

HBV VACCINES AND METHODS TREATING HBV

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit under 35 U.S.C. § 119(e) of U.S. provisional application no. 62/908,494, filed on September 30, 2019, which is hereby incorporated herein by reference in its entirety for all purposes.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on September 4, 2020, is named 1324_PF_SL.txt and is 296,675 bytes in size.

BACKGROUND

[0003] There have been many attempts to use vaccination to treat patients with chronic hepatitis B virus (HBV) infection to improve rates of HBV surface antigen (sAg) loss, the primary marker of functional cure. Such attempts have included vaccination with recombinant proteins (Dikici, *et al.*, *J Gastroenterol Hepatol.* (2003) 18(2):218-22; Pol, *et al.*, *J Hepatol.* (2001) 34(6):917-21; Vandepapeliere, *et al.*, *Vaccine* (2007) 25(51):8585-97; Yalcin, *et al.*, *J Clin Gastroenterol.* (2003) 37(4):330-5; Al-Mahtab, *Hepatol Int.* (2013) 7(4):981-9; Hoa, *et al.*, *Antimicrob Agents Chemother.* (2009) 53(12):5134-40; and Yalcin, *et al.*, *Infection.* (2003) 31(4):221-5), recombinant DNA (Mancini-Bourguine, *et al.*, *Hepatology.* (2004) 40(4):874-82; Yang, *et al.*, *World J Gastroenterol.* (2017) 23(2):306-17; Yang, *et al.*, *J Viral Hepat.* (2012) 19(8):581-93; Yoon, *et al.*, *Liver Int.* (2015) 35(3):805-15; Cavanaugh, *et al.*, *PLoS One.* (2011) 6(2):e14626; and Godon, *et al.*, *Mol Ther.* (2014) 22(3):675-84), dendritic cells (Luo, *et al.*, *Vaccine.* (2010) 28(13):2497-504; and Wei, *et al.*, *Int Immunopharmacol.* (2015) 27(2):238-43), a yeast vector (Gane, *et al.*, *J Hepatol.* (2019) Epub 2019/07/16. doi: 10.1016/j.jhep.2019.06.028. PubMed PMID: 31306680), and some viral vectors (Cavanaugh, *et al.*, *supra*; and Zoulim, *et al.*, *Hum Vaccin Immunother.* (2019) Epub 2019/08/03. doi: 10.1080/21645515.2019.1651141. PubMed PMID: 31373537). Despite these many attempts, to date no therapeutic vaccination approach has shown consistent benefit in chronic HBV infection (CHB). Deficits in previous vaccine approaches may explain the failures of previous vaccine approaches.

[0004] Such deficits include limitations in the antigen designs and in the vaccine technologies used. An optimal antigen will contain highly conserved portions of HBV proteins and exclude poorly conserved regions, because highly conserved regions can induce responses against epitopes that are identical in the vaccine antigen and in the virus present in the treated patient, while poorly conserved regions may elicit immunodominant T cell responses against epitopes that are not present in the patient's infecting virus strain (Swadling, *et al.*, *Vaccines* (Basel). (2016) 4(3). Epub 2016/08/05. doi: 10.3390/vaccines4030027. PubMed PMID: 27490575). However, some prior vaccines used antigen designs that do not meet these criteria (Yalcin, *et al.*, *J Clin Gastroenterol.* (2003) 37(4):330-5; Hoa, *et al.*, *supra*; Yalcin, *et al.*, *Infection.* (2003) 31(4):221-5; Mancini-Bourgine, *et al.*, *supra*; Yang, *et al.*, *J Viral Hepat.* (2012) 19(8):581-93; Cavanaugh, *et al.*, *supra*; Godon, *et al.*, *supra*; Gane. *et al.*, *supra*; and Obeng-Adjei, *et al.*, *Cancer Gene Ther.* (2013) 20(12):652-62). Additionally, many prior vaccines have failed to induce a full combination of virus-specific CD4⁺ T cells, CD8⁺ T cells, and antibody responses (Dikici, *et al.*, *supra*; Pol, *et al.*, *supra*; Vandepapeliere, *et al.*, *supra*; Yalcin, *et al.*, *J Clin Gastroenterol.* (2003) 37(4):330-5; Al-Mahtab, *supra*; Hoa, *et al.*, *supra*; Yalcin, *et al.*, *Infection.* (2003) 31(4):221-5; Mancini-Bourgine, *et al.*, *supra*; Yang, *et al.*, *J Viral Hepat.* (2012) 19(8):581-93; Gane. *et al.*, *supra*; and Zoulim, *et al.*, *supra*). These immune components are particularly important for curing chronic HBV infection as CD8⁺ T cells have been shown to be the main effector cells responsible for viral clearance during acute HBV infection in chimpanzees (Thimme, *et al.*, *J Virol.* (2003) 77(1):68-76). In addition, antibodies that bind to HBV surface antigen (HBsAg) facilitate HBsAg clearance and prevent spread of residual HBV. Moreover, a high magnitude of immune response is likely necessary to achieve a therapeutic effect, but many prior CHB vaccines have failed to induce such a robust response (Mancini-Bourgine, *et al.*, *supra*; Yang, *et al.*, *J Viral Hepat.* (2012) 19(8):581-93; Cavanaugh, *et al.*, *supra*; Gane. *et al.*, *supra*; and Zoulim, *et al.*, *supra*). Lastly, some prior CHB vaccine antigens have not been sufficiently stable in the delivery vectors to enable commercial-scale vaccine manufacture.

SUMMARY

[0005] In one aspect, provided are truncated hepatitis B virus (HBV) polymerase polypeptides, *e.g.*, capable of inducing or eliciting an immune response in a human upon administration. In some embodiments, the truncated HBV polymerase polypeptide comprises an inactivated reverse transcriptase domain and an inactivated RNase H, and does not comprise all of the terminal protein (TP) domain and all or part of the Spacer domain. In some embodiments, the polypeptide is no longer than 600 amino acids in length, *e.g.*, no longer than 595, 590, 585,

580, 575, 570, 565, 560, 555, 550, 545, 540 or 535 amino acids in length. In some embodiments, the reverse transcriptase domain does not comprise a YMDD motif (SEQ ID NO: 97) and the RNase H domain does not comprise an AELL motif (SEQ ID NO: 98). In some embodiments, the YMDD motif (SEQ ID NO: 97) in the reverse transcriptase domain is mutated to YMHD (SEQ ID NO: 99) and wherein the AELL motif (SEQ ID NO: 98) in the RNase H domain is mutated to AHLL (SEQ ID NO: 100). In some embodiments, the polypeptide is from an HBV genotype A, B, C or D. In some embodiments, (a) the polypeptide is from HBV genotype B and does not comprise a polypeptide sequence (*e.g.*, the sequence is removed or deleted or not included) of SEQ ID NO: 50, or a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NO: 50; or (b) the polypeptide is from HBV genotype D and does not comprise a polypeptide sequence of SEQ ID NO: 51, or a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NO: 51. In some embodiments, the truncated HBV polymerase polypeptide comprises or consists of an amino acid sequence of any one of SEQ ID NOs: 13-14, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 13-14.

[0006] In another aspect, provided are HBV polymerase deletion mutant polypeptides. In some embodiments, the HBV polymerase deletion mutant polypeptide comprises in sequential order from the N-terminus to the C-terminus, a terminal protein (TP) domain, an inactivated reverse transcriptase domain, and an inactivated RNase H, wherein the mutant polypeptide does not comprise all or part of a Spacer domain. In some embodiments, the polypeptide is no longer than 800 amino acids in length, *e.g.*, no longer than 795, 790, 785, 780, 775, 770, 765, 760, 755, 750, 745, 740, 735, 730, 725, 720, 715, 710 or 705 amino acids in length. In some embodiments, the reverse transcriptase domain does not comprise a YMDD motif (SEQ ID NO: 97) and the RNase H domain does not comprise an AELL motif (SEQ ID NO: 98). In some embodiments, the YMDD motif (SEQ ID NO: 97) in the reverse transcriptase domain is mutated to YMHD (SEQ ID NO: 99) and wherein the AELL motif (SEQ ID NO: 98) in the RNase H domain is mutated to AHLL (SEQ ID NO: 100). In some embodiments, the polypeptide is from an HBV genotype A, B, C or D. In some embodiments, (a) the polypeptide is from HBV genotype A and does not comprise a polypeptide of SEQ ID NO: 42 or 46, or a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NO: 42 or 46; (b) the polypeptide is from HBV genotype B and does not comprise a polypeptide of SEQ ID NO: 43 or 47, or a sequence that is at least 90%, 91%, 92%,

93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NO: 43 or 47; (c) the polypeptide is from HBV genotype C and does not comprise a polypeptide of SEQ ID NO: 44 or 48, or a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NO: 44 or 48; or (d) the polypeptide is from HBV genotype D and does not comprise a polypeptide of SEQ ID NO: 45 or 49, or a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NO: 45 or 49. In some embodiments, the HBV polymerase deletion mutant polypeptide comprises or consists of an amino acid sequence of any one of SEQ ID NOs: 5-12, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 5-12. In some embodiments, HBV polymerase deletion mutant polypeptide further comprises (*e.g.*, is a fusion protein including) an HBV core polypeptide. In some embodiments, the HBV polymerase deletion mutant polypeptide comprises in sequential order from the N-terminus to the C-terminus, an HBV core polypeptide and the HBV polymerase deletion mutant polypeptide, as described herein. In some embodiments, the HBV polymerase deletion mutant polypeptide comprises or consists of an amino acid sequence of any one of SEQ ID NOs: 19-26, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 19-26.

