

TITLE OF THE INVENTION
HEPATITIS B VIRUS (HBV) VACCINES AND USES THEREOF

5 CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to International Patent Application No. PCT/IB2017/058142, filed December 19, 2017, and U.S. Provisional Patent Application No. 62/607,426, filed December 19, 2017, the disclosures of which are incorporated herein by reference in their entireties.

10 REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0002] This application contains a sequence listing, which is submitted electronically via EFS-Web as an ASCII formatted sequence listing with a file name "688097-403 Sequence Listing," creation date of December 10, 2018, and having a size of 46.6 KB. The sequence listing submitted via EFS-Web is part of the specification and is herein incorporated by reference
15 in its entirety.

BACKGROUND OF THE INVENTION

[0003] Hepatitis B virus (HBV) is a small 3.2-kb hepatotropic DNA virus that encodes four open reading frames and seven proteins. About two billion people are infected with HBV, and approximately 240 million people have chronic hepatitis B infection (chronic HBV),
20 characterized by persistent virus and subvirus particles in the blood for more than 6 months (1). Persistent HBV infection leads to T-cell exhaustion in circulating and intrahepatic HBV-specific CD4+ and CD8+ T-cells through chronic stimulation of HBV-specific T-cell receptors with viral peptides and circulating antigens. As a result, T-cell polyfunctionality is decreased (i.e., decreased levels of IL-2, tumor necrosis factor (TNF)- α , IFN- γ , and lack of proliferation).
25 [0004] A safe and effective prophylactic vaccine against HBV infection has been available since the 1980s and is the mainstay of hepatitis B prevention (3). The World Health Organization recommends vaccination of all infants, and, in countries where there is low or intermediate hepatitis B endemicity, vaccination of all children and adolescents (<18 years of age), and of people of certain at risk population categories. Due to vaccination, worldwide infection rates
30 have dropped dramatically. However, prophylactic vaccines do not cure established HBV infection.

[0005] Chronic HBV is currently treated with IFN- α and nucleoside or nucleotide analogs, but there is no ultimate cure due to the persistence in infected hepatocytes of an intracellular viral replication intermediate called covalently closed circular DNA (cccDNA), which plays a fundamental role as a template for viral RNAs, and thus new virions. It is thought that induced virus-specific T-cell and B-cell responses can effectively eliminate cccDNA-carrying hepatocytes. Current therapies targeting the HBV polymerase suppress viremia, but offer limited effect on cccDNA that resides in the nucleus and related production of circulating antigen. The most rigorous form of a cure may be elimination of HBV cccDNA from the organism, which has neither been observed as a naturally occurring outcome nor as a result of any therapeutic intervention. However, loss of HBV surface antigens (HBsAg) is a clinically credible equivalent of a cure, since disease relapse can occur only in cases of severe immunosuppression, which can then be prevented by prophylactic treatment. Thus, at least from a clinical standpoint, loss of HBsAg is associated with the most stringent form of immune reconstitution against HBV.

[0006] For example, immune modulation with pegylated interferon (pegIFN)- α has proven better in comparison to nucleoside or nucleotide therapy in terms of sustained off-treatment response with a finite treatment course. Besides a direct antiviral effect, IFN- α is reported to exert epigenetic suppression of cccDNA in cell culture and humanized mice, which leads to reduction of virion productivity and transcripts (4). However, this therapy is still fraught with side-effects and overall responses are rather low, in part because IFN- α has only poor modulatory influences on HBV-specific T-cells. In particular, cure rates are low (< 10%) and toxicity is high. Likewise, direct acting HBV antivirals, namely the HBV polymerase inhibitors entecavir and tenofovir, are effective as monotherapy in inducing viral suppression with a high genetic barrier to emergence of drug resistant mutants and consecutive prevention of liver disease progression. However, cure of chronic hepatitis B, defined by HBsAg loss or seroconversion, is rarely achieved with such HBV polymerase inhibitors. Therefore, these antivirals in theory need to be administered indefinitely to prevent reoccurrence of liver disease, similar to antiretroviral therapy for human immunodeficiency virus (HIV).

[0007] Therapeutic vaccination has the potential to eliminate HBV from chronically infected patients (5). Many strategies have been explored, but to date therapeutic vaccination has not proven successful.

BRIEF SUMMARY OF THE INVENTION

[0008] Accordingly, there is an unmet medical need in the treatment of hepatitis B virus (HBV), particularly chronic HBV, for a finite well-tolerated treatment with a higher cure rate. The invention satisfies this need by providing immunogenic compositions and methods for inducing an immune response against hepatitis B virus (HBV) infection. The immunogenic compositions and methods of the invention can be used to provide therapeutic immunity to a subject, such as a subject having chronic HBV infection.

[0009] In a general aspect, the application relates to a non-naturally occurring nucleic acid molecule encoding an HBV antigen, such as a truncated HBV core antigen or a HBV polymerase antigen. An HBV antigen according to an embodiment of the application is a consensus antigen capable of inducing an immune response (humoral and cellular) in a mammal against at least two HBV genotypes, preferably inducing a T cell response in a mammal against at least HBV genotypes B, C and D, more preferably, a CD8 T cell response in a human subject against at least HBV genotypes A, B, C and D.

[0010] In an embodiment, a non-naturally occurring nucleic acid molecule of the application encodes a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 14, and the non-naturally occurring nucleic acid molecule comprises a polynucleotide sequence that is at least 90% identical to SEQ ID NO: 1 or SEQ ID NO: 15. Preferably, the non-naturally occurring nucleic acid molecule comprises the polynucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 15.

[0011] In an embodiment, a non-naturally occurring nucleic acid molecule of the application encodes HBV polymerase antigen comprising an amino acid sequence that is at least 98% identical to SEQ ID NO: 4, wherein the HBV polymerase antigen does not have reverse transcriptase activity and RNase H activity. Preferably, an HBV polymerase antigen comprises the amino acid sequence of SEQ ID NO: 4. More preferably, a non-naturally occurring nucleic acid molecule comprises a polynucleotide sequence at least 90% identical to SEQ ID NO: 3 or SEQ ID NO: 16, most preferably 100% identical to SEQ ID NO: 3 or SEQ ID NO: 16.

[0012] In another general aspect, the application relates to a vector, preferably a DNA plasmid or a viral vector, comprising a non-naturally occurring nucleic acid molecule of the application.

[0013] In another general aspect, the application relates to a recombinant host cell comprising a non-naturally occurring nucleic acid molecule or a vector of the application.

[0014] In another general aspect, the application relates to a non-naturally occurring polypeptide encoded by a non-naturally occurring nucleic acid molecule of the application.

5 [0015] In yet another general aspect, the application relates to a composition comprising at least one of a non-naturally occurring nucleic acid molecule, vector, recombinant host cell, and non-naturally occurring polypeptide of the application, and a pharmaceutically acceptable carrier.

[0016] In another general aspect, the application relates to an immunogenic combination,
10 particularly a kit, comprising:

(a) a first non-naturally occurring nucleic acid molecule comprising a first polynucleotide encoding a HBV polymerase antigen having an amino acid sequence that is at least 98% identical to SEQ ID NO: 4, wherein the HBV polymerase antigen does not have reverse transcriptase activity and RNase H activity;

15 (b) a second non-naturally occurring nucleic acid molecule comprising a second polynucleotide encoding a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 14; and

(c) a pharmaceutically acceptable carrier,

wherein the first non-naturally occurring nucleic acid molecule and the second non-naturally
20 occurring nucleic acid molecule are present in the same non-naturally occurring nucleic acid molecule or in two different non-naturally occurring nucleic acid molecules.

[0017] In some embodiments, an immunogenic combination, particularly a kit, of the application comprises the first polynucleotide present in a first vector and the second polynucleotide present in a second vector. Preferably, the first vector is different from the
25 second vector. More preferably, the vector is a plasmid vector or viral vector. More preferably, each of the first vector and the second vector is a plasmid DNA vector.

[0018] In an embodiment, an immunogenic combination, particularly a kit, of the application comprises:

30 a) a first plasmid DNA vector comprising a first polynucleotide sequence encoding a HBV polymerase antigen having the amino acid sequence of SEQ ID NO: 4;

- b) a second plasmid DNA vector comprising a second polynucleotide encoding a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 2 or 14; and
- c) a pharmaceutically acceptable carrier,
- 5 wherein each of the first plasmid DNA vector and the second plasmid DNA vector further comprises an antibiotic resistance gene, and an origin of replication, and wherein the first plasmid DNA vector and the second plasmid DNA vector are present in the same composition or in two different compositions.

[0019] In a particular embodiment, an immunogenic combination, particularly a kit, of the application comprises:

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- a) a first plasmid DNA vector comprising, from 3'-end to 5'-end, a promoter sequence comprising the polynucleotide sequence of SEQ ID NO: 7, an enhancer sequence comprising the polynucleotide sequence of SEQ ID NO: 8, a signal peptide coding sequence comprising the polynucleotide sequence of SEQ ID NO: 5, a first polynucleotide sequence comprising the polynucleotide sequence of SEQ ID NO: 3, and a polyadenylation signal sequence comprising the polynucleotide sequence of SEQ ID NO: 11;
- 15
- b) a second plasmid DNA vector comprising, from 3'-end to 5'-end, the promoter sequence comprising the polynucleotide sequence of SEQ ID NO: 7, the regulatory sequence comprising the polynucleotide sequence of SEQ ID NO: 8, the signal peptide coding sequence comprising the polynucleotide sequence of SEQ ID NO: 5, a second polynucleotide sequence comprising the polynucleotide sequence of SEQ ID NO: 1, and the polyadenylation signal sequence comprising the polynucleotide sequence of SEQ ID NO: 11; and
- 20
- c) a pharmaceutically acceptable carrier,
- 25 wherein each of the first plasmid DNA vector and the second plasmid DNA vector further comprises a kanamycin resistance gene having the polynucleotide sequence of SEQ ID NO: 12, and an origin of replication having the polynucleotide sequence of SEQ ID NO: 10, and
- 30 wherein the first plasmid DNA vector and the second plasmid DNA vector are present in the same composition or in two different compositions.

[0020] In other embodiments, an immunogenic combination, particularly a kit, of the application comprises the first polynucleotide and the second polynucleotide present in the same vector. Preferably, the vector is a plasmid vector or a viral vector. More preferably, the vector is an adenoviral vector, such as an Ad26 or Ad35 vector.

5 [0021] In an embodiment, an immunogenic combination, particularly a kit, of the application comprises:

- a) a vector comprising a first polynucleotide sequence encoding a HBV polymerase antigen having the amino acid sequence of SEQ ID NO: 4, and a second polynucleotide encoding a truncated HBV core antigen consisting of the amino acid
10 sequence of SEQ ID NO: 2 or SEQ ID NO: 14; and
- b) a pharmaceutically acceptable carrier.

[0022] In a particular embodiment, an immunogenic combination, particularly a kit, of the application comprises a viral vector, preferably an adenoviral vector, comprising, from 5' end to 3' end, a promoter sequence comprising the polynucleotide sequence of SEQ ID NO: 17, a
15 regulatory sequence comprising the polynucleotide sequence of SEQ ID NO: 23, a signal peptide coding sequence comprising the polynucleotide sequence of SEQ ID NO: 18, a second polynucleotide sequence comprising the polynucleotide sequence of SEQ ID NO: 15, a linker coding sequence comprising the polynucleotide sequence of SEQ ID NO: 22, a first polynucleotide sequence comprising the polynucleotide sequence of SEQ ID NO: 16, and a
20 polyadenylation signal sequence comprising the polynucleotide sequence of SEQ ID NO: 24, and a pharmaceutically acceptable carrier.

[0023] Other aspects of the application relate to methods of manufacturing polynucleotides, vectors, polypeptides, and compositions and immunogenic combinations or kits of the application.

25 [0024] And in yet another general aspect, the application relates to a method of inducing an immune response against hepatitis B virus (HBV) in a subject in need thereof, the method comprising administering to the subject an immunogenically effective amount of a composition or an immunogenic combination of the application. Preferably, the method induces an immune response, such as an antibody response and/or a T cell response, in the subject against at least
30 two HBV genotypes. Preferably, the method induces a T cell response in the subject against at least HBV genotypes B, C and D. More preferably, the method induces a CD8 T cell response in

a human subject against at least HBV genotypes A, B, C and D. In an embodiment, the method further comprises administering to the subject another immunogenic agent, preferably another HBV antigen.

[0025] In another aspect, the application relates to a method of treating a hepatitis B virus (HBV)-induced disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a composition or an immunogenic combination of the application. Preferably, the subject has chronic HBV infection, and the HBV-induced disease is selected from the group consisting of advanced fibrosis, cirrhosis and hepatocellular carcinoma (HCC). In an embodiment, the method further comprises administering to the subject another therapeutic agent, preferably another anti-HBV antigen.

[0026] The application also relates to a composition, an immunogenic combination or a kit of the application for use in inducing an immune response against hepatitis B virus (HBV); and use of a composition, an immunogenic combination or a kit of the application in the manufacture of a medicament for inducing an immune response against hepatitis B virus (HBV). The use can further comprise a combination with another immunogenic agent, preferably another HBV antigen. Preferably, the subject has chronic HBV infection.

[0027] The application further relates to a composition, an immunogenic combination or a kit of the application for use in treating a HBV- induced disease in a subject in need thereof; and use of a composition, an immunogenic combination or a kit of the application in the manufacture of a medicament for treating a HBV- induced disease in a subject in need thereof. The use can further comprise a combination with another therapeutic agent, preferably another anti-HBV antigen. Preferably, the subject has chronic HBV infection, and the HBV-induced disease is selected from the group consisting of advanced fibrosis, cirrhosis and hepatocellular carcinoma (HCC).

[0028] Other aspects, features and advantages of the invention will be apparent from the following disclosure, including the detailed description of the invention and its preferred embodiments and the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings. It

should be understood that the invention is not limited to the precise embodiments shown in the drawings.

[0030] In the drawings:

[0031] **FIGS. 1A-1B** depict the genome and viral life cycle of hepatitis B virus; **FIG. 1A** is a diagram of the genome of hepatitis B virus (HBV); in the native virus, the polymerase protein (Pol) contains the coding sequence for the envelope proteins in a different open reading frame; the envelope proteins (pre-S1, pre-S2, and S) are in the same open reading frame; **FIG. 1B** shows the viral life cycle of HBV;

[0032] **FIGS. 2A-2H** show the design and optimization of expression cassettes and DNA plasmids encoding HBV pol and core antigens as described in Example 1; **FIG. 2A** is a schematic representation of an expression strategy in which coding sequences of the HBV core and pol antigens are fused in frame; **FIG. 2B** is a schematic representation of an expression strategy in which coding sequences of both the core and pol antigens are expressed from a single plasmid by means of the ribosomal FA2 slippage site; **FIG. 2C** is a schematic representation of an expression strategy in which the core and pol antigens are expressed from two separate plasmids; **FIG. 2D** is a Western blot of core antigen expression in HEK293T cells transfected with a plasmid expressing core with and without the post-transcriptional regulatory element WPRE; expression was tested in cell lysate (left) and supernatant (sup; right) using an α -core antibody; **FIG. 2E** is a Western blot analysis showing a comparison of core expression in HEK293T cells transfected with a core expressing plasmid including the intron/exon sequence derived from human apolipoprotein A1 precursor ("AI intron"), untranslated R-U5 domain of the human T-cell leukemia virus type 1 (HTLV-1) long terminal repeat (LTR) ("HTLV R"), or triple enhancer composite sequence of the HTLV-1 LTR, synthetic rabbit β -globin intron, and a splicing enhancer ("triple"); the unlabeled lane is purified core protein as a size marker; expression was tested in both lysate (left) and supernatant (sup; right); core antigen expression was highest with the triple enhancer composite sequence; **FIG. 2F** is a Western blot analysis of core antigen secretion using different signal peptides fused to the N-terminus of the HBV core antigen; the most efficient protein secretion was observed with the Cystatin S signal peptide; **FIG. 2G** is a schematic representation of optimized HBV core/pol antigen expression cassettes for each of the three expression strategies illustrated in **FIGS. 2A-2C**; CMVpr: human CMV-IE promoter; TRE: triple enhancer sequence; SP: cystatin S signal peptide; FA2: FMDV ribosomal

slippage site; pA: BGH polyadenylation signal; **FIG. 2H** is a Western blot analysis of HBV core and pol antigen expression of pDK vectors containing each of the expression cassettes shown in **FIG. 2G**; lanes 1 and 2: pDK-core; lanes 3 and 4: pDK-pol; lanes 5 and 6: pDK-coreFA2Pol; lanes 7 and 8: pDK-core-pol fusion: the most consistent expression profile for cellular and secreted core and pol antigens was observed when the antigens were encoded by separate vectors;

[0033] **FIGS. 3A-3B** show schematic representations of DNA plasmids according to embodiments of the application; **FIG. 3A** shows a DNA plasmid encoding an HBV core antigen according to an embodiment of the application; **FIG. 3B** shows a DNA plasmid encoding an HBV polymerase (pol) antigen according to an embodiment of the application; the HBV core and pol antigens are expressed under control of a CMV promoter with an N-terminal cystatin S signal peptide that is cleaved from the expressed antigen upon secretion from the cell; transcriptional regulatory elements of the plasmid include an enhancer sequence located between the CMV promoter and the polynucleotide sequence encoding the HBV antigen and a bGH polyadenylation sequence located downstream of the polynucleotide sequence encoding the HBV antigen; a second expression cassette is included in the plasmid in reverse orientation including a kanamycin resistance gene under control of an Amp^r (bla) promoter; an origin of replication (pUC) is also included in reverse orientation;

[0034] **FIG. 4** shows ELISPOT responses of Balb/c mice immunized with different DNA plasmids expressing HBV core antigen or HBV pol antigen, as described in Example 2; peptide pools used to stimulate splenocytes isolated from the various vaccinated animal groups are indicated in gray scale; the number of responsive T-cells are indicated on the y-axis expressed as spot forming cells (SFC) per 10⁶ splenocytes;

[0035] **FIG. 5** shows ELISPOT responses of Balb/C mice immunized with a combination of DNA plasmids expressing HBV core antigen and HBV pol antigen according to the dose-finding study described in Example 3; peptide pools used to stimulate splenocytes isolated from the various vaccinated animal groups are indicated in gray scale; the number of responsive T-cells are indicated on the y-axis expressed as spot forming cells (SFC) per 10⁶ splenocytes;

[0036] **FIG. 6** shows ELISPOT responses of Balb/c mice immunized with DNA plasmids (pDNA) expressing HBV core antigen and HBV pol antigen according to the immune interference study as described in Example 4; Group 1, single *Core* pDNA; Group 2, single *Pol*

pDNA; Group 3, mixed *Core* and *Pol* pDNA; Group 4, *Core* and *Pol* pDNA applied separately at different sites; peptide pools used to stimulate splenocytes isolated from the various vaccinated animal groups are indicated in gray scale; the number of responsive T-cells are indicated on the y-axis expressed as spot forming cells (SFC) per 10^6 splenocytes;

5 [0037] **FIGS. 7A and 7B** show the immunogenicity of a DNA vaccine according to an embodiment of the application in NHPs as described in Example 5; **FIG. 7A** shows the IFN- γ cytokine response after immunization with DNA plasmids expressing HBV Core and Pol antigens; peptide pools used to stimulate PBMCs isolated from the vaccinated animal groups are indicated in gray scale; the number of responsive T-cells are indicated on the y-axis expressed as
10 spot forming cells (SFC) per 10^6 PBMC; **FIG. 7B** shows CD4 and CD8 T-cell memory immune response against Core, Pol-1, and Pol-2 peptide pools as measured by flow cytometry; the graph shows the results from Day 76 as % CD4 or CD8 T-cell response (IFN- γ , IL-2 and TNF- α) to the 3 pools after the DMSO media-only background was subtracted for each pool; CD4 response is shown on the left and CD8 response is shown on the right;

15 [0038] **FIGS. 8A and 8B** show the schematic representations of the expression cassettes in adenoviral vectors according to embodiments of the application; **FIG. 8A** shows the expression cassette for a truncated HBV core antigen, which contains a CMV promoter, an intron (a fragment derived from the human ApoAI gene - GenBank accession X01038 base pairs 295 – 523, harboring the ApoAI second intron), a human immunoglobulin secretion signal, followed
20 by a coding sequence for a truncated HBV core antigen and a SV40 polyadenylation signal; **FIG. 8B** shows the expression cassette for a fusion protein of a truncated HBV core antigen operably linked to a HBV polymerase antigen, which is otherwise identical to the expression cassette for the truncated HBV core antigen except the HBV antigen; and

[0039] **FIG. 9** shows ELISPOT responses in F1 mice (C57BL/6 x Balb/C) immunized with
25 HBV adenoviral vectors, as described in Example 8; HBV core or polymerase peptide pools used to stimulate splenocytes isolated from the various vaccinated animal groups are indicated in black (core) and grey (pol); Pol1 and pol2 responses were summed; the X-axis shows the adenovector dose and experimental groups. The number of responsive T-cells are indicated on the y-axis expressed as spot forming cells (SFC) per 10^6 splenocytes.

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DETAILED DESCRIPTION OF THE INVENTION

[0040] Various publications, articles and patents are cited or described in the background and throughout the specification; each of these references is herein incorporated by reference in its entirety. Discussion of documents, acts, materials, devices, articles or the like which has been
5 included in the present specification is for the purpose of providing context for the invention. Such discussion is not an admission that any or all of these matters form part of the prior art with respect to any inventions disclosed or claimed.

[0041] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention
10 pertains. Otherwise, certain terms used herein have the meanings as set forth in the specification. All patents, published patent applications and publications cited herein are incorporated by reference as if set forth fully herein.

[0042] It must be noted that as used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural reference unless the context clearly dictates otherwise.

15 [0043] Unless otherwise indicated, the term “at least” preceding a series of elements is to be understood to refer to every element in the series. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the invention.

20 [0044] Throughout this specification and the claims which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising”, will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integer or step. When used herein the term “comprising” can be substituted with the term “containing” or “including” or sometimes when
25 used herein with the term “having”.

[0045] When used herein “consisting of” excludes any element, step, or ingredient not specified in the claim element. When used herein, “consisting essentially of” does not exclude materials or steps that do not materially affect the basic and novel characteristics of the claim. Any of the aforementioned terms of “comprising”, “containing”, “including”, and “having”,
30 whenever used herein in the context of an aspect or embodiment of the application can be

replaced with the term “consisting of” or “consisting essentially of” to vary scopes of the disclosure.

[0046] As used herein, the conjunctive term “and/or” between multiple recited elements is understood as encompassing both individual and combined options. For instance, where two
5 elements are conjoined by “and/or,” a first option refers to the applicability of the first element without the second. A second option refers to the applicability of the second element without the first. A third option refers to the applicability of the first and second elements together. Any one of these options is understood to fall within the meaning, and therefore satisfy the requirement of the term “and/or” as used herein. Concurrent applicability of more than one of the options is also
10 understood to fall within the meaning, and therefore satisfy the requirement of the term “and/or.”

[0047] Unless otherwise stated, any numerical value, such as a concentration or a concentration range described herein, are to be understood as being modified in all instances by the term “about.” Thus, a numerical value typically includes $\pm 10\%$ of the recited value. For example, a concentration of 1 mg/mL includes 0.9 mg/mL to 1.1 mg/mL. Likewise, a
15 concentration range of 1 mg/mL to 10 mg/mL includes 0.9 mg/mL to 11 mg/mL. As used herein, the use of a numerical range expressly includes all possible subranges, and all individual numerical values within that range, including integers within such ranges and fractions of the values unless the context clearly indicates otherwise.

[0048] The phrases “percent (%) sequence identity” or “% identity” or “% identical to” when
20 used with reference to an amino acid sequence describe the number of matches (“hits”) of identical amino acids of two or more aligned amino acid sequences as compared to the number of amino acid residues making up the overall length of the amino acid sequences. In other terms, using an alignment, for two or more sequences the percentage of amino acid residues that are the same (e.g. 90%, 91%, 92%, 93%, 94%, 95%, 97%, 98%, 99%, or 100% identity over the full-
25 length of the amino acid sequences) may be determined, when the sequences are compared and aligned for maximum correspondence as measured using a sequence comparison algorithm as known in the art, or when manually aligned and visually inspected. The sequences which are compared to determine sequence identity may thus differ by substitution(s), addition(s) or deletion(s) of amino acids. Suitable programs for aligning protein sequences are known to the
30 skilled person. The percentage sequence identity of protein sequences can, for example, be

determined with programs such as CLUSTALW, Clustal Omega, FASTA or BLAST, e.g., using the NCBI BLAST algorithm (Altschul SF, et al (1997), *Nucleic Acids Res.* 25:3389-3402).

