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

Timo Vesikari, MD; Adam Finn, MD; Pierre van Damme, MD, PhD; Isabel Leroux-Roels, MD; Geert Leroux-Roels, MD, PhD; Nathan Segall, MD; Azhar Toma, MD; Gerald Vallieres, MD; Ronnie Aronson, MD; Dennis Reich, MD; Samir Arora, MD; Peter J. Ruane, MD; Clancy L. Cone, MD; Michael Manns, MD; Catherine Cosgrove, MD; Saul N. Faust, MD; Maheshi N. Ramasamy, MD; Nathalie Machluf, PhD; Johanna N. Spaans, MSc; Bebi Yassin-Rajkumar, MSc; David Anderson, PhD; Vlad Popovic, MD; Francisco Diaz-Mitoma, MD, PhD; for the CONSTANT Study Group

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 3 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 81.6% [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 [56.0%].

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.
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

JAMA Network Open. 2021;4(10):e2128652. doi:10.1001/jamanetworkopen.2021.28652

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 schedule7 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-B8-12 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 Methods 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 Methods 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 Methods 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 2A). 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 Pooled (n = 2124)* | Lot A (n = 711) | Lot B (n = 708) | Lot C (n = 705) | Total (N = 2836) |
|---------------------------------------------------------------|
| 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 +                                                  | 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                                              |
| 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, kg                                                 |
| 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                                            |
| ≤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).

* Pooled 3A-HBV includes lots A, B, and C.

+ Other race includes all racial groups not listed.

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

JAMA Network Open. 2021;4(10):e2128652. doi:10.1001/jamanetworkopen.2021.28652

October 12, 2021 5/13

Downloaded From: https://jamanetwork.com/ on 01/14/2022
1614 Excluded
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 m²
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
5 HIV
4 Inactivated vaccines
2 Secondary immunodeficiency disorder
2 Other clinical trials or investigational products
2 History of cancer
1 Study center staff
1 Inactivated vaccines

Figure 1. Study Flowchart

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.

* 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, 11 mIU/mL) compared with the 1A-HBV group (1567.2 mIU/mL; 95% CI, 1338.0-1834.0 mIU/mL; SE, 11 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...
(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.\textsuperscript{17,18} 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\textsuperscript{2} and among individuals with low socioeconomic status, incarcerated individuals, and those with drug use disorders.\textsuperscript{20} Even among travelers who are offered...
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. 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. Also, 3A-HBV is produced in mammalian CHO cells, which are used extensively in safe human biologics production; and unlike yeast-derived 1A-HBV, 3A-HBV has a mammalian protein folding and glycosylation pattern that enhances vaccine immunogenicity.

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%), 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%) 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, 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 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 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 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 and also in key subgroups of older adults with poor or delayed responses to standard-of-care HBV vaccines.
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.
Supervision: Finn, van Damme, I. Leroux-Roels, Reich, Arora, Cosgrove, Faust, Ramasamy, Anderson, Popovic, Diaz-Mitoma.

