**Name of Journal:** *World Journal of Gastroenterology*

**Manuscript NO:** 65987

**Manuscript Type:** EDITORIAL

**Nucleic acid vaccines: A taboo broken and prospect for a hepatitis B virus cure**

Tsounis EP *et al*. Nucleic acid vaccines for HBV

Efthymios P Tsounis, Athanasia Mouzaki, Christos Triantos

**Efthymios P Tsounis, Christos Triantos,** Division of Gastroenterology, Department of Internal Medicine, University of Patras, Patras 26504, Greece

**Athanasia Mouzaki,** Division of Hematology, Department of Internal Medicine, University of Patras, Patras 26504, Greece

**Author contributions:** Tsounis PE was responsible for the literature search and analysis, drafting of the manuscript, and interpretation of the data; Mouzaki A and Triantos C were responsible for revising the manuscript for important intellectual content; All authors gave their final approval of the submitted version.

**Corresponding author: Christos Triantos, FAASLD, MD, PhD, Assistant Professor,** Division of Gastroenterology, Department of Internal Medicine, University of Patras, D. Stamatopoulou 4, Patras 26504, Greece. chtriantos@hotmail.com

**Received:** March 18, 2021

**Revised:** July 7, 2021

**Accepted: October 14, 2021**

**Published online:**

**Abstract**

Although a prophylactic vaccine is available, hepatitis B virus (HBV) remains a major cause of liver-related morbidity and mortality. Current treatment options are improving clinical outcomes in chronic hepatitis B; however, true functional cure is currently the exception rather than the rule. Nucleic acid vaccines are among the emerging immunotherapies that aim to restore weakened immune function in chronically infected hosts. DNA vaccines in particular have shown promising results *in vivo* by reducing viral replication, breaking immune tolerance in a sustained manner, or even decimating the intranuclear covalently closed circular DNA reservoir, the hallmark of HBV treatment. Although DNA vaccines encoding surface antigens administered by conventional injection elicit HBV-specific T cell responses in humans, initial clinical trials failed to demonstrate additional therapeutic benefit when administered with nucleos(t)ide analogs. In an attempt to improve vaccine immunogenicity, several techniques have been used, including codon/promoter optimization, coadministration of cytokine adjuvants, plasmids engineered to express multiple HBV epitopes, or combinations with other immunomodulators. DNA vaccine delivery by electroporation is among the most efficient strategies to enhance the production of plasmid-derived antigens to stimulate a potent cellular and humoral anti-HBV response. Preliminary results suggest that DNA vaccination *via* electroporation efficiently invigorates both arms of adaptive immunity and suppresses serum HBV DNA. In contrast, the study of mRNA-based vaccines is limited to a few *in vitro* experiments in this area. Further studies are needed to clarify the prospects of nucleic acid vaccines for HBV cure.

**Key Words:** Chronic hepatitis B; Therapeutic vaccination; Nucleic acid vaccines; DNA vaccines; Electroporation; Immunotherapy

Tsounis EP, Mouzaki A, Triantos C. Nucleic acid vaccines: A taboo broken and prospect for a hepatitis B virus cure. *World J Gastroenterol* 2021; In press

**Core Tip:** A nucleic acid vaccine could be of particular value in the field of hepatitis B virus therapies. DNA vaccines have been studied more extensively over the past two decades and have been shown to overcome immune exhaustion in preclinical models of chronic infection. Although vaccination elicited robust humoral and cellular immune responses, it had negligible effects on clinical endpoints. Therefore, the scientific community has focused on optimizing vaccine design and delivery to improve immunogenicity. Electroporation-mediated delivery of multivalent plasmids in combination with molecular adjuvants could efficiently restore adaptive immunity in virally suppressed patients and be part of future combination therapy.

**INTRODUCTION**

Hepatitis B virus (HBV) is a relatively small (3.2 kb) DNA virus that causes acute or chronic liver infection leading to cirrhosis or hepatocellular carcinoma. Although an effective preventive vaccine containing hepatitis B surface antigen (HBsAg) produced from recombinant DNA has been available for more than two decades[1], chronic hepatitis B (CHB) remains a major burden of liver-related morbidity and mortality, with more than 292000000 infections worldwide[2]. Current treatment strategies, *i.e.* nucleoside or nucleotide (NA) drugs and interferon-α (IFN-α), effectively suppress viral replication, prevent progression of liver disease to end-stage, and improve patient quality of life. However, a truly functional cure, defined as undetectable HBV DNA with concomitant elimination of HBsAg over a limited therapeutic period, is rarely achieved with existing therapies[3]. HBV has evolved several mechanisms to become chronic, and the failure to eradicate HBV is largely due to the inability to address the following issues: (1) Organization of the viral genome into a highly stable, drug-resistant, chromosomal covalently closed circular DNA (cccDNA) conformation in the nuclei of infected cells; (2) Integration of HBV DNA into the host genome; (3) Disruption of innate immunity signaling; and (4) Depletion of HBV-specific T and B cells[4].

There is a growing consensus that elimination of HBV requires a combination of treatments that include traditional therapeutics, novel direct-acting antivirals that target different steps of the HBV life cycle, and immunotherapies to restore the host immune response. A number of immunomodulators are in clinical trials, including pattern recognition receptor agonists, immune checkpoint inhibitors, adaptive transfer of engineered T cells, and therapeutic vaccines[5]. Various vaccine delivery platforms are being explored to optimize immunogenicity and elicit robust responses to rejuvenate the exhausted immune system. In particular, viral vector technology, immune complex platforms, virus-like particle-based vaccines, and nucleic acid vaccines are some of the categories of therapeutic vaccines under development[6].

