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Journal Summary: Therapeutic Vaccines for Chronic Hepatitis B—Hope or Hype

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Ever since the first trail for assessing the therapeutic potential of hepatitis B vaccination by Pol et al[1] in 1994 using recombinant peptide vaccine (S and pre-S2 antigen), a fully functional therapeutic vaccine is yet to be translated from clinical trials to clinical practice. Wei et al[2] recently conducted a phase 2 clinical trial involving hepatitis B e antigen (HBeAg) positive chronic hepatitis B (CHB) patients who received a liposome-based nanoparticle vaccine (εPA-44). It had been shown previously by Wang et al[3] that nanoparticles as a delivery system target distinct receptors in T helper cells involved in the pathogenesis of CHB. Improving the delivery of peptide to designated receptor elicit greater immune response in CHB from SIGNR1$^+$ of T follicular helper cells. Li et al[4] have shown that liposome as a vaccine adjuvant promote antigen presentation in dendritic cells via NLRP3 inflammasome pathway. In contrast to previous recombinant vaccines, Wei et al have used a novel liposome-based nanoparticle vaccine delivering a synthetic peptide which was derived from hepatitis B core antigen (HBcAg), tetanus toxoid, and hepatitis B surface antigen (HBsAg), thus combining immunogen, adjuvant, and a delivery system.

Study was done in two stages: stage 1 was a double-blinded placebo-controlled trial where 360 patients were randomized to six doses of placebo arm (900 µg empty liposome), 600 µg (600 µg εPA-44 with 300 µg empty liposome), or 900 µg of εPA-44 and followed for 72 weeks. Stage 2 was an open label study where patients with serological response (HBeAg seroconversion) and virological response (hepatitis B virus deoxyribonucleic acid [HBV-DNA] level $< 2.93 \times 10^4$ IU/mL) in stage 1 were followed until week 144 without any intervention (follow-up group) and nonresponders were given additional 15 doses of 900 µg εPA-44 and followed until week 144 (extended treatment group), primary endpoint of study being HBeAg seroconversion at end of 76 weeks. The results suggest that HBeAg seroconversion rate in the 900 µg group was significantly higher when compared with placebo (38.8% vs. 20.2%, *p*-value 0.002) and no significant difference between the 600 µg group and placebo (28.6% vs. 20.2%, *p*-value 0.13), seroconversion rate of 20.2% in the placebo group was attributed to liposomal activation of immunity. The combined endpoint of HBeAg seroconversion, alanine aminotransferase normalization, and HBV DNA $<$ 2,000 IU/m was 5% in placebo which was significantly lower when compared with 900 µg (18.1%, *p*-value 0.002) and 600 µg (14.3%, *p*-value 0.02). Analysis of the individual endpoints at week 76 showed no significant difference in number of patients with HBV DNA $<$ 2000 IU/mL and undetectable HBV DNA among three groups. Study of change in serum HBsAg levels revealed significant reductions in HBsAg in the 600 µg group in comparison with placebo and 900 µg group at week 52, 64, and 76, although baseline levels were almost similar, which was not explained by authors. Stage 2 further showed 22.1% of patients in the extended treatment group additionally achieved seroconversion, although none achieved functional cure. However, these results cannot be generalized to other population groups as the individuals selected in the study were human leukocyte antigen-A2 positive whose frequency is greater in northern Asian groups as compared with Indian[5] (30.88%) or U.S.[6] (47.6%) population. The vaccine is now undergoing a phase 3 trial (ChiCTR number: ChiCTR2100043708).

Therapeutic vaccination has been gaining new frontiers in the era of precision medicine; it is based on the principle of stimulating the immune system with a target antigen

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Table 1 Summary of latest HBV vaccines