[0049] As used herein, the terms and phrases “in combination,” “in combination with,” “co-delivery,” and “administered together with” in the context of the administration of two or more therapies or components to a subject refers to simultaneous administration of two or more therapies or components, such as two vectors, e.g., DNA plasmids, or an immunogenic combination and an adjuvant. “Simultaneous administration” can be administration of the two components at least within the same day. When two components are “administered together with” or “administered in combination with,” they can be administered in separate compositions sequentially within a short time period, such as 24, 20, 16, 12, 8 or 4 hours, or within 1 hour, or they can be administered in a single composition at the same time. The use of the term “in combination with” does not restrict the order in which therapies or components are administered to a subject. For example, a first therapy or component (e.g. first DNA plasmid encoding an HBV antigen) can be administered prior to (e.g., 5 minutes to one hour before), concomitantly with or simultaneously with, or subsequent to (e.g., 5 minutes to one hour after) the administration of a second therapy or component (e.g., second DNA plasmid encoding an HBV antigen). In some embodiments, a first therapy or component (e.g. first DNA plasmid encoding an HBV antigen) and a second therapy or component (e.g., second DNA plasmid encoding an HBV antigen) are administered in the same composition. In other embodiments, a first therapy or component (e.g. first DNA plasmid encoding an HBV antigen) and a second therapy or component (e.g., second DNA plasmid encoding an HBV antigen) are administered in separate compositions.

[0050] As used herein, a “non-naturally occurring” nucleic acid or polypeptide, refers to a nucleic acid or polypeptide that does not occur in nature. A “non-naturally occurring” nucleic acid or polypeptide can be synthesized, treated, fabricated, and/or otherwise manipulated in a laboratory and/or manufacturing setting. In some cases, a non-naturally occurring nucleic acid or polypeptide can comprise a naturally-occurring nucleic acid or polypeptide that is treated, processed, or manipulated to exhibit properties that were not present in the naturally-occurring nucleic acid or polypeptide, prior to treatment. As used herein, a “non-naturally occurring” nucleic acid or polypeptide can be a nucleic acid or polypeptide isolated or separated from the natural source in which it was discovered, and it lacks covalent bonds to sequences with which it

was associated in the natural source. A “non-naturally occurring” nucleic acid or polypeptide can be made recombinantly or via other methods, such as chemical synthesis.

[0051] As used herein, “subject” means any animal, preferably a mammal, most preferably a human, to whom will be or has been treated by a method according to an embodiment of the application. The term “mammal” as used herein encompasses any mammal. Examples of mammals include, but are not limited to, cows, horses, sheep, pigs, cats, dogs, mice, rats, rabbits, guinea pigs, non-human primates (NHPs) such as monkeys or apes, humans, etc., more preferably a human.

[0052] As used herein, the term “operably linked” refers to a linkage or a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. For example, a regulatory sequence operably linked to a nucleic acid sequence of interest is capable of directing the transcription of the nucleic acid sequence of interest, or a signal sequence operably linked to an amino acid sequence of interest is capable of secreting or translocate the amino acid sequence of interest over a membrane.

[0053] In an attempt to help the reader of the application, the description has been separated in various paragraphs or sections, or is directed to various embodiments of the application. These separations should not be considered as disconnecting the substance of a paragraph or section or embodiments from the substance of another paragraph or section or embodiments. To the contrary, one skilled in the art will understand that the description has broad application and encompasses all the combinations of the various sections, paragraphs and sentences that can be contemplated. The discussion of any embodiment is meant only to be exemplary and is not intended to suggest that the scope of the disclosure, including the claims, is limited to these examples. For example, while embodiments of HBV vectors of the application (e.g., plasmid DNA or viral vectors) described herein may contain particular components, including, but not limited to, certain promoter sequences, enhancer or regulatory sequences, signal peptides, coding sequence of an HBV antigen, polyadenylation signal sequences, etc. arranged in a particular order, those having ordinary skill in the art will appreciate that the concepts disclosed herein may equally apply to other components arranged in other orders that can be used in HBV vectors of the application. The application contemplates use of any of the applicable components in any combination having any sequence that can be used in HBV vectors of the application, whether or not a particular combination is expressly described.

[0054] Hepatitis B Virus (HBV)

[0055] As used herein “hepatitis B virus” or “HBV” refers to a virus of the hepadnaviridae family. HBV is a small (e.g., 3.2 kb) hepatotropic DNA virus that encodes four open reading frames and seven proteins. See **FIG. 1A**. The seven proteins encoded by HBV include small (S), medium (M), and large (L) surface antigen (HBsAg) or envelope (Env) proteins, pre-Core protein, core protein, viral polymerase (Pol), and HBx protein. HBV expresses three surface antigens, or envelope proteins, L, M, and S, with S being the smallest and L being the largest. The extra domains in the M and L proteins are named Pre-S2 and Pre-S1, respectively. Core protein is the subunit of the viral nucleocapsid. Pol is needed for synthesis of viral DNA (reverse transcriptase, RNaseH, and primer), which takes place in nucleocapsids localized to the cytoplasm of infected hepatocytes. PreCore is the core protein with an N-terminal signal peptide and is proteolytically processed at its N and C termini before secretion from infected cells, as the so-called hepatitis B e-antigen (HBeAg). HBx protein is required for efficient transcription of covalently closed circular DNA (cccDNA). HBx is not a viral structural protein. All viral proteins of HBV have their own mRNA except for core and polymerase, which share an mRNA. With the exception of the protein pre-Core, none of the HBV viral proteins are subject to post-translational proteolytic processing.

[0056] The HBV virion contains a viral envelope, nucleocapsid, and single copy of the partially double-stranded DNA genome. The nucleocapsid comprises 120 dimers of core protein and is covered by a capsid membrane embedded with the S, M, and L viral envelope or surface antigen proteins. After entry into the cell, the virus is uncoated and the capsid-containing relaxed circular DNA (rcDNA) with covalently bound viral polymerase migrates to the nucleus. During that process, phosphorylation of the core protein induces structural changes, exposing a nuclear localization signal enabling interaction of the capsid with so-called importins. These importins mediate binding of the core protein to nuclear pore complexes upon which the capsid disassembles and polymerase/rcDNA complex is released into the nucleus. Within the nucleus the rcDNA becomes deproteinized (removal of polymerase) and is converted by host DNA repair machinery to a covalently closed circular DNA (cccDNA) genome from which overlapping transcripts encode for HBeAg, HBsAg, Core protein, viral polymerase and HBx protein. Core protein, viral polymerase, and pre-genomic RNA (pgRNA) associate in the cytoplasm and self-assemble into immature pgRNA-containing capsid particles, which further convert into mature

rcDNA-capsids and function as a common intermediate that is either enveloped and secreted as infectious virus particles or transported back to the nucleus to replenish and maintain a stable cccDNA pool. See **FIG. 1B**.

[0057] To date, HBV is divided into four serotypes (adr, adw, ayr, ayw) based on antigenic epitopes present on the envelope proteins, and into eight genotypes (A, B, C, D, E, F, G, and H) based on the sequence of the viral genome. The HBV genotypes are distributed over different geographic regions. For example, the most prevalent genotypes in Asia are genotypes B and C. Genotype D is dominant in Africa, the Middle East, and India, whereas genotype A is widespread in Northern Europe, sub-Saharan Africa, and West Africa.

10 [0058] **HBV Antigens**

[0059] As used herein, the terms “HBV antigen,” “antigenic polypeptide of HBV,” “HBV antigenic polypeptide,” “HBV antigenic protein,” “HBV immunogenic polypeptide,” and “HBV immunogen” all refer to a polypeptide capable of inducing an immune response, e.g., a humoral and/or cellular mediated response, against an HBV in a subject. The HBV antigen can be a polypeptide of HBV, a fragment or epitope thereof, or a combination of multiple HBV polypeptides, portions or derivatives thereof. An HBV antigen is capable of raising in a host a protective immune response, e.g., inducing an immune response against a viral disease or infection, and/or producing an immunity (i.e., vaccinates) in a subject against a viral disease or infection, that protects the subject against the viral disease or infection. For example, an HBV antigen can comprise a polypeptide or immunogenic fragment(s) thereof from any HBV protein, such as HBeAg, pre-core protein, HBsAg (S, M, or L proteins), core protein, viral polymerase, or HBx protein derived from any HBV genotype, e.g., genotype A, B, C, D, E, F, G, and/or H, or combination thereof.

20 [0060] **(1) HBV Core Antigen**

25 [0061] As used herein, each of the terms “HBV core antigen,” “HBcAg” and “core antigen” refers to an HBV antigen capable of inducing an immune response, e.g., a humoral and/or cellular mediated response, against an HBV core protein in a subject. Each of the terms “core,” “core polypeptide,” and “core protein” refers to the HBV viral core protein. Full-length core antigen is typically 183 amino acids in length and includes an assembly domain (amino acids 1 to 149) and a nucleic acid binding domain (amino acids 150 to 183). The 34-residue nucleic acid binding domain is required for pre-genomic RNA encapsidation. This domain also functions as

a nuclear import signal. It comprises 17 arginine residues and is highly basic, consistent with its function. HBV core protein is dimeric in solution, with the dimers self-assembling into icosahedral capsids. Each dimer of core protein has four α -helix bundles flanked by an α -helix domain on either side. Truncated HBV core proteins lacking the nucleic acid binding domain are also capable of forming capsids.

[0062] In an embodiment of the application, an HBV antigen is a truncated HBV core antigen. As used herein, a “truncated HBV core antigen,” refers to an HBV antigen that does not contain the entire length of an HBV core protein, but is capable of inducing an immune response against the HBV core protein in a subject. For example, an HBV core antigen can be modified to delete one or more amino acids of the highly positively charged (arginine rich) C-terminal nucleic acid binding domain of the core antigen, which typically contains seventeen arginine (R) residues. A truncated HBV core antigen of the application is preferably a C-terminally truncated HBV core protein which does not comprise the HBV core nuclear import signal and/or a truncated HBV core protein from which the C-terminal HBV core nuclear import signal has been deleted. In an embodiment, a truncated HBV core antigen comprises a deletion in the C-terminal nucleic acid binding domain, such as a deletion of 1 to 34 amino acid residues of the C-terminal nucleic acid binding domain, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, or 34 amino acid residues, preferably a deletion of all 34 amino acid residues. In a preferred embodiment, a truncated HBV core antigen comprises a deletion in the C-terminal nucleic acid binding domain, preferably a deletion of all 34 amino acid residues.

[0063] An HBV core antigen of the application can be a consensus sequence derived from multiple HBV genotypes (e.g., genotypes A, B, C, D, E, F, G, and H). As used herein, “consensus sequence” means an artificial sequence of amino acids based on an alignment of amino acid sequences of homologous proteins, e.g., as determined by an alignment (e.g., using Clustal Omega) of amino acid sequences of homologous proteins. It can be the calculated order of most frequent amino acid residues, found at each position in a sequence alignment, based upon sequences of HBV antigens (e.g., core, pol, etc.) from at least 100 natural HBV isolates. A consensus sequence can be non-naturally occurring and different from the native viral sequences. Consensus sequences can be designed by aligning multiple HBV antigen sequences from different sources using a multiple sequence alignment tool, and at variable alignment positions,

selecting the most frequent amino acid. Preferably, a consensus sequence of an HBV antigen is derived from HBV genotypes B, C, and D. The term “consensus antigen” is used to refer to an antigen having a consensus sequence.

[0064] An exemplary truncated HBV core antigen according to the application lacks the nucleic acid binding function, and is capable of inducing an immune response in a mammal against at least two HBV genotypes. Preferably a truncated HBV core antigen is capable of inducing a T cell response in a mammal against at least HBV genotypes B, C and D. More preferably, a truncated HBV core antigen is capable of inducing a CD8 T cell response in a human subject against at least HBV genotypes A, B, C and D.

[0065] Preferably, an HBV core antigen of the application is a consensus antigen, preferably a consensus antigen derived from HBV genotypes B, C, and D, more preferably a truncated consensus antigen derived from HBV genotypes B, C, and D. An exemplary truncated HBV core consensus antigen according to the application consists of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 14, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or 100% identical to SEQ ID NO: 2 or SEQ ID NO: 14. SEQ ID NO: 2 and SEQ ID NO: 14 are core consensus antigens derived from HBV genotypes B, C, and D. SEQ ID NO: 2 and SEQ ID NO: 14 contain a 34-amino acid C-terminal deletion of the highly positively charged (arginine rich) nucleic acid binding domain of the native core antigen.

[0066] In a particular embodiment of the application, an HBV core antigen is a truncated HBV antigen consisting of the amino acid sequence of SEQ ID NO: 2. In another particular embodiment, an HBV core antigen is a truncated HBV antigen consisting of the amino acid sequence of SEQ ID NO: 14.

[0067] (2) HBV Polymerase Antigen

[0068] As used herein, the term “HBV polymerase antigen,” “HBV Pol antigen” or “HBV pol antigen” refers to an HBV antigen capable of inducing an immune response, e.g., a humoral and/or cellular mediated response, against an HBV polymerase in a subject. Each of the terms “polymerase,” “polymerase polypeptide,” “Pol” and “pol” refers to the HBV viral DNA polymerase. The HBV viral DNA polymerase has four domains, including, from the N terminus to the C terminus, a terminal protein (TP) domain, which acts as a primer for minus-strand DNA

synthesis; a spacer that is nonessential for the polymerase functions; a reverse transcriptase (RT) domain for transcription; and a RNase H domain.

[0069] In an embodiment of the application, an HBV antigen comprises an HBV Pol antigen, or any immunogenic fragment or combination thereof. An HBV Pol antigen can contain further
5 modifications to improve immunogenicity of the antigen, such as by introducing mutations into the active sites of the polymerase and/or RNase domains to decrease or substantially eliminate certain enzymatic activities.

[0070] Preferably, an HBV Pol antigen of the application does not have reverse transcriptase activity and RNase H activity, and is capable of inducing an immune response in a mammal
10 against at least two HBV genotypes. Preferably, an HBV Pol antigen is capable of inducing a T cell response in a mammal against at least HBV genotypes B, C and D. More preferably, a HBV Pol antigen is capable of inducing a CD8 T cell response in a human subject against at least HBV genotypes A, B, C and D.

[0071] Thus, in some embodiments, an HBV Pol antigen is an inactivated Pol antigen. In an
15 embodiment, an inactivated HBV Pol antigen comprises one or more amino acid mutations in the active site of the polymerase domain. In another embodiment, an inactivated HBV Pol antigen comprises one or more amino acid mutations in the active site of the RNaseH domain. In a preferred embodiment, an inactivated HBV pol antigen comprises one or more amino acid mutations in the active site of both the polymerase domain and the RNaseH domain. For
20 example, the “YXDD” motif in the polymerase domain of an HBV pol antigen that can be required for nucleotide/metal ion binding can be mutated, e.g., by replacing one or more of the aspartate residues (D) with asparagine residues (N), eliminating or reducing metal coordination function, thereby decreasing or substantially eliminating reverse transcriptase function.

Alternatively, or in addition to mutation of the “YXDD” motif, the “DEDD” motif in the
25 RNaseH domain of an HBV pol antigen required for Mg^{2+} coordination can be mutated, e.g., by replacing one or more aspartate residues (D) with asparagine residues (N) and/or replacing the glutamate residue (E) with glutamine (Q), thereby decreasing or substantially eliminating RNaseH function. In a particular embodiment, an HBV pol antigen is modified by (1) mutating the aspartate residues (D) to asparagine residues (N) in the “YXDD” motif of the polymerase
30 domain; and (2) mutating the first aspartate residue (D) to an asparagine residue (N) and the first glutamate residue (E) to a glutamine residue (N) in the “DEDD” motif of the RNaseH domain,

thereby decreasing or substantially eliminating both the reverse transcriptase and RNaseH functions of the pol antigen.

[0072] In a preferred embodiment of the application, an HBV pol antigen is a consensus antigen, preferably a consensus antigen derived from HBV genotypes B, C, and D, more preferably an inactivated consensus antigen derived from HBV genotypes B, C, and D. An exemplary HBV pol consensus antigen according to the application comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 4, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 4, preferably at least 98% identical to SEQ ID NO: 4, such as at least 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 4. SEQ ID NO: 4 is a pol consensus antigen derived from HBV genotypes B, C, and D comprising four mutations located in the active sites of the polymerase and RNaseH domains. In particular, the four mutations include mutation of the aspartic acid residues (D) to asparagine residues (N) in the “YXDD” motif of the polymerase domain; and mutation of the first aspartate residue (D) to an asparagine residue (N) and mutation of the glutamate residue (E) to a glutamine residue (Q) in the “DEDD” motif of the RNaseH domain.

[0073] In a particular embodiment of the application, an HBV pol antigen comprises the amino acid sequence of SEQ ID NO: 4. In other embodiments of the application, an HBV pol antigen consists of the amino acid sequence of SEQ ID NO: 4.

[0074] (3) Fusion of HBV Core Antigen and HBV Polymerase Antigen

[0075] As used herein the term “fusion protein” or “fusion” refers to a single polypeptide chain having at least two polypeptide domains that are not normally present in a single, natural polypeptide.

[0076] In an embodiment of the application, an HBV antigen comprises a fusion protein comprising a truncated HBV core antigen operably linked to a HBV Pol antigen, or a HBV Pol antigen operably linked to a truncated HBV core antigen, preferably via a linker.

[0077] As used herein, the term “linker” refers to a compound or moiety that acts as a molecular bridge to operably link two different molecules, wherein one portion of the linker is operably linked to a first molecule, and wherein another portion of the linker is operably linked to a second molecule. For example, in a fusion protein containing a first polypeptide and a

second heterologous polypeptide, a linker serves primarily as a spacer between the first and second polypeptides. In an embodiment, a linker is made up of amino acids linked together by peptide bonds, preferably from 1 to 20 amino acids linked by peptide bonds, wherein the amino acids are selected from the 20 naturally occurring amino acids. In an embodiment, the 1 to 20 amino acids are selected from glycine, alanine, proline, asparagine, glutamine, and lysine. Preferably, a linker is made up of a majority of amino acids that are sterically unhindered, such as glycine and alanine. Exemplary linkers are polyglycines, particularly (Gly)₅, (Gly)₈; poly(Gly-Ala), and polyalanines. One exemplary suitable linker as shown in the Examples below is (AlaGly)_n, wherein n is an integer of 2 to 5.

10 **[0078]** Preferably, a fusion protein of the application is capable of inducing an immune response in a mammal against HBV core and HBV Pol of at least two HBV genotypes. Preferably, a fusion protein is capable of inducing a T cell response in a mammal against at least HBV genotypes B, C and D. More preferably, the fusion protein is capable of inducing a CD8 T cell response in a human subject against at least HBV genotypes A, B, C and D.

15 **[0079]** In an embodiment of the application, a fusion protein comprises a truncated HBV core antigen having an amino acid sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or 100% identical to SEQ ID NO: 2 or SEQ ID NO: 14, a linker, and a HBV Pol antigen having an amino acid sequence at least 90%, such as at least 90%, 20 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or 100%, identical to SEQ ID NO: 4.

[0080] In a preferred embodiment of the application, a fusion protein comprises a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 14, a linker comprising 25 (AlaGly)_n, wherein n is an integer of 2 to 5, and a HBV Pol antigen having the amino acid sequence of SEQ ID NO: 4. More preferably, a fusion protein according to an embodiment of the application comprises the amino acid sequence of SEQ ID NO: 20.

[0081] In an embodiment of the application, a fusion protein further comprises a signal sequence. Preferably, the signal sequence has the amino acid sequence of SEQ ID NO: 6 or SEQ 30 ID NO: 19. More preferably, a fusion protein comprises the amino acid sequence of SEQ ID NO: 21.

[0082] Polynucleotides and Vectors

[0083] In another general aspect, the application provides a non-naturally occurring nucleic acid molecule encoding an HBV antigen according to the application, and a vector comprising the non-naturally occurring nucleic acid. A non-naturally occurring nucleic acid molecule can

5 comprise any polynucleotide sequence encoding an HBV antigen of the application, which can be made using methods known in the art in view of the present disclosure. Preferably, a polynucleotide encodes at least one of a truncated HBV core antigen and an HBV polymerase antigen of the application. A polynucleotide can be in the form of RNA or in the form of DNA obtained by recombinant techniques (e.g., cloning) or produced synthetically (e.g., chemical

10 synthesis). The DNA can be single-stranded or double-stranded, or can contain portions of both double-stranded and single-stranded sequence. The DNA can, for example, comprise genomic DNA, cDNA, or combinations thereof. The polynucleotide can also be a DNA/RNA hybrid.

The polynucleotides and vectors of the application can be used for recombinant protein production, expression of the protein in a host cell, or the production of viral particles.

15 Preferably, a polynucleotide is DNA.

[0084] In an embodiment of the application, a non-naturally occurring nucleic acid molecule comprises a polynucleotide encoding a truncated HBV core antigen consisting of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 14, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%,

20 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 2, preferably 98%, 99% or 100% identical to SEQ ID NO: 2 or SEQ ID NO: 14. In a particular embodiment of the application, a non-naturally occurring nucleic acid molecule encodes a truncated HBV core antigen consisting the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 14.

25 **[0085]** Examples of polynucleotide sequences of the application encoding a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 14 include, but are not limited to, a polynucleotide sequence at least 90% identical to SEQ ID NO: 1 or SEQ ID NO: 15, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100%

30 identical to SEQ ID NO: 1 or SEQ ID NO: 15, preferably 98%, 99% or 100% identical to SEQ ID NO: 1 or SEQ ID NO: 15. Exemplary non-naturally occurring nucleic acid molecules

encoding a truncated HBV core antigen have the polynucleotide sequence of SEQ ID NOs: 1 or 15.

[0086] In an embodiment of the application, a non-naturally occurring nucleic acid molecule encodes a HBV polymerase antigen comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 4, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 4, preferably 100% identical to SEQ ID NO: 4. In a particular embodiment of the application, a non-naturally occurring nucleic acid molecule encodes a HBV polymerase antigen consisting of the amino acid sequence of SEQ ID NO: 4.

[0087] Examples of polynucleotide sequences of the application encoding a HBV Pol antigen comprising the amino acid sequence of SEQ ID NO: 4 include, but are not limited to, a polynucleotide sequence at least 90% identical to SEQ ID NO: 3 or SEQ ID NO: 16, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 3 or SEQ ID NO: 16, preferably 98%, 99% or 100% identical to SEQ ID NO: 3 or SEQ ID NO: 16. Exemplary non-naturally occurring nucleic acid molecules encoding a HBV pol antigen have the polynucleotide sequence of SEQ ID NOs: 3 or 16.

[0088] In another embodiment of the application, a non-naturally occurring nucleic acid molecule encodes a fusion protein comprising a truncated HBV core antigen operably linked to a HBV Pol antigen, or a HBV Pol antigen operably linked to a truncated HBV core antigen. In a particular embodiment, a non-naturally occurring nucleic acid molecule of the application encodes a truncated HBV core antigen consisting of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 14, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 2 or SEQ ID NO: 14, preferably 100% identical to SEQ ID NO: 2 or SEQ ID NO: 14, more preferably 100% identical to SEQ ID NO: 14; a linker; and a HBV polymerase antigen comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 4, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 4, preferably 98%, 99% or 100% identical to SEQ ID NO: 4. In a particular embodiment of the application, a non-naturally

occurring nucleic acid molecule encodes a fusion protein comprising a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 14, a linker comprising (AlaGly)_n, wherein n is an integer of 2 to 5; and a HBV Pol antigen comprising the amino acid sequence of SEQ ID NO: 4. In a particular embodiment of the application, a non-naturally occurring nucleic acid molecule encodes a fusion protein comprising the amino acid sequence of SEQ ID NO: 20.

[0089] Examples of polynucleotide sequences of the application encoding a fusion protein include, but are not limited to, a polynucleotide sequence at least 90% identical to SEQ ID NO: 1 or SEQ ID NO: 15, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 1 or SEQ ID NO: 15, preferably 98%, 99% or 100% identical to SEQ ID NO: 1 or SEQ ID NO: 15, operably linked to a linker coding sequence at least 90% identical to SEQ ID NO: 22, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 22, preferably 98%, 99% or 100% identical to SEQ ID NO: 22, which is further operably linked to a polynucleotide sequence at least 90% identical to SEQ ID NO: 3 or SEQ ID NO: 16, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 3 or SEQ ID NO: 16, preferably 98%, 99% or 100% identical to SEQ ID NO: 3 or SEQ ID NO: 16. In particular embodiments of the application, a non-naturally occurring nucleic acid molecule encoding a fusion protein comprises SEQ ID NO: 1 or SEQ ID NO: 15, operably linked to SEQ ID NO: 22, which is further operably linked to SEQ ID NO: 3 or SEQ ID NO: 16.

[0090] The application also relates to a vector comprising an isolated polynucleotide encoding an HBV antigen. As used herein, a “vector” is a nucleic acid molecule used to carry genetic material into another cell, where it can be replicated and/or expressed. Any vector known to those skilled in the art in view of the present disclosure can be used. Examples of vectors include, but are not limited to, plasmids, viral vectors (bacteriophage, animal viruses, and plant viruses), cosmids, and artificial chromosomes (e.g., YACs). Preferably, a vector is a DNA plasmid. A vector can be a DNA vector or an RNA vector. One of ordinary skill in the art can construct a vector of the application through standard recombinant techniques in view of the present disclosure.

