*Clinical Infectious Diseases*

MAJOR ARTICLE

# Efficacy and Safety of a 3-Antigen (Pre-S1/Pre-S2/S) Hepatitis B Vaccine: Results of a Phase 3 Randomized Clinical Trial in the Russian Federation

**Elena V. Esaulenko,[1] Aleksey A. Yakovlev,[2] Genady A. Volkov,[3] Anastasia A. Sukhoruk,[1] Kirill G. Surkov,[3] Peter V. Kruglyakov,[3] and Francisco Diaz-Mitoma[4]**

[1]Federal State Budgetary Educational Institution of Higher Education; Saint Petersburg State Paediatric Medical University of the Ministry of Health of the Russian Federation, Saint Petersburg, Russia, [2]St Petersburg State Budgetary Healthcare Institution; S.P. Botkin Clinical Infectious Diseases Hospital, Saint Petersburg, Russia, [3]Pharmsynthez PAO (Publichnoye Aktsionernoye Obshchestvo), Kuzmolovski, Russia and [4]VBI Vaccines Inc., Cambridge, Massachusetts, USA

***Background.*** This study compares the immunogenicity and safety of a 3-antigen (S/pre-S1/pre-S2) hepatitis B (HepB) vaccine (3AV), to a single antigen vaccine (1AV) in adults to support the registration of 3AV in Russia.

***Methods.*** We conducted a randomized, double-blind, comparative study of 3-dose regimens of 3AV (10 μg) and 1AV (20 μg) in adults aged 18–45 years. We evaluated immunogenicity based on hepatitis B surface (HBs) antibody titers at days 1, 28, 90, 180, and 210, adverse and serious adverse events (SAEs) to study day 210. The primary outcome was based on the difference in rates of seroconversion at day 210 (lower bound 95% confidence interval [CI]: > − 4%). Secondary outcomes were seroprotection rates (SPR), defined as anti-HBs ≥10 mIU/mL and anti-HBs geometric mean concentration (GMC).

***Results.*** Rate of seroconversion in 3AV (100%) was noninferior to 1AV (97.9%) at study day 210 (difference: 2.1%, 95% CI: −2.0, 6.3%]) but significantly higher at study day 28. SPR at study day 210 was >97% in both arms. Anti-HBs titers were significantly higher at study days 90 ($P$ = .001) and 180 ($P$ = .0001) with 3AV. Sex, age, and body mass index (BMI) had no impact on anti-HBs titers. The rates of local reactions related to vaccination were similar between vaccine arms (3AV vs 1AV) after the first (30% vs 18.8%, $P$ = .15), second (20.0% vs 14.6%, $P$ = .33), and third vaccination (14.9% vs 23.4%, $P$ = .22). No SAEs were reported.

***Conclusions.*** 3AV was noninferior to 1AV. 3AV induced high SPR, and there were no safety concerns.

**Clinical Trials Registration.** NCT04209400.

**Keywords.** hepatitis B; vaccine; seroconversion; seroprotection.

Approximately 2 billion people have been infected with hepatitis B virus (HBV) worldwide [1], who may be at an increased risk of chronic hepatitis, cirrhosis, and hepatic carcinoma [2]. The global prevalence of chronic HBV infection in 2016 was 3.5% with 257 million people living with chronic infection [3] resulting in nearly 900 000 deaths each year, primarily from sequelae of infection [4]. The World Health Organization (WHO) recommended the introduction of vaccination into national public immunization schedules as the most effective measure to eradicate HBV.

The first-generation HepB vaccine containing inactivated virus particles [5] was approved in some countries as a public health measure as early as 1981 [6]. With the success of recombinant technologies and the development of eukaryotic cell lines expressing the HBV surface antigen (HBsAg) protein, the plasma-derived vaccine was replaced by second-generation recombinant vaccines, consisting of a HBsAg expression plasmid, and produced in yeast or animal cells [7]. The first commercial recombinant single antigen HepB vaccine, Engerix-B®, was developed by GlaxoSmithKline and approved in 1986 [8]. Numerous second-generation vaccines have been developed and are currently used worldwide in children and adults [9]. As a result of increasing rates of HBV infection in Russia between 1993 and 1996, Russia implemented a prophylactic immunization schedule that included the vaccination of newborns against HBV, which came into effect in 1998 and was expanded to include the vaccination of adolescents in 2001 [10]. As of 2016, HepB vaccination had been incorporated in 190 countries' national infant immunization programs [11].

Despite widespread use of second-generation vaccines in children as part of the expanded programme on immunization, there has been limited success in the control of HBV in adults worldwide. It is estimated that only 10.5% of people

Received 20 July 2020; editorial decision 15 October 2020; published online 29 October 2020.
Correspondence: F. Diaz-Mitoma, VBI Vaccines Inc., 310 Hunt Club Rd, Ottawa ON K1V 1C1, Canada (fdiazmitoma@vbivaccines.com).

