Original Investigation | Infectious Diseases

Immunogenicity and Safety of a 3-Antigen Hepatitis B Vaccine vs a Single-Antigen Hepatitis B Vaccine
A Phase 3 Randomized Clinical Trial

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Abstract

IMPORTANCE There is a need for improved immunogenicity of hepatitis B virus (HBV) vaccines among young adults with risk of infection.

OBJECTIVES To demonstrate manufacturing equivalence of a 3-antigen (3A) HBV vaccine, evaluate noninferiority of seroprotection rate (SPR) of 3A-HBV vs single-antigen (1A) HBV after 2 and 3 vaccine doses, and compare safety and reactogenicity between 3A-HBV and 1A-HBV vaccines.

DESIGN, SETTING, AND PARTICIPANTS This phase 3, double-blinded, randomized clinical trial included healthy adults aged 18 to 45 years randomized to 1 of three 3A-HBV groups or 1 control group receiving 1A-HBV. The trial was conducted at 37 community clinics and academic hospitals in Canada, Europe, the United Kingdom, and the United States between December 2017 and October 2019. Participants were followed up for 48 weeks after the first vaccination.

INTERVENTIONS Intramuscular administration of 3A-HBV (10 µg) or 1A-HBV (20 µg) on days 0, 28, and 168.

MAIN OUTCOMES AND MEASURES Geometric mean concentration (GMC) of serum hepatitis B surface antibodies (anti-HBs) and proportion of participants achieving seroprotection.

RESULTS Of 2838 participants, 1638 (57.8%) were women, 2595 (91.5%) were White, and 161 (5.7%) were Black or African American. A total of 712 participants (25.1%) were randomized to the 1A-HBV group and 2126 (74.9%) to 3A-HBV. The mean (SD) age at informed consent was 33.5 (8.0) years. The study demonstrated 3A-HBV lot-to-lot consistency, as the 2-sided 95% CIs for each pairwise comparison for the anti-HBs GMC ratios were within 0.67 and 1.50 (eg, adjusted GMC ratio, lot A vs lot B: 0.82; 95% CI, 0.67-1.00; lot A vs lot C: 0.95; 95% CI, 0.78-1.15; lot B vs lot C: 1.16; 95% CI, 0.95-1.41). The SPR of the pooled 3A-HBV was noninferior to 1A-HBV and higher than 1A-HBV after 2 vaccinations at day 168 (90.4% [95% CI, 89.0%-91.8%] vs 51.6% [95% CI, 47.5%-55.6%]) and 3 vaccinations at day 196 (99.3% [95% CI, 98.7%-99.6%] vs 94.8% [95% CI, 92.7%-96.4%]). The mean GMC of anti-HBs with 3A-HBV was 7.9 times higher after 2 vaccinations at day 168 and 3.5 times higher after 3 vaccinations at day 196 compared with 1A-HBV (after 2 vaccinations, 3A-HBV: GMC, 118.7 mIU/mL; 95% CI, 108.0-129.0 mIU/mL; SE, 1.0 mIU/mL; 1A-HBV: GMC, 15.0 mIU/mL; 95% CI, 12.9-17.5 mIU/mL; SE, 1.0 mIU/mL; after 3 vaccinations, 3A-HBV: GMC, 5442.4 mIU/mL; 95% CI, 4967.0-5963.0 mIU/mL; SE, 1.0 mIU/mL; 1A-HBV: 1567.2 mIU/mL; 95% CI, 1338.0-1834.0 mIU/mL; SE, 1.0 mIU/mL). Rates of local and systemic reactogenicities were higher with 3A-HBV compared with 1A-HBV (local: 1805 of 2124 [85.0%] vs 469 of 712 [65.9%]; systemic: 1445 [68.0%] vs 428

(continued)

Key Points

Question What is the immunogenicity and safety of a 3-antigen hepatitis B virus (HBV) vs a single-antigen HBV vaccine among young adults?

Findings This randomized clinical trial of 2838 participants found that the 3-antigen HBV vaccine was noninferior to the single-antigen HBV vaccine. The 3-antigen HBV vaccine had higher seroprotection rates after the second and third vaccinations than the single-antigen HBV vaccine.

Meaning In this study, rapid and consistently high rates of seroprotection were achieved with 2 and 3 doses of the 3-antigen HBV vaccine in young adults.

➕ **Visual Abstract**

➕ **Supplemental content**

Author affiliations and article information are listed at the end of this article.

Abstract (continued)

[60.1%]). Vaccine discontinuation due to adverse events (AE) was uncommon, and serious AEs were infrequent, reported in 42 participants (2.0%) and 3 participants (0.4%) in the 3A-HBV and 1A-HBV groups, respectively.

CONCLUSIONS AND RELEVANCE In this study, consistently higher antibody concentrations and SPRs were found with 3A-HBV after 2 and 3 doses vs 1A-HBV in adults aged 18 to 45 years old. The safety and efficacy of 3A-HBV shows its usefulness for the prevention of hepatitis B in young healthy adults.

TRIAL REGISTRATION Clinicaltrials.gov Identifier: NCT03408730; EU Clinical Trials Number: 2017-001820-22

Introduction

Vaccination rates against hepatitis B virus (HBV), a leading cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma, remain low in adults. Adults who were not immunized as infants remain at risk of HBV infection. Noncompletion of a 3-dose vaccine over 6 months is frequent,[1,2] and a recent study found that a 2-dose vaccine has better adherence than a 3-dose vaccine among US adults.[3] New HBV infections in the United States are the highest among those aged 30 to 49 years,[4] with 33.2% of those aged 25 to 39 years, 32.0% of those aged 45 to 54 years, and 27.6% of those aged 55 years and older in 2016.[5] Health care workers, the military, and travelers to endemic regions are most in need of an HBV vaccine that ensures rapid seroprotection.

