Immunogenicity and safety of a tri-antigenic versus a mono-antigenic hepatitis B vaccine in adults (PROTECT): a randomised, double-blind, phase 3 trial

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Summary

Background The seroprotection rate (SPR) of hepatitis B vaccination in adults is suboptimal. The aim of this study was to compare the SPR of a tri-antigenic hepatitis B vaccine (TAV), with a mono-antigenic vaccine (MAV) in adults of all ages.

Methods This was a multicentre, double-blind, phase 3, randomised controlled trial (PROTECT) comparing the immunogenicity and safety of TAV with MAV in 28 community and hospital sites in the USA, Finland, Canada, and Belgium. Adults (aged ≥18 years) seronegative for hepatitis B virus (HBV), including those with well-controlled common chronic conditions, were randomly assigned (1:1) and stratified by study centre and age according to a web-based permuted blocked randomisation. Participants received either TAV or MAV which were administered as an intramuscular dose (1 mL) of TAV (10 µg; Sci-B-Vac, VBI Vaccines [SciVac, Rehovot, Israel]) or MAV (20 µg; Engerix-B [GlaxoSmithKline Biologicals, Rixensart, Belgium]) on days 0, 28, and 168 with six study visits and 24 weeks of follow-up after the third vaccination. Participants, investigators, and those assessing outcomes were masked to group assignment. The co-primary outcomes were to show non-inferiority of the SPRs 4 weeks after the third vaccination with TAV versus MAV in adults aged 18 years and older, as well as superiority in adults aged 45 years and older. SPR was defined as the percentage of participants attaining anti-HBs titres of 10 mIU/mL or higher. Non-inferiority of TAV to MAV was concluded if the lower limit of the 95% CI for the between-group difference was greater than –5%. Non-inferiority was assessed in the per-protocol set of participants (aged ≥18 years) and superiority was assessed in all participants (aged ≥45 years) who received at least one vaccination and had at least one evaluable immunogenicity sample after baseline (full analysis set). Safety analyses were a secondary outcome and included all participants who received at least one injection. This trial is registered at Clinicaltrials.gov (NCT03393754) and EudraCT (2017–001819–36) and is closed to new participants.

Findings Between Dec 13, 2017, and April 8, 2019, 1607 participants (796 allocated to TAV and 811 allocated to MAV) were randomly assigned and distributed across age cohorts of 18–44 years (299 of 1607; 18·6%), 45–64 years (716 of 1607; 44·6%), and 65 years and older (592 of 1607; 36·8%). In participants aged 18 years and older, SPR was 91·4% (656 of 718) in the TAV group versus 76·5% (553 of 723) in the MAV group (difference 14·9%, 95% CI 11·2–18·6), showing non-inferiority in the per-protocol set. In participants aged 45 years and older, SPR was 89·4% (559 of 625) in the TAV group versus 73·1% (458 of 627) in the MAV group (difference 16·4%, 95% CI 12·2–20·7), showing superiority in the full analysis set. TAV was associated with higher rates of mild or moderate injection site pain (63·2% [503 of 796] in TAV *vs* 36·3% [294 of 811] in MAV), tenderness (60·8% [484 of 796] in TAV *vs* 34·8% [282 of 811] in MAV), and myalgia (34·7% [276 of 796] *vs* 24·3% [197 of 811] in MAV). Otherwise, the safety profile of TAV was similar to that of MAV.

Interpretation The safety and efficacy of TAV shows its usefulness for the prevention of HBV infection in adults, including those with stable and controlled chronic conditions.

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Introduction

Hepatitis B virus (HBV) infection can cause liver inflammation, fibrosis, and liver injury, resulting in potentially life-threatening conditions through acute illness and chronic disease, including liver failure, cirrhosis, and cancer. Globally, up to 350 million people are chronically affected with HBV,[1] resulting in about 800 000 deaths annually from sequelae of infection.[2] Although the risk of acquiring chronic HBV infection is approximately 5% in adulthood, acute HBV progresses to chronic HBV in 20–40% of individuals with an impaired immune response.[3] Millions living with HBV

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Research in context

Evidence before this study

Viral hepatitis continues to pose a serious threat to the health of millions of people worldwide. Despite the highly infectious nature of the hepatitis B virus (HBV), millions of people living with HBV do not know they are infected since the initial infection is often asymptomatic—it is estimated that as few as 10·5% of people globally, and 33% with chronic HBV in the USA are aware of their infection, increasing their likelihood of transmitting the virus. We searched PubMed, Medline, and EMBASE for articles published in English from Jan 1, 1990, to June 1, 2020, to identify any published phase 3 clinical trials assessing the efficacy of hepatitis B vaccines, containing small (HBsAg), medium (pre-S2), and large surface antigens (pre-S1) of the HBV envelope. We used the search terms "hepatitis B virus", "pre-S1", pre-S2", "HepB vaccines", "prophylaxis", "vaccine", and "clinical trials". Hepatitis B vaccines, based on immunostimulatory adjuvants including aluminium hydroxide and synthetic cytosine phosphoguanine oligonucleotide, have been studied in phase 3 trials. The mono-antigenic hepatitis B vaccine, Engerix-B (MAV), containing the small HBsAg is associated with lower seroprotection rates (SPR) in men than in women, which progressively decreases with age. Furthermore, up to 40% of vaccinees miss the third injection, resulting in inadequate clinical protection against HBV infection.

Before the phase 3 clinical development programme of Sci-B-Vac (TAV) in Europe and North America, a total of 23 clinical trials has been completed in neonates, children, and adults since 1989 using the current or previous formulations of TAV. Of the completed studies, 11 were done in adults, of which ten were in generally healthy seronegative adults and one in adult non-responders to other hepatitis B vaccines. Data on these studies indicate that immunisations with TAV lead to high rates of seroprotection and development of high levels of anti-HBs titres. The tolerability and safety profile of TAV are favourable and comparable to other currently approved hepatitis B vaccines and the benefit–risk ratio continues to be positive and favourable for TAV vaccinations. A second phase 3 pivotal study CONSTANT (n=2838) was done to show TAV lot-to-lot manufacturing consistency and to compare safety and immunogenicity of TAV and MAV in 18–45 year olds. To our knowledge, PROTECT is the only phase 3 trial of TAV in the USA, Canada, and Europe that has reported results.

Added value of this study

Our trial is the first to investigate the non-inferiority and superiority of TAV compared with a standard dose of MAV in a primarily older adult population. A more potent hepatitis B vaccine that is more immunogenic, induces seroprotection faster, eliminates the need for re-vaccinations, and has a favourable safety profile has important public health implications. The significance of this study is the recognition that TAV is immunogenic in an older population that is not adequately protected following vaccination with current yeast-derived alum-adjuvanted MAVs.