**Clinical Infectious Diseases® 2021;73(9):e3333–9**

DOI: 10.1093/cid/ciaa1649

living with HBV are aware of their disease status [3], and adults who were not immunized against HBV as children remain at risk of becoming infected. The most affected age group for both acute and chronic HBV infection are 25- to 34-year-olds, representing 30% of the 26 907 cases reported in 2017 by the 30 EU/EEA Member States [12]. In Russia, the rates of acute HBV infection decreased to 1.3 cases/100 000 people in 2014 following the implementation of the HepB immunization campaign [10]. Despite the immunization of over half of all Russian adults 18–59 years [10], the incidence of chronic hepatitis B in 2014 continued to be high at 11.3 cases/100 000 people [13]. Consequently, there is a significant HBV reservoir in Russia and a persistently high risk of infection, particularly among adults. Vaccines able to induce high rates of seroconversion and seroprotection in the adult population, comparable to those achieved in children, have the potential to dramatically reduce HBV transmission and prevalence in the Russian Federation and elsewhere.

Seroconversion is defined as the development of detectable and specific HBs antibodies, against HBsAg, above an established threshold of 2.1 mIU/mL [14–16]. The development of specific anti-HBs titers of at least 10 mIU/mL is an established surrogate of seroprotection and correlate of protective immunity against HBV [17, 18]. Although second-generation recombinant HepB vaccines are safe and highly effective in preventing HBV infection in infants, children, and young adults, evidence suggests decreased or delayed immunity to HepB in older adults [19], and those with impaired immune responses related to chronic conditions and comorbidities [20] following vaccination. Specifically, the SPR 1 month following the third vaccination can be as low as 70% in adults >40–70 years old [21] and has been shown to be only 75.4% in adults with diabetes [22] and 73% in obese individuals [23].

HBV surface antigens are composed of 3 envelope proteins: the "small" antigen S, "medium" pre-S2, and "large" pre-S1 surface antigens [24]. Recombinant second-generation HepB vaccines contain only the small surface antigen but not the pre-S1 and pre-S2 domains. Because both these domains are known to contain antigenic determinants specific for T-lymphocytes that enhance immunogenicity [25], their absence in second-generation HepB vaccines may reduce the overall immunogenicity of the vaccines.

Sci-B-Vac® (3AV) is a 3-antigen HepB vaccine that contains all 3 recombinant HBV envelope proteins—the S, pre-S1, and pre-S2 surface antigens [15]—and has been shown to induce high rates of seroprotection in infants, children, and adults and is currently approved for the prevention of HBV infection in Israel and Hong Kong [16, 24, 26–30].

The purpose of this study was to compare the immunogenicity and safety of the second-generation, standard-of-care, recombinant single antigen HepB vaccine (Engerix-B®; 1AV) to 3AV, in Russian adults who have not previously been vaccinated against HBV, to support the registration of 3AV in Russia.

## PARTICIPANTS AND METHODS

### Study Design

This was a phase 3, 2-arm, randomized, double-blind trial conducted at 3 research centers (State Budgetary Healthcare Institution Medius and K LLC, and Saint Petersburg State Budgetary Healthcare) in the Russian Federation between 18 April 2014 and 20 April 2015. Participants, aged 18–45 years of age, were recruited from a healthy volunteer internal database, enrolled into the study, randomized in a double-blind manner via the envelope method with 3-digit numbers, to receive 10 µg 3AV or 20 µg 1AV, both administered as a 1.0 mL intramuscular dose. Participants, investigators, and assessors were blinded as to treatment assignment. Because masking of the vaccines was not feasible, randomization, allocation, and administration of the vaccines were conducted by the unblinded study nurse who did not participate in the clinical evaluation process. Allocation documentation was stored in a separate study file that was accessible only by an unblinded study staff.

The study complied with pertinent national laws and standards set according to Good Clinical Practices and international ethics and was approved by the Ethics Board of the Department of Regulation of Medicines of the Russian Ministry of Health and local independent ethics committees at the study sites (State Budgetary Healthcare Institution, Medius and K LLC, and Saint Petersburg State Budgetary Healthcare). Informed consent was obtained from participants prior to study participation.

### Inclusion and Exclusion Criteria

Volunteers were healthy individuals, age 18–45 years, who had no previous exposure to HepB vaccination, and were seronegative with respect to antibodies to HBs and HB core antigen, and HBsAg on screening. Volunteers with congenital or inherited immunodeficiency disorders, blood or cardiac disorders, tumors, current use of immune-altering medications, HBV infection, anaphylaxis, severe allergy, history of alcoholism, drug abuse, pregnancy, and breast-feeding were excluded. A total of 100 volunteers who met the inclusion criteria were randomized into 3AV or 1AV arms in a 1 : 1 ratio. At screening, serodiagnosis for human immunodeficiency virus (HIV), syphilis, HBV, and hepatitis C virus was performed.