Limitations with single-antigen (1A), yeast-derived HBV vaccines include prolonged time to achieve seroprotection, given that only 30% to 40% of adults are seroprotected after 2 doses.[6] At least 10% of all adults fail to achieve seroprotection after a 3-dose schedule[7] and are considered nonresponders to HBV vaccination. The proportion of adult nonresponders is higher in individuals 30 years or older, among whom there is a well-documented age-dependent decline in response rate to conventional single-antigen vaccines (1A-HBV) such as Engerix-B[8-12] with seroprotection rates (SPRs) in adults falling to less than 75% after age 40 years.[9,12,13] HBV vaccines that are more immunogenic than conventional vaccines and optimally designed to safely provide robust and rapid seroprotection are required.[14] Sci-B-Vac contains 3 HBV surface antigens, pre-S1, pre-S2, and S, unlike currently available HBV vaccines that only contain the small S antigen (HBsAg). The pivotal phase 3 study, PROTECT, showed that this 3-antigen HBV (3A-HBV) vaccine is highly immunogenic for adults, including older adults and those with well-controlled chronic conditions.[15] The 3A-HBV vaccine may provide more opportunities for the immune system to respond with antibodies to the virus, helping the host to overcome limitations of 1A-HBV.

Supported by clinical studies that reinforced its safety and efficacy in neonates, children, and adults, 3A-HBV received marketing authorization in Israel in 2000. In this study, we aimed to demonstrate the consistency of 3 consecutively manufactured lots of 3A-HBV in terms of antibody response 4 weeks after completion of the 3-dose regimen and the noninferiority of seroprotection achieved with 3A-HBV vs 1A-HBV to support regulatory approval of 3A-HBV in North America and Europe.

Methods

Study Design

The study design was a phase 3, double-blinded, randomized, multicenter, lot-to-lot consistency study with 3 parallel groups of 3A-HBV and a comparator group of 1A-HBV. All participants provided written informed consent. The study protocol, written informed consent, and other information requiring preapproval were reviewed and approved by regional or investigational center institutional review boards. The study was conducted in accordance with the Declaration of Helsinki[16] and the principles of Good Clinical Practice. The trial protocol and statistical analysis plan are provided in Supplement 1. This study followed the Consolidated Standards of Reporting Trials (CONSORT) reporting guideline.

Participants

Participants were aged 18 to 45 years at the time of the first vaccination and in stable health. A complete list of exclusion criteria is presented in eMethods 1 in Supplement 2. We collected data on race and ethnicity that are relevant for public health reasons to investigate immunogenicity in subgroups of interest using the categories used at ClinicalTrials.gov.

Intervention

The 3A-HBV vaccine contains a virus-like particle (VLP) formed by the full set of the 3 HBV envelope proteins or surface antigens (HBsAg), ie, S, pre-S1, and pre-S2, in their glycosylated and nonglycosylated forms, manufactured in Chinese hamster ovary (CHO) mammalian cells. Each 1-mL adult dose is formulated to contain 10 μg of pre-S1/pre-S2/S VLP adsorbed on aluminum hydroxide [Al(OH)3] as an adjuvant (aluminum content of 0.5 mg/mL). Preclinical and nonclinical data support critical roles for pre-S1 and pre-S2 domains in the pathogenesis of HBV infection and in the immunity against HBV,[13-15,17-19] which may account for the immunogenicity and enhanced overall antibody response observed with 3A-HBV. The comparator 1A-HBV was provided as 1-mL vials containing 20 μg of HBsAg-S adsorbed onto 0.5 mg of Al^{3+} as aluminum hydroxide adjuvant and was sourced commercially.

Study Periods and Randomization

Participants were followed up between December 2017 and September 2019 at 37 community and hospital sites in Finland, the United Kingdom, Belgium, Germany, Canada, and the United States. Participants were randomized (1:1:1:1) using an interactive web-based response system to receive 3 doses from 1 of the 3 independent consecutive lots (A, B, and C) of 3A-HBV or 1A-HBV. The randomization algorithm accounted for study center. All study personnel providing clinical assessments and participants were blind to the vaccine allocation. Study participants received a 1-mL dose of 3A-HBV or 1A-HBV by intramuscular injection on study days 0, 28, and 168.

Primary Outcome

The primary efficacy endpoint was the manufacturing equivalence of 3 independent consecutive 3A-HBV lots, in terms of immunogenicity. Immunogenicity was measured by the geometric mean concentration (GMC) of anti-HBs concentrations 4 weeks after the third injection (day 196).

Secondary Outcomes, Immunogenicity, and Safety Assessment

The secondary end points were (1) to demonstrate that the SPR of the 3-dose regimen of 3A-HBV (pooled) was noninferior to that of a 3-dose regimen of 1A-HBV, 4 weeks after the third injection (day 196) and (2) to evaluate the safety and reactogenicity of 3A-HBV compared with 1A-HBV. Exploratory end points are fully described in eMethods 2 in Supplement 2 and include GMC and SPRs after 2 or 3 vaccinations and the proportion of participants achieving anti-HBs concentrations of at least 100

mIU/mL. The detailed methods for immunogenicity and safety are provided in eMethods 3 in
Supplement 2.