[0091] A vector of the application can be an expression vector. As used herein, the term “expression vector” refers to any type of genetic construct comprising a nucleic acid coding for an RNA capable of being transcribed. Expression vectors include, but are not limited to, vectors for recombinant protein expression, such as a DNA plasmid or a viral vector, and vectors for
5 delivery of nucleic acid into a subject for expression in a tissue of the subject, such as a DNA plasmid or a viral vector. It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc.

[0092] Vectors of the application can contain a variety of regulatory sequences. As used
10 herein, the term “regulatory sequence” refers to any sequence that allows, contributes or modulates the functional regulation of the nucleic acid molecule, including replication, duplication, transcription, splicing, translation, stability and/or transport of the nucleic acid or one of its derivative (i.e. mRNA) into the host cell or organism. In the context of the disclosure, this term encompasses promoters, enhancers and other expression control elements (e.g.,
15 polyadenylation signals and elements that affect mRNA stability).

[0093] In some embodiments of the application, a vector is a non-viral vector. Examples of non-viral vectors include, but are not limited to, DNA plasmids, bacterial artificial chromosomes, yeast artificial chromosomes, bacteriophages, etc. Examples of non-viral vectors include, but are not limited to, RNA replicon, mRNA replicon, modified mRNA replicon or self-amplifying
20 mRNA, closed linear deoxyribonucleic acid, e.g., a linear covalently closed DNA, e.g., a linear covalently closed double stranded DNA molecule. Preferably, a non-viral vector is a DNA plasmid. A “DNA plasmid”, which is used interchangeably with “DNA plasmid vector,” “plasmid DNA” or “plasmid DNA vector,” refers to a double-stranded and generally circular DNA sequence that is capable of autonomous replication in a suitable host cell. DNA plasmids
25 used for expression of an encoded polynucleotide typically comprise an origin of replication, a multiple cloning site, and a selectable marker, which for example, can be an antibiotic resistance gene. Examples of suitable DNA plasmids that can be used include, but are not limited to, commercially available expression vectors for use in well-known expression systems (including both prokaryotic and eukaryotic systems), such as pSE420 (Invitrogen, San Diego, Calif.), which
30 can be used for production and/or expression of protein in *Escherichia coli*; pYES2 (Invitrogen, Thermo Fisher Scientific), which can be used for production and/or expression in

Saccharomyces cerevisiae strains of yeast; MAXBAC[®] complete baculovirus expression system (Thermo Fisher Scientific), which can be used for production and/or expression in insect cells; pcDNA[™] or pcDNA3[™] (Life Technologies, Thermo Fisher Scientific), which can be used for high level constitutive protein expression in mammalian cells; and pVAX or pVAX-1 (Life Technologies, Thermo Fisher Scientific), which can be used for high-level transient expression of a protein of interest in most mammalian cells. The backbone of any commercially available DNA plasmid can be modified to optimize protein expression in the host cell, such as to reverse the orientation of certain elements (e.g., origin of replication and/or antibiotic resistance cassette), replace a promoter endogenous to the plasmid (e.g., the promoter in the antibiotic resistance cassette), and/or replace the polynucleotide sequence encoding transcribed proteins (e.g., the coding sequence of the antibiotic resistance gene), by using routine techniques and readily available starting materials. (See e.g., Sambrook et al., Molecular Cloning a Laboratory Manual, Second Ed. Cold Spring Harbor Press (1989)).

[0094] Preferably, a DNA plasmid is an expression vector suitable for protein expression in mammalian host cells. Expression vectors suitable for protein expression in mammalian host cells include, but are not limited to, pcDNA[™], pcDNA3[™], pVAX, pVAX-1, ADVAX, NTC8454, etc. Preferably, an expression vector is based on pVAX-1, which can be further modified to optimize protein expression in mammalian cells. pVAX-1 is a commonly used plasmid in DNA vaccines, and contains a strong human immediate early cytomegalovirus (CMV-IE) promoter followed by the bovine growth hormone (bGH)-derived polyadenylation sequence (pA). pVAX-1 further contains a pUC origin of replication and a kanamycin resistance gene driven by a small prokaryotic promoter that allows for bacterial plasmid propagation.

[0095] A vector of the application can also be a viral vector. In general, viral vectors are genetically engineered viruses carrying modified viral DNA or RNA that has been rendered non-infectious, but still contains viral promoters and transgenes, thus allowing for translation of the transgene through a viral promoter. Because viral vectors are frequently lacking infectious sequences, they require helper viruses or packaging lines for large-scale transfection. Examples of viral vectors that can be used include, but are not limited to, adenoviral vectors, adeno-associated virus vectors, pox virus vectors, enteric virus vectors, Venezuelan Equine Encephalitis virus vectors, Semliki Forest Virus vectors, Tobacco Mosaic Virus vectors, lentiviral vectors, arenavirus viral vectors, replication-deficient arenavirus viral vectors or replication-competent

arenavirus viral vectors, bi-segmented or tri-segmented arenavirus, infectious arenavirus viral vectors, nucleic acids which comprise an arenavirus genomic segment wherein one open reading frame of the genomic segment is deleted or functionally inactivated (and replaced by a nucleic acid encoding a HBV antigen as described herein), arenavirus such as lymphocytic choriomeningitis virus (LCMV), e.g., clone 13 strain or MP strain, and arenavirus such as Junin virus e.g., Candid #1 strain, etc. The vector can also be a non-viral vector.

[0096] Preferably, a viral vector is an adenovirus vector, e.g., a recombinant adenovirus vector. A recombinant adenovirus vector can for instance be derived from a human adenovirus (HAdV, or AdHu), or a simian adenovirus such as chimpanzee or gorilla adenovirus (ChAd, AdCh, or SAdV) or rhesus adenovirus (rhAd). Preferably, an adenovirus vector is a recombinant human adenovirus vector, for instance a recombinant human adenovirus serotype 26, or any one of recombinant human adenovirus serotype 5, 4, 35, 7, 48, etc. In other embodiments, an adenovirus vector is a rhAd vector, e.g. rhAd51, rhAd52 or rhAd53. A recombinant viral vector useful for the application can be prepared using methods known in the art in view of the present disclosure. For example, in view of the degeneracy of the genetic code, several nucleic acid sequences can be designed that encode the same polypeptide. A polynucleotide encoding an HBV antigen of the application can optionally be codon-optimized to ensure proper expression in the host cell (e.g., bacterial or mammalian cells). Codon-optimization is a technology widely applied in the art, and methods for obtaining codon-optimized polynucleotides will be well known to those skilled in the art in view of the present disclosure.

[0097] A vector of the application, e.g., a DNA plasmid or a viral vector (particularly an adenoviral vector), can comprise any regulatory elements to establish conventional function(s) of the vector, including but not limited to replication and expression of the HBV antigen(s) encoded by the polynucleotide sequence of the vector. Regulatory elements include, but are not limited to, a promoter, an enhancer, a polyadenylation signal, translation stop codon, a ribosome binding element, a transcription terminator, selection markers, origin of replication, etc. A vector can comprise one or more expression cassettes. An "expression cassette" is part of a vector that directs the cellular machinery to make RNA and protein. An expression cassette typically comprises three components: a promoter sequence, an open reading frame, and a 3'-untranslated region (UTR) optionally comprising a polyadenylation signal. An open reading frame (ORF) is a reading frame that contains a coding sequence of a protein of interest (e.g., HBV antigen) from

a start codon to a stop codon. Regulatory elements of the expression cassette can be operably linked to a polynucleotide sequence encoding an HBV antigen of interest. As used herein, the term “operably linked” is to be taken in its broadest reasonable context, and refers to a linkage of polynucleotide elements in a functional relationship. A polynucleotide is “operably linked” when it is placed into a functional relationship with another polynucleotide. For instance, a promoter is operably linked to a coding sequence if it affects the transcription of the coding sequence. Any components suitable for use in an expression cassette described herein can be used in any combination and in any order to prepare vectors of the application.

[0098] A vector can comprise a promoter sequence, preferably within an expression cassette, to control expression of an HBV antigen of interest. The term “promoter” is used in its conventional sense, and refers to a nucleotide sequence that initiates the transcription of an operably linked nucleotide sequence. A promoter is located on the same strand near the nucleotide sequence it transcribes. Promoters can be a constitutive, inducible, or repressible. Promoters can be naturally occurring or synthetic. A promoter can be derived from sources including viral, bacterial, fungal, plants, insects, and animals. A promoter can be a homologous promoter (i.e., derived from the same genetic source as the vector) or a heterologous promoter (i.e., derived from a different vector or genetic source). For example, if the vector to be employed is a DNA plasmid, the promoter can be endogenous to the plasmid (homologous) or derived from other sources (heterologous). Preferably, the promoter is located upstream of the polynucleotide encoding an HBV antigen within an expression cassette.

[0099] Examples of promoters that can be used include, but are not limited to, a promoter from simian virus 40 (SV40), a mouse mammary tumor virus (MMTV) promoter, a human immunodeficiency virus (HIV) promoter such as the bovine immunodeficiency virus (BIV) long terminal repeat (LTR) promoter, a Moloney virus promoter, an avian leukosis virus (ALV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter (CMV-IE), Epstein Barr virus (EBV) promoter, or a Rous sarcoma virus (RSV) promoter. A promoter can also be a promoter from a human gene such as human actin, human myosin, human hemoglobin, human muscle creatine, or human metallothionein. A promoter can also be a tissue specific promoter, such as a muscle or skin specific promoter, natural or synthetic.

[0100] Preferably, a promoter is a strong eukaryotic promoter, preferably a cytomegalovirus immediate early (CMV-IE) promoter. A nucleotide sequence of an exemplary CMV-IE promoter is shown in SEQ ID NO: 7 and SEQ ID NO: 17.

[0101] A vector can comprise additional polynucleotide sequences that stabilize the expressed transcript, enhance nuclear export of the RNA transcript, and/or improve transcriptional-translational coupling. Examples of such sequences include polyadenylation signals and enhancer sequences. A polyadenylation signal is typically located downstream of the coding sequence for a protein of interest (e.g., an HBV antigen) within an expression cassette of the vector. Enhancer sequences are regulatory DNA sequences that, when bound by transcription factors, enhance the transcription of an associated gene. An enhancer sequence is preferably located upstream of the polynucleotide sequence encoding an HBV antigen, but downstream of a promoter sequence within an expression cassette of the vector.

[0102] Any polyadenylation signal known to those skilled in the art in view of the present disclosure can be used. For example, the polyadenylation signal can be a SV40 polyadenylation signal (e.g., SEQ ID NO: 24), LTR polyadenylation signal, bovine growth hormone (bGH) polyadenylation signal, human growth hormone (hGH) polyadenylation signal, or human β -globin polyadenylation signal. Preferably, a polyadenylation signal is a bovine growth hormone (bGH) polyadenylation signal or a SV40 polyadenylation signal. A nucleotide sequence of an exemplary bGH polyadenylation signal is shown in SEQ ID NO: 11. A nucleotide sequence of an exemplary SV40 polyadenylation signal is shown in SEQ ID NO: 24.

[0103] Any enhancer sequence known to those skilled in the art in view of the present disclosure can be used. For example, an enhancer sequence can be a human actin, human myosin, human hemoglobin, human muscle creatine, or a viral enhancer, such as one from CMV, HA, RSV, or EBV. Examples of particular enhancers include, but are not limited to, Woodchuck HBV Post-transcriptional regulatory element (WPRE), intron/exon sequence derived from human apolipoprotein A1 precursor (ApoAI), untranslated R-U5 domain of the human T-cell leukemia virus type 1 (HTLV-1) long terminal repeat (LTR), a splicing enhancer, a synthetic rabbit β -globin intron, or any combination thereof. Preferably, an enhancer sequence is a composite sequence of three consecutive elements of the untranslated R-U5 domain of HTLV-1 LTR, rabbit β -globin intron, and a splicing enhancer, which is referred to herein as “a triple enhancer sequence.” A nucleotide sequence of an exemplary triple enhancer sequence is shown

in SEQ ID NO: 8. Another exemplary enhancer sequence is an ApoAI gene fragment shown in SEQ ID NO: 23.

[0104] A vector can comprise a polynucleotide sequence encoding a signal peptide sequence. Preferably, the polynucleotide sequence encoding the signal peptide sequence is located
5 upstream of the polynucleotide sequence encoding an HBV antigen. Signal peptides typically direct localization of a protein, facilitate secretion of the protein from the cell in which it is produced, and/or improve antigen expression and cross-presentation to antigen-presenting cells. A signal peptide can be present at the N-terminus of an HBV antigen when expressed from the vector, but is cleaved off by signal peptidase, e.g., upon secretion from the cell. An expressed
10 protein in which a signal peptide has been cleaved is often referred to as the “mature protein.” Any signal peptide known in the art in view of the present disclosure can be used. For example, a signal peptide can be a cystatin S signal peptide; an immunoglobulin (Ig) secretion signal, such as the Ig heavy chain gamma signal peptide SPIgG or the Ig heavy chain epsilon signal peptide SPIgE.

[0105] Preferably, a signal peptide sequence is a cystatin S signal peptide. Exemplary nucleic acid and amino acid sequences of a cystatin S signal peptide are shown in SEQ ID NOs: 5 and 6, respectively. Exemplary nucleic acid and amino acid sequences of an immunoglobulin secretion signal are shown in SEQ ID NOs: 18 and 19, respectively.

[0106] A vector, such as a DNA plasmid, can also include a bacterial origin of replication
20 and an antibiotic resistance expression cassette for selection and maintenance of the plasmid in bacterial cells, e.g., *E. coli*. Bacterial origins of replication and antibiotic resistance cassettes can be located in a vector in the same orientation as the expression cassette encoding an HBV antigen, or in the opposite (reverse) orientation. An origin of replication (ORI) is a sequence at which replication is initiated, enabling a plasmid to reproduce and survive within cells.
25 Examples of ORIs suitable for use in the application include, but are not limited to ColE1, pMB1, pUC, pSC101, R6K, and 15A, preferably pUC. An exemplary nucleotide sequence of a pUC ORI is shown in SEQ ID NO: 10.

[0107] Expression cassettes for selection and maintenance in bacterial cells typically include a promoter sequence operably linked to an antibiotic resistance gene. Preferably, the promoter
30 sequence operably linked to an antibiotic resistance gene differs from the promoter sequence operably linked to a polynucleotide sequence encoding a protein of interest, e.g., HBV antigen.

The antibiotic resistance gene can be codon optimized, and the sequence composition of the antibiotic resistance gene is normally adjusted to bacterial, e.g., *E. coli*, codon usage. Any antibiotic resistance gene known to those skilled in the art in view of the present disclosure can be used, including, but not limited to, kanamycin resistance gene (Kan^r), ampicillin resistance gene (Amp^r), and tetracycline resistance gene (Tet^r), as well as genes conferring resistance to chloramphenicol, bleomycin, spectinomycin, carbenicillin, etc.

[0108] Preferably, an antibiotic resistance gene in the antibiotic expression cassette of a vector is a kanamycin resistance gene (Kan^r). The sequence of Kan^r gene is shown in SEQ ID NO: 13. Preferably, the Kan^r gene is codon optimized. An exemplary nucleic acid sequence of a codon optimized Kan^r gene is shown in SEQ ID NO: 12. The Kan^r can be operably linked to its native promoter, or the Kan^r gene can be linked to a heterologous promoter. In a particular embodiment, the Kan^r gene is operably linked to the ampicillin resistance gene (Amp^r) promoter, known as the bla promoter. An exemplary nucleotide sequence of a bla promoter is shown in SEQ ID NO: 9.

[0109] In a particular embodiment of the application, a vector is a DNA plasmid comprising an expression cassette including a polynucleotide encoding at least one of an HBV antigen selected from the group consisting of an HBV pol antigen comprising an amino acid sequence at least 98% identical to SEQ ID NO: 4, such as at least 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 4, and a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 2; an upstream sequence operably linked to the polynucleotide encoding the HBV antigen comprising, from 5' end to 3' end, a promoter sequence, preferably a CMV promoter sequence of SEQ ID NO: 7, an enhancer sequence, preferably a triple enhancer sequence of SEQ ID NO: 8, and a polynucleotide sequence encoding a signal peptide sequence, preferably a cystatin S signal peptide having the amino acid sequence of SEQ ID NO: 6; and a downstream sequence operably linked to the polynucleotide encoding the HBV antigen comprising a polyadenylation signal, preferably a bGH polyadenylation signal of SEQ ID NO: 11. Such vector further comprises an antibiotic resistance expression cassette including a polynucleotide encoding an antibiotic resistance gene, preferably a Kan^r gene, more preferably a codon optimized Kan^r gene that is at least 90% identical to SEQ ID NO: 12, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%,

99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 12, preferably 100% identical to SEQ ID NO: 12, operably linked to an Amp^r (bla) promoter of SEQ ID NO: 9, upstream of and operably linked to the polynucleotide encoding the antibiotic resistance gene; and an origin of replication, preferably a pUC ori of SEQ ID NO: 10. Preferably, the antibiotic resistance cassette and the origin of replication are present in the plasmid in the reverse orientation relative to the HBV antigen expression cassette. Exemplary DNA plasmids comprising the above mentioned features are shown in **FIGS. 2A and 2B**.

[0110] In another particular embodiment of the application, a vector is a viral vector, preferably an adenoviral vector, more preferably an Ad26 or Ad35 vector, comprising an expression cassette including a polynucleotide encoding at least one of an HBV antigen selected from the group consisting of an HBV pol antigen comprising an amino acid sequence at least 98% identical to SEQ ID NO: 4, such as at least 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 4, and a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 14; an upstream sequence operably linked to the polynucleotide encoding the HBV antigen comprising, from 5' end to 3' end, a promoter sequence, preferably a CMV promoter sequence of SEQ ID NO: 17, an enhancer sequence, preferably an ApoAI gene fragment sequence of SEQ ID NO: 23, and a polynucleotide sequence encoding a signal peptide sequence, preferably an immunoglobulin secretion signal having the amino acid sequence of SEQ ID NO: 19; and a downstream sequence operably linked to the polynucleotide encoding the HBV antigen comprising a polyadenylation signal, preferably a SV40 polyadenylation signal of SEQ ID NO: 24.

[0111] In an embodiment of the application, a vector, such as a plasmid DNA vector or a viral vector (preferably an adenoviral vector, more preferably an Ad26 or Ad35 vector), encodes an HBV Pol antigen having the amino acid sequence of SEQ ID NO: 4. Preferably, the vector comprises a coding sequence for the HBV Pol antigen that is at least 90% identical to the polynucleotide sequence of SEQ ID NO: 3, such as 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 3, preferably 100% identical to SEQ ID NO: 3.

- [0112] In an embodiment of the application, a vector, such as a plasmid DNA vector or a viral vector (preferably an adenoviral vector, more preferably an Ad26 or Ad35 vector), encodes a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 14. Preferably, the vector comprises a coding sequence for the truncated HBV core antigen that is at least 90% identical to the polynucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 15, such as 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 1 or SEQ ID NO: 15, preferably 100% identical to SEQ ID NO: 1 or SEQ ID NO: 15.
- 10 [0113] In yet another embodiment of the application, a vector, such as a plasmid DNA vector or a viral vector (preferably an adenoviral vector, more preferably an Ad26 or Ad35 vector), encodes a fusion protein comprising an HBV Pol antigen having the amino acid sequence of SEQ ID NO: 4 and a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 14. Preferably, the vector comprises a coding sequence for the fusion,
- 15 which contains a coding sequence for the truncated HBV core antigen at least 90% identical to SEQ ID NO: 1 or SEQ ID NO: 15, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 1 or SEQ ID NO: 15, preferably 98%, 99% or 100% identical to SEQ ID NO: 1 or SEQ ID NO: 15, more preferably SEQ ID NO: 15,
- 20 operably linked to a coding sequence for the HBV Pol antigen at least 90% identical to SEQ ID NO: 3 or SEQ ID NO: 16, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 3 or SEQ ID NO: 16, preferably 98%, 99% or 100% identical to SEQ ID NO: 3 or SEQ ID NO: 16, more preferably SEQ ID NO: 16.
- 25 Preferably, the coding sequence for the truncated HBV core antigen is operably linked to the coding sequence for the HBV Pol antigen via a coding sequence for a linker at least 90% identical to SEQ ID NO: 22, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 22, preferably 98%, 99% or 100% identical to
- 30 SEQ ID NO: 22. In particular embodiments of the application, a vector comprises a coding

sequence for the fusion having SEQ ID NO: 15 operably linked to SEQ ID NO: 22, which is further operably linked to SEQ ID NO: 16.

[0114] The polynucleotides and expression vectors encoding the HBV antigens of the application can be made by any method known in the art in view of the present disclosure. For example, a polynucleotide encoding an HBV antigen can be introduced or “cloned” into an expression vector using standard molecular biology techniques, e.g., polymerase chain reaction (PCR), etc., which are well known to those skilled in the art.

[0115] **Cells, Polypeptides and Antibodies**

[0116] The application also provides cells, preferably isolated cells, comprising any of the polynucleotides and vectors described herein. The cells can, for instance, be used for recombinant protein production, or for the production of viral particles.

[0117] Embodiments of the application thus also relate to a method of making an HBV antigen of the application. The method comprises transfecting a host cell with an expression vector comprising a polynucleotide encoding an HBV antigen of the application operably linked to a promoter, growing the transfected cell under conditions suitable for expression of the HBV antigen, and optionally purifying or isolating the HBV antigen expressed in the cell. The HBV antigen can be isolated or collected from the cell by any method known in the art including affinity chromatography, size exclusion chromatography, etc. Techniques used for recombinant protein expression will be well known to one of ordinary skill in the art in view of the present disclosure. The expressed HBV antigens can also be studied without purifying or isolating the expressed protein, e.g., by analyzing the supernatant of cells transfected with an expression vector encoding the HBV antigen and grown under conditions suitable for expression of the HBV antigen.

[0118] Thus, also provided are non-naturally occurring or recombinant polypeptides comprising an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 14, or SEQ ID NO: 4. As described above and below, isolated nucleic acid molecules encoding these sequences, vectors comprising these sequences operably linked to a promoter, and compositions comprising the polypeptide, polynucleotide, or vector are also contemplated by the application.

[0119] In an embodiment of the application, a recombinant polypeptide comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 2, such as

90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 2.

Preferably, a non-naturally occurring or recombinant polypeptide consists of SEQ ID NO: 2.

[0120] In another embodiment of the application, a non-naturally occurring or recombinant

5 polypeptide comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 4, such as 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 4. Preferably, a non-naturally occurring or recombinant polypeptide comprises SEQ ID NO: 4.

10 **[0121]** In another embodiment of the application, a non-naturally occurring or recombinant

polypeptide comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 14, such as 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 14. Preferably, a non-naturally occurring or

15 recombinant polypeptide consists of SEQ ID NO: 14.

[0122] Also provided are antibodies or antigen binding fragments thereof that specifically

bind to a non-naturally occurring polypeptide of the application. In an embodiment of the application, an antibody specific to a non-naturally occurring HBV antigen of the application does not bind specifically to another HBV antigen. For example, an antibody of the application

20 that binds specifically to an HBV Pol antigen having the amino acid sequence of SEQ ID NO: 4 will not bind specifically to an HBV Pol antigen not having the amino acid sequence of SEQ ID NO: 4.

[0123] As used herein, the term “antibody” includes polyclonal, monoclonal, chimeric,

humanized, Fv, Fab and F(ab')₂; bifunctional hybrid (e.g., Lanzavecchia et al., Eur. J. Immunol.

25 17:105, 1987), single-chain (Huston et al., Proc. Natl. Acad. Sci. USA 85:5879, 1988; Bird et al., Science 242:423, 1988); and antibodies with altered constant regions (e.g., U.S. Pat. No. 5,624,821).

[0124] As used herein, an antibody that “specifically binds to” an antigen refers to an

antibody that binds to the antigen with a K_D of 1×10^{-7} M or less. Preferably, an antibody that

30 “specifically binds to” an antigen binds to the antigen with a K_D of 1×10^{-8} M or less, more preferably 5×10^{-9} M or less, 1×10^{-9} M or less, 5×10^{-10} M or less, or 1×10^{-10} M or less. The term

“KD” refers to the dissociation constant, which is obtained from the ratio of Kd to Ka (i.e., Kd/Ka) and is expressed as a molar concentration (M). KD values for antibodies can be determined using methods known in the art in view of the present disclosure. For example, the KD of an antibody can be determined by using surface plasmon resonance, such as by using a biosensor system, e.g., a Biacore® system, or by using bio-layer interferometry technology, such as a Octet RED96 system.

[0125] The smaller the value of the KD of an antibody, the higher affinity that the antibody binds to a target antigen.