Polypeptide vaccine							
Name	**Vaccine composition**	**Groups**	**Target group**	**Subjects**	**Phase**	**Endpoint/Outcome**	**References**
NASVAC	HBsAg with HBcAg	Peg-IFN vs. NASVAC	HBeAg positive CHB patients	160	3	HBV DNA control was significantly more in NASVAC group ($p < 0.05$) at 24 weeks. HBeAg clearance was more frequent in NASVAC group	Al Mahtab M, Akbar SMF, Aguilar JC, et al. Treatment of chronic hepatitis B naïve patients with a therapeutic vaccine containing HBs and HBc antigens (a randomized, open and treatment controlled phase III clinical trial). *PLoS One*. 2018;13(8):e0201236. Published 2018 Aug 22. doi:10.1371/journal.pone.0201236
GS-4774	Whole yeast cells expressing conserved regions of HBs, HBcAg, and X	GS-4774 + nivolumab vs. nivolumab	ENCI	24	1	No significant difference in mean decline in HBsAg in nivolumab group (-0.30) and nivolumab plus GS-4774 group (-0.16)	Gane E, Verdon DJ, Brooks AE, et al. Anti-PD-1 blockade with nivolumab with and without therapeutic vaccination for virally suppressed chronic hepatitis B: A pilot study. *J Hepatol*. 2019;71(5):900–907. doi:10.1016/j.jhep.2019.06.028
		Tenofovir alone vs. tenofovir with GS-4774	Treatment-naive CHB	168	2	No significant decrease in HBsAg levels	Boni C, Janssen HLA, Rossi M, et al. Combined GS-4774 and Tenofovir Therapy Can Improve HBV-Specific T cell Responses in Patients With Chronic Hepatitis. *Gastroenterology*. 2019;157(1):227–241.e7. doi:10.1053/j.gastro.2019.03.044
BRII-179 (VBI-2601)	3 HBV surface envelope proteins (pre-S1, pre-S2, and S) coadjuvant IFN-α	BRII-179 with or without INF-α	Virally suppressed CHB under NA therapy	49	Ib/IIa	No notable reduction in HBsAg, anti-HBs responses in > 30% patients in all treatment cohorts	Ma H, Lim TH, Leerapun A, et al. Therapeutic vaccine BRII-179 restores HBV-specific immune responses in patients with chronic HBV in a phase Ib/IIa study. *JHEP Rep*. 2021;3(6):100361. Published 2021 Sep 8. doi:10.1016/j.jhepr.2021.100361
HepTcell	9 synthetic HBV derived peptides with TLR 9 adjuvant	HepTcell vaccine vs. placebo	ENCI	Recruiting estimates 80	2 (on-going)	Proportion of patients achieving serologic clearance of HBsAg and HBV DNA	NCT04684914

(*Continued*)

Table 1 (*Continued*)

Polypeptide vaccine							
Name	**Vaccine composition**	**Groups**	**Target group**	**Subjects**	**Phase**	**Endpoint/Outcome**	**References**
Genetic vaccines							
HB110	Improved plasmid of HB-100 encoding S and L HBsAg, Pol, and HBcAg coencoded with IL-12	Adefovir alone or adefovir in combination with HB-110	CHB	27	1b	No HBsAg seroconversion, HBeAg seroconversion in four, HBV DNA $<$ 200 copies in 5	Yoon SK, Seo YB, Im SJ, et al. Safety and immunogenicity of therapeutic DNA vaccine with antiviral drug in chronic HBV patients and its immunogenicity in mice. *Liver Int*. 2015;35(3):805–815. doi:10.1111/liv.12530
TG1050	Nonreplicative adenovirus serotype 5 encoding a fusion protein of truncated HBV Core, modified HBV Polymerase and 2 HBV envelope domains	Single dose and multidose TG1050 vs. placebo	CHB on NT	48	Ib	Both single and multi-dose cohort group decrease of HBsAg remains minor ($<$ 0.2 log), TG1050 was shown to induce IFN-γ producing T-cells	Zoulim F, Fournier C, Habersetzer F, et al. Safety and immunogenicity of the therapeutic vaccine TG1050 in chronic hepatitis B patients: a phase 1b placebo-controlled trial. *Hum Vaccin Immunother*. 2020;16 (2):388–399. doi:10.1080/ 21645515.2019.1651141
TherVacB	DNA plasmid encoding pre- S2 and S, MVAencoding pre-S2 and S	Vaccine alone vs. lamivudine alone vs. vaccine plus lamivudine	CHB	48	Ib	No noticeable sustained effect on viral load, no HBsAg seroconversion. One lost HBeAg in lamivudine plus vaccine cohort	Cavenaugh JS, Awi D, Mendy M, Hill AV, Whittle H, McConkey SJ. Partially randomized, non-blinded trial of DNA and MVA therapeutic vaccines based on hepatitis B virus surface protein for chronic HBV infection. *PLoS One*. 2011;6(2): e14626. Published 2011 Feb 15. doi:10.1371/journal. pone.0014626
Dual plasmid HBV DNA vaccine	Plasmid delivered by electroporation encoding M HBsAg and coencoded IL-12 and IFN-γ	Lamivudine with vaccine vs. lamivudine with placebo	CHB	225	IIb	Significant more cases with $>$ 2 $\log_{10}$ loss of HBV DNA in vaccine group at week 12 after EOT, no significant difference in HBeAg seroconversion and HBV DNA loss	Yang FQ, Rao GR, Wang GQ, et al. Phase IIb trial of in vivo electroporation mediated dual-plasmid hepatitis B virus DNA vaccine in chronic hepatitis B patients under lamivudine therapy. *World J Gastroenterol*. 2017;23(2):306–317. doi:10.3748/wjg.v23.i2.306
Dendritic cell vaccine							
HBV pulsed DC	Dendritic cells exposed ex vivo to HBV peptides including surface antigen, core protein, and polymerase	HBV pulsed DC vs. entecavir vs. entecavir with HBV pulsed DC	HBeAg positive CHB	80	II	Significant reduction in HBV DNA levels with combination therapy than on either monotherapy, nonsignificant greater reduction of	Wei MJ, Pan XN, Wei KP, et al. Efficacy of HBV-pulsed DCs in combination with entecavir in patients with chronic hepatitis B infection. *Int Immunopharmacol*. 2015;27(2):238–243.