**Conflict of Interest Disclosures:** Dr Vesikari reported being the majority shareholder of Nordic Research Network Oy. Dr Finn reported receiving grants from VBIVaccines during the conduct of the study; receiving grants from Pfizer, Sanofi, GlaxoSmithKline, AstraZeneca, and Valneva outside the submitted work; being a member of the UK NITAG (Joint Committee for Vaccination and Immunisation); and serving as chair of the World Health Organization Euro Technical Advisory Group of Experts on Immunisation. Dr van Damme reported that the University of Antwerp received grants from GlaxoSmithKline, Sanofi, Janssen Vaccines, Curevac, Merck, and Merck Sharp & Dohme for the conduct of vaccine trials and grants from PATH, the Bill & Melinda Gates Foundation, the Belgian Centre for Expertise, and the Flemish Research Fund for the conduct of research and vaccine trials. Dr Aronson reported receiving grants from VBIVaccines during the conduct of the study; receiving personal fees from Sanofi, Eli Lilly and Co, Novo Nordisk, Boehringer Ingelheim, HTL Strefa, Gilead, BD Technologies, Takeda, and Merck and receiving grants from Xeris, Medpace, Kowa, and Zealand outside the submitted work. Dr Manns reported receiving consulting fees from Roche, Bristol Myers Squibb, Gilead, Enyo Pharma, and Curevac and receiving lecture honoraria and travel support from Roche, Bristol Myers Squibb, and Gilead outside the submitted work. Dr Faust reported receiving grants from VBIVaccines during the conduct of the study; serving on the advisory boards of Medimmune, Sanofi, Pfizer, Seqirus, Sandoz, and Merck; and receiving grants Pfizer, Sanofi, GlaxoSmithKline, Johnson & Johnson, Merck, AstraZeneca, and Valneva outside the submitted work. Dr Ramasamy reported receiving grants from VBIVaccines during the conduct of the study. Dr Machluf reported being an employee of VBIVaccines during the conduct and outside the submitted work. Ms Spans reported being employee of VBIVaccines during the conduct of the study. Dr Anderson reported receiving personal fees from VBIVaccines during the conduct of the study and outside the submitted work. Dr Popovic reported receiving personal fees from VBIVaccines during the conduct of the study and outside the submitted work. Dr Diaz-Mitoma reported receiving personal fees from VBIVaccines during the conduct of the study; owning shares in VBIVaccines outside the submitted work; and being the cited inventor of patents owned by VBIVaccines. No other disclosures were reported.

**Funding/Support:** Funding for this research was provided by VBIVaccines Inc.

**Role of the Funder/Sponsor:** Ms Spans, Dr Machluf, Ms Yassin-Rajkumar, and Drs Anderson, Popovic, and Diaz-Mitoma are employees of the funding source and played a role in the design and conduct of the study; interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

**Group Members:** See Supplement 3.

**Data Sharing Statement:** See Supplement 4.

**Additional Contributions:** Study authors graciously acknowledge the contribution of participants and clinical, regulatory, and research staff at VBIVaccines Inc.

**REFERENCES**

1. Ghaswala PK, Patterson BJ, Cheng WY, Duchesneau E, Macheca M, Duh MS. Hepatitis A, B, and A/B vaccination series completion among US adults: a claims-based analysis. *Hum Vaccin Immunother*. 2018;14(11):2780-2785. doi:10.1080/21645515.2018.1489189

2. Hechter RC, Qian L, Luo Y, et al. Impact of an electronic medical record reminder on hepatitis B vaccine initiation and completion rates among insured adults with diabetes mellitus. Vaccine. 2019;37(1):195-201. doi:10.1016/j.vaccine.2018.06.035

3. Bruxvoort K, Sleazak J, Huang R, et al. Association of number of doses with hepatitis B vaccine series completion in US adults. *JAMA Netw Open*. 2020;3(11):e2027577. doi:10.1001/jamanetworkopen.2020.27577

4. US Department of Health and Human Services. Hepatitis B basic information. Reviewed August 31, 2021. Accessed April 5, 2020. [www.hhs.gov/hepatitis/learn-about-viral-hepatitis/hepatitis-b-basics/index.html](http://www.hhs.gov/hepatitis/learn-about-viral-hepatitis/hepatitis-b-basics/index.html)

5. US Centers for Disease Control and Prevention. Viral hepatitis surveillance: United States, 2016. Accessed April 5, 2020. [cdc.gov/hepatitis/statistics/2016surveillance/pdfs/2016HepSurveillanceRpt.pdf](https://cdc.gov/hepatitis/statistics/2016surveillance/pdfs/2016HepSurveillanceRpt.pdf)

6. Mast EE, Margolis HS, Fiore AE, et al; Advisory Committee on Immunization Practices (ACIP). A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP) part I: immunization of infants, children, and adolescents. *MMWR Recomm Rep*. 2005;54(RR-16):1-31.

