

HEPATITIS B VIRUS VACCINES

STATEMENT OF GOVERNMENTAL INTEREST

[0001] The United States government has certain rights in this invention pursuant to Contract No. 89233218CNA000001 between the United States Department of Energy and TRIAD National Security, LLC for the operation of Los Alamos National Laboratory.

STATEMENT REGARDING SEQUENCE LISTING

[0001] The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is 930185_414WO_SEQUENCE_LISTING.txt. The text file is 186 KB, was created on August 21, 2020, and is being submitted electronically via EFS-Web.

BACKGROUND

Field of the Invention

[0002] The present subject matter relates, in general, to Hepatitis B virus (HBV) and, in particular, to HBV vaccines.

Description of the Related Art

[0003] Hepatitis B virus (HBV) infection represents a major global health burden. HBV infection results in chronic liver disease in 5 – 10% of infected adults, while the rate is inverted for perinatal transmission with >90% progressing to chronic disease. Untreated chronic hepatitis B (CHB) infection frequently progresses to necrotic inflammation and ongoing liver damage leading to cirrhosis and hepatocellular carcinoma (HCC). CHB is estimated to increase the risk of developing HCC by 20-fold and accounts for about 54% of HCC cases. HCC is the third most lethal form of cancer with about 800,000 new cases diagnosed annually. Highly effective (>95%) prophylactic vaccines were implemented in the early 1980's; however these HBV

vaccines are inefficient once infection is established. Despite the wide use of prophylactic vaccines, there are 240-340 million chronic HBV carriers and over 780,000 related deaths per year worldwide. The vast majority of new HBV infections occur in highly endemic regions, such as China, Southeast Asia, and sub-Saharan Africa. HBV infection occurs through sexual, nosocomial, or blood-borne transmission.

[0005] The HBV genome is a 3.2 kB double-stranded DNA molecule that is organized into four overlapping open reading frames: a polymerase (P, or Pol), a Core (C), a surface antigen (HBsAg) (S), and a gene called "X," whose function is not fully understood but which has been implicated in development of liver cancer. Locarnini *et al. Antivir Ther.* 2010;15 Suppl 3:3-14. And, unlike other small chronic viruses such as HIV or HCV that display high mutation rates resulting in quasispecies, the small (3.2 kb) DNA genome of HBV is more constrained in its ability to mutate due to overlapping open reading frames (ORFs). While there is limited intra-host mutation, distinct genotypes of HBV are found globally that need to be taken into account when designing treatments, such as a vaccine.

[0006] The current standard of care for chronic HBV infection is treatment with antivirals and interferon- α .

[0007] To date, therapeutic vaccinations for HBV have been ineffective. There are a number of potential reasons. Identifying the HBV sequence that infects an individual is useful in determining which specific vaccines will provide efficacious treatment. An HBV database called "HBVdb," developed as a collaborative consortium that sequences and examines HBV genomes, has identified about 5000 HBV complete genomes. See Hayer *et al. Nucleic Acids Res.*, 41:D566-D570 2013, the entirety of which is herein incorporated by reference. However, because of general overlap of sequences, and because multiple sequences were isolated from the same patient or from highly tight clusters, the number of truly unique total HBV complete genomes that HBVdb has identified is roughly 3000. The HBV genomes identified by HBVdb can be categorized into different genotypes (A, B, C, CB, D, DC, DE, E, and F). The distribution of genotypes varies throughout the world population. For example, nearly

75% of the dataset sequences available on HBVdb in samples of individuals from Asia and in particular China comprise genotypes B and C. On the other hand, more than half of HBV infections in Europe represent infections by genotypes A and D.

[0008] While treatments with antivirals and interferon- α inhibit viral replication and stimulate the innate immune system, they rarely clear the virus (1-4% per year) and patients often require life-long treatment. Moreover, individual responses to these therapies vary as the disease progresses, and prolonged treatments can result in both resistance mutations and a wide spectrum of side effects. The most effective direct antiviral therapies, tenofovir and entecavir, are expensive, and are only used in a subset of patients using expert guidelines and algorithms. As therapies fail or are not used, liver inflammation becomes chronic, and the damage and regeneration cycle can lead to fibrosis, abnormal liver architecture, and possibly HCC. Thus, there is an urgent need for an effective immunotherapy that mounts an effective immune response leading to elimination of HBV and ultimate cure.

[0009] A minority of individuals with chronic HBV will have spontaneous clearance of their infection, as documented by loss of measured surface antigen in the blood. The rate is as low as 1% per year and is minimally improved with direct acting antiviral agents. The most effective clearance occurs in individuals with a low quantitative level of circulating surface antigens who undergo treatment with type 1 interferons, which have severe side effects and are often poorly tolerated.

[0010] Therapeutic vaccination has also been in part ineffective, as the T cells that are needed to clear infection have become exhausted or tolerized and do not lead to effective clearance of HBV in the liver. Thus there is a need to elicit T cells that are not induced by natural infection, but that still recognize peptide sequences displayed on the surface of infected hepatocytes.

[0011] CMV/HBV vaccines offer an avenue to mount an effective response leading to elimination of HBV and ultimate cure. The induction of active liver resident effector CD8⁺ T cell (TEM) responses is crucial for HBV clearance. Thus, the induction of sustained, effector HBV-specific CD8⁺ T cells recognizing novel epitopes in the liver of CHB patients should suffice to control and eventually eliminate HBV.

[0012] Therefore, there remains a need in the art to identify effective vaccinations for people infected with different genotypes of HBV. And, because of the ineffectiveness of currently available vaccines against chronic HBV infections, there is a need in the art to develop a vaccine that could be used to treat such infections. There
5 also remains a need to design, manufacture, and test therapeutic HBV vaccines in preparation for clinical testing. The compositions and methods disclosed herein address these needs.

BRIEF SUMMARY

[0013] In certain aspects, the present disclosure provides a polypeptide
10 comprising the amino acid sequence as set forth in SEQ ID NOs:1-11 or SEQ ID NOs:14-36. In some embodiments, the polypeptide comprises two or more amino acid sequences as set forth in SEQ ID NOs:16-36. In some embodiments, the aforementioned polypeptides, or polynucleotides encoding the polypeptides, may be used in an HBV vaccine. In some embodiments, the present disclosure provides an
15 immunogenic composition comprising a polypeptide comprising the amino acid sequence as set forth in SEQ ID NOs:1-11 or SEQ ID NOs:14-36. In still further embodiments, the present disclosure provides for the use of the aforementioned polypeptides or immunogenic compositions comprising the polypeptides generating an immune response to HBV, or treating or preventing an HBV infection.

20 [0014] In some embodiments, the present disclosure provides a viral vector comprising a cytomegalovirus (CMV) vector comprising a polynucleotide comprising the sequence encoding one or more amino acid sequences as set forth in SEQ ID NOs:1-36. In some embodiments, the polynucleotide encodes one or more amino acid sequences that comprise one or more of SEQ ID NOs:1-11, SEQ ID NOs:14-15, and
25 SEQ ID NOs:24-26. In some embodiments, the sequences encoded by the polynucleotide are ordered for improved expression. In some embodiments, the present disclosure provides an immunogenic composition comprising a CMV vector that encodes a polynucleotide comprising the sequence encoding one or more amino acid sequences as set forth in SEQ ID NOs:1-36. In still further embodiments, the present

disclosure provides for the use of the aforementioned vectors or immunogenic compositions comprising the vectors in generating an immune response to HBV, or treating or preventing an HBV infection.

[0015] Also provided herein is an immunogenic composition or vaccine comprising one or more HBV episensus antigens. In some embodiments, the antigens of the vaccine are provided as polypeptides. In some embodiments the antigens are encoded by a polynucleotide. In some embodiments, a viral vector comprises the polynucleotides. In some embodiments, the antigens are encoded by two or more polynucleotides, which may be expressed by the same or different promoters. In some
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embodiments, the antigens are encoded by different viral vectors. In some embodiments, the HBV vaccine comprises two or more HBV episensus antigens. In some embodiments, the HBV vaccine comprises a cytomegalovirus (CMV) vector and a polynucleotide encoding one or more HBV episensus antigens.

[0016] In some embodiments, the vaccine is a prophylactic vaccine. In some
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embodiments, the vaccine is a therapeutic vaccine.

[0017] In some embodiments, the immunogenic composition or vaccine further comprises a pharmaceutically acceptable carrier or excipient.

[0018] In certain aspects, the present disclosure provides a composition, *e.g.*, an immunogenic composition, comprising two or more of the aforementioned
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polypeptides, polynucleotides, vectors, or vaccines.

[0019] Also provided herein are methods of treating HBV in a subject comprising administering an effective amount of the aforementioned polypeptides, vectors, vaccines, or compositions to a subject in need thereof. Further provided herein are methods of protecting a subject from an HBV infection comprising administering an
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effective amount of the aforementioned polypeptides, vectors, vaccines, or compositions to a subject in need thereof. Further provided herein are methods of generating an immune response to HBV comprising administering an effective amount of the aforementioned polypeptides, vectors, vaccines, or compositions to a subject in need thereof.

[0020] Also provided herein are some embodiments wherein the aforementioned polypeptides, vectors, vaccines, or compositions are for use in treating HBV, protecting a subject from an HBV infection, or inducing an immune response to HBV.

5 [0021] The present disclosure also provides for the use of the aforementioned polypeptides, vectors, vaccines, or compositions for the manufacture of a medicament for use in treatment of an HBV infection. The present disclosure also provides for the use of the aforementioned polypeptides, vectors, vaccines, or compositions for the manufacture of a medicament for use in protecting a subject from an HBV infection.
10 The present disclosure also provides for the use of the aforementioned polypeptides, vectors, vaccines, or compositions for the manufacture of a medicament for use in generating or inducing an immune response to HBV.

[0022] Provided herein are HBV polypeptides comprising one or more episensus antigens that have amino acid sequences derived from HBV C, S, P,
15 including full-length sequences, regions thereof, or any combination thereof.

[0023] Further provided herein are immunogenic compositions or vaccines comprising one or more pharmaceutically acceptable carriers and one or more episensus antigens. Also provided herein are immunogenic compositions or vaccines comprising a vector capable of expressing one or more episensus antigens. In some embodiments,
20 the HBV episensus antigen comprises two or more episensus sequences. In some embodiments, the HBV vaccines are prophylactic vaccines. In some embodiments, the HBV vaccines are therapeutic vaccines.

[0024] The present disclosure further provides methods of preventing or treating HBV infection in a subject comprising administering an effective amount of the
25 aforementioned immunogenic compositions or vaccines to the subject in need thereof. Further provided are methods of designing and producing an immunogenic composition or vaccine for a subject comprising sequencing HBV viruses in the subject, selecting vaccine antigens designed to cover the diversity within the viruses present in the subject, and inserting the vaccine antigens into a vector. Also provided herein are
30 methods of treating an HBV infection in a subject, comprising administering an

effective amount of the disclosed immunogenic compositions or vaccines to the subject in need thereof.

[0025] Also provided herein is a method of inducing an effector memory T cell response comprising: (a) designing one or more episensus antigens; (b) producing an immunogenic composition or vaccine comprising a CMV backbone and a polynucleotide encoding the one or more episensus antigens; and (c) administering the vaccine to a subject in need thereof.

[0026] In certain embodiments, the episensus antigens of the methods provided herein comprise one or more of the amino acid sequences selected from the group consisting of SEQ ID NOs:1-11 and SEQ ID NOs:14-36.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] **Fig. 1.** HBV Genome Organization. Unlike other small chronic viruses such as HIV or HCV that display high mutations rates resulting in a quasispecies, the small (3.2 kb) DNA genome of HBV is more constrained in its ability to mutate due to overlapping open reading frames (ORFs).

[0028] **Fig. 2A-2B.** World distribution of 3041 HBV sequences available from HBVdb. Genotypes of HBV are distributed throughout the world. Only sequences with a known sampling country are shown on the map.

[0029] **Fig. 3A-3B.** Distribution of HBV sequences in Asia and in China that are available from HBVdb. Approximately 75% of the genotypes in Asia and particularly in China are genotypes B and C.