[0007] In a further aspect, provided is an HBV core-sAg fusion protein. In some embodiments, the core-sAg fusion protein comprises in sequential order from the N-terminus to the C-terminus, an HBV core polypeptide and an HBV small surface antigen (sAg) polypeptide. In various embodiments, the core polypeptide is from an HBV genotype B or C and the sAg polypeptide is from an HBV genotype C. In some embodiments, the core polypeptide is from an HBV genotype D and the sAg polypeptide is from an HBV genotype D. In some embodiments, the core-sAg fusion protein comprises: (a) a core polypeptide comprising or consisting of an amino acid sequence of SEQ ID NO: 65, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 65, and a sAg polypeptide comprising or consisting of an amino acid sequence of SEQ ID NO: 3, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 3; or (b) a core polypeptide comprising or consisting of an amino acid sequence of SEQ ID NO: 66, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 66, and a sAg polypeptide comprising or consisting of an amino acid sequence of SEQ ID NO:

4, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical SEQ ID NO: 4. In some embodiments, the core polypeptide comprises a serine (S) residue at the amino acid position corresponding to position 12, and an asparagine (N) residue at the amino acid position corresponding to position 67, wherein the position numbers are with reference to SEQ ID NO:65 or SEQ ID NO:66. In some embodiments, the sAg polypeptide comprises an isoleucine (I) residue at the amino acid position corresponding to position 68, wherein the position numbers are with reference to SEQ ID NO:3 or SEQ ID NO:4. In some embodiments, the sAg polypeptide comprises one or more of a serine (S) residue at the amino acid position corresponding to position 53, an isoleucine (I) residue at the amino acid position corresponding to position 68, a threonine (T) residue at the amino acid position corresponding to position 125, a proline (P) residue at the amino acid position corresponding to position 127, an phenylalanine (F) residue at the amino acid position corresponding to position 161, a tyrosine (Y) residue at the amino acid position corresponding to position 200, a serine (S) residue at the amino acid position corresponding to position 210, and a leucine (L) residue at the amino acid position corresponding to position 213, wherein the position numbers are with reference to SEQ ID NO:3 or SEQ ID NO:4. In various embodiments, the sAg polypeptide does not comprise a pre-S1 polypeptide. In various embodiments, the sAg polypeptide does not comprise a pre-S2 polypeptide. In some embodiments, the sAg polypeptide does not comprise an HBV pre-S2 polypeptide comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 79-83, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 79-83. In some embodiments, the sAg polypeptide does not comprise both of an HBV pre-S1 polypeptide and an HBV pre-S2 polypeptide. In some embodiments, the sAg polypeptide does not comprise an HBV pre-S1-pre-S2 polypeptide comprising or consisting of an amino acid sequence of any one of SEQ ID NO: 84-88, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 84-88. In various embodiments, the core-sAg fusion protein comprises a cleavable linker operably linked to and positioned between the HBV core polypeptide and the HBV sAg polypeptide. In some embodiments, the cleavable linker is a 2A cleavable peptide. In some embodiments, the cleavable linker is a 2A cleavable peptide selected from foot-and-mouth disease virus (F2A), equine rhinitis A virus (E2A), porcine teschovirus-1 (P2A) and Thosea asigna virus (T2A). In some embodiments, the cleavable linker is a porcine teschovirus-1 (P2A) linker. In some embodiments, the cleavable linker comprises or consists of an amino acid sequence of ATNFSLLKQAGDVEENPGP (SEQ ID NO: 56),

APVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 57), QCTNYALLKLAGDVESNPGP (SEQ ID NO: 58), or EGRGSLLTCGDVEENPGP (SEQ ID NO: 59), or an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or at least 99% identical to ATNFSLLKQAGDVEENPGP (SEQ ID NO: 56),

5 APVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 57), QCTNYALLKLAGDVESNPGP (SEQ ID NO: 58), or EGRGSLLTCGDVEENPGP (SEQ ID NO: 59). In some embodiments, the cleavable linker comprises or consists of an amino acid sequence of ATNFSLLKQAGDVEENPGP (SEQ ID NO: 56), or an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or at least 99% identical to

10 ATNFSLLKQAGDVEENPGP (SEQ ID NO: 56). In some embodiments, the core-sAg fusion protein comprises a flexible linker and/or a furin recognition/cleavage site operably linked to and positioned N-terminal to the cleavable linker and C-terminal to the HBV core polypeptide. In some embodiments, the furin recognition/cleavage site comprises or consists of an amino acid sequence selected from RAKR (SEQ ID NO: 60), REKR (SEQ ID NO: 61) and RRKR (SEQ ID

15 NO: 62). In some embodiments, the flexible linker comprises a polyglycine or polyalanine sequence. In some embodiments, the flexible linker comprises or consists of a polyglycine or polyalanine sequence selected from AA, AAA, AAY, GG, GGG, GGS, GSG and GGGS (SEQ ID NO: 63). In some embodiments, the core-sAg fusion protein is no longer than 450 amino acids in length, *e.g.*, no longer than 445, 440, 435, 430, 425, 420, 415 or 410 amino acids in

20 length. In some embodiments, the core-sAg fusion protein comprises or consists of an amino acid sequence of any one of SEQ ID NOs: 38-41, *e.g.*, SEQ ID NO: 41, or a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 38-41, *e.g.*, SEQ ID NO:41. In some embodiments, the fusion polypeptide comprises one or more of a serine (S) residue at the amino acid position corresponding to position 12, an

25 asparagine (N) residue at the amino acid position corresponding to position 67, a valine (V) residue at the amino acid position corresponding to position 74, a phenylalanine (F) residue at the amino acid position corresponding to position 97, a threonine (T) residue at the amino acid position corresponding to position 249, a threonine (T) residue at the amino acid position corresponding to position 250, a serine (S) residue at the amino acid position corresponding to

30 position 317, a serine (S) residue at the amino acid position corresponding to position 318, an arginine (R) residue at the amino acid position corresponding to position 326, a tyrosine (Y) residue at the amino acid position corresponding to position 338, a glycine (G) residue at the amino acid position corresponding to position 363, and an alanine (A) residue at the amino acid position corresponding to position 372, wherein the position numbers are with reference to SEQ

35 ID NO:41. In various embodiments, the core-sAg fusion polypeptide does not comprise an

amino sequence or fragment thereof from an HBV protein selected from the group consisting of X, pre-core, pre-S1 and pre-S2.

[0008]

With respect to the immunogenic HBV polypeptides, in some embodiments, the truncated HBV polymerase polypeptide, the HBV polymerase deletion mutant polypeptide, or the core-sAg fusion protein, as described herein, further comprise an N-terminal signal peptide or leader sequence. In various embodiments, the signal peptide or leader sequence is from a source protein selected from a serum protein, a cytokine, a chemokine, a chaperone protein, an invariant protein, and a protein that directs proteins to the lysosomal compartment. In various embodiments, the signal peptide or leader sequence is from a source protein selected from colony stimulating factor 2 (CSF2, GM-CSF), tissue type plasminogen activator (PLAT, t-PA), C-C motif chemokine ligand 7 (CCL7, MCP-3), C-X-C motif chemokine ligand 10 (CXCL10, IP-10), catenin beta 1 (CTNNB1), CD74 (p33; DHLAG; HLADG; Ia-GAMMA, invariant chain), serum albumin (ALB), polyubiquitin B/C (UBB/UBC), calreticulin (CALR), vesicular stomatitis virus G protein (VSV-G), lysosomal associated membrane protein 1 (LAMP-1) and lysosomal associated membrane protein 2 (LAMP-2). In some embodiments, the signal peptide or leader sequence is selected from an amino acid sequence of any one of SEQ ID NOs: 67-78, or a sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to any one of SEQ ID NOs: 67-78. In various embodiments, the truncated HBV polymerase polypeptide, the HBV polymerase deletion mutant polypeptide, and/or the core-sAg fusion protein, as described herein, can be recombinantly produced or chemically synthesized. In various embodiments, the truncated HBV polymerase polypeptide, the HBV polymerase deletion mutant polypeptide, and/or the core-sAg fusion protein, as described herein, are capable of inducing, promoting or stimulating an immune response (*e.g.*, expansion and/or activation of CD8+ and/or CD4+ T cells; production of antibodies that bind to and/or neutralize one or more of HBV polymerase, HBV core and HBV sAg) in a human. In various embodiments, the truncated HBV polymerase polypeptide, the HBV polymerase deletion mutant polypeptide, and/or the core-sAg fusion protein, as described herein, are capable of inducing, promoting or stimulating an immune response against HBV (*e.g.*, that prevents, delays progression of, inhibits and/or reverses HBV infection) in a human. In various embodiments, the truncated HBV polymerase polypeptide, the HBV polymerase deletion mutant polypeptide, and/or the core-sAg fusion protein, as described herein, are capable of inducing, promoting or stimulating proliferation and/or activation of one or more cell types selected from monocyte-derived dendritic cells (DCs), CD8+ T cells and CD4+ T cells.

[0009] In a further aspect, provided are polynucleotides encoding the immunogenic HBV polypeptides, as described herein. For example, provided are polynucleotides encoding one or more of the truncated HBV polymerase polypeptides, the HBV polymerase deletion mutant polypeptide, or the core-sAg fusion protein, as described herein. In some embodiments, the polynucleotide comprises cDNA, mRNA, self-amplifying RNA (SAM), self-replicating RNA, or self-amplifying replicon RNA (RepRNA). In some embodiments, polynucleotide comprises self-replicating or self-amplifying alphavirus replicons. In some embodiments, the polynucleotide comprises or consists of a nucleic acid sequence of any one of SEQ ID NOs: 27-37, *e.g.*, SEQ ID NOs: 37 and 89-94, *e.g.*, SEQ ID NOs: 29, 89, 90 or 92, or that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 27-37, *e.g.*, SEQ ID NO:37 and 89-94, *e.g.*, SEQ ID NOs: 29, 89, 90 or 92.

[0010] In another aspect, provided is a lipid nanoparticle (LNP) comprising one or more of the polynucleotides encoding an immunogenic HBV polypeptide, as described herein.

[0011] In another aspect, provided are expression cassettes comprising one or more of the polynucleotides encoding an immunogenic HBV polypeptide, as described herein, operably linked to one or more regulatory sequences. In some embodiments, the polynucleotide is operably linked to and under the control of a constitutive promoter. In some embodiments, the promoter is selected from cytomegalovirus major immediate-early (CMV), the CMV enhancer fused to the chicken beta-actin promoter (CAG), human elongation factor-1 α (HEF-1 α), mouse cytomegalovirus (mouse CMV), Chinese hamster elongation factor-1 α (CHEF-1 α), and phosphoglycerate kinase (PGK).