7. Coates T, Wilson R, Patrick G, André F, Watson V. Hepatitis B vaccines: assessment of the seroprotective efficacy of two recombinant DNA vaccines. *Clin Ther*. 2001;23(3):392-403. doi:10.1016/S0149-2918(01)80044-8

8. Fisman DN, Agrawal D, Leder K. The effect of age on immunologic response to recombinant hepatitis B vaccine: a meta-analysis. *Clin Infect Dis*. 2002;35(11):1368-1375. doi:10.1086/344271
9. Averhoff F, Mahoney F, Coleman P, Schatz G, Hurwitz E, Margolis H. Immunogenicity of hepatitis B vaccines: implications for persons at occupational risk of hepatitis B virus infection. Am J Prev Med. 1998;15(1):1-8. doi:10.1016/S0749-3797(98)00003-8

10. Shaw FE Jr, Guess HA, Roets JM, et al. Effect of anatomic injection site, age and smoking on the immune response to hepatitis B vaccination. Vaccine. 1989;7(5):425-430. doi:10.1016/0264-410X(89)90157-6

11. Williams RE, Sena AC, Moorman AC, et al. Hepatitis B vaccination of susceptible elderly residents of long term care facilities during a hepatitis B outbreak. Vaccine. 2012;30(21):3147-3150. doi:10.1016/j.vaccine.2012.02.078

12. Janssen JM, Heyward WL, Martin JT, Janssen RS. Immunogenicity and safety of an investigational hepatitis B vaccine with a Toll-like receptor 9 agonist adjuvant (HBsAg-1018) compared with a licensed hepatitis B vaccine in patients with chronic kidney disease and type 2 diabetes mellitus. Vaccine. 2015;33(7):833-837. doi:10.1016/j.vaccine.2014.12.060

13. Young MD, Rosenthal MH, Dickson B, Du W, Maddrey WC. A multi-center controlled study of rapid hepatitis B vaccination using a novel triple antigen recombinant vaccine. Vaccine. 2001;19(25-26):3437-3443. doi:10.1016/S0264-410X(01)00054-8

14. Gerlich WH. Do we need better hepatitis B vaccines? Indian J Med Res. 2017;145(4):414-419.

15. Vesikari T. Immunogenicity and safety of a tri-antigenic versus a mono-antigenic hepatitis B vaccine in adults (PROTECT): a randomised, double-blind, phase 3 trial. Lancet. Published online May 11, 2021. doi:10.1016/S1473-3099(20)30780-5

16. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191-2194. doi:10.1001/jama.2013.281053

17. Diaz-Mitoma F, Spaans J, Machluf N, Anderson D, Mazaltov A. High seroprotection rates achieved with two doses of Sci-B-Vac, a third generation hepatitis B vaccine containing preS1, preS2 and S Antigens. VBI Vaccines. Accessed September 14, 2021. https://www.vbivaccines.com/document/high-seroprotection-rates-achieved-with-two-doses-of-sci-b-vac-a-third-generation-hepatitis-b-vaccine-containing-pre-s1-pre-s2-and-s-antigens/

18. Raz R, Koren R, Bass D. Safety and immunogenicity of a new mammalian cell-derived recombinant hepatitis B vaccine containing Pre-S1 and Pre-S2 antigens in adults. For Med Assoc J. 2001;3(5):328-332.

