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Efficacy and safety of therapeutic vaccines for the treatment of chronic hepatitis B

A systematic review and meta-analysis of randomized controlled trials update

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Abstract

Background: Most people diagnosed with chronic hepatitis B (CHB) need treatment to help reduce the risk of liver disease and limit disease transmission. Therapeutic vaccine (TV) candidates have been under study for their clinical effects on inducing HBV-specific host immune responses. This review aimed to systematically synthesize updated evidence on the efficacy and safety of TVs in patients with CHB.

Methods: This systematic review was performed by searching different databases from January to February 2021. Completed randomized controlled trials that reported TVs' efficacy and/or safety for treating CHB compared with the standard of care (SOC) or placebo were included. Efficacy and safety estimates were reported as the logarithm of the odds ratio and risk differences, respectively. $I^2 > 50\%$ was considered significant heterogeneity. Significant publication bias was considered when Egger's test P value < .10. The risk of bias was assessed using the Cochrane Risk of Bias tool. The GRADE methodology was used to assess the certainty of the evidence for each outcome.

Results: Twenty-four articles with 2889 pooled samples were included. TVs made a significant difference in hepatitis B envelope antigen (HBeAg) SC (log OR = 0.76, P = .01) and (log OR = 0.40, P = .03) compared to placebo and combination therapy, respectively. HBeAg SC was significantly affected by TVs at the end of follow up (log OR = 0.49, P = .01), with significant HBsAg mean difference (MD = –0.62, P = .00). At the end of treatment, the TVs had no significant effect on HBV DNA negativity over the SOC (log OR = 0.62, P = .09) or placebo (log OR = –0.07, P = .91). TVs do not significantly affect the risk of serious adverse events (RD 0.02, 95% CI 0.00–0.04).

Conclusion: In patients with CHB, TVs had significant effects on HBeAg SC compared to the SOC or placebo. There was no significant difference between serious adverse events. TVs are promising treatment strategy to overcome CHB.

Abbreviations: cccDNA = covalently closed circular deoxyribonucleic acid, EOF = end of follow up, EOT = end of treatment, HBeAg = hepatitis B envelope antigen, HBsAg = hepatitis B surface antigen, HBV = hepatitis B virus, LAM = lamivudine, NAs = nucleotide analogues, RCT = randomized clinical trial, SAE = serious adverse events, SOC = standard of care, TV = therapeutic vaccine, YIC = yeast based immunocomplex vaccine.

Keywords: chronic hepatitis B, randomized clinical trial, therapeutic vaccine

1. Introduction

Hepatitis B virus (HBV) infection remains a life-threatening and leading cause of liver disease worldwide.[1,2] Each year, an estimated 1.5 million people are infected with HBV, 820,000 die, and 296 million live with chronic hepatitis B (CHB).[3] HBV has a non-cytopathic nature, so the host immune response determines whether the virus is cleared or induces immunopathology and liver damage.[4] CHB depends upon defective cell-mediated immunity, an exhausted phenotype of HBV-specific T cells,

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impaired dendritic cell functions, and an imbalance of cytokine production.[5] The immunotherapeutic approach remains the most effective way to induce the host immune response to overcome the defects, terminate viral persistence, and hasten the functional cure of CHB.[6]

A safe and effective prophylactic vaccine against HBV infection has been available since the 1980s and is the pillar of hepatitis B prevention.[7] However, preventive vaccines do not cure established HBV infections. Most approved approaches to treat CHB aim to control viral replication with several nucleos(t)ide analogues such as tenofovir disoproxil fumarate (TDF), entecavir, lamivudine, adefovir, telbivudine, and tenofovir, or standard and pegylated interferon-alpha and prevention with prophylactic vaccines that generate humoral responses.[8–10] However, these antiviral therapies are ineffective for HBV elimination; they require long treatments and induce undesirable side effects. The nucleos(t)ide analogues-based polymerase inhibitors such as entecavir and TDF result in losing hepatitis B surface antigen (HBsAg) in less than 10% of subjects after many years of therapy, even though they effectively inhibit HBV genome replication. These require a life-long treatment to maintain viral suppression but do not eliminate the risk of developing hepatocellular carcinoma.[11]

HBV virus-infected cells possess a stable pool of covalently closed circular viral DNA (cccDNA) reservoirs for viral replication and antigen production.[12,13] The cccDNA, which persists in the host cell nucleus, drives the viral rebound and recurrent disease once therapy is discontinued.[14] Therefore, these antivirals must be administered indefinitely to prevent the reoccurrence of liver disease. Alternatively, therapeutic vaccination using prophylactic vaccines alone or combined with interferon-alpha or nucleos(t)ide analogues was designed to improve or modulate host immune responses in patients with CHB.[15,16] Therapeutic vaccination can potentially eliminate HBV in chronically infected patients.[16] Various formulations satisfy this need by providing therapeutic combinations or compositions and methods for inducing an immune response against HBV infection. Immunogenic compositions/combinations and immunotherapeutic approaches can provide immunity to a subject with CHB. Several clinical trials have been performed using different vaccine formulations; however, none has demonstrated sufficient clinical efficacy.[16,17]

Some previous reviews assessed the efficacy of therapeutic vaccines against CHB; however, they are relatively outdated to inform recent advances in the area. This systematic and meta-analysis aimed to synthesize contemporary evidence about the efficacy and safety of therapeutic vaccines against CHB.

2. Methods

2.1. Search strategy

This systematic review and meta-analysis followed the Preferred Reporting Items for Systematic Review and Meta-Analysis guidelines.[18] The databases PubMed, EMBASE, Cochrane Library, Clinical Trials.gov, and Google Scholar were searched from January to February 2021 for completed randomized controlled trials (RCTs) published in English that reported the efficacy or safety of therapeutic vaccines for treatment against CHB. Search terms used were (Chronic Hepatitis B) AND (therapeutic vaccination) AND (Clinical Trial) ("hepatitis B, chronic"[MeSH Terms]) OR ("chronic hepatitis B"[All Fields]) AND (("therapeutics"[MeSH Terms]) OR ("therapeutics"[All Fields]) AND ("vaccination"[MeSH Terms]) OR ("vaccination"[All Fields]) OR ("vaccines"[MeSH Terms]) OR ("vaccines"[All Fields])). Reference lists of key articles were searched manually to find additional eligible trials.

2.2. Eligibility

Eligibility criteria were designed using the PICOS (participants, interventions, comparison, outcomes, and study designs)[19]:

Participants: patients with CHB.

Intervention: HBV therapeutic vaccine of any type used with curative intent, including vaccines with various HBV antigens, various adjuvants, various routes of administration, DNA vaccines and modified live vaccines.

Comparator: receiving no treatment, placebo, or standard-of-care.

Outcome: end-of-treatment hepatitis B envelope antigen (HBeAg) seroconversion and/or undetectable HBV DNA, the incidence of adverse events.

Study design: randomized controlled trials.

Studies were excluded if their data were unreliable, had only an abstract or were conference papers, reviews, and in vitro or animal studies.

2.3. Study selection

Two independent authors read and reviewed the title and abstract of the studies identified. The full texts of the included articles were downloaded and screened to select relevant articles. Studies that were duplicated and did not meet the inclusion criteria were excluded. Two authors reviewed the full texts of the remaining studies, and disagreements were resolved by consensus.

2.4. Data extraction and outcomes

Data extracted from the RCTs include the name of the first author, study year of publication, baseline characteristics of participants, type/name of the HBV therapeutic vaccines with their composition, dosage, and delivery systems, and efficacy and safety outcomes, including undetectable HBV DNA, HBeAg seroconversion, and HBeAg loss. Undetectable HBV DNA was the primary endpoint, but not all studies reported or achieved this. Therefore, a reduction in HBV DNA was used as an alternative. For each efficacy endpoint, 2 time points were reported, status at the end of the treatment (EOT) period (measured ± 4 weeks from the last dose) and at the end of the post-treatment period follow-up (EOF). Safety outcomes were assessed by the incidence of adverse events (AEs).

2.5. Statistical analysis

The meta-analysis was performed using STATA version 16 (StataCorp LLC, Texas) with a random-effects model. Efficacy effect estimates were reported as the logarithm of odds ratio or risk differences. Safety estimates were reported as risk differences (risk in the therapeutic vaccine [TV] group minus risk in the Control group) with 95% confidence intervals (CI).

The heterogeneity of studies was evaluated using the I^2 test, which describes the variability in effect estimates because of heterogeneity beyond sampling error.[20] The extent of heterogeneity between studies was checked using the I^2 tests, where $P < .10$ or $I^2 > 50\%$, indicating significant heterogeneity.[21] A sensitivity analysis was performed to confirm that any single study did not drive our findings. A P value $< .05$ was considered statistically significant. Publication bias was checked with Egger's regression test and represented graphically by a standard funnel plot with 10 or more studies. Egger's regression test with a P value $< .10$ was considered significant publication bias. The trim and fill methods were applied to add studies that appeared to be missing to enhance the symmetry whenever publication bias was found.

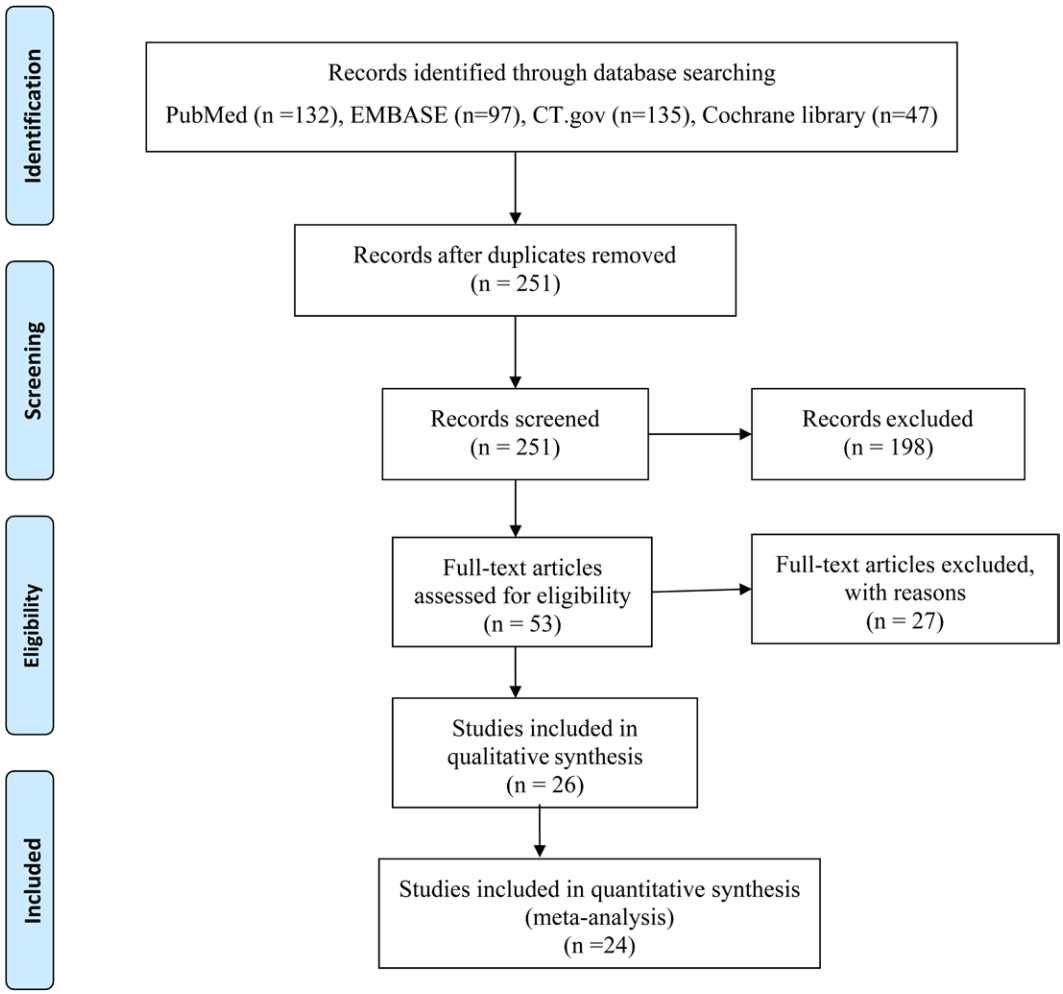

Figure 1. PRISMA flow diagram screening and selection process. PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

2.6. Risk of bias assessment

The Cochrane Risk of Bias tool version 2.0 was used to assess the study and outcome level risk of bias.[22] An overall risk of bias (RoB) judgment for each outcome was formed by adopting the worst-case judgment from any of the RoB domains (sequence generation, allocation concealment, blinding of participants and investigators, blinding of outcome assessment, incomplete outcome data, selective outcome reporting, and other sources of bias). The judgment of each reviewer on each domain is categorized as low risk, high risk, or some concerns of bias.