The study met both its co-primary endpoints. TAV was non-inferior to MAV and induced higher SPR in adults aged 18 years or older, when compared with MAV after three doses, with statistical and clinical superiority in adults aged 45 years or older. The SPR of TAV compared with MAV in adults (aged ≥18 years) was higher and almost double at each timepoint in the first 6 months after the first injection.

Implications of all the available evidence

PROTECT showed that rapid and high SPRs are achievable with TAV, which highlights its potential use in at-risk populations in an accelerated manner, and is particularly desirable for unvaccinated health-care workers, public service sector workers, and the military. Higher SPRs that were consistent across key subgroups included in our analyses support the use of TAV in adults, including older adults with controlled chronic conditions. TAV is a vaccine with a 20-year history of safe and effective use in the prevention of HBV in Israel, and the results of the PROTECT study, along with the results of our second pivotal phase 3 study, CONSTANT, support public health efforts to eliminate new HBV infections.

are unaware of their infection, since it is often asymptomatic. It is estimated that as few as 10·5% globally, and 34% of chronically infected patients in the USA are aware of their infection status, increasing the likelihood of spreading HBV.[4] Moreover, increasing rates of injection drug users have contributed to the rise in HBV infection in North America and Europe. In the EU or European Economic Area, an estimated 4·7 million people are chronically infected, which combined with the persistently low adult vaccination rates, poses a serious threat of hepatitis B in Europe.[5] The acute HBV infection rate in the USA increased by 20·7% in 2015, rising for the first time since 2006, with the sharpest increases occurring largely in states that have been affected most by the ongoing opioid epidemic.[6]

The best way to prevent adult transmission of HBV is through successful vaccination. The adult hepatitis B vaccination rates, however, remain low in the USA and Europe, with vaccination rates of only about 25% among all adults (aged ≥19 years) in the USA,[7] and ranges from 8% to 46% in Europe.[5] By contrast with adults, neonatal and childhood hepatitis B vaccination programmes have been successful in most countries, meeting global targets to eliminate HBV infection.[8] Improved hepatitis B vaccines are needed to ensure safe and effective seroprotection against HBV for all adults. Vaccination of adults with current standard mono-antigenic yeast-derived alum-adjuvanted hepatitis B vaccines (MAVs) have important limitations, including reduced immunogenicity in older adults, obese individuals, smokers, patients with diabetes,

chronic kidney or liver failure,[9] and low response rates after two doses, which prolongs the time to protection against HBV infection.[10] Studies have shown that standard yeast-derived alum-adjuvanted hepatitis B vaccines are able to elicit seroprotection rates (SPRs) of 98·6% in healthy participants aged 16–40 years,[11] but only 59% in those aged 40 years and older,[12] underscoring that age is a major factor in vaccine response.[9–12]

The tri-antigenic hepatitis B vaccine (TAV) is an alum-adjuvanted vaccine produced in mammalian cells that contains pre-S1, pre-S2, and S HBV surface antigens that resemble the naturally occurring HBV particles in terms of protein composition, glycosylation patterns, and harbours all antigenic epitopes and domains of the HBV envelope. Unlike currently available yeast-derived MAVs that only contain the small non-glycosylated S antigen (HBsAg), TAV expresses highly immunogenic T and B cell epitopes present in the pre-S1 and pre-S2 antigens, which might enhance immunogenicity[13–16] in populations with reduced immune responses to MAVs.

Previous studies of TAV have consistently shown that a three-dose regimen (administered at 0, 1, and 6 months) elicits very high SPRs (>98%) and high anti-HBs titres. Comparative studies in children and adults have shown that antibody responses with TAV after each dose are higher than MAVs, with high SPRs noted after the first, second, or third dose.[16–18] In addition, TAV induced cellular immunity as well as protective anti-HBs titres in previously vaccinated individuals that were non-responders and low-responders.[19] TAV had comparable SPR to MAV in young adults;[20,21] studies in older adults vaccinated with TAV were not available. The purpose of this phase 3 study (PROTECT) was to compare SPRs induced by TAV and MAV in adults (aged ≥18 years), including older adults with stable chronic comorbidities.

Methods

Study design and participants

This was a multicentre, phase 3, double-blind randomised controlled trial done at 28 community and hospital sites in the USA (ten sites), Finland (ten), Canada (seven), and Belgium (one). Eligible participants were adults (aged ≥18 years) in stable health, determined by history and physical examination and laboratory tests (complete blood count, liver and renal function tests, and urinalysis) at screening. Participants with well-controlled common chronic conditions including, but not limited to, type 2 diabetes, high blood pressure, chronic obstructive pulmonary disease, and asthma, were eligible for enrolment. Participants aged older than 65 years were required to have a clinical frailty score of 3 or less.[22] Exclusion criteria included current or past HBV infection or vaccination as evidenced by HBV markers (anti-HBc, anti-HBs, or HBsAg) at screening, hepatitis C virus or HIV infection or positive serology at screening, administration of live attenuated vaccines within 4 weeks before enrolment, or administration of inactivated vaccines within 2 weeks before enrolment. A complete list of all inclusion and exclusion criteria is detailed in the appendix (pp 2–3). Approval of the study protocol was obtained from each country's regulatory agency and appropriate institutional ethics review boards. Study participants provided written informed consent to participate in the study. The protocol is available online.

See Online for appendix

For the **protocol** see https://clinicaltrials.gov/ProvidedDocs/54/NCT03393754/Prot_000.pdf

Randomisation and masking

Vaccine allocation through an interactive web response system used permuted block randomisation with a block size of four. Participants were randomly assigned (1:1) to one of the two study groups and stratified by age and study centre. To obtain a good representation across the spectrum of older adults, the targeted enrolment of adults aged 45 years and older was 80%, with approximately 20% in the 18–44 year strata, 40% in the 45–64 year strata, and 40% in the 65 years and older strata. Unblinded study personnel obtained the randomisation assignment and administered vaccines, but had no other role in the study (appendix pp 3–4); participants, investigators, and those assessing outcomes were masked to group assignment. Immunogenicity data were blinded until database lock on May 17, 2019.

Procedures

Screening was done within 28 days (4 weeks) of the first visit. Upon confirmation of enrolment, all participants were asked to come for a total of six visits. Participants were followed up for a minimum of 48 weeks after receiving the first vaccination, with at least a 24-week follow-up after receiving the third injection. At each of the three hepatitis B vaccinations, study participants received one injection of 1·0 mL of TAV (10 µg; Sci-B-Vac, VBI Vaccines [SciVac, Rehovot, Israel]) or MAV (20 µg; Engerix-B [GlaxoSmithKline, Rixensart, Belgium]). Vaccines were administered in the deltoid muscle of the non-dominant arm on day 0, and subsequent injections on days 28 and 168 alternated between the non-dominant and dominant arms. Study participants were monitored for 30 minutes after vaccination. Vital signs were recorded pre and post vaccination, with abnormal recordings contributing to other solicited events adverse events.

