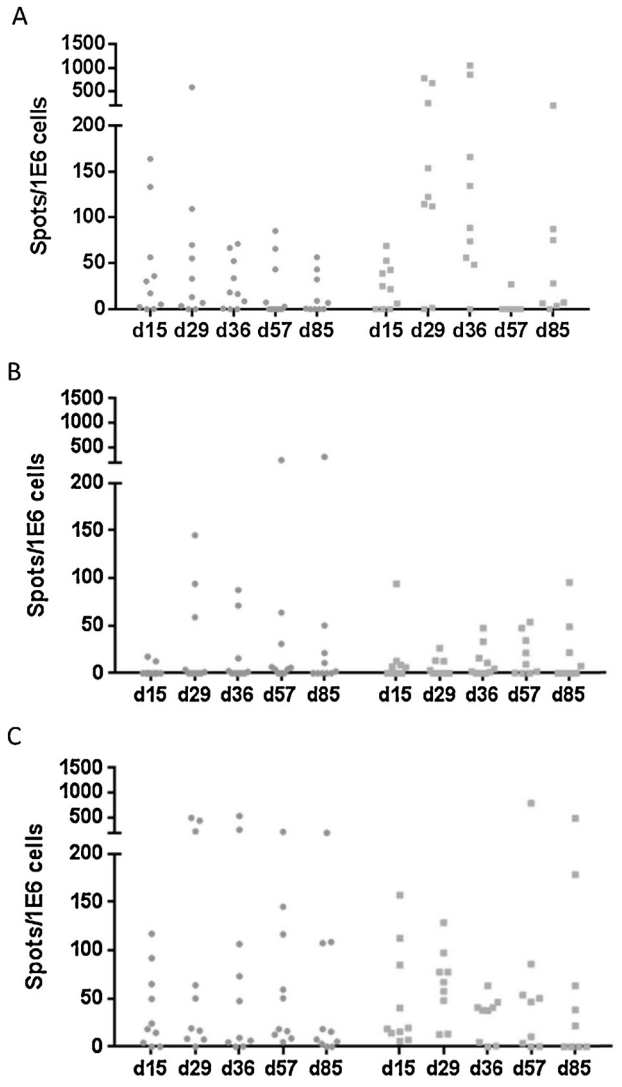

Fig. 2. Combined responses to HBsAg, HBcAg, and HBx as determined by IFN-γ ELISpot after weekly (circles) and monthly (squares) immunization of healthy subjects with 10 YU (A), 40 YU (B) and 80 YU (C) GS-4774. Responses are shown after background and baseline adjustment as described in the text.

In the 10 YU group, ELISpot responses were preferentially seen when PBMCs were stimulated with HBV recombinant antigens. In contrast, in the 40 and 80 YU groups, there was a 2-fold higher frequency of ELISpot responders to peptide pools. The production of IFN-γ in response to stimulation with HBV recombinant antigens was mainly directed to HBsAg and HBcAg (43% to HBsAg or HBcAg versus 20% to HBx). Across dose groups, the majority of ELISpot responses after stimulation of PBMCs with peptide pools were directed to overlapping pools of 15-residue peptides representing the HBV insert sequence.

3.5. Lymphocyte proliferation assay

Lymphocyte proliferation in response to HBV recombinant antigens was observed in most (90%) subjects. The number of LPA responders was slightly higher in the two highest dose groups (40 and 80 YU: 100% and 90%, respectively) compared with the lowest dose group (10 YU: 77%) (Table 3). The frequency and magnitude of LPA responses were similar regardless of the immunization regimen (Cohort A: 92%; Cohort B: 88%) (Fig. 3). In contrast to the ELISpot results, approximately 50% of subjects across dose groups displayed responses by day 15 (Fig. 3). However, the number of LPA responders was lowest at this time-point compared with later times.

All three HBV antigens were able to stimulate lymphocyte proliferation; HBcAg elicited the highest number of LPA responders across dose groups and HBx the lowest. The breadth of the response (defined as those displaying LPA responses to all three antigens) was similar for the 10 and 40 YU groups (50% and 56%, respectively) and higher than for the 80 YU group (29%). The magnitude of proliferation was similar across groups: 25 subjects had an SI > 5 and 12 subjects had an SI > 10 at any time-point compared with baseline. Proliferative responses of greatest magnitude (SI > 10) across dose groups were elicited by HBcAg.