[0126] **Compositions, Immunogenic Combinations, and Vaccines**

[0127] The application also relates to compositions, immunogenic combinations, more particularly kits, and vaccines comprising one or more HBV antigens, polynucleotides, and/or vectors encoding one more HBV antigens according to the application. Any of the HBV antigens, polynucleotides (including RNA and DNA), and/or vectors of the application described herein can be used in the compositions, immunogenic combinations or kits, and vaccines of the application.

[0128] The application provides a composition comprising an isolated or non-naturally occurring nucleic acid molecule (DNA or RNA) encoding a truncated HBV core antigen consisting of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 14, or a HBV polymerase antigen comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 4, a vector comprising the isolated or non-naturally occurring nucleic acid molecule, and/or an isolated or non-naturally occurring polypeptide encoded by the isolated or non-naturally occurring nucleic acid molecule.

[0129] In an embodiment of the application, a composition comprises an isolated or non-naturally occurring nucleic acid molecule (DNA or RNA) encoding a truncated HBV core antigen consisting of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 14, preferably 100% identical to SEQ ID NO: 2 or SEQ ID NO: 14.

[0130] In an embodiment of the application, a composition comprises an isolated or non-naturally occurring nucleic acid molecule (DNA or RNA) encoding a HBV Pol antigen comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 4, preferably 100% identical to SEQ ID NO: 4.

[0131] In an embodiment of the application, a composition comprises an isolated or non-naturally occurring nucleic acid molecule (DNA or RNA) comprising a polynucleotide sequence encoding a truncated HBV core antigen consisting of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 14, preferably 100% identical to SEQ ID NO: 2 or
5 SEQ ID NO: 14; and an isolated or non-naturally occurring nucleic acid molecule (DNA or RNA) comprising a polynucleotide sequence encoding a HBV Pol antigen comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 4, preferably 100% identical to SEQ ID NO: 4. The coding sequences for the truncated HBV core antigen and the HBV Pol antigen can be present in the same isolated or non-naturally occurring nucleic acid molecule (DNA or
10 RNA), or in two different isolated or non-naturally occurring nucleic acid molecules (DNA or RNA).

[0132] In an embodiment of the application, a composition comprises a vector, preferably a DNA plasmid or a viral vector (such as an adenoviral vector) comprising a polynucleotide encoding a truncated HBV core antigen consisting of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 14, preferably 100% identical to SEQ ID NO: 2 or
15 SEQ ID NO: 14.

[0133] In an embodiment of the application, a composition comprises a vector, preferably a DNA plasmid or a viral vector (such as an adenoviral vector), comprising a polynucleotide encoding a HBV Pol antigen comprising an amino acid sequence that is at least 90% identical to
20 SEQ ID NO: 4, preferably 100% identical to SEQ ID NO: 4.

[0134] In an embodiment of the application, a composition comprises a vector, preferably a DNA plasmid or a viral vector (such as an adenoviral vector), comprising a polynucleotide encoding a truncated HBV core antigen consisting of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 14, preferably 100% identical to SEQ ID NO: 2 or
25 SEQ ID NO: 14; and a vector, preferably a DNA plasmid or a viral vector (such as an adenoviral vector), comprising a polynucleotide encoding a HBV Pol antigen comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 4, preferably 100% identical to SEQ ID NO: 4. The vector comprising the coding sequence for the truncated HBV core antigen and the vector comprising the coding sequence for the HBV Pol antigen can be the same vector, or two
30 different vectors.

- [0135] In an embodiment of the application, a composition comprises a vector, preferably a DNA plasmid or a viral vector (such as an adenoviral vector), comprising a polynucleotide encoding a fusion protein comprising a truncated HBV core antigen consisting of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 14, preferably 100% identical to SEQ ID NO: 2 or SEQ ID NO: 14, operably linked to a HBV Pol antigen comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 4, preferably 100% identical to SEQ ID NO: 4, or vice versa. Preferably, the fusion protein further comprises a linker that operably links the truncated HBV core antigen to the HBV Pol antigen, or vice versa. Preferably, the linker has the amino acid sequence of (AlaGly)_n, wherein n is an integer of 2 to 5.
- 10 [0136] In an embodiment of the application, a composition comprises an isolated or non-naturally occurring truncated HBV core antigen consisting of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 14, preferably 100% identical to SEQ ID NO: 2 or SEQ ID NO: 14.
- 15 [0137] In an embodiment of the application, a composition comprises an isolated or non-naturally occurring HBV Pol antigen comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 4, preferably 100% identical to SEQ ID NO: 4.
- [0138] In an embodiment of the application, a composition comprises an isolated or non-naturally occurring truncated HBV core antigen consisting of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 14, preferably 100% identical to SEQ ID NO: 2 or SEQ ID NO: 14; and an isolated or non-naturally occurring HBV Pol antigen comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 4, preferably 100% identical to SEQ ID NO: 4.
- 20 [0139] In an embodiment of the application, a composition comprises an isolated or non-naturally occurring fusion protein comprising a truncated HBV core antigen consisting of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 14, preferably 100% identical to SEQ ID NO: 2 or SEQ ID NO: 14, operably linked to a HBV Pol antigen comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 4, preferably 100% identical to SEQ ID NO: 4, or vice versa. Preferably, the fusion protein further comprises a linker that operably links the truncated HBV core antigen to the HBV Pol antigen, or vice versa. Preferably, the linker has the amino acid sequence of (AlaGly)_n, wherein n is an integer of 2 to 5.
- 30

[0140] The application also relates to an immunogenic combination or a kit comprising polynucleotides expressing a truncated HBV core antigen and an HBV pol antigen according to embodiments of the application. Any polynucleotides and/or vectors encoding HBV core and pol antigens of the application described herein can be used in the immunogenic combinations or kits of the application.

[0141] According to embodiments of the application, a vaccine combination or kit comprises:

- (a) a first non-naturally occurring nucleic acid molecule comprising a first polynucleotide encoding a HBV polymerase antigen having an amino acid sequence that is at least 98% identical to SEQ ID NO: 4, wherein the HBV polymerase antigen does not have reverse transcriptase activity and RNase H activity;
- (b) a second non-naturally occurring nucleic acid molecule comprising a second polynucleotide encoding a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 14; and
- (c) a pharmaceutically acceptable carrier,

wherein the first non-naturally occurring nucleic acid molecule and the second non-naturally occurring nucleic acid molecule are present in the same non-naturally occurring nucleic acid molecule or in two different non-naturally occurring nucleic acid molecules.

[0142] According to embodiments of the application, the polynucleotides in a vaccine combination or kit can be linked or separate, such that the HBV antigens expressed from such polynucleotides are fused together or produced as separate proteins, whether expressed from the same or different polynucleotides. In an embodiment, the first and second polynucleotides are present in separate vectors, e.g., DNA plasmids or viral vectors, used in combination either in the same or separate compositions, such that the expressed proteins are also separate proteins, but used in combination. In another embodiment, the HBV antigens encoded by the first and second polynucleotides can be expressed from the same vector, such that an HBV core-pol fusion antigen is produced. Optionally, the core and pol antigens can be joined or fused together by a short linker. Alternatively, the HBV antigens encoded by the first and second polynucleotides can be expressed independently from a single vector using a ribosomal slippage site (also known as cis-hydrolase site) between the core and pol antigen coding sequences. This strategy results in a bicistronic expression vector in which individual core and pol antigens are produced

from a single mRNA transcript. The core and pol antigens produced from such a bicistronic expression vector can have additional N or C-terminal residues, depending upon the ordering of the coding sequences on the mRNA transcript. Examples of ribosomal slippage sites that can be used for this purpose include, but are not limited to, the FA2 slippage site from foot-and-mouth disease virus (FMDV). Another possibility is that the HBV antigens encoded by the first and second polynucleotides can be expressed independently from two separate vectors, one encoding the HBV core antigen and one encoding the HBV pol antigen.

[0143] In a preferred embodiment, the first and second polynucleotides are present in separate vectors, e.g., DNA plasmids or viral vectors. Preferably, the separate vectors are present in the same composition.

[0144] According to preferred embodiments of the application, an immunogenic combination or kit comprises a first polynucleotide present in a first vector and a second polynucleotide present in a second vector. The first and second vectors can be the same or different. Preferably the vectors are DNA plasmids.

[0145] In a particular embodiment of the application, an immunogenic combination or kit comprises: a first vector comprising a polynucleotide encoding a truncated HBV core antigen consisting of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2, preferably 100% identical to SEQ ID NO: 2; and a second vector comprising a polynucleotide encoding a HBV polymerase antigen comprising an amino acid sequence that is at least 98% identical to SEQ ID NO: 4, preferably 100% identical to SEQ ID NO: 4.

[0146] In a particular embodiment of the application, the first vector is a first DNA plasmid and the second vector is a second DNA plasmid. Each of the first and second DNA plasmids comprises an origin of replication, preferably pUC ORI of SEQ ID NO: 10, and an antibiotic resistance cassette, preferably comprising a codon optimized Kan^r gene having a polynucleotide sequence that is at least 90% identical to SEQ ID NO: 12, preferably under control of a bla promoter, for instance the bla promoter shown in SEQ ID NO: 9. Each of the first and second DNA plasmids independently further comprises at least one of a promoter sequence, enhancer sequence, and a polynucleotide sequence encoding a signal peptide sequence operably linked to the first polynucleotide sequence or the second polynucleotide sequence. Preferably, each of the first and second DNA plasmids comprises an upstream sequence operably linked to the first polynucleotide or the second polynucleotide, wherein the upstream sequence comprises, from 5'

end to 3' end, a promoter sequence of SEQ ID NO: 7, an enhancer sequence of SEQ ID NO: 8, and a polynucleotide sequence encoding a signal peptide sequence having the amino acid sequence of SEQ ID NO: 6. Each of the first and second DNA plasmids can also comprise a polyadenylation signal located downstream of the coding sequence of the HBV antigen, such as the bGH polyadenylation signal of SEQ ID NO: 11.

[0147] In one particular embodiment of the application, the first vector is a first viral vector and the second vector is a second viral vector. Preferably, each of the first and second viral vector is an adenoviral vector, more preferably an Ad26 or Ad35 vector, comprising an expression cassette including the polynucleotide encoding an HBV pol antigen or a truncated HBV core antigen of the application; an upstream sequence operably linked to the polynucleotide encoding the HBV antigen comprising, from 5' end to 3' end, a promoter sequence, preferably a CMV promoter sequence of SEQ ID NO: 17, an enhancer sequence, preferably an ApoAI gene fragment sequence of SEQ ID NO: 23, and a polynucleotide sequence encoding a signal peptide sequence, preferably an immunoglobulin secretion signal having the amino acid sequence of SEQ ID NO: 19; and a downstream sequence operably linked to the polynucleotide encoding the HBV antigen comprising a polyadenylation signal, preferably a SV40 polyadenylation signal of SEQ ID NO: 24.

[0148] In another preferred embodiment, the first and second polynucleotides are present in a single vector, e.g., DNA plasmid or viral vector. Preferably, the single vector is an adenoviral vector, more preferably an Ad26 vector, comprising an expression cassette including a polynucleotide encoding an HBV pol antigen and a truncated HBV core antigen of the application, preferably encoding an HBV pol antigen and a truncated HBV core antigen of the application as a fusion protein; an upstream sequence operably linked to the polynucleotide encoding the HBV pol and truncated core antigens comprising, from 5' end to 3' end, a promoter sequence, preferably a CMV promoter sequence of SEQ ID NO: 17, an enhancer sequence, preferably an ApoAI gene fragment sequence of SEQ ID NO: 23, and a polynucleotide sequence encoding a signal peptide sequence, preferably an immunoglobulin secretion signal having the amino acid sequence of SEQ ID NO: 19; and a downstream sequence operably linked to the polynucleotide encoding the HBV antigen comprising a polyadenylation signal, preferably a SV40 polyadenylation signal of SEQ ID NO: 24

[0149] When an immunogenic combination of the application comprises a first vector, such as a DNA plasmid or viral vector, and a second vector, such as a DNA plasmid or viral vector, the amount of each of the first and second vectors is not particularly limited. For example, the first DNA plasmid and the second DNA plasmid can be present in a ratio of 10:1 to 1:10, by weight, such as 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, or 1:10, by weight. Preferably, the first and second DNA plasmids are present in a ratio of 1:1, by weight.

[0150] Compositions and immunogenic combinations of the application can comprise additional polynucleotides or vectors encoding additional HBV antigens and/or additional HBV antigens or immunogenic fragments thereof. However, in particular embodiments, the compositions and immunogenic combinations of the application do not comprise certain antigens.

[0151] In a particular embodiment, a composition or immunogenic combination or kit of the application does not comprise a HBsAg or a polynucleotide sequence encoding the HBsAg.

[0152] In another particular embodiment, a composition or immunogenic combination or kit of the application does not comprise a HBV L protein or a polynucleotide sequence encoding the HBV L protein.

[0153] In yet another particular embodiment of the application, a composition or immunogenic combination of the application does not comprise a HBV envelope protein or a polynucleotide sequence encoding the HBV envelope protein.

[0154] Compositions and immunogenic combinations of the application can also comprise a pharmaceutically acceptable carrier. A pharmaceutically acceptable carrier is non-toxic and should not interfere with the efficacy of the active ingredient. Pharmaceutically acceptable carriers can include one or more excipients such as binders, disintegrants, swelling agents, suspending agents, emulsifying agents, wetting agents, lubricants, flavorants, sweeteners, preservatives, dyes, solubilizers and coatings. Pharmaceutically acceptable carriers can include vehicles, such as lipid (nano)particles. The precise nature of the carrier or other material can depend on the route of administration, e.g., intramuscular, intradermal, subcutaneous, oral, intravenous, cutaneous, intramucosal (e.g., gut), intranasal or intraperitoneal routes. For liquid injectable preparations, for example, suspensions and solutions, suitable carriers and additives include water, glycols, oils, alcohols, preservatives, coloring agents and the like. For solid oral

preparations, for example, powders, capsules, caplets, gelcaps and tablets, suitable carriers and additives include starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like. For nasal sprays/inhalant mixtures, the aqueous solution/suspension can comprise water, glycols, oils, emollients, stabilizers, wetting agents, preservatives, aromatics, flavors, and the like as suitable carriers and additives.

[0155] Compositions and immunogenic combinations of the application can be formulated in any matter suitable for administration to a subject to facilitate administration and improve efficacy, including, but not limited to, oral (enteral) administration and parenteral injections. The parenteral injections include intravenous injection or infusion, subcutaneous injection, intradermal injection, and intramuscular injection. Compositions of the application can also be formulated for other routes of administration including transmucosal, ocular, rectal, long acting implantation, sublingual administration, under the tongue, from oral mucosa bypassing the portal circulation, inhalation, or intranasal.

[0156] In a preferred embodiment of the application, compositions and immunogenic combinations of the application are formulated for parental injection, preferably subcutaneous, intradermal injection, or intramuscular injection, more preferably intramuscular injection.

[0157] According to embodiments of the application, compositions and immunogenic combinations for administration will typically comprise a buffered solution in a pharmaceutically acceptable carrier, e.g., an aqueous carrier such as buffered saline and the like, e.g., phosphate buffered saline (PBS). The compositions and immunogenic combinations can also contain pharmaceutically acceptable substances as required to approximate physiological conditions such as pH adjusting and buffering agents. For example, a composition or immunogenic combination of the application comprising plasmid DNA can contain phosphate buffered saline (PBS) as the pharmaceutically acceptable carrier. The plasmid DNA can be present in a concentration of, e.g., 0.5 mg/mL to 5 mg/mL, such as 0.5 mg/mL, 1 mg/mL, 2 mg/mL, 3 mg/mL, 4 mg/mL, or 5 mg/mL, preferably at 1 mg/mL.

[0158] Compositions and immunogenic combinations of the application can be formulated as a vaccine (also referred to as an “immunogenic composition”) according to methods well known in the art. Such compositions can include adjuvants to enhance immune responses. The optimal ratios of each component in the formulation can be determined by techniques well known to those skilled in the art in view of the present disclosure.

- [0159] In a particular embodiment of the application, a composition or immunogenic combination is a DNA vaccine. DNA vaccines typically comprise bacterial plasmids containing a polynucleotide encoding an antigen of interest under control of a strong eukaryotic promoter. Once the plasmids are delivered to the cell cytoplasm of the host, the encoded antigen is produced and processed endogenously. The resulting antigen typically induces both humoral and cell-mediated immune responses. DNA vaccines are advantageous at least because they offer improved safety, are temperature stable, can be easily adapted to express antigenic variants, and are simple to produce. Any of the DNA plasmids of the application can be used to prepare such a DNA vaccine.
- 10 [0160] In other particular embodiments of the application, a composition or immunogenic combination is an RNA vaccine. RNA vaccines typically comprise at least one single-stranded RNA molecule encoding an antigen of interest, e.g., HBV antigen. Once the RNA is delivered to the cell cytoplasm of the host, the encoded antigen is produced and processed endogenously, inducing both humoral and cell-mediated immune responses, similar to a DNA vaccine. The RNA sequence can be codon optimized to improve translation efficiency. The RNA molecule can be modified by any method known in the art in view of the present disclosure to enhance stability and/or translation, such by adding a polyA tail, e.g., of at least 30 adenosine residues; and/or capping the 5-end with a modified ribonucleotide, e.g., 7-methylguanosine cap, which can be incorporated during RNA synthesis or enzymatically engineered after RNA transcription. An RNA vaccine can also be a self-replicating RNA vaccine developed from an alphavirus expression vector. Self-replicating RNA vaccines comprise a replicase RNA molecule derived from a virus belonging to the alphavirus family with a subgenomic promoter that controls replication of the HBV antigen RNA followed by an artificial poly A tail located downstream of the replicase.
- 25 [0161] In certain embodiments, an adjuvant is included in a composition or immunogenic combination of the application, or co-administered with a composition or immunogenic combination of the application. Use of an adjuvant is optional, and can further enhance immune responses when the composition is used for vaccination purposes. Adjuvants suitable for co-administration or inclusion in compositions in accordance with the application should preferably be ones that are potentially safe, well tolerated and effective in humans. An adjuvant can be a small molecule or antibody including, but not limited to, immune checkpoint inhibitors (e.g.,
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anti-PD1, anti-TIM-3, etc.), toll-like receptor agonists (e.g., TLR7 agonists and/or TLR8 agonists), RIG-1 agonists, IL-15 superagonists (Altor Bioscience), mutant IRF3 and IRF7 genetic adjuvants, STING agonists (Aduro), FLT3L genetic adjuvant, IL-12 genetic adjuvant, and IL-7-hyFc. Adjuvants can also e.g., be chosen from among the following anti-HBV agents:

5 HBV DNA polymerase inhibitors; immunomodulators; toll-like receptor 7 modulators; toll-like receptor 8 modulators; toll-like receptor 3 modulators; interferon alpha receptor ligands; hyaluronidase inhibitors; modulators of IL-10; HBsAg inhibitors; toll-like receptor 9 modulators; cyclophilin inhibitors; HBV prophylactic vaccines; HBV therapeutic vaccines; HBV viral entry inhibitors; antisense oligonucleotides targeting viral mRNA, more particularly anti-HBV

10 antisense oligonucleotides; short interfering RNAs (siRNA), more particularly anti-HBV siRNA; endonuclease modulators; inhibitors of ribonucleotide reductase; hepatitis B virus E antigen inhibitors; HBV antibodies targeting the surface antigens of the hepatitis B virus; HBV antibodies; CCR2 chemokine antagonists; thymosin agonists; cytokines, such as IL12; capsid assembly modulators, nucleoprotein inhibitors (HBV core or capsid protein inhibitors); nucleic

15 acid polymers (NAPs); stimulators of retinoic acid-inducible gene 1; stimulators of NOD2; recombinant thymosin alpha-1; hepatitis B virus replication inhibitors; PI3K inhibitors; cccDNA inhibitors; immune checkpoint inhibitors, such as PD-L1 inhibitors, PD-1 inhibitors, TIM-3 inhibitors, TIGIT inhibitors, Lag3 inhibitors, and CTLA-4 inhibitors; agonists of co-stimulatory receptors that are expressed on immune cells (more particularly T cells), such as CD27, CD28,

20 etc.; BTK inhibitors; other drugs for treating HBV; IDO inhibitors; arginase inhibitors; and KDM5 inhibitors.

[0162] The application also provides methods of making compositions and immunogenic combinations of the application. A method of producing a composition or immunogenic combination comprises mixing an isolated polynucleotide encoding an HBV antigen, vector,

25 and/or polypeptide of the application with one or more pharmaceutically acceptable carriers. One of ordinary skill in the art will be familiar with conventional techniques used to prepare such compositions.

[0163] **Methods of Inducing an Immune Response**

[0164] The application also provides methods of inducing an immune response against

30 hepatitis B virus (HBV) in a subject in need thereof, comprising administering to the subject an immunogenically effective amount of a composition or immunogenic composition of the

application. Any of the compositions and immunogenic combinations of the application described herein can be used in the methods of the application.

[0165] As used herein, the term “infection” refers to the invasion of a host by a disease causing agent. A disease causing agent is considered to be “infectious” when it is capable of invading a host, and replicating or propagating within the host. Examples of infectious agents include viruses, e.g., HBV and certain species of adenovirus, prions, bacteria, fungi, protozoa and the like. “HBV infection” specifically refers to invasion of a host organism, such as cells and tissues of the host organism, by HBV.

[0166] The phrase “inducing an immune response” when used with reference to the methods described herein encompasses causing a desired immune response or effect in a subject in need thereof against an infection, e.g., an HBV infection. “Inducing an immune response” also encompasses providing a therapeutic immunity for treating against a pathogenic agent, e.g., HBV. As used herein, the term “therapeutic immunity” or “therapeutic immune response” means that the vaccinated subject is able to control an infection with the pathogenic agent against which the vaccination was done, for instance immunity against HBV infection conferred by vaccination with HBV vaccine. In an embodiment, “inducing an immune response” means producing an immunity in a subject in need thereof, e.g., to provide a therapeutic effect against a disease, such as HBV infection. In certain embodiments, “inducing an immune response” refers to causing or improving cellular immunity, e.g., T cell response, against HBV infection. In certain embodiments, “inducing an immune response” refers to causing or improving a humoral immune response against HBV infection. In certain embodiments, “inducing an immune response” refers to causing or improving a cellular and a humoral immune response against HBV infection.

[0167] As used herein, the term “protective immunity” or “protective immune response” means that the vaccinated subject is able to control an infection with the pathogenic agent against which the vaccination was done. Usually, the subject having developed a “protective immune response” develops only mild to moderate clinical symptoms or no symptoms at all. Usually, a subject having a “protective immune response” or “protective immunity” against a certain agent will not die as a result of the infection with said agent.

[0168] Typically, the administration of compositions and immunogenic combinations of the application will have a therapeutic aim to generate an immune response against HBV after HBV

infection or development of symptoms characteristic of HBV infection, e.g., for therapeutic vaccination.

[0169] As used herein, “an immunogenically effective amount” or “immunologically effective amount” means an amount of a composition, polynucleotide, vector, or antigen sufficient to induce a desired immune effect or immune response in a subject in need thereof. An immunogenically effective amount can be an amount sufficient to induce an immune response in a subject in need thereof. An immunogenically effective amount can be an amount sufficient to produce immunity in a subject in need thereof, e.g., provide a therapeutic effect against a disease such as HBV infection. An immunogenically effective amount can vary depending upon a variety of factors, such as the physical condition of the subject, age, weight, health, etc.; the particular application, e.g., providing protective immunity or therapeutic immunity; and the particular disease, e.g., viral infection, for which immunity is desired. An immunogenically effective amount can readily be determined by one of ordinary skill in the art in view of the present disclosure.

[0170] In particular embodiments of the application, an immunogenically effective amount refers to the amount of a composition or immunogenic combination which is sufficient to achieve one, two, three, four, or more of the following effects: (i) reduce or ameliorate the severity of an HBV infection or a symptom associated therewith; (ii) reduce the duration of an HBV infection or symptom associated therewith; (iii) prevent the progression of an HBV infection or symptom associated therewith; (iv) cause regression of an HBV infection or symptom associated therewith; (v) prevent the development or onset of an HBV infection, or symptom associated therewith; (vi) prevent the recurrence of an HBV infection or symptom associated therewith; (vii) reduce hospitalization of a subject having an HBV infection; (viii) reduce hospitalization length of a subject having an HBV infection; (ix) increase the survival of a subject with an HBV infection; (x) eliminate an HBV infection in a subject; (xi) inhibit or reduce HBV replication in a subject; and/or (xii) enhance or improve the prophylactic or therapeutic effect(s) of another therapy.

[0171] An immunogenically effective amount can also be an amount sufficient to reduce HBsAg levels consistent with evolution to clinical seroconversion; achieve sustained HBsAg clearance associated with reduction of infected hepatocytes by a subject’s immune system; induce HBV-antigen specific activated T-cell populations; and/or achieve persistent loss of

HBsAg within 12 months. Examples of a target index include lower HBsAg below a threshold of 500 copies of HBsAg international units (IU) and/or higher CD8 counts.