At select sites, participants were asked to come for three additional visits to assess clinical laboratory parameters (haematology and biochemistry) 1 week after each vaccination (days 7, 35, and 175), as part of a clinical laboratory substudy, which included at least 10% of the total number of participants enroled in the trial. Blood samples for immunogenicity testing were obtained before vaccination on days 0, 28, and 168, and on days 56, 196, and 336. Measurement of anti-HBs titres was done using a validated VITROS anti-HBs quantitative assay (Ortho Vitros 5600; Ortho-Clinical Diagnostics, NY, USA). In addition, blood samples were collected to explore pre-S1 and pre-S2 antibody levels and characteristics, and

cell-mediated immunity. The results of this work will be reported in a separate publication.

Outcomes

The co-primary endpoints were: non-inferiority of SPR of TAV compared with MAV in adults (aged ≥18 years), 4 weeks after the third vaccination (day 196); and superiority of SPR of TAV compared with MAV in adults (aged ≥45 years), at day 196. Vaccine-induced seroprotection, considered a surrogate of protection against infection, is defined as anti-HBs titres 10 mIU/mL or higher; SPR was defined as the percentage of participants attaining seroprotection.

Secondary endpoints included SPRs at days 56 and 168, 4 and 20 weeks after receiving the second TAV dose and the SPR at day 196, 4 weeks after receiving the third MAV dose; percentage of participant-reported, local and systemic solicited adverse events (erythema, pain, tenderness, oedema, pruritis, nausea or vomiting, diarrhoea, headache, fatigue, myalgia, fever, tachycardia, bradycardia, hypertension, hypotension, and changes in respiratory rate on the day of vaccination and the following 6 days), unsolicited adverse events (on the day of vaccination and the following 27 days); safety follow-up for 48 weeks after the first vaccination for serious adverse events; medically significant events or new onset of chronic illness until day 336; percentage of participants with abnormal vital signs or physical examination findings compared with baseline; and changes in concomitant medication.

Exploratory endpoints included SPRs in both study groups at days 0, 28, 56, 168, and 336; geometric mean concentration (GMC) of anti-HBs titres and the proportion of participants having anti-HBs titres of at least 100 mIU/mL in serum at baseline and 4 weeks after each vaccination on days 28, 56, and 196, and at days 168 and 336; rate of non-response (proportion of participants not attaining anti-HBs titres ≥10 mIU/mL) at day 196, 4 weeks after the final study vaccine; and comparison of SPR, GMC, and the rate of non-response in subgroups of interest, at day 196.

In correlative studies, cell-mediated immunity directed against pre-S1 and pre-S2 will be measured and correlated with anti-HBs immune responses in a subset of the study population. The results from the correlative studies will be the focus of a forthcoming publication and are not presented herein.

Statistical analysis

The overall sample size for the study was driven by the superiority co-primary endpoint in study participants aged 45 years and older. Assuming an SPR of 0·81 for MAV and 0·96 for TAV, a minimum of 540 participants (270 per treatment group) being 45 years or older provided 90% power to show superiority of SPR. Based on a targeted enrolment of 80% of study participants aged 45 years or older, an additional 180 (20%) 18–44 years old study participants were enrolled, for a total of at least 680 participants in the full study. This sample size provided at least 90% power to show non-inferiority of TAV compared with MAV in participants aged 18 years and older (co-primary endpoint). For superiority and non-inferiority analyses, we assumed a 5% margin with a two-sided type 1 error of 0·05. Given the desire to have robust immunogenicity estimates of SPR in the adult population following a three-dose regimen of TAV and to guard against a better than expected SPR for MAV (up to 84% in participants aged ≥45 years), a total of 1564 participants were targeted to be enrolled to the trial, as planned for statistical purposes.

The two co-primary analyses were tested hierarchically, such that the test for superiority in participants aged 45 years or older was only done after non-inferiority in participants aged 18 years or older was shown. Non-inferiority of TAV to MAV was concluded if the lower limit of the 95% CI for the between-group difference was greater than –5%. Upon establishment of non-inferiority, statistical superiority in participants was established if the lower limit of the 95% CI was more than 0%, and for clinical superiority, if more than 5%. The Miettinen & Nurminen[23] formula was adopted to calculate a two-sided 95% CI for the difference in adjusted proportion. Non-inferiority was assessed in the per-protocol set of participants aged 18 years and older; superiority was assessed in the full analysis set (participants who received at least one vaccination and had at least one evaluable immunogenicity sample after baseline) for the study population of participants aged 45 years and older. The secondary and exploratory immunogenicity analyses were assessed in the per-protocol set of participants aged 18 years and older. Sensitivity analysis was done using the full analysis and the intent-to-treat data sets. Safety analyses were based on the safety set (all enrolled participants who received at least one injection). All analyses were done with the SAS software (version 9.3). An independent data and safety monitoring board reviewed blinded safety data throughout the trial.

Role of the funding source

NM, JNS, BY-R, DEA, VP, and FDM are employees of the funder and were involved in the study design, data analysis, data interpretation, and the writing of the report. The funder of the study had no role in data collection.

Results

Participant visits ran from Dec 13, 2017, to April 8, 2019. Of the 2472 volunteers screened for study eligibility, 1607 (796 assigned to TAV and 811 assigned to MAV) volunteers were assigned to an intervention group (figure 1). The most common reasons for screen failure were previous HBV vaccination (71 [8·2%] of 865) or HBV infection markers (anti-HBc, anti-HBs, and HBsAg)

at screening (139 (16·0%) of 865) and uncontrolled hypertension (137 [16·0%] of 865). A total of 1543 (96%) participants received three study vaccinations, and rates of study completion were similar for both vaccine groups (TAV 756 [95·0%] of 796; MAV 769 [94·8%] of 811). 82 (5·1%) participants withdrew before completing the study; the most common reasons were loss to follow-up (35 [2·2%] of 1607) and consent withdrawn (20 [1·2%] of 1607), with similar rates of withdrawal in both study groups. The most common major protocol deviations were related to procedures or tests not done as per protocol (323 [20·1%] of 1607) and study visits attended outside of windows (110 [6·8%] of 1607).

A total of 1607 adults were randomly assigned. Participants were from the USA (680 [42·3%] of 1607), Finland and Belgium (668 [41·6%] of 1607), and Canada (259 [16·1%] of 1607). The randomised population comprised the following age cohorts: 18·6% (299 of 1607) were aged 18–44 years, 44·6% (716 of 1607) were 45–64 years, and 36·8% (592 of 1607) 65 years and older. Demographic variables and comorbidities known to affect immunogenicity such as age, gender (38% males *vs* 62% females), diabetes (7·8%), and body-mass index (BMI 29·3 kg/m², SD 6·52) were similar across vaccine groups (table 1).