Although the world is still struggling with a global health crisis, coronavirus disease 2019 has been at the forefront of scientific breakthroughs in the field of vaccinology, bringing nucleic acid vaccine technology to the forefront. Recent technological innovations have improved the delivery, tolerability, and efficacy of DNA- and mRNA-based therapeutics. Two prophylactic mRNA vaccines against severe acute respiratory syndrome coronavirus 2 were the first drugs in this category to be approved for human use and represent the pinnacle of progress[7,8]. Clearly, the role of nucleic acid-based vaccines in restoring immune dysfunction in CHB and their prospects as part of future combination therapy need to be re-evaluated.

**HBV-INDUCED IMMUNE DYSREGULATION**

Resolution of acute HBV infection requires an alert innate immune system and polyclonal and multispecific cellular and humoral responses. The human innate immune system is equipped with several pattern recognition receptors that recognize pathogen- or damage-associated patterns and initiate intracellular signal transduction leading to the production of antiviral IFNs and proinflammatory cytokines. Previous studies in humans and chimpanzees reported limited IFN type I production during the initial phase of the logarithmic rise in viremia, supporting the view that HBV is a “stealth virus”[9,10]. However, recent data show that hepatocytes express pattern recognition receptors that recognize HBV components, highlighting the role of nucleic acid signaling and the associated activation of NF-κB-dependent pathways[11]. Therefore, it seems more likely that HBV impairs intrinsic immunity to establish chronic infection. Indeed, HBV alters the functional phenotype of monocytes/macrophages by inducing the secretion of anti-inflammatory cytokines interleukin (IL)-10 and transforming growth factor-β and suppressing the production of tumor necrosis factor-α and IL-12 by inhibiting the toll-like receptor-2 downstream signaling pathway[12,13]. In parallel, myeloid-derived suppressive cells are recruited to CHB and contribute to the immunosuppressive cascade by secreting IL-10 and arginase and downregulating IFN-γ expression by T cells[14]. In addition, dendritic cells, which are critical for generating effective adaptive immune responses, exhibit decreased antigen presentation capacity, cell migration capacity, phagocytic activity, and cytokine production, possibly due to inhibition of costimulatory molecule expression[15]. Therefore, HBV creates a tolerogenic microenvironment in the liver infiltrated with IFN-γ-deficient natural killer cells and T regulatory cell populations[16]. This dysregulated milieu has a significant impact on T and B cell maturation and differentiation, resulting in impaired adaptive immune responses. Moreover, prolonged exposure to high concentrations of viral antigens contributes to T cell exhaustion, characterized by reduced cytotoxic capacity, impaired proliferative capacity, and upregulation of inhibitory molecules (programmed death-1 (PD-1), CTLA-4, T cell immunoglobulin and mucin-domain containin-3). Clearly, approaches aimed at restoring or stimulating sustained HBV-specific cellular and humoral responses may be central to the treatment of chronically infected patients.

**DNA VACCINES**

DNA vaccine technology is based on genetically engineered plasmids containing a potent promoter that triggers increased transcriptional activity *in vivo*,followed by a sequence encoding the preselected immunogenic antigen(s). The corresponding plasmid is administered either systemically or primarily topically *via* intramuscular injection. Taking advantage of the host cellular apparatus, the exogenous DNA is delivered to the nuclei of transfected cells, including resident antigen-presenting cells (APCs).

The expression of plasmid-encoded antigens and their presentation by major histocompatibility complex (MHC) class I and MHC class II molecules are key elements of adaptive immunity. In particular, transfected myocytes or keratinocytes express the plasmid-derived proteins, which are later released into the bloodstream *via* exosomes or apoptotic bodies[17]. APCs play an important role in this process by mediating the presentation of vaccine peptides both on MHC-I molecules, either by direct transfection or cross-presentation, and on MHC-II molecules after uptake of circulating antigens. Subsequently, afferent lymphatic vessels transport APCs to lymph nodes, where they present the antigenic epitopes to naïve T and B cells and provide essential costimulatory signals. This interaction leads to clonal expansion of CD8+ T cells, which elicit a strong cytotoxic response, and CD4+ T cells, which regulate the differentiation of antigen-presenting B cells[18].

DNA vaccine platforms have many advantages: they are fast to develop, easy to replicate, and very stable at room temperature, resulting in low manufacturing and storage costs[19]. Theoretically, they are safer than conventional live attenuated vaccines because they do not elicit potential anti-vector immune responses and by continuously expressing antigens elicit a long-lasting response without being infectious[20]. Moreover, the intracellular synthesis of the encoded antigens enables the endogenous post-translational modifications that generate proteins in their native conformation[18]. In addition, the expression of a broader repertoire of antigenic epitopes is possible by combining two or more plasmids or by constructing polycistronic carriers[19]. Indeed, DNA vaccination has shown promising results in preclinical models of chronic hepatitis virus infection. In HBV transgenic mice, vaccination broke immune tolerance and caused a significant decrease in viral replication[21], while in a duck HBV model it was able to reduce the intranuclear cccDNA pool[22]. Moreover, immunization with a DNA prime adenovirus boost vaccine in transgenic mice showed strong synergistic effects with NAs, eliciting sustained suppression of viral replication and strong and specific CD8+ T cell responses[23].