Statistical Analysis

Adjusted estimates of GMCs and their associated 95% CIs were each determined using an analysis of
covariance model with a factor for vaccine lot and a covariate for the log-transformed prevaccination
(baseline) titer. The ratio of GMCs between each 3A-HBV vaccine lot group, including their associated
2-sided 95% CIs were calculated. If the upper and lower bound of the 2-sided 95% CI of the GMC of
anti-HBs ratios 4 weeks after the third vaccination for all 3 pairwise comparisons were between 0.67
and 1.50, lot-to-lot consistency (manufacturing equivalence) was demonstrated. Statistical analyses
were performed on the logarithmically (base 10) transformed values. Data from the three 3A-HBV
groups were combined to compute the 95% CIs for the difference in proportions (ie, SPR of 3A-HBV
minus SPR of 1A-HBV). To address the noninferiority to 1A-HBV, the lower bound of the 2-sided 95%
CI of the difference between the SPR for 3A-HBV and 1A-HBV (ie, SPR of 3A-HBV minus SPR of
1A-HBV) needed to be greater than −5%. Safety and reactogenicity of 3A-HBV compared with
1A-HBV were assessed in all participants who received at least 1 vaccine dose. Demographic
characteristics were summarized by group using descriptive statistics. The detailed methods are
provided in eMethods 4 in Supplement 2. All analyses were conducted in SAS version 9.3 (SAS
Institute).

Results

Of 2838 participants, 1638 (57.8%) were women. The mean (SD) age of participants was 33.5 (8.0)
years, and most participants were White (2596 [91.5%]; 161 [5.7%] Black or African American). The
median (IQR) body mass index (BMI; calculated as weight in kilograms divided by height in meters
squared) in the safety set was 25.4 (13.9-34.9), and 2332 participants (82.2%) had BMI of 30 or less.
Most participants (1748 [61.6%]) did not smoke, and 2645 (93.3%) consumed 0 to 1 alcoholic drinks
per day at baseline. Demographic and baseline characteristics were comparable between groups. Of
the 2836 participants in the safety set, most participants were enrolled in Europe and the United
Kingdom (1965 [69.3%]) followed by the United States (750 [26.4%]) and Canada (121 [4.3%]). A
complete summary of demographic characteristics is provided in **Table 1**.

The study was conducted between December 2017 and October 2019. A total of 2838 adults
were randomized: 712 participants (25.1%) to the 1A-HBV group and 2126 (74.9%) to 3A-HBV. There
were 2511 participants in per-protocol set 1 (ie, those who received all 3 vaccinations, had evaluable
serum immunogenicity samples at baseline and at the point of interest, were seronegative at
baseline, and had no major protocol deviations leading to exclusion) and 2381 in per-protocol set 2
(ie, those in per-protocol set 1, except participants who attended study visits 3 and 4 outside of the
defined windows). Vaccination compliance was assessed by number of vaccinations received. High
3-dose completion rates were observed across the vaccine groups. All but 2 participants received
their assigned vaccine (99.9%). Overall, 2638 (93.0%) received all 3 injections, 135 (4.8%) received
2, and 63 (2.2%) received 1 injection, and 2541 (89.5%) completed the study (**Figure 1**). Vaccine
exposure was similar across vaccine groups. Vaccine discontinuation due to nonserious AEs or SAEs
was uncommon, reported in 11 participants (0.5%) in the pooled 3A-HBV group and 2 participants
(0.3%) in the 1A-HBV group. Three participants (0.1%) receiving 3A-HBV had unsolicited AEs
assessed as vaccine related, resulting in vaccine discontinuation. These vaccine-related AEs included
osteoarthritis, dizziness, oropharyngeal pain, and injection site pain.

Lot-to-lot consistency based on immunogenicity was demonstrated, as the 2-sided 95% CIs of
the GMC ratios of anti-HBs concentrations 4 weeks after the third injection of 3A-HBV were within
the prespecified margin of 0.67 and 1.50 for all 3 pairwise comparisons (lot A vs lot B: 0.82; 95% CI,
0.67-1.00; lot A vs lot C: 0.95; 95% CI, 0.78-1.15; lot B vs lot C: 1.16; 95% CI, 0.95-1.41) (**Table 2**). The
difference in SPR and 2-sided 95% CIs between the pooled 3A-HBV (99.3%; 95% CI, 98.8%-99.6%)

and the 1A-HBV (94.8%; 95% CI, 92.7%-96.4%) was 4.5% (95% CI, 2.9%-6.6%). Since the lower bound of the 2-sided 95% CI of the difference in SPR was greater than the preset margin of −5%, noninferiority of 3A-HBV compared with 1A-HBV at study day 196 was demonstrated, and the secondary end point was met (**Table 3**). Markedly higher SPR was noted in the pooled 3A-HBV group as compared with the 1A-HBV group at study day 168 (90.4% [95% CI, 89.0%-91.8%] vs 51.6% [95% CI, 47.5%-55.6%]) (**Figure 2**A). In exploratory analysis, SPRs after 2 doses of 3A-HBV and 3 doses of 1A-HBV were compared. The SPR after 2 doses of 3A-HBV was 90.4% (95% CI, 89.0%-91.8%) and SPR after 3 doses of 1A-HBV was 94.8% (95% CI, 92.7%-96.4%) with a difference of −4.3% (95% CI,