### Vaccine Administration

Vaccines were administered intramuscularly in the deltoid region in a 3-dose regimen at days 1, 28, and 180. Each 3AV and 1AV dose contained 20 µg and 10 µg of HBsAg in 1 mL formulations, respectively.

### Efficacy Analysis

The primary efficacy endpoint of the study was noninferiority of the seroconversion rate, defined as the percent of individuals who acquired anti-HBs titers ≥2.1 mIU/mL with 3AV compared to 1AV at study day 210, 30 days after the third vaccination. If the estimated confidence interval around the proportional difference (3AV–1AV) contained zero, the differences were considered statistically insignificant. Noninferiority of 3AV compared to 1AV was confirmed if the lower limit of the 95% confidence interval (CI) was at least −4%, based on a 2-sided 5% level of significance. Based on WHO recommendations to assure the quality, safety, and efficacy of recombinant HepB vaccines [9], a vaccine is considered effective if seroconversion is achieved in at least 95% of participants [9, 31]. Secondary efficacy endpoints included seroprotection rates (anti-HBs ≥ 10 mIU/mL) and anti-HBs titer geometric mean concentrations (GMC) after the first, second, and third vaccinations, reactogenicity for 5 days after each vaccination and serious adverse events (SAEs) until the end of the study. Anti-HBs titers, seroconversion and seroprotection, were evaluated at study days 28, 90, 180, and 210 of the study. Anti-HBs titer was measured by enzyme immunoassay using a test system manufactured by Abbott Laboratories (Chicago, IL USA).

### Safety Analysis

To assess safety, the rate, intensity, and association with vaccination of all SAEs were considered throughout the study to study day 210 by the site investigator. In addition, the rate and severity of local signs and systemic symptoms reported within a 5-day follow-up period after each vaccination were evaluated (Figure 3), along with routine clinical and laboratory testing throughout the study. An independent data monitoring committee evaluated safety and efficacy.

### Statistical Analysis

To verify that that the statistical hypothesis for testing 3AV for the entire duration was noninferior to the comparator 1AV given that the anticipated proportion of participants who reached seroconversion levels was about 95% in the 1AV group, and the differences in the proportions of the compared groups, 3AV and 1AV were −4%, we assumed 5% probability of type 1 error for the limit of −20% when testing the noninferiority hypothesis, and for a power of 80%. Thus, the number of participants for each group comparison was at least 45 individuals. Considering potential withdrawal, we planned to enroll 100 healthy volunteers, 50 per group for the primary outcome event of noninferiority between 3AV and 1AV. The primary analysis was performed on the intent-to-treat (ITT) set, which included all participants randomized. Percentages, including rates of seroconversion and seroprotection, and the proportion of participants demonstrating AEs, were compared between arms using the $\chi^2$test. The impact of sex, age, body weight, and type of vaccine received on anti-HBs titers was compared using 2-way analysis of variance (ANOVA). Pairwise comparisons between the vaccine arms were performed using Tukey test. ANOVA and Tukey test were conducted using the log-transformed (to base 10) anti-HBs titers. For safety analysis, the 95% CIs of the percentages of participants with AEs were calculated using angular transformation