[0030] **Fig. 4A-4B.** Fig. 4A: Whole proteome coverage of HBV using one, two, and three episensus sequences. Fig. 4A shows that single episensus sequences provide varying and limited coverage over the genome of HBV (top panel). Coverage is improved with the use of two episensus sequences (middle panel) or three episensus sequences (bottom panel). Fig. 4B: Three episensus sequences provide better coverage than one or two episensus sequences, as shown by the number of exact 9-mer matches.

[0031] **Fig. 5A-5C.** Whole proteome coverage when one, two, or three episensus sequences developed using the Global HBV population are used. Use of two

or three episensus sequences developed using the Global HBV population substantially improve coverage of all major genotypes, while providing comparable coverage of B and C, as compared to episensus sequences developed using B and C HBV populations.

[0032] **Fig. 6.** Whole proteome coverage of HBV using two episensus sequences, in relation to four conserved regions of the HBV genome. Hypervariable regions, including 29 N-terminal amino acids and the N-terminus of the P protein, are illustrated with windows. The hypervariable regions are removed from further analysis.

[0033] **Fig. 7A-7B.** Coverage of potential T cell epitopes (PTE) when one (Fig. 7A) or two (Fig. 7B) episensus sequences are used in samples from China, samples not from China, and a cohort of Global samples. X-axis indicates percentage of coverage. The grey histograms indicate the potential T cell epitopes coverage by 1000 randomly selected natural strains.

[0034] **Fig. 8.** Comparison of coverage for various episensus sequences. The 2_ChinaGlobal_Epi (2_CHGL_epi) sequences provides 0.809 (80.9%) exact full sequence coverage of Global samples. The 2_CHGL_epi sequence provides 0.879 (87.9%) exact full sequence coverage in samples from China and 0.772 (77.2%) exact full sequence coverage in samples not from China.

[0035] **Fig. 9A-9B.** Comparison of conserved region coverage of 1_D.vac, 1_C.ref, 1_CH_epi, 1_GL_epi, 2_CH_epi, 2_CHGL_epi, and 3_GL_epi in samples from China (Fig. 9A, left), samples not from China (Fig. 9A, middle), and a cohort of Global samples (Fig. 9A, right). Fig. 9B depicts conserved region coverage of 1_D.vac, 1_GL_epi, and 2_CHGL_epi in all major genotypes.

[0036] **Fig. 10A-10B.** Conserved region coverage of 1089 Chinese HBV sequences (Fig. 10A) and of all 3041 Global HBV Sequences (Fig. 10B) using the 2_CHGL_epi sequences compared to for the wild type Genotype D sequence and the 1_GL_epi sequence. The Genotype D sequence is used in vaccines by Transgene and GlobeImmune.

[0037] **Fig. 11A-11D.** Fig. 11A and Fig. 11B show HBV genotype D episensus antigens developed for efficient epitope coverage in the U.S. and Europe; the antigen constructs begin in Fig. 11A and continue in Fig. 11B. The HBV genotype D episensus

antigens derived from the core (C), the PreS1 and PreS2, the surface antigen (S), and the polymerase (P) sequences are shown in the indicated orders. Variants of the episensus antigens derived from sequences with deletions in transmembrane (TM) domains or having the antigens reordered relative to the order of the proteins in HBV are developed to minimize aggregation and maximize expression. Fig. 11C shows constructs for the same antigens, each including a hemagglutinin (HA) epitope tag inserted at the C-terminus. Fig. 11D shows viral expression of the antigens as measured by SDS poly-acrylamide gel electrophoresis (SDS-page) followed by immunoblots developed with antibodies directed to the HA epitope tag engineered into each construct.

[0038] Fig. 12A-12B. Fig. 12A shows HBV genotype D episensus antigens derived from the PreS1 and PreS2, the surface antigen (S), the polymerase (P), and/or the core (C) sequences. Four of the antigens have deletions in the sequence derived from the P protein, as shown. V5 or hemagglutinin (HA) epitope tags engineered into the constructs; the V5 tag was inserted at the N'-terminus and the HA tag was inserted at the C'-terminus. Fig. 12B shows viral expression of the antigens as measured by SDS-page followed by immunoblots developed with antibodies directed to the V5 or HA epitope tag engineered into each construct.

DETAILED DESCRIPTION

I. Definitions

[0039] Various terms relating to aspects of the description are used throughout the specification and claims. Such terms are to be given their ordinary meaning in the art unless otherwise indicated. Other specifically defined terms are to be construed in a manner consistent with the definitions provided herein.

[0040] As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a cell" includes a combination of two or more cells, and the like. The use of the alternative (*e.g.*, "or") should be understood to

mean either one, both, or any combination thereof of the alternatives, and may be used synonymously with “and/or”. As used herein, the terms “include” and “have” are used synonymously, which terms and variants thereof are intended to be construed as non-limiting.

5 **[0041]** The term “about” as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of up to $\pm 20\%$ from the specified value, as such variations are appropriate to perform the disclosed methods. Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth used
10 in the specification and claims are to be understood as being modified in all instances by the term “about.” Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained. At the very least, and not as an attempt to limit the application of the doctrine
15 of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

[0042] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of this disclosure are approximations, the numerical values set forth in
20 the specific examples are reported as precisely as possible. Any numerical values, however, inherently contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

[0043] The term “comprise” means the presence of the stated features, integers, steps, or components as referred to in the claims, but does not preclude the presence or
25 addition of one or more other features, integers, steps, components, or groups thereof. The term “consisting essentially of” limits the scope of a claim to the specified materials or steps and those that do not materially affect the basic and novel characteristics of the claimed subject matter.

[0044] The word “substantially” does not exclude “completely”; *e.g.*, a
30 composition which is “substantially free” from Y may be completely free from Y.

Where necessary, the word “substantially” may be omitted from definitions provided herein.

[0045] As employed above and throughout the disclosure the term “effective amount” refers to an amount effective, at dosages, and for periods of time necessary, to achieve the desired result with respect to the treatment of the relevant disorder, condition, or side effect. It will be appreciated that the effective amount of components will vary from patient to patient not only with the particular vaccine, component or composition selected, the route of administration, and the ability of the components to elicit a desired result in the individual, but also with factors such as the disease state or severity of the condition to be alleviated, hormone levels, age, sex, weight of the individual, the state of being of the patient, and the severity of the pathological condition being treated, concurrent medication or special diets then being followed by the particular patient, and other factors which those skilled in the art will recognize, with the appropriate dosage being at the discretion of the attending physician. Dosage regimes may be adjusted to provide the improved therapeutic response. An effective amount is also one in which any toxic or detrimental effects of the components are outweighed by the therapeutically beneficial effects.

[0046] The term “administering” means either directly administering a compound or composition, or administering a prodrug, derivative or analog which will form an equivalent amount of the active compound or substance within the body.

[0047] The terms “subject,” “individual,” and “patient” are used interchangeably herein, and refer to an animal, for example a human, to whom treatment, including prophylactic treatment, with the pharmaceutical compositions disclosed herein, is provided. The term “subject” as used herein refers to human and non-human animals. The term “non-human animals” includes all vertebrates, *e.g.*, mammals, such as non-human primates, (particularly higher primates), sheep, dog, rodent, (*e.g.*, mouse or rat), guinea pig, goat, pig, cat, rabbits, cows, horses and non-mammals such as reptiles, amphibians, chickens, and turkeys.

[0048] The term “episensus” refers to an epitope-based consensus sequence. It is a sequence whose epitopes match, as closely as possible, the epitopes in a reference

set of natural sequences. The terms “epitope” and “potential epitope” refer to a sequence of k characters (typically k is in the range of 8-12), often in the context of a k-character subsequence of a much longer natural or vaccine antigen sequence. T cells can recognize such peptides in an immune response.

5 **[0049]** The term “EpiGraph” refers to a computational strategy developed to create sequences that provide an optimal episensus sequence, or a set of sequences that when combined provide optimal coverage of a population of diverse viral sequences. The EpiGraph method was previously described in PCT Application No. WO 2016/054654 A1, and in Theiler, *et al.*, *Sci. Rep.* 6:33987 (2016), the entireties of which
10 are herein incorporated by reference. The EpiGraph method produces sets of artificial but intact antigens with maximized coverage of potential T cell epitopes (PTE, typically 9mer peptide sequences) found in a diverse viral population. The EpiGraph is the next step over previous mosaic vaccine design methods. The graph-based EpiGraph method is much more computationally powerful than mosaics that use genetic algorithm, thus
15 allowing an improved PTE coverage through substantially more combinations considered. Both EpiGraph and mosaic methods produce protein antigens with greater coverage of T cell epitope diversity than natural strains. HBV, HIV, and HCV mosaics elicited cellular immune responses of greater breadth and depth than combinations of natural strains or consensus immunogens. HBV mosaics, and HIV mosaics are in phase
20 I clinical trials. In some embodiments, the EpiGraph algorithm is used to design “episensus” sequences corresponding to the conserved regions of HBV.

[0050] The term “episensus sequence” refers to the amino acid sequence of an artificial antigen that is designed using the EpiGraph algorithm based on a population of HBV sequences. An episensus sequence that is “central” to a population of HBV
25 sequences is a computationally derived sequence that provides the maximal average epitope coverage of the population. An “episensus antigen” is an antigen comprising an episensus amino acid sequence.

[0051] As used herein, the terms “treatment” or “therapy” (as well as different forms thereof, including curative or palliative) refer to treatment of an infected person.
30 As used herein, the term “treating” includes alleviating or reducing at least one adverse

or negative effect or symptom of a condition, disease, or disorder. This condition, disease, or disorder can be HBV infection.

[0052] As used herein, the terms “prevention” or “prophylaxis” refer to preventing a subject from becoming infected with, or reducing the risk of a subject from becoming infected with, or halting transmission of, or the reducing the risk of transmission of, for example, HBV, or a related virus.

[0053] “Pharmaceutically acceptable” refers to those compounds, materials, compositions, and/or dosage forms that are, within the scope of sound medical judgment, suitable for contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem complications commensurate with a reasonable benefit/risk ratio.

[0054] As used herein, the terms “vaccine,” “immunogenic compound,” and “immunogenic composition” are used interchangeably to refer to a compound or composition that induces an immune response in a subject. A prophylactic vaccine provides some degree of protection against new infections. A therapeutic vaccine assists in the treatment of an existing infection.

II. Antigens

[0055] Provided herein are polypeptides comprising sequences derived from certain HBV populations. In some embodiments, the polypeptide comprises the amino acid sequence as set forth in SEQ ID NOs:1-11 or SEQ ID NOs:14-36. In some embodiments, the polypeptide comprises two or more amino acid sequences as set forth in SEQ ID NOs:16-36. In some embodiments, the polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:19 or 29, the amino acid sequence as set forth in SEQ ID NOs:16, 21, 27, 28, or 34, and the amino acid sequence as set forth in SEQ ID NOs:20, 22, 23, 30, 31, 33, 35, or 36. In some embodiments, the polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:1. In some embodiments, the polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:2. In some embodiments, the polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:3. In some embodiments, the polypeptide comprises the amino

acid sequence as set forth in SEQ ID NO:4. In some embodiments, the polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:5. In some embodiments, the polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:6. In some embodiments, the polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:7. In some embodiments, the polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:8. In some embodiments, the polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:9. In some embodiments, the polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:10. In some embodiments, the polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:11. In some embodiments, the polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:14. In some embodiments, the polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:15. In some embodiments, the polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:24. In some embodiments, the polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:25. In some embodiments, the polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:26. In some embodiments, the polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:3 and the amino acid sequence as set forth in SEQ ID NO:4. In some embodiments, the polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:5 and the amino acid sequence as set forth in SEQ ID NO:6. In some embodiments, the polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:7 and the amino acid sequence as set forth in SEQ ID NO:8. In some embodiments, the polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:9, the amino acid sequence as set forth in SEQ ID NO:10, and the amino acid sequence as set forth in SEQ ID NO:11. In any of the aforementioned embodiments, the polypeptide may further comprise one or more amino acid sequences as set forth in SEQ ID NOs:17 and 18.