[0012] In another aspect, provided are comprising one or more of the polynucleotides encoding an immunogenic HBV polypeptide, as described herein, or one or more expression cassettes comprising such polynucleotides. In various embodiments, the vector is a plasmid vector, a bacterial vector or a viral vector. In some embodiments, the vector is a viral vector. In various embodiments, the viral vector is a DNA virus or an RNA virus. In some embodiments, the viral vector is from a virus selected from adenovirus, adeno-associated virus, arenavirus, alphavirus, poxvirus, cytomegalovirus, rhabdovirus, vesicular stomatitis virus, flavivirus, maraba virus and vaccinia virus. In some embodiments, the viral vector is from a virus from a taxonomic family selected from Adenoviridae, Arenaviridae, Herpesviridae (*e.g.* Cytomegalovirus), Poxviridae (*e.g.* Vaccinia virus, *e.g.* modified vaccinia Ankara (MVA)), Flaviviridae (*e.g.* Yellow fever virus), Rhabdoviridae (*e.g.* Vesiculovirus, *e.g.* Maraba vesiculovirus), Togaviridae (*e.g.*, Alphavirus). In some embodiments, the viral vector is an

arenavirus vector selected from Lymphocytic choriomeningitis mammarenavirus (LCMV), Cali
 mammarenavirus (*a.k.a.*, Pichinde mammarenavirus or Pichinde arenavirus (PICV)), Guanarito
 virus (GTOV), Junin virus (JUNV), Lassa virus (LASV), Lujo virus (LUJV), Machupo virus
 (MACV), Sabia virus (SABV), and Whitewater Arroyo virus (WWAV). In some embodiments,
 5 the viral vector is an arenavirus vector selected from Lymphocytic choriomeningitis
 mammarenavirus (LCMV) or Cali mammarenavirus (*a.k.a.*, Pichinde mammarenavirus or
 Pichinde arenavirus (PICV)). In some embodiments, the viral vector is a human adenovirus or a
 simian adenovirus (*e.g.*, a chimpanzee adenovirus, a gorilla adenovirus or a rhesus adenovirus).
 In some embodiments, the viral vector is an adenovirus vector selected from adenovirus
 10 serotype 5 (Ad5), adenovirus serotype 26 (Ad26), adenovirus serotype 34 (Ad34), adenovirus
 serotype 35 (Ad35), adenovirus serotype 48 (Ad48), chimpanzee adenovirus (*e.g.* ChAdOx1,
 ChAdOx2, ChAd3 (AdC3), ChAd5 (AdC5), ChAd6 (AdC6), ChAd7 (AdC7), ChAd8 (AdC8),
 ChAd9 (AdC9), ChAd10 (AdC10), ChAd11 (AdC11), ChAd17 (AdC17), ChAd16 (AdC16),
 ChAd19 (AdC19), ChAd20 (AdC20), ChAd22 (AdC22), ChAd24 (AdC24), ChAdY25, ChAd26
 15 (AdC26), ChAd28 (AdC28), ChAd30 (AdC30), ChAd31 (AdC31), ChAd37 (AdC37), ChAd38
 (AdC38), ChAd43 (AdC43), ChAd44 (AdC44), ChAd55 (AdC55), ChAd63 (AdC63),
 ChAdV63, ChAd68 (AdC68), ChAd73 (AdC73), ChAd82 (AdC82), ChAd83 (AdC83),
 ChAd143 (AdC143), ChAd144 (AdC144), ChAd145 (AdC145), ChAd147 (AdC147)), gorilla
 adenovirus (*e.g.* GC44, GC45, GC46) and rhesus adenovirus (*e.g.*, RhAd51, RhAd52, RhAd53,
 20 RhAd54, RhAd55, RhAd56, RhAd57, RhAd58, RhAd59, RhAd60, RhAd61, RhAd62, RhAd63,
 RhAd64, RhAd65, RhAd66). In some embodiments, the viral vector is replication-defective,
 replication-deficient, replication-attenuated or replication-competent. In some embodiments, the
 viral vector is a replication-defective arenavirus having a bi-segmented genome. In some
 embodiments, the viral vector is a replication-attenuated arenavirus having a tri-segmented
 25 genome.

[0013] In a further aspect, provided are arenavirus vectors. In one embodiment,
 provided is an arenavirus vector comprising a polynucleotide encoding an HBV core-sAg fusion
 polypeptide comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 38-
 41, *e.g.*, SEQ ID NO:41, or a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%,
 30 97%, 98% or 99% identical to any one of SEQ ID NOs: 38-41, *e.g.*, SEQ ID NO:41, and
 wherein the sAg polypeptide does not comprise an HBV pre-S1 polypeptide and/or an HBV pre-
 S2 polypeptide. In some embodiments, the core polypeptide comprises a serine (S) residue at
 the amino acid position corresponding to position 12, and an asparagine (N) residue at the amino
 acid position corresponding to position 67, wherein the position numbers are with reference to

SEQ ID NO:65 or SEQ ID NO:66. In some embodiments, the sAg polypeptide comprises an isoleucine (I) residue at the amino acid position corresponding to position 68, wherein the position numbers are with reference to SEQ ID NO:3 or SEQ ID NO:4. In some embodiments, the sAg polypeptide comprises one or more of a serine (S) residue at the amino acid position corresponding to position 53, an isoleucine (I) residue at the amino acid position corresponding to position 68, a threonine (T) residue at the amino acid position corresponding to position 125, a proline (P) residue at the amino acid position corresponding to position 127, an phenylalanine (F) residue at the amino acid position corresponding to position 161, a tyrosine (Y) residue at the amino acid position corresponding to position 200, a serine (S) residue at the amino acid position corresponding to position 210, and a leucine (L) residue at the amino acid position corresponding to position 213, wherein the position numbers are with reference to SEQ ID NO:3 or SEQ ID NO:4. In some embodiments, the core-sAg fusion polypeptide comprises one or more of a serine (S) residue at the amino acid position corresponding to position 12, an asparagine (N) residue at the amino acid position corresponding to position 67, a valine (V) residue at the amino acid position corresponding to position 74, a phenylalanine (F) residue at the amino acid position corresponding to position 97, a threonine (T) residue at the amino acid position corresponding to position 249, a threonine (T) residue at the amino acid position corresponding to position 250, a serine (S) residue at the amino acid position corresponding to position 317, a serine (S) residue at the amino acid position corresponding to position 318, an arginine (R) residue at the amino acid position corresponding to position 326, a tyrosine (Y) residue at the amino acid position corresponding to position 338, a glycine (G) residue at the amino acid position corresponding to position 363, and an alanine (A) residue at the amino acid position corresponding to position 372, wherein the position numbers are with reference to SEQ ID NO:41. In some embodiments, the polynucleotide comprises or consists of a nucleic acid sequence of any one of SEQ ID NOs: 33-37, or that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 33-37. In some embodiments, the polynucleotide comprises or consists of a nucleic acid sequence of SEQ ID NO: 37, or that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 37. In some embodiments, the arenavirus vector has a bisegmented genome and further comprises a polynucleotide encoding a truncated HBV polymerase comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 13-14, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 13-14, and wherein the truncated HBV polymerase does not comprise all of an HBV polymerase terminal

protein (TP) domain and does not comprise all or part of an HBV polymerase Spacer domain.

In some embodiments, the truncated HBV polymerase does not comprise a polypeptide sequence of SEQ ID NO: 50 or SEQ ID NO:51, or a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NO: 50 or SEQ ID

5 NO: 51. In some embodiments, the polynucleotide comprises or consists of a nucleic acid sequence of any one of SEQ ID NOs: 29 and 89-94, *e.g.*, SEQ ID NOs: 29, 89, 90 or 92, or that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 29 and 89-94, *e.g.*, SEQ ID NOs: 29, 89, 90 or 92. In some embodiments, the arenavirus vector is a Lymphocytic

10 choriomeningitis mammarenavirus (LCMV) vector and the polynucleotide comprises or consists of a nucleic acid sequence of SEQ ID NO: 29, or that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%

identical to any one of SEQ ID NO: 29. In some embodiments, the arenavirus vector is a Cali mammarenavirus (*a.k.a.*, Pichinde mammarenavirus or Pichinde arenavirus (PICV)) vector and

15 the polynucleotide comprises or consists of a nucleic acid sequence of SEQ ID NO: 90, or that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 90.

[0014] Further provided is an arenavirus vector comprising a polynucleotide encoding a truncated HBV polymerase comprising or consisting of an amino acid sequence of any one of

20 SEQ ID NOs: 13-14, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 13-14, and wherein the truncated HBV polymerase does not comprise all of an HBV polymerase terminal protein (TP) domain and does not comprise all or part of an HBV polymerase Spacer domain. In some embodiments, the truncated HBV polymerase does not

25 comprise a polypeptide sequence of SEQ ID NO: 50 or SEQ ID NO:51, or a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NO: 50 or SEQ ID NO: 51. In some embodiments, the polynucleotide comprises or consists of a nucleic acid sequence of any one of SEQ ID NOs: 29 and 89-94, *e.g.*, SEQ ID NOs: 29, 89, 90 or 92, or that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%,

30 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 29 and 89-94, *e.g.*, SEQ ID NOs: 29, 89, 90 or 92. In some embodiments, the arenavirus vector is a Lymphocytic choriomeningitis mammarenavirus (LCMV) vector and the polynucleotide comprises or consists of a nucleic acid sequence of SEQ ID NO: 29, or that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,

98% or 99% identical to any one of SEQ ID NO: 29. In some embodiments, the arenavirus vector is a Cali mammarenavirus vector and the polynucleotide comprises or consists of a nucleic acid sequence of SEQ ID NO: 90, or that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to

5 SEQ ID NO: 90. In some embodiments, the arenavirus vector is replication-defective, replication-deficient, or replication-incompetent.

[0015] In a further aspect, provided are host cells comprising one or more polynucleotides encoding one or more immunogenic HBV polypeptides, as described herein, or one or more vectors comprising such polynucleotides. In some embodiments, the one or more
10 polynucleotides encoding one or more immunogenic HBV polypeptides, as described herein, are not integrated into the host cell genome, *e.g.*, are episomal. In some embodiments, the one or more polynucleotides are integrated into the host cell genome. In some embodiments, the host cell is a mammalian cell, *e.g.*, a human cell. In various embodiments, the host cell can be *in vitro* or *in vivo*.