The initial clinical trials investigating therapeutic vaccination in CHB patients showed moderate efficacy in restoring host T cell responses but dampened enthusiasm by demonstrating moderate immunogenicity. In a phase I clinical trial, administration of a DNA vaccine expressing envelope proteins (S and preS2/S) resulted in transient activation of IFN-γ-producing T cells and a reduction in HBV DNA in 10 viremic patients[24]. Repeated immunization doses were well tolerated, resulted in proliferative responses against HBsAg, and achieved hepatitis B e antigen (HBeAg) seroconversion in 2 participants[24,25]. In another study, DNA-based vaccination resulted in changes in peripheral NK cell populations, with a relative increase in CD56bright NK cells correlating with HBV-specific T cell activity, indicating the importance of CD56bright NK cells in shaping the adaptive immune response[26].

To determine whether therapeutic vaccines are unable to reactivate pre-existing HBV-specific T cells due to persistent antigenemia, DNA vaccines were administered in combination with conventional therapeutics. The efficacy of a preS2/S-expressing DNA vaccine was evaluated in a phase I/II clinical trial in virus-suppressed patients on stable NA therapy. Addition of the vaccine to NA treatment elicited multispecific, polyfunctional, and far more sustained CD4+ T cell responses compared with monotherapy. Nevertheless, the immunostimulatory effect was not strong enough to influence relapse rates after discontinuation of NA[27]. Accordingly, five intramuscular injections of an envelope-expressing DNA vaccine failed to sufficiently restore immune function or reduce relapse risk after treatment discontinuation in a prospective multicenter study of 70 virus-suppressed CHB patients[28]. These results have underscored the need to optimize vaccine immunogenicity, use new delivery systems, and re-evaluate vaccination regimens.

Recently, a new generation of DNA-based vaccines has demonstrated significant immunostimulatory activity with a favorable safety profile. Their preventive or therapeutic value has already been investigated in clinical trials on infectious diseases (Ebola virus, ZIKA virus, HIV, influenza virus) or malignancies (prostate cancer, cervical cancer, and human papillomavirus-related head and neck tumors)[29]. This progress is mainly due to the breakthroughs in biomedical engineering and nanotechnology, which have introduced novel delivery platforms to improve DNA uptake compared to previous needle approaches, *e.g.*, gene gun, jet injection, advanced electroporation (EP), and chemical or biological adjuvant systems[19]. In addition, various molecular tools have been used to improve the immunogenicity of DNA vaccines, *e.g.*, codon optimization, plasmid vector backbone optimization, attachment of virus-derived nuclear localization signals to facilitate nuclear entry, or DNA-complexing nanocarriers to prevent extracellular DNA degradation[17,30]. The development of target-specific plasmid-encoded chimeric molecules and polycistronic vectors that contain genetic loci encoding cytokines or other signaling molecules in addition to immunogenic epitopes are alternative strategies to further stimulate immune responses[19,29].

In this regard, Wang *et al*[31] demonstrated the superior protective effect of a bicistronic DNA vaccine encoding the core protein plus IFN-γ gene sequences compared to a monovalent vaccine expressing the core antigen in a model of HBV infection in marmosets. Experimental *in vivo* models have also shown that coadministration of plasmids encoding cytokine sequences (IL-2, IFN-γ) with core-expressing DNA vaccines increases the production of HBV-specific neutralizing antibodies[32] and that the cccDNA reservoir was depleted in the majority of subjects receiving IFN-γ as an adjuvant[33]. In an attempt to optimize antigen delivery to APCs and thereby increase vaccine efficacy, plasmids were combined with a protein that targets dendritic cells *via* electrostatic coupling (pSVK-HBVA vaccine). In HBV transgenic mice, the pSVK-HBVA vaccine significantly reduced HBV DNA copy number as well as circulating HBsAg[34].

These encouraging preclinical data formed the basis for transferring these techniques from the laboratory bench to clinical trials. Yang *et al*[35] showed that the DNA-based vaccine HB-100, which consists of five different plasmids expressing most HBV antigens as well as a human IL-12 mutant (hIL-12N222L) induced durable CD4+ memory T cell responses when administered to 12 chronically infected Caucasian patients receiving regular lamivudine therapy. Moreover, the DNA vaccine in combination with an oral antiviral resulted in HBeAg seroconversion in 4 of 6 participants, whereas HBsAg clearance was achieved in only 1 patient, who had the highest concentration of HBV-specific IFN-γ-secreting memory T cells[35]. Long-term control of viremia also correlated with an increase in plasma IL-12 and IL-12/p40 ratio[36].

These positive observations promoted the development of HB-110, a second generation multivalent HBV DNA vaccine comprising three plasmids encoding envelope proteins, core protein/polymerase, and the human IL-12 mutant. HB-110 was administered in a randomized, dose-escalated phase I clinical trial to Korean patients with CHB treated with adefovir dipivoxil. Although HB-110 elicited robust humoral and T cell responses in the HBV mouse model, vaccination in the Korean cohort resulted in weaker HBV-specific T cell responses and lower HBeAg seroconversion rates than HB-100 immunization in Caucasian patients[37]. These unexpected results may be due in part to the fact that HB-110 was tested in Asian patients, most of whom acquire HBV by vertical transmission and have high immune tolerance. It is clear that in addition to improving the immunogenicity of the vaccine, a combination of therapies is required to address compromised immune function and effectively eradicate chronic HBV infection.

In this context, the advent of immune checkpoint inhibitors has paved the way for alternative strategies to overcome T cell exhaustion or anergy. Remarkably, the addition of a PD-1 receptor inhibitor to treatment with NA plus DNA vaccine resulted in significant expansion and activation of virus-specific CD8+ T cells and prolonged suppression of viral replication in marmosets[38]. A phase I clinical trial of the PD-1 blocker nivolumab alone or in combination with the yeast-derived vaccine GS-4774 in 24 HBeAg-negative virally suppressed patients demonstrated not only high-affinity binding of nivolumab to its ligand but also sustained occupancy of the receptor. In addition, significant HBsAg responses were observed without serious adverse events, and HBsAg levels decreased below the limit of detection in 1 participant[39].