At day 196, immunogenicity samples were evaluable for 718 of 718 (TAV) and 723 of 729 (MAV) study participants. SPR in participants (aged ≥18 years) at day 196, 4 weeks after the third vaccination, was 91·4% (656 of 718; 95% CI 89·1 to 93·3) for TAV compared with 76·5% (553 of 723; 73·2 to 79·5) for MAV in the per-protocol set. The mean SPR difference was 14·9% (95% CI 11·2 to 18·6), thereby meeting the non-inferiority endpoint in the per-protocol set. Results were consistent in the full analysis set. SPR in TAV recipients (aged ≥45 years) at day 196 was 89·4% (559 of 625; 95% CI 86·8 to 91·7) compared with 73·1% (458 of 627; 69·4 to 76·5) for MAV, with a mean SPR difference of 16·4% (12·2 to 20·7), exceeding the pre-set margins for statistical superiority (lower limit of 95% CI >0%) and clinical superiority (lower limit of 95% CI >5%) in the full analysis set. At day 196, the rate of non-response in the per-protocol set (≥18 years) was higher in the MAV group (23·5%; 170 of 723) compared with TAV (8·6%; 62 of 718) with a difference in the rate of non-response of –14·9% (95% CI –18·6 to –11·2).

The safety population was comprised of 1607 participants (796 assigned to TAV and 811 assigned to MAV). Early discontinuation of vaccination due to non-serious

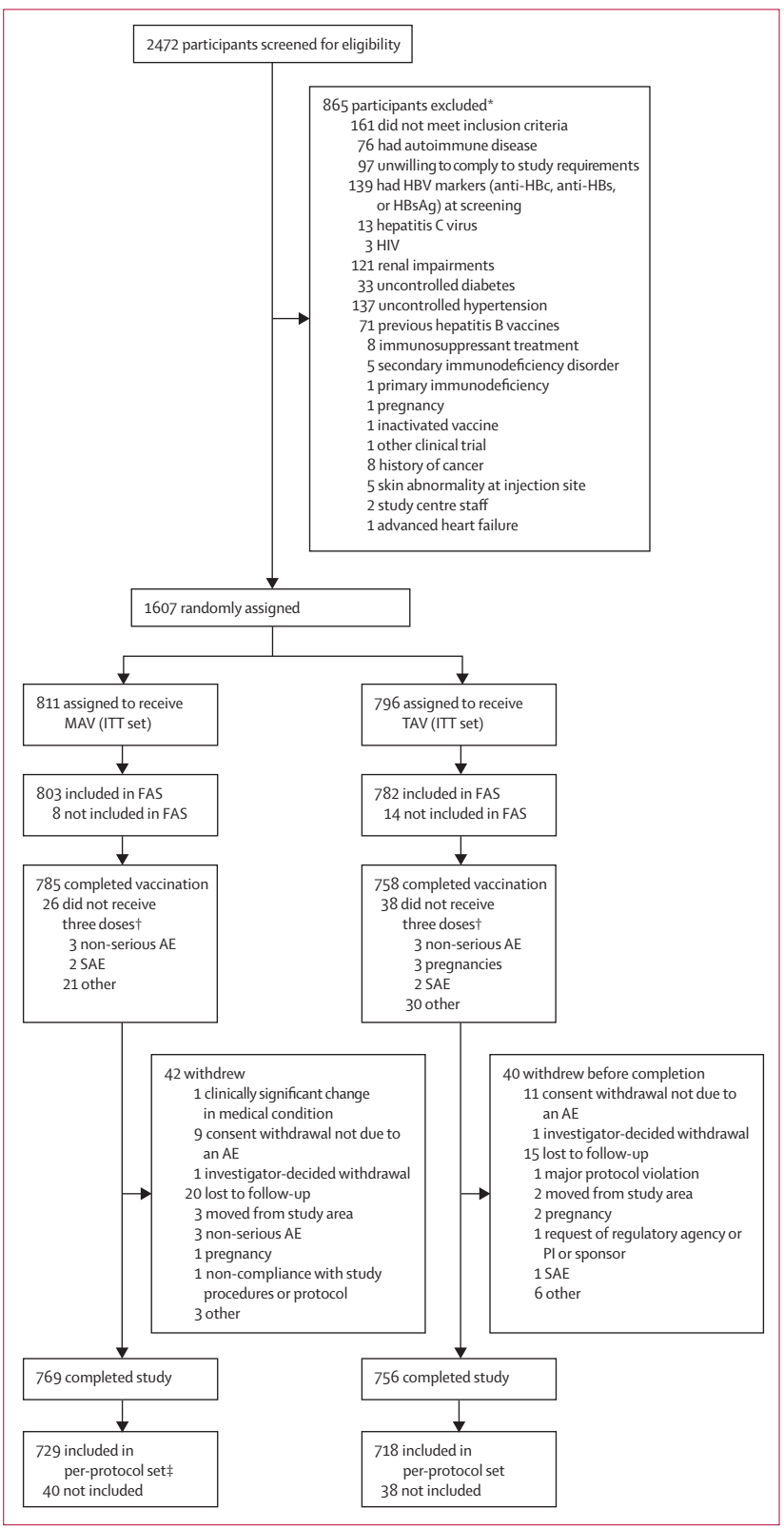


Figure 1: **Trial profile**
MAV=mono-antigenic vaccine. ITT=intention-to-treat. TAV=tri-antigenic vaccine. FAS=full analysis set. AE=adverse events. SAE=serious adverse events. PI=principal investigator. *If a participant had multiple reasons for screen failure, the participant was counted more than once. †37 participants received two doses (21 TAV, 16 MAV) and 27 received one dose (17 TAV, 10 MAV). ‡Final analysis included 723 participants because six participants did not have evaluable immunogenicity samples at day 196.

	TAV (n=796)	MAV (n=811)
Sex		
Male	315 (39·6%)	303 (37·4%)
Female	481 (60·4%)	508 (62·6%)
Mean age, years	56·6 (18–86)	56·6 (18–90)
Body-mass index*, kg/m²		
>30	297 (37·3%)	292 (36·0%)
≤30	499 (62·7%)	519 (64·0%)
Race and ethnicity		
White	715 (89·8%)	730 (90·0%)
Black or African American	66 (8·3%)	65 (8·0%)
Others (Asian, Native Hawaiian or other Pacific Islander, American Indian, or Alaska Native)	15 (1·8%)	16 (2·0%)
Hispanic or Latino	79 (9·9%)	75 (9·2%)
Non-Hispanic or Latino	714 (89·7%)	732 (90·3%)
Unknown	3 (0·4%)	4 (0·5%)
Smoking status		
Current	104 (13·1%)	113 (13·9%)
Former	203 (25·5%)	224 (27·6%)
Non-smoker	489 (61·4%)	474 (58·4%)
Type 2 diabetes status		
Yes	60 (7·5%)	65 (8·0%)
No	736 (92·5%)	746 (92·0%)
Average daily alcohol consumption†		
≥4	4 (0·5%)	4 (0·5%)
2–3	59 (7·4%)	63 (7·8%)
0–1	733 (92·1%)	744 (91·7%)
Country or region		
USA	338 (42·5%)	342 (42·2%)
Canada	126 (15·8%)	133 (16·4%)
Europe	332 (41·7%)	336 (41·4%)