[0056] In some embodiments, the present disclosure provides an immunogenic composition or vaccine comprising one or more of the aforementioned polypeptides. In certain embodiments, the immunogenic compositions comprise one of the

aforementioned polypeptides. In other embodiments, the immunogenic compositions comprise two or more of the aforementioned polypeptides, which may be provided as a single polypeptide or as two or more polypeptides.

[0057] In certain embodiments, the polypeptides are episensus antigens, which
5 comprise sequences derived from wild type HBV sequences using the EpiGraph method. In the EpiGraph algorithm, the natural sequences are characterized by a large graph of nodes, each node corresponding to an epitope that appears in the natural sequences. Directed edges connect two nodes when the corresponding two epitope strings are “consistent”, meaning the last k-1 characters in the first string agree with the
10 first k-1 characters in the second string. If two strings are consistent, then together they form a string of length k+1. More generally, a path through this graph of nodes and edges corresponds to a single string that contains k-mer substrings corresponding to each of the nodes in the graph. Each node is weighted according to how many sequences in the reference set exhibit a substring corresponding to that node. The
15 EpiGraph algorithm uses a dynamic programming scheme to find the path through this full graph that maximizes the sum of these weights, and therefore provides the greatest coverage.

[0058] In some embodiments, the episensus antigen is derived from the HBV C protein. In some embodiments, the episensus antigen is derived from the HBV Pol (P)
20 or S protein, or another HBV protein. The episensus antigens may be derived from full-length protein sequences, regions of full-length protein sequences, or any combination thereof. In some embodiments, the episensus antigen comprises epitopes derived from two or more of the HBV C, P, and S protein. In some embodiments, the episensus antigen further comprises one or more epitopes derived from the PreS1 domain or the
25 PreS2 domain. In some embodiments, the episensus antigen is derived from a conserved region of HBV. In some embodiments, the episensus antigen is derived from a conserved region of one or more of HBV C, S, and P.

[0059] In some embodiments, the episensus antigen comprises sequences derived from two or more of the HBV C, P, and S proteins, with the sequences
30 reordered relative to their order in the HBV genome. Reordering of the sequences

within the episensus antigen may provide for more efficient or otherwise improved *in vitro* expression of the antigen.

[0060] In some embodiments, the episensus antigen is derived from an HBV protein that has contains one or more mutations, such as deletions or substitutions of amino acids. In some embodiments, the mutation inactivates at least one function present in the wild type protein. For example, PreS1 may be inactivated by removing the myristoylation sequence in the N terminus of the protein, or Pol may be inactivated by deleting or mutating enzymatic active sites. In some embodiments, one or more mutations are present in the active site of the HBV ribonuclease H (RNaseH) and result in loss of RNaseH activity. Exemplary mutations are shown in Table 1.

[0061] In certain embodiments, the episensus antigen is developed using a selected HBV population, which results in an episensus antigen that is central to that population. For example, the HBV population may be HBV genotypes present in a selected geographic location, such as Asia, North America, South America, Europe, Africa, or Australia. In some embodiments, the episensus antigen is central to the HBV B regional epidemic in Asia or, more specifically, in China. In other embodiments, the episensus antigen is central to the HBV C regional epidemic in Asia or, more specifically, in China. In some embodiments, the episensus antigen is central to the HBV A regional epidemic in Asia or, more specifically, in China. In some embodiments, the episensus antigen is central to the HBV CB regional epidemic in Asia or, more specifically, in China. In some embodiments, the episensus antigen is central to the HBV D regional epidemic in Asia or, more specifically, in China. In some embodiments, the episensus antigen is central to the HBV DC regional epidemic in Asia or, more specifically, in China. In some embodiments, the episensus antigen is central to the HBV DE regional epidemic in Asia or, more specifically, in China. In some embodiments, the episensus antigen is central to the HBV E regional epidemic in Asia or, more specifically, in China. In some embodiments, the episensus antigen is central to the HBV F regional epidemic in Asia or, more specifically, in China.

[0062] In other embodiments, the episensus antigen is central to the HBV multi-genotype regional epidemic in North America. In some embodiments, the episensus

antigen is central to the HBV A regional epidemic in North America. In some
embodiments, the population episensus antigen is central to the HBV B regional
epidemic in North America. In some embodiments, the episensus antigen is central to
the HBV C regional epidemic in North America. In some embodiments, the episensus
5 antigen is central to the HBV CB regional epidemic in North America. In some
embodiments, the episensus antigen is central to the HBV D regional epidemic in North
America. In some embodiments, the episensus antigen is central to the HBV DC
regional epidemic in North America. In some embodiments, the episensus antigen is
central to the HBV DE regional epidemic in North America. In some embodiments, the
10 episensus antigen is central to the HBV E regional epidemic in North America. In some
embodiments, the episensus antigen is central to the HBV F regional epidemic in North
America.

[0063] In other embodiments, the episensus antigen is central to the HBV multi-
genotype regional epidemic in South America. In some embodiments, the episensus
15 antigen is central to the HBV A regional epidemic in South America. In some
embodiments, the episensus antigen is central to the HBV B regional epidemic in South
America. In some embodiments, the episensus antigen is central to the HBV C regional
epidemic in South America. In some embodiments, the episensus antigen is central to
the HBV CB regional epidemic in South America. In some embodiments, the episensus
20 antigen is central to the HBV D regional epidemic in South America. In some
embodiments, the episensus antigen is central to the HBV DC regional epidemic in
South America. In some embodiments, the episensus antigen is central to the HBV DE
regional epidemic in South America. In some embodiments, the episensus antigen is
central to the HBV E regional epidemic in South America. In some embodiments, the
25 episensus antigen is central to the HBV F regional epidemic in South America.

[0064] In other embodiments, the episensus antigen is central to the HBV multi-
genotype regional epidemic in Europe. In some embodiments, the episensus antigen is
central to the HBV A regional epidemic in Europe. In some embodiments, the
episensus antigen is central to the HBV B regional epidemic in Europe. In some
30 embodiments, the episensus antigen is central to the HBV C regional epidemic in

Europe. In some embodiments, the episensus antigen is central to the HBV CB regional epidemic in Europe. In some embodiments, the episensus antigen is central to the HBV D regional epidemic in Europe. In some embodiments, the population episensus antigen is central to the HBV DC regional epidemic in Europe. In some embodiments, the episensus antigen is central to the HBV DE regional epidemic in Europe. In some embodiments, the episensus antigen is central to the HBV E regional epidemic in Europe. In some embodiments, the episensus antigen is central to the HBV F regional epidemic in Europe.

[0065] In other embodiments, the episensus antigen is central to the HBV multi-genotype regional epidemic in Africa. In some embodiments, the episensus antigen is central to the HBV A regional epidemic in Africa. In some embodiments, the episensus antigen is central to the HBV B regional epidemic in Africa. In some embodiments, the episensus antigen is central to the HBV C regional epidemic in Africa. In some embodiments, the episensus antigen is central to the HBV CB regional epidemic in Africa. In some embodiments, the episensus antigen is central to the HBV D regional epidemic in Africa. In some embodiments, the episensus antigen is central to the HBV DC regional epidemic in Africa. In some embodiments, the episensus antigen is central to the HBV DE regional epidemic in Africa. In some embodiments, the episensus antigen is central to the HBV E regional epidemic in Africa. In some embodiments, the episensus antigen is central to the HBV F regional epidemic in Africa.

[0066] In other embodiments, the episensus antigen is central to the HBV multi-genotype regional epidemic in Australia. In some embodiments, the episensus antigen is central to the HBV A regional epidemic in Australia. In some embodiments, the episensus antigen is central to the HBV B regional epidemic in Australia. In some embodiments, the episensus antigen is central to the HBV C regional epidemic in Australia. In some embodiments, the episensus antigen is central to the HBV CB regional epidemic in Australia. In some embodiments, the episensus antigen is central to the HBV D regional epidemic. In some embodiments, the episensus antigen is central to the HBV DC regional epidemic. In some embodiments, the episensus antigen is central to the HBV DE regional epidemic. In some embodiments, the episensus

antigen is central to the HBV E regional epidemic. In some embodiments, the episensus antigen is central to the HBV F regional epidemic.

[0067] In other embodiments, the episensus antigen is central to the HBV Global set in the disclosed vaccines.

5 [0068] In some embodiments, the HBV episensus antigen is 1_CH_epi (SEQ ID NO:1), which was developed using 1044 HBV sequences from China. In some
embodiments, the HBV episensus antigen is 1_GL_epi (SEQ ID NO:2), developed
using the Global set of 3041 HBV sequences. In some embodiments, the episensus
antigen is a combination of two or more individual episensus antigens. For example, in
10 some embodiments, the episensus antigen comprises 2_CH_epi, which comprises two
episensus antigens developed using 1044 HBV sequences from China (SEQ ID NO:3
and SEQ ID NO:4). In some embodiments, the episensus antigen comprises
2_CHGL_epi, which comprises a first episensus antigen, Epi1 (SEQ ID NO:5),
developed using 1044 HBV sequences from China, and a second episensus antigen,
15 Epi2 (SEQ ID NO:6), developed using the Global set of 3041 HBV sequences, with
Epi1 already fixed in the solution. In other words, Epi2 is complementary to Epi1,
developed with Epi1 already present. In some embodiments, the episensus antigen
comprises a modified version of 2_CHGL_epi, which comprises Epi3 (SEQ ID NO:7)
and Epi4 (SEQ ID NO:8). In some embodiments, the episensus antigen comprises
20 3_GL_epi, which comprises three episensus antigens (SEQ ID NO:9; SEQ ID NO:10;
and SEQ ID NO:11), developed using the Global set of 3041 HBV sequences. In some
embodiments, the episensus antigen comprises natural D subtype sequence (SEQ ID
NO:12), GenBank accession number Y07587. In some embodiments, the episensus
antigen comprises natural C subtype reference sequence (SEQ ID NO:13), GenBank
25 accession number GQ358158. Each of the reference genes of accession numbers
Y07587 and GQ358158 is incorporated by reference herein in its entirety. In some
embodiments, the episensus antigen is a modified genotype D sequence of SEQ ID
NO:14. In some embodiments, the episensus antigen is a re-ordered genotype D
sequence of SEQ ID NO:15. In some embodiments, the episensus antigen comprises
30 one or more of the sequences set forth in SEQ ID NOs:16-23 and SEQ ID NOs: 27-36.

In some embodiments, the HBV episensus antigen comprises two or more of the sequences set forth in SEQ ID NO:1 – SEQ ID NO:36.

[0069] Further provided herein are immunogenic compositions or vaccines comprising one or more of the aforementioned episensus antigens. The episensus antigens may be delivered as DNA, RNA, or polypeptides. In certain embodiments, the vaccines comprise a single antigen. In other embodiments, the vaccines comprise two or more antigens, which may be provided as a single polypeptide or as two or more polypeptides. In certain embodiments, the vaccines comprise episensus antigens that provide efficient epitope coverage for selected HBV genotypes, such as the genotypes present in a selected geographic location. The vaccines provided herein may be prophylactic vaccines or therapeutic vaccines. In some embodiments, the vaccine comprises one or more polypeptides and a pharmaceutically acceptable carrier or excipient.

[0070] In some embodiments, the vaccine comprises two episensus antigens developed using HBV sequences from China. In certain embodiments, the vaccine comprises episensus antigens comprising the sequences set forth in SEQ ID NO:3 and SEQ ID NO:4, which may be provided as one polypeptide or as two polypeptides. In some embodiments, the vaccine comprises an episensus antigen developed using HBV sequences from China and an episensus antigen developed using HBV sequences from the Global set. In certain embodiments, the vaccine comprises episensus antigens comprising the sequences set forth in SEQ ID NO:5 and SEQ ID NO:6, which may be provided as one polypeptide or as two polypeptides. In certain embodiments, the vaccine comprises episensus antigens comprising the sequences set forth in SEQ ID NO:7 and SEQ ID NO:8, which may be provided as one polypeptide or as two polypeptides. In some embodiments, the vaccine comprises three episensus antigens developed using HBV sequences from the Global set. In certain embodiments, the vaccine comprises episensus antigens comprising the sequences set forth in SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11, which may be provided as one polypeptide, as two polypeptides, or as three polypeptides.