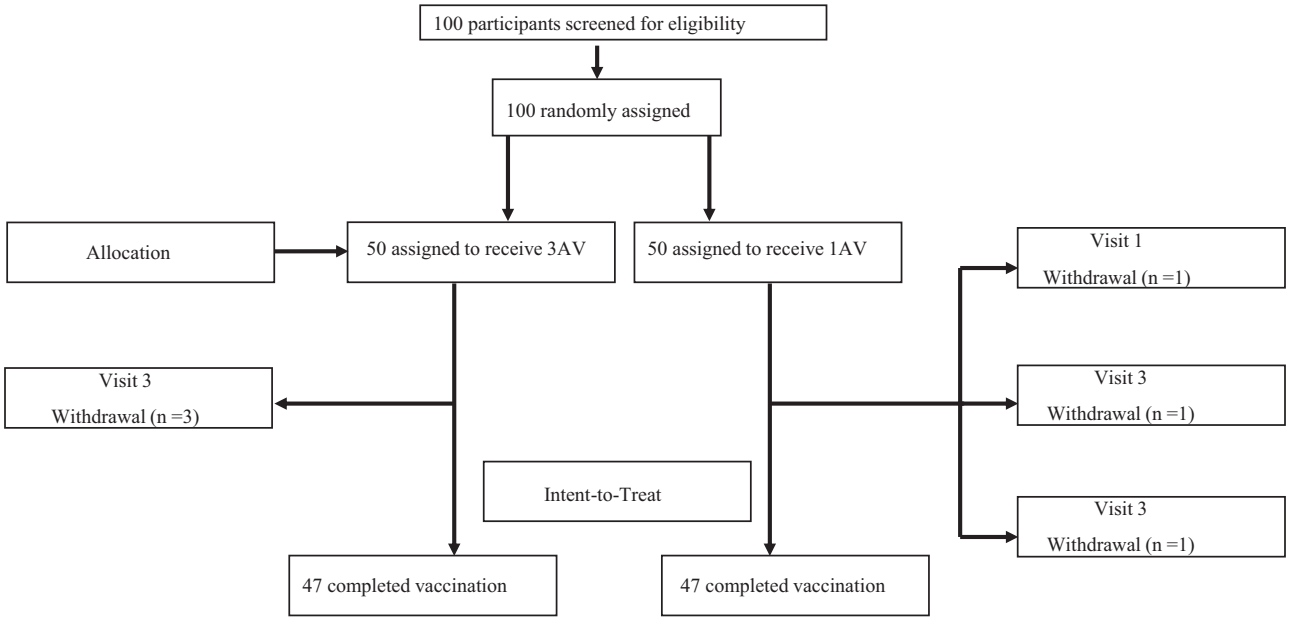


**Figure 1.** Consolidated Standards of Reporting Trials flowchart depicting the study events. Abbreviations: 1AV, single antigen vaccine; 3AV, 3-antigen vaccine.

**Table 1. Demographics of the Study Participants**

| Parameter | | 3AV n | 1AV N | *P* value |
|---|---|---|---|---|
| No. participants | | 50 | 50 | NS |
| Sex | Male | 18 | 21 | NS |
| | Female | 32 | 29 | NS |
| | | Mean ± SE | Mean ± SE | |
| Age, y | | 28.38 ± 7.72 | 30.56 ± 8.13 | NS |
| Height, cm | | 170.24 ± 9.56 | 173.10 ± 9.20 | NS |
| Weight, kg | | 70.11 ± 12.20 | 71.49 ± 15.91 | NS |
| Body mass index, kg/m$^2$ | | 24.18 ± 3.71 | 23.64 ± 3.69 | NS |
| Caucasian race | | + | + | |

*P* > .05; all participants were Caucasian.

Abbreviations: 1AV, single antigen vaccine; 3AV, 3-antigen vaccine; NS, not significant; SE, standard error.

and Wald methods. When assessing a 1-sided variant of *z*-criterion for the frequency of local reactions to any injections, and for determining statistically significant differences, the significance level was set at $P < .05$.

## RESULTS

In total, 100 participants were randomized into the 2 arms of the study (Figure 1), and 99 participants were vaccinated. Participant demographics are shown in Table 1. Median body mass index (BMI) in the 3AV group was 24.2 ± 3.7 kg/m$^2$, and 23.6 ± 3.7 kg/m$^2$ in the 1AV group, with no differences between groups ($P = .46$); obesity (>30 kg/m$^2$) was noted in 6% (n = 6; 1 participant in the Engerix-B and 5 in the Sci-B-Vac arm) of participants. Smoking status was not captured. There were no significant differences between vaccine arms in demographic and other baseline characteristics. One participant randomized to the 1AV arm withdrew at the first visit and did not receive any vaccination. Five participants (2 in the 1AV arm and 3 in the 3AV arm) withdrew consent; in all, 6 participants were excluded from the analysis.

At study day 28, after 1 vaccination, seroconversion was higher with 3AV (93.9%) compared to 1AV (76.6%) ($P < .05$). At the end of the complete vaccination regimen at study day 210, the seroconversion rate was 100% in 3AV and 97.9% in 1AV arms (difference 2.1, 95% CI: −2.0%, 6.3%). As the lower limit of the 95% CI was higher than the stated value of −4%, 3AV was considered to be noninferior to 1AV.

High rates of seroprotection were observed with 3AV (100%) and 1AV (97.9%) at study day 210, 1 month after completing the 3-dose regimen. The seroconversion and seroprotection rates obtained in this study are summarized in Table 2. Although the rates of seroconversion and seroprotection were not statistically different between vaccine arms after the second and third vaccination, the HBs antibodies induced by 3AV were significantly higher than those induced by 1AV after the second vaccination at study days 90 (618.3 ± 404.2 vs 378.7 ± 417.9, $P = .001$) and 180 (757.7 ± 378.0 vs 441.3 ± 437.6, $P = .0001$), and trended toward significance at study day 210 (891.4 ± 253.7 vs 787.0 ± 351.9, $P = .057$) based on the log-transformed (base 10) anti-HBs titers (Figure 2). Subgroup analysis showed that age, body weight, and sex had no significant effect on anti-HBs titers in either vaccine arm (Table 3).