Table 1. Demographic and Other Baseline Characteristics in the Safety Set

Characteristic	Participants, No. (%)					
	1A-HBV (n = 712)	3A-HBV				Total (N = 2836)
		Pooled (n = 2124)[a]	Lot A (n = 711)	Lot B (n = 708)	Lot C (n = 705)	
Gender						
Male	291 (40.9)	907 (42.7)	303 (42.6)	313 (44.2)	291 (41.3)	1198 (42.2)
Female	421 (59.1)	1217 (57.3)	408 (57.4)	395 (55.8)	414 (58.7)	1638 (57.8)
Race						
White	654 (91.9)	1941 (91.4)	650 (91.4)	641 (90.5)	650 (92.2)	2595 (91.5)
Asian	9 (1.3)	37 (1.7)	9 (1.3)	15 (2.1)	13 (1.8)	46 (1.6)
Black or African American	38 (5.3)	123 (5.8)	46 (6.5)	43 (6.1)	34 (4.8)	161 (5.7)
American Indian or Alaska Native	2 (0.3)	6 (0.3)	2 (0.3)	1 (0.1)	3 (0.4)	8 (0.3)
Other[b]	9 (1.3)	17 (0.8)	4 (0.6)	8 (1.1)	5 (0.7)	26 (0.9)
Ethnicity						
Hispanic or Latino	74 (10.4)	195 (9.2)	64 (9.0)	70 (9.9)	61 (8.7)	269 (9.5)
Non-Hispanic or Latino	636 (89.3)	1924 (90.6)	643 (90.4)	638 (90.1)	643 (91.2)	2560 (90.3)
Not collected per local guidelines	2 (0.3)	5 (0.2)	4 (0.6)	0	1 (0.1)	7 (0.2)
Age at informed consent, y						
Mean (SD)	33.4 (8.10)	33.5 (7.97)	33.8 (7.96)	32.9 (8.00)	33.9 (7.91)	33.5 (8.00)
Median (range)	35.0 (18-45)	35.0 (18-45)	36.0 (18-45)	34.0 (18-45)	36.0 (18-45)	35.0 (18-45)
Weight, kg[c]						
Mean (SD)	75.00 (14.389)	76.16 (14.942)	76.12 (15.102)	76.23 (14.765)	76.14 (14.978)	75.87 (14.812)
Median (range)	73.95 (42.4-119.4)	75.00 (32.2-135.0)	75.00 (42.0-135.0)	75.00 (45.6-125.0)	75.20 (32.2-126.1)	74.90 (32.2-135.0)
BMI[c]						
Mean (SD)	25.69 (4.103)	25.88 (4.118)	25.92 (4.215)	25.75 (3.968)	25.97 (4.170)	25.83 (4.114)
Median (range)	24.97 (16.3-34.9)	25.55 (13.9-34.9)	25.68 (16.1-34.9)	25.37 (16.3-34.9)	25.73 (13.9-34.9)	25.43 (13.9-34.9)
BMI category[c]						
≤30	595 (83.6)	1737 (81.8)	576 (81.0)	591 (83.5)	570 (80.9)	2332 (82.2)
>30	117 (16.4)	387 (18.2)	135 (19.0)	117 (16.5)	135 (19.1)	504 (17.8)
Smoking status and tobacco use						
Current use	136 (19.1)	406 (19.1)	139 (19.5)	142 (20.1)	125 (17.7)	542 (19.1)
Former use	141 (19.8)	404 (19.0)	137 (19.3)	131 (18.5)	136 (19.3)	545 (19.2)
No use	435 (61.1)	1313 (61.8)	435 (61.2)	435 (61.4)	443 (62.8)	1748 (61.6)
Average daily alcohol consumption, drinks/d						
0-1	653 (91.7)	1992 (93.8)	673 (94.7)	660 (93.2)	659 (93.5)	2645 (93.3)
2-3	54 (7.6)	120 (5.6)	32 (4.5)	45 (6.4)	43 (6.1)	174 (6.1)
≥4	5 (0.7)	12 (0.6)	6 (0.8)	3 (0.4)	3 (0.4)	17 (0.6)
Country/region						
United States	188 (26.4)	562 (26.5)	191 (26.9)	186 (26.3)	185 (26.2)	750 (26.4)
Canada	31 (4.4)	90 (4.2)	31 (4.4)	29 (4.1)	30 (4.3)	121 (4.3)
Europe and the UK	493 (69.2)	1472 (69.3)	489 (68.8)	493 (69.6)	490 (69.5)	1965 (69.3)