[0016] In another aspect, provided are immunogenic compositions comprising one or more of the immunogenic HBV polypeptides, as described herein. In some embodiments, the immunogenic composition comprises one or more, *e.g.*, two or more, of the truncated HBV polymerase polypeptides, one or more, *e.g.*, two or more, of the HBV polymerase deletion mutant polypeptides, and/or one or more, *e.g.*, two or more, of the core-sAg fusion protein, as
20 described herein. In some embodiments, the immunogenic composition comprises one or more, *e.g.*, two or more, polynucleotides encoding one or more, *e.g.*, two or more, of the truncated HBV polymerase polypeptides, one or more, *e.g.*, two or more, of the HBV polymerase deletion mutant polypeptides, and/or one or more, *e.g.*, two or more, of the core-sAg fusion protein, as described herein. In some embodiments, the immunogenic composition comprises one or more,
25 *e.g.*, two or more, one or more, *e.g.*, two or more, vectors comprising one or more, *e.g.*, two or more, polynucleotides encoding one or more, *e.g.*, two or more, of the truncated HBV polymerase polypeptides, one or more, *e.g.*, two or more, of the HBV polymerase deletion mutant polypeptides, and/or one or more, *e.g.*, two or more, of the core-sAg fusion protein, as described herein. The immunogenic compositions further comprise a pharmaceutically
30 acceptable carrier. In some embodiments, the immunogenic composition comprises one or more polynucleotides in the form of DNA, cDNA, mRNA, or self-replicating RNA. In various embodiments, the immunogenic composition comprises a first viral expression vector and a second viral expression vector, wherein: (a) the first viral expression vector comprises a polynucleotide encoding a truncated HBV polymerase polypeptide or an HBV polymerase

deletion mutant polypeptide, as described herein; and (b) the second viral expression vector comprises a polynucleotide encoding the core-sAg fusion protein, as described. In some embodiments, the immunogenic composition comprises a first viral expression vector and a second viral expression vector, wherein: (a) the first viral expression vector comprises a

5 polynucleotide encoding an HBV polymerase polypeptide mutant comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 5-14, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 5-14; and (b) the second viral expression vector comprises a polynucleotide encoding the core-sAg fusion protein comprising or

10 consisting of an amino acid sequence of any one of SEQ ID NOs: 38-41, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 38-41. In some embodiments, the immunogenic composition comprises a first viral expression vector and a second viral expression vector, wherein: (a) the first viral expression vector comprises a

15 polynucleotide encoding an HBV polymerase polypeptide mutant comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 13-14, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 13-14; and (b) the second viral expression vector comprises a polynucleotide encoding the core-sAg fusion protein comprising or

20 consisting of an amino acid sequence of any one of SEQ ID NOs: 38-41, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 38-41. In some embodiments, the immunogenic compositions comprise a first viral expression vector and a second viral expression vector, wherein: (a) the first viral expression vector comprises a

25 polynucleotide encoding an HBV polymerase polypeptide mutant comprising or consisting of an amino acid sequence of SEQ ID NO: 13, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 13; and (b) the second viral expression vector comprises a polynucleotide encoding the core-sAg fusion protein comprising or consisting of an amino acid

30 sequence of SEQ ID NO: 41, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 41. In some embodiments, the core polypeptide comprises a serine (S) residue at the amino acid position corresponding to position 12, and an asparagine (N) residue at the amino acid position corresponding to position 67, wherein the position numbers are with reference to

35 SEQ ID NO:65 or SEQ ID NO:66. In some embodiments, the sAg polypeptide comprises an

isoleucine (I) residue at the amino acid position corresponding to position 68, wherein the position numbers are with reference to SEQ ID NO:3 or SEQ ID NO:4. In some embodiments, the sAg polypeptide comprises one or more of a serine (S) residue at the amino acid position corresponding to position 53, an isoleucine (I) residue at the amino acid position corresponding to position 68, a threonine (T) residue at the amino acid position corresponding to position 125, a proline (P) residue at the amino acid position corresponding to position 127, an phenylalanine (F) residue at the amino acid position corresponding to position 161, a tyrosine (Y) residue at the amino acid position corresponding to position 200, a serine (S) residue at the amino acid position corresponding to position 210, and a leucine (L) residue at the amino acid position corresponding to position 213, wherein the position numbers are with reference to SEQ ID NO:3 or SEQ ID NO:4. In some embodiments, the core-sAg fusion polypeptide comprises one or more of a serine (S) residue at the amino acid position corresponding to position 12, an asparagine (N) residue at the amino acid position corresponding to position 67, a valine (V) residue at the amino acid position corresponding to position 74, a phenylalanine (F) residue at the amino acid position corresponding to position 97, a threonine (T) residue at the amino acid position corresponding to position 249, a threonine (T) residue at the amino acid position corresponding to position 250, a serine (S) residue at the amino acid position corresponding to position 317, a serine (S) residue at the amino acid position corresponding to position 318, an arginine (R) residue at the amino acid position corresponding to position 326, a tyrosine (Y) residue at the amino acid position corresponding to position 338, a glycine (G) residue at the amino acid position corresponding to position 363, and an alanine (A) residue at the amino acid position corresponding to position 372, wherein the position numbers are with reference to SEQ ID NO:41. In some embodiments, the immunogenic compositions comprise a first viral expression vector and a second viral expression vector, wherein: (a) the first viral expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of any one of SEQ ID NOs: 27-32 and 89-94 *e.g.*, SEQ ID NOs: 29, 89, 90 or 92, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 27-32 and 89-94 *e.g.*, SEQ ID NOs: 29, 89, 90 or 92; and (b) the second viral expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of any one of SEQ ID NOs: 33-37 or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 33-37. In some embodiments, the immunogenic composition comprises a first viral expression vector and a second viral expression vector, wherein: (a) the first viral expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 29 or 90, or

a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 29 or 90; and (b) the second viral expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 37 or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NO: 37. In various embodiments, the first viral expression vector and the second viral expression vector are independently from a taxonomic family selected from Adenoviridae, Arenaviridae, Herpesviridae (*e.g.* Cytomegalovirus), Poxviridae (*e.g.* Vaccinia virus, *e.g.* modified vaccinia Ankara (MVA)), Flaviviridae (*e.g.* Yellow fever virus), Rhabdoviridae (*e.g.* Vesiculovirus, *e.g.* Maraba vesiculovirus), Togaviridae (*e.g.*, Alphavirus). In various embodiments, the first viral expression vector and the second viral expression vector in the immunogenic composition can be from the same taxonomic family or different taxonomic families. In some embodiments, the first viral expression vector and the second viral expression vector in the immunogenic composition are from Arenaviridae. In some embodiments, the first viral expression vector and the second viral expression vector in the immunogenic composition are independently from an arenavirus vector selected from Lymphocytic choriomeningitis mammarenavirus (LCMV), Cali mammarenavirus (*a.k.a.*, Pichinde mammarenavirus or Pichinde arenavirus (PICV)), Guanarito virus (GTOV), Junin virus (JUNV), Lassa virus (LASV), Lujo virus (LUJV), Machupo virus (MACV), Sabia virus (SABV), and Whitewater Arroyo virus (WWAV). In some embodiments, the first viral expression vector and the second viral expression vector are independently from an arenavirus vector selected from Lymphocytic choriomeningitis mammarenavirus (LCMV) or Cali mammarenavirus (*a.k.a.*, Pichinde mammarenavirus or Pichinde arenavirus (PICV)). In some embodiments, the first viral expression vector and the second viral expression vector are replication-defective or replication-deficient. In some embodiments, the first viral expression vector and the second viral expression vector are replication-attenuated. In some embodiments, the immunogenic composition comprises a first LCMV arenavirus expression vector and a second LCMV arenavirus expression vector, wherein: (a) the first LCMV arenavirus expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 29, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 29; and (b) the second LCMV arenavirus expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 37 or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NO: 37. In some embodiments, the immunogenic

composition comprises a first Pichinde arenavirus expression vector and a second Pichinde arenavirus expression vector, wherein: (a) the first Pichinde arenavirus expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 90, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 90; and (b) the second Pichinde arenavirus expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 37 or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NO: 37. In various embodiments, the first viral expression vector and the second viral expression vector are provided in the immunogenic composition in a ratio in the range of from 1:10 to 10:1, *e.g.*, 1:9 to 9:1, 1:8 to 8:1, 1:7 to 7:1, 1:6 to 6:1, 1:5 to 5:1, 1:4 to 4:1, 1:3 to 3:1, 1:2 to 2:1 or 1:1. In some embodiments, the immunogenic composition comprises in the range of about 10^3 to about 10^{12} viral focus forming units (FFU) or plaque forming units (PFU) or infectious units (IU) or viral particles (vp) per milliliter, *e.g.* from about 10^4 to about 10^7 viral FFU or PFU or IU or vp per milliliter, *e.g.* from about 10^3 to about 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} or 10^{12} viral FFU or PFU or IU or vp per milliliter, of each of the first viral expression vector and the second viral expression vector. In some embodiments, the immunogenic composition further comprises one or more of an adjuvant, a detergent, a micelle-forming agent, and an oil. In various embodiments, the immunogenic composition is formulated for administration via a route selected from intravenous, intramuscular, intradermal, subcutaneous and mucosal (*e.g.* buccal, intranasal, intrarectal, intravaginal). In some embodiments, the immunogenic composition is an aqueous solution or suspension, *e.g.*, is formulated as a liquid. In some embodiments, the immunogenic composition is lyophilized.