**ELECTROPORATION-MEDIATED DELIVERY**

One of the most efficient methods of DNA vaccine delivery is EP, which uses an electrical pulse to create a potential difference across the cell membrane that creates transient pores, thereby increasing membrane permeability[40]. Compared to conventional injection of plasmid DNA with a syringe, a pulsed electric field dramatically increases cellular uptake, approximately by a factor of 500, resulting in profound immune responses[29]. At the same time, low inflammation and altered vascular permeability cause a local influx of APCs and other immune cells, suggesting robust antigen processing and presentation at the injection site[41]. Cova[42] gave a concise overview of the progress of EP-mediated therapeutic vaccination from experimental models to clinical reality. Initial studies of EP-based DNA vaccination showed that it is capable of eliciting *in vivo* multispecific cytotoxic T cell responses and more effective immune stimulation, resulting in a dose-sparing effect of the vaccine[43]. EP-mediated administration of HB-110 accelerated antigen expression, increased anti-HBs antibody production, and elicited a broader repertoire of multispecific cellular responses in mice against all antigens, including subdominant epitopes[44]. Comparable conclusions emerged from high-pressure injection in combination with EP, to deliver codon-optimized HBc and IL-12 expressing plasmids that elicit polyfunctional T cell responses[45].

In the duck hepatitis B virus model, EP-mediated vaccination increased the production of neutralizing HBV-specific antibodies, expanded the spectrum of targeted epitopes[46] and resulted in T helper type 1 polarization[47]. Short non-coding DNA fragments appear to increase the immunopotency of EP-mediated HBV DNA vaccination[48]. DNA vaccine platforms based on EP show an enhanced ability to activate both arms of adaptive immunity and thus represent an important tool to overcome immunological exhaustion in CHB.

Regarding human studies, a dual plasmid-ΗΒV vaccine consisting of a therapeutic plasmid encoding the preS2/S antigen and an adjuvant plasmid containing the fused sequence of IL-12 and IFN-γ was the first to be administered *via* electroporation against CHB. In total, 6 of 39 HBeAg-positive participants received vaccine monotherapy, while the remaining patients were randomized 1:2 to receive either lamivudine plus placebo or the experimental treatment, lamivudine plus vaccination. The EP-mediated immunization stimulated HBV-addressing IFN-γ-producing T cells, and the combination therapy produced a more profound suppression of viral replication and a lower risk of virologic breakthrough compared with NA monotherapy[49].

The satisfactory immunostimulatory efficacy followed by a very consistent safety record as demonstrated in the pilot study has led to a phase IIb trial of the EP-mediated dual plasmid DNA vaccine. In this study, 225 previously untreated HBeAg-positive patients were divided 1:1 into groups treated with either lamivudine plus vaccine or lamivudine plus placebo. Although the primary endpoint of undetectable HBV DNA rate or HBeAg seroconversion was not met, four intramuscular doses of the vaccine showed a modest therapeutic effect, with more vaccinated patients achieving a > 2 log10 IU/mL decrease in HBV DNA compared with the control group[50].

Other HBV DNA vaccines administered *via* EP are currently being investigated in clinical trials with different eligibility criteria, combination regimens, or dosing schedules. An open-label phase I study (NCT02431312) evaluating the safety and reactogenicity of dose combinations of INO-1800 (DNA plasmid expressing core and surface antigens) and INO-9112 (DNA plasmid containing IL-12 sequences) in patients who have received stable NA therapy for at least 1 year has been completed, and final results are pending. In addition, a phase I study (NCT03463369) is underway to evaluate the efficacy of the DNA vaccine JNJ-64300535 administered by EP-mediated intramuscular injection in virally suppressed CHB patients on NA treatment. Overall, administration of the DNA vaccine by EP resulted in multispecific humoral and long-lasting memory T cell responses, although efficacy on clinical endpoints was subpar. These results are important for future studies in which careful patient selection is performed and EP-mediated vaccination realizes its full potential to efficiently restore adaptive immune responses in CHB.

**mRNA VACCINES**

mRNA vaccines are an attractive alternative to traditional vaccine platforms because they allow a rapid and scalable manufacturing process without being infectious or carrying the risk of integration. Compared to plasmid vaccines, mRNA vaccines have a stronger immunostimulatory effect on innate immunity and result in the desired vaccine responses[51]. Optimization of mRNA stability and translation as well as advances in vaccine delivery have led to increased use of mRNA therapeutics in basic research and clinical trials for the treatment of infectious diseases and cancer[52]. Nevertheless, research on mRNA-based vaccines in the field of anti-HBV prevention or control strategies has been sparse.

In an attempt to develop an anti-HBV mRNA vaccine for prophylactic or immunotherapeutic purposes, Lamb[53] developed and implemented an mRNA production process containing all critical HBsAg epitopes. The mRNA lipoplex nanoparticles were designed to protect against exonuclease degradation, promote endocytosis-mediated cellular uptake, and facilitate endosomal escape of the entrapped mRNA. Upon release into the cytoplasm, the mRNA is used by the host translational machinery as a template for the production of S-HBsAg or L-HBsAg, which are amenable to intrinsic post-translational modifications and are either secreted or degraded in a proteasome-dependent manner. *In situ* processing and presentation of the mRNA-derived antigen products would then resemble that of DNA vaccines and elicit robust pathogen-specific humoral and cell-mediated immune responses[53,54]. Interestingly, an mRNA-based vaccine formulation that was efficiently translated into detectable L-HBs and S-HBs in cultured hepatoma cells was produced using a highly reproducible method. However, L-HBs was expressed at lower levels than expected, and its secretion was modest, possibly reducing its immunostimulatory effect[53]. Clearly, in later stages of development, optimization of downstream processes is required to fully exploit the immunogenic benefits of the vaccine.