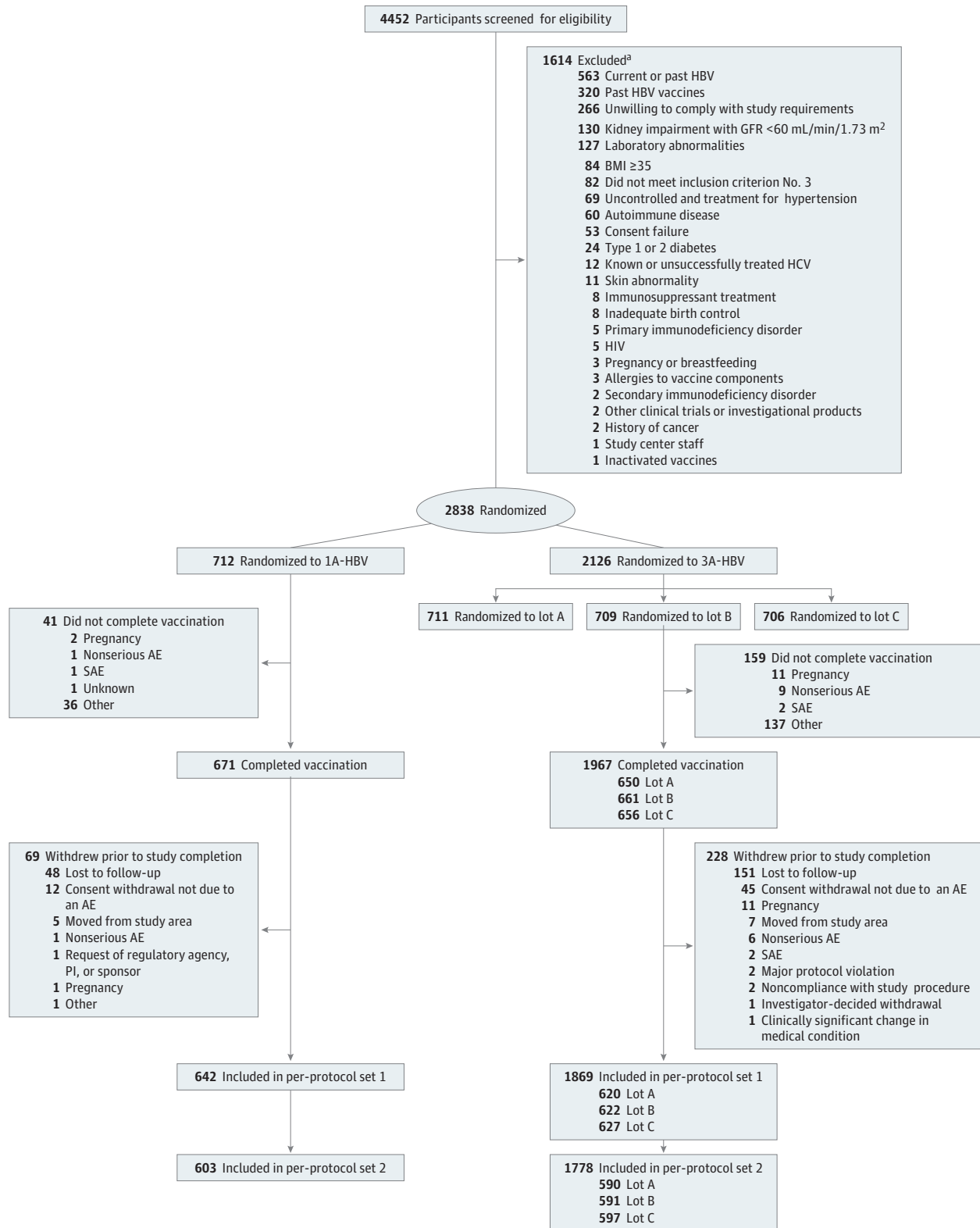Abbreviation: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared).

[a] Pooled 3A-HBV includes lots A, B, and C.

[b] Other race includes all racial groups not listed.

[c] Baseline for body weight and BMI was defined as the last measurement prior to the first vaccination.

Figure 1. Study Flowchart

4452 Participants screened for eligibility

1614 Excluded[a]
- **563** Current or past HBV
- **320** Past HBV vaccines
- **266** Unwilling to comply with study requirements
- **130** Kidney impairment with GFR <60 mL/min/1.73 m^2
- **127** Laboratory abnormalities
- **84** BMI ≥35
- **82** Did not meet inclusion criterion No. 3
- **69** Uncontrolled and treatment for hypertension
- **60** Autoimmune disease
- **53** Consent failure
- **24** Type 1 or 2 diabetes
- **12** Known or unsuccessfully treated HCV
- **11** Skin abnormality
- **8** Immunosuppressant treatment
- **8** Inadequate birth control
- **5** Primary immunodeficiency disorder
- **5** HIV
- **3** Pregnancy or breastfeeding
- **3** Allergies to vaccine components
- **2** Secondary immunodeficiency disorder
- **2** Other clinical trials or investigational products
- **2** History of cancer
- **1** Study center staff
- **1** Inactivated vaccines

2838 Randomized

712 Randomized to 1A-HBV

2126 Randomized to 3A-HBV

711 Randomized to lot A

709 Randomized to lot B

706 Randomized to lot C

41 Did not complete vaccination
- **2** Pregnancy
- **1** Nonserious AE
- **1** SAE
- **1** Unknown
- **36** Other

159 Did not complete vaccination
- **11** Pregnancy
- **9** Nonserious AE
- **2** SAE
- **137** Other

671 Completed vaccination

1967 Completed vaccination
- **650** Lot A
- **661** Lot B
- **656** Lot C

69 Withdrew prior to study completion
- **48** Lost to follow-up
- **12** Consent withdrawal not due to an AE
- **5** Moved from study area
- **1** Nonserious AE
- **1** Request of regulatory agency, PI, or sponsor
- **1** Pregnancy
- **1** Other

228 Withdrew prior to study completion
- **151** Lost to follow-up
- **45** Consent withdrawal not due to an AE
- **11** Pregnancy
- **7** Moved from study area
- **6** Nonserious AE
- **2** SAE
- **2** Major protocol violation
- **2** Noncompliance with study procedure
- **1** Investigator-decided withdrawal
- **1** Clinically significant change in medical condition