[0017] In a further aspect, provided are kits. In various embodiments, the kit comprises one or more, *e.g.*, two or more, unitary doses of one or more, *e.g.*, two or more, of a truncated HBV polymerase polypeptide, one or more, *e.g.*, two or more, of an HBV polymerase deletion mutant polypeptide and/or one or more, *e.g.*, two or more, of a core-sAg fusion protein, as described herein. In some embodiments, the kit comprises one or more, *e.g.*, two or more, unitary doses of one or more, *e.g.*, two or more, polynucleotides encoding one or more, *e.g.*, two or more, of a truncated HBV polymerase polypeptide, one or more, *e.g.*, two or more, of an HBV polymerase deletion mutant polypeptide and/or one or more, *e.g.*, two or more, of a core-sAg fusion protein, as described herein. In some embodiments, the kit comprises one or more, *e.g.*, two or more, unitary doses of one or more, *e.g.*, two or more, vectors comprising one or

more, *e.g.*, two or more, polynucleotides encoding one or more, *e.g.*, two or more, of a truncated HBV polymerase polypeptide, one or more, *e.g.*, two or more, of an HBV polymerase deletion mutant polypeptide and/or one or more, *e.g.*, two or more, of a core-sAg fusion protein, as described herein. In various embodiments, the kit comprises one or more, *e.g.*, two or more, unitary doses of one or more, *e.g.*, two or more, immunogenic compositions, as described herein. In some embodiments, the one or more unitary doses in the kit are in a single container. In some embodiments, the one or more unitary doses in the kit are in two or more separate containers. In some embodiments, the kit comprises one or more containers selected from vials, ampules and pre-loaded syringes. In some embodiments, the kit comprises one or more containers comprising the one or more polypeptides, one or more polynucleotides, one or more vectors or one or more immunogenic compositions in an aqueous solution or suspension, or as a lyophilized preparation. In various embodiments, the one or more unitary doses can be the same or different. In some embodiments, the kit comprises one or more unitary doses of one or more viral vectors, as described herein, wherein the unitary doses are in the range of about 10^3 to about 10^{12} viral focus forming units (FFU) or plaque forming units (PFU) or infectious units (IU) or viral particles (vp), *e.g.* from about 10^4 to about 10^7 viral FFU or PFU or IU or vp, *e.g.* from about 10^3 to about 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} or 10^{12} viral FFU or PFU or IU or vp. In some embodiments, the kit comprises one or more polynucleotides encoding, or one or more vectors expressing, or an immunogenic composition comprising, at least two immunogenic polypeptides, the immunogenic polypeptides comprising: (a) an HBV polymerase polypeptide mutant comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 5-14, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 5-14; and (b) an HBV core-sAg fusion protein comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 38-41, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 38-41. In some embodiments, the kit comprises one or more polynucleotides encoding, or one or more vectors expressing, or an immunogenic composition comprising, at least two immunogenic polypeptides, the immunogenic polypeptides comprising: (a) an HBV polymerase polypeptide mutant comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 13-14, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 13-14; and (b) an HBV core-sAg fusion protein comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 38-41, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%,

95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 38-41. In some embodiments, the kit comprises one or more polynucleotides encoding, or one or more vectors expressing, or an immunogenic composition comprising, at least two immunogenic polypeptides, the immunogenic polypeptides comprising: (a) an HBV polymerase polypeptide mutant comprising or consisting of an amino acid sequence of SEQ ID NO: 13, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 13; and (b) an HBV core-sAg fusion protein comprising or consisting of an amino acid sequence of SEQ ID NO: 41, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 41. In some embodiments, the core polypeptide comprises a serine (S) residue at the amino acid position corresponding to position 12, and an asparagine (N) residue at the amino acid position corresponding to position 67, wherein the position numbers are with reference to SEQ ID NO:65 or SEQ ID NO:66. In some embodiments, the sAg polypeptide comprises an isoleucine (I) residue at the amino acid position corresponding to position 68, wherein the position numbers are with reference to SEQ ID NO:3 or SEQ ID NO:4. In some embodiments, the sAg polypeptide comprises one or more of a serine (S) residue at the amino acid position corresponding to position 53, an isoleucine (I) residue at the amino acid position corresponding to position 68, a threonine (T) residue at the amino acid position corresponding to position 125, a proline (P) residue at the amino acid position corresponding to position 127, an phenylalanine (F) residue at the amino acid position corresponding to position 161, a tyrosine (Y) residue at the amino acid position corresponding to position 200, a serine (S) residue at the amino acid position corresponding to position 210, and a leucine (L) residue at the amino acid position corresponding to position 213, wherein the position numbers are with reference to SEQ ID NO:3 or SEQ ID NO:4. In some embodiments, the core-sAg fusion polypeptide comprises one or more of a serine (S) residue at the amino acid position corresponding to position 12, an asparagine (N) residue at the amino acid position corresponding to position 67, a valine (V) residue at the amino acid position corresponding to position 74, a phenylalanine (F) residue at the amino acid position corresponding to position 97, a threonine (T) residue at the amino acid position corresponding to position 249, a threonine (T) residue at the amino acid position corresponding to position 250, a serine (S) residue at the amino acid position corresponding to position 317, a serine (S) residue at the amino acid position corresponding to position 318, an arginine (R) residue at the amino acid position corresponding to position 326, a tyrosine (Y) residue at the amino acid position corresponding to position 338, a glycine (G) residue at the amino acid position corresponding to position 363, and an alanine (A) residue at the amino acid

position corresponding to position 372, wherein the position numbers are with reference to SEQ ID NO:41. In some embodiments, the kit comprises first and second vectors encoding first and second immunogenic polypeptides, respectively, the first and second immunogenic polypeptides comprising, respectively: (a) an HBV polymerase polypeptide mutant comprising or consisting of an amino acid sequence of SEQ ID NO: 13, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 13; and (b) an HBV core-sAg fusion protein comprising or consisting of an amino acid sequence of SEQ ID NO: 41, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 41. In some embodiments, the kit comprises a first viral expression vector and a second viral expression vector, wherein: (a) the first viral expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of any one of SEQ ID NOs: 27-32 and 89-94, *e.g.*, SEQ ID NOs: 29, 89, 90 or 92, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 27-32 and 89-94, *e.g.*, SEQ ID NOs: 29, 89, 90 or 92; and (b) the second viral expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of any one of SEQ ID NOs: 33-37, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 33-37. In some embodiments, the kit comprises a first viral expression vector and a second viral expression vector, wherein: (a) the first viral expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 29 or 90, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 29 or 90; and (b) the second viral expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 37, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 37. In some embodiments, the kit comprises one or more unitary doses of an immunogenic composition comprising first and second viral expression vectors, as described herein, wherein the first and second viral expression vectors comprise a replication-deficient or replication-defective Cali mammarenavirus (*a.k.a.*, Pichinde mammarenavirus or Pichinde arenavirus (PICV)). In some embodiments, the kit comprises one or more unitary doses of an immunogenic composition comprising first and second viral expression vectors, as described herein, wherein the first and second viral expression vectors comprise a replication-deficient or replication-defective Lymphocytic choriomeningitis mammarenavirus (LCMV). In

some embodiments, the kit comprises (a) one or more unitary doses of an immunogenic composition, as described herein, wherein the first and second viral expression vectors are from Adenoviridae; and (b) one or more unitary doses of an immunogenic composition, as described herein, wherein the first and second viral expression vectors are from Poxviridae (*e.g.*, Vaccinia virus, *e.g.*, modified vaccinia Ankara (MVA)). In some embodiments, the kit comprises (a) one or more unitary doses of an immunogenic composition, as described herein, wherein the first and second viral expression vectors are from Arenaviridae; and (b) one or more unitary doses of an immunogenic composition, as described herein, wherein the first and second viral expression vectors are from Adenoviridae. In some embodiments, the kit comprises a first LCMV arenavirus expression vector and a second LCMV arenavirus expression vector, wherein: (a) the first LCMV arenavirus expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 29, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 29; and (b) the second LCMV arenavirus expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 37, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 37. In some embodiments, the kit comprises a first Pichinde arenavirus expression vector and a second Pichinde arenavirus expression vector, wherein: (a) the first Pichinde arenavirus expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 90, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 90; and (b) the second Pichinde arenavirus expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 37, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 37. In various embodiment, the kit further comprises one or more unitary doses of one or more additional therapeutic agents. In some embodiments, the kit further comprises one or more agonists or activators of one or more toll-like receptors (TLRs). In some embodiments, the kit further comprises one or more TLR agonists or activators selected from a TLR2 agonist, a TLR3 agonist, a TLR4 agonist, a TLR5 agonist, a TLR7 agonist, a TLR8 agonist and a TLR9 agonist. In some embodiments, the kit further comprises a TLR7 agonist selected from GS 9620 (vesatolimod), R848 (Resiquimod), DS-0509, LHC-165 and TMX-101 (imiquimod). In some embodiments, the kit further comprises a TLR8 agonist selected from GS-9688, R848 (Resiquimod) and NKTR-262 (dual TLR7/TLR8 agonist). In some embodiments, the kit further comprises one or more interleukin

receptor agonists of an interleukin receptor selected from IL-2, IL-7, IL-12 and IL-15. In some embodiments, the kit further comprises one or more cytokines selected from IL-2, IL-7, IL-12, IL-15, and variants thereof. In some embodiments, the kit further comprises one or more innate immune activators. In some embodiments, the kit further comprises one or more innate immune

5 activators comprising an agonist of a receptor selected from fms related tyrosine kinase 3 (FLT3), stimulator of interferon genes (STING) receptor, DExD/H-box helicase 58 (DDX58; *a.k.a.*, RIG-I), nucleotide binding oligomerization domain containing 2 (NOD2). In some embodiments, the kit further comprises one or more unitary doses of GS-3583 and/or GS-9992. In some embodiments, the kit further comprises one or more antagonists or inhibitors of an

10 inhibitory immune checkpoint protein or receptor and/or one or more activators or agonists of a stimulatory immune checkpoint protein or receptor. In some embodiments, the kit further comprises one or more immune checkpoint proteins or receptors selected from CD27, CD70; CD40, CD40LG; CD47, CD48 (SLAMF2), transmembrane and immunoglobulin domain containing 2 (TMIGD2, CD28H), CD84 (LY9B, SLAMF5), CD96, CD160, MS4A1 (CD20),

15 CD244 (SLAMF4); CD276 (B7H3); V-set domain containing T cell activation inhibitor 1 (VTCN1, B7H4); V-set immunoregulatory receptor (VSIR, B7H5, VISTA); immunoglobulin superfamily member 11 (IGSF11, VSIG3); natural killer cell cytotoxicity receptor 3 ligand 1 (NCR3LG1, B7H6); HERV-H LTR-associating 2 (HHLA2, B7H7); inducible T cell co-stimulator (ICOS, CD278); inducible T cell co-stimulator ligand (ICOSLG, B7H2); TNF