Table 1 (*Continued*)

Polypeptide vaccine							
Name	**Vaccine composition**	**Groups**	**Target group**	**Subjects**	**Phase**	**Endpoint/Outcome**	**References**
						HBeAg and anti-HBeAg seroconversion in combination group. None had HBsAg loss	doi:10.1016/j.intimp.2015.06.019
HBV pulsed DC	Dendritic cells exposed ex vivo to peptides of HBcAg and pre-S2	–	CHB	380	Pilot	At the end of 48 weeks, in HBeAg negative CHB 46.36% had had undetectable DNA levels, 10.26% had HBsAg loss. In HBeAg positive CHB 3.13% of had undetectable DNA levels, 29.73% had HBeAg loss and none had HBsAg loss	Luo J, Li J, Chen RL, et al. Autologus dendritic cell vaccine for chronic hepatitis B carriers: a pilot, open label, clinical trial in human volunteers. *Vaccine*. 2010;28(13):2497–2504. doi:10.1016/j.vaccine.2010.01.038

Abbreviations: CHB, chronic hepatitis B; DC, dendritic cells; ENCI, HBeAg negative chronic infection; EOT, end of treatment; HBcAg, hepatitis B core antigen; HBeAg, hepatitis B e antigen; HBIG, hepatitis B immunoglobulin; HBsAg, hepatitis B surface antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid; IFN-α, interferon α; IFN-γ, interferon gamma; IL-12, interleukin 12; MHBsAg, middle hepatitis B surface antigen; MVA, modified Vaccinia virus Ankara; NASVAC, nasal vaccine candidate; NT, nucleos(t)ide analogues; peg-IFN, pegylated interferon; pol, polymerase; TLR-9, Toll-like receptor 9.

Table 2 Advantages and disadvantages of each vaccine type

Vaccine type	Advantages	Disadvantages
Recombinant peptide vaccine	• Immune stimulation and specific response to HBsAg mounted	• Short lasting immune response • HBsAg clearance could not be achieved
Recombinant DNA vaccine	• Can simultaneously express multiple viral peptides along with cytokines • Robust Induction of both humoral and cell mediated immunity • Achieved functional cure in woodchuck and mouse models	• No significant HBsAg clearance achieved in the clinical studies thus far
Viral vector vaccines	• Effective delivery of DNA vaccine • Superiority over other vaccines in terms of T cell induction	• Risk of immune response to virus on repeated vaccination
DC vaccines	• Restimulation of dysfunctional HBV specific CD-8 T cells	• Laborious • No long lasting effect seen with the present available studies

Abbreviations: DC, dendritic cell; DNA, deoxyribonucleic acid; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