III. Polynucleotides and Vectors

[0071] In some embodiments, the present disclosure provides a polynucleotide sequence that encodes a polypeptide. The polynucleotide may be DNA or RNA, and may encode any of the aforementioned antigens. In some embodiments, the
5 polynucleotide encodes a polypeptide comprising the amino acid sequence as set forth in one or more of SEQ ID NOs:1-36. In some embodiments, the polynucleotide sequence is codon optimized for expression in a particular host.

[0072] In some embodiments, the polynucleotide encodes a polypeptide comprising the amino acid sequence as set forth in SEQ ID NOs:1-11 or SEQ ID
10 NOs:14-36. In some embodiments, the polynucleotide encodes a polypeptide comprising two or more amino acid sequences as set forth in SEQ ID NOs:16-36. In some embodiments, the polynucleotide encodes a polypeptide comprising the amino acid sequence as set forth in SEQ ID NOs:19 or 29, the amino acid sequence as set forth in SEQ ID NOs:16, 21, 27, 28, or 34, and the amino acid sequence as set forth in SEQ
15 ID NOs:20, 22, 23, 30, 31, 33, 35, or 36. In some embodiments, the polynucleotide encodes a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:1. In some embodiments, the polynucleotide encodes a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:2. In some embodiments, the polynucleotide encodes a polypeptide comprising the amino acid sequence as set forth
20 in SEQ ID NO:3. In some embodiments, the polynucleotide encodes a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:4. In some embodiments the polynucleotide encodes a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:5. In some embodiments, the polynucleotide encodes a polypeptide comprising the amino acid sequence as set forth in SEQ ID
25 NO:6. In some embodiments, the polynucleotide encodes a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:7. In some embodiments, the polynucleotide encodes a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:8. In some embodiments, the polynucleotide encodes a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:9. In some
30 embodiments, the polynucleotide encodes a polypeptide comprising the amino acid

sequence as set forth in SEQ ID NO:10. In some embodiments, the polynucleotide encodes a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:11. In some embodiments, the polynucleotide encodes a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:14. In some embodiments, the polynucleotide encodes a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:15. In some embodiments, the polynucleotide encodes a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:24. In some embodiments, the polynucleotide encodes a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:25. In some embodiments, the polynucleotide encodes a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:26. In some embodiments, the polynucleotide encodes a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:3 and the amino acid sequence as set forth in SEQ ID NO:4. In some embodiments, the polynucleotide encodes a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:5 and the amino acid sequence as set forth in SEQ ID NO:6. In some embodiments, the polynucleotide encodes a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:7 and the amino acid sequence as set forth in SEQ ID NO:8. In some embodiments, the polynucleotide encodes a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:9, the amino acid sequence as set forth in SEQ ID NO:10, and the amino acid sequence as set forth in SEQ ID NO:11. In any of the aforementioned embodiments, the polynucleotide may further encode a polypeptide comprising one or more amino acid sequences as set forth in SEQ ID NOs:17 and 18.

[0073] In some embodiments, the present disclosure provides a composition, *e.g.*, an immunogenic composition, comprising two or more of the aforementioned polynucleotides.

[0074] In some embodiments, the present disclosure provides a vaccine comprising one or more of the aforementioned polynucleotides. In certain embodiments, the vaccine further comprises a pharmaceutically acceptable carrier or excipient.

[0075] Further provided herein are vectors that comprise any of the aforementioned polynucleotides. Vectors that can be used include, but are not limited to, plasmids, bacterial vectors, and viral vectors. Viral vectors include cytomegalovirus vectors. An advantage of CMV vectors for use in therapeutic vaccine delivery is that they can be used to elicit particular CD8⁺ T cell responses and induce more potent and enduring responses. It has been shown in animal models that vaccines based on these viral vectors can clear viral infections (Hansen *et al.*, *Science* 340:1237874 (2013)), and so these approaches have promise for a therapeutic vaccine (also: Hansen *et al.*, *Science* 351:714-720 (2016)).

10 [0076] Other viral vectors can include poxvirus, including vaccinia Ankara and canary pox; adenoviruses, including adenovirus type 5 (Ad5); rubella; sendai virus; rhabdovirus; alphaviruses; and adeno-associated viruses. Alternatively, vaccine antigens could be delivered as DNA, RNA or protein components of a vaccine. Episensus antigens would be compatible with essentially any mode of vaccine antigen
15 delivery.

[0077] In some aspects, the present disclosure provides vector-based immunogenic compositions or vaccines in which an expression vector is used to deliver a nucleic acid encoding one or more antigens disclosed herein. For example, the expression vector may be a poxvirus, adenovirus, rubella, sendai virus, rhabdovirus, alphavirus, or adeno-associated virus backbone.

[0078] In some embodiments, a CMV vector is used in the compositions and methods disclosed herein. Several aspects render chronic HBV infection particularly suited for immunotherapeutic intervention by CMV vectors.

[0079] First, the vast majority of individuals with a mature immune system readily mount an effective immune response and clear acute infection. Since this immune clearance is mostly mediated by cytokine-producing CD8⁺ T cells, it appears that the induction of active liver resident effector CD8⁺ T cell (TEM) responses is crucial for HBV clearance. Thus, the induction of sustained, effector HBV-specific CD8⁺ T cells in the liver of CHB patients should suffice to control and eventually
25 eliminate HBV. TEM-frequencies induced by CMV-based vectors are particularly high
30

in the liver of inoculated rhesus macaques (RM) (Hansen *et al.*, *Nature* 473:523-527 (2011), at supplemental Figure 1).

[0080] Second, unlike other small chronic viruses such as HIV or HCV that display high mutations rates resulting in a quasispecies, the small (3.2 kb) DNA genome of HBV is more constrained in its ability to mutate due to overlapping open reading frames (ORFs) (Figure 1).

[0081] Unfortunately, T cells elicited by traditional vaccination approaches (*e.g.*, peptide-based, protein-based, DNA, and even heterologous prime-boost T cell inducing regimens involving both DNA and pox viral vectors) are no longer detectable in the periphery after a few months, which may be too short to completely prevent the survival of residual HBV genomes that can potentially provide a source for viral rebound. In contrast, only CMV-vector induced TEM are persistently maintained in circulation and in the liver at high frequencies for years (Hansen *et al.*, *Nature* 2011).

[0082] In certain embodiments, the vector comprises a human CMV (HCMV) vector or a rhesus CMV (RhCMV) vector comprising a HCMV or RhCMV backbone and one or more polynucleotides encoding an antigen. The one or more polynucleotides may encode any of the aforementioned antigens. In some embodiments, the polynucleotide encodes two or more of the aforementioned antigens. In some embodiments, the vector comprises two or more polynucleotides, each polynucleotide encoding one or more of the aforementioned antigens.

[0083] The present disclosure further provides for compositions comprising two or more vectors, each comprising one or more polynucleotides encoding one or more of the aforementioned antigens.

[0084] In some embodiments, the CMV vector lacks the UL82 gene, which encodes the tegument protein pp71. In some embodiments, the UL82 gene is replaced with one or more polynucleotides encoding one or more of the aforementioned antigens. In some embodiments, the CMV vector lacks the UL128-UL130 gene region. In some embodiments, the CMV vector lacks the UL146-UL147 gene region. In some embodiments, the CMV vector lacks the UL128-UL130 gene region and lacks the UL146-147 gene region. In some embodiments, the CMV vector has an intact UL128-

UL130 gene region and an intact UL146-UL147 gene region. The UL128-UL130 gene region includes the UL128 gene, the UL130 gene, and any region in between the UL128 gene and the UL130 gene. The UL146-UL147 gene region includes the UL146 gene, the UL147 gene, and any region in between the UL146 gene and the UL147 gene.

5 **[0085]** In certain aspects, the present disclosure provides for immunogenic compositions or vaccines comprising the aforementioned vectors. Immune responses elicited by CMV vectors are not affected by pre-existing anti-vector immunity, thus enabling the sequential use of the same vector for different antigens (Hansen *et al.*, *Nature Medicine* 15:293-299 (2009)). In part, the ability to super-infect is due to viral
10 inhibitors of MHC-I-mediated antigen presentation to CD8⁺ T cells (Hansen *et al.*, *Science* 328:102-106 (2010)). Since almost the entire human population, including most CHB patients, is chronically infected with HCMV, super-infection is an important feature, enabling the use of CMV vectors regardless of recipient CMV-status. CMV vectors have unique immunology. A surprising and unexpected feature of certain
15 modified RhCMV/SIV vectors was the finding that these vectors elicited CD8⁺ T cells that did not recognize any of the epitopes recognized by conventional MHC-Ia-restricted CD8⁺ T cells in response to SIV infection itself, or in response to any other vector platform expressing SIV antigens. Nevertheless, CMV-vector- elicited CD8⁺ T cells recognized 3x as many peptides within a given antigen, as shown in
20 PCT/US2016/017373, the entirety of which is herein incorporated by reference (*see also* Hansen *et al.*, *Science* 2013, 2016).

[0086] The underlying reason for this remarkable breadth was determined by analyzing the restriction elements for individual peptides of given antigens (CMV, SIV, TB) in more than 100 animals, and demonstrates an astonishing feature of CMV
25 vectors: each of the peptides induced by the Rh 68-1 vector was either presented by MHC-II, which is normally recognized by CD4⁺ T cells, or by MHC-E, a non-polymorphic MHC-I molecule that normally binds the MHC-I-derived peptide VMAPRTLTL (VL9) and acts as a ligand for inhibitory NKG2A NK cell receptors. (Of note, the CD4⁺ T cells are all conventional, *i.e.*, restricted by MHC-II.)
30 Remarkably, strain 68-1 RhCMV-induced CD8⁺ T cell responses to overlapping

peptide pools covering entire antigens can be completely blocked by addition of VL9 (inhibiting MHC-E) or invariant-chain derived CLIP (inhibiting MHC-II), thus demonstrating that all CD8⁺ T cell epitopes are “unconventional” (Hansen *et al.*, *Science* 2016). While both MHC-II and MHC-E-restricted CD8⁺ T cells have been
5 observed occasionally in other infectious diseases and cancer, the abundant presence of such T cells in RhCMV-immunized animals is unprecedented and truly paradigm-breaking. Importantly, RhCMV-vector induced MHC-II and MHC-E and MHC-Ia-non-canonical (discussed below) restricted CD8⁺ T cells recognize SIV-infected CD4⁺ T cells, suggesting that unconventional antigen presentation can occur in SIV-infected
10 cells, even if SIV is unable to prime such a response. Since many chronic viruses, including HCV, upregulate HLA-E, presumably as a defense against NK cells, this highly conserved MHC molecule represents a new target for immunotherapy. (The expression of HLA-E in HBV-infected hepatocytes is less well known compared to HCV, but high levels of circulating HLA-E are found in chronic HBV carriers). The
15 ability to elicit MHC-E restricted CD8⁺ T cells thus opens the possibility to target HBV via this highly conserved restriction element.

[0087] Particular patterns of gene modifications in CMV vectors are associated with various T cell responses. Unconventional CD8⁺ T cells are induced by vectors that lack the UL128-UL130 gene region and the UL146-UL147 gene region (Hansen *et al.*, *Science* 2013, 2016, OHSU2346). In contrast, CD8⁺ T cell responses induced by
20 natural RhCMV infection or to a UL128-UL130-repaired version of RhCMV (Rh68-1) are conventional, *i.e.*, MHC-I restricted (Hansen *et al.*, *Science* 2013). However, even the conventional CD8⁺ T cell responses elicited by UL128-UL130-intact vectors are still significantly broader than those induced by non-CMV vectors (Hansen *et al.*,
25 *Science* 2013). Moreover, this broad conventional CD8⁺ T cell response is entirely directed to subdominant (“non-canonical”) epitopes, as the immune evasion gene US11 prevents induction of T cells recognizing “canonical” (*i.e.*, immunodominant) MHC-I epitopes (Hansen *et al.*, *Science* 2013). Thus, genetically modified CMV vectors are able to elicit four different CD8⁺ T cell populations that each recognize a set of non-
30 overlapping epitopes and classified as follows: 1. Unconventional, MHC-II restricted;

2. Unconventional, MHC-E restricted; 3. Conventional MHC-I restricted, non-canonical (=subdominant); 4. Conventional, MHC-I restricted, canonical (=immunodominant).