3.6. Anti-S. cerevisiae antibody

The frequency of ASCA responders was low, although there were more responders in Cohort A (seven subjects, 12%) than Cohort B (one subject, 2%). There was also a slight trend toward higher IgA and IgG levels in Cohort A. The total number of responders (IgA plus IgG) was the highest in Cohort A 80 YU (five subjects, 8%). Generally, IgA and IgG levels were low at baseline with only six subjects showing a baseline response ≥25 U. These low levels were maintained during treatment. Seven of the eight ASCA responders were also defined as responders in the ELISpot. In addition, for 80 YU, all ASCA responders also displayed ELISpot and LPA responses.

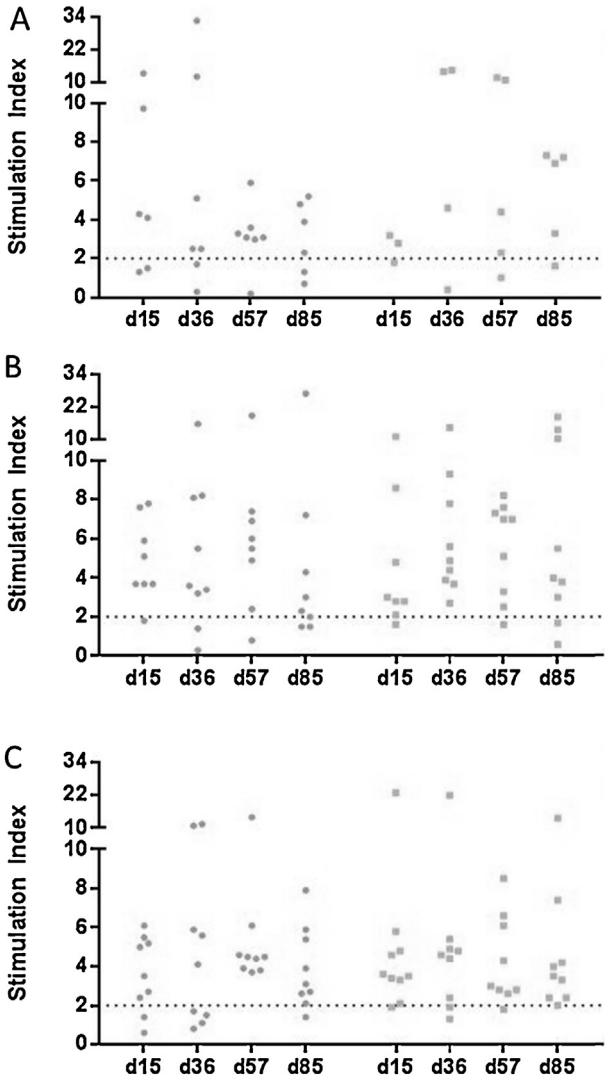

Fig. 3. Combined responses to HBsAg, HBcAg, and HBx as determined by LPA after weekly (circles) and monthly (squares) immunization of healthy subjects with 10 YU (A), 40 YU (B) and 80 YU (C) GS-4774. Dotted line: SI = 2, denotes threshold for response. SI was adjusted for background and baseline as described in the text.

3.7. Anti-HBcAg and anti-HBsAg

No anti-HBcAg antibodies were detected at any time during the study and no anti-HBsAg antibody levels >8.4 IU/mL were determined. Two subjects, both in Cohort A 80 YU, had anti-HBsAg levels ≥3.5 IU/L during the study.

3.8. HLA

HLA testing was performed to evaluate for any HLA restriction of immune responses to GS-4774. The most frequent HLA alleles were A*02, C*07, DQB1*03, and DRB1*04. No association was found between common HLA alleles and the IFN-γ ELISpot response to peptides or recombinant antigens (Supplementary Table 7).

4. Discussion

In the present study, GS-4774 was generally safe and well-tolerated. The most common adverse events were injection-site reactions. Adverse events occurred more frequently in both cohorts of the highest dose group, 80 YU, and the number of individual adverse events was higher after weekly than monthly immunization. Immunization with GS-4774 led to HBV antigen-specific and treatment-emergent T-cell responses. The majority of subjects showed a response when assessed by at least one of the assays. GS-4774 was immunogenic at all three doses tested and both immunization regimens, weekly and monthly dosing, induced T-cell-mediated immune responses. Immunogenicity was independent of HLA alleles.