2.7. Certainty of evidence

For each outcome, the Grading of Recommendations, Assessment, Development and Evaluations methodology was used to assess the certainty of the evidence across all contributing studies.[23] The reporting of vaccine efficacy and safety incorporates the Grading of Recommendations, Assessment, Development and Evaluations of certainty of evidence ratings and adopts standardized expressions recommended by the Cochrane Collaboration.[24]

2.8. Ethical approval

Ethical approval is not necessary because it is a systematic review and meta-analysis.

3. Results

3.1. Study characteristics and types of vaccines

We identified 251 unique records after the removal of duplicates. The titles and abstracts were screened, and 198 records were excluded. After the full-text screening, 26 articles were finally included in the qualitative analysis[25–50] and 24 studies were included in the quantitative analysis. Two studies were included only for qualitative analysis as they did not report efficacy outcomes[49,50] (Fig. 1). Funnel plots were generated for some outcomes since the number of studies per outcome for most results was too small, and the influence of other biases could not be ruled out.

The pooled sample size of the 24 articles included in the meta-analysis was 2889. Among those studies included in this review, 14 of them compared therapeutic vaccines with the standard of care, such as nucleosi(t)ide analogues and interferon-alpha. The others were comparing therapeutic vaccines with no treatment and placebo. Most of the studies focused on adults as the targeted population. A summary of the included studies and the characteristics of their patients are presented in Table 1.

An overview of the vaccine types, dosages, routes of administration, dosing schedules, and sample size is provided in Table 2. Various vaccines were tested, ranging from yeast-derived immunocomplexes to DNA vaccines. The HBV antigenic structures used to formulate the vaccines were S, pre-S1, pre-S2, core, X, and HB Immunoglobulin G (HBIG). The number of administrations varied from 3 to 12 over 8 to 52 weeks, with an average

Table 1

General characteristics of the studies included in this review.

Author (year) country	Patient characteristics	Vaccine type	Outcomes	EOT	EOF
Zoulim (2020)[50]	18–65 yr receiving nucleo(s)ides treatment, undetectable HBV DNA > 6 mo, and 3 were HBeAg+, ALT ≤ ULN	TG1050	HBeAg Sc HBsAg loss HBV DNA reduction SAE	Na Na Na	Na Na Na
Wu (2019) China[25]	Adults 18–65 yr, HBeAg + prior to ETV treatment, HBsAg+ ≥ 6 mo prior to enrollment, HBV DNA ≤ 1000 copies/mL	Hep B + IFN and rhIL-2,	HBeAg Sc HBsAg loss HBV DNA reduction SAE	+ + + +	+ + + –
Boni (2019)[26]	18–69 yr HBsAg > 6 mo, not received antiviral treatment for HBV within 3 mo of screening, HBV DNA 2000 IU/mL	GS-4774	HBeAg Sc HBsAg loss HBV DNA reduction SAE	+ - na -	+ - na -
Gane (2019)[49]	18–69 yr HBsAg + for 6 mo an HBeAg (-) and HBV DNA < 20 IU/mL undetectable at screening, receiving either tenofovir disoproxil fumarate or entecavir	Heat-inactivated yeast	HBeAg Sc HBsAg loss HBV DNA reduction SAE		Na + na -
Mahtab (2018)[27]	18–65 yr treatment naïve, HBsAg+ and HBV DNA in Blood > 6 mo, ALT > ULN, HBV levels were more than 103 copies/mL for hepatitis HBeAg (-) patients and 104 for HBeAg (+)	NASVAC	HBeAg Sc HBsAg loss HBV DNA reduction SAE	+ na + –	+ na + –
Yang (2017) China[28]	18–55 yr HBsAg+ and HBeAg+ >6 mo, ALT 2–10	Electroporation-mediated	HBeAg SC		+ -
	Times ULN, HBV DNA > 106 Copies/mL (1.79 × 105 IU/mL)	Dual-plasmid HBV DNA Vaccine	HBsAg loss HBV DNA reduction SAE		+ +
Lok (2016) USA[29]	18–65 yr HBsAg+ for >6 mo, virally suppressed HBV DNA < 29 IU/mL at screening	GS-4774	HBeAg SC HBsAg loss HBV DNA reduction SAE	+ + Na –	+ + Na +
Fontaine (2014) France[30]	18–75 yr, HBeAg-negative (62) and HBeAg positive (8), HBV DNA > 6 log10 IU/mL	DNA vaccine	HBeAg SC HBsAg loss HBV DNA reduction SAE		+ + + +
Yoon (2014) Korea[31]	19–55 yr HBsAg+ ≥6 mo, HBeAg+, no antiviral	HB-110	HBeAg SC		+
	Therapy for ≥6 mo before screening, HBV DNA >105 copies/mL, ALT 1.5–5 times ULN		HBsAg loss HBV DNA reduction SAE		+ +
Xu (2013) China[32]	18–65 yr, HBsAg+ and HBeAg+ >6 mo, ALT 2–10 Times ULN, anti-HBeAb(-), HBV viral load >100,000 copies/mL	YIC	HBeAg SC HBsAg loss HBV DNA reduction SAE	+ - + -	+ - + +
Yang (2012) China[33]	18–47 yr, HBsAg+ and HBeAg+ >6 mo, ALT of 2–10 times ULN, HBV DNA > 104 copies/mL	dual-plasmid HBV DNA vaccine	HBeAg SC HBsAg loss HBV DNA reduction SAE		+ – +
Wang (2010) China[34]	Adults, HBsAg+, HBeAg+ and HBeAg-, HBV DNA > 7 log 10 copies/mL, ALT > 3 times ULN	YIC	HBeAg SC HBsAg loss HBV DNA reduction SAE		– + - +
Hoa (2009) Vietnam[35]	16–70 yr, HBsAg+ >6 mo, HBeAg+ and anti-HBeAb-, elevated ALT 2x ULN	Recombinant HBsAg	HBeAg SC HBsAg loss HBV DNA reduction SAE		Nr + + +
Xu (2008) China[36]	18–65 yr, HBsAg + and HBeAg+ >6 mo, HBeAb- with, ALT of 2–10 × ULN, HBV DNA. 100,000 copies/mL	YIC	HBeAg SC HBsAg loss HBV DNA reduction SAE	+ - + -	– + - + +
Vandepapeli`ere (2007) Belgium[37]	Adults (18–60 yr) HBsAg+, HBeAg+ >6 mo, ALT 2-5 × ULN	Recombinant HBsAg with AS02B liquid adjuvant	HBeAg SC HBsAg loss HBV DNA reduction SAE	+ – + +	+ – + +
Ishikawa (2007) Japan[38]	HBeAg+ and HBeAg-, DNA detectable	HB vaccine	HBeAg SC HBsAg loss HBV DNA reduction SAE	+ + + –	+ + + –

(*Continued*)

Table 1

(*Continued*)

Author (year) country	Patient characteristics	Vaccine type	Outcomes	EOT	EOF
Karaoglan (2006)[39]	Children, Naive patients of CHB and hepatitis-B carriers, HBeAg+ and HBeAg-, DNA+ and DNA-, ALT> 1.5ULN	Recombinant HBsAg	HBeAg SC		+
			HBsAg loss		+
			HBV DNA reduction SAE		+
					–
Horiike (2005) Japan[40]	Adults, positive HBsAg and HBV DNA. HBeAg+ and HBeAg-, elevated levels of serum ALT	Recombinant HBsAg * (S and pre-S2 proteins)	HBeAg SC	**+**	+
			HBsAg loss	nr	nr
			HBV DNA reduction SAE	+	+
				nr	nr
Helvac (2004) Turkey[41]	2–13 yr, HBsAg, HBeAg and HBV-DNA positivity > 6 mo; elevated ALT	Hepvac with aluminum hydroxide as adjuvant	HBeAg SC	EOT9: at	+
			HBsAg loss	mo 9	+
			HBV DNA reduction SAE	(week 39)	+
					–
Yalcin (2003) Turkey[42]	14–60 yr; positive for HBsAg, HBeAg and HBV DNA; normal ALT on at least 3 occasions	Genhevac-B with aluminum hydroxide as adjuvant	HBeAg SC	+	+
			HBsAg loss	+	+
			HBV DNA reversion	+	+
			SAE	-	+
Yalcin (2003a) Turkey[43]	16–54 yr, HBsAg+ and anti-HBeAb+, HBeAg-, undetectable HBV-DNA, normal ALT >6 mo	Genhevac-B	HBeAg SC	na	na
			HBsAg loss	+	+
			HBV DNA reduction SAE	+	+
				-	+
DIKICI (2003) Turkey[44]	Children, HBsAg+ >6 mo and HBeAg, HBV-DNA detectable, ALT <1.5 times ULN	GenHevac B	HBeAg SC		-
			HBsAg loss		+
			HBV DNA reduction SAE		+
					-
DIKICI (2003a) Turkey[45]	4–15 yr, HBsAg+ >6 mo and HBeAg, HBV-DNA detectable, ALT < 1.5 times ULN	Gen-Hevac B	HBeAg SC		+
			HBsAg loss		+
			HBV DNA reduction SAE		+
					-
Dahmen (2002)[46]	HBsAg+, HBeAg+ and HBeAg-,	ENGINERX	HBeAg SC		+
			HBsAg loss		nr
			HBV DNA reduction SAE		+
					nr
Jung (2002)[47]	Adults, HBsAg+, Detactable DNA, ALT > 3 times ULN	Hepacare	HBeAg SC		+
			HBsAg loss		nr
			HBV DNA reduction SAE		+
					nr
Pol (2001) France[48]	Adults	GenHevac B and Recombivax	HBeAg SC		+
			HBsAg loss		nr
			HBV DNA reduction SAE		+
					nr

ALT = alanine transaminase, DNA = deoxyribonucleic acid, EOF = end of follow up, EOT = end of treatment, GeneVac-B = recombinant DNA hepatitis B vaccine, HB-110 = hepatitis B 110 vaccine, HBeAg = hepatitis B envelope antigen, HBsAg = hepatitis B surface antigen, HBV = hepatitis B virus, Hep B = hepatitis B vaccine, Hepacare, HepVac = hepatitis vaccine, IFN = interferon, na = not available, NASVAC = intranasal vaccine, Recombivax = recombinant hepatitis B vaccine, rhIL-2 = Recombinant Human Interleukin-2, SAE = serious adverse events, TG1050 = Transgene1050, UNL = upper normal limit.

follow-up of 54 weeks (16–96 weeks). TVs administered within 4 weeks of dosing schedules had a significant effect on HBeAg SC (Log OR = –0.53, $P = .04$) compared to 2 weeks, 8 weeks, and 12 weeks schedules (Fig. 2). Moreover, different nucleos(t)ide analogues were used with the HB vaccine (combination therapy) or only as control. The dose of vaccine monotherapy varied from 10 µg to 20 mg.