The presence or absence of the UL128-UL130 and UL146-UL147 gene regions determines the switch from conventional to unconventional CD8⁺ T cells, whereas
5 canonical CD8⁺ T cells are induced in the absence of US11 in both vectors in which UL128-UL130 and UL146-UL147 are present and vectors in which UL128-130 and UL146-UL147 are absent.

[0088] In some embodiments, the present disclosure provides an immunogenic composition or vaccine comprising any of the aforementioned vectors and one or more
10 polynucleotides encoding an antigen. The one or more polynucleotides may encode any of the aforementioned antigens. In some embodiments, the polynucleotide encodes two or more of the aforementioned antigens. The two or more polypeptides may be expressed by the same or different promoters. In some embodiments, the vector comprises two or more polynucleotides, each polynucleotide encoding one or more of
15 the aforementioned antigens. In certain embodiments, the vaccine comprises one or more of the aforementioned vectors and a pharmaceutically acceptable carrier or excipient.

III. Methods of Treatment

[0089] In certain embodiments, methods of treating HBV in a subject
20 comprising administering an effective amount of an immunogenic composition or vaccine comprising one or more of the aforementioned polypeptides, polynucleotides, vectors, or compositions to the subject in need thereof are provided. In some embodiments, the vaccine comprises one or more antigens or polynucleotides encoding antigens that are selected to efficiently cover the HBV genotype diversity within a
25 geographical area. In some embodiments, the vaccine is provided in the form of one or more polypeptides. In other embodiments, the vaccine is provided in the form of one or more polynucleotides, which may be provided in a recombinant viral vector. In any of the aforementioned embodiments, the vaccine may further comprise one or more pharmaceutically acceptable carriers or excipients.

[0090] In some embodiments, methods of inducing or generating an immune response in a subject are provided. Such methods comprise administering an effective amount of an immunogenic composition or vaccine comprising one or more of the aforementioned polypeptides, polynucleotides, vectors, or compositions to the subject
5 in need thereof are provided. In some embodiments, the vaccine comprises one or more antigens or polynucleotides encoding antigens that are selected to efficiently cover the HBV genotype diversity within a geographical area. In some embodiments, the vaccine is provided in the form of one or more polypeptides. In other embodiments, the vaccine is provided in the form of one or more polynucleotides, which may be provided in a
10 recombinant viral vector. In any of the aforementioned embodiments, the vaccine may further comprise one or more pharmaceutically acceptable carriers or excipients.

[0091] Some embodiments include methods of treating an HBV infection in a subject comprising administering an effective amount of an immunogenic composition or vaccine comprising one or more of the aforementioned polypeptides,
15 polynucleotides, vectors, or compositions to the subject in need thereof. In some embodiments, the vaccine comprises one or more antigens or polynucleotides encoding antigens that are selected to efficiently cover the HBV genotype diversity within a geographical area. In some embodiments, the vaccine is provided in the form of one or more polypeptides. In other embodiments, the vaccine is provided in the form of one or
20 more polynucleotides, which may be provided in a recombinant viral vector. In any of the aforementioned embodiments, the vaccine may further comprise one or more pharmaceutically acceptable carriers or excipients.

[0092] The present disclosure further provides for methods of protecting a subject from an HBV infection comprising administering an effective amount of an
25 immunogenic composition or vaccine comprising one or more of the aforementioned polypeptides, polynucleotides, vectors, or compositions to the subject in need thereof. In some embodiments, the vaccine comprises one or more antigens or polynucleotides encoding antigens that are selected to efficiently cover the HBV genotype diversity within a geographical area. In some embodiments, the vaccine is provided in the form
30 of one or more polypeptides. In other embodiments, the vaccine is provided in the form

of one or more polynucleotides, which may be provided in a recombinant viral vector. In any of the aforementioned embodiments, the vaccine may further comprise one or more pharmaceutically acceptable carriers or excipients.

[0093] Some embodiments include methods of inducing an effector memory T cell response comprising determining one or more episensus sequences, generating a vaccine comprising a vector comprising one or more polynucleotides encoding the one or more episensus sequences, and administering the vaccine to a subject in need thereof. Some embodiments include methods of inducing an effector memory T cell response comprising determining one or more episensus sequences, generating a vaccine comprising one or more antigens having the amino acid sequence of the one or more episensus sequences, and administering the vaccine to a subject in need thereof. In some embodiments, methods are provided of inducing an effector memory T cell response wherein the one or more episensus sequences comprises 1_CH_epi (SEQ ID NO:1), 1_GL_epi (SEQ ID NO:2), 2_CH_epi, which comprises the amino acid sequences set forth in SEQ ID NO:3 and SEQ ID NO:4, 2_CHGL_epi, which comprises the amino acid sequences set forth in (SEQ ID NO:5 and SEQ ID NO:6, 2_CHGL_epi, which comprises the amino acid sequences set forth in SEQ ID NO:7 and SEQ ID NO:8, 3_GL_epi, which comprises the amino acid sequences set forth in SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11, natural D subtype sequence (SEQ ID NO:12), natural C subtype reference sequence (SEQ ID NO:13), the modified genotype D sequence of SEQ ID NO:14, and the re-ordered genotype D sequence of SEQ ID NO:15. In some embodiments, methods are provided of inducing an effector memory T cell response wherein the one or more episensus sequences comprises one or more of the sequences set forth in SEQ ID NOs:16-23 and SEQ ID NOs:27-36. In some embodiments, methods are provided of inducing an effector memory T cell response wherein the one or more episensus sequences comprise two or more of SEQ ID NO:1 – SEQ ID NO:36.

[0094] Some embodiments include methods of inducing an effector memory T cell response comprising generating an immunogenic composition or vaccine comprising one or more of the aforementioned polynucleotides encoding one or more

episensus antigens and administering the immunogenic composition or vaccine to a subject in need thereof. Some embodiments include methods of inducing an effector memory T cell response comprising generating an immunogenic composition or vaccine comprising one or more of the aforementioned episensus antigens and administering the immunogenic composition or vaccine to a subject in need thereof. In some embodiments, methods are provided of inducing an effector memory T cell response wherein the one or more episensus sequences comprises 1_CH_epi (SEQ ID NO:1), 1_GL_epi (SEQ ID NO:2), 2_CH_epi, which comprises the amino acid sequences set forth in SEQ ID NO:3 and SEQ ID NO:4, 2_CHGL_epi, which comprises the amino acid sequences set forth in (SEQ ID NO:5 and SEQ ID NO:6, 2_CHGL_epi, which comprises the amino acid sequences set forth in SEQ ID NO:7 and SEQ ID NO:8, 3_GL_epi, which comprises the amino acid sequences set forth in SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11, natural D subtype sequence (SEQ ID NO:12), natural C subtype reference sequence (SEQ ID NO:13), the modified genotype D sequence of SEQ ID NO:14, and the re-ordered genotype D sequence of SEQ ID NO:15. In some embodiments, methods are provided of inducing an effector memory T cell response wherein the one or more episensus sequences comprises one or more of the sequences set forth in SEQ ID NOs:16-23 and SEQ ID NOs:27-36. In some embodiments, methods are provided of inducing an effector memory T cell response wherein the one or more episensus sequences comprise two or more of SEQ ID NO:1 – SEQ ID NO:36.

[0095] In any of the aforementioned embodiments, the immunogenic composition or vaccine may be a prophylactic vaccine or a therapeutic vaccine.

[0096] Recent breakthroughs in HBV vaccine research include the concept of an effector memory T cell (TEM)-inducing vaccine to prevent HBV infection. Unlike central memory T cells (TCM) induced by traditional vaccine approaches, TEM are persistently maintained in lymphoid tissues and extralymphoid effector sites and are immediately ready to mediate anti-viral effector function, thus providing a constant immune shield at the portals of viral entry and sites of viral reactivation. The most qualified vector system to induce and indefinitely maintain TEM is derived from CMV.

Presumably due to continuous, low-level reactivation and/or gene expression in persistently infected cells, CMV maintains just the right amount of persistent, low level immune stimulation required for TEM maintenance without triggering T cell exhaustion.

5 **[0097]** In certain embodiments, the immunogenic composition or vaccine can be used in a therapeutic vaccine setting. For example, the vaccine can be designed using a k-means clustering strategy to identify a set of 6-10 antigen sequences that provide good coverage of epitopes in a population of people that are infected with a highly variable pathogen, such as HBV. In some embodiments, the virus infecting a subject is
10 sequenced and two or three vaccines (or one or more vaccine comprising two or three polypeptides or one or more polynucleotides encoding two or three polypeptides) are delivered that provide good coverage of epitopes in the infecting virus. Thus, efficient epitope coverage is provided, while epitope mismatches between the vaccine and the infecting strain are minimized.

15 **[0098]** Certain embodiments provided include an immunogenic composition or vaccine comprising a HCMV backbone vector, which lacks certain CMV gene regions, and a polynucleotide encoding an episensus antigen. In certain embodiments, the HCMV backbone lacks the UL128-UL130 gene region (which includes the UL128 gene, the UL130 gene, and any region in between the UL128 gene and the UL130 gene)
20 and the UL146-UL147 gene region (which includes the UL146 gene, the UL147 gene, and any region in between the UL146 gene and the UL147 gene). Certain embodiments can also include deletion of the tegument protein pp71 (UL82) gene. (U.S. Patent Application Publication Nos. 2014/0141038; 2008/0199493; 2013/0142823; and International Application Publication No. WO/2014/138209).

25 **[0099]** In some embodiments, the present disclosure provides any of the aforementioned polypeptides, polynucleotides, vectors, vaccines, or compositions for use in treating HBV infection. In some embodiments, the present disclosure provides any of the aforementioned polypeptides, polynucleotides, vectors, vaccines, or compositions for use in protecting a subject from an HBV infection.

[0100] The present disclosure further provides for the use of any of the aforementioned polypeptides, polynucleotides, vectors, vaccines, or compositions for the manufacture of a medicament for use in the treatment of an HBV infection. In some embodiments, present disclosure further provides for the use of any of the

5 aforementioned polypeptides, polynucleotides, vectors, immunogenic compositions, vaccines, or compositions for the manufacture of a medicament for use in protecting a subject from an HBV infection.

[0101] Typical routes of administering the polypeptides, polynucleotides, vector, vaccines, or compositions described herein include, without limitation, oral,

10 topical, transdermal, inhalation, parenteral, sublingual, buccal, rectal, vaginal, and intranasal. The term “parenteral”, as used herein, includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. In certain embodiments, administering comprises administering by a route that is selected from oral, intravenous, parenteral, intragastric, intrapleural, intrapulmonary, intrarectal,

15 intradermal, intraperitoneal, intratumoral, subcutaneous, topical, transdermal, intracisternal, intrathecal, intranasal, and intramuscular.

[0102] Pharmaceutical compositions according to certain embodiments of the present invention are formulated so as to allow the active ingredients contained therein to be bioavailable upon administration of the composition to a patient. Compositions

20 that will be administered to a subject or patient may take the form of one or more dosage units, where for example, a tablet may be a single dosage unit, and a container of a herein described polypeptides, polynucleotides, vector, vaccines, or compositions in liquid form may hold a plurality of dosage units. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example,

25 *see* Remington: The Science and Practice of Pharmacy, 20th Edition (Philadelphia College of Pharmacy and Science, 2000). The composition to be administered will, in any event, contain an effective amount of polypeptide, polynucleotide, vector, vaccine, or composition of the present disclosure, for treatment or prevention of HBV in accordance with teachings herein.