(vaccine) and overcoming the immune tolerance to better recognize deleterious organisms or cells. Given its novel way of stimulating the immunity several phase 1 and 2 clinical trials are being performed in infectious diseases (human immunodeficiency virus, CHB, tuberculosis, urinary tract infections), deaddiction, autoimmune diseases (arthritis, diabetes, multiple sclerosis), degenerative diseases (Alzheimer's disease), malignancies, chronic conditions like hypertension, atherosclerosis, and allergies.[5]

Approximately 5 to 10% of acute HBV infection in adults becomes chronic due to defective functioning of HBV-specific T cells.[6] This is due to multiple mechanisms: induction of immune tolerance, expression of immune checkpoint inhibitors, and increased apoptosis. T cells play a major role in HBV clearance.[6] Concordantly, T cell responses are far more abundant and of higher quality in those who achieve clearance after acute HBV infection. Hence, basis of newer modalities for treating HBV infection is to boosting or introducing HBV-directed T cell responses on which therapeutic vaccination is based.

Approaches Used for Therapeutic Vaccination

Therapeutic vaccination has been attempted with different types of vaccines: recombinant vaccine, DNA vaccine, yeast-derived vaccine, and adenoviral vectored vaccines (later 3 can be grouped together as genetic vaccines). Initial trials targeted HBsAg-specific T cells with peptide and ►**Fig. 1** DNA-based vaccine which lead to significant reduction in HBV DNA levels and loss of HbeAg although the responses

were transient with no HBsAg loss, and the T cell response was transient and low showing that vaccination was not solely enough to stimulate dysfunctional T cells.[8] This lead to usage of combination of peptide antigens (NASVAC [nasal vaccine candidate]), DNA vaccine co-encoding interleukin-12 along with viral peptides (HB-110), dendritic cells modified ex vivo pulsed with either HBsAg or HBcAg, viral vector vaccines encoding multiple peptides (TG1050), and yeast-