**CONCLUSION**

Recently, numerous nucleic acid vaccines have been used in clinical trials to prevent or treat infectious diseases as innovations in biotechnology have improved their immunogenicity and tolerability. Compared to mRNA-based formulations, plasmid vaccines have been studied more intensively in the field of HBV therapeutics. Pioneering preclinical studies have shown that DNA vaccination could suppress HBV transcriptional activity and even affect the cccDNA pool and was able to break CHB immune tolerance. In parallel, the introduction of *in vivo* EP as a delivery platform has dramatically improved the immunostimulatory effect of DNA vaccines without serious adverse events.

Given these data and despite the moderate results of the initial clinical trials, the design of new studies to clarify the role of DNA vaccination in this field is essential. An ideal vaccine candidate would contain multiple dominant and subdominant HBV epitopes in combination with appropriate adjuvants to elicit potent and multispecific responses and should be administered *via* EP to enhance immunogenicity. Add-on or sequential treatment regimens could be applied to progressively repair the dysregulated adaptive immune responses and eventually achieve HBV eradication. Identifying patients who benefit most from immunization strategies should be a priority of future studies. Preferably, vaccination should be studied in virus-suppressed CHB patients on stable therapy with second-generation NAs that are less susceptible to drug resistance, such as entecavir or tenofovir. In summary, nucleic acid-based immunotherapies still have a long way to go before market approval but may retain a place in the context of combination therapy aimed at a functional cure for HBV.

**REFERENCES**

1 **McAleer WJ**, Buynak EB, Maigetter RZ, Wampler DE, Miller WJ, Hilleman MR. Human hepatitis B vaccine from recombinant yeast. *Nature* 1984; **307**: 178-180 [PMID: 6318124 DOI: 10.1038/307178a0]

2 **Polaris Observatory Collaborators.**. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. *Lancet Gastroenterol Hepatol* 2018; **3**: 383-403 [PMID: 29599078 DOI: 10.1016/S2468-1253(18)30056-6]

3 **Cornberg M**, Lok AS, Terrault NA, Zoulim F; 2019 EASL-AASLD HBV Treatment Endpoints Conference Faculty. Guidance for design and endpoints of clinical trials in chronic hepatitis B - Report from the 2019 EASL-AASLD HBV Treatment Endpoints Conference‡. *J Hepatol* 2020; **72**: 539-557 [PMID: 31730789 DOI: 10.1016/j.jhep.2019.11.003]

4 **Tsai KN**, Kuo CF, Ou JJ. Mechanisms of Hepatitis B Virus Persistence. *Trends Microbiol* 2018; **26**: 33-42 [PMID: 28823759 DOI: 10.1016/j.tim.2017.07.006]

5 **Fanning GC**, Zoulim F, Hou J, Bertoletti A. Therapeutic strategies for hepatitis B virus infection: towards a cure. *Nat Rev Drug Discov* 2019; **18**: 827-844 [PMID: 31455905 DOI: 10.1038/s41573-019-0037-0]

6 **Hoogeveen RC**, Boonstra A. Checkpoint Inhibitors and Therapeutic Vaccines for the Treatment of Chronic HBV Infection. *Front Immunol* 2020; **11**: 401 [PMID: 32194573 DOI: 10.3389/fimmu.2020.00401]

7 **Polack FP**, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, Perez JL, Pérez Marc G, Moreira ED, Zerbini C, Bailey R, Swanson KA, Roychoudhury S, Koury K, Li P, Kalina WV, Cooper D, Frenck RW Jr, Hammitt LL, Türeci Ö, Nell H, Schaefer A, Ünal S, Tresnan DB, Mather S, Dormitzer PR, Şahin U, Jansen KU, Gruber WC; C4591001 Clinical Trial Group. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med* 2020; **383**: 2603-2615 [PMID: 33301246 DOI: 10.1056/NEJMoa2034577]

8 **Baden LR**, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, Diemert D, Spector SA, Rouphael N, Creech CB, McGettigan J, Khetan S, Segall N, Solis J, Brosz A, Fierro C, Schwartz H, Neuzil K, Corey L, Gilbert P, Janes H, Follmann D, Marovich M, Mascola J, Polakowski L, Ledgerwood J, Graham BS, Bennett H, Pajon R, Knightly C, Leav B, Deng W, Zhou H, Han S, Ivarsson M, Miller J, Zaks T; COVE Study Group. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *N Engl J Med* 2021; **384**: 403-416 [PMID: 33378609 DOI: 10.1056/NEJMoa2035389]

9 **Wieland S**, Thimme R, Purcell RH, Chisari FV. Genomic analysis of the host response to hepatitis B virus infection. *Proc Natl Acad Sci U S A* 2004; **101**: 6669-6674 [PMID: 15100412 DOI: 10.1073/pnas.0401771101]