There were no SAEs reported in this study. One 1AV vaccinee showed clinically significant increases in the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT) that were deemed to be a symptom of steatohepatitis, which was moderate in intensity, and not investigated further. The rates of local reactions (erythema, itching, pain) related to vaccination (Figure 3) were similar between vaccine arms (3AV vs 1AV) after the first (30% vs 18.8%, $P = .15$), second (20.0% vs 14.6%, $P = .33$), and third vaccination (14.9% vs 23.4%, $P = .22$). Of the 16 AEs reported by 9 study participants, 13 AEs were reported by 7 participants in the 1AV arm (hyperthermia up to 37.2°C (1), drowsiness (2), nausea (2), metallic taste (2), acute respiratory viral infection (3), rotavirus infection (1), steatohepatitis (1), weakness (1)), and 3 AEs were reported by 2 participants in the 3AV arm (drowsiness (3) (Table 4). Of these, steatohepatitis, acute respiratory viral infection, and rotavirus infection were deemed

**Table 2. Time Course of Seroconversion and Seroprotection Rates Achieved With Vaccination**

| Study Day | Seroconversion Rate (%) 3AV | 1AV | *P* value | Seroprotection Rate (%) 3AV | 1AV | *P* value |
|---|---|---|---|---|---|---|
| 1 | 0 | 0 | | 0 | 0 | |
| 28 | 93.88 | 76.60 | <.05 | 61.22 | 51.06 | NS |
| 90 | 100.00 | 95.75 | NS | 95.92 | 87.23 | NS |
| 180 | 100.00 | 95.75 | NS | 100.00 | 89.36 | NS |
| 210 | 100.00 | 97.87 | NS | 100.00 | 97.87 | NS |

*P* > .05.

Abbreviations: 1AV, single antigen vaccine; 3AV, 3-antigen vaccine, NS, not significant.

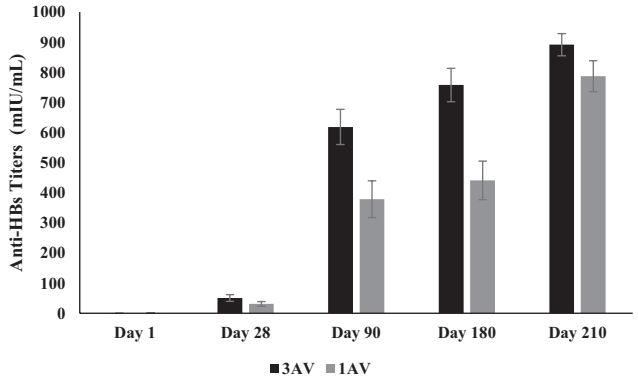


**Figure 2.** Time course of anti-HBs titers (mIU/mL). Concentrations of antibodies induced by 3AV were significantly higher than those induced by 1AV at days 90 ($P = .001$) and 180 ($P = .0001$). Abbreviations: 1AV, single antigen vaccine; 3AV, 3-antigen vaccine; HBs, hepatitis B surface.

unrelated to vaccination, although the remaining were potentially related. 3AV was potentially related to 3 AEs (27%) in 2 participants and 1AV to 8 AEs (73%) in 3 participants, as shown in Table 4. The severities of all AEs were classified as "mild" in 81.3% and as "moderate" in 18.7%. There were no grade 3 or grade 4 AEs. The $\chi^2$ test for the proportion of participants with AEs at the time of completion was 2.05, with the significance level at $P > .05$ of $P = .153$. Both vaccines demonstrated a good safety profile.

## DISCUSSION

Comparison of the seroconversion results at study days 28, 90, 180, and 210 demonstrated that more than 95% of the participants achieved seroconversion after 2 doses, which exceeded the WHO requirements for the efficacy of recombinant preventive HepB vaccines. The significant differences in seroconversion rates between vaccine arms after the first dose were particularly noteworthy, indicating achievement of a more rapid immune response with 3AV. The SPR was >97% for both 3AV and 1AV following completion of the 3-dose regimen. Though SPRs achieved with 3AV were numerically higher than 1AV at all timepoints examined, these differences did not reach statistical significance likely due to the small study sample size. The robust immunogenicity and high rates of seroprotection achieved by 3AV, which were not shown to be influenced by sex or weight, are consistent with the high rates of seroconversion and seroprotection observed in previous 3AV studies in adults [32, 33], and differ from reports of conventional HepB vaccines, suggesting decreased immunogenicity in males and those with BMI ≥25 kg/m$^2$ [34].