[0172] As general guidance, an immunogenically effective amount when used with reference to a DNA plasmid can range from about 0.1 mg/mL to 10 mg/mL of DNA plasmid total, such as 0.1 mg/mL, 0.25 mg/mL, 0.5 mg/mL, 0.75 mg/mL, 1 mg/mL, 1.5 mg/mL, 2 mg/mL, 3 mg/mL, 4 mg/mL, 5 mg/mL, 6 mg/mL, 7 mg/mL, 8 mg/mL, 9 mg/mL, or 10 mg/mL. Preferably, an immunogenically effective amount of DNA plasmid is less than 8 mg/mL, more preferably less than 6 mg/mL, even more preferably 3-4 mg/mL. An immunogenically effective amount can be from one vector or plasmid, or from multiple vectors or plasmids. An immunogenically effective amount can be administered in a single composition, or in multiple compositions, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 compositions (e.g., tablets, capsules or injectables, or any composition adapted to intradermal delivery, e.g., to intradermal delivery using an intradermal delivery patch), wherein the administration of the multiple capsules or injections collectively provides a subject with an immunogenically effective amount. For example, when two DNA plasmids are used, an immunogenically effective amount can be 3-4 mg/mL, with 1.5-2 mg/mL of each plasmid. It is also possible to administer an immunogenically effective amount to a subject, and subsequently administer another dose of an immunogenically effective amount to the same subject, in a so-called prime-boost regimen. This general concept of a prime-boost regimen is well known to the skilled person in the vaccine field. Further booster administrations can optionally be added to the regimen, as needed.

[0173] An immunogenic combination comprising two DNA plasmids, e.g., a first DNA plasmid encoding an HBV core antigen and second DNA plasmid encoding an HBV pol antigen can be administered to a subject by mixing both plasmids and delivering the mixture to a single anatomic site. Alternatively, two separate immunizations each delivering a single expression plasmid can be performed. In such embodiments, whether both plasmids are administered in a single immunization as a mixture or in two separate immunizations, the first DNA plasmid and the second DNA plasmid can be administered in a ratio of 10:1 to 1:10, by weight, such as 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, or 1:10, by weight. Preferably, the first and second DNA plasmids are administered in a ratio of 1:1, by weight.

[0174] Preferably, a subject to be treated according to the methods of the application is an HBV-infected subject, particular a subject having chronic HBV infection. Acute HBV infection

is characterized by an efficient activation of the innate immune system complemented with a subsequent broad adaptive response (e.g., HBV-specific T-cells, neutralizing antibodies), which usually results in successful suppression of replication or removal of infected hepatocytes. In contrast, such responses are impaired or diminished due to high viral and antigen load, e.g., HBV envelope proteins are produced in abundance and can be released in sub-viral particles in 1,000-fold excess to infectious virus.

[0175] Chronic HBV infection is described in phases characterized by viral load, liver enzyme levels (necroinflammatory activity), HBeAg, or HBsAg load or presence of antibodies to these antigens. cccDNA levels stay relatively constant at approximately 10 to 50 copies per cell, even though viremia can vary considerably. The persistence of the cccDNA species leads to chronicity. More specifically, the phases of chronic HBV infection include: (i) the immune-tolerant phase characterized by high viral load and normal or minimally elevated liver enzymes; (ii) the immune activation HBeAg-positive phase in which lower or declining levels of viral replication with significantly elevated liver enzymes are observed; (iii) the inactive HBsAg carrier phase, which is a low replicative state with low viral loads and normal liver enzyme levels in the serum that may follow HBeAg seroconversion; and (iv) the HBeAg-negative phase in which viral replication occurs periodically (reactivation) with concomitant fluctuations in liver enzyme levels, mutations in the pre-core and/or basal core promoter are common, such that HBeAg is not produced by the infected cell.

[0176] As used herein, “chronic HBV infection” refers to a subject having the detectable presence of HBV for more than 6 months. A subject having a chronic HBV infection can be in any phase of chronic HBV infection. Chronic HBV infection is understood in accordance with its ordinary meaning in the field. Chronic HBV infection can for example be characterized by the persistence of HBsAg for 6 months or more after acute HBV infection. For example, a chronic HBV infection referred to herein follows the definition published by the Centers for Disease Control and Prevention (CDC), according to which a chronic HBV infection can be characterized by laboratory criteria such as: (i) negative for IgM antibodies to hepatitis B core antigen (IgM anti-HBc) and positive for hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), or nucleic acid test for hepatitis B virus DNA, or (ii) positive for HBsAg or nucleic acid test for HBV DNA, or positive for HBeAg two times at least 6 months apart.

[0177] Preferably, an immunogenically effective amount refers to the amount of a composition or immunogenic combination of the application which is sufficient to treat chronic HBV infection.

[0178] In some embodiments, a subject having chronic HBV infection is undergoing nucleoside analog (NUC) treatment, and is NUC-suppressed. As used herein, “NUC-suppressed” refers to a subject having an undetectable viral level of HBV and stable alanine aminotransferase (ALT) levels for at least six months. Examples of nucleoside/nucleotide analog treatment include HBV polymerase inhibitors, such as entecavir and tenofovir. Preferably, a subject having chronic HBV infection does not have advanced hepatic fibrosis or cirrhosis. Such subject would typically have a METAVIR score of less than 3 for fibrosis and a fibroscan result of less than 9 kPa. The METAVIR score is a scoring system that is commonly used to assess the extent of inflammation and fibrosis by histopathological evaluation in a liver biopsy of patients with hepatitis B. The scoring system assigns two standardized numbers: one reflecting the degree of inflammation and one reflecting the degree of fibrosis.

[0179] It is believed that elimination or reduction of chronic HBV may allow early disease interception of severe liver disease, including virus-induced cirrhosis and hepatocellular carcinoma. Thus, the methods of the application can also be used as therapy to treat HBV-induced diseases. Examples of HBV-induced diseases include, but are not limited to cirrhosis, cancer (e.g., hepatocellular carcinoma), and fibrosis, particularly advanced fibrosis characterized by a METAVIR score of 3 or higher for fibrosis. In such embodiments, an immunogenically effective amount is an amount sufficient to achieve persistent loss of HBsAg within 12 months and significant decrease in clinical disease (e.g., cirrhosis, hepatocellular carcinoma, etc.).

[0180] Methods according to embodiments of the application further comprise administering to the subject in need thereof another immunogenic agent (such as another HBV antigen or other antigen) or another anti-HBV agent (such as a nucleoside analog or other anti-HBV agent) in combination with a composition of the application. For example, another anti-HBV agent or immunogenic agent can be a small molecule or antibody including, but not limited to, immune checkpoint inhibitors (e.g., anti-PD1, anti-TIM-3, etc.), toll-like receptor agonists (e.g., TLR7 agonists and/or TLR8 agonists), RIG-1 agonists, IL-15 superagonists (Altor Bioscience), mutant IRF3 and IRF7 genetic adjuvants, STING agonists (Aduro), FLT3L genetic adjuvant, IL-12 genetic adjuvant, IL-7-hyFc; CAR-T which bind HBV env (S-CAR cells); capsid assembly

modulators; cccDNA inhibitors, HBV polymerase inhibitors (e.g., entecavir and tenofovir). The one or more other anti-HBV active agents can be, for example, a small molecule, an antibody or antigen binding fragment thereof, a polypeptide, protein, or nucleic acid. The one or more other anti-HBV agents can e.g., be chosen from among HBV DNA polymerase inhibitors;

5 immunomodulators; toll-like receptor 7 modulators; toll-like receptor 8 modulators; toll-like receptor 3 modulators; interferon alpha receptor ligands; hyaluronidase inhibitors; modulators of IL-10; HBsAg inhibitors; toll-like receptor 9 modulators; cyclophilin inhibitors; HBV prophylactic vaccines; HBV therapeutic vaccines; HBV viral entry inhibitors; antisense oligonucleotides targeting viral mRNA, more particularly anti-HBV antisense oligonucleotides;

10 short interfering RNAs (siRNA), more particularly anti-HBV siRNA; endonuclease modulators; inhibitors of ribonucleotide reductase; hepatitis B virus E antigen inhibitors; HBV antibodies targeting the surface antigens of the hepatitis B virus; HBV antibodies; CCR2 chemokine antagonists; thymosin agonists; cytokines, such as IL12; capsid assembly modulators, nucleoprotein inhibitors (HBV core or capsid protein inhibitors); nucleic acid polymers (NAPs);

15 stimulators of retinoic acid-inducible gene 1; stimulators of NOD2; recombinant thymosin alpha-1; hepatitis B virus replication inhibitors; PI3K inhibitors; cccDNA inhibitors; immune checkpoint inhibitors, such as PD-L1 inhibitors, PD-1 inhibitors, TIM-3 inhibitors, TIGIT inhibitors, Lag3 inhibitors, and CTLA-4 inhibitors; agonists of co-stimulatory receptors that are expressed on immune cells (more particularly T cells), such as CD27, CD28, etc.; BTK

20 inhibitors; other drugs for treating HBV; IDO inhibitors; arginase inhibitors; and KDM5 inhibitors.

[0181] Methods of Delivery

[0182] Compositions and immunogenic combinations of the application can be administered to a subject by any method known in the art in view of the present disclosure, including, but not

25 limited to, parenteral administration (e.g., intramuscular, subcutaneous, intravenous, or intradermal injection), oral administration, transdermal administration, and nasal administration. Preferably, compositions and immunogenic combinations are administered parenterally (e.g., by intramuscular injection or intradermal injection) or transdermally.

[0183] In some embodiments of the application in which a composition or immunogenic

30 combination comprises one or more DNA plasmids, administration can be by injection through the skin, e.g., intramuscular or intradermal injection, preferably intramuscular injection.

Intramuscular injection can be combined with electroporation, i.e., application of an electric field to facilitate delivery of the DNA plasmids to cells. As used herein, the term “electroporation” refers to the use of a transmembrane electric field pulse to induce microscopic pathways (pores) in a bio-membrane. During *in vivo* electroporation, electrical fields of appropriate magnitude and duration are applied to cells, inducing a transient state of enhanced cell membrane permeability, thus enabling the cellular uptake of molecules unable to cross cell membranes on their own. Creation of such pores by electroporation facilitates passage of biomolecules, such as plasmids, oligonucleotides, siRNAs, drugs, etc., from one side of a cellular membrane to the other. *In vivo* electroporation for the delivery of DNA vaccines has been shown to significantly increase plasmid uptake by host cells, while also leading to mild-to-moderate inflammation at the injection site. As a result, transfection efficiency and immune response are significantly improved (e.g., up to 1,000 fold and 100 fold respectively) with intradermal or intramuscular electroporation, in comparison to conventional injection.

[0184] In a typical embodiment, electroporation is combined with intramuscular injection. However, it is also possible to combine electroporation with other forms of parenteral administration, e.g., intradermal injection, subcutaneous injection, etc.

[0185] Administration of a composition, immunogenic combination or vaccine of the application via electroporation can be accomplished using electroporation devices that can be configured to deliver to a desired tissue of a mammal a pulse of energy effective to cause reversible pores to form in cell membranes. The electroporation device can include an electroporation component and an electrode assembly or handle assembly. The electroporation component can include one or more of the following components of electroporation devices: controller, current waveform generator, impedance tester, waveform logger, input element, status reporting element, communication port, memory component, power source, and power switch. Electroporation can be accomplished using an *in vivo* electroporation device. Examples of electroporation devices and electroporation methods that can facilitate delivery of compositions and immunogenic combinations of the application, particularly those comprising DNA plasmids, include CELLECTRA® (Inovio Pharmaceuticals, Blue Bell, PA), Elgen electroporator (Inovio Pharmaceuticals, Inc.) Tri-Grid™ delivery system (Ichor Medical Systems, Inc., San Diego, CA 92121) and those described in U.S. Patent No. 7,664,545, U.S. Patent No. 8,209,006, U.S. Patent No. 9,452,285, U.S. Patent No. 5,273,525, U.S. Patent No. 6,110,161, U.S. Patent No. 6,261,281,

U.S. Patent No. 6,958,060, and U.S. Patent No. 6,939,862, U.S. Patent No. 7,328,064, U.S. Patent No. 6,041,252, U.S. Patent No. 5,873,849, U.S. Patent No. 6,278,895, U.S. Patent No. 6,319,901, U.S. Patent No. 6,912,417, U.S. Patent No. 8,187,249, U.S. Patent No. 9,364,664, U.S. Patent No. 9,802,035, U.S. Patent No. 6,117,660, and International Patent Application
5 Publication WO2017172838, all of which are herein incorporated by reference in their entireties. Other examples of *in vivo* electroporation devices are described in International Patent Application entitled "Method and Apparatus for the Delivery of Hepatitis B Virus (HBV) Vaccines," filed on the same day as this application with the Attorney Docket Number 688097-405WO1, the contents of which are hereby incorporated by reference in their entireties. Also
10 contemplated by the application for delivery of the compositions and immunogenic combinations of the application are use of a pulsed electric field, for instance as described in, e.g., U.S. Patent No. 6,697,669, which is herein incorporated by reference in its entirety.

[0186] In other embodiments of the application in which a composition or immunogenic combination comprises one or more DNA plasmids, the method of administration is transdermal.
15 Transdermal administration can be combined with epidermal skin abrasion to facilitate delivery of the DNA plasmids to cells. For example, a dermatological patch can be used for epidermal skin abrasion. Upon removal of the dermatological patch, the composition or immunogenic combination can be deposited on the abraded skin.

[0187] Methods of delivery are not limited to the above described embodiments, and any
20 means for intracellular delivery can be used. Other methods of intracellular delivery contemplated by the methods of the application include, but are not limited to, liposome encapsulation, nanoparticles, etc.

[0188] Adjuvants

[0189] In some embodiments of the application, a method of inducing an immune response
25 against HBV further comprises administering an adjuvant. The terms "adjuvant" and "immune stimulant" are used interchangeably herein, and are defined as one or more substances that cause stimulation of the immune system. In this context, an adjuvant is used to enhance an immune response to HBV antigens and antigenic HBV polypeptides of the application.

[0190] According to embodiments of the application, an adjuvant can be present in an
30 immunogenic combination or composition of the application, or administered in a separate composition. An adjuvant can be, e.g., a small molecule or an antibody. Examples of adjuvants

suitable for use in the application include, but are not limited to, immune checkpoint inhibitors (e.g., anti-PD1, anti-TIM-3, etc.), toll-like receptor agonists (e.g., TLR7 and/or TLR8 agonists), RIG-1 agonists, IL-15 superagonists (Altor Bioscience), mutant IRF3 and IRF7 genetic adjuvants, STING agonists (Aduro), FLT3L genetic adjuvant, IL-12 genetic adjuvant, and IL-7-hyFc. Examples of adjuvants can e.g., be chosen from among the following anti-HBV agents: HBV DNA polymerase inhibitors; immunomodulators; toll-like receptor 7 modulators; toll-like receptor 8 modulators; toll-like receptor 3 modulators; interferon alpha receptor ligands; hyaluronidase inhibitors; modulators of IL-10; HBsAg inhibitors; toll-like receptor 9 modulators; cyclophilin inhibitors; HBV prophylactic vaccines; HBV therapeutic vaccines; HBV viral entry inhibitors; antisense oligonucleotides targeting viral mRNA, more particularly anti-HBV antisense oligonucleotides; short interfering RNAs (siRNA), more particularly anti-HBV siRNA; endonuclease modulators; inhibitors of ribonucleotide reductase; hepatitis B virus E antigen inhibitors; HBV antibodies targeting the surface antigens of the hepatitis B virus; HBV antibodies; CCR2 chemokine antagonists; thymosin agonists; cytokines, such as IL12; capsid assembly modulators, nucleoprotein inhibitors (HBV core or capsid protein inhibitors); nucleic acid polymers (NAPs); stimulators of retinoic acid-inducible gene 1; stimulators of NOD2; recombinant thymosin alpha-1; hepatitis B virus replication inhibitors; PI3K inhibitors; cccDNA inhibitors; immune checkpoint inhibitors, such as PD-L1 inhibitors, PD-1 inhibitors, TIM-3 inhibitors, TIGIT inhibitors, Lag3 inhibitors, and CTLA-4 inhibitors; agonists of co-stimulatory receptors that are expressed on immune cells (more particularly T cells), such as CD27, CD28, etc.; BTK inhibitors; other drugs for treating HBV; IDO inhibitors; arginase inhibitors; and KDM5 inhibitors.

[0191] Compositions and immunogenic combinations of the application can also be administered in combination with at least one other anti-HBV agent. Examples of anti-HBV agents suitable for use with the application include, but are not limited to small molecules, antibodies, and/or CAR-T therapies which bind HBV env (S-CAR cells), capsid assembly modulators, TLR agonists (e.g., TLR7 and/or TLR8 agonists), cccDNA inhibitors, HBV polymerase inhibitors (e.g., entecavir and tenofovir), and/or immune checkpoint inhibitors, etc. The at least one anti-HBV agent can e.g., be chosen from among HBV DNA polymerase inhibitors; immunomodulators; toll-like receptor 7 modulators; toll-like receptor 8 modulators; toll-like receptor 3 modulators; interferon alpha receptor ligands; hyaluronidase inhibitors;

modulators of IL-10; HBsAg inhibitors; toll-like receptor 9 modulators; cyclophilin inhibitors; HBV prophylactic vaccines; HBV therapeutic vaccines; HBV viral entry inhibitors; antisense oligonucleotides targeting viral mRNA, more particularly anti-HBV antisense oligonucleotides; short interfering RNAs (siRNA), more particularly anti-HBV siRNA; endonuclease modulators; inhibitors of ribonucleotide reductase; hepatitis B virus E antigen inhibitors; HBV antibodies targeting the surface antigens of the hepatitis B virus; HBV antibodies; CCR2 chemokine antagonists; thymosin agonists; cytokines, such as IL12; capsid assembly modulators, nucleoprotein inhibitors (HBV core or capsid protein inhibitors); nucleic acid polymers (NAPs); stimulators of retinoic acid-inducible gene 1; stimulators of NOD2; recombinant thymosin alpha-1; hepatitis B virus replication inhibitors; PI3K inhibitors; cccDNA inhibitors; immune checkpoint inhibitors, such as PD-L1 inhibitors, PD-1 inhibitors, TIM-3 inhibitors, TIGIT inhibitors, Lag3 inhibitors, and CTLA-4 inhibitors; agonists of co-stimulatory receptors that are expressed on immune cells (more particularly T cells), such as CD27, CD28, etc.; BTK inhibitors; other drugs for treating HBV; IDO inhibitors; arginase inhibitors; and KDM5 inhibitors. Such anti-HBV agents can be administered with the compositions and immunogenic combinations of the application simultaneously or sequentially.

[0192] Methods of Prime/Boost Immunization

[0193] Embodiments of the application also contemplate administering an immunogenically effective amount of a composition or immunogenic combination to a subject, and subsequently administering another dose of an immunogenically effective amount of a composition or immunogenic combination to the same subject, in a so-called prime-boost regimen. Thus, in an embodiment, a composition or immunogenic combination of the application is a primer vaccine used for priming an immune response. In another embodiment, a composition or immunogenic combination of the application is a booster vaccine used for boosting an immune response. The priming and boosting vaccines of the application can be used in the methods of the application described herein. This general concept of a prime-boost regimen is well known to the skilled person in the vaccine field. Any of the compositions and immunogenic combinations of the application described herein can be used as priming and/or boosting vaccines for priming and/or boosting an immune response against HBV.

[0194] In some embodiments of the application, a composition or immunogenic combination of the application can be administered for priming immunization. The composition or

immunogenic combination can be re-administered for boosting immunization. Further booster administrations of the composition or vaccine combination can optionally be added to the regimen, as needed. An adjuvant can be present in a composition of the application used for boosting immunization, present in a separate composition to be administered together with the composition or immunogenic combination of the application for the boosting immunization, or administered on its own as the boosting immunization. In those embodiments in which an adjuvant is included in the regimen, the adjuvant is preferably used for boosting immunization.

[0195] An illustrative and non-limiting example of a prime-boost regimen includes administering a single dose of an immunogenically effective amount of a composition or immunogenic combination of the application to a subject to prime the immune response; and subsequently administering another dose of an immunogenically effective amount of a composition or immunogenic combination of the application to boost the immune response, wherein the boosting immunization is first administered about two to six weeks, preferably four weeks after the priming immunization is initially administered. Optionally, about 10 to 14 weeks, preferably 12 weeks, after the priming immunization is initially administered, a further boosting immunization of the composition or immunogenic combination, or other adjuvant, is administered.

[0196] Kits

[0197] Also provided herein is a kit comprising an immunogenic combination of the application. A kit can comprise the first polynucleotide and the second polynucleotide in separate compositions, or a kit can comprise the first polynucleotide and the second polynucleotide in a single composition. A kit can further comprise one or more adjuvants or immune stimulants, and/or other anti-HBV agents.

[0198] The ability to induce or stimulate an anti-HBV immune response upon administration in an animal or human organism can be evaluated either *in vitro* or *in vivo* using a variety of assays which are standard in the art. For a general description of techniques available to evaluate the onset and activation of an immune response, see for example Coligan et al. (1992 and 1994, Current Protocols in Immunology; ed. J Wiley & Sons Inc, National Institute of Health). Measurement of cellular immunity can be performed by measurement of cytokine profiles secreted by activated effector cells including those derived from CD4⁺ and CD8⁺ T-cells (e.g. quantification of IL-10 or IFN gamma-producing cells by ELISPOT), by determination of the

activation status of immune effector cells (e.g. T cell proliferation assays by a classical [³H] thymidine uptake or flow cytometry-based assays), by assaying for antigen-specific T lymphocytes in a sensitized subject (e.g. peptide-specific lysis in a cytotoxicity assay, etc.).

[0199] The ability to stimulate a cellular and/or a humoral response can be determined by antibody binding and/or competition in binding (see for example Harlow, 1989, Antibodies, Cold Spring Harbor Press). For example, titers of antibodies produced in response to administration of a composition providing an immunogen can be measured by enzyme-linked immunosorbent assay (ELISA). The immune responses can also be measured by neutralizing antibody assay, where a neutralization of a virus is defined as the loss of infectivity through reaction/inhibition/neutralization of the virus with specific antibody. The immune response can further be measured by Antibody-Dependent Cellular Phagocytosis (ADCP) Assay.

EMBODIMENTS

[0200] Embodiments Section 1

[0201] Embodiment 1 comprises an isolated or non-naturally occurring nucleic acid molecule comprising a polynucleotide sequence encoding a truncated HBV core antigen consisting of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 14, preferably 100% identical to SEQ ID NO: 2 or SEQ ID NO: 14; preferably the truncated HBV core antigen is capable of inducing an immune response in a mammal against at least two HBV genotypes; more preferably the truncated HBV core antigen is capable of inducing a T cell response in a mammal against at least HBV genotypes B, C and D; further more preferably the truncated HBV core antigen is capable of inducing a CD8 T cell response in a human subject against at least HBV genotypes A, B, C and D; and most preferably, the truncated HBV core antigen coding sequence comprises a polynucleotide that is at least 90%, such as 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 1 or SEQ ID NO: 15.

[0202] Embodiment 2 comprises the non-naturally occurring nucleic acid molecule of embodiment 1, wherein the truncated HBV core antigen consists of the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 14.

[0203] Embodiment 3 comprises the non-naturally occurring nucleic acid molecule of embodiment 1 or embodiment 2, further comprising a polynucleotide sequence encoding a signal sequence operably linked to the HBV polymerase antigen.

[0204] Embodiment 4 comprises the non-naturally occurring nucleic acid molecule of embodiment 3, wherein the signal sequence comprises the amino acid sequence of SEQ ID NO: 6 or SEQ ID NO: 19, preferably the signal sequence is encoded by the polynucleotide sequence of SEQ ID NO: 5 or SEQ ID NO: 18.

[0205] Embodiment 5 comprises the non-naturally occurring nucleic acid molecule of any one of embodiments 1 to 4, wherein the polynucleotide sequence encoding the truncated core HBV antigen is at least 90% identical to SEQ ID NO: 1 or SEQ ID NO: 15.

[0206] Embodiment 6 comprises the non-naturally occurring nucleic acid molecule of embodiment 5, wherein the polynucleotide sequence encoding the truncated core HBV antigen comprises the polynucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 15.

[0207] Embodiment 7 comprises a non-naturally occurring nucleic acid molecule comprising a first polynucleotide sequence encoding an HBV polymerase antigen comprising an amino acid sequence that is at least 98% identical to SEQ ID NO: 4, wherein the HBV polymerase antigen does not have reverse transcriptase activity and RNase H activity.

[0208] Embodiment 8 comprises the non-naturally occurring nucleic acid molecule of embodiment 7, wherein the HBV polymerase antigen is capable of inducing an immune response in a mammal against at least two HBV genotypes, preferably the HBV polymerase antigen is capable of inducing a T cell response in a mammal against at least HBV genotypes B, C and D, and more preferably the HBV polymerase antigen is capable of inducing a CD8 T cell response in a human subject against at least HBV genotypes A, B, C and D.