20 receptor superfamily member 4 (TNFRSF4, OX40); TNF superfamily member 4 (TNFSF4, OX40L); TNFRSF8 (CD30), TNFSF8 (CD30L); TNFRSF10A (CD261, DR4, TRAILR1), TNFRSF9 (CD137), TNFSF9 (CD137L); TNFRSF10B (CD262, DR5, TRAILR2), TNFRSF10 (TRAIL); TNFRSF14 (HVEM, CD270), TNFSF14 (HVEML); CD272 (B and T lymphocyte associated (BTLA)); TNFRSF17 (BCMA, CD269), TNFSF13B (BAFF); TNFRSF18 (GITR),

25 TNFSF18 (GITRL); MHC class I polypeptide-related sequence A (MICA); MHC class I polypeptide-related sequence B (MICB); CD274 (CD274, PDL1, PD-L1); programmed cell death 1 (PDCD1, PD1, PD-1); cytotoxic T-lymphocyte associated protein 4 (CTLA4, CD152); CD80 (B7-1), CD28; nectin cell adhesion molecule 2 (NECTIN2, CD112); CD226 (DNAM-1); Poliovirus receptor (PVR) cell adhesion molecule (PVR, CD155); PVR related immunoglobulin

30 domain containing (PVRIG, CD112R); T cell immunoreceptor with Ig and ITIM domains (TIGIT); T cell immunoglobulin and mucin domain containing 4 (TIMD4; TIM4); hepatitis A virus cellular receptor 2 (HAVCR2, TIMD3, TIM3); galectin 9 (LGALS9); lymphocyte activating 3 (LAG3, CD223); signaling lymphocytic activation molecule family member 1 (SLAMF1, SLAM, CD150); lymphocyte antigen 9 (LY9, CD229, SLAMF3); SLAM family

35 member 6 (SLAMF6, CD352); SLAM family member 7 (SLAMF7, CD319); UL16 binding

protein 1 (ULBP1); UL16 binding protein 2 (ULBP2); UL16 binding protein 3 (ULBP3); retinoic acid early transcript 1E (RAET1E; ULBP4); retinoic acid early transcript 1G (RAET1G; ULBP5); retinoic acid early transcript 1L (RAET1L; ULBP6); lymphocyte activating 3 (CD223); killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 1 (KIR, CD158E1); killer cell lectin like receptor C1 (KLRC1, NKG2A, CD159A); killer cell lectin like receptor K1 (KLRK1, NKG2D, CD314); killer cell lectin like receptor C2 (KLRC2, CD159c, NKG2C); killer cell lectin like receptor C3 (KLRC3, NKG2E); killer cell lectin like receptor C4 (KLRC4, NKG2F); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 1 (KIR2DL1); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 2 (KIR2DL2); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 3 (KIR2DL3); killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 1 (KIR3DL1); killer cell lectin like receptor D1 (KLRD1); and SLAM family member 7 (SLAMF7). In some embodiments, the kit further comprises one or more blockers or inhibitors of one or more T-cell inhibitory immune checkpoint proteins or receptors. In some embodiments, the kit further comprises one or more T-cell inhibitory immune checkpoint proteins or receptors selected from CD274 (CD274, PDL1, PD-L1); programmed cell death 1 ligand 2 (PDCD1LG2, PD-L2, CD273); programmed cell death 1 (PDCD1, PD1, PD-1); cytotoxic T-lymphocyte associated protein 4 (CTLA4, CD152); CD276 (B7H3); V-set domain containing T cell activation inhibitor 1 (VTCN1, B7H4); V-set immunoregulatory receptor (VSIR, B7H5, VISTA); immunoglobulin superfamily member 11 (IGSF11, VSIG3); TNFRSF14 (HVEM, CD270), TNFSF14 (HVEM); CD272 (B and T lymphocyte associated (BTLA)); PVR related immunoglobulin domain containing (PVRIG, CD112R); T cell immunoreceptor with Ig and ITIM domains (TIGIT); lymphocyte activating 3 (LAG3, CD223); hepatitis A virus cellular receptor 2 (HAVCR2, TIMD3, TIM3); galectin 9 (LGALS9); killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 1 (KIR, CD158E1); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 1 (KIR2DL1); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 2 (KIR2DL2); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 3 (KIR2DL3); and killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 1 (KIR3DL1). In some embodiments, the kit further comprises one or more agonists or activators of one or more T-cell stimulatory immune checkpoint proteins or receptors. In some embodiments, the kit further comprises one or more T-cell stimulatory immune checkpoint proteins or receptors selected from CD27, CD70; CD40, CD40LG; inducible T cell co-stimulator (ICOS, CD278); inducible T cell co-stimulator ligand (ICOSLG, B7H2); TNF receptor superfamily member 4 (TNFRSF4, OX40); TNF superfamily

member 4 (TNFSF4, OX40L); TNFRSF9 (CD137), TNFSF9 (CD137L); TNFRSF18 (GITR), TNFSF18 (GITRL); CD80 (B7-1), CD28; nectin cell adhesion molecule 2 (NECTIN2, CD112); CD226 (DNAM-1); Poliovirus receptor (PVR) cell adhesion molecule (PVR, CD155). In some embodiments, the kit further comprises one or more unitary doses of AGEN-2373 and/or

5 AGEN-1223. In some embodiments, the kit further comprises one or more blockers or inhibitors of one or more NK-cell inhibitory immune checkpoint proteins or receptors. In some embodiments, the kit further comprises one or more NK-cell inhibitory immune checkpoint proteins or receptors selected from killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 1 (KIR, CD158E1); killer cell immunoglobulin like receptor, two Ig

10 domains and long cytoplasmic tail 1 (KIR2DL1); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 2 (KIR2DL2); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 3 (KIR2DL3); killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 1 (KIR3DL1); killer cell lectin like receptor C1 (KLRC1, NKG2A, CD159A); and killer cell lectin like receptor D1 (KLRD1, CD94). In

15 some embodiments, the kit further comprises one or more agonists or activators of one or more NK-cell stimulatory immune checkpoint proteins or receptors. In some embodiments, the kit further comprises one or more NK-cell stimulatory immune checkpoint proteins or receptors selected from CD16, CD226 (DNAM-1); killer cell lectin like receptor K1 (KLRK1, NKG2D, CD314); and SLAM family member 7 (SLAMF7). In some embodiments, the kit further

20 comprises one or more proteinaceous inhibitors of PD-L1 (CD274), PD-1 (PDCD1) and/or CTLA4. In some embodiments, the kit further comprises one or more proteinaceous inhibitors of CTLA4 selected from ipilimumab, tremelimumab, BMS-986218, AGEN1181, AGEN1884, BMS-986249, MK-1308, REGN-4659, ADU-1604, CS-1002, BCD-145, APL-509, JS-007, BA-3071, ONC-392, AGEN-2041, JHL-1155, KN-044, CG-0161, ATOR-1144, PBI-5D3H5, FPT-

25 155 (CTLA4/PD-L1/CD28), PF-06936308 (PD-1/CTLA4), MGD-019 (PD-1/CTLA4), KN-046 (PD-1/CTLA4), MEDI-5752 (CTLA4/PD-1), XmAb-20717 (PD-1/CTLA4) and AK-104 (CTLA4/PD-1). In some embodiments, the kit further comprises one or more proteinaceous inhibitors of PD-L1 (CD274) or PD-1 (PDCD1) selected from zimberelimab (AB122), pembrolizumab, nivolumab, cemiplimab, pidilizumab, AMP-224, MEDI0680 (AMP-514),

30 spartalizumab, atezolizumab, avelumab, ASC22, durvalumab, BMS-936559, CK-301, PF-06801591, BGB-A317 (tisnelizumab), GLS-010 (WBP-3055), AK-103 (HX-008), AK-105, CS-1003, HLX-10, MGA-012, BI-754091, AGEN-2034, JS-001 (toripalimab), JNJ-63723283, genolimzumab (CBT-501), LZM-009, BCD-100, LY-3300054, SHR-1201, SHR-1210 (camrelizumab), Sym-021, ABBV-181, PD1-PIK, BAT-1306, (MSB0010718C), CX-072, CBT-

35 502, TSR-042 (dostarlimab), MSB-2311, JTX-4014, BGB-A333, SHR-1316, CS-1001 (WBP-

3155, KN-035, IBI-308 (sintilimab), HLX-20, KL-A167, STI-A1014, STI-A1015 (IMC-001), BCD-135, FAZ-053, TQB-2450, MDX1105-01, FPT-155 (CTLA4/PD-L1/CD28), PF-06936308 (PD-1/CTLA4), MGD-013 (PD-1/LAG-3), FS-118 (LAG-3/PD-L1) MGD-019 (PD-1/CTLA4), KN-046 (PD-1/CTLA4), MEDI-5752 (CTLA4/PD-1), RO-7121661 (PD-1/TIM-3), XmAb-20717 (PD-1/CTLA4), AK-104 (CTLA4/PD-1), M7824 (PD-L1/TGFβ-EC domain), CA-170 (PD-L1/VISTA), CDX-527 (CD27/PD-L1), LY-3415244 (TIM3/PDL1), and INBRX-105 (4-1BB/PDL1). In some embodiments, the kit further comprises one or more small molecule inhibitors of CD274 (PDL1, PD-L1), programmed cell death 1 (PDCD1, PD1, PD-1) and/or CTLA4. In some embodiments, the kit further comprises one or more small molecule inhibitors of CD274 or PDCD1 selected from GS-4224, GS-4416, INCB086550 and MAX10181. In some embodiments, the kit further comprises the small molecule inhibitor of CTLA4, BPI-002. In some embodiments, the kit further comprises one or more anti-viral agents. In some embodiments, the kit further comprises one or more antiviral agents selected from lamivudine (LAM), adefovir dipivoxil (ADV), entecavir (ETV), telbivudine (LdT), tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF or VEMSIDY®) and ledipasvir + sofosbuvir (HARVONI®). In some embodiments, the kit further comprises one or more therapeutic agents selected from HBV antigen inhibitors (*e.g.*, HBV core antigen (HBcAg) inhibitors, HBV surface antigen (HBsAg) inhibitors, HBx inhibitors, HBV E antigen inhibitors), anti-HBV antigen antibodies, inhibitory nucleic acids targeting HBV (*e.g.*, antisense oligonucleotide, short interfering RNA (siRNA), DNA-directed RNA interference (ddRNAi)), gene editors targeting HBV (*e.g.*, CRISPR-Cas (*e.g.*, Cas9, Cas12, Cascade, Cas13), zinc finger nucleases, homing endonucleases, homing meganucleases (*e.g.*, ARCUS), synthetic nucleases, TALENs), covalently closed circular DNA (cccDNA) inhibitors and HBsAg secretion or assembly inhibitors and HBV viral entry inhibitors.