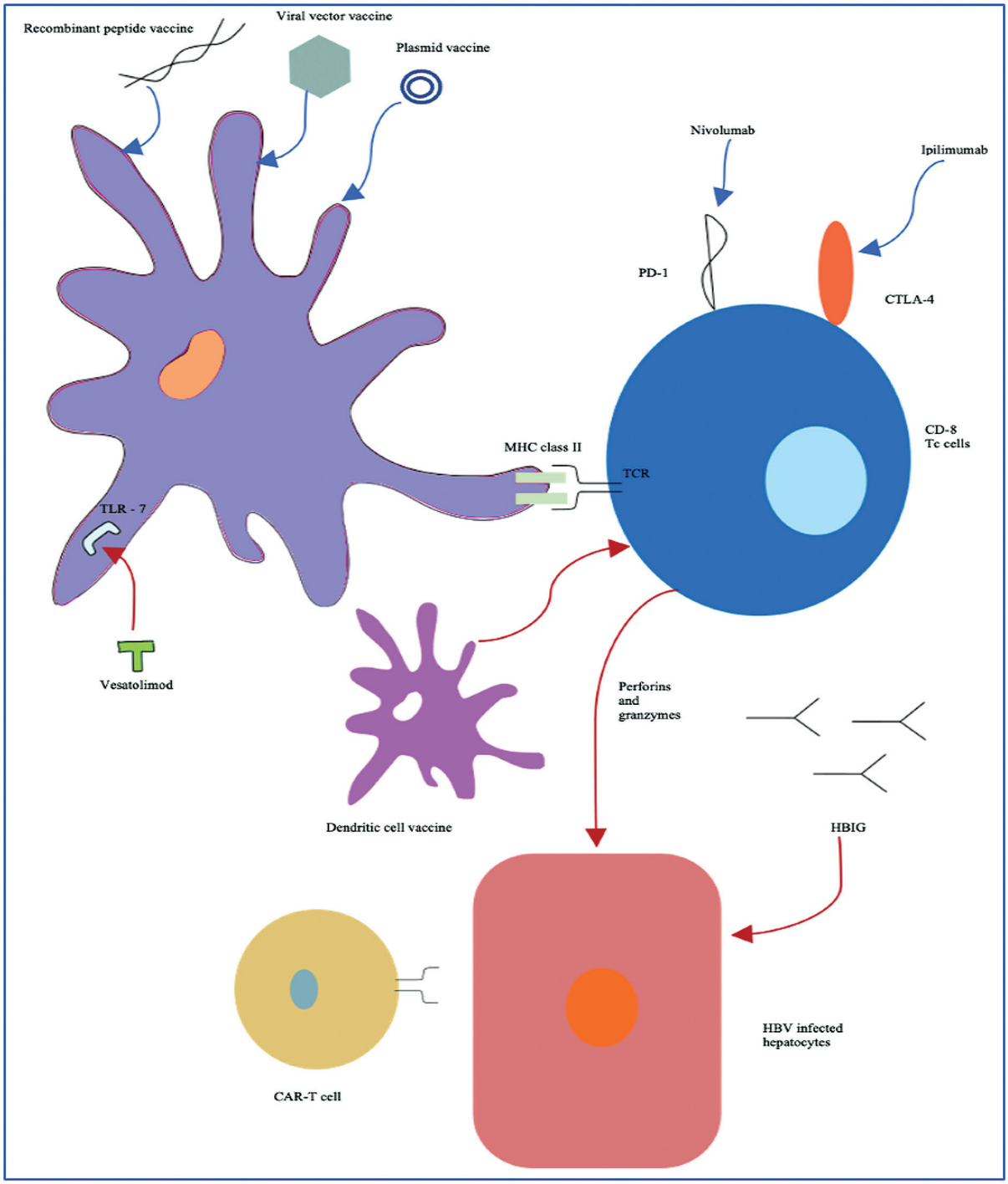

Fig. 1 Potential targets, infected hepatocytes can be targeted by appropriately stimulating both the innate immunity with Toll-like receptor agonist (vesatolimod), and adaptive immunity with therapeutic vaccination which stimulate CD8 T cell by antigen presentation via major histocompatibility complex (MHC) class II, modified dendritic cells pulsed with hepatitis B virus (HBV) peptides ex vivo, stimulation of dysfunctional T cells via ipilimumab which binds to CTLA-4 blocking the T cell inhibitory signals and nivolumab which binds to PD-1 receptor blocking its interaction with PD-L1 and PD-L2, usage of hepatitis B immunoglobulin which directly attacks infected hepatocytes, all of the proposed therapies can be used in conjunction at various stages with the goal of overcoming the dysfunctional immune response and achieving a functional cure. CTLA-4, cytotoxic t-lymphocyte associated protein 4, HBIG, hepatitis B immune globulin, PD-1, programmed cell death protein 1, TCR, T cell receptor, TLR -7, Toll-like receptor 7.

based vaccine expressing combination of viral peptides (GS-4774), most of the studies have shown a significant reduction in the HBV DNA levels, HBeAg loss, and improved T cell response; however, none of them lead to functional cure (HBsAg loss). Therapeutic vaccination can also cause beneficial off-target effects as highlighted in the study by Boni et al[9] on yeast-based vaccine where polymerase (pol)-specific T cell response was seen although it was not a part of vaccine which the authors attributed to adjuvant effect of yeast on pol presenting dendritic cells which can be taken advantage of in future studies. Combination of different forms of genetic vaccine was also studied, TherVacB[10] used combination of DNA and viral vector vaccine is bound to undergo a large-scale multicenter trial starting from June 2022.

However, we would like to point out the recent phase 1 study by Gane et al[11] involving virally suppressed HBeAg negative CHB patients using GS-4774 plus nivolumab showed HBsAg loss in one subject (10%, $n = 10$), although it was not statistically significant it paves the way for the combination of approaches. Newer therapies which when used in conjunction act at various levels of adaptive and innate immune system supporting survival and activation of T cells (CD 8) which play a central role in CHB infection.

Full potential of therapeutic vaccine is yet to be completely explored, using a combination of right antigens, antivirals, and immunoadjuvants at different phases of HBV infection might lead to a functional cure. A summary of the latest HBV vaccines is given in ►**Table 1** and specific advantages and disadvantages of each vaccine type mentioned in brief in ►**Table 2**.

Ethical Statement
Not applicable.

Author Contributions
P.B.N. wrote the initial draft and performed literature search; S.T. did critical revisions; both authors approved the final version.

Data Availability Statement
There are no associated data.

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Conflict of Interest
None declared.

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