642 Included in per-protocol set 1

1869 Included in per-protocol set 1
- **620** Lot A
- **622** Lot B
- **627** Lot C

603 Included in per-protocol set 2

1778 Included in per-protocol set 2
- **590** Lot A
- **591** Lot B
- **597** Lot C

Per-protocol set 1 included those who received all 3 vaccinations, had evaluable serum immunogenicity samples at baseline and at the point of interest, were seronegative at baseline, and had no major protocol deviations leading to exclusion. Per-protocol set 2 included those in per-protocol set 1, except those who attended study visits 3 and 4 outside of the defined windows. 1A-HBV indicates single-antigen hepatitis B virus vaccine; 3A-HBV, 3-antigen HBV; AE, adverse event; and SAE, serious AE.

[a] Individuals may have multiple reasons for exclusion.

−6.5% to −1.9%); the lower limit of the 95% CI was greater than −10%. At each time point, the proportion of participants who achieved anti-HBs concentrations of at least 100 mIU/mL was also higher in the pooled 3A-HBV group vs the 1A-HBV group (981 of 1775 [55.3%] vs 100 of 603 [16.6%] at study day 168, 1679 of 1753 [95.8%] vs 511 of 592 [86.3%] at study day 196, and 1592 of 1718 [92.7%] vs 429 of 580 [74.0%] at study day 336). At study day 196, the proportion of nonresponders after 3 doses of vaccine was 7 times higher with 1A-HBV (31 of 592 [5.2%]) compared with 3A-HBV (13 of 1753 [0.7%]) with a difference of −4.5% (95% CI, −6.6% to −2.9%).

Anti-HBs concentrations increased markedly between the second and third vaccinations with both 3A-HBV lots and 1A-HBV (Figure 2B). Mean GMC of anti-HBs at study day 168 was 118.8 mIU/mL [95% CI, 108.0-129.0 mIU/mL; SE, 1.0 mIU/mL] in the 3A-HBV group and 15.1 mIU/mL [95% CI, 13.0-17.5 mIU/mL; SE, 1.1 mIU/mL] in the 1A-HBV group. GMC peaked at study day 196, 4 weeks after the third vaccination, with 3.5 times higher mean GMC in the 3A-HBV group (5442.4 mIU/mL; 95% CI, 4967.0-5963.0 mIU/mL; SE, 1.1 mIU/mL) compared with the 1A-HBV group (1567.2 mIU/mL; 95% CI, 1338.0-1834.0 mIU/mL; SE, 1.1 mIU/mL) (eTable 1 in Supplement 2). The GMC ratio (ie, 3A-HBV divided by 1A-HBV) and corresponding 95% CI, based on the adjusted GMC was 7.9 (95% CI, 6.6-9.4), 3.5 (95% CI, 2.9-4.4), and 4.4 (95% CI, 3.6-5.4) for study days 168, 196 and 336, respectively.

Incidence of solicited local AEs (pain, tenderness, pruritus, erythema, swelling) within 7 days of any vaccination was higher with 3A-HBV vs 1A-HBV. The difference was largely attributable to a higher frequency of injection site pain and tenderness with 3A-HBV than with 1A-HBV (eTable 2 in Supplement 2), which was mostly of mild or moderate severity and short duration; median duration of local symptoms ranged between 1 and 2 days. Solicited systemic AEs were reported in 1445 participants (68.0%) in the 3A-HBV group and 428 participants (60.1%) in the 1A-HBV group within 7 days of any injection (eTable 3 in Supplement 2). The median duration of systemic symptoms was 2 days or less. Overall, 186 participants (8.8%) in the pooled 3A-HBV group and 54 participants

Table 2. GMC of Hepatitis B Surface Antibodies at Day 196 for Lot-to-Lot Consistency in the Per-Protocol Set 1

| Statistic | 3A-HBV | | |
	Lot A (n = 620)	Lot B (n = 622)	Lot C (n = 627)
Participants evaluated, No.	611	610	619
GMC, mean (SD)[a]	5883.9 (5.4)	4824.1 (6.3)	5506.0 (6.0)
GMC, median (range)	12 200.0 (2.1-20 000.0)	10 700.0 (2.1-20 000.0)	12 000.0 (2.1-20 000.0)
Mean adjusted GMC (SE) [95% CI][b]	5882.3 (1.1) [5112.4-6768.0]	4821.7 (1.1) [4190.1-5548.4]	5570.0 (1.1) [4844.63-6403.7]
Adjusted GMC ratio (95% CI)[b]			
Lot A vs lot B	0.82 (0.67-1.00)		
Lot A vs lot C	0.95 (0.78-1.15)		
Lot B vs lot C	1.16 (0.95-1.41)		

Abbreviation: GMC, geometric mean concentration.

[a] The mean and SD are based on $\log_{10}$-transformed data, then transformed back to hepatitis B surface antibody concentrations.

[b] Adjusted GMC, GMC ratio, and corresponding 95% CI were analyzed using analysis of covariance with a factor for vaccine lot group, and a covariate for the log transformed prevaccination (baseline) titer.