[0018] In a further aspect, provided are methods for eliciting an immune response to human hepatitis B virus (HBV) in a subject in need thereof. Also provided are methods of treating or preventing human hepatitis B virus (HBV) in a subject in need thereof. In some embodiments, the methods comprise administering to the subject a therapeutically effective amount of one or more immunogenic compositions, as described herein. In some embodiments, the methods entail administering one or more immunogenic compositions comprising a mixture comprising a first viral expression vector and a second viral expression vector, wherein: (a) the first viral expression vector comprises a polynucleotide encoding a truncated HBV polymerase polypeptide or a HBV polymerase deletion mutant polypeptide, as described herein; and (b) the second viral expression vector comprises a polynucleotide encoding the core-sAg fusion protein,

as described herein. In some embodiments, the methods entail administering to the subject a therapeutically effective amount of one or more immunogenic compositions comprising a mixture comprising a first viral expression vector and a second viral expression vector, wherein:

(a) the first viral expression vector comprises a polynucleotide encoding an HBV polymerase polypeptide mutant comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 5-14, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 5-14; and (b) the second viral expression vector comprises a polynucleotide encoding the core-sAg fusion protein comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 38-41, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 38-41. In some embodiments, the methods entail administering to the subject a

therapeutically effective amount of one or more immunogenic compositions comprising a mixture comprising a first viral expression vector and a second viral expression vector, wherein:

(a) the first viral expression vector comprises a polynucleotide encoding an HBV polymerase polypeptide mutant comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 13-14, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 13-14; and (b) the second viral expression vector comprises a polynucleotide encoding the core-sAg fusion protein comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 38-41, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 38-41. In some embodiments, the methods entail administering to the subject a

therapeutically effective amount of one or more immunogenic compositions comprising a mixture comprising a first viral expression vector and a second viral expression vector, wherein:

(a) the first viral expression vector comprises a polynucleotide encoding an HBV polymerase polypeptide mutant comprising or consisting of an amino acid sequence of SEQ ID NO: 13, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 13; and (b) the second

viral expression vector comprises a polynucleotide encoding the core-sAg fusion protein comprising or consisting of an amino acid sequence of SEQ ID NO: 41, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 41. In some embodiments, the core polypeptide comprises a serine (S) residue at the amino acid position corresponding to position

12, and an asparagine (N) residue at the amino acid position corresponding to position 67,

wherein the position numbers are with reference to SEQ ID NO:65 or SEQ ID NO:66. In some embodiments, the sAg polypeptide comprises an isoleucine (I) residue at the amino acid position corresponding to position 68, wherein the position numbers are with reference to SEQ ID NO:3 or SEQ ID NO:4. In some embodiments, the sAg polypeptide comprises one or more of a serine (S) residue at the amino acid position corresponding to position 53, an isoleucine (I) residue at the amino acid position corresponding to position 68, a threonine (T) residue at the amino acid position corresponding to position 125, a proline (P) residue at the amino acid position corresponding to position 127, an phenylalanine (F) residue at the amino acid position corresponding to position 161, a tyrosine (Y) residue at the amino acid position corresponding to position 200, a serine (S) residue at the amino acid position corresponding to position 210, and a leucine (L) residue at the amino acid position corresponding to position 213, wherein the position numbers are with reference to SEQ ID NO:3 or SEQ ID NO:4. In some embodiments, the core-sAg fusion polypeptide comprises one or more of a serine (S) residue at the amino acid position corresponding to position 12, an asparagine (N) residue at the amino acid position corresponding to position 67, a valine (V) residue at the amino acid position corresponding to position 74, a phenylalanine (F) residue at the amino acid position corresponding to position 97, a threonine (T) residue at the amino acid position corresponding to position 249, a threonine (T) residue at the amino acid position corresponding to position 250, a serine (S) residue at the amino acid position corresponding to position 317, a serine (S) residue at the amino acid position corresponding to position 318, an arginine (R) residue at the amino acid position corresponding to position 326, a tyrosine (Y) residue at the amino acid position corresponding to position 338, a glycine (G) residue at the amino acid position corresponding to position 363, and an alanine (A) residue at the amino acid position corresponding to position 372, wherein the position numbers are with reference to SEQ ID NO:41. In some embodiments, the methods entail administering to the subject a therapeutically effective amount of one or more immunogenic compositions comprising a mixture comprising a first viral expression vector and a second viral expression vector, wherein: (a) the first viral expression vector comprises a polynucleotide comprising or consisting of a nucleic sequence of any one of SEQ ID NOs: 27-32 and 89-94, *e.g.*, SEQ ID NOs: 29, 89, 90 or 92, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 27-32 and 89-94, *e.g.*, SEQ ID NOs: 29, 89, 90 or 92; and (b) the second viral expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of any one of SEQ ID NOs: 33-37, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 33-37. In some embodiments, the

methods comprise administering to the subject a therapeutically effective amount of one or more immunogenic compositions comprising a mixture comprising a first viral expression vector and a second viral expression vector, wherein: (a) the first viral expression vector comprises a polynucleotide comprising or consisting of a nucleic sequence of SEQ ID NO: 29 or 90, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 29 or 90; and (b) the second viral expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 37, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 37. In some embodiments of the methods, the first viral expression vector and the second viral expression vector are from Arenaviridae. In some embodiments of the methods, the first viral expression vector and the second viral expression vector are from an arenavirus vector selected from Lymphocytic choriomeningitis mammarenavirus (LCMV), Cali mammarenavirus (*a.k.a.*, Pichinde mammarenavirus or Pichinde arenavirus (PICV)), Guanarito virus (GTOV), Junin virus (JUNV), Lassa virus (LASV), Lujo virus (LUJV), Machupo virus (MACV), Sabia virus (SABV), and Whitewater Arroyo virus (WWAV). In some embodiments of the methods, the first viral expression vector and the second viral expression vector are from an arenavirus vector selected from Lymphocytic choriomeningitis mammarenavirus (LCMV) or Cali mammarenavirus (*a.k.a.*, Pichinde mammarenavirus or Pichinde arenavirus (PICV)). In some embodiments of the methods, the first viral expression vector and the second viral expression vector are replication-defective or replication-deficient. In some embodiments of the methods, the first viral expression vector and the second viral expression vector are replication-attenuated. In some embodiments, the methods comprise administering to the subject a therapeutically effective amount of one or more immunogenic compositions comprising a mixture comprising a first LCMV arenavirus expression vector and a second LCMV arenavirus expression vector, wherein: (a) the first LCMV arenavirus expression vector comprises a polynucleotide comprising or consisting of a nucleic sequence of SEQ ID NO: 29, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 29; and (b) the second LCMV arenavirus expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 37, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 37. In some embodiments, the methods comprise administering to the subject a therapeutically effective amount of one or more immunogenic compositions comprising a mixture comprising a first Pichinde arenavirus expression vector and a second

Pichinde arenavirus expression vector, wherein: (a) the first Pichinde arenavirus expression vector comprises a polynucleotide comprising or consisting of a nucleic sequence of SEQ ID NO: 90, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 90; and (b)

5 the second Pichinde arenavirus expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 37, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 37. In some embodiments of the methods, the subject is infected with HBV, is suspected of being infected with HBV, or is at risk of being

10 infected with HBV. In some embodiments of the methods, the subject is asymptomatic. In some embodiments of the methods, the subject is chronically infected with HBV. In some embodiments of the methods, the subject is exhibiting or experiencing one or more symptoms selected from hepatic failure, hepatic cancer, hepatic fibrosis and hepatic cirrhosis. In some

15 embodiments of the methods, the subject is acutely infected with HBV. In some embodiments of the methods, the subject is exhibiting or experiencing one or more symptoms selected from jaundice, visible webs of swollen blood vessels in the skin, dark-colored (*e.g.*, orange or brown) urine, light-colored feces, fever, persistent fatigue, malaise, abdominal pain, abdominal fluid, loss of appetite, nausea, and vomiting. In some embodiments of the methods, the subject is co-

20 infected with hepatitis D virus (HDV). In some embodiments of the methods, the composition is administered via a route selected from intravenous, intramuscular, intradermal, subcutaneous and mucosal (*e.g.* buccal, intranasal, intrarectal, intravaginal). In some embodiments, the methods entail administering to the subject from about 10^3 to about 10^{12} viral focus forming units (FFU) or plaque forming units (PFU) or infectious units (IU) or viral particles (vp), *e.g.* from about 10^4 to about 10^7 viral FFU or PFU or IU or vp, *e.g.* from about 10^3 to about 10^4 , 10^5 ,

25 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} or 10^{12} viral FFU or PFU or IU or vp, per administration. In some embodiments of the methods, the one or more compositions are administered multiple times. In some embodiments, the methods entail administering intravenously or intramuscularly from about 10^6 to about 10^8 viral FFU or PFU or IU or vp per administration every other week (Q2W) or monthly (Q4W). In some embodiments, the methods entail multiple administrations of the

30 one or more immunogenic compositions over a time period of at least about 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 19 months, 20 months, 21 months, 22 months, 23 months, 24 months, or longer, or until sAg is not detectable in the serum or plasma of the subject. In some embodiments, the methods