[0103] A composition may be in the form of a solid or liquid. In some embodiments, the carrier(s) are particulate, so that the compositions are, for example, in tablet or powder form. The carrier(s) may be liquid, with the compositions being, for example, an oral oil, injectable liquid or an aerosol, which is useful in, for example, inhalatory administration. When intended for oral administration, the pharmaceutical composition is preferably in either solid or liquid form, where semi solid, semi liquid, suspension and gel forms are included within the forms considered herein as either solid or liquid.

[0104] As a solid composition for oral administration, the pharmaceutical composition may be formulated into a powder, granule, compressed tablet, pill, capsule, chewing gum, wafer, or the like. Such a solid composition will typically contain one or more inert diluents or edible carriers. In addition, one or more of the following may be present: binders such as carboxymethylcellulose, ethyl cellulose, microcrystalline cellulose, gum tragacanth, or gelatin; excipients such as starch, lactose or dextrins, disintegrating agents such as alginic acid, sodium alginate, Primogel, corn starch, and the like; lubricants such as magnesium stearate or Sterotex; glidants such as colloidal silicon dioxide; sweetening agents such as sucrose or saccharin; a flavoring agent such as peppermint, methyl salicylate, or orange flavoring; and a coloring agent. When the composition is in the form of a capsule, for example, a gelatin capsule, it may contain, in addition to materials of the above type, a liquid carrier such as polyethylene glycol or oil.

[0105] The composition may be in the form of a liquid, for example, an elixir, syrup, solution, emulsion, or suspension. The liquid may be for oral administration or for delivery by injection, as two examples. When intended for oral administration, preferred compositions contain, in addition to the present compounds, one or more of a sweetening agent, preservatives, dye/colorant, and flavor enhancer. In a composition intended to be administered by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer, and isotonic agent may be included.

[0106] Liquid pharmaceutical compositions, whether they be solutions, suspensions or other like form, may include one or more of the following adjuvants: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol, or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates, or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes, or multiple dose vials made of glass or plastic. Physiological saline is a preferred adjuvant. An injectable pharmaceutical composition is preferably sterile. A liquid composition intended for either parenteral or oral administration should contain an amount of a polypeptide, polynucleotide, vector, vaccine, or composition as herein disclosed such that a suitable dosage will be obtained.

[0107] The composition may be intended for topical administration, in which case the carrier may suitably comprise a solution, emulsion, ointment, or gel base. The base, for example, may comprise one or more of the following: petrolatum, lanolin, polyethylene glycols, bee wax, mineral oil, diluents such as water and alcohol, and emulsifiers and stabilizers. Thickening agents may be present in a composition for topical administration. If intended for transdermal administration, the composition may include a transdermal patch or iontophoresis device. The pharmaceutical composition may be intended for rectal administration, in the form, for example, of a suppository, which will melt in the rectum and release the drug. The composition for rectal administration may contain an oleaginous base as a suitable nonirritating excipient. Such bases include, without limitation, lanolin, cocoa butter, and polyethylene glycol.

[0108] A composition may include various materials that modify the physical form of a solid or liquid dosage unit. For example, the composition may include materials that form a coating shell around the active ingredients. The materials that form the coating shell are typically inert, and may be selected from, for example, sugar,

shellac, and other enteric coating agents. Alternatively, the active ingredients may be encased in a gelatin capsule. The composition in solid or liquid form may include an agent that binds to the polypeptide, polynucleotide, vector, vaccine, or composition of the disclosure and thereby assists in the delivery of the compound. Suitable agents that may act in this capacity include monoclonal or polyclonal antibodies, one or more proteins, or a liposome. The composition may consist essentially of dosage units that can be administered as an aerosol. The term aerosol is used to denote a variety of systems ranging from those of colloidal nature to systems consisting of pressurized packages. Delivery may be by a liquefied or compressed gas or by a suitable pump system that dispenses the active ingredients. Aerosols may be delivered in single phase, bi phasic, or tri phasic systems in order to deliver the active ingredient(s). Delivery of the aerosol includes the necessary container, activators, valves, subcontainers, and the like, which together may form a kit. One of ordinary skill in the art, without undue experimentation, may determine preferred aerosols.

15 **[0109]** It will be understood that compositions of the present disclosure also encompass carrier molecules for polynucleotides, as described herein (*e.g.*, lipid nanoparticles, nanoscale delivery platforms, and the like).

[0110] The pharmaceutical compositions may be prepared by methodology well known in the pharmaceutical art. For example, a composition intended to be administered by injection can be prepared by combining a composition that comprises a polypeptide, polynucleotide, vector, vaccine, or composition as described herein and optionally, one or more of salts, buffers, and/or stabilizers, with sterile, distilled water so as to form a solution. A surfactant may be added to facilitate the formation of a homogeneous solution or suspension. Surfactants are compounds that non-covalently interact with a molecule so as to facilitate dissolution or homogeneous suspension of the molecule in the aqueous delivery system.

25 **[0111]** In general, an appropriate dose and treatment regimen provide the composition(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit.

[0112] Compositions are administered in an effective amount, which will vary depending upon a variety of factors including the activity of the specific compound employed; the metabolic stability and length of action of the compound; the age, body weight, general health, sex, and diet of the subject; the mode and time of
5 administration; the rate of excretion; the drug combination; the severity of the particular disorder or condition; and the subject undergoing therapy.

[0113] The following examples are provided to describe the embodiments described herein with greater detail. They are intended to illustrate, not to limit, the embodiments.

10 [0114] All documents, patent, and patent applications cited herein are hereby incorporated by reference, and may be employed in the practice of the methods and compositions disclosed herein.

EXAMPLES

EXAMPLE 1:

15 IDENTIFICATION OF EPISENSUS ANTIGENS FOR USE AS THERAPEUTIC VACCINES

[0115] Episensus antigens for HBV were developed using the EpiGraph method, as described in PCT Application No. WO 2016/054654 A1 and in Theiler, *et al.*, *Sci. Rep.* 6:33987 (2016), which are fully incorporated herein by reference. A tool for using the EpiGraph algorithm is also available at
20 hiv.lanl.gov/content/sequence/EPIGRAPH/epigraph.html.

[0116] While it is not feasible to build a designer vaccine for each subject, it is feasible to sequence virus from that subject to try to get a good match from within a small reference set of vaccine options. A China-based HBV B genotype trial population was used to identify vaccines that would provide therapeutic effects. An
25 Asian-based reference vaccine set and a Global vaccine set were designed, as well as an updated Asian-based genotypes. Conserved regions of the HBV genome are found in the C protein (minus the first 29 amino acids on the N-terminus), the S protein, and the

P protein. The highly variable region of the N-terminus of the P protein was removed from consideration (see Figure 6).

[0117] Unlike in the case of a prophylactic vaccine, where it is not known which viruses might be encountered by the subject, in the case of a therapeutic vaccine, the infecting virus sequence can be obtained and matched.

[0118] Selection of the antigen for use in an immunogenic composition or vaccine takes two factors into account: the antigen epitopes should match as many epitopes of the subject's infecting viruses as possible and the epitope mismatches between the antigen and the subject's infecting viruses should be as few as possible, so that the vaccine response is as targeted as possible on the relevant epitopes.

[0119] The phylogeny within HBV major genotypes tends to have little clear structure. Rather it is a "starburst" with very long external branches, and very short, poorly defined internal branches near the base. Part of this structure is likely due to intra-subtype recombination. While that is hard to quantify, recombination is certainly occurring relatively frequently, and by analogy with what is seen in terms of inter-subtype recombination, it is likely to be extensive. Given the structure of the tree, simply using clustering on a phylogenetic tree to define the reference set of possible vaccines will not be as effective because within-genotype associations are of limited meaning from an epitope perspective. Instead, sequence relationships should be considered by the relevant measure, and the reference set should be selected based on potential epitope similarities between natural strains and putative vaccine designs.

[0120] Epigraph sequences were identified using amino acid 9-mers (see Figure 4A), but the method can be used with any length k -mers as a reference point, where k is the putative potential epitope length. In past work with mosaics, the optimal solution for 9-mers has been very nearly optimal for other nearby lengths (8, 10, 11, 12), and that is expected to also be true for the EpiGraph method.

[0121] The epitope coverage of vaccines comprising a) one episensus sequence; b) two episensus sequences; c) three episensus sequences; d) one natural HBV genotype sequence; and e) two natural HBV genotype sequences are compared in Figures 4A-7B. Generally, genotype-based vaccines provide excellent coverage of that genotype, but

poor coverage of other genotypes. The data in Figures 5A-5C demonstrate that two and three valent Global EpiGraph sequences substantially improve coverage of all major genotypes, while providing comparable coverage of B and C, compared to B and C specific vaccines. For example, as seen in Figures 5A to 5C, Genotype D coverage is about 90% when three episensus antigens are used, as opposed to coverage of about 50% for B or C specific vaccines, or even B+C specific vaccines.

EXAMPLE 2:

TRANSIENT EXPRESSION OF EPISENSUS ANTIGENS

[0122] Antigens designed to maximize epitope matches using the EpiGraph method resemble natural sequences but no longer code for native proteins. While the theoretical guidelines for expression of these artificial amino acid sequences are adhered to in the construction of these sequences, the proteins may exhibit unanticipated expression profiles or fail to express a stable full-length protein.

[0123] To evaluate the expression profile of these sequences in the context of mammalian cells, polynucleotides encoding episensus antigens were synthesized and cloned for transient transfection using methods described in WO 2016/054654, the entirety of which is herein incorporated by reference. Briefly, DNA encoding the constructs was synthesized (Genscript, Piscataway, NJ) to contain compatible cloning sites for plasmid vectors (pcDNA3.1 and pOri). All inserts were codon optimized for the host. Each construct was also modified to eliminate residual enzyme activity of the native sequence as described in Kulkarni *et al.* Vaccine (2011), the entirety of which is herein incorporated by reference. The plasmid vector was linearized with compatible endonucleases and treated with calf intestinal phosphatase to prevent recircularization of empty vector. Vector and insert fragments were resolved by agarose gel electrophoresis to confirm digest fragment sizes and cleaned for ligation by PCR purification kit (Thermo Scientific). Inserts were ligated to linearized vector at approximately 3:1 insert to vector ratio for 15 minutes at room temperature using a rapid ligation kit (Roche, Indianapolis, IN), transformed into chemically competent *E.*

coli (DH5-alpha), and plated on antibiotic selection plates. DNA from resulting colonies was screened by restriction digestion for inserts.

[0124] Clones containing each of the correct inserts in the appropriate orientation relative to vector promoter and poly(A) sequences were grown in liquid culture for plasmid DNA purification. Actively growing sub-confluent 293T or HELA cells in 12-well tissue culture plates received 500µl of fresh media (DMEM 10% FBS) while liposomes are prepared. To generate liposomes containing plasmid DNA, 250µl of serum free media was mixed with 500ng of plasmid DNA, and 250µl of serum free media was mixed with 2µl of lipid (Lipofectamine 2000, Invitrogen). After 5 minutes incubation at room temperature these solutions were combined, mixed, and incubated for 20 minutes. The DNA-containing liposomes (500µl) formed during this process were added drop-wise to the culture and allowed to incubate 12-16 hours, after which time the transfection mixture is replaced with fresh media. After an additional day of incubation, cultures were harvested by scraping and centrifugation. Supernatants were removed by aspiration and cell pellets lysed by resuspension in 100µl gel loading dye containing 5% SDS and 10% 2-mercaptoethanol and centrifugation through QiaShred column (Qiagen, Valencia, CA).