Table 3. Analysis of SPR 4 Weeks After the Third Injection at Study Day 196[a]

Parameter	1A-HBV (n = 603)	Pooled 3A-HBV (n = 1778)
Participants evaluated, No.	592	1753
Participants who achieved seroprotection, No.	561	1740
SPR, % (95% CI)[b]	94.8 (92.7-96.4)	99.3 (98.7-99.6)
Estimated difference in SPR (95% CI)[c]	4.5 (2.9-6.6)	

Abbreviation: SPR, seroprotection rate.

[a] Seroprotection was defined as a hepatitis B surface antibody concentration of at least 10 mIU/mL in serum.

[b] Exact (Clopper-Pearson) 2-sided CI based on the observed proportion of participants.

[c] The estimated difference in proportions and 2-sided 95% CIs were calculated using the Miettinen and Nurminen method.

(7.6%) in the 1A-HBV group experienced solicited AEs that continued beyond day 7. Overall, rates of local and systemic reactogenicities were higher with 3A-HBV compared with 1A-HBV (local: 1805 of 2124 [85.0%] vs 469 of 712 [65.9%]; systemic: 1445 [68.0%] vs 428 [60.1%]). The incidence of solicited local and systemic AEs did not increase with successive injections. The proportion of participants reporting unsolicited AEs within 28 days following any injection was similar in the 3A-HBV and 1A-HBV groups (eTable 4 in Supplement 2).

During the study, 51 SAEs were reported by 45 participants. There were 47 events among 42 participants in the 3A-HBV group (2.0%) and 4 events among 3 participants in the 1A-HBV group (0.4%). One fatal SAE (sudden cardiac death) was reported 7 days after the first dose (3A-HBV group) in a participant with a history of open-heart surgery and biventricular hypertrophy. The investigator assessed the event as unrelated to vaccination. There were no vaccine-related SAEs during the study (eTable 5 in Supplement 2). After the database lock, an SAE of mild congenital ankyloglossia (tongue-tie) was reported in an offspring of a participant who received 3A-HBV that was possibly related to study vaccine.

Discussion

This trial found that 3A-HBV can consistently induce a robust immune response across vaccine lots and was immunologically noninferior to 1A-HBV in healthy adults aged 18 to 45 years following 3 doses of 3A-HBV, meeting both the primary and secondary immunogenicity end points. Additionally, 3A-HBV was found to be highly immunogenic in young healthy adults, with higher SPRs after both 2 and 3 doses compared with 1A-HBV. The high SPRs reported in this study are consistent with previous reports of 3A-HBV in young adults, which have reported SPRs greater than 98% following a 3-dose regimen, with higher SPR noted after the first and second doses compared with conventional yeast-derived HBV vaccines.[17-19] The rapid induction of protective antibody levels in more than 90% of participants after 2 doses of 3A-HBV in the current study is noteworthy, particularly for populations in whom rapid seroprotection is required. Vaccination rates against HBV are generally low, particularly with a 3-dose schedule[2] and among individuals with low socioeconomic status, incarcerated individuals, and those with drug use disorders.[20] Even among travelers who are offered

Figure 2. Seroprotection Rates and Serum Hepatitis B Surface Antibody (Anti-HB) Concentrations in Pooled 3-Antigen Hepatitis B Virus Vaccine (3A-HBV) and Single-Antigen (1A-HBV) Groups
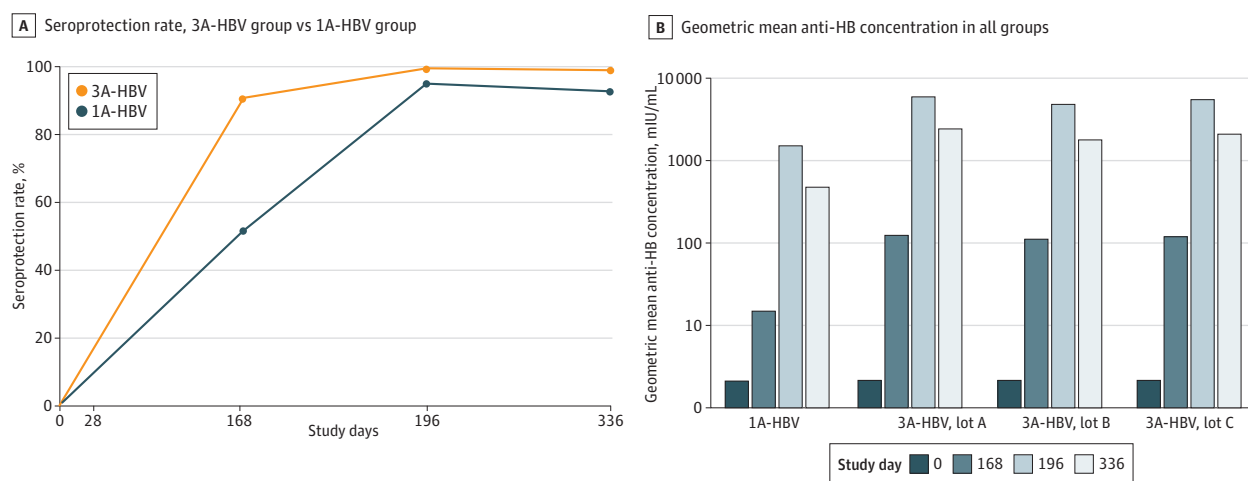
A, Comparison of seroprotection rate in the pooled 3A-HBV group to 1A-HBV group in the per-protocol set 2, at study days 168, 196, and 336. B, The antibody response, as measured by geometric mean concentration of anti-HBs, at study day 168 (ie, 20 weeks following the second vaccination and prior to the third vaccination), study day 196 (4 weeks after the third vaccination), and study day 336 as well as adjusted estimates of geometric mean concentrations by vaccine group and visit in per-protocol set 2. There were no meaningful differences in GMCs at study days 168, 196, and 336 across the three lots of 3A-HBV.

a 2-visit vaccination schedule that consists of a double-dose HBV vaccine at day 0 followed by a single dose in 4 to 12 months, most participants did not return to complete their vaccinations, and therefore, limited data exist on whether they were protected against HBV during their trip.[21] Nevertheless, our results indicate that there is high seroprotection after 2 doses of 3A-HBV, which will protect against HBV infection in young adults as old as 45 years.