**Table 3. Subgroup Analysis for Sex, Age, and Body Weight on Anti-HBs Titers at Study Day 210**

| | 3AV | 1AV |
|---|---|---|
| Sex | | |
| Male | 836.4 ± 313.8 | 844.6 ± 307.3 |
| Female | 836.4 ± 312.2 | 832.8 ± 314.7 |
| Age, y | | |
| 18–22 | 829.9 ± 318.1 | 841.1 ± 309.9 |
| 23–28 | 828.0 ± 318.2 | 838.0 ± 312.8 |
| 29–35 | 833.0 ± 313.1 | 836.5 ± 312.4 |
| 36–45 | 834.8 ± 315.2 | 825.0 ± 319.9 |
| Weight, kg | | |
| 47–59 | 828.2 ± 320.7 | 838.0 ± 312.8 |
| 60–70 | 829.0 ± 317.3 | 837.7 ± 310.7 |
| 71–79 | 840.5 ± 309.6 | 830.2 ± 316.7 |
| 80–110 | 836.4 ± 313.8 | 836.1 ± 314.1 |

$P > .05$; all values are in mIU/mL.

Abbreviations: 1AV, single antigen vaccine; 3AV, 3-antigen vaccine; HBs, hepatitis B surface.

Consistent with previous clinical data [26, 27, 30, 33, 35], 3AV was safe and well tolerated. Overall, the safety and tolerability observed in this study were consistent with the known safety profile and post-market use of 3AV with no new safety risks identified. There were no patterns or concerns in the distribution of AEs in concordance with the lack of causal association between various diseases and HepB vaccine.

3AV has previously been shown to induce higher serum concentrations of antibodies to HBsAg compared to those induced by 1AV. This characteristic may be predictive of long-term persistence of circulating HBs antibodies [36, 37] and may be particularly important in the vaccination of poor responders, such as dialysis patients, who generally have suboptimal immune responses to HepB vaccines [38]. This study conducted in the Russian Federation contributes to the existing literature on 3AV in children and adults [16, 26–29, 32, 38–40], demonstrating rapid onset of SPR following fewer vaccinations. Hepatitis B is one of the most serious global infectious disease burdens warranting the need for successful vaccination. The high immunogenicity of 3AV compared to other HepB vaccines indicates its potential role in addressing a significant unmet need in the adult population, including those that remain at risk in the Russian Federation.

There are several strengths and limitations to this study. Our study is the first RCT to investigate a 3-antigen HepB vaccine in the Russian Federation. The favorable safety outcomes observed with 3AV are relevant to improved compliance particularly when it is known that noncompliance to the third dose of standard HepB vaccine is very high globally. Limitations include the homogeneity of the ethnic Russian population investigated in this study, which may not be comparable to that in North America. Second, the limited sample size affects generalizability of the results. Third, despite the nonrestrictive inclusion criteria allowing a representative cohort of eligible participants, most participants were young, healthy, and nonobese, and participants with any comorbidities were too few to conduct meaningful subgroup analyses that may have been useful for hypothesis testing in a larger population of participants.

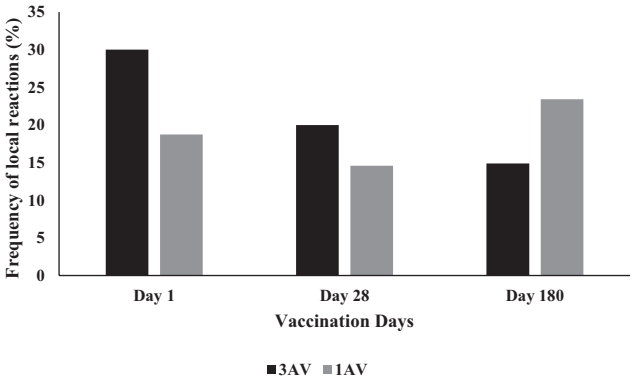


**Figure 3.** Frequency of local reactions (erythema, itching, pain) in both study arms for each injection administered. Frequency of local reactions due to 3AV and 1AV, assessed using the 1-sided variant of *z*-criterion, was similar throughout the study. Abbreviations: 1AV, single antigen vaccine; 3AV, 3-antigen vaccine.

## CONCLUSION

This study showed that 3AV and 1AV demonstrated high rates of seroconversion and rapid seroprotection in young healthy adults in the Russian Federation without any safety concerns. 3AV was noninferior to 1AV, 1 month after the third vaccination. 3AV demonstrated enhanced immunogenicity as illustrated by higher rates of seroconversion and seroprotection following the first vaccination and significantly higher anti-HBs titers after the second vaccination. The enhanced immunogenicity of 3AV may be attributable to the inclusion of all three antigenic determinants of the HBV envelope—S, pre-S2, and pre-S1. This unique feature of 3AV may enable it to play a critical public health role in prevention of HBV infection, including for those requiring quick and effective HBV protection (eg, healthcare workers) and those who are immunocompromised and at-risk of delayed or suboptimal response to conventional second-generation vaccines.