[0125] Expression of episensus antigens was demonstrated by SDS poly-acrylamide gel electrophoresis (SDS-page) and western blotting developed with antibodies to the V5 or hemagglutinin epitope tag engineered into each construct. Briefly, NuPAGE 4-12% Bis-Tris gels were prepared and loaded with 20-50ug total protein and electrophoresed at 110 – 120 volts for 90 minutes. The resolved proteins were transferred to PVDF membranes by semi-dry or wet transfer at 30 volts for 90 minutes. Non-specific binding was blocked with a solution of 10% nonfat dry milk in tris buffered saline with 0.1% tween-20 (PBS-T) for 60 minutes. HA (Sigma) or V5 (Invitrogen) antibodies were diluted in 5% milk solution and incubated with membranes for 1 hour followed by 3 washes with TBS-T prior to addition of 1:10000 dilution of horseradish peroxidase conjugated goat anti-mouse (Invitrogen) secondary antibody for 1 hour. Subsequently blots were washed three times in TBS-T and developed with

enzyme linked chemi-luminescence (ECL kit (Thermo-Pierce) and visualized with a digital gel imaging system.

EXAMPLE 3:

ENGINEERING OF EPISENSUS ANTIGENS INTO CMV VECTOR BAC CONSTRUCTS AND 5 EXPRESSION FROM RECONSTITUTED VIRUS

[0126] Episensus antigens were designed to provide good coverage of T-cell epitopes representative of the spectrum of viral sequences and genotypes of HBV from which they are generated. To utilize these antigens most effectively, polynucleotides encoding the antigens have been engineered into CMV vectors that have been
10 demonstrated to produce three times the CD8⁺ T cell spectrum of competing platforms. Broad antigen presentation and lifelong expression profiles of CMV vectors have demonstrated the capacity to protect and cure rhesus monkeys infected with SIV. The episensus antigen design algorithm in combination with CMV vectors may provide even greater coverage of HBV within and across genotypes when applied to broadly
15 prophylactic vaccines.

[0127] Polynucleotides encoding episensus sequences that demonstrated expression in transient transfection systems were transferred to CMV backbones using BAC recombineering with galactokinase and kanamycin selection. (Warming et al. Nucleic Acids Research. 2005. 33(4):1-12; and Paredes and Yu Curr Protoc Microbiol.
20 2012. Feb; Chapter 14:Unit14E.4; both of which are herein incorporated by reference in their entirety). Further, the episensus sequences derived from HBV proteins or protein domains, such as the core (C) protein, the surface antigen (S) protein, the PreS1 domain, the PreS2 domain, and the polymerase (P) protein, can be re-ordered relative to the order in which they occur in wild type HBV, as shown in Figures 11A to 11B, for
25 improved expression.

[0128] BAC recombineering facilitates the manipulation of large DNA sequences utilizing temperature and metabolite regulated recombination enzymes in the context of *E. coli* strain SW105 containing a parental BAC. Recombination is a sequential two-step process consisting of Step 1: insertion of the galactokinase (galK)

sequence with the antibiotic resistance gene kanamycin (kan) into the target region, followed by Step 2: replacement of the galK/kan cassette with the antigen of interest. Step 1 results in recombinants containing the galK/kan insert after positive selection in kan and step 2 results in recombinants containing the antigen of interest after negative selection in 2-deoxy-galactose (DOG). For both steps, the sequences to be inserted are amplified by standard PCR from template DNA containing either the galK/kan cassette or the antigen of interest using primers with long (50+ bp) homology arms to the HCMV sequences flanking the insertion site.

[0129] To prepare the bacterial cells for the galK/kan cassette insertion (Step 1), 5 mL cultures were grown overnight at 30°C in 2 x YT or Terrific Broth with 12.5ug/mL chloramphenicol, and diluted 1:50 the following morning. Bacteria were grown for approximately 2-4 additional hours at 30°C (to an OD₆₀₀ = 0.5-0.6), and then heat shocked by shaking at 42°C for 15 minutes to induce the recombination enzymes. Following this induction, bacteria were pelleted (3000rpm, 10 minutes, 4°C) and then washed three times in ice-cold water. The *E. coli* cells were rendered electro-competent to receive the PCR product and recombination competent for insertion of the sequence into the target region of the BAC. Purified insert (300 ng) was combined with 40 µl competent *E. coli* on ice, moved to a 1mm cuvette, and electroporated using the Bio-rad Gene Pulser Xcell. Following electroporation, the bacteria were diluted by addition of 5mL culture media and allowed to recover by shaking at 30°C for 2 hours prior to plating on chloramphenicol/kanamycin plates. Plates were incubated at 30°C for two days and colonies screened by restriction digest and PCR for recombination events.

[0130] BAC constructs positive for the galK/kan insert proceeded to the second step, where the galK/kan cassette was replaced by recombination with the PCR fragment containing the antigen of interest. To prepare the bacterial cells for the replacement of the galK/kan cassette in Step 2, the bacteria were grown and electroporated as described above. For electroporation recovery, the bacteria were diluted by addition of 5mL culture media and allowed to recover by shaking at 30°C for at least 4 to 4.5 hours. The cells were then pelleted (3000rpm, 10 minutes, 4°C) and washed three times with 1x M9 media prior to plating on M63 minimal media plates

with added glycerol, leucine, biotin, DOG and chloramphenicol. Colonies were then screened by PCR to confirm replacement of the galK/kan cassette with the antigen of interest. Positive clones were re-streaked on DOG plates and BAC DNA is isolated for further characterization by restriction digest and sequencing.

5 **[0131]** Viral Reconstitution: To regenerate virus, the BAC DNA was transferred into mammalian host cells permissive for viral growth. In brief, BAC DNA was purified using an endotoxin-free plasmid DNA kit (Macherey-Nagel) and transfected (16-24ug BAC DNA/T150) into a confluent flask of primary human fibroblasts grown in DMEM plus 1x glutamax and 9% FBS at 37°C with 5% CO₂. Transfection was
10 achieved using Lipofectamine 3000 following the manufacturer's protocol (ThermoFisher). The following day, the media was changed and the cells were then monitored daily for the formation of plaques. Once full cytopathic effect (CPE) was reached, the viral supernatant was harvested, clarified via centrifugation at 2500 x g for 5 min at room temperature and stored at -80°C. The remaining attached cells were
15 harvested by cell scraper in DPBS, pelleted by centrifugation (2,500 rpm, 5 minutes) and stored at -80°C.

[0132] Viral Episensus Antigen Expression: Expression of episensus antigens was tested by SDS poly-acrylamide gel electrophoresis (SDS-page) followed by immunoblots developed with either HBV specific antibodies or antibodies directed to
20 the V5 or hemagglutinin (HA) epitope tag engineered into each construct. Briefly, cell pellets were lysed by resuspension in radioimmunoprecipitation assay (RIPA) buffer and protein is quantified using a standard bicinchoninic acid assay (BCA). NuPAGE 4-12% Bis-Tris gels were prepared and loaded with 20-50ug total protein and electrophoresed at 110 – 130 volts for 90 minutes. The resolved proteins were
25 transferred to PVDF membranes by semi-dry or wet transfer at 30 volts for 90 minutes or 15V overnight. Non-specific binding was blocked with a solution of 5% nonfat dry milk in tris buffered saline with 0.1% tween-20 (PBS-T) for 60 minutes. Primary antibodies were diluted in 5% milk solution and incubated with membranes for 1 hour, followed by 3 washes with TBS-T prior to addition of 1:10,000 dilution of horseradish
30 peroxidase conjugated goat anti-mouse secondary antibody for 1 hour. Subsequently,

blots were washed three times in TBS-T and developed with enzyme linked chemiluminescence (ECL kit (Thermo-Pierce) and visualized with a digital gel imaging system.

EXAMPLE 4:

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POPULATION EPISSENSUS VACCINES

[0133] The EpiGraph method was used to create a set of vaccines comprising polynucleotides encoding episensus antigens using CMV vectors initially, however, other vaccine delivery systems can be utilized.

[0134] A total of 58 strategies of different episensus antigens and combinations of antigens were tested to determine the most comprehensive vaccine treatment. HBV samples from dbHBV were divided into three groups: samples from China, samples Not from China, and Global samples. Global samples comprise the combination of samples from China and samples Not from China. Vaccines against natural D and natural C subtypes were used as controls. Vaccines with one, two, or three episensus antigens were tested against each group.

[0135] Controls included vaccines comprising polynucleotides encoding natural D subtype sequence (SEQ ID NO:12), GenBank accession number Y07587; and natural C subtype reference sequence (SEQ ID NO:13), GenBank accession number GQ358158. The vaccines comprising polynucleotides encoding the D and C subtype sequences were then compared to vaccines comprising polynucleotides encoding: (a) 1_CH_epi (SEQ ID NO:1), developed using 1044 HBV sequences from China; (b) 1_GL_epi (SEQ ID NO:2), developed using the Global set of 3041 HBV sequences; (c) 2_CH_epi, which comprises two episensus antigens (SEQ ID NO:3 and SEQ ID NO:4) developed using 1044 HBV sequences from China; (d) 2_CHGL_epi, which comprises two episensus antigens – a first episensus antigen, Epi1 (SEQ ID NO:5), that was developed using 1044 HBV sequences from China, and a second episensus antigen, Epi2 (SEQ ID NO:6), that was developed using the Global set of 3041 HBV sequences, with Epi1 already fixed in the solution; (e) Epi7 and Epi8 (SEQ ID NO:7 and SEQ ID NO:8, respectively), which are variants of Epi1 and Epi2 modified to induce better

expression; and (f) 3_GL_epi, which comprises three episensus antigens (SEQ ID NO:9; SEQ ID NO:10; and SEQ ID NO:11), developed using the Global set of 3041 HBV sequences.

5 [0136] As shown in Figure 8, a two episensus antigen vaccine that comprises a polynucleotide encoding a first sequence developed using samples from China and a second sequence developed using the Global set provides coverage of ~88% of China sequences, ~77% of NOT China sequences, and ~80% of Global sequences.

EXAMPLE 5:

EPITOPE COVERAGE

10 [0137] HBV epitope coverage for vaccines comprising certain episensus antigen sequences was computationally analyzed. The vaccine arms for initial testing in CMV included: 1) a single population episensus antigen, central to the China genotype epidemic; 2) a single population episensus antigen that provides coverage to all HBV Global samples; 3) two population episensus antigens that provide coverage to HBV
15 samples from China; 4) two population episensus antigens that provide coverage to all HBV Global samples; 5) three population episensus antigens that provide coverage to all HBV Global samples; and 6) two population episensus antigens: a first population episensus antigen that provides coverage to HBV samples from China and a second population episensus antigen that provides coverage to HBV samples from the Global
20 set.

[0138] Controls included vaccines comprising polynucleotides encoding natural D subtype sequence (SEQ ID NO:12), GenBank accession number Y07587; and natural C subtype reference sequence (SEQ ID NO:13), GenBank accession number GQ358158. We then compared the vaccines comprising polynucleotides encoding the
25 D and C subtype sequences to vaccines comprising polynucleotides encoding: (a) 1_CH_epi (SEQ ID NO:1), developed using 1044 HBV sequences from China; (b) 1_GL_epi (SEQ ID NO:2), developed using the Global set of 3041 HBV sequences; (c) 2_CH_epi, which comprises two episensus antigens (SEQ ID NO:3 and SEQ ID NO:4) developed using 1044 HBV sequences from China; (d) 2_CHGL_epi, which comprises

two episensu antigens – a first episensu antigen, Epi1 (SEQ ID NO:5), that was developed using 1044 HBV sequences from China, and a second episensu antigen, Epi2 (SEQ ID NO:6), that was developed using the Global set of 3041 HBV sequences, with Epi1 already fixed in the solution; (e) Epi7 and Epi8 (SEQ ID NO:7 and SEQ ID NO:8, respectively), which are variants of Epi1 and Epi2 modified to induce better expression; and (f) 3_GL_epi, which comprises three episensu antigens (SEQ ID NO:9; SEQ ID NO:10; and SEQ ID NO:11), developed using the Global set of 3041 HBV sequences.

[0139] As seen in Figures 9A to 10B, the vaccine comprising 2_CHGL_epi provides over 80 percent exact coverage against sequences from China, over 75% exact coverage against sequences Not from China, and 80% exact Global coverage. These data demonstrate superior coverage of all HBV sequences compared to the control vaccines.