LPA responses were observed in the majority of subjects with no increase in the frequency of responders related to dose or timing of dose. LPA responses were measured using recombinant HBV proteins which preferentially utilize an MHC Class II pathway resulting in a bias toward $CD4^+$ T-cell activation [12]. The responses, therefore, may represent early $CD4^+$ T-cell activation with GS-4774 in these subjects. The higher magnitude LPA responses with SI > 5, breadth of proliferative responses to recombinant antigens, and timing of response emergence suggested an increase in LPA responses from 10 to 40 YU doses but not from 40 to 80 YU.

IFN-γ ELISpot responses were seen in fewer subjects and at later time-points than LPA responses. This assay did not differentiate $CD4^+$ from $CD8^+$ T-cell responses, although as performed the assay biases toward $CD4^+$ T-cell responses. Because there were more ELISpot responses at later time-points, further protracting treatment may augment the $CD8^+$ T-cell response. IFN-γ ELISpot responses were comparable between all weekly and monthly regimens. Also, responses were similar in the monthly 10 and 80 YU dose groups, suggesting a dose-independent response on monthly regimens. The slightly lower ELISpot response rate in the 40 YU compared with 10 or 80 YU dose groups is puzzling but may be an artifact of sample variability or inter-subject differences. Our results show promise that immunization with GS-4774 may successfully clear viral loads in patients with chronic HBV infection, although the influence of altered immune function in these individuals on vaccine activity remains unknown in the absence of clinical trials.

Injection-site reactions after administration of an HBV vaccine are commonly reported in studies conducted in healthy subjects [13–15]. Based on its mechanism of action, GS-4774 is likely to interact with antigen-presenting cells in the subcutaneous layer of the skin and elicit a local immune response. Furthermore, the highest dose group required four injections per dose and this likely contributed to the increased number of injection site reactions in this group. Therefore, the injection-site reactions (i.e. local immune responses) observed in the present study were not unexpected and are similar to those seen in prior studies evaluating the yeast platform for vaccination [16–18].

Our safety and immunogenicity data provide the rationale for the selection of dose and immunization regimens in future studies with GS-4774. The safety analysis revealed a clear dose-dependent increase in the frequency of adverse events. Compared with monthly immunization, weekly immunization was associated with a higher incidence of adverse events, including injection-site reactions, and with increased ASCA responses. The impact of ASCA responses on the anti-HBV immune response to GS-4774 is not known and should be evaluated in longer dosing regimens with GS-4774. The LPA data indicated no apparent benefit in increasing the GS-4774 dose from 40 to 80 YU.

Prior attempts at therapeutic vaccines for chronic infection with HBV have mainly used recombinant proteins or peptides coupled with an adjuvant to induce a B-cell response and have largely been unsuccessful [19–21]. GS-4774 was developed to include more portions of the HBV genome than prior vaccine candidates and is developed with a platform that allows MHC Class I and Class II display of processed peptides. The ability to induce or augment the CD4+ and CD8+ T-cell responses to HBV may allow for stable control of HBV DNA within hepatocytes, resulting in no detectable

serum HBV proteins and DNA, allowing antiviral treatment to be discontinued. Confirmation of these concepts in patients with CHB will require further evaluation of GS-4774 in clinical trials.

In conclusion, GS-4774 was safe and well-tolerated in healthy subjects with injection-site reactions being the most frequently reported adverse events. GS-4774 was immunogenic and both weekly and monthly regimens led to rigorous immune responses at all doses evaluated. Further evaluation of GS-4774 is ongoing in patients with chronic HBV infection.

Author contribution statement

Claire Coeshott, David Apelian, and Timothy Rodell were involved in the conception and design of the study and on data acquisition, analysis, and interpretation. Anuj Gaggar, Gong Shen, G. Mani Subramanian, and John G. McHutchison participated in the analysis and interpretation of data. All authors critically reviewed draft versions of the manuscript and approved the final version.

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Conflict of interest statement: Anuj Gaggar, Gong Shen, Mani Subramanian and John McHutchison are Gilead Sciences, Inc. employees. Claire Coeshott, David Apelian and Timothy Rodell are employees of GlobeImmune, Inc., the company that developed GS-4774 before it was licensed by Gilead Sciences, Inc.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2014.07.027.

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