Data are n (%) or mean (range), unless otherwise specified. MAV=mono-antigenic vaccine. TAV=tri-antigenic vaccine. *Body-mass index was calculated for all participants. †The average daily alcohol consumption was according to standard classification of drinks as per the National Institutes of Health (12 ounces of regular beer; 5% alcohol; 5 ounces of wine, approximately 12% alcohol; 1·5 ounces of distilled spirits; 40% alcohol).[24]

Table 1: **Baseline characteristics**

	TAV (n=796)	MAV (n=811)	P value
Solicited local adverse event			
Any solicited local adverse event	572 (71·9%)	379 (46·7%)	<0·0001
Pain	503 (63·2%)	294 (36·3%)	<0·0001
Tenderness	484 (60·8%)	282 (34·8%)	<0·0001
Pruritus or itching	76 (9·5%)	66 (8·1%)	0·614
Redness or erythema	18 (2·3%)	15 (1·8%)	0·539
Swelling or oedema	18 (2·3%)	12 (1·5%)	0·559
Solicited systemic adverse event			
Any systemic	445 (55·9%)	396 (48·8%)	0·02
Fatigue	242 (30·4%)	249 (30·7%)	0·469
Headache	249 (31·3%)	238 (29·3%)	0·678
Myalgia	276 (34·7%)	197 (24·3%)	0·008
Diarrhoea	82 (10·3%)	96 (11·8%)	0·375
Nausea or vomiting	56 (7·0%)	73 (9·0%)	0·327

Data are n (%). Implausible erythema measurements of ≥900 mm or <0 mm, and swelling measurements of ≥500 mm or <0 mm were removed from the analysis but included in listings. MAV=mono-antigenic vaccine. TAV=tri-antigenic vaccine.

Table 2: **Incidence of solicited local and systemic adverse events**

adverse events was 0·4% (3 of 796) in the TAV group and 0·4% (3 of 811) in the MAV group; and serious adverse events was 0·3% (2 of 796) in the TAV and 0·2% (2 of 811) in the MAV group, which were low in both groups. In the TAV group, there were higher rates of local (71·9% [572 of 796] *vs* 46·7% [379 of 811]) and systemic (55·9% [445 of 796] *vs* 48·8% [396 of 811]) reactogenicities (table 2); they were mostly of mild or moderate severity. The higher rate of solicited adverse events was largely due to higher rates of injection site pain (63·2% [503 of 796] *vs* 36·3% [294 of 811]), tenderness (60·8% [484 of 796] *vs* 34·8% [282 of 811]), and myalgia (34·7% [276 of 796] *vs* 24·3% [197 of 811]; table 2). The severity of local and systemic reactogenicities was similar between vaccine groups (appendix p 6).

Solicited adverse events were not found to increase with increasing age of recipient or with successive vaccinations (appendix pp 7–9). A total of 12 grade 4 solicited adverse events, erythema and swelling at the injection site, was reported by 11 participants—three in TAV and eight in MAV; however, none of these events were medically attended. Median duration of solicited adverse events was 1–2 days, with solicited adverse events that continued beyond day 7 (local, systemic, or other) experienced by 10·2% (81 of 796) in the TAV group and 11·5% (93 of 811) in MAV group.

There were no differences in the rates of unsolicited adverse events within 28 days of any vaccination (369 [46·4%] of 796 in TAV *vs* 389 [48·0%] of 811 in MAV; appendix pp 10–11). Most adverse events reported during the study were of mild or moderate severity, and were assessed as unrelated to study vaccination. No clear clusters or unusual patterns of unsolicited adverse events were observed during the study. Over the course of the study, medically attended events were reported by 25·4% (202 of 796) participants in the TAV group and 28·5% (231 of 811) participants in the MAV group (appendix pp 12–13), with the most common events being urinary tract infection (2·1%; 17 in each group), sinusitis (nine [1·1%] of 796 *vs* 14 [1·7%] of 811), and upper respiratory infection (ten [1·3%] of 796] *vs* seven [0·9%] of 811]). New onset of chronic illness was reported by 3·3% (26 of 796) of participants in the TAV group and 3·7% (30 of 811) of participants in the MAV group, with the most common event being hypertension (two [0·3%] of 796 *vs* six [0·7%] of 811), hypothyroidism (0% *vs* four [0·5%] of 811]), and hypercholesterolaemia (one [0·1%] of 796 *vs* three [0·4%] of 811; appendix pp 14–15).

Serious adverse events were reported by 4·0% (32 of 796) participants in the TAV group and 2·6% (21 of 811) participants in the MAV group (appendix pp 16–17). Of the 62 serious adverse events reported during the study, only three occurred in more than one participant in either study group. These were atrial fibrillation (one [0·1%] of 796 in TAV *vs* two [0·2%] of 811 in MAV), congestive cardiac failure (two [0·3%] of 796 in TAV *vs* 0 in MAV), and colon cancer (0 in TAV *vs* two [0·2%] of 811 in MAV). All serious adverse events, except one, were reported as unrelated or unlikely related to study vaccines. One serious adverse event of viral gastroenteritis in the TAV group was assessed by the investigator (WH) as probably related and resulted in study withdrawal. There were no deaths reported in the study.

In the secondary immunogenicity analysis in participants aged 18 years and older, SPR after two doses of TAV (at day 168) was 66·0% (473 of 717), compared with 76·5% (553 of 723) after three doses of MAV (at day 196) in the per-protocol set. The mean SPR difference was –10·5% (95% CI –15·2 to –5·9), thereby not achieving non-inferiority. However, in adults aged 18–44 years, the SPR after two doses of TAV was comparable to three doses of MAV (87·2% [109 of 125] *vs* 91·1% [123 of 135]), a difference of –3·9% (95% CI –11·9 to 3·8).

The SPR of the TAV group was higher than that of the MAV group at each post-vaccination timepoint in participants aged 18 years and older and in each age strata (figure 2), at days 28, 56, 168, 196, and 336. Peak SPRs were reached at day 196 and decreased at the end of the study (day 336), which was less for TAV (from 91·4% [656 of 718] at day 196 to 89·0% [631 of 709] at day 336) than for MAV (76·5% [553 of 723] at day 196 to 68·8% [492 of 715] at day 336) in participants aged 18 years and older (day 196 difference 14·9%, 95% CI 11·2–8·6; day 336 difference 20·2%; 16·1–24·3).