10 **Dunn C**, Peppa D, Khanna P, Nebbia G, Jones M, Brendish N, Lascar RM, Brown D, Gilson RJ, Tedder RJ, Dusheiko GM, Jacobs M, Klenerman P, Maini MK. Temporal analysis of early immune responses in patients with acute hepatitis B virus infection. *Gastroenterology* 2009; **137**: 1289-1300 [PMID: 19591831 DOI: 10.1053/j.gastro.2009.06.054]

11 **Thomas E**, Baumert TF. Hepatitis B Virus-Hepatocyte Interactions and Innate Immune Responses: Experimental Models and Molecular Mechanisms. *Semin Liver Dis* 2019; **39**: 301-314 [PMID: 31266064 DOI: 10.1055/s-0039-1685518]

12 **Wang S**, Chen Z, Hu C, Qian F, Cheng Y, Wu M, Shi B, Chen J, Hu Y, Yuan Z. Hepatitis B virus surface antigen selectively inhibits TLR2 ligand-induced IL-12 production in monocytes/macrophages by interfering with JNK activation. *J Immunol* 2013; **190**: 5142-5151 [PMID: 23585678 DOI: 10.4049/jimmunol.1201625]

13 **Li H**, Zhai N, Wang Z, Song H, Yang Y, Cui A, Li T, Wang G, Niu J, Crispe IN, Su L, Tu Z. Regulatory NK cells mediated between immunosuppressive monocytes and dysfunctional T cells in chronic HBV infection. *Gut* 2018; **67**: 2035-2044 [PMID: 28899983 DOI: 10.1136/gutjnl-2017-314098]

14 **Pallett LJ**, Gill US, Quaglia A, Sinclair LV, Jover-Cobos M, Schurich A, Singh KP, Thomas N, Das A, Chen A, Fusai G, Bertoletti A, Cantrell DA, Kennedy PT, Davies NA, Haniffa M, Maini MK. Metabolic regulation of hepatitis B immunopathology by myeloid-derived suppressor cells. *Nat Med* 2015; **21**: 591-600 [PMID: 25962123 DOI: 10.1038/nm.3856]

15 **Yonejima A**, Mizukoshi E, Tamai T, Nakagawa H, Kitahara M, Yamashita T, Arai K, Terashima T, Iida N, Fushimi K, Okada H, Yamashita T, Sakai Y, Honda M, Kaneko S. Characteristics of Impaired Dendritic Cell Function in Patients With Hepatitis B Virus Infection. *Hepatology* 2019; **70**: 25-39 [PMID: 30938456 DOI: 10.1002/hep.30637]

16 **Li TY**, Yang Y, Zhou G, Tu ZK. Immune suppression in chronic hepatitis B infection associated liver disease: A review. *World J Gastroenterol* 2019; **25**: 3527-3537 [PMID: 31367154 DOI: 10.3748/wjg.v25.i27.3527]

17 **Hobernik D**, Bros M. DNA Vaccines-How Far From Clinical Use? *Int J Mol Sci* 2018; **19** [PMID: 30445702 DOI: 10.3390/ijms19113605]

18 **Kutzler MA**, Weiner DB. DNA vaccines: ready for prime time? *Nat Rev Genet* 2008; **9**: 776-788 [PMID: 18781156 DOI: 10.1038/nrg2432]

19 **Ghaffarifar F**. Plasmid DNA vaccines: where are we now? *Drugs Today (Barc)* 2018; **54**: 315-333 [PMID: 29911696 DOI: 10.1358/dot.2018.54.5.2807864]

20 **Gulce-Iz S,** Saglam-Metiner P. Current state of the art in DNA vaccine delivery and molecular adjuvants: Bcl-xL anti-apoptotic protein as a molecular adjuvant. In: Tyagi RK, Bisen PS (Ed). Immune Response Activation and Immunomodulation. IntechOpen, 2019 [DOI: 10.5772/intechopen.82203]

21 **Mancini M**, Hadchouel M, Davis HL, Whalen RG, Tiollais P, Michel ML. DNA-mediated immunization in a transgenic mouse model of the hepatitis B surface antigen chronic carrier state. *Proc Natl Acad Sci U S A* 1996; **93**: 12496-12501 [PMID: 8901610 DOI: 10.1073/pnas.93.22.12496]

22 **Thermet A**, Buronfosse T, Werle-Lapostolle B, Chevallier M, Pradat P, Trepo C, Zoulim F, Cova L. DNA vaccination in combination or not with lamivudine treatment breaks humoral immune tolerance and enhances cccDNA clearance in the duck model of chronic hepatitis B virus infection. *J Gen Virol* 2008; **89**: 1192-1201 [PMID: 18420797 DOI: 10.1099/vir.0.83583-0]

23 **Siegel F**, Lu M, Roggendorf M. Coadministration of gamma interferon with DNA vaccine expressing woodchuck hepatitis virus (WHV) core antigen enhances the specific immune response and protects against WHV infection. *J Virol* 2001; **75**: 5036-5042 [PMID: 11333883 DOI: 10.1128/JVI.75.11.5036-5042.2001]

24 **Mancini-Bourgine M**, Fontaine H, Scott-Algara D, Pol S, Bréchot C, Michel ML. Induction or expansion of T-cell responses by a hepatitis B DNA vaccine administered to chronic HBV carriers. *Hepatology* 2004; **40**: 874-882 [PMID: 15382173 DOI: 10.1002/hep.20408]

25 **Mancini-Bourgine M**, Fontaine H, Bréchot C, Pol S, Michel ML. Immunogenicity of a hepatitis B DNA vaccine administered to chronic HBV carriers. *Vaccine* 2006; **24**: 4482-4489 [PMID: 16310901 DOI: 10.1016/j.vaccine.2005.08.013]