The 3A-HBV vaccine was able to rapidly elicit higher anti-HBs titers, which were more than 7.5 greater after 2 doses and almost 3.5 times greater after the third dose compared with 1A-HBV. This robust antibody response might obviate revaccination due to persistence and durability of seroprotection, as demonstration of a titer of at least 10 mIU/mL is required in the health care setting and in first responders. An expected 5% of the participants receiving the 1A-HBV vaccine were nonresponders, compared with 0.7% of the participants receiving the 3A-HBV vaccine, providing evidence for immunogenicity of pre-S1 and pre-S2. T-helper epitopes in the pre–S1/S2 domains overcome genetic nonresponsiveness to induce antibodies to S.[22,23] Also, 3A-HBV is produced in mammalian CHO cells, which are used extensively in safe human biologics production[24]; and unlike yeast-derived 1A-HBV, 3A-HBV has a mammalian protein folding and glycosylation pattern that enhances vaccine immunogenicity.[25]

The SPRs reported for 3A-HBV following a 3-dose regimen in this study (99.3%) are slightly higher than those reported in PROTECT (91.4%),[15] which enrolled individuals aged 18 to 90 years in stable health, including those with well-controlled chronic conditions. Of note, the SPR in age subgroup of those aged 18 to 44 years in PROTECT (99.2%)[15] was almost identical to overall SPR of the pooled 3A-HBV in this study's participants, who were aged 18 to 45 years (99.4%). Similar to this study, higher SPR of 3A-HBV compared with 1A-HBV was noted at each postvaccination point in PROTECT. The peak mean anti-HBs GMCs were orders of magnitude higher than the levels required for seroprotection in both studies,[15] although the concentrations achieved in this trial were somewhat higher, given that the study population was younger. Importantly, a preplanned exploratory analysis adopting a statistical margin of noninferiority for vaccine studies[26] between 2 doses of 3A-HBV and 3 doses of 1A-HBV demonstrated the ability of 3A-HBV to induce more rapid seroprotection compared with 1A-HBV in healthy individuals.

The higher reactogenicity of 3A-HBV noted in this study, which was mostly of mild or moderate severity and short duration, is consistent with the safety profile known from previous clinical trials of 3A-HBV[15,18,19,27] and postmarketing experience. Completion of the 3-dose schedule for 3A-HBV was high (93.0%), and study discontinuation due to SAEs or AEs was rare (0.4%). Although the frequency of SAEs was higher in the 3A-HBV group than the 1A-HBV group, there were no unusual patterns or clustering of SAEs by type, frequency, or timing with respect to vaccination, and there were no vaccine-related SAEs during the study.

The strengths of our study are that the humoral response to 3A-HBV was measured using highly reproducible and well-established methods to demonstrate consistency of immunogenicity across consecutively manufactured vaccine lots. Second, the study was well powered, and the validity of our findings was reinforced by randomization to study center and to the vaccine lots, and the addition of a comparative arm (1A-HBV) to assess immunogenicity and safety.

The 3A-HBV vaccine is a recombinant, 3-antigen vaccine that has shown, in clinical trials, to induce high antibody concentrations resulting in high SPRs[15,18,19,28] against HBV, which can cause a lifelong chronic infection with a high risk of liver fibrosis, cirrhosis, and hepatocellular carcinoma if left untreated. The 3A-HBV vaccine has been shown to achieve high SPRs and induce anti-HBs concentrations across diverse healthy adult populations in Asia, Europe, and North America[15,17,19] and also in key subgroups of older adults with poor or delayed responses to standard-of-care HBV vaccines.[15]

Limitations

This study has limitations. A limitation of the study was the use of seroprotection, defined as attaining an anti-HBs concentration of at least 10 mIU/mL as the immunological surrogate of clinical protection against HBV infection, although it is a widely accepted correlate of immune protection.[29]

Conclusions

This study demonstrated robust, consistent, and strong humoral response induced after 2 and 3 doses of 3A-HBV, thus establishing consistency of the 3A-HBV lots tested. We also demonstrated noninferiority based on the SPR of 3A-HBV compared with 1A-HBV 4 weeks after the third dose. The 3A-HBV vaccine was highly immunogenic in young healthy adults, with higher SPRs after 2 and 3 doses compared with 1A-HBV. The rapid induction of protective antibody levels in more than 90% of participants after 2 doses of 3A-HBV and prior to the third vaccination was a significant finding. The good safety profile of 3A-HBV supports its use in young adults and those at risk of infection who may require accelerated seroprotection.

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SUPPLEMENT 1.
Trial Protocol and Statistical Analysis Plan

SUPPLEMENT 2.
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SUPPLEMENT 3.
Nonauthor Collaborators

SUPPLEMENT 4.
Data Sharing Statement