Higher SPRs in the TAV group compared with the MAV group at day 196 were noted in all subgroups of interest (figure 3A). The proportion of participants aged 18 years or older who had anti-HBs titres of at least 100 mIU/mL at day 196 was higher with TAV (80·8% [580 of 718]) than MAV (60·7% [439 of 723]; difference 20·1%, 95% CI 15·5–24·6) and was consistent for BMI of more than 30 kg/m² (79·6% [214 of 269] *vs* 55·5% [141 of 254]), current smokers (70·7% [65 of 92] *vs* 54·7% [52 of 95]), and diabetics (59·3% [32 of 54] *vs* 45·0% [27 of 60]; figure 3B). Regardless of age, BMI, or diabetes status, the antibody GMC of participants who received TAV was five to eight times higher than MAV at day 196 (appendix p 18; figure 1).

Discussion

The study data presented herein from the phase 3 study, PROTECT trial, indicate that TAV might be able to overcome some of the limitations of conventional MAVs in the adult population, particularly in those aged 45 years and older where TAV showed superior seroprotection compared with MAV. TAV was shown to be a highly effective hepatitis B vaccine with favourable safety profile in this older population and across key subgroups of interest based on age, sex, obesity, diabetes, and

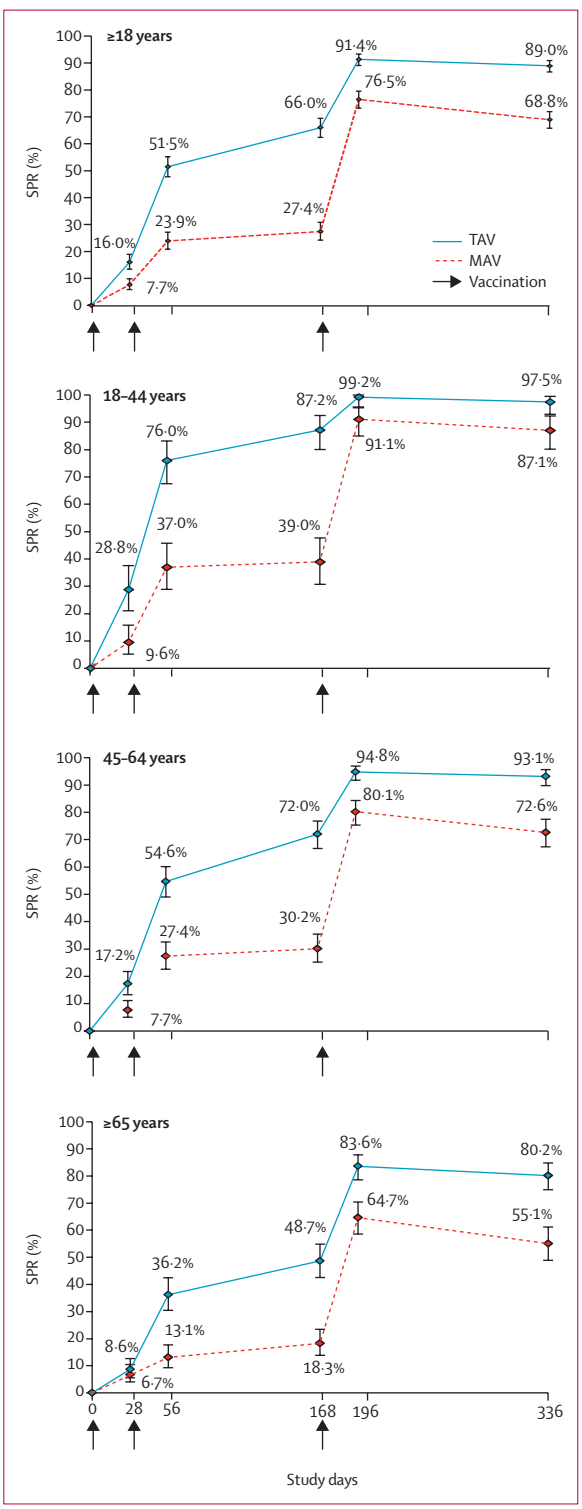
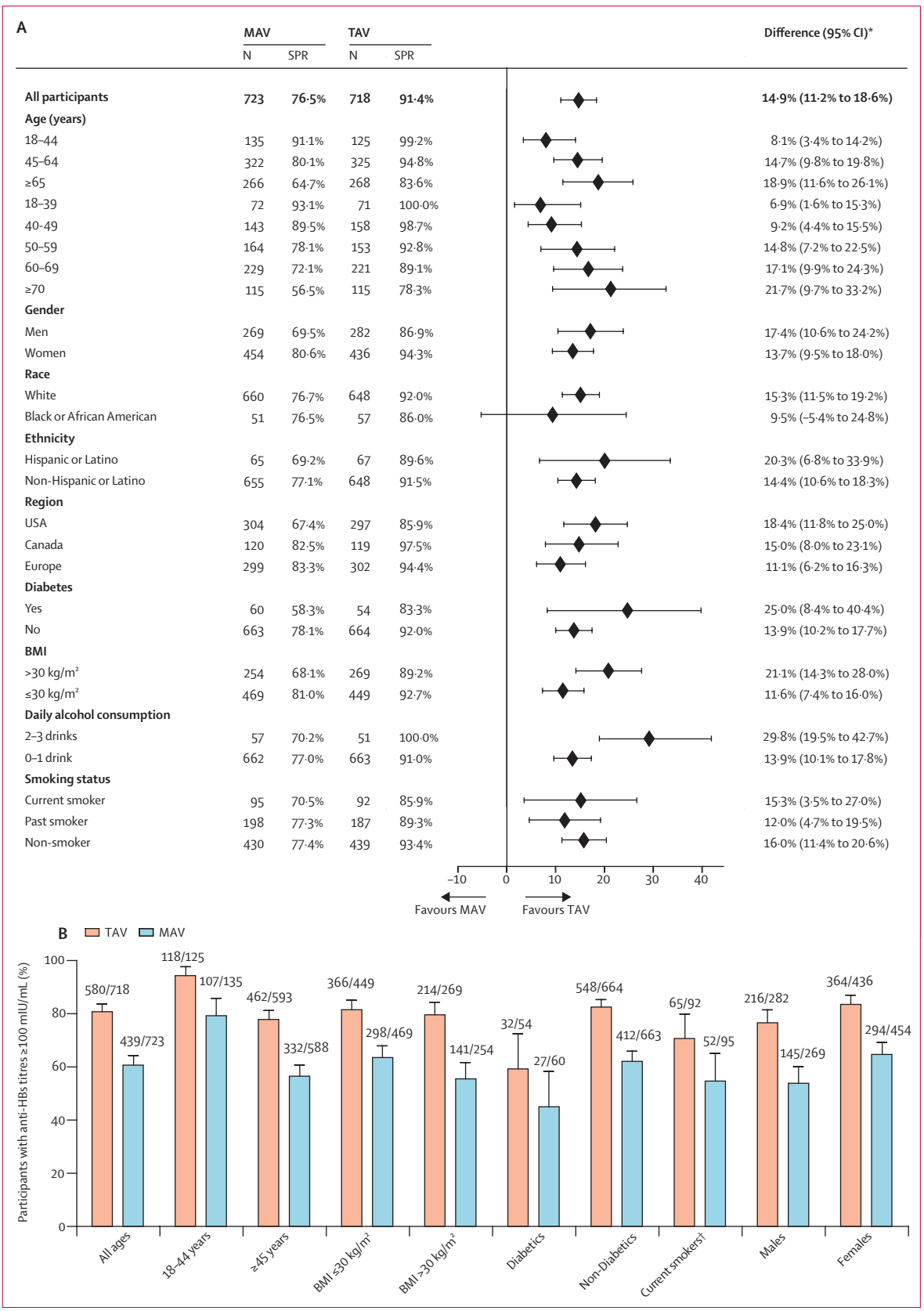