3.2. Risk of bias assessment

The risk of bias in efficacy outcomes overall judgment was 54.2% for studies with some concerns but 16.7% for those with some and serious concerns. This difference emanated primarily because of the measurement differences in the outcome and selection of the reported results (Fig. 3). Moreover, the randomization process was the only item or domain completely at low risk of bias for all the studies. There were some serious concerns in 15 studies (62.5%) for adverse events due to the combined concerns of selective reporting and incomplete outcome data. Seven studies had a high risk of bias, while 8 studies had some concern about the risk of bias. Nine studies had a low risk of bias for adverse events (Fig. 4).

3.3. Treatment effects

From the total articles analyzed in this review, 20 reported HBeAg seroconversion, 10 reported HBeAg loss, 17 reported HBV DNA negativity, 5 reported HBsAg loss, and 11 reported ALT normalization at the EOF. At the EOT, 13 articles reported HBeAg SC, 4 articles reported undetectable HBV DNA, 12 reported HBV DNA reduction, and 7 reported HBeAg loss.

The EOT pooled data showed that there was no significant difference between therapeutic vaccines and the comparator in terms of HBeAg seroconversion (log OR = 0.37, $P = .23$). Pooled studies were found to be significantly heterogeneous ($I^2 = 49.04\%$, $P = .03$) (Fig. 5). Additionally, the overall effect estimates did not favor the TV groups over the comparator group regarding HBeAg loss at the EOT (log OR 0.21, $P = .26$); and the

Table 2

Types of therapeutic vaccines with their composition, dosage and delivery systems included in this review.

Author (year) country	Follow up (wk)	CT phase	Vaccine type	Vaccine composition	Vaccine dose (number)	Delivery	Doses interval	Vaccine sample size	Control sample size	SOC doses (duration)
Zoulim (2020)[50]	52	I	TG1050 SD	Ad5V, encoding C, P, e, a fusion Protein	VP = 10^9, 10^{10}, 10^{11}	Null	Null	9	Placebo (3)	Null
Zoulim (2020)[50]	52	I	TG1050 MD	Ad5V, encoding C, P, e, fusion Protein	VP = 10^9, 10^{10}, 10^{11}	Null	Null	27	Placebo (9)	Null
Wu (2019) China[25]	72	IV	Hep B + IFN and rhIL-2		60 µg + 6 mIU + 0.25 mIU (12×)	IM	4	Hep B + IFN and rhIL-2 (32), IFN a-2b (33)	ETV (27)	0.5 mg ETV (72 wk) + 6mIUIFN SC (48 wk)
Boni (2019)[26]	72	II	GS-4774	Heat-inactivated yeast containing S, core, X proteins	2 YU (6×) 10 YU (6×) 40 YU (6×)	SC	4	GS-4774 (168)	TDF (27)	300 mg (48 wk)
Gane (2019) New Zealand[49]	16	Pilot-study	GS-4774	Heat-inactivated yeast containing S, core, X proteins	40 YU + 0.3 mg Nivo (2×)	SC	4	40 YU GS-4774 (10)	Nivo (12)	0.3 mg/kg (4 wk)
Mahtab (2018) Bangladesh[27]	48	III	NASVAC	HBsAg and HBcAg	100 µg + 100 µg (10×)	Nasal + SC	2	NASVAC	Peg-IFN	48 wk
Yang (2017) China[28]	72	IIb	DNA + LAM	pS2.S HBV DNA vaccine plasmid encoding the HBV envelope M and a pFP adjuvant plasmid	2 mg DNA vaccine plasmid + 2 mg adjuvant plasmid (7×)	IM Prime-boost	4	DNA	LAM	100 mg (36 wk)
Lok (2016) USA[29]	48	II	GS-4774 + OAV	Heat-inactivated yeast containing S, core, X proteins	2 YU (5×) 10 YU (5×) 40 YU (5×)	SC	2	GS-4774 (151,11)	Oral antiviral therapy (27, 5)	48 wk
Fontaine (2014) France[30]	72	I/II	CT	pCMV-S2.S DNA vaccine produced encodes the small (S) and middle (pre-S2 + S) proteins of the HBV envelope	Null (5×)	IM		HB02-VAC-ADN DNA (34, 1)	No Rx (36, 1)	48 wk
Yoon (2014) Korea[31]	48	RCT	ADV + HB-110	DNA plasmids encoding HBs, PreS1HBc, Hbpol and hIL-12	2, 4, 8 mg respective (12×)	IM	4	HB-110 (18)	ADV (9)	10 mg/d (48 wk)
xu (2013) China[32]	76	III	YIC	HBsAg-HBIG; Alum as adjuvant	60 µg (12×)	IM	4	YIC	Placebo	0.1% Alum
Yang (2012) China[33]	72	RCT	VM and CT	EP-mediated dual-plasmid HBVDNAvaccine	4 mg (4×)	IM 1prime 3 boost	4	DNA (6,1), DNA + LAM (22, 2)	LAM (11, 2)	100 mg/d (72 wk)
Wang (2010) China[34]	44	RCT	YIC	HBsAg-HBIG	60 µg (6×)	IM	4	YIC (78)	Placebo (78)	Null
Hoa (2009) Vietnam[35]	72	RCT	Sci-B-Vac	Recombinant HBsAg of S, Pre-S1 and Pre-S2 with alum as an adjuvant	20 µg (8×)	IM	4	(VM = 60, 3) (V + LAM = 60, 2)	LAM (60, 9)	100 mg/d (72 wk)
Xu (2008) China[36]	44	IIb	YIC	HBsAg-HBIG	30 µg (6×), 60 µg (6×)	IM	4 and 8	YIC 81, 78 (8)	Placebo 78 (5)	Alum for 20 wk
Vandepapeli`ere (2007) Belgium[37]	52	RCT	HBsAg/AS02	Recombinant HBsAg to be reconstituted into the AS02B liquid adjuvant	100 µg (12×)	IM	2 and 8	HBsAg/AS02 (87, 2)	LAM (98, 4)	100 mg/d (52 wk)
Ishikawa (2007) Japan[38]	48	RCT	HB vaccine	HBsAg vaccine including a part of pre-S2	10 µg (6×) 24 wk	SC	4	28	LAM (25)	100 mg (48 wk)

(*Continued*)

Table 2

(*Continued*)

Author (year) country	Follow up (wk)	CT phase	Vaccine type	Vaccine composition	Vaccine dose (number)	Delivery	Doses interval	Vaccine sample size	Control sample size	SOC doses (duration)
Karaoglan (2006)[39]	12	RCT	VM and CT Vacc + Lami-visol	Recombinant HBsAg (S and pre-S2 proteins)	20, 30, and 40 µg	SC	4	VM (28), LV (33)	Levamisole (32)	2.5 mg/kg 3 times a wk
Horiike (2005) Japan[40]	52	RCT	Combination therapy	Recombinant HBsAg (S and pre-S2 proteins)	20 µg (12×)	ID	2	15	LAM (57)	100 mg/d (52 wk)
Helvac (2004) Turkey[41]	36	RCT	Hepvac + IFN	22 mg of HBsAg particle (M and S Ag) with aluminum hydroxide as adjuvant	20 µg (3×)	IM	4	Hepvac + IFN (25)	IFN (25)/ placebo	Null
Yalcin (2003) Turkey[42]	48	RCT	Genhevac-B	22 mg of HBsAg particle (M and S Ag) with aluminum hydroxide as Adjuvant	20 µg (3×)	IM	4	30	No any medica-tion (17)	null
Yalcin (2003a) Turkey[43]	72	RCT	Genhevac-B	22 mg of HBsAg particle (M and S Ag) with aluminum hydroxide as Adjuvant	20 mg (3×)	IM	4	Genhevac-B (31)	40	null
DIKICI (2003) Turkey[44]	52	RCT	Gen-Hevac B	22 mg of HBsAg particle (M and S Ag) with aluminum hydroxide as adjuvant	20 mg (3×)	IM	4	VM (43)	Placebo (31)	Null
DIKICI (2003a) Turkey[45]	52	RCT	Gen-Hevac B	22 mg of HBsAg particle (M and S Ag) with alum as adjuvant	20 mg (3×)	IM	2 and 4	Gen-Hevac B (23)	28	Null
Dahmen (2002)[46]	36	RCT	ENGINERX	20 µg Recombinant HBsAg with alum as an adjuvant	20 µg VM (1 mL + 300 mg), CT (1 mL + 300 m + 1 ml U) (6×)	SC/ID	2 and4 respec-tive	ENGINERX (9), ENGIN-ERX + IL-2 (5)	LAM (11)	100–150 mg (12 wk)
Jung (2002)[47]	22	RCT	Hepacare	Recombinant HBsAg of S, Pre-S1 and Pre-S2 with alum as an adjuvant	20 µg/mL (8×)	IM 4	4	Hepacare (11)	Placebo (11)	Null
Pol (2001) France[48]	52	RCT	GenHevac B, Recombivax	22 mg of HBsAg particle (M and S Ag) with aluminum hydroxide as adjuvant	20 µg (6×)	IM	4 and 12	GenHevac B (46), Recombivax (34)	Control (38)	20 µg

HBeAg = hepatitis B envelope antigen, HBsAg = hepatitis B surface antigen, ID = intradermal, IFN = interferon, IM = intramuscular, LAM = lamivudine, RCT = randomized clinical trial, SC = subcutaneous, VP = viral particle, YU = Yeast unit.

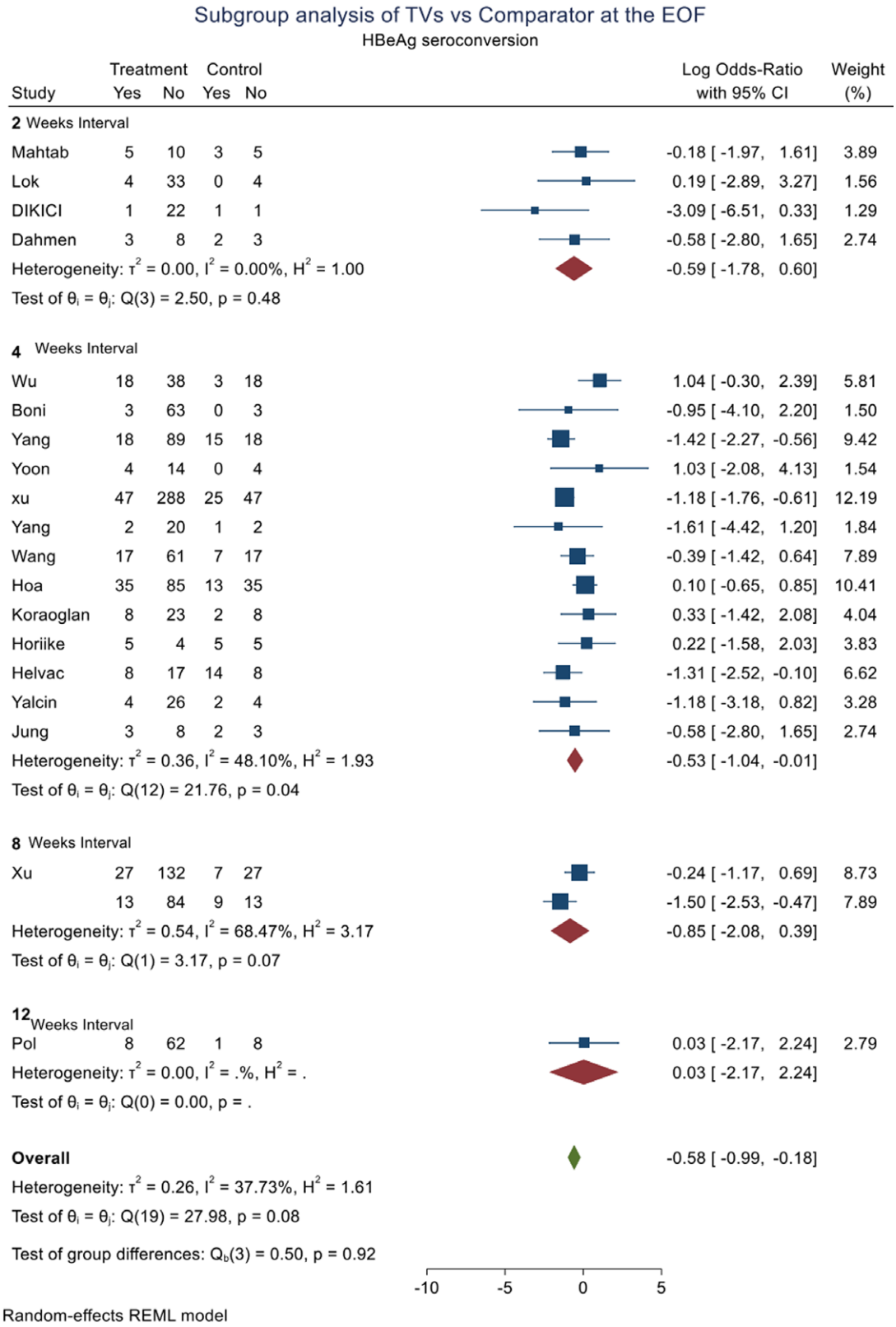

Figure 2. Subgroup analysis by vaccination schedules forest plot of HBeAg seroconversion outcome of therapeutic vaccines group versus comparator group at the EOF. EOF = end of follow up, HBeAg = hepatitis B envelope antigen.