[0209] Embodiment 9 comprises the non-naturally occurring nucleic acid molecule of embodiment 7, wherein the HBV polymerase antigen comprises the amino acid sequence of SEQ ID NO: 4.

[0210] Embodiment 10 comprises the non-naturally occurring nucleic acid molecule of any one of embodiments 7-9, further comprising a polynucleotide sequence encoding a signal sequence operably linked to the HBV polymerase antigen.

[0211] Embodiment 11 comprises the non-naturally occurring nucleic acid molecule of embodiment 10, wherein the signal sequence comprises the amino acid sequence of SEQ ID NO:

6 or SEQ ID NO: 19, preferably the signal sequence is encoded by the polynucleotide sequence of SEQ ID NO: 5 or SEQ ID NO: 18.

[0212] Embodiment 12 comprises the non-naturally occurring nucleic acid molecule of any one of embodiments 7 to 11, wherein the first polynucleotide sequence is at least 90% identical to SEQ ID NO: 3 or SEQ ID NO: 16.

[0213] Embodiment 13 comprises the non-naturally occurring nucleic acid molecule of embodiment 12, wherein the first polynucleotide sequence comprises the polynucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 16.

[0214] Embodiment 14 comprises the non-naturally occurring nucleic acid molecule of any one of embodiments 7 to 13, further comprising a second polynucleotide sequence encoding a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 14.

[0215] Embodiment 15 comprises the non-naturally occurring nucleic acid molecule of embodiment 14, wherein the second polynucleotide sequence is at least 90% identical to SEQ ID NO: 1 or SEQ ID NO: 15.

[0216] Embodiment 16 comprises the non-naturally occurring nucleic acid molecule of embodiment 15, wherein the second polynucleotide sequence comprises the polynucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 15.

[0217] Embodiment 17 comprises the non-naturally occurring nucleic acid molecule of any one of embodiments 14 to 16, encoding a fusion protein comprising the truncated HBV core antigen operably linked to the HBV polymerase antigen.

[0218] Embodiment 18 comprises the non-naturally occurring nucleic acid molecule of embodiment 17, wherein the fusion protein comprises the truncated HBV core antigen operably linked to the HBV polymerase antigen via a linker.

[0219] Embodiment 19 comprises the non-naturally occurring nucleic acid molecule of embodiment 18, wherein the linker comprises the amino acid sequence of (AlaGly)_n, and n is an integer of 2 to 5, preferably the linker is encoded by a polynucleotide sequence comprising SEQ ID NO: 22.

[0220] Embodiment 20 comprises the non-naturally occurring nucleic acid molecule of embodiment 19, wherein the fusion protein comprises the amino acid sequence of SEQ ID NO: 20.

[0221] Embodiment 21 comprises the non-naturally occurring nucleic acid molecule of any one of embodiments 17 to 20, wherein the fusion protein further comprises a signal sequence, preferably the signal sequence comprises the amino acid sequence of SEQ ID NO: 6 or SEQ ID NO: 19, more preferably the signal sequence is encoded by the polynucleotide sequence of SEQ ID NO: 5 or SEQ ID NO: 18.

[0222] Embodiment 22 comprises the non-naturally occurring nucleic acid molecule of any one of embodiments 7 to 21 further comprising a promoter sequence, optionally one or more additional regulatory sequences, preferably the promoter sequence comprises the polynucleotide sequence of SEQ ID NO: 7 or SEQ ID NO: 17, and the additional regulatory sequence is selected from the group consisting of an enhancer sequences of SEQ ID NO: 8 or SEQ ID NO: 23, and a polyadenylation signal sequence of SEQ ID NO: 11 or SEQ ID NO: 24.

[0223] Embodiment 23 comprises the non-naturally occurring nucleic acid molecule of any one of embodiments 7 to 22, wherein the non-naturally occurring nucleic acid molecule does not encode a HBV antigen selected from the group consisting of a Hepatitis B surface antigen (HBsAg), a HBV envelope (Env) antigen, and a HBV L protein antigen.

[0224] Embodiment 24 comprises a vector comprising the non-naturally occurring nucleic acid molecule of any one of embodiments 1 to 24.

[0225] Embodiment 25 comprises a vector of embodiment 24, wherein the non-naturally occurring nucleic acid molecule comprises, from 5' end to 3' end, a promoter sequence, an enhancer sequence, a signal peptide coding sequence, the first polynucleotide sequence, and a polyadenylation signal sequence, optionally, the non-naturally occurring nucleic acid molecule further comprises the second polynucleotide sequence.

[0226] Embodiment 26 comprises the vector of embodiment 24 or 25, wherein the vector is a plasmid DNA vector, and the plasmid DNA vector further comprises an origin of replication and an antibiotic resistance gene.

[0227] Embodiment 27 comprises the vector of embodiment 26, wherein the plasmid DNA vector contains the origin of replication comprising the polynucleotide sequence of SEQ ID NO: 10, the antibiotic resistance gene comprising the polynucleotide sequence of SEQ ID NO: 12, the promoter sequence comprising the polynucleotide sequence of SEQ ID NO: 7, the enhancer sequence comprising the polynucleotide sequence of SEQ ID NO: 8, the signal peptide coding sequence comprising the polynucleotide sequence of SEQ ID NO: 5, the first polynucleotide

sequence comprising the polynucleotide sequence of SEQ ID NO: 3, and the polyadenylation signal sequence comprising the polynucleotide sequence of SEQ ID NO: 11, and optionally the second polynucleotide sequence comprising the polynucleotide sequence of SEQ ID NO: 1.

5 [0228] Embodiment 28 comprises the vector of embodiment 24 or 25, wherein the vector is an adenoviral vector, preferably an Ad26 or Ad35 vector.

[0229] Embodiment 29 comprises the vector of embodiment 28, wherein the vector is an Ad26 vector comprising the non-naturally occurring nucleic acid encoding the truncated core HBV antigen of any one of embodiments 1 to 6.

10 [0230] Embodiment 30 comprises the vector of embodiment 28, wherein the vector is an Ad26 vector comprising the non-naturally occurring nucleic acid encoding the HBV polymerase antigen of any one of embodiments 7 to 13.

[0231] Embodiment 31 comprises the vector of embodiment 28, wherein the vector is an Ad26 vector comprising the non-naturally occurring nucleic acid encoding the fusion protein of any one of embodiments 17-21.

15 [0232] Embodiment 31a comprises the vector of any one of embodiments 28 to 31, wherein the adenoviral vector contains the promoter sequence comprising the polynucleotide sequence of SEQ ID NO: 17, the regulatory sequence comprising the polynucleotide sequence of SEQ ID NO: 23, the signal peptide coding sequence comprising the polynucleotide sequence of SEQ ID NO: 18, the second polynucleotide sequence comprising the polynucleotide sequence of SEQ ID NO: 15, the linker coding sequence comprising the polynucleotide sequence of SEQ ID NO: 22, the first polynucleotide sequence comprising the polynucleotide sequence of SEQ ID NO: 16, and the polyadenylation signal sequence comprising the polynucleotide sequence of SEQ ID NO: 24.

25 [0233] Embodiment 32 comprises the non-naturally occurring polypeptide encoded by the non-naturally occurring nucleic acid molecule of any embodiments 1 to 32.

[0234] Embodiment 33 comprises a host cell comprising the non-naturally occurring nucleic acid molecule of any one of embodiments 1 to 23 or the vector of any one of embodiments 24 to 32.

30 [0235] Embodiment 34 comprises a composition comprising the non-naturally occurring nucleic acid molecule of any one of embodiments 1 to 23, the vector of any one of embodiments

24 to 32, or the non-naturally occurring polypeptide of embodiment 33, and a pharmaceutically acceptable carrier.

[0236] Embodiment 35 comprises the composition of embodiment 34, comprising the first polynucleotide of any one of embodiments 7 to 13, the second polynucleotide of any one of
5 embodiments 14-16, and a pharmaceutically acceptable carrier, wherein the first and second polynucleotides are not comprised in the same nucleic acid molecule or in the same nucleic acid vector.

[0237] Embodiment 36 comprises the composition of embodiment 35, wherein the first and second polynucleotides are comprised in two separate vectors, preferably adenovirus vectors,
10 more preferably Ad26 vectors.

[0238] Embodiment 37 comprises a kit comprising:

(a) a first non-naturally occurring nucleic acid molecule comprising a first polynucleotide encoding a HBV polymerase antigen having an amino acid sequence that is at least 98% identical to SEQ ID NO: 4, wherein the HBV polymerase antigen does not have
15 reverse transcriptase activity and RNase H activity;

(b) a second non-naturally occurring nucleic acid molecule comprising a second polynucleotide encoding a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 14; and

(c) a pharmaceutically acceptable carrier,

20 wherein the first non-naturally occurring nucleic acid molecule and the second non-naturally occurring nucleic acid molecule are present in the same non-naturally occurring nucleic acid molecule or in two different non-naturally occurring nucleic acid molecules.

[0239] Embodiment 38 comprises the kit of embodiment 37, wherein the HBV polymerase antigen is capable of inducing an immune response in a mammal against at least two HBV
25 genotypes, preferably the HBV polymerase antigen is capable of inducing a T cell response in a mammal against at least HBV genotypes B, C and D, and more preferably the HBV polymerase antigen is capable of inducing a CD8 T cell response in a human subject against at least HBV genotypes A, B, C and D.

[0240] Embodiment 39 comprises the kit of embodiment 37, wherein the HBV polymerase
30 antigen comprises the amino acid sequence of SEQ ID NO: 4.

- [0241] Embodiment 40 comprises the kit of any one of embodiments 37 to 39, wherein at least one of the first non-naturally occurring nucleic acid molecule and the second non-naturally occurring nucleic acid molecule further comprises a polynucleotide sequence encoding a signal sequence operably linked to at least one of the HBV polymerase antigen and the truncated HBV core antigen.
- [0242] Embodiment 41 comprises the kit of embodiment 40, wherein the signal sequence independently comprises the amino acid sequence of SEQ ID NO: 6 or SEQ ID NO: 19, preferably the signal sequence is independently encoded by the polynucleotide sequence of SEQ ID NO: 5 or SEQ ID NO: 18.
- [0243] Embodiment 42 comprises the kit of any one of embodiments 37 to 41, wherein the first polynucleotide sequence is at least 90% identical to SEQ ID NO: 3 or SEQ ID NO: 16.
- [0244] Embodiment 43 comprises the kit of embodiment 42, wherein the first polynucleotide sequence comprises the polynucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 16.
- [0245] Embodiment 44 comprises the kit of any one of embodiments 37 to 43, wherein the second polynucleotide sequence is at least 90% identical to SEQ ID NO: 1 or SEQ ID NO: 15.
- [0246] Embodiment 45 comprises the kit of embodiment 44, wherein the second polynucleotide sequence comprises the polynucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 15.
- [0247] Embodiment 46 comprises the kit of any one of embodiments 37 to 45, wherein at least one of the first non-naturally occurring nucleic acid molecule and the second non-naturally occurring nucleic acid molecule further comprises a promoter sequence, optionally an enhancer sequence, and further optionally a polyadenylation signal sequence, preferably the promoter sequence has the polynucleotide sequence of SEQ ID NO: 7 or SEQ ID NO: 17, the enhancer sequence independently has the polynucleotide sequence of SEQ ID NO: 8 or SEQ ID NO: 23, and the polyadenylation signal sequence independently has the polynucleotide sequence of SEQ ID NO: 11 or SEQ ID NO: 24.
- [0248] Embodiment 47 comprises the kit of any one of embodiments 37 to 46, wherein the kit does not contain a nucleic acid molecule encoding a HBV antigen selected from the group consisting of a Hepatitis B surface antigen (HBsAg), a HBV envelope (Env) antigen, and a HBV L protein antigen, nor a HBV antigen selected from the group consisting of a Hepatitis B surface antigen (HBsAg), a HBV envelope (Env) antigen, and a HBV L protein antigen.

- [0249] Embodiment 48 comprises the kit of any one of embodiments 37 to 47, wherein the first non-naturally occurring nucleic acid molecule and the second non-naturally occurring nucleic acid molecule are present in the same vector.
- [0250] Embodiment 49 comprises the kit of embodiment 48, wherein the vector encodes the HBV polymerase antigen and the truncated HBV core antigen as two separate proteins.
- 5 [0251] Embodiment 50 comprises the kit of embodiment 49, wherein the vector encodes a fusion protein comprising the truncated HBV core antigen operably linked to the HBV polymerase antigen.
- [0252] Embodiment 51 comprises the kit of embodiment 50, wherein the fusion protein
- 10 comprises the truncated HBV core antigen operably linked to the HBV polymerase antigen via a linker.
- [0253] Embodiment 52 comprises the kit of embodiment 51, wherein the linker comprises the amino acid sequence of (AlaGly)_n, and n is an integer of 2 to 5, preferably the linker is encoded by a polynucleotide sequence comprising SEQ ID NO: 22.
- 15 [0254] Embodiment 53 comprises the kit of embodiment 52, wherein the fusion protein comprises the amino acid sequence of SEQ ID NO: 20.
- [0255] Embodiment 54 comprises the kit of embodiment 53, wherein the fusion protein further comprises a signal sequence, preferably the signal sequence has the amino acid sequence of SEQ ID NO: 6 or SEQ ID NO: 19, more preferably the signal sequence is encoded by the
- 20 polynucleotide sequence of SEQ ID NO: 5 or SEQ ID NO: 18.
- [0256] Embodiment 55 comprises the kit of embodiment 54, wherein the fusion protein comprises the amino acid sequence of SEQ ID NO: 21.
- [0257] Embodiment 56 comprises the kit of any one of embodiments 48 to 55, wherein the vector contains, from 5' end to 3' end, a promoter sequence, an enhancer sequence, a signal
- 25 peptide coding sequence, the second polynucleotide sequence, a linker coding sequence, the first polynucleotide sequence, and a polyadenylation signal sequence.
- [0258] Embodiment 57 comprises the kit of embodiment 56, wherein the vector is an adenoviral vector, preferably an Ad26 or Ad35 vector.
- [0259] Embodiment 58 comprises the kit of embodiment 57, wherein the adenoviral vector
- 30 contains the promoter sequence comprising the polynucleotide sequence of SEQ ID NO: 17, the regulatory sequence comprising the polynucleotide sequence of SEQ ID NO: 23, the signal

peptide coding sequence comprising the polynucleotide sequence of SEQ ID NO: 18, the second polynucleotide sequence comprising the polynucleotide sequence of SEQ ID NO: 15, the linker coding sequence comprising the polynucleotide sequence of SEQ ID NO: 22, the first polynucleotide sequence comprising the polynucleotide sequence of SEQ ID NO: 16, and the polyadenylation signal sequence comprising the polynucleotide sequence of SEQ ID NO: 24.

[0260] Embodiment 59 comprises the kit of embodiment 58, wherein the kit does not contain a nucleic acid molecule encoding a HBV antigen selected from the group consisting of a Hepatitis B surface antigen (HBsAg), a HBV envelope (Env) antigen, and a HBV L protein antigen, nor a HBV antigen selected from the group consisting of a Hepatitis B surface antigen (HBsAg), a HBV envelope (Env) antigen, and a HBV L protein antigen.

[0261] Embodiment 60 comprises the kit of any one of embodiments 37 to 59, wherein the first non-naturally occurring nucleic acid molecule and the second non-naturally occurring nucleic acid molecule are present in two different vectors.

[0262] Embodiment 61 comprises the kit of embodiment 60, wherein the first non-naturally occurring nucleic acid molecule is present in a first plasmid DNA vector, and the second non-naturally occurring nucleic acid molecule is present in a second plasmid DNA vector.

[0263] Embodiment 62 comprises the kit of embodiment 61, wherein each of the first and second plasmid DNA vectors comprises an origin of replication, an antibiotic resistance gene, and from 5' end to 3' end, a promoter sequence, a regulatory sequence, a signal peptide coding sequence, the first polynucleotide sequence or the second polynucleotide sequence, and a polyadenylation signal sequence.

[0264] Embodiment 63 comprises the kit of embodiment 62, wherein the antibiotic resistance gene is a kanamycin resistance gene having a polynucleotide sequence at least 90% identical to SEQ ID NO: 12, preferably 100% identical to SEQ ID NO: 12.

[0265] Embodiment 64 comprises the kit of embodiment 63, comprising,
(a) a first plasmid DNA vector comprising, from 3'-end to 5'-end, the promoter sequence comprising the polynucleotide sequence of SEQ ID NO: 7, the regulatory sequence comprising the polynucleotide sequence of SEQ ID NO: 8, the signal peptide coding sequence comprising the polynucleotide sequence of SEQ ID NO: 5, the first polynucleotide sequence comprising the polynucleotide sequence of SEQ ID NO: 3,

and the polyadenylation signal sequence comprising the polynucleotide sequence of SEQ ID NO: 11;

5 (b) a second plasmid DNA vector comprising, from 3'-end to 5'-end, the promoter sequence comprising the polynucleotide sequence of SEQ ID NO: 7, the regulatory sequence comprising the polynucleotide sequence of SEQ ID NO: 8, the signal peptide coding sequence comprising the polynucleotide sequence of SEQ ID NO: 5, the second polynucleotide sequence comprising the polynucleotide sequence of SEQ ID NO: 1, and the polyadenylation signal sequence comprising the polynucleotide sequence of SEQ ID NO: 11; and

10 (c) a pharmaceutically acceptable carrier,

wherein each of the first plasmid DNA vector and the second plasmid DNA vector further comprises a kanamycin resistance gene having the polynucleotide sequence of SEQ ID NO: 12, and an origin of replication having the polynucleotide sequence of SEQ ID NO: 10, and wherein the first plasmid DNA vector and the second plasmid DNA vector are in the same composition or two different compositions.

15 **[0266]** Embodiment 65 comprises the kit of embodiment 60, wherein the first non-naturally occurring nucleic acid molecule is present in a first adenovirus vector, preferably Ad26 vector, and the second non-naturally occurring nucleic acid molecule is present in a second adenovirus vector, preferably Ad26 vector.

20 **[0267]** Embodiment 66 comprises the kit of embodiment 65, wherein each of the first and second adenovirus vectors comprises from 5' end to 3' end, a promoter sequence, a regulatory sequence, a signal peptide coding sequence, the first polynucleotide sequence or the second polynucleotide sequence, and a polyadenylation signal sequence.

[0268] Embodiment 67 comprises the kit of embodiment 66, comprising,

25 (a) a first Ad26 vector comprising, from 3'-end to 5'-end, the promoter sequence comprising the polynucleotide sequence of SEQ ID NO: 17, the regulatory sequence comprising the polynucleotide sequence of SEQ ID NO: 23, the signal peptide coding sequence comprising the polynucleotide sequence of SEQ ID NO: 17, the first polynucleotide sequence comprising the polynucleotide sequence of SEQ ID NO: 15, and the polyadenylation signal sequence comprising the polynucleotide sequence of SEQ ID NO: 24;

30

(b) a second Ad26 vector comprising, from 3'-end to 5'-end, the promoter sequence comprising the polynucleotide sequence of SEQ ID NO: 17, the regulatory sequence comprising the polynucleotide sequence of SEQ ID NO: 23, the signal peptide coding sequence comprising the polynucleotide sequence of SEQ ID NO: 17, the second polynucleotide sequence comprising the polynucleotide sequence of SEQ ID NO: 16, and the polyadenylation signal sequence comprising the polynucleotide sequence of SEQ ID NO: 24; and

(c) a pharmaceutically acceptable carrier, wherein each of the first Ad26 vector and the second Ad26 vector are in the same composition or two different compositions.

[0269] Embodiment 68 comprises the kit of any one of embodiments 64 to 67, wherein the kit does not contain a nucleic acid molecule encoding a HBV antigen selected from the group consisting of a Hepatitis B surface antigen (HBsAg), a HBV envelope (Env) antigen, and a HBV L protein antigen, nor a HBV antigen selected from the group consisting of a Hepatitis B surface antigen (HBsAg), a HBV envelope (Env) antigen, and a HBV L protein antigen.

[0270] Embodiment 69 comprises the composition of any one of embodiments 34 to 36 or the kit of any one of embodiments 37 to 68 for use in inducing an immune response against a hepatitis B virus (HBV) in a subject in need thereof, preferably the subject has chronic HBV infection.

[0271] Embodiment 70 comprises a combination of another immunogenic agent, preferably another HBV antigen, with the composition of any one of embodiments 34 to 36 or the kit of any one of embodiments 37 to 68 for use in inducing an immune response against a hepatitis B virus (HBV) in a subject in need thereof, preferably the subject has chronic HBV infection.

[0272] Embodiment 71 comprises the composition of any one of embodiments 34 to 36 or the kit of any one of embodiments 37 to 68 for use in treating a hepatitis B virus (HBV)-induced disease in a subject in need thereof, preferably the subject has chronic HBV infection, and the HBV-induced disease is selected from the group consisting of advanced fibrosis, cirrhosis and hepatocellular carcinoma (HCC).

[0273] Embodiment 72 comprises a combination of another therapeutic agent, preferably another anti-HBV agent, with the composition of any one of embodiments 34 to 36 or the kit of any one of embodiments 37 to 68 for use in treating a hepatitis B virus (HBV)-induced disease in

a subject in need thereof, preferably the subject has chronic HBV infection, and the HBV-induced disease is selected from the group consisting of advanced fibrosis, cirrhosis and hepatocellular carcinoma (HCC).

[0274] Embodiments Section 2

- 5 **[0275]** Embodiment 1 comprises a non-naturally occurring nucleic acid molecule comprising a first polynucleotide sequence encoding an HBV polymerase antigen comprising an amino acid sequence that is at least 98% identical to SEQ ID NO: 4, wherein the HBV polymerase antigen does not have reverse transcriptase activity and RNase H activity.

- 10 **[0276]** Embodiment 2 comprises the non-naturally occurring nucleic acid molecule of embodiment 1, wherein the HBV polymerase antigen is capable of inducing an immune response in a mammal against at least two HBV genotypes, preferably the HBV polymerase antigen is capable of inducing a T cell response in a mammal against at least HBV genotypes B, C and D, and more preferably the HBV polymerase antigen is capable of inducing a CD8 T cell response in a human subject against at least HBV genotypes A, B, C and D.

- 15 **[0277]** Embodiment 3 comprises the non-naturally occurring nucleic acid molecule of embodiment 1, wherein the HBV polymerase antigen comprises the amino acid sequence of SEQ ID NO: 4.

- 20 **[0278]** Embodiment 4 comprises the non-naturally occurring nucleic acid molecule of any one of embodiments 1-3, further comprising a polynucleotide sequence encoding a signal sequence operably linked to the HBV polymerase antigen.

[0279] Embodiment 5 comprises the non-naturally occurring nucleic acid molecule of embodiment 4, wherein the signal sequence comprises the amino acid sequence of SEQ ID NO: 6 or SEQ ID NO: 19, preferably the signal sequence is encoded by the polynucleotide sequence of SEQ ID NO: 5 or SEQ ID NO: 18.

- 25 **[0280]** Embodiment 6 comprises the non-naturally occurring nucleic acid molecule of any one of embodiments 1 to 5, wherein the first polynucleotide sequence is at least 90% identical to SEQ ID NO: 3 or SEQ ID NO: 16.

- 30 **[0281]** Embodiment 7 comprises the non-naturally occurring nucleic acid molecule of embodiment 6, wherein the first polynucleotide sequence comprises the polynucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 16.

[0282] Embodiment 8 comprises the non-naturally occurring nucleic acid molecule of any one of embodiments 1 to 7, further comprising a second polynucleotide sequence encoding a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 14.

5 [0283] Embodiment 9 comprises the non-naturally occurring nucleic acid molecule of embodiment 8, wherein the second polynucleotide sequence is at least 90% identical to SEQ ID NO: 1 or SEQ ID NO: 15.

[0284] Embodiment 10 comprises the non-naturally occurring nucleic acid molecule of embodiment 9, wherein the second polynucleotide sequence comprises the polynucleotide
10 sequence of SEQ ID NO: 1 or SEQ ID NO: 15.

[0285] Embodiment 11 comprises the non-naturally occurring nucleic acid molecule of any one of embodiments 8 to 10, encoding a fusion protein comprising the truncated HBV core antigen operably linked to the HBV polymerase antigen.

[0286] Embodiment 12 comprises the non-naturally occurring nucleic acid molecule of
15 embodiment 11, wherein the fusion protein comprises the truncated HBV core antigen operably linked to the HBV polymerase antigen via a linker.

[0287] Embodiment 13 comprises the non-naturally occurring nucleic acid molecule of embodiment 12, wherein the linker comprises the amino acid sequence of (AlaGly)_n, and n is an integer of 2 to 5, preferably the linker is encoded by a polynucleotide sequence comprising SEQ
20 ID NO: 22.