Figure 2: **TAV and MAV SPRs over time by age group**
Results for the per-protocol set at study days 28, 56, 168, 196, and 336 are graphically shown for adults aged ≥18 years of age (A), 18–44 years of age (B), 45–64 years of age (C), and ≥65 years of age (D). TAV induced higher seroprotection compared with MAV after the first and second vaccinations and at day 168 just before the third vaccination. The decay after peak seroprotection (day 196) to the end of the study (day 336) was less for TAV than for MAV. Vertical dark arrows indicate the vaccination timepoints. MAV=mono-antigenic vaccine. SPR=seroprotection rate. TAV=tri-antigenic vaccine.


A
MAV
TAV
N
SPR
N
SPR
Difference (95% CI)*
All participants 723 76·5% 718 91·4% 14·9% (11·2% to 18·6%)
Age (years)
18–44 135 91·1% 125 99·2% 8·1% (3·4% to 14·2%)
45–64 322 80·1% 325 94·8% 14·7% (9·8% to 19·8%)
≥65 266 64·7% 268 83·6% 18·9% (11·6% to 26·1%)
18–39 72 93·1% 71 100·0% 6·9% (1·6% to 15·3%)
40–49 143 89·5% 158 98·7% 9·2% (4·4% to 15·5%)
50–59 164 78·1% 153 92·8% 14·8% (7·2% to 22·5%)
60–69 229 72·1% 221 89·1% 17·1% (9·9% to 24·3%)
≥70 115 56·5% 115 78·3% 21·7% (9·7% to 33·2%)
Gender
Men 269 69·5% 282 86·9% 17·4% (10·6% to 24·2%)
Women 454 80·6% 436 94·3% 13·7% (9·5% to 18·0%)
Race
White 660 76·7% 648 92·0% 15·3% (11·5% to 19·2%)
Black or African American 51 76·5% 57 86·0% 9·5% (–5·4% to 24·8%)
Ethnicity
Hispanic or Latino 65 69·2% 67 89·6% 20·3% (6·8% to 33·9%)
Non-Hispanic or Latino 655 77·1% 648 91·5% 14·4% (10·6% to 18·3%)
Region
USA 304 67·4% 297 85·9% 18·4% (11·8% to 25·0%)
Canada 120 82·5% 119 97·5% 15·0% (8·0% to 23·1%)
Europe 299 83·3% 302 94·4% 11·1% (6·2% to 16·3%)
Diabetes
Yes 60 58·3% 54 83·3% 25·0% (8·4% to 40·4%)
No 663 78·1% 664 92·0% 13·9% (10·2% to 17·7%)
BMI
>30 kg/m² 254 68·1% 269 89·2% 21·1% (14·3% to 28·0%)
≤30 kg/m² 469 81·0% 449 92·7% 11·6% (7·4% to 16·0%)
Daily alcohol consumption
2–3 drinks 57 70·2% 51 100·0% 29·8% (19·5% to 42·7%)
0–1 drink 662 77·0% 663 91·0% 13·9% (10·1% to 17·8%)
Smoking status
Current smoker 95 70·5% 92 85·9% 15·3% (3·5% to 27·0%)
Past smoker 198 77·3% 187 89·3% 12·0% (4·7% to 19·5%)
Non-smoker 430 77·4% 439 93·4% 16·0% (11·4% to 20·6%)
–10
0
10
20
30
40
Favours MAV
Favours TAV
B
TAV
MAV
Participants with anti-HBs titres ≥100 mIU/mL (%)
100
80
60
40
20
0
580/718
439/723
118/125
107/135
462/593
332/588
366/449
298/469
214/269
141/254
32/54
27/60
548/664
412/663
65/92
52/95
216/282
145/269
364/436
294/454
All ages
18–44 years
≥45 years
BMI ≤30 kg/m²
BMI >30 kg/m²
Diabetics
Non-Diabetics
Current smokers†
Males
Females

smoking status. Notably, with an overall SPR above 90% in this predominantly older study population, and with successful demonstration of superiority in adults aged 45 years and older, the immunogenicity of TAV was shown to be less impacted by age than the comparator MAV. The study did not meet the secondary objective of non-inferiority of two doses of TAV compared with three doses of MAV in this predominantly older population of participants aged 18 years and older, suggesting that in older adults, a full three-dose regimen of MAV is probably required to reach adequate levels of seroprotection. Nevertheless, the SPR of TAV was significantly higher at each time point on a per-visit basis compared with MAV. The results obtained in this study suggest that TAV offers seroprotection to more adults earlier than MAV.

Thus, the higher SPRs seen with TAV, compared with MAV, observed across key subgroups supports its effectiveness in the general population of older adults and those with controlled chronic conditions.[14,19] This finding might be of particular clinical relevance to adults with pre-diabetes and diabetes who are at a higher risk of HBV infection and more severe complications associated with HBV infection.[25] Finally, the higher SPRs observed with TAV in the 6 months after the first vaccination, which at each timepoint was more than double the SPR reached with MAV, suggest that more adults are seroprotected earlier following fewer vaccinations, which could be particularly relevant for adults at high risk of infection.