EXAMPLE 6:

SECOND GENERATION HBV EPISENSUS ANTIGENS

[0140] For most HBV genotypes, the HBV N-terminal assembly domain (NTD) of the core (C) is involved in core particle assembly and the C-terminal domain is involved in packaging of the pregenome/reverse transcriptase complex. The surface (S) proteins are products of a single open reading frame and distinguished by three domains: PreS1, PreS2, and S. The polymerase (P) protein exhibits both DNA-dependent DNA polymerase and RNA-dependent DNA polymerase (reverse transcriptase) activities. P protein replicates the HBV genome from an encapsidated pregenomic RNA template. The P protein is composed of 4 domains: (1) Terminal Protein (TP) domain, involved in the protein-priming mechanism through a conserved tyrosine; (2) non-conserved spacer domain; (3) Reverse Transcriptase domain (RNA-dependent DNA polymerase (RT) and DNA-dependent DNA polymerase (active site: YMDD conserved motif)); and (4) RNase H domain (ribonuclease H activity).

[0141] Episensu antigens are designed to provide good coverage of T-cell epitopes representative of the spectrum of viral sequences from which they are

generated. Infection by HBV genotype D represents one of the most prevalent HBV infections in the U.S. and Europe. Therapeutic vaccines comprising genotype D episensus (EpiD) antigens or nucleotides encoding EpiD antigens may be beneficial for patients who have been pre-screened for the particular genotype. Provided herein are

5 examples of HBV genotype D episensus antigen (SEQ ID NO: 14) that provide good coverage for HBV genotypes in the U.S. and Europe. In one variant, as shown in Figure 11A and 11B, the HBV genotype D episensus antigens derived from the core (C) protein, the PreS1 and PreS2 domains, the surface antigen (S) protein, and the polymerase (P) protein were initially used in the order in which they appear in the HBV

10 genome: C-S-P (“CSP”) (SEQ ID NO: 14), and were reordered as S-P-C (“SPC”) (SEQ ID NO: 15). Additional antigen variants were generated by applying the EpiGraph method to HBV protein sequences in which transmembrane domains 1-2 or 1-4 of the S protein were deleted (“CSP ΔTM1-2,” “CSP ΔTM1-4,” “SPC ΔTM1-2,” or “SPC ΔTM1-4”) to reduce aggregation and improve expression. Therefore, the HBV

15 episensus antigens may also be derived from HBV protein sequences comprising one or more of the aforementioned variants.

[0142] Further, episensus antigens derived from P protein variants with mutations and/or deletion in the active sites of the polymerase domain were generated to reduce potential toxicity and improve safety. Examples of such episensus antigens

20 are provided in, for instance, SEQ ID NOs: 7, 8, and 15. The mutations present in the sequences used to develop these episensus antigens are provided in Table 1, below. Other P protein variants having different mutations or deletions may also be used.

Table 1: Mutational Modification of Polymerase in HBV Genotype D

Position	Amino Acid(s)	Modification
538-541	YMDD	Deleted
689	D	Replaced with A
718	E	Replaced with A
737	D	Replaced with V
777	D	Replaced with A

[0143] The positions in the left column of the table refer to the amino acid numbers in the full-length polymerase in HBV genotype D. The YMDD sequence is the reverse transcriptase active site. (See Radziwill, *et al.*, *J Virol.* 1990 Feb;64(2):613-20, which is incorporated herein by reference in its entirety.) The other 4 amino acids (D, E, D, D) are the RNase H active sites. (See Tavis *et al.*, *PLoS Pathog.* 2013 Jan;9(1):e1003125, which is incorporated herein by reference in its entirety.)

[0144] Expression of the episensus antigens derived from these variant sequences was tested by SDS-page and western blotting as described above (Figures 11A to 11D). Removing the transmembrane (TM) domains did not affect expression (Figure 11D).

[0145] No significant aggregation was observed with any of the EpiD antigen variants. Protein order of the antigen fusion was related to efficient expression. SPC re-ordered antigen fusion proteins show significantly increased expression.

[0146] HBV episensus antigens may be derived from one or more of the following HBV proteins or protein domains: the core (C) protein, the surface (S) protein, the PreS1 protein, the PreS2 protein, the transmembrane domains 1-4 (TM1-4) of the S protein, the determinant, and the polymerase (P) protein. The episensus antigens may be derived from various HBV proteins and protein domains having deletions or mutations, and/or the episensus antigens may be re-ordered relative to their order in HBV, for improved expression or activity. For illustration purposes, an episensus sequence derived from core protein of HBV subtype D is provided in SEQ ID NO:16, an episensus sequence derived from PreS1 protein of HBV subtype D is provided in SEQ ID NO:17, an episensus sequence derived from PreS2 protein of HBV subtype D is provided in SEQ ID NO:18, an episensus sequence derived from surface protein (S) of HBV subtype D is provided in SEQ ID NO:19, an episensus sequence derived from polymerase (P) protein of HBV subtype D is provided in SEQ ID NOs:20 or 22, and an episensus sequence derived from polymerase (P) protein of HBV subtype D containing the mutations and deletion shown in Table 1 is provided in SEQ ID NO:23. An example of an episensus sequence derived from HBV subtype C surface protein (S), polymerase protein (P), and core protein (C), with the antigen sequences in

the order “SPC” is provided in SEQ ID NO:24. Examples of episensus sequences derived from P protein that has a larger deleted region than that shown in Table 1 include the episensus sequence derived from HBV subtype C surface protein (S), polymerase protein (P) having a deletion of amino acids 612-838, and core protein (C),
5 provided in SEQ ID NO:25; and the episensus sequence derived from HBV subtype D surface protein (S), polymerase protein (P) having a deletion of amino acids 601-827, and core protein (C), provided in SEQ ID NO:26. Both SEQ ID NOs:25 and 26 are reordered, providing the antigen sequences in the order “SPC.”

[0147] Additional examples of episensus sequences derived from P protein with
10 deleted regions are shown in Figure 12A, and include deletions of P protein amino acids 1-200, 201-400, or 401-600. Expression data for the constructs of Figure 12A are shown in Figure 12B.

[0148] Further examples of episensus sequences derived from core protein of HBV are provided in SEQ ID NOs:27, 28, and 34. Further examples of episensus
15 sequences derived from surface protein of HBV are provided in SEQ ID NO:29. Further examples of episensus sequences derived from polymerase protein of HBV are provided in SEQ ID NOs:30-32 and SEQ ID NOs:35-36.

[0149] The sequences of HBV proteins and protein domains can be determined for any HBV subtype by sequence alignment with the sequences disclosed herein. For
20 example, the complete genomic sequence of the natural D subtype of HBV is provided in GenBank accession number Y07587 (incorporated by reference herein in its entirety). The P protein is encoded by nucleotides of 1-1625 and 2309-3182 of GenBank reference gene Y07587. The PreS protein is encoded by nucleotides of 1-837 and 2850-3182 of GenBank reference gene Y07587. The S domain is encoded by
25 nucleotides 157-837 of GenBank reference gene Y07587. The PreC/C protein is encoded by nucleotides 1816-2454 of GenBank reference gene Y07587. The C protein is encoded by nucleotides of 1903-2451 of GenBank reference gene Y07587. In addition, the complete genomic sequence of the natural C subtype of HBV is provided in GenBank accession number GQ358158 (incorporated by reference herein in its
30 entirety). The P protein is encoded by nucleotides of 1-1623 and 2307-3215 of

GenBank reference gene GQ358158. The S protein is encoded by nucleotides of 1-835 and 2848-3215 of GenBank reference gene GQ358158. The PreC/C protein is encoded by nucleotides of 1814-2452 of GenBank reference gene GQ358158. The C protein is encoded by nucleotides of 1901-2452 of GenBank reference gene GQ358158.

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EXAMPLE 7:

VACCINE TESTING

[0150] Vaccines comprising computationally designed episensus antigens are tested in Rhesus macaques (RM). CMV-based T cell responses are expected to be much broader and therefore cover a much higher percentage of sequences than reported previously for other vectors. Thus, even with a relatively small number of animals there should be sufficient epitope responses to evaluate the impact of sequence variation on the cross-reactive potential of the responses. The number and magnitude of all responses to the vaccines is determined by using vaccine-matched sets of peptides. Once the targeted peptides are determined, using just those peptides that are positive in each animal, the impact of natural variation on each vaccine-responsive peptide is determined. The natural variants that are tested are based on the variation found in a reference panel. Nonparametric and computational re-sampling statistical methods are used as the primary tools to evaluate the impact of epitope variation on diminishing magnitude or abrogation of recognition. These analyses are complemented, however, by using generalized linear models as needed to explore the impact of more complex interactions on T cell response cross-reactivity.

[0151] The vaccine arms for initial testing in CMV include vectors encoding: 1) a single population episensus antigen, central to the China genotype epidemic; 2) a single population episensus antigen that provides coverage to all HBV Global samples; 3) two population episensus antigens that provide coverage to HBV samples from China; 4) two population episensus antigens that provide coverage to all HBV Global samples; 5) three population episensus antigens that provide coverage to all HBV Global samples; and 6) two population episensus antigens: a first population episensus antigen that provides coverage to HBV samples from China and a second population

episensus antigen that provides coverage to HBV samples from the Global set, 7) an episensus antigen generated using a truncated Pol sequence that provides coverage to HBV C subtype epitopes (SEQ ID NO: 25), and 8) an episensus antigen generated using a truncated Pol sequence that provides coverage to HBV D subtype epitopes (SEQ ID NO:26). Controls included vaccines comprising polynucleotides encoding natural D subtype sequence (SEQ ID NO:12), GenBank accession number Y07587; and natural C subtype reference sequence (SEQ ID NO:13), GenBank accession number GQ358158.

[0152] Up to ten cohorts of 5 Rhesus macaques (RM) are inoculated with 10^6 PFU of HCMV vectors as follows: up to eight cohorts each receive one of vaccines 1-8, listed above; a single cohort receives a vaccine comprising a polynucleotide encoding natural D subtype; and a single cohort receives a vaccine comprising a polynucleotide encoding natural C subtype. Cohort 3 receives the episensus plus a different tailored vaccine vector and cohort 4 receives the episensus plus both tailored vaccine vectors.

[0153] Rhesus macaques (RM) are inoculated subcutaneously at day 0 and week 12 and followed longitudinally for one year. Since vaccination by HCMV-vectors is not affected by pre-existing anti-RhCMV immunity, animals naturally infected with RhCMV are used for these experiments. Flow cytometric intracellular cytokine analysis (ICS) is used to determine the CD4⁺ and CD8⁺ T cell response to individual consecutive 15mer peptides comprising the vaccine sequences within the vaccine inserts administered to each animal (which will comprise the total vaccine-elicited responses). It is then determined whether these epitope-specific T cells recognize epitope variants in both the target strain and the non-target strains. For peptides that show responses to strain-specific epitopes, the magnitude, functional avidity, and functional characteristics (IFN- γ , TNF- α , IL-2 and MIP-1 β production and CD107 externalization) of these responses to the “parent” (vaccine insert sequences) peptide variants are compared to determine the degree of functional cross-reactivity. In selected cases, truncation analysis is used to identify the core epitope for similar comparative analysis. To determine the percentage of MHC-II restricted CD8⁺ T cells present, “blocking” mAbs specific for MHC-I and MHC-II, and the invariant chain-

derived, MHC-II-specific binding peptide CLIP is used to inhibit influenza-specific CD8⁺ T cell responses in PBMC.

5 **[0154]** While specific embodiments have been illustrated and described, it will be readily appreciated that the various embodiments described above can be combined to provide further embodiments, and that various changes can be made therein without departing from the spirit and scope of the invention.

10 **[0155]** All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications, and non-patent publications referred to in the specification and/or listed in the Application Data Sheet, including U.S. Provisional Patent Application No. 62/893,546, filed August 29, 2019, and U.S. Provisional Patent Application No. 62/941,125, filed November 27, 2019, are incorporated herein by reference, in their entirety, unless explicitly stated otherwise. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, applications and publications to provide yet further embodiments.

15 **[0156]** These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled.

20 Accordingly, the claims are not limited by the disclosure.