[0288] Embodiment 14 comprises the non-naturally occurring nucleic acid molecule of embodiment 13, wherein the fusion protein comprises the amino acid sequence of SEQ ID NO: 20.

[0289] Embodiment 15 comprises the non-naturally occurring nucleic acid molecule of any
25 one of embodiments 11 to 14, wherein the fusion protein further comprises a signal sequence, preferably the signal sequence comprises the amino acid sequence of SEQ ID NO: 6 or SEQ ID NO: 19, more preferably the signal sequence is encoded by the polynucleotide sequence of SEQ ID NO: 5 or SEQ ID NO: 18.

[0290] Embodiment 16 comprises the non-naturally occurring nucleic acid molecule of any
30 one of embodiments 1 to 15 further comprising a promoter sequence, optionally one or more additional regulatory sequences, preferably the promoter sequence comprises the polynucleotide

sequence of SEQ ID NO: 7 or SEQ ID NO: 17, and the additional regulatory sequence is selected from the group consisting of SEQ ID NO: 8 or SEQ ID NO: 23, and a polyadenylation signal sequence of SEQ ID NO: 11 or SEQ ID NO: 24.

[0291] Embodiment 17 comprises the non-naturally occurring nucleic acid molecule of any one of embodiments 1 to 16, wherein the non-naturally occurring nucleic acid molecule does not encode a HBV antigen selected from the group consisting of a Hepatitis B surface antigen (HBsAg), a HBV envelope (Env) antigen, and a HBV L protein antigen.

[0292] Embodiment 18 comprises a vector comprising the non-naturally occurring nucleic acid molecule of any one of embodiments 1 to 17.

10 **[0293]** Embodiment 19 comprises the vector of embodiment 18, wherein the non-naturally occurring nucleic acid molecule comprises, from 5' end to 3' end, a promoter sequence, an enhancer sequence, a signal peptide coding sequence, the first polynucleotide sequence, and a polyadenylation signal sequence, optionally, the non-naturally occurring nucleic acid molecule further comprises the second polynucleotide sequence.

15 **[0294]** Embodiment 20 comprises the vector of embodiment 18 or 19, wherein the vector is a plasmid DNA vector, and the plasmid DNA vector further comprises an origin of replication and an antibiotic resistance gene.

[0295] Embodiment 21 comprises the vector of embodiment 20, wherein the plasmid DNA vector contains the origin of replication comprising the polynucleotide sequence of SEQ ID NO: 20 10, the antibiotic resistance gene comprising the polynucleotide sequence of SEQ ID NO: 12, the promoter sequence comprising the polynucleotide sequence of SEQ ID NO: 7, the enhancer sequence comprising the polynucleotide sequence of SEQ ID NO: 8, the signal peptide coding sequence comprising the polynucleotide sequence of SEQ ID NO: 5, the first polynucleotide sequence comprising the polynucleotide sequence of SEQ ID NO: 3, and the polyadenylation 25 signal sequence comprising the polynucleotide sequence of SEQ ID NO: 11, and optionally the second polynucleotide sequence comprising the polynucleotide sequence of SEQ ID NO: 1.

[0296] Embodiment 22 comprises the vector of embodiment 18 or 19, wherein the vector is an adenoviral vector, preferably an Ad26 or Ad35 vector.

30 **[0297]** Embodiment 23 comprises the vector of embodiment 22, wherein the adenoviral vector contains the promoter sequence comprising the polynucleotide sequence of SEQ ID NO: 17, the regulatory sequence comprising the polynucleotide sequence of SEQ ID NO: 23, the

signal peptide coding sequence comprising the polynucleotide sequence of SEQ ID NO: 18, the second polynucleotide sequence comprising the polynucleotide sequence of SEQ ID NO: 15, the linker coding sequence comprising the polynucleotide sequence of SEQ ID NO: 22, the first polynucleotide sequence comprising the polynucleotide sequence of SEQ ID NO: 16, and the polyadenylation signal sequence comprising the polynucleotide sequence of SEQ ID NO: 24.

[0298] Embodiment 24 comprises a non-naturally occurring polypeptide encoded by the non-naturally occurring nucleic acid molecule of any one of embodiments 1 to 17.

[0299] Embodiment 25 comprises a host cell comprising the non-naturally occurring nucleic acid molecule of any one of embodiments 1 to 17 or the vector of any one of embodiments 18 to 23.

[0300] Embodiment 26 comprises a composition comprising the non-naturally occurring nucleic acid molecule of any one of embodiments 1 to 17, the vector of any one of embodiments 18 to 23, or the non-naturally occurring polypeptide of embodiment 24, and a pharmaceutically acceptable carrier.

[0301] Embodiment 27 comprises the composition of embodiment 26, comprising the first polynucleotide of any one of embodiments 1-7, the second polynucleotide of any one of embodiments 8-10, and a pharmaceutically acceptable carrier, wherein the first and second polynucleotides are not comprised in the same nucleic acid molecule or in the same nucleic acid vector.

[0302] Embodiment 28 comprises a kit comprising:

(a) a first non-naturally occurring nucleic acid molecule comprising a first polynucleotide encoding a HBV polymerase antigen having an amino acid sequence that is at least 98% identical to SEQ ID NO: 4, wherein the HBV polymerase antigen does not have reverse transcriptase activity and RNase H activity;

(b) a second non-naturally occurring nucleic acid molecule comprising a second polynucleotide encoding a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 14; and

(c) a pharmaceutically acceptable carrier,

wherein the first non-naturally occurring nucleic acid molecule and the second non-naturally occurring nucleic acid molecule are present in the same non-naturally occurring nucleic acid molecule or in two different non-naturally occurring nucleic acid molecules.

[0303] Embodiment 29 comprises the kit of embodiment 28, wherein the HBV polymerase antigen is capable of inducing an immune response in a mammal against at least two HBV genotypes, preferably the HBV polymerase antigen is capable of inducing a T cell response in a mammal against at least HBV genotypes B, C and D, and more preferably the HBV polymerase antigen is capable of inducing a CD8 T cell response in a human subject against at least HBV genotypes A, B, C and D.

[0304] Embodiment 30 comprises the kit of embodiment 29, wherein the HBV polymerase antigen comprises the amino acid sequence of SEQ ID NO: 4.

[0305] Embodiment 31 comprises the kit of any one of embodiments 28 to 30, wherein at least one of the first non-naturally occurring nucleic acid molecule and the second non-naturally nucleic acid molecule further comprises a polynucleotide sequence encoding a signal sequence operably linked to the at least one of the HBV polymerase antigen and the truncated HBV core antigen.

[0306] Embodiment 32 comprises the kit of embodiment 31, wherein the signal sequence independently comprises the amino acid sequence of SEQ ID NO: 6 or SEQ ID NO: 19, preferably the signal sequence is independently encoded by the polynucleotide sequence of SEQ ID NO: 5 or SEQ ID NO: 18.

[0307] Embodiment 33 comprises the kit of any one of embodiment s 28 to 32, wherein the first polynucleotide sequence is at least 90% identical to SEQ ID NO: 3 or SEQ ID NO: 16.

[0308] Embodiment 34 comprises the kit of embodiment 33, wherein the first polynucleotide sequence comprises the polynucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 16.

[0309] Embodiment 35 comprises the kit of any one of embodiments 28 to 34, wherein the second polynucleotide sequence is at least 90% identical to SEQ ID NO: 1 or SEQ ID NO: 15.

[0310] Embodiment 36 comprises the kit of embodiment 35, wherein the second polynucleotide sequence comprises the polynucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 15.

[0311] Embodiment 37 comprises the kit of any one of embodiments 28 to 36, wherein at least one of the first non-naturally occurring nucleic acid molecule and the second non-naturally nucleic acid molecule further comprises a promoter sequence, optionally an enhancer sequence, and further optionally a polyadenylation signal sequence, preferably the promoter sequence has the polynucleotide sequence of SEQ ID NO: 7 or SEQ ID NO: 17, the enhancer sequence

independently has the polynucleotide sequence of SEQ ID NO: 8 or SEQ ID NO: 23, and the polyadenylation signal sequence independently has the polynucleotide sequence of SEQ ID NO: 11 or SEQ ID NO: 24.

[0312] Embodiment 38 comprises the kit of any one of embodiments 28 to 37, wherein the kit does not contain a nucleic acid molecule encoding a HBV antigen selected from the group consisting of a Hepatitis B surface antigen (HBsAg), a HBV envelope (Env) antigen, and a HBV L protein antigen, nor a HBV antigen selected from the group consisting of a Hepatitis B surface antigen (HBsAg), a HBV envelope (Env) antigen, and a HBV L protein antigen.

[0313] Embodiment 39 comprises the kit of any one of embodiment 28 to 38, wherein the first non-naturally occurring nucleic acid molecule and the second non-naturally occurring nucleic acid molecule are present in the same vector.

[0314] Embodiment 40 comprises the kit of embodiment 39, wherein the vector encodes the HBV polymerase antigen and the truncated HBV core antigen as two separate proteins.

[0315] Embodiment 41 comprises the kit of embodiment 39, wherein the vector encodes a fusion protein comprising the truncated HBV core antigen operably linked to the HBV polymerase antigen.

[0316] Embodiment 42 comprises the kit of embodiment 41, wherein the fusion protein comprises the truncated HBV core antigen operably linked to the HBV polymerase antigen via a linker.

[0317] Embodiment 43 comprises the kit of embodiment 42, wherein the linker comprises the amino acid sequence of (AlaGly)_n, and n is an integer of 2 to 5, preferably the linker is encoded by a polynucleotide sequence comprising SEQ ID NO: 22.

[0318] Embodiment 44 comprises the kit of embodiment 43, wherein the fusion protein comprises the amino acid sequence of SEQ ID NO: 20.

[0319] Embodiment 45 comprises the kit of embodiment 44, wherein the fusion protein further comprises a signal sequence, preferably the signal sequence has the amino acid sequence of SEQ ID NO: 6 or SEQ ID NO: 19, more preferably the signal sequence is encoded by the polynucleotide sequence of SEQ ID NO: 5 or SEQ ID NO: 18.

[0320] Embodiment 46 comprises the kit of embodiment 45, wherein the fusion protein comprises the amino acid sequence of SEQ ID NO: 21.

- [0321] Embodiment 47 comprises the kit of any one of embodiments 41 to 46, wherein the vector contains, from 5' end to 3' end, a promoter sequence, an enhancer sequence, a signal peptide coding sequence, the second polynucleotide sequence, a linker coding sequence, the first polynucleotide sequence, and a polyadenylation signal sequence.
- 5 [0322] Embodiment 48 comprises the kit of embodiment 47, wherein the vector is an adenoviral vector, preferably an Ad26 or Ad35 vector.
- [0323] Embodiment 49 comprises the kit of embodiment 48, wherein the adenoviral vector contains the promoter sequence comprising the polynucleotide sequence of SEQ ID NO: 17, the regulatory sequence comprising the polynucleotide sequence of SEQ ID NO: 23, the signal
- 10 peptide coding sequence comprising the polynucleotide sequence of SEQ ID NO: 18, the second polynucleotide sequence comprising the polynucleotide sequence of SEQ ID NO: 15, the linker coding sequence comprising the polynucleotide sequence of SEQ ID NO: 22, the first polynucleotide sequence comprising the polynucleotide sequence of SEQ ID NO: 16, and the polyadenylation signal sequence comprising the polynucleotide sequence of SEQ ID NO: 24.
- 15 [0324] Embodiment 50 comprises the kit of embodiment 49, wherein the kit does not contain a nucleic acid molecule encoding a HBV antigen selected from the group consisting of a Hepatitis B surface antigen (HBsAg), a HBV envelope (Env) antigen, and a HBV L protein antigen, nor a HBV antigen selected from the group consisting of a Hepatitis B surface antigen (HBsAg), a HBV envelope (Env) antigen, and a HBV L protein antigen.
- 20 [0325] Embodiment 51 comprises the kit of any one of embodiments 28 to 38, wherein the first non-naturally occurring nucleic acid molecule and the second non-naturally occurring nucleic acid molecule are present in two different vectors.
- [0326] Embodiment 52 comprises the kit of embodiment 51, wherein the first non-naturally occurring nucleic acid molecule is present in a first plasmid DNA vector, and the second non-
- 25 naturally occurring nucleic acid molecule is present in a second plasmid DNA vector.
- [0327] Embodiment 53 comprises the kit of embodiment 52, wherein each of the first and second plasmid DNA vectors comprises an origin of replication, an antibiotic resistance gene, and from 5' end to 3' end, a promoter sequence, a regulatory sequence, a signal peptide coding sequence, the first polynucleotide sequence or the second polynucleotide sequence, and a
- 30 polyadenylation signal sequence.

[0328] Embodiment 54 comprises the kit of embodiment 53, wherein the antibiotic resistance gene is a kanamycin resistance gene having a polynucleotide sequence at least 90% identical to SEQ ID NO: 12, preferably 100% identical to SEQ ID NO: 12.

[0329] Embodiment 55 comprises the kit of embodiment 54, comprising,

- 5 (a) a first plasmid DNA vector comprising, from 3'-end to 5'-end, the promoter sequence comprising the polynucleotide sequence of SEQ ID NO: 7, the regulatory sequence comprising the polynucleotide sequence of SEQ ID NO: 8, the signal peptide coding sequence comprising the polynucleotide sequence of SEQ ID NO: 5, the first polynucleotide sequence comprising the polynucleotide sequence of SEQ ID NO: 3,
10 and the polyadenylation signal sequence comprising the polynucleotide sequence of SEQ ID NO: 11;
- (b) a second plasmid DNA vector comprising, from 3'-end to 5'-end, the promoter sequence comprising the polynucleotide sequence of SEQ ID NO: 7, the regulatory sequence comprising the polynucleotide sequence of SEQ ID NO: 8, the signal
15 peptide coding sequence comprising the polynucleotide sequence of SEQ ID NO: 5, the second polynucleotide sequence comprising the polynucleotide sequence of SEQ ID NO: 1, and the polyadenylation signal sequence comprising the polynucleotide sequence of SEQ ID NO: 11; and
- (c) a pharmaceutically acceptable carrier,
20 wherein each of the first plasmid DNA vector and the second plasmid DNA vector further comprises a kanamycin resistance gene having the polynucleotide sequence of SEQ ID NO: 12, and an origin of replication having the polynucleotide sequence of SEQ ID NO: 10, and
wherein the first plasmid DNA vector and the second plasmid DNA vector are in the
25 same composition or two different compositions.

[0330] Embodiment 56 comprises the kit of embodiment 55, wherein the kit does not contain a nucleic acid molecule encoding a HBV antigen selected from the group consisting of a Hepatitis B surface antigen (HBsAg), a HBV envelope (Env) antigen, and a HBV L protein antigen, nor a HBV antigen selected from the group consisting of a Hepatitis B surface antigen
30 (HBsAg), a HBV envelope (Env) antigen, and a HBV L protein antigen.

[0331] Embodiment 57 comprises the composition of embodiment 26 or the kit of any one of embodiments 27 to 55 for use in inducing an immune response against a hepatitis B virus (HBV) in a subject in need thereof, preferably the subject has chronic HBV infection.

[0332] Embodiment 58 comprises a combination of another immunogenic agent, preferably another HBV antigen, with the composition of embodiment 26 or the kit of any one of embodiments 27 to 55 for use in inducing an immune response against a hepatitis B virus (HBV) in a subject in need thereof, preferably the subject has chronic HBV infection.

[0333] Embodiment 59 comprises the composition of embodiment 26 or 27 or the kit of any one of embodiments 28 to 56 for use in treating a hepatitis B virus (HBV)-induced disease in a subject in need thereof, preferably the subject has chronic HBV infection, and the HBV-induced disease is selected from the group consisting of advanced fibrosis, cirrhosis and hepatocellular carcinoma (HCC).

[0334] Embodiment 60 comprises a combination of another therapeutic agent, preferably another anti-HBV agent, with the composition of embodiment 26 or 27 or the kit of any one of embodiments 28 to 56 for use in treating a hepatitis B virus (HBV)-induced disease in a subject in need thereof, preferably the subject has chronic HBV infection, and the HBV-induced disease is selected from the group consisting of advanced fibrosis, cirrhosis and hepatocellular carcinoma (HCC).

EXAMPLES

[0335] The following examples of the application are to further illustrate the nature of the application. It should be understood that the following examples do not limit the application and the scope of the application is to be determined by the appended claims.

[0336] Example 1: Generation of HBV Core and Pol Antigen Sequences and Plasmid Optimization

[0337] T-cell epitopes on the hepatitis core protein are considered important for elimination of hepatitis B infection and hepatitis B viral proteins, such as polymerase, may serve to improve the breadth of the response. Thus, hepatitis B core and polymerase proteins were selected as antigens for the design of a therapeutic hepatitis B virus (HBV) vaccine.

[0338] Derivation of HBV Core and Polymerase Antigen Consensus Sequences

[0339] HBV pol and core antigen consensus sequences were generated based on HBV genotypes B, C, and D. Different HBV sequences were obtained from different sources and aligned separately for core and polymerase proteins. Original sequence alignments for all subtypes (A to H) were subsequently limited to HBV genotypes, B, C, and D. Consensus sequences were defined for each protein alignment in each subtype separately and in all joint BCD sequences. In variable alignment positions, the most frequent amino acid was used in the consensus sequence.

[0340] Optimization of HBV Core Antigen

[0341] The HBV core antigen consensus sequence was optimized by a deletion in the native viral protein. In particular, a deletion of thirty-four amino acids corresponding to the C-terminal highly positively charged segment was made, which is required for pre-genomic RNA encapsidation.

[0342] Optimization of the HBV Pol Antigen

[0343] The HBV pol antigen consensus sequence was optimized by changing four residues to remove reverse transcriptase and RNaseH enzymatic activities. In particular, the aspartate residues (D) were changed to asparagine residues (N) in the "YXDD" motif of the reverse transcriptase domain to eliminate any coordination function, and thus nucleotide/metal ion binding. Additionally, the first aspartate residue (D) was changed to an asparagine residue (N) and the first glutamate residue (E) was changed to a glutamine residue (A) in the "DEDD" motif of the RNaseH domain to eliminate Mg^{2+} coordination. Additionally, the sequence of the HBV pol antigen was codon optimized to scramble the internal open reading frames for the envelope proteins, including the S protein and versions of the S protein with the N-terminal extensions pre-S1 and pre-S2. As a result, open reading frames for the envelope proteins (pre-S1, pre-S2, and S protein) and the X protein were removed.

[0344] Optimization of HBV Core and Pol Antigen Expression Strategies

[0345] Three different strategies were tested to obtain maximum and equal expression of both core and pol antigens from plasmid vectors: (1) fusion of HBV core and pol antigens in frame with a small AGAG inserted between the coding sequences to produce a single Core-Pol fusion protein (**FIG. 2A**); (2) expression of both core and pol antigens from one plasmid by means of a ribosomal slippage site, particularly the FA2 slippage site from foot-and-mouth disease (FMDV) to produce a bicistronic expression vector expressing individual core and pol

proteins from a single mRNA (**FIG. 2B**); and (3) two separate plasmids encoding for HBV core and pol antigens, respectively (**FIG. 2C**).

[0346] *In vitro* Expression Analysis

[0347] The coding sequences of consensus HBV core and pol antigens according to each of the above three expression strategies were cloned into the commercially available expression plasmid pcDNA3.1. HEK-293T cells were transfected with the vectors and protein expression was evaluated by Western blot using a HBV core-specific antigen.

[0348] Optimization of Post-Transcriptional Regulatory Elements

[0349] Four different post-transcriptional regulatory elements were evaluated for enhancement of protein expression by stabilizing the primary transcript, facilitating its nuclear export, and/or improving transcriptional-translational coupling: (1) Woodchuck HBV post-transcriptional regulatory element (WPRE) (GenBank: J04514.1); (2) intron/exon sequence derived from human apolipoprotein A1 precursor (GenBank: X01038.1); (3) untranslated R-U5 domain of the human T-cell leukemia virus type 1 (HTLV-1) long terminal repeat (LTR) (GenBank: KM023768.1); and (4) composite sequence of the HTLV-1 LTR, synthetic rabbit β -globin intron (GenBank: V00882.1), and a splicing enhancer (triple composite sequence). The enhancer sequences were introduced between a CMV promoter and the HBV antigen coding sequences in the plasmids. No significant difference was observed by Western blot in the expression of the core antigen when expressed from a plasmid in the presence and absence of the WPRE element (**FIG. 2D**). However, when core antigen expression in HEK293T transfected cells from plasmids having the other three post-transcriptional regulatory sequences was evaluated by Western blot, the triple enhancer sequence resulted in the strongest core antigen expression (**FIG. 2E**).

[0350] Selection of Signal Peptide for Efficient Protein Secretion

[0351] Three different signal peptides introduced in frame at the N-terminus of the HBV core antigen were evaluated: (1) Ig heavy chain gamma signal peptide SPIgG (BAA75024.1); (2) the Ig heavy chain epsilon signal peptide SPIgE (AAB59424.1); and (3) the Cystatin S precursor signal peptide SPCS (NP_0018901.1). Signal peptide cleavage sites were optimized *in silico* for core fusion using the Signal P prediction program. Secretion efficiency was determined by analyzing core protein levels in the supernatant. Western blot analysis of core antigen secretion

using the three different signal peptides fused at the N-terminus demonstrated that the Cystatin S signal peptide resulted in the most efficient protein secretion (**FIG. 2F**).

[0352] DNA Vaccine Vector Optimization

[0353] The optimized expression cassettes containing the triple composite enhancer sequence upstream of the HBV antigen coding sequence with an N-terminal Cystatin S signal peptide sequence were cloned in the DNA vaccine vector pVax-1 (Life Technologies, Thermo Fisher Scientific, Waltham, MA). The expression cassette in pVax-1 contains a human CMV-IE promoter followed by the bovine growth hormone (BGH)-derived polyadenylation sequence (pA). Bacterial propagation is driven by the pUC ori replicon and kanamycin resistance gene (Kan R) driven by a small eukaryotic promoter. The pUC ori replication, KanR expression cassette, and expression cassette driven by the CMV-IE promoter are all in the same orientation within the plasmid backbone. However, a marked reduction in core antigen expression was observed in the pVax-1 vector as compared to the expression level observed in the pcDNA3.1 vector.

[0354] Several strategies were employed to improve protein expression: (1) reversal of the entire pUCori-KanR cassette into counterclockwise orientation (pVD-core); and (2) changing the codon usage of the KanR gene along with replacement of the Kan promoter with the Amp promoter from the pcDNA3.1 vector (pDK-core). Both strategies restore core antigen expression, but core antigen expression was highest with the pDK vector, which contained the codon-adjusted Kan R gene, AmpR promoter (instead of KanR promoter), and inverse orientation of the pUCori-KanR cassette.

[0355] The four different HBV core/pol antigen optimized expression cassettes as shown in **FIG. 2G** were introduced into the pDK plasmid backbone to test each of the three expression strategies illustrated in **FIGS. 2A-2C**. The plasmids were tested *in vitro* for core and pol antigen expression by Western blot analysis using core and pol specific antibodies. The most consistent expression profile for cellular and secreted core and pol antigens was achieved when the core and pol antigens were encoded by separate vectors, namely the individual DNA vectors pDK-core and pDK-pol (**FIG. 2H**). A schematic representation of the pDK-pol and pDK-core vectors is shown in **FIGS. 3A and 3B**, respectively.

[0356] **Example 2: Generation of Adenoviral Vectors Expressing a Fusion of Truncated HBV Core Antigen with HBV Pol Antigen**

[0357] The creation of an adenovirus vector has been designed as a fusion protein expressed from a single open reading frame. Additional configurations for the expression of the two proteins, e.g. using two separate expression cassettes, or using a 2A-like sequence to separate the two sequences, can also be envisaged.

5 [0358] Design of expression cassettes for adenoviral vectors

[0359] The expression cassettes (diagrammed in **FIG. 8 A and FIG. 8B**) are comprised of the CMV promoter (SEQ ID NO: 17), an intron (SEQ ID NO: 23) (a fragment derived from the human ApoAI gene - GenBank accession X01038 base pairs 295 – 523, harboring the ApoAI second intron), followed by the optimized coding sequence – either core alone or the core and
10 polymerase fusion protein preceded by a human immunoglobulin secretion signal coding sequence (SEQ ID NO: 18), and followed by the SV40 polyadenylation signal (SEQ ID NO: 24).

[0360] A secretion signal was included because of past experience showing improvement in the manufacturability of some adenoviral vectors harboring secreted transgenes, without influencing the elicited T-cell response (mouse experiments).

15 [0361] The last two residues of the Core protein (VV) and the first two residues of the Polymerase protein (MP) if fused results in a junction sequence (VVMPP) that is present on the human dopamine receptor protein (D3 isoform), along with flanking homologies.

[0362] The interjection of an AGAG linker between the core and the polymerase sequences eliminates this homology and returned no further hits in a Blast of the human proteome.