19. Yap I, Guan R, Chan SH. Study on the comparative immunogenicity of a recombinant DNA hepatitis B vaccine containing pre-S components of the HBV coat protein with non pre-S containing vaccines. J Gastroenterol Hepatol. 1995;10(1):51-55. doi:10.1111/j.1440-1746.1995.tb01047.x

20. Nyamathi AM, Marlow E, Branson C, Marfisee M, Nandy K. Hepatitis A/B vaccine completion among homeless adults with history of incarceration. J Forensic Nurs. 2012;8(1):13-22. doi:10.1111/j.1939-3938.2011.01123.x

21. Wong J, Payne M, Hollenberg S. A double-dose hepatitis B vaccination schedule in travelers presenting for late consultation. J Travel Med. 2014;21(4):260-265. doi:10.1111/jtm.12123

22. Milich DR, Leroux-Roels GG, Louie RE, Chisari FV. Genetic regulation of the immune response to hepatitis B surface antigen (HBsAg), IV: distinct H-2-linked Irg genes control antibody responses to different HBsAg determinants on the same molecule and map to the I-A and I-C subregions. J Exp Med. 1984;159(1):41-56. doi:10.1084/jem.159.1.41

23. Milich DR, Thornton GB, Neurath AR, et al. Enhanced immunogenicity of the pre-S region of hepatitis B surface antigen. Science. 1985;228(4704):1195-1199. doi:10.1126/science.2408336

24. Kim JY, Kim Y-G, Lee GM. CHO cells in biotechnology for production of recombinant proteins: current state and further potential. Appl Microbiol Biotechnol. 2012;93(3):917-930. doi:10.1007/s00253-011-3758-5

25. Gerlich WH. Prophylactic vaccination against hepatitis B: achievements, challenges and perspectives. Med Microbiol Immunol. 2015;204(1):39-55. doi:10.1007/s00430-014-0373-y

26. Donken R, de Melker HE, Rots NY, Berbers G, Knol MJ. Comparing vaccines: a systematic review of the use of the non-inferiority margin in vaccine trials. Vaccine. 2015;33(12):1426-1432. doi:10.1016/j.vaccine.2015.01.072

27. Rendi-Wagner P, Shouval D, Genton B, et al. Comparative immunogenicity of a PreS/S hepatitis B vaccine in non- and low responders to conventional vaccine. Vaccine. 2006;24(15):2781-2789. doi:10.1016/j.vaccine.2006.01.007

28. Yerushalmi B, Raz R, Blondheim O, Shumov E, Koren R, Dagan R. Safety and immunogenicity of a novel mammalian cell-derived recombinant hepatitis B vaccine containing pre-S1 and pre-S2 antigens in neonates. Pediatr Infect Dis J. 1997;16(6):587-592. doi:10.1097/00006454-199706000-00009

29. Leuridan E, Van Damme P. Hepatitis B and the need for a booster dose. Clin Infect Dis. 2011;53(1):68-75. doi:10.1093/cid/cir270
SUPPLEMENT 1.
Trial Protocol and Statistical Analysis Plan

SUPPLEMENT 2.
  eMethods 1. Exclusion Criteria
  eMethods 2. Exploratory End Points Assessed
  eMethods 3. Immunogenicity and Safety Assessments
  eMethods 4. Statistical Analysis
  eTable 1. Geometric Mean Concentration (GMC) of Anti-HBs and GMC Ratio at Study Days 168, 196, and 336 by Vaccine Group
  eTable 2. Solicited Local Adverse Events by Vaccine Group and Severity—Interval of Onset: Day 1 to Day 7 of Any Vaccination (Safety Set)
  eTable 3. Solicited Systemic Adverse Events by Vaccine Group and Severity—Interval of Onset: Day 1 to Day 7 of Any Vaccination (Safety Set)
  eTable 4. Summary of Unsolicited TEAEs Reported in at Least 1% of Participants in Either 1A-HBV or Pooled 3A-HBV Group by Standard of Care and Preferred Term—Interval of Onset: Day 1 to Day 28 of Any Injection (Safety Set)
  eTable 5. Summary of All Serious Unsolicited Adverse Events Reported During the Entire Study Period—Interval of Onset: Day 1 to Day 336 (Safety Set)

SUPPLEMENT 3.
  Nonauthor Collaborators

SUPPLEMENT 4.
  Data Sharing Statement