TAV also induced higher anti-HBs GMC across subgroups compared with MAV, which might be predictive of long-term persistence of circulating HBs antibodies and durable protection.[25] Peak titres for TAV were significantly higher than that of MAV across all key subgroups and a higher percentage of adults treated with TAV had anti-HBs titres of at least 100 mIU/mL, the optimal threshold for persistent and durable immune response and protection. This threshold is not often reached by older individuals or those with impaired immune response to vaccinations with MAV. The results of our study show the durability of seroprotection where the proportion of participants who had anti-HBs titres of at least 100 mIU/mL was higher in TAV compared with MAV, in adults aged 18 years or older, those aged 45 years or older, and also in key subgroups, whose immune responses might be impaired. Moreover, the SPRs induced with TAV remained stable to the end of the study following completion of the three-dose regimen compared with MAV, where there was a notable decline in SPR. This finding might be of particular significance when considering the duration of protection in all adults, including in individuals with impaired immune responses such as renal failure patients, who have a faster antibody decline than healthy individuals.[26] Although the SPR reached with MAV was low compared with some historical studies in the general population, it has done as expected in the older population studied in the PROTECT trial. SPRs as low as 70% have been observed in older populations vaccinated with MAV.[12,27]

The safety and tolerability seen in this study were consistent with the known safety profile of TAV. No safety signals were observed in either vaccine group, and no new safety risks were identified. Overall, higher local and systemic reactogenicities were associated with TAV compared with MAV, which were primarily due to higher incidence of mild or moderate pain and tenderness at the injection site and myalgia. This result was expected and consistent with previous TAV studies.[14–20,28,29] There were no notable differences in the incidence of unsolicited adverse events in the 28 days following injection between the vaccine groups and there were no observed clusters or unusual patterns of adverse events or serious adverse events. The type and frequency of protocol deviations were well balanced across vaccine groups and were considered unlikely to affect the study conclusions.

The PROTECT data showed the favourable safety and efficacy profile of TAV in adults, based on anti-HBs titres at least 10 mIU/mL and at least 100 mIU/mL. The ability of TAV to safely seroprotect more adults earlier following fewer vaccinations in adults aged 18–44 years, including those with impaired vaccine immune responses in individuals with well-controlled comorbidities, shows its potential to overcome the limitations of current standard MAVs and to help address ongoing unmet medical needs in the prevention of HBV infection.

TAV might be beneficial to the substantial number of adult non-responders to yeast-derived conventional hepatitis B vaccines in whom a more immunogenic HBV vaccine is required. TAV received initial marketing authorisation in Israel in 2000 for indications against HBV infection in healthy children and adults. An evaluation of TAV in various populations in previous clinical trials suggest higher doses might be required in individuals with impaired immune responses.[19,28,30] Our

***Figure 3*: Difference in SPRs 4 weeks afer the final vaccine dose in subgroups (per-protocol set)**

(A) Analyses were done to compare the seroprotection induced by TAV versus MAV within subgroups of age, sex, BMI, diabetes, smoking, alcohol consumption, race, ethnicity, and country or region and to show whether seroprotection induced by TAV or MAV differs by these demographic and baseline parameters. SPRs at study day 196 from the key subgroup analysis and differences in the SPR between MAV and TAV groups are displayed. Seroprotection is defined as anti-HBs titres ≥10 mIU/mL; SPR was defined as the percentage of participants attaining seroprotection. Consistently higher SPRs were noted in all participants, including those known to have a reduced immune response to a MAV. (B) Proportion of participants who had anti-HBs titres of ≥100 mIU/mL. The proportions of these participants were higher with TAV than MAV in participants aged 18 years or older (80·8% *vs* 60·7%) at day 196 (difference 20·1%, 95% CI 15·5–24·6) and was consistent across key subgroups including BMI >30 kg/m² (79·6% *vs* 55·5%), current smokers (70·7% *vs* 54·7%), and diabetics (59·3% *vs* 45·0 %). Validated VITROS anti-HBs quantitative assay was used to measure anti-HBs titres in serum, which was collected from participants on the day of their in-clinic visit. BMI=body-mass index. MAV=mono-antigenic vaccine. SPR=seroprotection rate. TAV=tri-antigenic vaccine. *Difference in SPR (ie, SPR TAV – SPR MAV). †Participants who were smoking tobacco regularly during the study.

results corroborate previous findings showing higher and faster rates of seroprotection after the second and third dose of TAV,[14] and comparable safety profile with MAV in various populations.[17,28,30,31]

Strengths of the study included the double-blind, randomised design, large sample size, geographical diversity, and enrolment of healthy older individuals as well as those with common chronic conditions in whom vaccine immunogenicity might be suboptimal. Older individuals with chronic conditions are not usually targeted in vaccine clinical trials and in universal vaccination programmes. The results of PROTECT expand the evidence of efficacy and safety of hepatitis B vaccination in older individuals and those with chronic conditions known to be associated with impaired response to immunisations. The results of CONSTANT (NCT03408730), which have not been published yet, will expand the evidence of efficacy and safety of hepatitis B vaccination in young adults. Also, our findings are relevant to populations in the USA, Canada, and Europe, where TAV has not been extensively studied before. The efficacy and safety of TAV in neonates, children, and adults in countries that have licensed TAV for over two decades show its potential use in all ages and in other jurisdictions as well.

A potential limitation of this study include the use of a surrogate marker for protection against HBV infection, anti-HBs titres (≥10 mIU/mL).[21] Consistent with previous studies of hepatitis B vaccine,[21] participants were followed to 48 weeks after the first vaccination. As such, the long-term durability of seroprotection with TAV could not be evaluated. A high percentage of adults treated with TAV had anti-HBs titres of at least 100 mIU/mL, which in some countries in Europe is considered a better surrogate marker for protection than measurements of at least 10 mIU/mL[32] and the higher GMC antibody titres warrant future studies to explore the duration of protection. The exclusion of patients with known immunodeficiencies is a limitation that should be considered in future clinical trials.

The robust immunogenicity generated with TAV was non-inferior in healthy participants aged 18 years or older and superior in participants aged 45 years or older compared with MAV, meeting both co-primary endpoints of the PROTECT trial. Higher immunogenicity was seen with TAV compared with MAV at all timepoints and in all age groups, including those individuals with concurrent medical conditions known to be associated with impaired immune response to immunisations. TAV proved to be well tolerated with no new safety risks identified.

Contributors

GL-R, PvD, JNS, NM, BY-R, DEA, VP, and FD-M determined the concept and design of the study. GL-R, IL-R, JNS, NM, BY-R, DEA, VP, and FD-M acquired, analysed, and interpreted the data. JNS, VP, and FD-M drafted the manuscript. TV, JML, NS, BJW, CC, GP, BS, SG, JEM, MD, PvD, IL-R, GL-R, JNS, NM, BY-R, DEA, VP, and FD-M critically revised the manuscript for important intellectual content. JNS, VP, and FD-M did the statistical analyses. JNS, NM, BY-R, DEA, VP, and FD-M were responsible for administrative, technical, or material support. All authors vouch for the completeness of data, analyses, and fidelity of this report to the protocol. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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*For the Canadian Immunization Research Network.

Declaration of interests

JNS, NM, BY-R, DA, VP, and FDM are employees of VBI Vaccines. TV, JML, NS, BJW, CC, GP, BS, SG, JEM, MD, PvD, IL-R, and GL-R received funding from VBI Vaccines for the conduct of the study.

Data sharing

Data will not be available for general use because of intellectual property and commercial reasons. However, the sponsor will consider requests for dataset sharing for scientific and clinical data analysis with the intent of peer-reviewed publication. The datasets, study protocol, and statistical analysis plan might be available after approval of a proposal and after signing a data access agreement.

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