20 [0363] **Example 3: *In Vivo* Immunogenicity Study of DNA Vaccine in Mice**

[0364] An immunotherapeutic DNA vaccine containing DNA plasmids encoding an HBV core antigen or HBV polymerase antigen was tested in mice. The purpose of the study was designed to detect T-cell responses induced by the vaccine after intramuscular delivery via electroporation into BALB/c mice. Initial immunogenicity studies focused on determining the
25 cellular immune responses that would be elicited by the introduced HBV antigens.

[0365] In particular, the plasmids tested included a pDK-Pol plasmid and pDK-Core plasmid, as shown in **FIGS. 3A and 3B**, respectively, and as described above in Example 1. The pDK-Pol plasmid encoded a polymerase antigen having the amino acid sequence of SEQ ID NO: 4, and the pDK-Core plasmid encoding a Core antigen having the amino acid sequence of SEQ
30 ID NO: 2. First, T-cell responses induced by each plasmid individually were tested. The DNA plasmid (pDNA) vaccine was intramuscularly delivered via electroporation to Balb/c mice using

a commercially available TriGridTM delivery system-intramuscular (TDS-IM) adapted for application in the mouse model in cranialis tibialis. See International Patent Application Publication WO2017172838, and U.S. Patent Application entitled “Method and Apparatus for the Delivery of Hepatitis B Virus (HBV) Vaccines,” filed on the same day as this application

5 with the Attorney Docket Number 688097-405U1 for additional description on methods and devices for intramuscular delivery of DNA to mice by electroporation, the disclosures of which are hereby incorporated by reference in their entireties. In particular, the TDS-IM array of a TDS-IM v1.0 device having an electrode array with a 2.5 mm spacing between the electrodes and an electrode diameter of 0.030 inch was inserted percutaneously into the selected muscle,

10 with a conductive length of 3.2 mm and an effective penetration depth of 3.2 mm, and with the major axis of the diamond configuration of the electrodes oriented in parallel with the muscle fibers. Following electrode insertion, the injection was initiated to distribute DNA (e.g., 0.020 ml) in the muscle. Following completion of the IM injection, a 250 V/cm electrical field (applied voltage of 59.4 -65.6 V, applied current limits of less than 4 A, 0.16 A/sec) was locally applied

15 for a total duration of about 400 ms at a 10% duty cycle (i.e., voltage is actively applied for a total of about 40 ms of the about 400 ms duration) with 6 total pulses. Once the electroporation procedure was completed, the TriGridTM array was removed and the animals were recovered. High-dose (20 µg) administration to BALB/c mice was performed as summarized in Table 1. Six mice were administered plasmid DNA encoding the HBV core antigen (pDK-core; Group 1), six

20 mice were administered plasmid DNA encoding the HBV pol antigen (pDK-pol; Group 2), and two mice received empty vector as the negative control. Animals received two DNA immunizations two weeks apart and splenocytes were collected one week after the last immunization.

[0366] **Table 1: Mouse immunization experimental design of the pilot study.**

Group	N	pDNA	Unilateral Admin Site (alternate sides)	Dose	Vol	Admin Days	Endpoint (spleen harvest) Day
1	6	Core	CT + EP	20 µg	20 µL	0, 14	21
2	6	Pol	CT + EP	20 µg	20 µL	0, 14	21
3	2	Empty Vector (neg control)	CT + EP	20 µg	20 µL	0, 14	21

25 CT, cranialis tibialis muscle; EP, electroporation.

[0367] Antigen-specific responses were analyzed and quantified by IFN- γ enzyme-linked immunospot (ELISPOT). In this assay, isolated splenocytes of immunized animals were incubated overnight with peptide pools covering the Core protein, the Pol protein, or the small peptide leader and junction sequence (2 μ g/ml of each peptide). These pools consisted of 15 mer peptides that overlap by 11 residues matching the Genotypes BCD consensus sequence of the Core and Pol vaccine vectors. The large 94 kDa HBV Pol protein was split in the middle into two peptide pools. Antigen-specific T cells were stimulated with the homologous peptide pools and IFN- γ -positive T cells were assessed using the ELISPOT assay. IFN- γ release by a single antigen-specific T cell was visualized by appropriate antibodies and subsequent chromogenic detection as a colored spot on the microplate referred to as spot-forming cell (SFC).

[0368] Substantial T-cell responses against HBV Core were achieved in mice immunized with the DNA vaccine plasmid pDK-Core (Group 1) reaching 1,000 SFCs per 10^6 cells (**FIG. 4**). Pol T-cell responses towards the Pol 1 peptide pool were strong (~1,000 SFCs per 10^6 cells). The weak Pol-2-directed anti-Pol cellular responses were likely due to the limited MHC diversity in mice, a phenomenon called T-cell immunodominance defined as unequal recognition of different epitopes from one antigen. A confirmatory study was performed confirming the results obtained in this study (data not shown).

[0369] The above results demonstrate that vaccination with a DNA plasmid vaccine encoding HBV antigens induces cellular immune responses against the administered HBV antigens.

[0370] Example 4: Dose-finding study of combined pDK-Core / pDK-Pol plasmids in Mice

[0371] The purpose of this dose-finding study with combined plasmids was to evaluate the immune responses in mice of a mixture of DNA plasmid (pDNA) vectors encoding HBV core and pol antigens applied in one site using different DNA doses. In this study, an immunotherapeutic DNA vaccine containing a 1:1 (w/v) mixture of two plasmids, the pDK-pol and pDK-core plasmids described in Example 1, was tested in mice. The DNA vaccine was delivered to Balb/c mice in one anatomic site intramuscularly via electroporation as described above in Example 3. Vaccination of the combined *Core*- and *Pol*-expressing plasmids at 10 μ g, 1 μ g, and 0.1 μ g DNA of each plasmid per site was performed as summarized in Table 2. Eight

mice were tested in each group, and two mice were administered empty vector as the negative control. Animals received two DNA immunizations three weeks apart and splenocytes were collected one week after the last immunization.

[0372] Table 2: Mouse immunization experimental design of the dose-finding study with combined plasmids.

Group	N	pDNA	Unilateral admin site (alternate sides)	Dose of each pDNA per site	Dose total pDNA per site	Admin days	Endpoint (spleen harvest) Day
1	8	Core and Pol	CT + EP	10 µg	20 µg	0, 21	28
2	8	Core and Pol	CT + EP	1 µg	2 µg	0, 21	28
3	8	Core and Pol	CT + EP	0.1 µg	0.2 µg	0, 21	28
4	2	Empty Vector (neg. control)	CT + EP	20 µg	20 µg	0, 21	28

pDNA, plasmid DNA; CT, cranialis tibialis muscle; EP, electroporation.

[0373] Antigen-specific responses were analyzed and quantified by IFN- γ enzyme-linked immunospot (ELISPOT) as described in Example 1. Considerable T-cell responses against Core and Pol were achieved in BALB/c mice immunized with the combined DNA vaccine consisting of plasmid pDK-Core and pDK-Pol (**FIG. 5**). There was no statistical difference in terms of the magnitude of immune responses between Group 1, immunized with 10 µg of each plasmid, and Group 2, immunized with only 1 µg of each plasmid. This result suggested that T-cell responses reached a maximum level at around 1 µg Core- and Pol-antigen-expressing plasmids. However, at 10-fold lower DNA exposure, i.e., at 0.1 µg of each plasmid, a significant decrease in SFCs was observed. Pol T-cell responses towards the Pol 1 peptide pool were dominant. The weak Pol-2-directed anti-Pol cellular responses were likely due to the limited MHC diversity in inbred mice, a phenomenon called T-cell immunodominance defined as unequal recognition of different epitopes from one antigen.

[0374] The above results demonstrate that in mice immunized with a combination of DNA plasmids expressing HBV core and pol antigens, considerable T-cell responses were found at doses of 1 µg of each plasmid, and some immune response was still observed at a dose 0.1 µg per plasmid.

[0375] Example 5: Immune Interference Study in Mice

[0376] For practical reasons, it would be desirable to develop the combination HBV core and pol antigen DNA vaccine as a combined (mixed) vector formulation. However, immunodominance might occur with multivalent vaccines and immune responses against subdominant antigens could be blunted. Therefore, immune interference, i.e., decreased Core- and/or Pol-specific cellular responses from administration of a combination of the two antigen-expression plasmids mixed together when compared to immunization of either vector in different anatomic sites, was assessed.

[0377] Balb/c mice were vaccinated with the pDK-core and/or pDK-pol DNA plasmids intramuscularly via electroporation as described in Example 3. The DNA plasmids (pDNA) were administered at a dose of 5 µg per site applied either individually, combined (mixed) at one site, or combined in separate sites, as summarized in Table 3. Animals received two DNA immunizations three weeks apart and splenocytes were collected one week after the last immunization.

[0378] Table 3: Mouse immunization experimental design of the immune interference study.

Group	N	pDNA	Unilateral admin site (alternate sides)	Dose each pDNA per site	Dose total pDNA per site	Admin days	Endpoint (spleen harvest) Day
1	6	Core	Bilateral CT	5 µg	10 µg	0, 21	28
2	6	Pol	Bilateral CT	5 µg	10 µg	0, 21	28
3	6	Core and Pol <i>mixed</i>	Bilateral CT	10 µg	20 µg	0, 21	28
4	6	Core and Pol <i>individual</i>	Core in left CT Pol in right CT	10 µg	20 µg	0, 21	28
5	2	Empty Vector (neg. control)	Bilateral CT	10 µg	20 µg	0, 21	28

CT, cranialis tibialis muscle.

[0379] Antigen-specific responses were analyzed and quantified by IFN-γ enzyme-linked immunospot (ELISPOT) as described in Example 1. Strong Core- and Pol-specific antigen responses were confirmed in BALB/c mice in this experiment (**FIG. 6**). No significant immune interference was observed based on the substantially identical T-cell responses obtained for Group 3, in which both plasmids were mixed and applied in the same site, and Group 4, in which

pDNA expressing core and pol antigens were individually electroporated in two different sites. One animal in Group 1 showed a low abnormal Pol-2-pool-directed response. The same experiment was repeated in C57/Bl6 mice with comparable results.

[0380] The above results demonstrate that substantially no immune interference was observed when combining the two HBV antigen-expression plasmids pDK-Core and pDK-Pol.

[0381] **Example 6: Evaluation of the Efficacy of a DNA Vaccine in Non-Human Primates**

[0382] The purpose of this study was to evaluate the efficacy of a therapeutic HBV DNA vaccine delivered intramuscularly with electroporation, and to induce and measure a HBV-specific T cell response/cell activation in Cynomolgus monkeys (*Macaca fascicularis*).

[0383] Vaccine

[0384] The vaccine used in this study was a combination of two separate DNA plasmids encoding an HBV core antigen and HBV polymerase antigen, respectively. In particular, the DNA plasmids were pDK-Pol plasmid (encoding an HBV polymerase antigen having the amino acid sequence of SEQ ID NO: 4) and pDK-Core plasmid (encoding an HBV core antigen having the amino acid sequence of SEQ ID NO: 2), as shown in **FIGS. 3A and 3B**, respectively, and described in Example 1.

[0385] The DNA plasmids were administered in a 1:1 (w/w) mixture of both plasmids delivered in one anatomic site. Non-human Primates (NHP) were electroporated with a TriGrid™ delivery system-intramuscular (TDS-IM) adapted for application in the NHP model. See International Patent Application Publication WO2017172838, and U.S. Patent Application entitled "Method and Apparatus for the Delivery of Hepatitis B Virus (HBV) Vaccines," filed on the same day as this application with the Attorney Docket Number 688097-405U1 for additional description on methods and devices for intramuscular delivery of DNA to NHP by

electroporation, the disclosures of which are hereby incorporated by reference in their entireties. In particular, the TDS-IM array of a TDS-IM v1.0 or TDS-IM v2.0 having an electrode array with a 6.0 mm spacing between the electrodes and an electrode diameter of 0.021 or 0.023 inch, respectively, was inserted percutaneously into the selected muscle with the major axis of the diamond configuration of the electrodes oriented in parallel with the muscle fibers. The conductive length was 5.0 mm for TDS-IM v1.0 or TDS-IM v2.0, while the effective penetration depth was 15.5 mm for TDS-IM v1.0 and 9.0 mm for TDS-IM v2.0. Following electrode

insertion, the injection was initiated to distribute DNA (e.g., 1.0 ml) in the muscle. Following completion of the IM injection, a 250 V/cm electrical field (applied voltage of 142.4 – 157.6 V, applied current limits of 0.6 - 4 A, 0.16 A/sec) was locally applied for a total duration of about 400 ms at a 10% duty cycle (i.e., voltage is actively applied for a total of about 40 ms of the about 400 ms duration) with 6 total pulses. Once the electroporation procedure was completed, the TriGrid™ array was removed and the animals were recovered. The initial immunogenicity study focused on determining the cellular immune responses that would be elicited by the introduced HBV antigens.

[0386] Non-human primates

10 **[0387]** Cynomolgus macaques (n=30) were sourced from China (Covance Research Products Inc. USA), at 2.5 to 5 years of age and weighing 3.0 to 5.0 kg at start of study. They were socially housed in enriched environment according to veterinary guidelines and National Research Council, *Guide for the Care and Use of Laboratory Animals*, 8th Edition, Washington DC: National Academies Press (2011). Animals were acclimatized for a period of 2 weeks before starting the study. Monkeys were anesthetized with ketamine prior to each plasmid electroporation administration. Blood was collected 2 weeks after each immunization in vials containing sodium heparin. PBMCs were isolated using ficoll gradient and stored in liquid nitrogen tanks until analysis.

[0388] Intramuscular/Electroporation Administration in the Non-Human Primates

20 **[0389]** Plasmid administration was performed three times (group 1) at days 0, 36 and 62, as summarized in Table 4. pDK-Core (1.0 mg) and pDK-Pol (1.0 mg) were administered via electroporation with the delivery system set to 19 mm (short) injection depth in the quadriceps (*vastus lateralis*) muscle. For each injection, an alternate leg muscle was administered. The syringe containing DNA plasmid was equipped with an injection depth limiter suitable for NHP quadriceps muscle, for an injection target depth of about 10 mm into the muscle, with the major axis of the diamond configuration oriented in parallel with the muscle fibers. Immediately after the IM injection was completed, the electroporation apparatus was activated, resulting in the electrical stimulation of the muscle at an amplitude of up to 250 V per cm of electrode spacing for a total of up to 40 mS duration over a 400 mS interval. Samples were collected on days 0, 14, 50, and 76, and analyzed by ELISPOT and intracellular cytokine staining.

[0390] **Table 4:** NHP vaccination experimental design

Group	N	pDNA	Unilateral admin site (alternate sides)	Dose each pDNA per site	Dose total pDNA per site	Admin days	Sample days
1	5	pDK-Core pDK-Pol	& CT + EP	1.0 mg	2.0 mg	0, 36 and 62	0, 14, 50 and 76

[0391] ELISPOT Analysis

[0392] Antigen-specific responses were analyzed and quantified by IFN- γ enzyme-linked immunospot (ELISPOT) using Primate IFN- γ ELISpot kit (R&D Systems, USA, Cat No. EL961). In this assay, isolated PBMCs of immunized animals were incubated in triplicate wells overnight with peptide pools (2 μ g/ml) covering the Core protein and the Pol protein. These pools consist of 15 mer peptides that overlap by 11 residues matching the Genotypes ABCD consensus sequence of the Core and Pol vaccine vectors. The peptides were synthesized at 90% purity (JPT, Germany). The large 94 kDa HBV Pol protein was split in the middle into two peptide pools. IFN- γ release by a single antigen-specific T cell was visualized by appropriate antibodies and subsequent chromogenic detection as a colored spot on the microplate referred to as spot-forming cell (SFC). The results are shown in **FIG. 7A**.

[0393] Intracellular Cytokine Staining (ICS)

[0394] Intracellular cytokine staining (ICS) was used to study the vaccine-induced T-cell responses. Frozen PBMCs were thawed and rested overnight in 10% FBS, RPMI medium before stimulation with vaccine-insert matched Core, Pol-1 or Pol-2 peptide pools (2 μ g/ μ l), DMSO or Leukocyte Activation cocktail for 6 hours in 10% FBS, RPMI medium containing Golgiplug Protein Transport Inhibitor (1 μ g/ μ l). Stimulated cells were stained with fixable viability dye eFluor 780 (65-0865-14, eBioscience), and treated for 20 minutes with Fixation/Permeabilization solution (554714, BD Biosciences) before staining for 30 minutes with intracellular stain mix as shown in Table 5 below. Stained cells were acquired using Fortessa flowcytometer with the appropriate single color compensation controls. Response magnitudes were reported as the percentage of CD4⁺ or CD8⁺ T cells expressing IFN- γ , TNF- α or IL-2 after stimulation. The results are shown in **FIG. 7B**.

[0395] **Table 5:** Antibody panel used for intracellular cytokine staining assay

BD Biosciences			
Cat no	Antibody	Fluorescence	Clone

557705	CD3	Alexa fluor 488	SP34
563823	CD8	BUV786	RPA-T8
564107	CD4	BUV395	L200
554701	IFN γ	PE	B27
554514	TNF	APC	MAb11
564164	IL-2	BV421	MQ1-17H12

[0396] Results

[0397] ELISPOT data (**FIG. 7A**) showed strong Core and Pol-2 responses after two immunizations. A third immunization greatly increased the IFN- γ magnitude. The Pol-1 peptide pool elicited an intermediate response that was also improved with a third immunization, although not as greatly improved as with Core and Pol-2. Day 76 data includes only the results from four NHPs, as blood draw from the fifth monkey was not successful. The high variation within each group is due to the NHPs being sourced from an outbred stock, and genetic diversity could account for the differing immune response.

[0398] The ICS assay data (**FIG. 7B**) showed that cytokine response from HBV peptide stimulation is CD8 driven and is specific to the Core and Pol-2 peptide pools, as previously observed with ELISPOT. The responding NHPs in the ICS assay are the same responding individuals as with the ELISPOT assay. Although a few individual ICS responses do not show positive as seen in the ELISPOT data, this may be attributed to the higher sensitivity of the ELISPOT assay.

[0399] Conclusion

[0400] The above results demonstrate that in NHPs immunized with a combination of pDK-Core and pDK-Pol vaccine by intramuscular injection via electroporation, considerable T-cell responses were found at doses of 1.0 mg of each plasmid, with peptide specific responses detected after two immunizations and even greater responses after three immunizations. At Day 76, ELISPOT assay results showed that peptide pools Core, Pol-1 and Pol-2 induced positive IFN- γ T cell responses in every tested NHP (4/5 NHP). The ICS assay on PBMCs from immunized NHPs show that the HBV peptide specific response is CD8 driven, with the highest responses against Core and Pol-2 peptide pools.

[0401] **Example 7: Evaluation of the Efficacy of a DNA Vaccine in Human Subjects**

[0402] The efficacy of a therapeutic HBV DNA vaccine delivered intramuscularly with electroporation is evaluated in human subjects.

[0403] Human Subjects

[0404] The human subjects are adult patients having chronic HBV infection that are HBsAg-positive. The human subjects are being treated with an HBV polymerase inhibitor (entecavir or tenofovir).

[0405] Vaccine

[0406] Human patients are administered a combination of two separate DNA plasmids encoding an HBV core antigen and HBV polymerase antigen, respectively. In particular, the DNA plasmids were pDK-Pol plasmid (encoding an HBV polymerase antigen having the amino acid sequence of SEQ ID NO: 4) and pDK-Core plasmid (encoding an HBV core antigen having the amino acid sequence of SEQ ID NO: 2), as shown in **FIGS. 3A and 3B**, respectively, and described in Example 1. The DNA plasmids are administered in a 1:1 mixture of both plasmids at different dosages, particularly dosages of 0.25 mg, 1 mg, and 6 mg of total plasmid according to a randomized, placebo-controlled escalating dose trial.

[0407] Intramuscular/Electroporation Administration in the Human Subjects

[0408] The DNA plasmids are administered to the human subjects by electroporation in 2 to 3 intramuscular immunizations using a TriGridTM delivery system-intramuscular (TDS-IM) adapted for application in humans. Some patients are administered placebo (i.e., plasmids lacking the coding sequences for HBV antigens) as control. A TriGridTM delivery system-intramuscular (TDS-IM) adapted for application in the human is used for the delivery of the plasmid DNA by electroporation. See International Patent Application Publication WO2017172838, and U.S. Patent Application entitled "Method and Apparatus for the Delivery of Hepatitis B Virus (HBV) Vaccines," filed on the same day as this application with the Attorney Docket Number 688097-405U1 for additional description on methods and devices for intramuscular delivery of DNA to humans by electroporation, the disclosures of which are hereby incorporated by reference in their entireties. For example, the TDS-IM array of TDS-IM v2.0 having an electrode array with a 6.0 mm spacing between the electrodes and an electrode diameter of 0.023 inch, respectively, can be inserted percutaneously into the selected muscle with the major axis of the diamond configuration of the electrodes oriented in parallel with the muscle fibers. The conductive length can be 5.0 mm, while the effective penetration depth can

be 19 mm. Following electrode insertion, the injection is initiated to distribute DNA (e.g., 1.0 ml) in the muscle. Following completion of the IM injection, a 250 V/cm electrical field (applied voltage of 142.4 – 157.6 V, applied current limits of 0.6 - 4 A, 0.16 A/sec) is locally applied for a total duration of about 400 ms at a 10% duty cycle (i.e., voltage is actively applied for a total of about 40 ms of the about 400 ms duration) with 6 total pulses. Once the electroporation procedure is completed, the TriGrid™ array is removed and the human subject is recovered.

[0409] Blood samples are collected from the patients at various time points post-immunization. The development of HBsAg levels post immunization, particularly for levels consistent with evolution to clinical seroconversion are evaluated in the patients 3 to 6 months post-immunization. The persistent loss of HBsAg and a decrease in clinical disease (e.g., cirrhosis, hepatocellular carcinoma) are evaluated in the patients 6 to 12 months post-immunization.

[0410] **Example 8: *In Vivo* Immunogenicity Study of Adenoviral Vectors in Mice**

[0411] An immunotherapeutic vaccine containing adenoviral vectors encoding an HBV core antigen or an HBV polymerase antigen was tested in mice. The purpose of the study was to detect T-cell responses induced by the vaccine after intramuscular delivery into F1 mice (C57BL/6 x Balb/C). Initial immunogenicity studies focused on determining the cellular immune responses that would be elicited by the introduced HBV antigens. In particular, the adenovectors tested contained the expression cassettes as shown in FIGS. 8A and 8B.

[0412] *In vivo* Immunogenicity Study

[0413] To evaluate the *in vivo* immunogenicity of the adenoviral vaccine, HBV adenoviral vectors were administered intramuscularly into F1 mice. These immunogenicity studies focused on determining the cellular immune responses elicited by the HBV antigens Core and Polymerase. The administration to F1 mice was performed as summarized in Table 6. Animals received one HBV adenoviral vector immunization. Splenocytes were collected nine weeks later.

[0414] **Table 6:** Experimental Design for Mouse Immunization with Adenoviral Vectors

Group	N	Prime Day 0	R	Dose (vp)	Endpt Day
1	4	Core Pol fusion + Core	IM	10 ⁸	63
2	4	Core Pol fusion + Core	IM	10 ⁹	63
3	4	Core Pol fusion + Core	IM	10 ¹⁰	63
7	4	Core Pol fusion	IM	10 ⁸	63
8	4	Core Pol fusion	IM	10 ⁹	63
9	4	Core Pol fusion	IM	10 ¹⁰	63

IM: intramuscular; vp: viral particles;

[0415] Evaluation of immunogenicity of HBV adenoviral vectors

[0416] Antigen-specific responses were analyzed and quantified by IFN- γ enzyme-linked immunospot (ELISPOT). In this assay, isolated splenocytes of immunized animals were
5 incubated with peptide pools covering the Core and the Pol protein (2 μ g/ml of each peptide). The pools consist of 15-mer peptides that overlap by 11 residues matching the genotypes ABCD consensus sequences of the Core and Pol adenoviral vectors. The large 94 kDa HBV Pol protein was split in the middle into two peptide pools. In ELISPOT, IFN- γ release by a single antigen-specific T-cell was visualized by appropriate antibodies and subsequent chromogenic detection
10 as a colored spot on the microplate referred to as spot-forming cell (SFC).

[0417] The results are shown in **FIG. 9**. From the results, it can be seen that HBV adenoviral vectors, especially the combination of Core Pol fusion and Core adenovectors gave rise to Core and Pol specific T cell responses. These data indicate that adenoviral vectors encoding HBV core and pol antigens give rise to robust T cell responses against core and pol in
15 F1 mice.

[0418] It is understood that the examples and embodiments described herein are for illustrative purposes only, and that changes could be made to the embodiments described above without departing from the broad inventive concept thereof. It is understood, therefore, that this invention is not limited to the particular embodiments disclosed, but it is intended to cover
20 modifications within the spirit and scope of the invention as defined by the appended claims.

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