pooled studies were heterologous (I^2 = 33.27%, P = .13) (Figure S1A, Supplemental Digital Content, http://links.lww.com/MD/N360). TVs also had no statistically significant HBV DNA negativity when compared with the comparator group at the EOT (log OR = 0.95, P = .12), but the reduction of HBV DNA level was statistically significant (log OR = 0.04, P = .03) (Figure S1B and C, Supplemental Digital Content, http://links.lww.com/MD/N360). Besides, when HBsAg loss was assessed, our pooled efficacy data at the EOT was not statistically significant between TVs and control groups (log OR = 0.61, P = .45); and the pooled

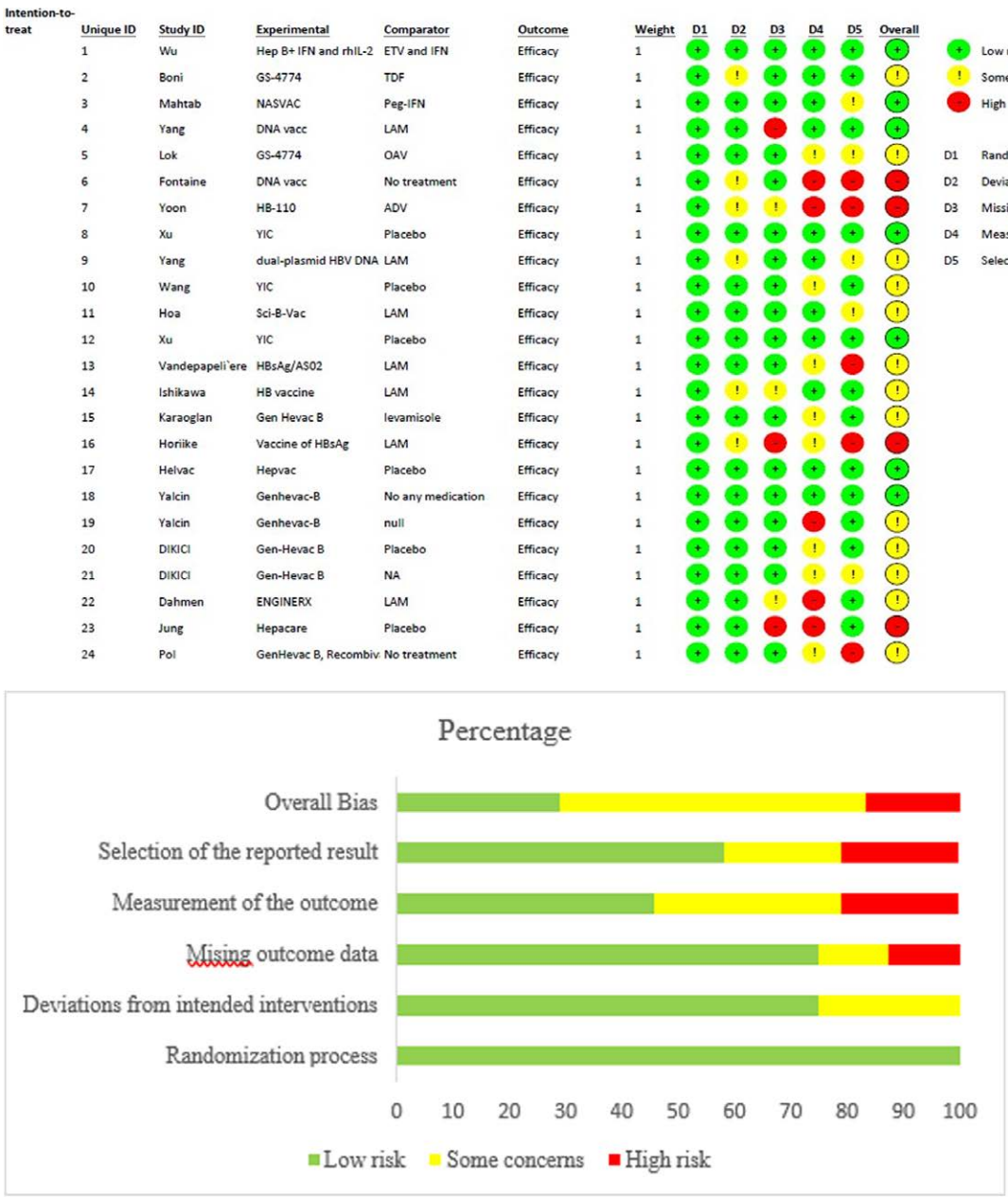

Figure 3. Risk of bias assessment for the efficacy of therapeutic vaccines of hepatitis B virus with the percentage of each domain using Cochrane risk of bias tool.

studies were homogenous (I^2 = 0.00%, P = .89) (Figure S1D, Supplemental Digital Content, http://links.lww.com/MD/N360).

Our pooled data comparing TVs versus the comparator group at the EOF for HBeAg seroconversion showed a significant difference (log OR = 0.49, P = .01); pooled studies showed no significant heterogeneity (I^2 = 39.57%, P = .07) (Fig. 6). Egger's test for small-study effects was performed to detect publication bias. The results showed no publication bias (P = .12) (Fig. 7). The sensitivity analysis indicated no single study effect (Fig. 8). Additionally, the overall effect estimate favored the therapeutic vaccine group over the comparator group regarding HBeAg loss at the EOF (log OR = 0.64, P = .00); pooled studies were homogenous (P = .61, I^2 = 0.01%) (Figure S2C, Supplemental Digital Content, http://links.lww.com/MD/N361). However, the efficacy of therapeutic vaccines at the EOF for HBsAg loss did not have a statistically significant effect difference compared to the control (log OR = 0.75, P = .24); pooled studies were homogenous (I^2 = 0.00%, P = .68). In contrast, the mean difference in HBsAg level was statistically significant in favor of the therapeutic vaccine group over the comparator group (MD = −0.62, P = .00); the pooled studies were homogenous (I^2 = 17.79%, P = .27) (Figure S2D and E, Supplemental Digital Content, http://links.lww.com/MD/N361).

The HBV DNA level reduction of greater than 2log revealed no significant effect difference (log OR = 0.62, P = .09); pooled studies showed significant heterogeneity (I^2 = 72.01%, P = .00) (Figure S2B, Supplemental Digital Content, http://links.lww.com/MD/N361), HBV DNA negativity revealed no significant difference (log OR = 0.13, P = .70); pooled studies showed significant heterogeneity (I^2 = 73.48%, P = .00) (Figure S2A, Supplemental Digital Content, http://links.lww.com/MD/N361). Despite performing subgroup analysis on the country, follow-up, route of administration, and number of doses, none of these factors contributed to the heterogeneity. Egger's test showed

Intention-to-treat	Unique ID	Study ID	Experimental	Comparator	Outcome	Weight	D1	D2	D3	D4	D5	Overall
	1	Wu	Hep B+ IFN and rhIL-2	ETV and IFN	Safety	1	+	+	+	+	!	+
	2	Boni	GS-4774	TDF	Safety	1	+	!	+	+	+	+
	3	Mahtab	NASVAC	Peg-IFN	Safety	1	+	+	+	+	+	+
	4	Yang	DNA vacc	LAM	Safety	1	+	+	-	+	!	!
	5	Lok	GS-4774	OAV	Safety	1	+	+	+	!	+	+
	6	Fontaine	DNA vacc	No treatment	Safety	1	+	!	+	!	!	!
	7	Yoon	HB-110	ADV	Efficacy	1	+	!	!	!	!	-
	8	Xu	YIC	Placebo	Safety	1	+	+	+	+	!	+
	9	Yang	dual-plasmid HBV DNA vaccine	LAM	Safety	1	+	!	+	+	!	!
	10	Wang	YIC	Placebo	Safety	1	+	+	+	!	-	!
	11	Hoa	Sci-B-Vac	LAM	Safety	1	+	+	+	-	-	-
	12	Xu	YIC	Placebo	Safety	1	+	+	+	+	!	!
	13	Vandepapelière	HBsAg/AS02	LAM	Safety	1	+	+	+	!	!	!
	14	Ishikawa	HB vaccine	LAM	Safety	1	+	!	!	-	-	-
	15	Karaoğlan	Gen Hevac B	levamisole	Safety	1	+	+	+	!	+	!
	16	Horiike	Vaccine of HBsAg	LAM	Safety	1	+	!	-	!	-	-
	17	Helvac	Hepvac	Placebo	Safety	1	+	+	+	+	+	+
	18	Yalcin	Genhevac-B	No any medication	Safety	1	+	+	+	+	!	+
	19	Yalcin	Genhevac-B	null	Safety	1	+	+	+	-	!	+
	20	DIKICI	Gen-Hevac B	Placebo	Safety	1	+	+	+	!	!	+
	21	DIKICI	Gen-Hevac B	NA	Safety	1	+	+	+	!	!	!
	22	Dahmen	ENGINERX	LAM	Safety	1	+	+	!	-	+	-
	23	Jung	Hepacare	Placebo	Safety	1	+	+	-	-	+	-
	24	Pol	GenHevac B, Recombivax	No treatment	Safety	1	+	+	+	!	-	-

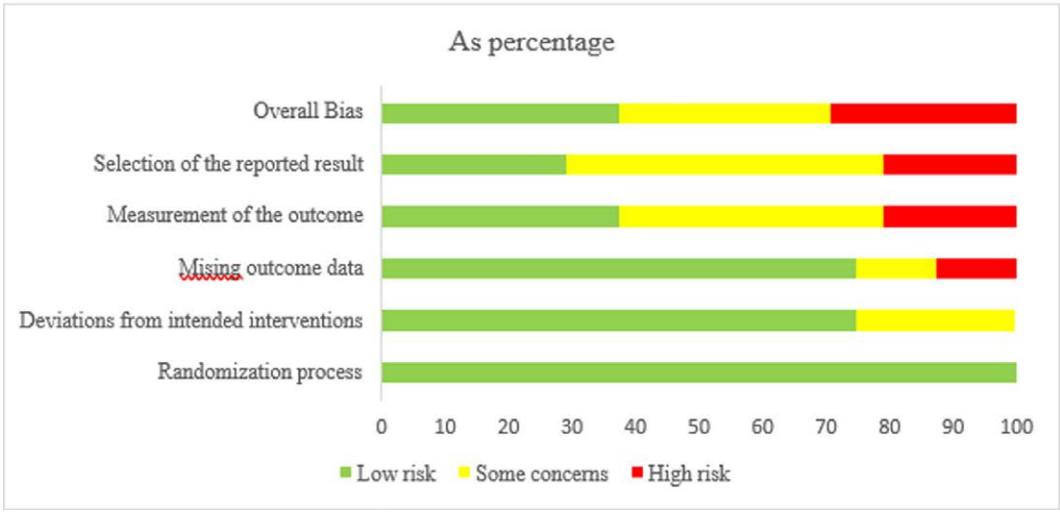

Figure 4. Risk of bias of safety of therapeutic vaccines of chronic hepatitis B with the percentage of each domain.

no small study effects (P = .58) (Figure S3, Supplemental Digital Content, http://links.lww.com/MD/N362).