26 **Scott-Algara D**, Mancini-Bourgine M, Fontaine H, Pol S, Michel ML. Changes to the natural killer cell repertoire after therapeutic hepatitis B DNA vaccination. *PLoS One* 2010; **5**: e8761 [PMID: 20090916 DOI: 10.1371/journal.pone.0008761]

27 **Godon O**, Fontaine H, Kahi S, Meritet JF, Scott-Algara D, Pol S, Michel ML, Bourgine M; ANRS HB02 study group. Immunological and antiviral responses after therapeutic DNA immunization in chronic hepatitis B patients efficiently treated by analogues. *Mol Ther* 2014; **22**: 675-684 [PMID: 24394187 DOI: 10.1038/mt.2013.274]

28 **Fontaine H**, Kahi S, Chazallon C, Bourgine M, Varaut A, Buffet C, Godon O, Meritet JF, Saïdi Y, Michel ML, Scott-Algara D, Aboulker JP, Pol S; ANRS HB02 study group. Anti-HBV DNA vaccination does not prevent relapse after discontinuation of analogues in the treatment of chronic hepatitis B: a randomised trial--ANRS HB02 VAC-ADN. *Gut* 2015; **64**: 139-147 [PMID: 24555998 DOI: 10.1136/gutjnl-2013-305707]

29 **Gary EN**, Weiner DB. DNA vaccines: prime time is now. *Curr Opin Immunol* 2020; **65**: 21-27 [PMID: 32259744 DOI: 10.1016/j.coi.2020.01.006]

30 **Li L**, Petrovsky N. Molecular mechanisms for enhanced DNA vaccine immunogenicity. *Expert Rev Vaccines* 2016; **15**: 313-329 [PMID: 26707950 DOI: 10.1586/14760584.2016.1124762]

31 **Wang J**, Gujar SA, Cova L, Michalak TI. Bicistronic woodchuck hepatitis virus core and gamma interferon DNA vaccine can protect from hepatitis but does not elicit sterilizing antiviral immunity. *J Virol* 2007; **81**: 903-916 [PMID: 17079319 DOI: 10.1128/JVI.01537-06]

32 **Saade F**, Buronfosse T, Pradat P, Abdul F, Cova L. Enhancement of neutralizing humoral response of DNA vaccine against duck hepatitis B virus envelope protein by co-delivery of cytokine genes. *Vaccine* 2008; **26**: 5159-5164 [PMID: 18554756 DOI: 10.1016/j.vaccine.2008.03.086]

33 **Saade F**, Buronfosse T, Guerret S, Pradat P, Chevallier M, Zoulim F, Jamard C, Cova L. In vivo infectivity of liver extracts after resolution of hepadnaviral infection following therapy associating DNA vaccine and cytokine genes. *J Viral Hepat* 2013; **20**: e56-e65 [PMID: 23490390 DOI: 10.1111/jvh.12023]

34 **Wang Y**, Wu S, Wang ZC, Zhu XM, Yin XT, Gao K, Du ZY, Chen GZ, Yu JY. Enhanced immunity and antiviral effects of an HBV DNA vaccine delivered by a DC-targeting protein. *J Viral Hepat* 2016; **23**: 798-804 [PMID: 27126208 DOI: 10.1111/jvh.12542]

35 **Yang SH**, Lee CG, Park SH, Im SJ, Kim YM, Son JM, Wang JS, Yoon SK, Song MK, Ambrozaitis A, Kharchenko N, Yun YD, Kim CM, Kim CY, Lee SH, Kim BM, Kim WB, Sung YC. Correlation of antiviral T-cell responses with suppression of viral rebound in chronic hepatitis B carriers: a proof-of-concept study. *Gene Ther* 2006; **13**: 1110-1117 [PMID: 16525482 DOI: 10.1038/sj.gt.3302751]

36 **Im SJ**, Yang SH, Yoon SK, Sung YC. Increase of Plasma IL-12/p40 Ratio Induced by the Combined Therapy of DNA Vaccine and Lamivudine Correlates with Sustained Viremia Control in CHB Carriers. *Immune Netw* 2009; **9**: 20-26 [PMID: 20107534 DOI: 10.4110/in.2009.9.1.20]

37 **Yoon SK**, Seo YB, Im SJ, Bae SH, Song MJ, You CR, Jang JW, Yang SH, Suh YS, Song JS, Kim BM, Kim CY, Jeong SH, Sung YC. Safety and immunogenicity of therapeutic DNA vaccine with antiviral drug in chronic HBV patients and its immunogenicity in mice. *Liver Int* 2015; **35**: 805-815 [PMID: 24620920 DOI: 10.1111/liv.12530]

38 **Liu J**, Zhang E, Ma Z, Wu W, Kosinska A, Zhang X, Möller I, Seiz P, Glebe D, Wang B, Yang D, Lu M, Roggendorf M. Enhancing virus-specific immunity in vivo by combining therapeutic vaccination and PD-L1 blockade in chronic hepadnaviral infection. *PLoS Pathog* 2014; **10**: e1003856 [PMID: 24391505 DOI: 10.1371/journal.ppat.1003856]

39 **Gane E**, Verdon DJ, Brooks AE, Gaggar A, Nguyen AH, Subramanian GM, Schwabe C, Dunbar PR. Anti-PD-1 blockade with nivolumab with and without therapeutic vaccination for virally suppressed chronic hepatitis B: A pilot study. *J Hepatol* 2019; **71**: 900-907 [PMID: 31306680 DOI: 10.1016/j.jhep.2019.06.028]