**Table 4. Adverse Events (AEs) Reported in the Study**

| AE Description | Number of AEs | | Relatedness to Vaccine |
|---|---|---|---|
| | 3AV | 1AV | |
| Hyperthermia | 0 | 1 | Potential |
| Drowsiness | 3 | 2 | Potential |
| Nausea | 0 | 2 | Potential |
| Metallic taste in mouth | 0 | 2 | Potential |
| Acute respiratory viral infections | 0 | 3 | Unrelated |
| Steatohepatitis | 0 | 1 | Unrelated |
| Weakness | | 1 | Potential |
| Rotavirus | | 1 | Unrelated |
| Total | 3 | 13 | |

Abbreviations: 1AV, single antigen vaccine; 3AV, 3-antigen vaccine.

## Notes

***Financial support.*** This study was supported by Pharmsynthez PAO and VBI Vaccines Inc.

***Potential conflicts of interest.*** F. D. M. is the Chief Medical Officer of and has equity ownership with VBI Vaccines Inc. G. A. V., K. G. S., and P. V. K. report to Pharmsynthez PAO. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

## References

1. MacLachlan JH, Cowie BC. Hepatitis B virus epidemiology. Cold Spring Harb Perspect Med **2015**; 5:a021410.
2. Ott JJ, Stevens GA, Groeger J, Wiersma ST. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. Vaccine **2012**; 30:2212–9.
3. Hutin Y, Nasrullah M, Easterbrook P, et al. Access to treatment for hepatitis B virus infection—worldwide, 2016. MMWR Morb Mortal Wkly Rep **2018**; 67:773–7.
4. World Health Organization. Global hepatitis report, 2017 [Internet]. Geneva, Switzerland; **2017**. Available at: http://apps.who.int/iris/bitstream/handle/10665/255016/9789241565455-eng. pdf?sequence=1. Accessed 15 January 2020.
5. Szmuness W, Stevens CE, Harley EJ, et al. Hepatitis B vaccine: demonstration of efficacy in a controlled clinical trial in a high-risk population in the United States. N Engl J Med **1980**; 303:833–41.
6. Szmuness W, Stevens CE, Zang EA, Harley EJ, Kellner A. A controlled clinical trial of the efficacy of the hepatitis B vaccine (Heptavax B): a final report. Hepatology **1981**; 1:377–85.
7. Heermann KH, Goldmann U, Schwartz W, Seyffarth T, Baumgarten H, Gerlich WH. Large surface proteins of hepatitis B virus containing the pre-S sequence. J Virol **1984**; 52:396–402.
8. Hauser P, Voet P, Simoen E, et al. Immunological properties of recombinant HBsAg produced in yeast. Postgrad Med J **1987**; 63 Suppl 2:83–91.
9. WHO. WHO Prequalified Vaccines. Geneva, Switzerland: WHO [Internet]. **2017**. https://extranet.who.int/gavi/PQ_Web/. Accessed 3 February 2020.
10. Klushkina VV, Kyuregyan KK, Kozhanova TV, et al. Impact of universal hepatitis B vaccination on prevalence, infection-associated morbidity and mortality, and circulation of immune escape variants in Russia. PLoS One **2016**; 11:e0157161.
11. Loharikar A, Dumolard L, Chu S, Hyde T, Goodman T, Mantel C. Status of new vaccine introduction—worldwide, September 2016. MMWR Morb Mortal Wkly Rep **2016**; 65:1136–40.
12. ECDC. Hepatitis B—Annual Epidemiological Report for 2017 [Internet]. Stockholm: European Centre for Disease Prevention and Control; **2019** June. Available at: https://www.ecdc.europa.eu/sites/default/files/documents/hepatitis-B-annual-epidemiological-report-2017.pdf. Accessed 3 February 2020.
13. Federal Center of Hygeine and Epidemiology. Infectious and parasitic diseases morbidity in Russian Federation. Moscow: Federal Center of Hygeine and Epidemiology; **2010**. Report No.: Federal statistical form 1 [in Russian].
14. Karpuch J, Scapa E, Eshchar J, Waron M, Bar-Shany S, Shwartz T. Vaccination against hepatitis B in a general hospital in Israel: antibody level before vaccination and immunogenicity of vaccine. Isr J Med Sci **1993**; 29:449–52.
15. Harsono SHA, Anwar Z, Pardede N. Comparison of seroconversion rates betweenlow-dose intradermal and recommended doseintramuscular hepatitis B vaccination in children. Paediatr Indones **2003**; 43:140–6.
16. Raz R, Dagan R, Gallil A, Brill G, Kassis I, Koren R. Safety and immunogenicity of a novel mammalian cell-derived recombinant hepatitis B vaccine containing Pre-S1 and Pre-S2 antigens in children. Vaccine **1996**; 14:207–11.
17. WHO. Recommendations to Assure the Quality, Safety and Efficacy of Recombinant Hepatitis B Vaccines, WHO, Adopted by the 61st meeting of the WHO Expert Committee on Biological Standardization, 18 to 22 October 2010. **2010**.
18. 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**; 23:392–403.
19. 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**; 35:1368–75.
20. Yao ZQ, Moorman JP. Immune exhaustion and immune senescence: two distinct pathways for HBV vaccine failure during HCV and/or HIV infection. Arch Immunol Ther Exp (Warsz) **2013**; 61:193–201.