3.4. Therapeutic vaccine versus placebo/no treatment

Therapeutic vaccines make a significant difference in HBeAg SC in HBeAg + patients (7 studies; 669 participants Log OR = 0.76, P = .01), high certainty of evidence; the vaccine monotherapy had a significant effect over placebo/no treatment (log OR = 0.76, P = .01) and these studies were homogenous (I^2 = 0.00%, P = .94) (Fig. 9). Therapeutic vaccines make no statistically significant difference in HBsAg loss (3 studies; 202 participants, log OR 1.01, P = .21), moderate certainty of evidence, and HBV DNA negativity in CHB patients (7 studies; 614 participants log OR 0.07, P = .91), low certainty evidence (Figure S4A and B, Supplemental Digital Content, http://links.lww.com/MD/N364). In addition, the vaccine monotherapy had no significant effect on HBV DNA reduction in CHB patients (4 studies; 348 participants) over placebo/no treatment (log OR 0.44, P = .09) (Figure S4C, Supplemental Digital Content, http://links.lww.com/MD/N364).

3.4.1. End of treatment. The vaccine monotherapy in HBeAg-positive patients (3 studies; 407 participants) had no significant effect of HBeAg SC over placebo/no treatment (log OR = 0.44, P = .32), and these studies were homogenous (I^2 = 0.00%, P = .51) (Fig. 10). Besides, TVs make no difference in HBsAg loss (one study; 70 participants; log OR = 0.76, P = .54) and HBV DNA negativity in CHB patients at EOT (one study; 70 participants; log OR = 0.03, P = .98) (Figure S5A and B, Supplemental Digital Content, http://links.lww.com/MD/N365). The vaccine monotherapy had no significant effect over placebo/no treatment for HBV DNA reduction (log OR = 0.55, P = .10) (Figure S5C, Supplemental Digital Content, http://links.lww.com/MD/N365).

3.4.2. End of follow-up. Therapeutic vaccines had a significant effect on HBeAg SC at EOF in HBeAg-positive patients (6 studies, 636 participants) over placebo or no therapy (log

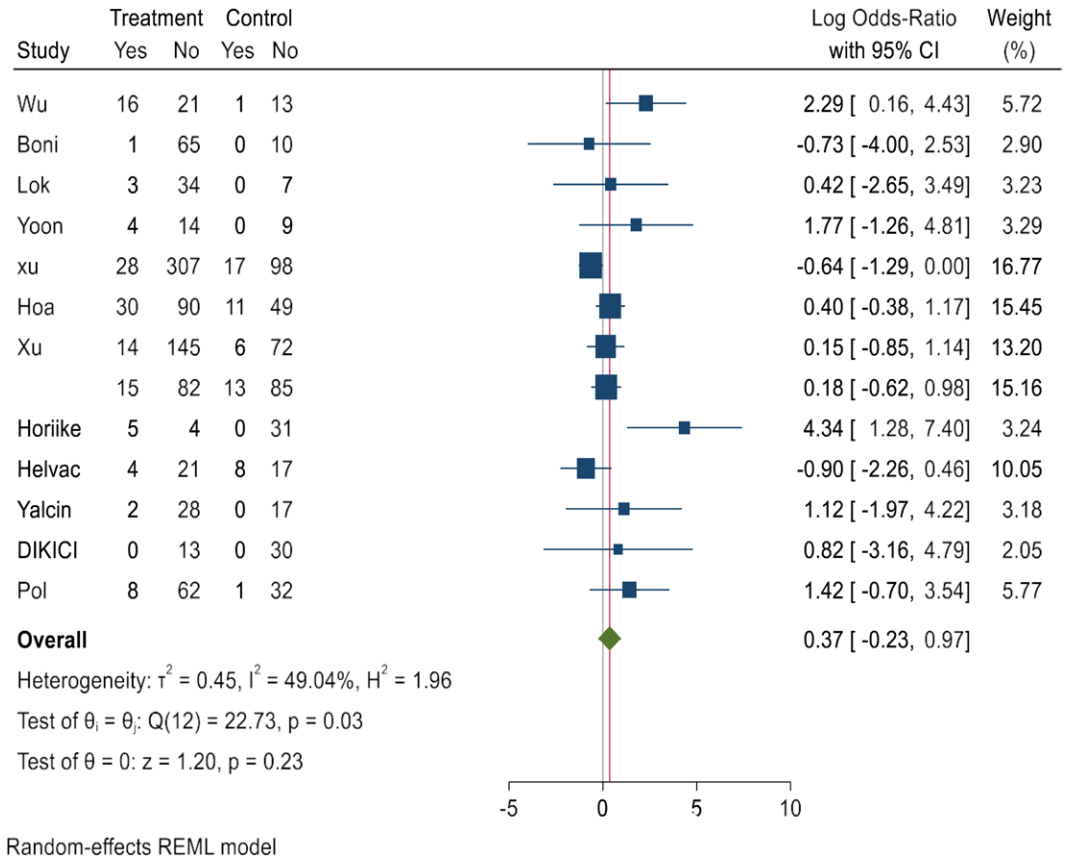

Figure 5. Forest plot of HBeAg seroconversion outcome of therapeutic vaccines group versus comparator group at the EOT. EOT = end of treatment, HBeAg = hepatitis B envelope antigen.

OR = 0.79, $P < .001$). The pooled data from these studies were homogeneous ($I^2 = 0.00\%$, $P = .93$) (Fig. 11). HBsAg loss was evaluated, and TVs made no difference in HBsAg loss (3 studies; 239 participants; log OR = 1.01, $P = .21$) compared to the control group. This showed no significant effect of TVs over placebo or no treatment. The studies regarding the report of HBsAg loss were homogenous ($I^2 = 0.00\%$, $P = .59$) (Figure S6A, Supplemental Digital Content, http://links.lww.com/MD/N366). TVs had no significant effect on HBV DNA negativity in CHB patients (6 studies, 583 participants; log OR = –0.07, $P = .91$) over placebo and/or no therapy. TV also showed no significant effect on HBV DNA reduction over placebo/treatment at the EOF (log OR = 0.35, $P = .20$); the pooled data revealed that the studies reported HBV DNA negativity had significant heterogeneity ($I^2 = 59.44\%$, $P = .01$) but for HBV DNA reduction were homogenous ($I^2 = 0.00\%$, $P = .91$) (Figure S6B and C, Supplemental Digital Content, http://links.lww.com/MD/N366).

3.5. Therapeutic vaccines versus standard of care

Therapeutic vaccines had a significant effect of HBeAg SC in HBeAg positive patients (12 studies; 1453 participants; log OR = 0.39, $P = .03$) compared with standard of care (SOC) taking patients (significant heterogeneity, $I^2 = 68.68\%$, $P = .01$) (Fig. 12), with high certainty of evidence. No significant difference in HBV DNA clearance was found in 10 studies (998 participants; log OR = 0.12, $P = .80$), with moderate certainty of evidence and significant heterogeneity ($I^2 = 79.22\%$, $P = .00$). In addition, TVs had no significant effect on HBV DNA reduction (log OR = 1.06, $P = .10$) with homogeneity of $I^2 = 0.00\%$ and $P = .54$. Besides, HBsAg loss was reported in 2 studies. TVs had no significant effect on HBsAg loss over SOC (log OR = 0.31, $P = .76$) with high certainty of evidence (Figure S7A–C, Supplemental Digital Content, http://links.lww.com/MD/N367).

3.5.1. End of treatment. Combined therapy was no more effective than standard-of-care monotherapy for HBeAg SC in HBeAg + patients in 9 studies of 1439 participants (log OR = 0.40, $P = .36$) with significant heterogeneity ($I^2 = 68.68\%$, $P = .01$) (Fig. 13). Similarly, no more effectiveness was found in HBV DNA reduction in 9 studies of 1294 participants (log OR = 0.14, $P = .53$) with a low heterogeneity ($I^2 = 41.21\%$, $P = .05$) and HBV DNA negativity in 3 studies of 217 participants (log OR = 0.40, $P = .68$) with a moderate heterogeneity ($I^2 = 55.09\%$, $P = .11$). Besides, HBsAg loss at the EOT was reported in one study. Therapeutic vaccines did not significantly affect HBsAg loss over nucleotide monotherapy (log OR = 0.53, $P = .64$) (Figure S8A–C, Supplemental Digital Content, http://links.lww.com/MD/N368).

3.5.2. End of follow-up. Combination therapy significantly affected HBeAg SC over nucleotide monotherapy in thirteen studies of 1525 participants (log OR = 0.54, $P = .02$) (Fig. 14). Besides, TVs significantly affected HBeAg SC compared to nucleotide analogues (log OR = 0.55, $P = .01$) monotherapy but not compared to interferon monotherapy (log OR = 0.36, $P = .63$) (Table 3). The pooled data from these studies were homogeneous ($I^2 = 5.81\%$, $P = .10$). Therapeutic vaccines had no significant effect on HBsAg loss over nucleotide monotherapy in 2 studies of 185 participants (log OR = 0.31, $P = .76$) with significant heterogeneity ($I^2 = 64.04\%$, $P = .10$). TVs had no significant effect on HBV DNA reduction over nucleotide monotherapy in 7 studies with 702 participants (log OR = 1.06, $P = .10$) with a significant heterogeneity ($I^2 = 75.17\%$, $P = .02$). On the other hand, TVs significantly affected HBV DNA

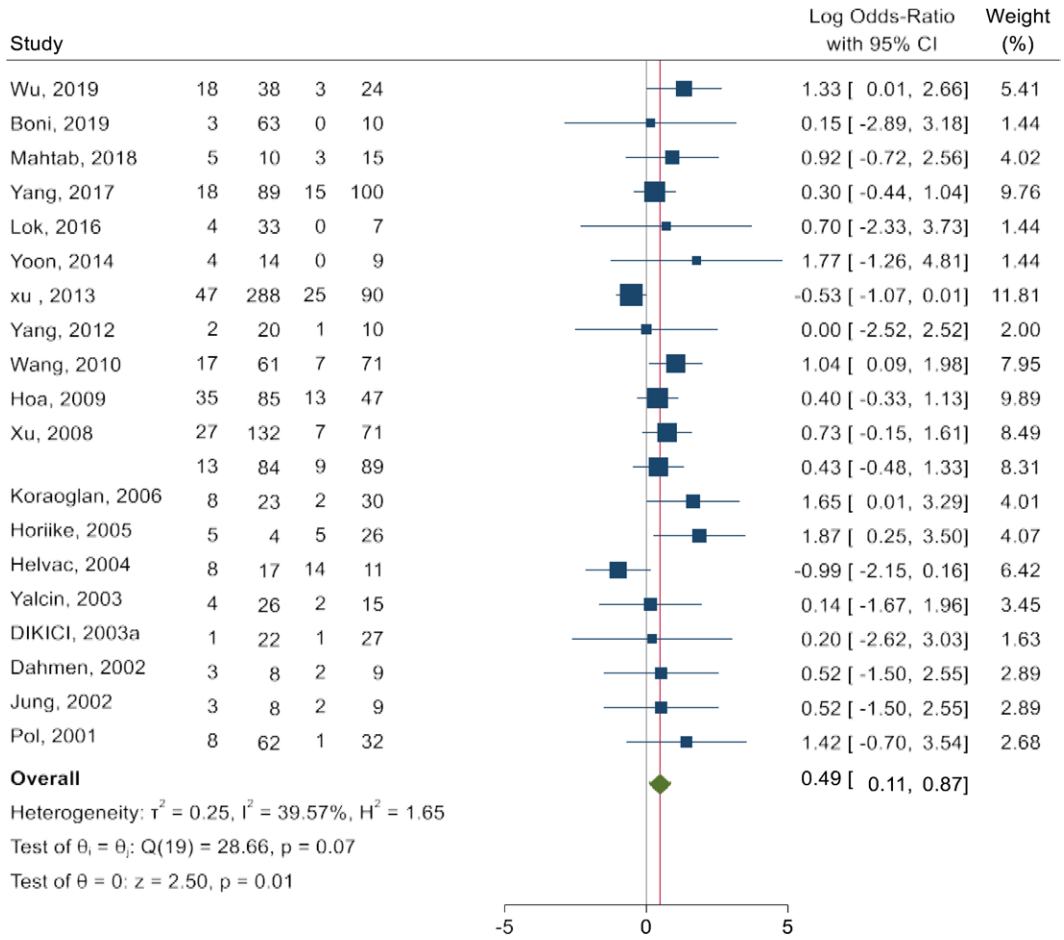

Figure 6. Forest plot of HBeAg seroconversion outcome of therapeutic vaccines group versus comparator group EOF. EOF = end of follow up, HBeAg = hepatitis B envelope antigen.