40 **Shi J**, Ma Y, Zhu J, Chen Y, Sun Y, Yao Y, Yang Z, Xie J. A Review on Electroporation-Based Intracellular Delivery. *Molecules* 2018; **23** [PMID: 30469344 DOI: 10.3390/molecules23113044]

41 **van Drunen Littel-van den Hurk S**, Hannaman D. Electroporation for DNA immunization: clinical application. *Expert Rev Vaccines* 2010; **9**: 503-517 [PMID: 20450325 DOI: 10.1586/erv.10.42]

42 **Cova L**. Present and future DNA vaccines for chronic hepatitis B treatment. *Expert Opin Biol Ther* 2017; **17**: 185-195 [PMID: 27892722 DOI: 10.1080/14712598.2017.1265940]

43 **Luxembourg A**, Hannaman D, Ellefsen B, Nakamura G, Bernard R. Enhancement of immune responses to an HBV DNA vaccine by electroporation. *Vaccine* 2006; **24**: 4490-4493 [PMID: 16140436 DOI: 10.1016/j.vaccine.2005.08.014]

44 **Kim CY**, Kang ES, Kim SB, Kim HE, Choi JH, Lee DS, Im SJ, Yang SH, Sung YC, Kim BM, Kim BG. Increased in vivo immunological potency of HB-110, a novel therapeutic HBV DNA vaccine, by electroporation. *Exp Mol Med* 2008; **40**: 669-676 [PMID: 19116452 DOI: 10.3858/emm.2008.40.6.669]

45 **Brass A**, Frelin L, Milich DR, Sällberg M, Ahlén G. Functional aspects of intrahepatic hepatitis B virus-specific T cells induced by therapeutic DNA vaccination. *Mol Ther* 2015; **23**: 578-590 [PMID: 25492563 DOI: 10.1038/mt.2014.233]

46 **Khawaja G**, Buronfosse T, Jamard C, Guerret S, Zoulim F, Luxembourg A, Hannaman D, Evans C, Hartmann D, Cova L. Enhanced magnitude and breadth of neutralizing humoral response to a DNA vaccine targeting the DHBV envelope protein delivered by in vivo electroporation. *Virology* 2012; **425**: 61-69 [PMID: 22284894 DOI: 10.1016/j.virol.2012.01.001]

47 **Khawaja G**, Buronfosse T, Jamard C, Abdul F, Guerret S, Zoulim F, Luxembourg A, Hannaman D, Evans CF, Hartmann D, Cova L. In vivo electroporation improves therapeutic potency of a DNA vaccine targeting hepadnaviral proteins. *Virology* 2012; **433**: 192-202 [PMID: 22921316 DOI: 10.1016/j.virol.2012.07.014]

48 **Peng J**, Shi S, Yang Z, Ding Q, Hai W, Tang H, Yang Y, Bernstein JR, Peyda P, Xu Y. Short noncoding DNA fragments improve the immune potency of electroporation-mediated HBV DNA vaccination. *Gene Ther* 2014; **21**: 703-708 [PMID: 24830435 DOI: 10.1038/gt.2014.44]

49 **Yang FQ**, Yu YY, Wang GQ, Chen J, Li JH, Li YQ, Rao GR, Mo GY, Luo XR, Chen GM. A pilot randomized controlled trial of dual-plasmid HBV DNA vaccine mediated by in vivo electroporation in chronic hepatitis B patients under lamivudine chemotherapy. *J Viral Hepat* 2012; **19**: 581-593 [PMID: 22762143 DOI: 10.1111/j.1365-2893.2012.01589.x]

50 **Yang FQ**, Rao GR, Wang GQ, Li YQ, Xie Y, Zhang ZQ, Deng CL, Mao Q, Li J, Zhao W, Wang MR, Han T, Chen SJ, Pan C, Tan DM, Shang J, Zhang MX, Zhang YX, Yang JM, Chen GM. 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**: 306-317 [PMID: 28127204 DOI: 10.3748/wjg.v23.i2.306]

51 **Liu MA**. A Comparison of Plasmid DNA and mRNA as Vaccine Technologies. *Vaccines (Basel)* 2019; **7** [PMID: 31022829 DOI: 10.3390/vaccines7020037]

52 **Pardi N**, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - a new era in vaccinology. *Nat Rev Drug Discov* 2018; **17**: 261-279 [PMID: 29326426 DOI: 10.1038/nrd.2017.243]

53 **Lamb C.** Development of an mRNA Vaccination Strategy for the Prevention and Treatment of HBV Infection. The University of the Witwatersrand, Johannesburg, South Africa, 2017. Available from: https://core.ac.uk/download/pdf/188774903.pdf

54 **Zhang C**, Maruggi G, Shan H, Li J. Advances in mRNA Vaccines for Infectious Diseases. *Front Immunol* 2019; **10**: 594 [PMID: 30972078 DOI: 10.3389/fimmu.2019.00594]

**Footnotes**

**Conflict-of-interest statement:** The authors declare that there are noconflicts of interest.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/Licenses/by-nc/4.0/

**Manuscript source:** Invited manuscript

**Peer-review started:** March 18, 2021

**First decision:** July 3, 2021

**Article in press:**

**Specialty type:** Infectious diseases

**Country/Territory of origin:** Greece

**Peer-review report’s scientific quality classification**

Grade A (Excellent): A

Grade B (Very good): 0

Grade C (Good): 0

Grade D (Fair): 0

Grade E (Poor): 0

**P-Reviewer:** Martin Mateos RM **S-Editor:** Ma YJ **L-Editor:** Filipodia **P-Editor:**