21. Heyward WL, Kyle M, Blumenau J, et al. Immunogenicity and safety of an investigational hepatitis B vaccine with a Toll-like receptor 9 agonist adjuvant (HBsAg-1018) compared to a licensed hepatitis B vaccine in healthy adults 40-70 years of age. Vaccine **2013**; 31:5300–5.
22. Van Der Meeren O, Peterson JT, Dionne M, et al. Prospective clinical trial of hepatitis B vaccination in adults with and without type-2 diabetes mellitus. Hum Vaccin Immunother **2016**; 12:2197–203.
23. Young KM, Gray CM, Bekker LG. Is obesity a risk factor for vaccine non-responsiveness? PLoS One **2013**; 8:e82779.
24. Shouval D. Hepatitis B vaccines. J Hepatol **2003**; 39:70–6.
25. Neurath AR, Kent SBH. Antigenic structure of human hepatitis viruses. In: Immunochemistry of viruses: the basis for serodiagnosis and vaccines. Amsterdam: Elsevier Science Ltd, **1990**.
26. 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**; 10:51–5.
27. Yap I, Guan R, Chan SH. Recombinant DNA hepatitis B vaccine containing pre-S components of the HBV coat protein: a preliminary study on immunogenicity. Vaccine **1992**; 10:439–42.
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**; 16:587–92.
29. Hourvitz A, Mosseri R, Solomon A, et al. Reactogenicity and immunogenicity of a new recombinant hepatitis B vaccine containing pre-S antigens: a preliminary report. J Viral Hepat **1996**; 3:37–42.
30. Shouval D, Roggendorf H, Roggendorf M. Enhanced immune response to hepatitis B vaccination through immunization with a pre-S1/pre-S2/S vaccine. Med Microbiol Immunol **2015**; 204:57–68.
31. Monograph "Hepatitis B vaccine (rDNA)," European Pharmacopoeia 7.0, 01/2008:1056 [Internet]. Available at: http://www.fptl.ru/biblioteka/farmacop/EP-7.0-2.pdf. Accessed 5 February 2020.
32. 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. Isr Med Assoc J **2001**; 3:328–32.
33. Shapira MY, Zeira E, Adler R, Shouval D. Rapid seroprotection against hepatitis B following the first dose of a pre-S1/pre-S2/S vaccine. J Hepatol **2001**; 34:123–7.
34. Yang S, Tian G, Cui Y, et al. Factors influencing immunologic response to hepatitis B vaccine in adults. Sci Rep **2016**; 6:27251.
35. Shouval D, Ilan Y, Hourvitz A, et al. Immunogenicity of a mammalian cell-derived recombinant hepatitis B vaccine containing pre-S2 and pre-S1 antigens: a preliminary report. In: K. Nishioka, H. Suzuki, S. Mishiro, et al, eds. Viral Hepatitis and Liver Disease. Tokyo: Springer Verlag; **1993**:543–6.
36. Schillie S, Vellozzi C, Reingold A, et al. Prevention of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices. MMWR Recomm Rep **2018**; 67:1–31.
37. Green Book: immunisation against infectious disease. London, UK: Public Health England. **2016**:161–85.
38. Elhanan E, Boaz M, Schwartz I, et al. A randomized, controlled clinical trial to evaluate the immunogenicity of a pre-S/S hepatitis B vaccine Sci-B-Vac™, as compared to Engerix B®, among vaccine naive and vaccine non-responder dialysis patients. Clin Exp Nephrol **2018**; 22:151–8.
39. Hellström UB, Madalinski K, Sylvan SP. Pre-S1 epitope recognition in newborns after vaccination with the third-generation Sci-B-Vac vaccine and their relation to the antibody response to hepatitis B surface antigen. Virol J **2009**; 6:7.
40. Krawczyk A, Ludwig C, Jochum C, et al. Induction of a robust T- and B-cell immune response in non- and low-responders to conventional vaccination against hepatitis B by using a third generation preS/S vaccine. Vaccine **2014**; 32:5077–82.