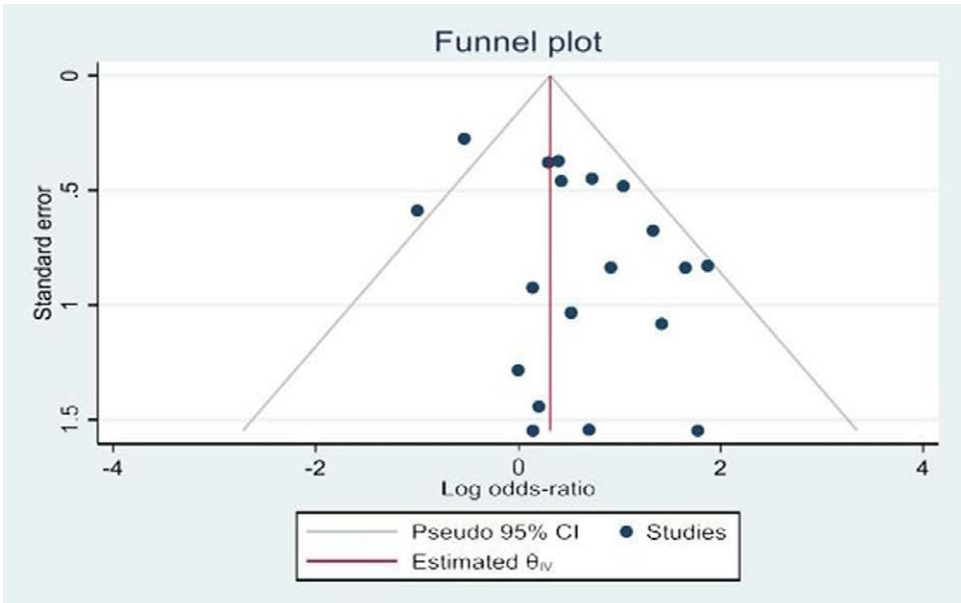

Figure 7. Funnel plot of HBeAg seroconversion of TVs and comparator at the EOF. EOF = end of follow up, HBeAg = hepatitis B envelope antigen, TVs = therapeutic vaccines.

reduction compared to nucleotide analogues (log OR = 1.58, *P* = .01) (Table 3). Combination therapy had no significant effect on HBV DNA clearance over nucleotide monotherapy in 10 studies of 998 participants (log OR = 0.14, *P* = .76) with significant heterogeneity (I^2 = 79.83%, *P* = .00) (Figure S9A–C, Supplemental Digital Content, http://links.lww.com/MD/N369).

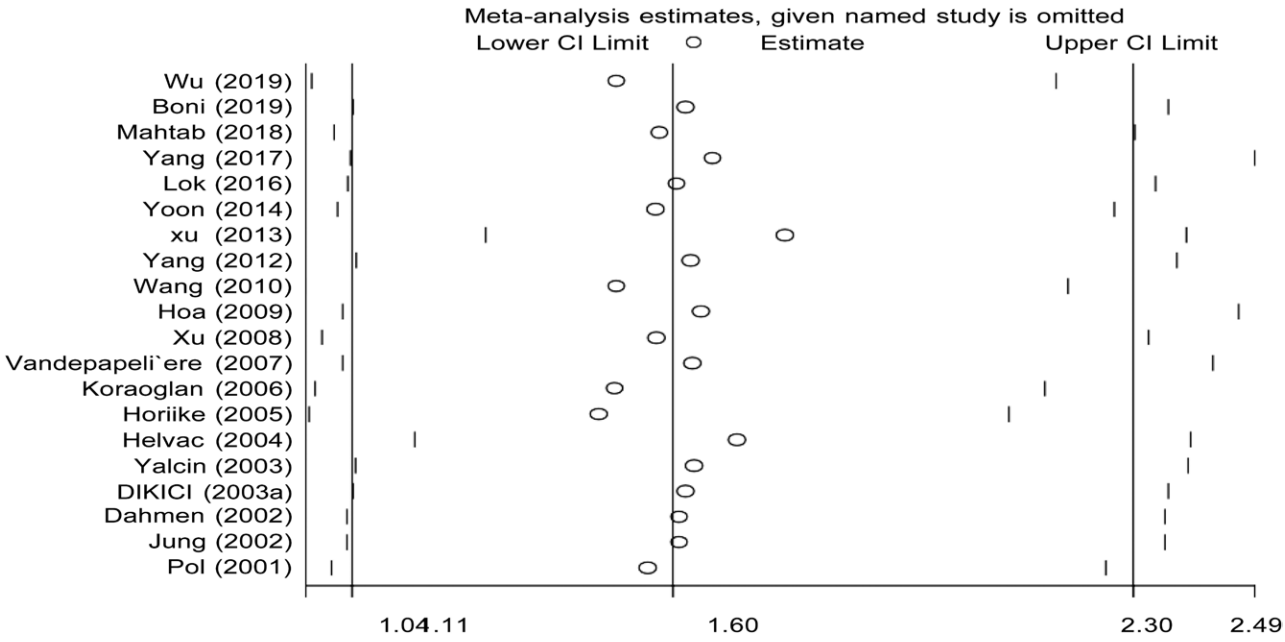

Figure 8. Sensitivity analysis of HBeAg seroconversion at the EOF. EOF = end of follow up, HBeAg = hepatitis B envelope antigen.

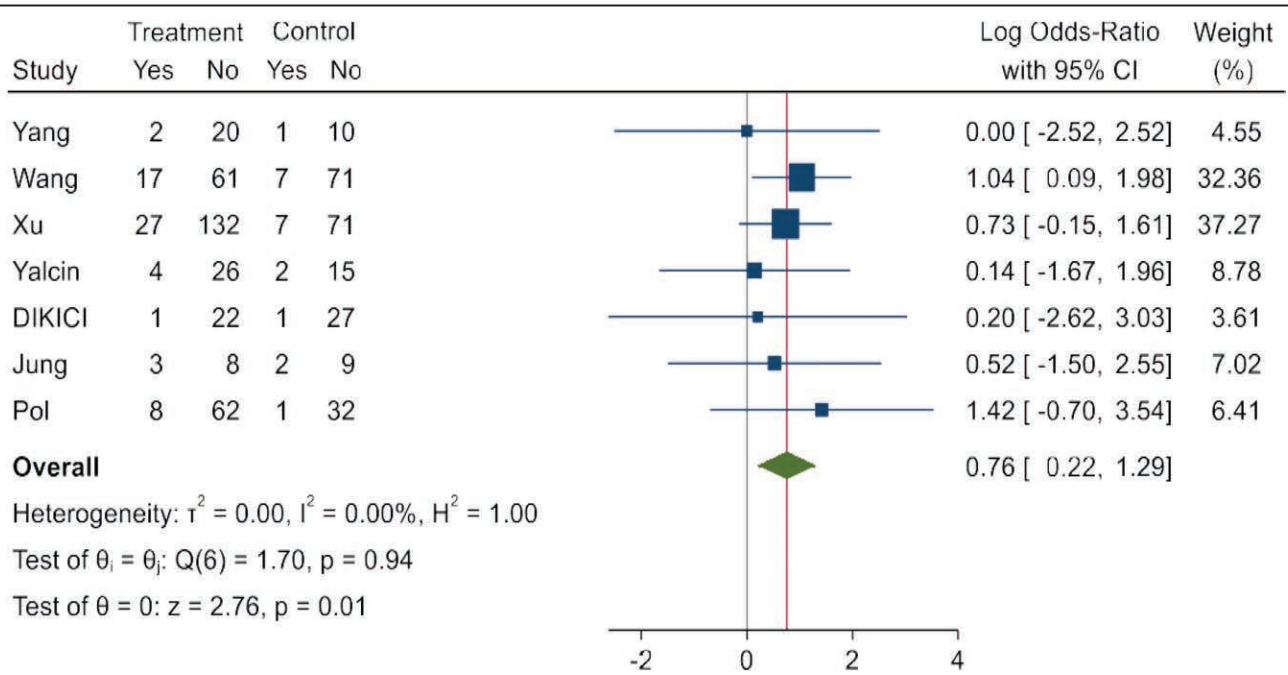

Figure 9. Forest plot of HBeAg seroconversion outcome of therapeutic vaccines group versus placebo/no treatment group. HBeAg = hepatitis B envelope antigen.

TVs significantly affected HBeAg loss compared to interferon-alpha monotherapy (log OR = 1.17, P = .03) (Table 3).

3.6. Adverse events

TVs do not significantly affect the risk of serious adverse events evaluated in 3 studies (870 participants, RD 0.02, 95% CI 0.00–0.04), with high certainty of evidence (Fig. 15). The overall risk of bias regarding the safety of the TV compared with the comparator was moderate. The TV group showed a significant increase in both local and systemic adverse effects compared to the comparator group. Local adverse effects include erythema (RD = 0.43, P < .001), injection site pain (RD = 0.38, P = .01), injection site induration (RD = 0.26, P < .001), and pruritic (RD = 0.25, P = .0001). Systemic adverse effects include chills (RD = 0.08, P = .0001), fatigue (RD = 0.14, P = .04), myalgia (RD = 0.25, P = .0001), and headache (RD = 0.23, P = .0001) (Figure S10A–I, Supplemental Digital Content, http://links.lww.com/MD/N370).

4. Discussion

This systematic review and meta-analysis provided an update on the efficacy and safety of therapeutic vaccines in patients with CHB, including the potential benefit of combining

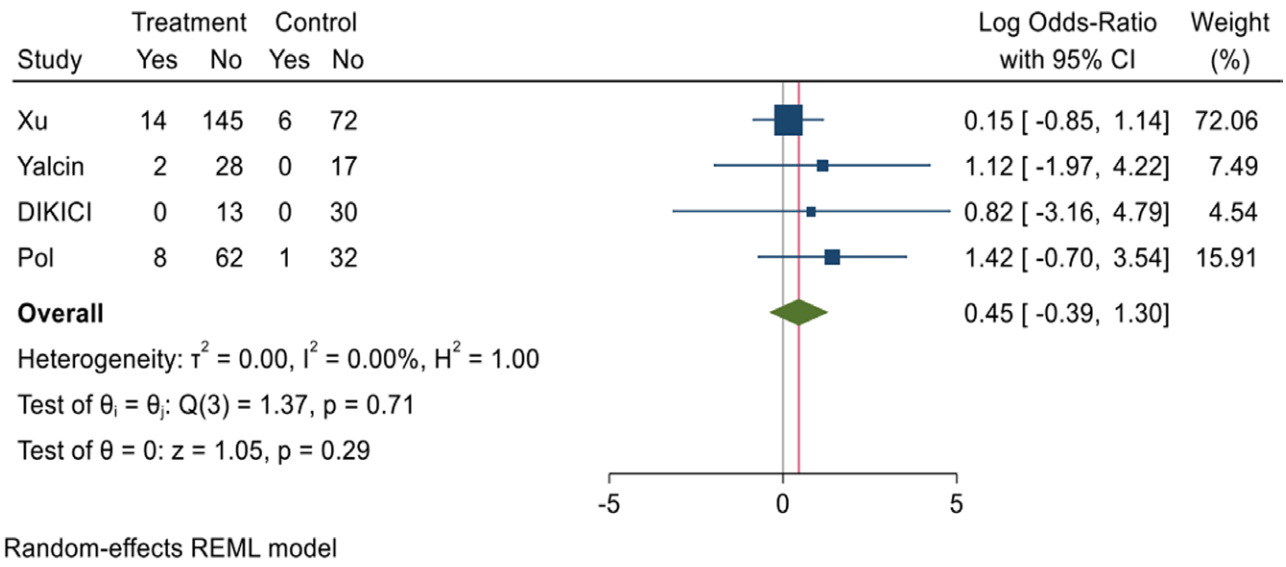

Figure 10. Forest plot of HBeAg seroconversion of therapeutic vaccines group versus placebo/no treatment group at the EOT. EOT = end of treatment, HBeAg = hepatitis B envelope antigen.

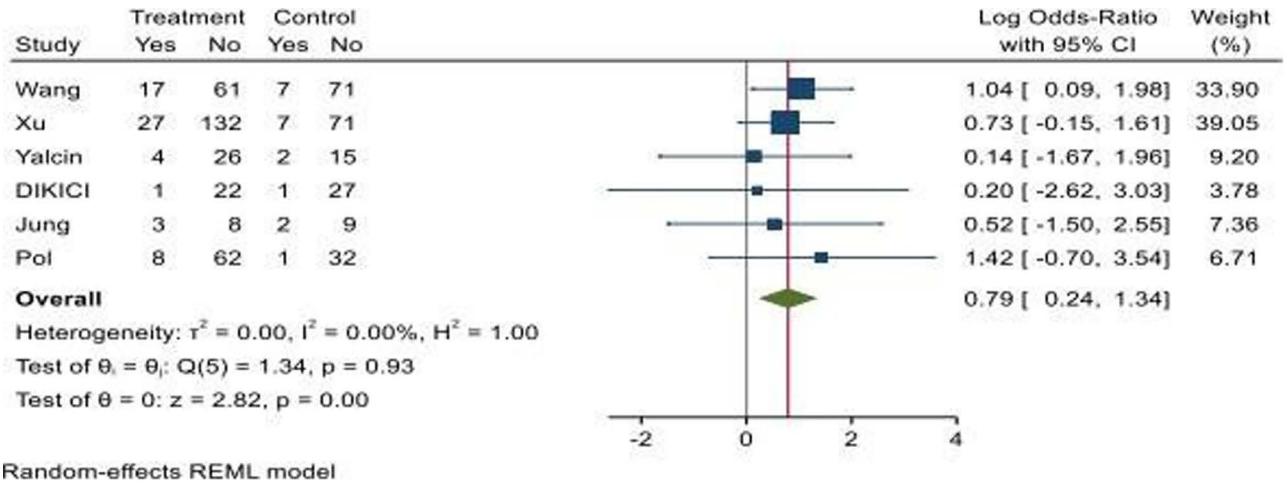

Figure 11. Forest plot of HBeAg seroconversion outcome of therapeutic vaccines group versus placebo/no therapy group at the EOF. EOF = end of follow up, HBeAg = hepatitis B envelope antigen.

hepatitis B vaccines with nucleos(t)ide analogues and vaccine monotherapy. For the treatment of CHB, a standard of care such as interferon-alpha and pegylated interferon-alpha, 3 nucleoside analogues (lamivudine, entecavir, and telbivudine), and 2 nucleoside analogue prodrugs (adefovir dipivoxil and tenofovir disoproxil fumarate) were used for the combination therapy. HBV DNA polymerase is the main target for nucleoside or nucleotide analogues.[51] Treatment of CHB with viral inhibitors has led to significant retardation in HBV-related cirrhosis or liver damage. However, CHB remains uncured due to the intrinsic stability of cccDNA in the liver and extrahepatic sites.[52,53]

The reduction of DNA level and HBeAg seroconversion were the focus of currently available therapeutic strategies. All of these endpoints would imply reducing liver disease progression and damage to hepatocytes. The dysfunction of HBV-specific antiviral immunity persists despite potent suppression of HBV replication in the livers of the treated patients. To achieve a functional cure for CHB, immunotherapy is believed to be a promising strategy, either alone or in combination with SOC.[52] Thus, this systematic review was conducted to summarize the best existing evidence about the role of therapeutic vaccination. The interpretation of this review emphasizes the results reported at EOF and EOT as an evaluation of efficacy.

In the present review, the efficacy outcomes of HBeAg SC, HBsAg loss, HBV DNA clearance, and an alternative HBV DNA reduction were used as surrogate markers or endpoints of efficacy. HBsAg loss is an essential endpoint for a functional cure, even though few studies have reported it. In contrast, HBeAg SC is the most consistent endpoint reported in most of the included studies for assessing TVs, which are likely to be influenced by immunotherapy. The other endpoints for efficacy are HBV DNA clearance and possibly HBV DNA reduction, although quantitative HBsAg and the pretreatment HBV DNA level can affect it after therapy.[54] The outcomes of this review might be influenced by clinical heterogeneity, varied types of vaccine formulations, compositions, and adjuvants, HBeAg status at baseline, different categories of vaccine types, the use of combination therapies and vaccine monotherapy in multiple studies, HBV DNA levels at baseline, and HBsAg levels. Thus, using consistent baseline information in various RCTs may have a significant value for the decision of therapeutic vaccines to have a potent effect on the therapy of CHB. Statistical heterogeneity influenced different therapeutic outcomes, but a

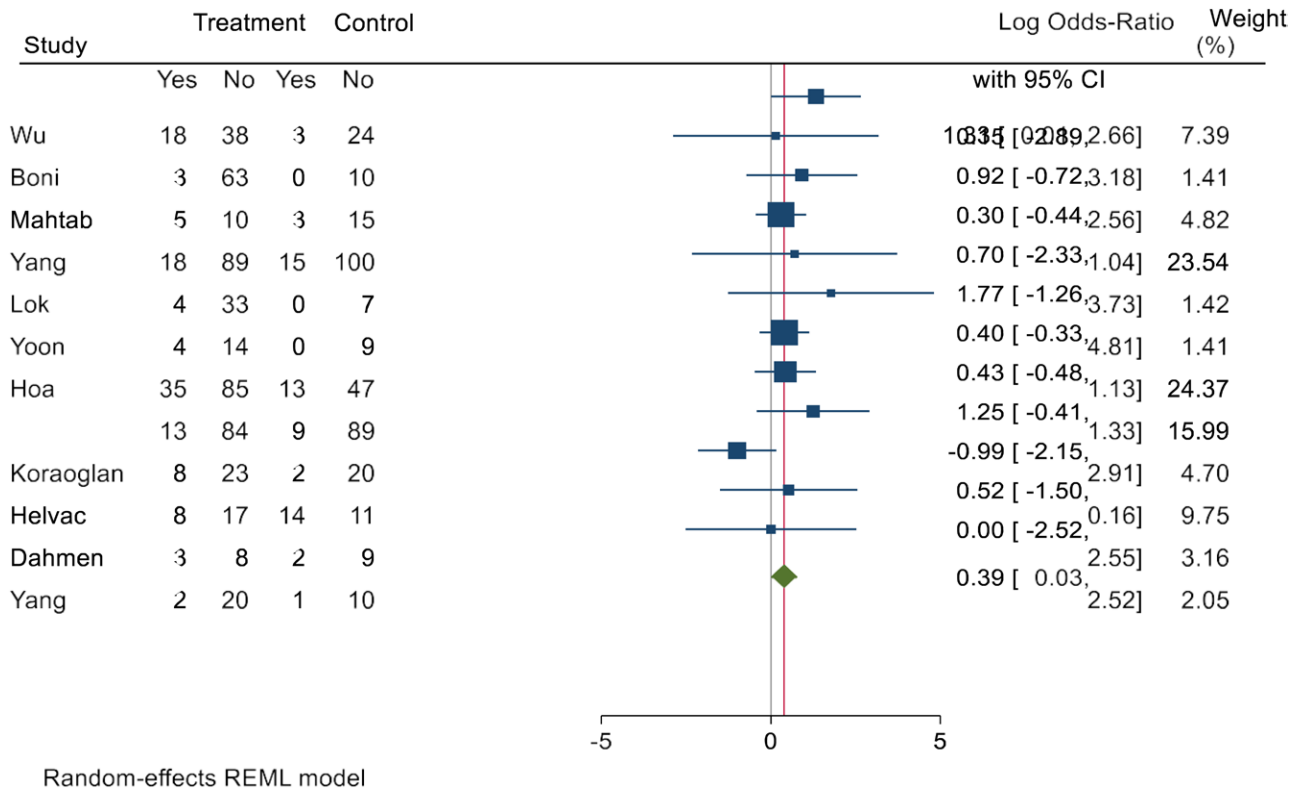

Figure 12. Forest plot of HBeAg seroconversion outcome of therapeutic vaccines group versus standard of care group. HBeAg = hepatitis B envelope antigen.

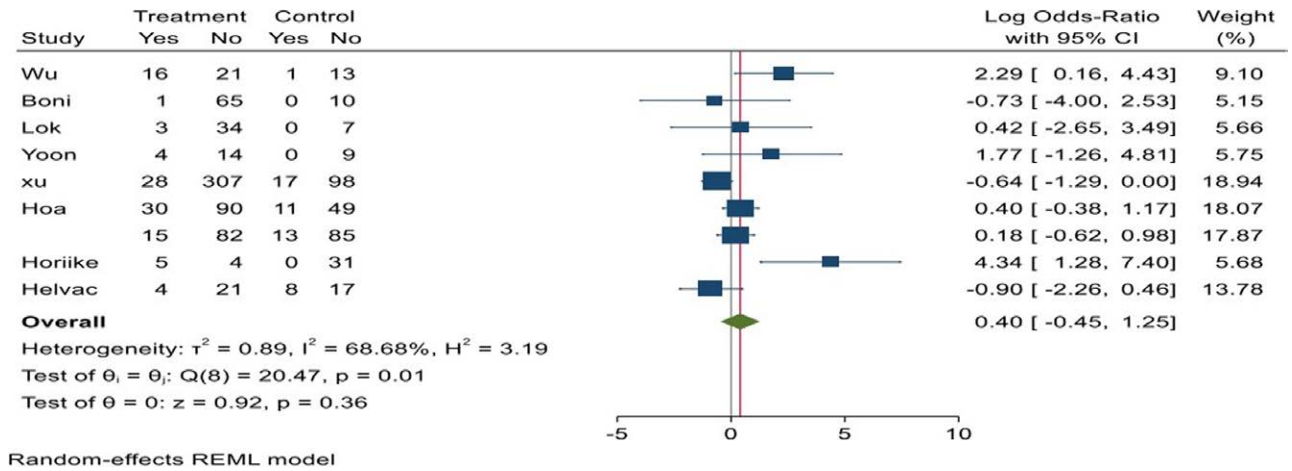

Figure 13. Forest plot of HBeAg seroconversion outcome of therapeutic vaccines group versus standard of care group at EOT. EOT = end of treatment, HBeAg = hepatitis B envelope antigen.

random effects model was employed to remove or reduce the heterogeneity.

The pooled data analysis of this review presented the virological response in the TV group and the comparator group at EOF. TVs had a statistically significant effect on HBeAg seroconversion and HBeAg loss compared to the comparator group at this endpoint. Wu et al,[25] Wang et al,[34] Ishikawa et al,[38] and Karaoglan et al[39] had the same effect of HBeAg seroconversion as the pooled result. Wu et al and Hoa et al also had the same impact on HBeAg loss with the pooled effect. However, TVs had not significantly reduced HBV DNA and cleared HBV DNA compared to the comparator group, even though one study showed a significant effect of HBV DNA reduction[36] and HBV DNA negativity.[35] This meta-analysis also showed no significant loss of HBeAg in TVs compared to the comparator group. At the EOT, HBeAg seroconversion, HBeAg loss, HBsAg loss, and HBV DNA reduction and clearance were not statistically significant different between TVs and comparator groups. Contrarily, a statistically significant difference in HBeAg seroconversion has been reported by Wu et al,[25] and Le Hoa et al,[35] HBeAg loss (Wu et al),[25] and DNA reduction (Le Hoa et al).[35]

The pooled data analysis also revealed that the virological response of combination therapy was statistically significant in reducing HBV DNA levels but not for HBV DNA clearance compared to single nucleotide analogue treatment. At EOT and EOF, combination therapy was found to be less statistically significant in reducing HBV DNA level and clearance than single nucleotide analogue treatment. Therapy with antiviral agents only had poor efficacy because

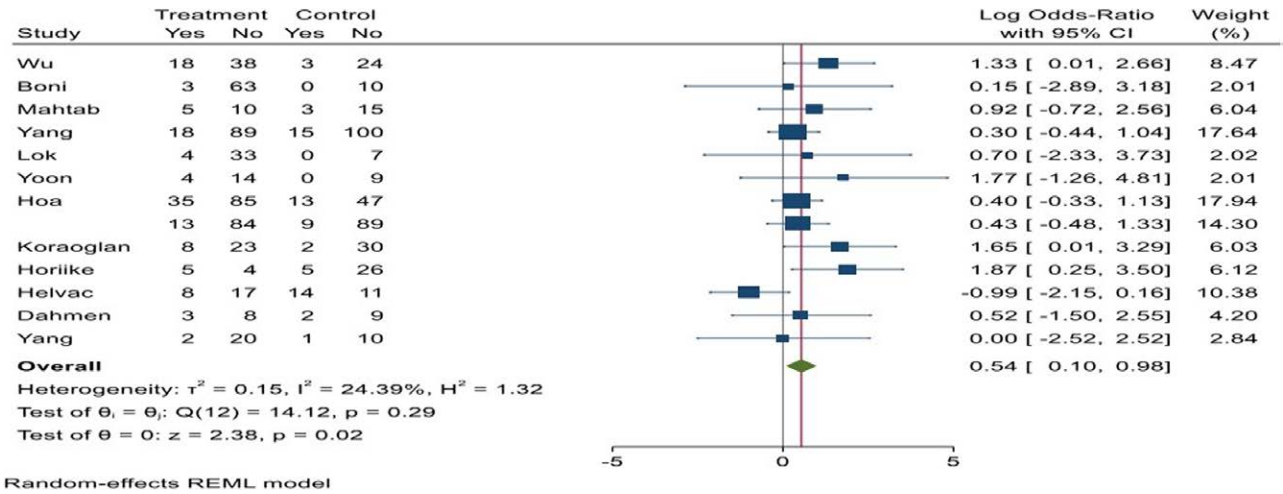

Figure 14. Forest plot of HBeAg seroconversion outcome of therapeutic vaccines group versus standard of care group at the EOF. EOF = end of follow up, HBeAg = hepatitis B envelope antigen.

Table 3

Efficacy parameters of therapeutic vaccine group compared with comparator group, standard of care, interferon, and neuclos(t)ide analogues.

		TVs vs comparator			TVs vs SOC			TVs vs IFN			TVs vs NUC		
Outcomes	**Endpoint**	**Studies**	**Log OR**	***P* value**	**Studies**	**Log OR**	***P* value**	**Studies**	**Log OR**	***P* value**	**Studies**	**Log OR**	***P* value**
HBeAg SC	EOT	13	0.17	.55	9	0.40	.36	2	0.58	.71	7	0.43	.11
	EOF	20	0.49	**.01***	13	0.54	**.02***	3	0.36	.63	10	0.55	**.01***
HBeAg loss	EOT	7	0.16	.37	5	0.23	.37	1	2.29	**.04***	4	0.11	.67
	EOF	10	0.64	**.00***	7	0.63	.05*	2	1.17	**.03***	5	0.42	.25
HBsAg loss	EOT	2	0.64	.45	1	0.53	.64	–	–	–	1	0.53	.64
	EOF	5	0.75	.24	2	0.31	.76	1	1.39	.36	1	–0.66	.64
HBV DNA negativity	EOT	4	0.95	.12	3	0.4	.68	1	–2.10	.16	2	1.28	.06
	EOF	17	0.13	.70	11	0.16	.69	3	–1.01	.43	8	0.49	.30
HBV DNA reduction	EOT	12	0.21	.26	9	0.14	.53	3	–0.10	.93	6	0.07	.76
	EOF	11	0.62	.09	7	1.06	.10	2	–0.63	.73	5	1.58	**.01***

DNA = deoxyribonucleic acid, EOF = end of follow up, EOT = end of treatment, HBeAg = hepatitis B envelope antigen, HBsAg = hepatitis B surface antigen, HBV = hepatitis B virus, IFN = interferon, Log OR = Logarithms of Odds Ratio, NUC = Nuclos/tide analogues, SOC = standard of care, TV = therapeutic vaccine.
*Bold indicates statistically significant effect.

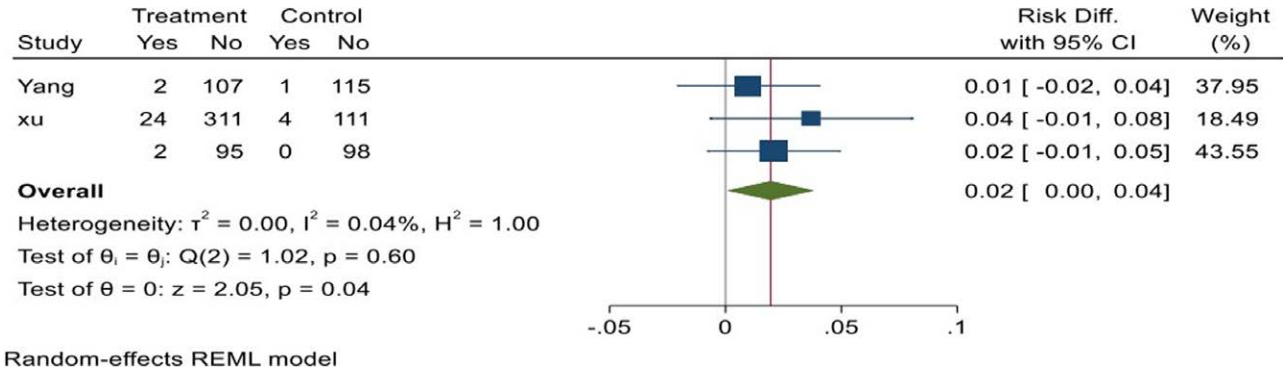

Figure 15. Forest plot of serious adverse events of therapeutic vaccines group versus comparator group.

HBV is noncytopathic for hepatocytes, or the pathogenesis of HBV infection is primarily mediated immunologically.[55] The production of cytokines by HBV-specific T lymphocytes may reduce serum HBV DNA levels via cytopathogenic and non-cytopathogenic pathways.[35] Vaccine monotherapy was also found not to significantly reduce HBV DNA levels and clear HBV DNA at the EOF and EOT compared to the placebo/no treatment group. This result is consistent with the data from each study included in this study.

The pooled data of combination therapy was found to be less statistically significant in HBsAg loss than single nucleotide analogue treatment. Besides, the effect of vaccine monotherapy on HBsAg loss was not statistically significant compared to placebo or no treatment. Additionally, the pooled analysis showed that

HBeAg loss did not favor CT over nucleotide analogues (NAs) monotherapy and VM over placebo or no treatment. In contrast, a significant difference was observed in HBeAg seroconversion between CT and NAs and between the VM and placebo. The result suggests the ability of TVs to induce HBeAg seroconversion (HBeAg loss and development of anti-HBe). HBeAg seroconversion is considered a desired and valuable endpoint in treating HBeAg-positive patients with chronic hepatitis B and marks a transition from the immune-active phase of the disease to the inactive carrier state.[56] Thus, early HBeAg seroconversion with NAs therapy is crucial in managing CHB.[57]

TVs have relevant clinically significant efficacy and acceptable safety compared to no therapy/placebo and SOC. The best estimate of strong evidence of a clinically significant effect of TVs was HBeAg SC from the efficacy outcomes. Vaccine monotherapy substantially impacts placebo/no therapy for HBeAg SC but not for HBV DNA negativity or HBsAg loss. On the other hand, for HBeAg SC, combination therapy makes a significant difference compared to SOC monotherapy. However, for HBsAg loss and HBV DNA clearance, combined therapy had no significant effect over SOC therapy. There was heterogeneity in HBV DNA clearance in this treatment group. A sensitivity analysis showed that no single study was the cause of the positive finding.

The included studies used various types of vaccines as therapeutic vaccines. GenHevac B vaccine comprises HBsAg and pre-S2 protein; Sci-B-Vac24 comprises pre-S1, pre-S2 and S antigen; and the GS4774 vaccine comprises HBsAg, hepatitis B core antigen, and hepatitis B X (HBX) antigen, while the ABX203 vaccine comprises HBsAg and HBcAg. Different types of adjuvants were also used in each category of vaccine. Aluminum hydroxide, known to stimulate B cells, was the most common adjuvant used, and AS02B, which contained monophosphoryl lipid (MPL), was designed as a T-cell adjuvant. Novel TVs, including DNA vaccination, a yeast-derived immune complex vaccine enhanced with IL-2, and a DNA plasmid prime followed by a vector boost vaccine, were also used. While these novel TVs were used, there was no convincing efficacy over the standard TVs. Subgroup analysis of different types of TVs indicated that there was no a significant difference among the TVs.

The TVs in CHB patients can induce anti-HBV immune responses to remove and cure infected hepatocytes without host cell damage, with subsequent prevention of viral spread to new hepatocytes and long-term viral control. Different vaccination frequencies and doses, different viral components as the vaccine, and different delivery approaches were used to prime-boost HBV-specific T-cell responses with TV.[58] Efficient immune responses have been reported in all articles included in this systematic review, except for the functional cure for CHB. For a potentially successful strategy, patients on antiviral therapy or without therapy with an undetectable viral load at baseline showed a seroclerance of HBsAg, suggesting the most promising candidates for future studies of TVs.

Therapeutic vaccinations resulted in acceptable safety, with no statistically significant higher incidence of adverse effects than the comparator. The local side effects of vaccine therapy were injection site pain, erythema, and induration. Furthermore, systemic adverse effects such as myalgia, headache, fatigue, and chills have also been recognized. Proper injection technique plays an essential role in reducing local cutaneous reactions.[59] According to the Centers for Disease Control and Prevention (CDC), these adverse events are common, usually mild, lasting 1 or 2 days.[60] Only 3 studies reported serious adverse events,[33,36,37] which was not a statistically significant risk difference between TVs and the comparator group.

The limitations of this review are that only papers published in English were included. The included studies consist of different vaccines and standards of care. In addition, different populations and sample sizes, various doses and diverse vaccination schedules, and delivery mechanisms make our analysis challenging to conclude the homogenous type of therapeutic vaccines. Although only 7 of the included studies have a low risk of bias concerning the targeted outcome measures, such as HBV DNA clearance and HBsAg loss, increased risk of bias (selective reporting, use of different units of measurement) was also noted. Not all articles reported serious adverse events as counts of events, which restricted the analysis and conclusion of safety.

5. Conclusions

In patients with CHB, therapeutic vaccines, either combination or monotherapy, had significant effects on HBeAg seroconversion at the end of treatment compared to the standard of care or placebo/no treatment. Besides, TVs significantly affected HBeAg SC and HBV DNA reduction greater than 2log compared to nucleotide analogues monotherapy at the end of follow-up. TVs also had the ability to cause HBsAg loss and HBV DNA negativity; however, there was no significant effect on these outcomes. TVs also had no significant effect on serious adverse events. As therapeutic vaccination is a promising treatment strategy to overcome CHB, further clinical trials should be performed to evaluate their efficacy and effectiveness.

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