35 comprise a prime-boost regimen comprising administering a priming composition at a first time

point and administering one or more boosting compositions at one or more subsequent time points. As appropriate, the methods can entail repeating the prime-boost regimen one or more iterations. In some embodiments of the methods, the administrations of the priming composition and the one or more boosting compositions are spaced at least 1 week and up to at least 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months or 6 months apart. In some embodiments of the methods, the priming composition and the boosting composition can comprise the same immunogenic composition or can comprise different immunogenic compositions. In some embodiments of the methods, the priming composition and the boosting composition comprise the same one or more polypeptides and same viral expression vector. In some embodiments of the methods, the priming composition and the boosting composition comprise different polypeptides and/or different viral expression vectors. In some embodiments, the methods entail priming with a priming composition comprising one or more (*e.g.*, first and second) viral expression vectors, and boosting with a boosting composition comprising one or more (*e.g.*, third and fourth) viral expression vectors. In various embodiments, the prime-boost regimen comprises: (a) Priming with a priming composition comprising one or more viral expression vectors and boosting with a boosting composition comprising one or more polynucleotides, wherein the one or more polynucleotides comprise DNA, cDNA, mRNA or self-replicating RNA; (b) Priming with a priming composition comprising one or more polynucleotides, wherein the one or more polynucleotides comprise DNA, cDNA, mRNA or self-replicating RNA, and boosting with a boosting composition comprising one or more viral expression vectors; (c) Priming with a priming composition comprising one or more viral expression vectors, and boosting with a boosting composition comprising one or more viral expression vectors, wherein the one or more viral expression vectors in the priming composition and the one or more viral expression vectors in the boosting composition are from identical, related or unrelated taxonomical families; (d) Priming with a priming composition comprising one or more replication-deficient viral expression vectors and boosting with a boosting composition comprising one or more replication-deficient viral expression vectors, wherein the one or more replication-deficient viral expression vectors in the priming composition and the one or more replication-deficient viral expression vectors in the boosting composition are from identical, related or unrelated taxonomical families; (e) Priming with a priming composition comprising one or more replication-attenuated viral expression vectors and boosting with a boosting composition comprising one or more replication-attenuated viral expression vectors, wherein the one or more replication-attenuated viral expression vectors in the priming composition and the one or more replication-attenuated viral expression vectors in the boosting composition are from identical, related or unrelated taxonomical families; (f) Priming with a

priming composition comprising one or more replication-deficient viral expression vectors and boosting with a boosting composition comprising one or more replication-attenuated viral expression vectors; (g) Priming with a priming composition comprising one or more replication-attenuated viral expression vectors and boosting with a boosting composition comprising one or more replication-deficient viral expression vectors; (h) Priming with a priming composition comprising one or more Lymphocytic choriomeningitis mammarenavirus (LCMV) viral expression vectors and boosting with a boosting composition comprising one or more Pichinde mammarenavirus (PICV) viral expression vectors; (i) Priming with a priming composition comprising one or more Pichinde mammarenavirus (PICV) viral expression vectors and boosting with a boosting composition comprising one or more Lymphocytic choriomeningitis mammarenavirus (LCMV) viral expression vectors; (j) Priming with a priming composition comprising one or more replication deficient Pichinde mammarenavirus (PICV) viral expression vectors and boosting with a boosting composition comprising one or more replication deficient Lymphocytic choriomeningitis mammarenavirus (LCMV) viral expression vectors; (k) Priming with a priming composition comprising one or more replication deficient Lymphocytic choriomeningitis mammarenavirus (LCMV) viral expression vectors and boosting with a boosting composition comprising one or more replication deficient Pichinde mammarenavirus (PICV) viral expression vectors; (l) Priming with a priming composition comprising one or more arenavirus viral expression vectors and boosting with a boosting composition comprising one or more adenovirus viral expression vectors; (m) Priming with a priming composition comprising one or more adenovirus viral expression vectors and boosting with boosting composition comprising one or more arenavirus viral expression vectors; (n) Priming with a priming composition comprising one or more poxvirus viral expression vectors and boosting with a boosting composition comprising one or more arenavirus viral expression vectors; (o) Priming with a priming composition comprising one or more arenavirus viral expression vectors and boosting with boosting composition comprising one or more poxvirus viral expression vectors; (p) Priming with a priming composition comprising one or more poxvirus viral expression vectors and boosting with a boosting composition comprising one or more adenovirus viral expression vectors; or (q) Priming with a priming composition comprising one or more adenovirus viral expression vectors and boosting with boosting composition comprising one or more poxvirus viral expression vectors. In some embodiments, the methods entail a prime-boost regimen that comprises: (a) Priming with a priming composition comprising one or more Lymphocytic choriomeningitis mammarenavirus (LCMV) viral expression vectors and boosting with a boosting composition comprising one or more Pichinde mammarenavirus (PICV) viral expression vectors; (b) Priming with a priming composition comprising one or more Pichinde

mammarenavirus (PICV) viral expression vectors and boosting with a boosting composition comprising one or more Lymphocytic choriomeningitis mammarenavirus (LCMV) viral expression vectors; (c) Priming with a priming composition comprising one or more replication deficient Pichinde mammarenavirus (PICV) viral expression vectors and boosting with a

5 boosting composition comprising one or more replication deficient Lymphocytic choriomeningitis mammarenavirus (LCMV) viral expression vectors; or (d) Priming with a priming composition comprising one or more replication deficient Lymphocytic choriomeningitis mammarenavirus (LCMV) viral expression vectors and boosting with a

10 (PICV) viral expression vectors. In some embodiments, the priming composition and the boosting composition comprise an immunogenic composition as described herein. In some embodiments, the subject is not receiving antiviral therapy or antiviral therapy is discontinued prior to administration of the one or more immunogenic compositions. In some embodiments of the methods, antiviral therapy is discontinued after one or more administrations of the one or

15 more immunogenic compositions. In some embodiments, the methods further comprise administering to the subject one or more additional therapeutic agents, *e.g.* two, three, four, or more additional therapeutic agents. In some embodiments, the methods comprise co-administering one or more agonists or activators of one or more toll-like receptors (TLRs). In some embodiments, the methods comprise co-administering one or more TLR agonists or

20 activators selected from a TLR2 agonist, a TLR3 agonist, a TLR4 agonist, a TLR5 agonist, a TLR7 agonist, a TLR8 agonist and a TLR9 agonist. In some embodiments, the methods entail co-administering a TLR7 agonist selected from GS-9620 (vesatolimod), R848 (Resiquimod), DS-0509, LHC-165 and TMX-101 (imiquimod). In some embodiments, the methods entail co-administering a TLR8 agonist selected from GS-9688, R848 (Resiquimod) and NKTR-262 (dual

25 TLR7/TLR8 agonist). In some embodiments, the methods entail co-administering one or more interleukin receptor agonists of an interleukin receptor selected from IL-2, IL-7, IL-12 and IL-15. In some embodiments, the methods entail co-administering one or more cytokines selected from IL-2, IL-7, IL-12, IL-15, and variants thereof. In some embodiments, the methods entail co-administering one or more innate immune activators. In some embodiments, the methods

30 entail co-administering one or more innate immune activators comprising an agonist of a receptor selected from fms related tyrosine kinase 3 (FLT3), stimulator of interferon genes (STING) receptor, DExD/H-box helicase 58 (DDX58; *a.k.a.*, RIG-I), nucleotide binding oligomerization domain containing 2 (NOD2). In some embodiments, the methods entail co-administering GS-3583 and/or GS-9992. In some embodiments, the methods entail co-

35 administering one or more antagonists or inhibitors of an inhibitory immune checkpoint protein

or receptor and/or one or more activators or agonists of a stimulatory immune checkpoint protein or receptor. In some embodiments, the methods entail co-administering one or more immune checkpoint proteins or receptors selected from: CD27, CD70; CD40, CD40LG; CD47, CD48 (SLAMF2), transmembrane and immunoglobulin domain containing 2 (TMIGD2, CD28H), CD84 (LY9B, SLAMF5), CD96, CD160, MS4A1 (CD20), CD244 (SLAMF4);

5 CD276 (B7H3); V-set domain containing T cell activation inhibitor 1 (VTCN1, B7H4); V-set immunoregulatory receptor (VSIR, B7H5, VISTA); immunoglobulin superfamily member 11 (IGSF11, VSIG3); natural killer cell cytotoxicity receptor 3 ligand 1 (NCR3LG1, B7H6);

10 HERV-H LTR-associating 2 (HHLA2, B7H7); inducible T cell co-stimulator (ICOS, CD278); inducible T cell co-stimulator ligand (ICOSLG, B7H2); TNF receptor superfamily member 4 (TNFRSF4, OX40); TNF superfamily member 4 (TNFSF4, OX40L); TNFRSF8 (CD30), TNFSF8 (CD30L); TNFRSF10A (CD261, DR4, TRAILR1), TNFRSF9 (CD137), TNFSF9 (CD137L); TNFRSF10B (CD262, DR5, TRAILR2), TNFRSF10 (TRAIL); TNFRSF14 (HVEM, CD270), TNFSF14 (HVEML); CD272 (B and T lymphocyte associated (BTLA));

15 TNFRSF17 (BCMA, CD269), TNFSF13B (BAFF); TNFRSF18 (GITR), TNFSF18 (GITRL); MHC class I polypeptide-related sequence A (MICA); MHC class I polypeptide-related sequence B (MICB); CD274 (CD274, PDL1, PD-L1); programmed cell death 1 (PDCD1, PD1, PD-1); cytotoxic T-lymphocyte associated protein 4 (CTLA4, CD152); CD80 (B7-1), CD28; nectin cell adhesion molecule 2 (NECTIN2, CD112); CD226 (DNAM-1); Poliovirus receptor

20 (PVR) cell adhesion molecule (PVR, CD155); PVR related immunoglobulin domain containing (PVRIG, CD112R); T cell immunoreceptor with Ig and ITIM domains (TIGIT); T cell immunoglobulin and mucin domain containing 4 (TIMD4; TIM4); hepatitis A virus cellular receptor 2 (HAVCR2, TIMD3, TIM3); galectin 9 (LGALS9); lymphocyte activating 3 (LAG3, CD223); signaling lymphocytic activation molecule family member 1 (SLAMF1, SLAM,

25 CD150); lymphocyte antigen 9 (LY9, CD229, SLAMF3); SLAM family member 6 (SLAMF6, CD352); SLAM family member 7 (SLAMF7, CD319); UL16 binding protein 1 (ULBP1); UL16 binding protein 2 (ULBP2); UL16 binding protein 3 (ULBP3); retinoic acid early transcript 1E (RAET1E; ULBP4); retinoic acid early transcript 1G (RAET1G; ULBP5); retinoic acid early transcript 1L (RAET1L; ULBP6); lymphocyte activating 3 (CD223); killer cell immunoglobulin

30 like receptor, three Ig domains and long cytoplasmic tail 1 (KIR, CD158E1); killer cell lectin like receptor C1 (KLRC1, NKG2A, CD159A); killer cell lectin like receptor K1 (KLRK1, NKG2D, CD314); killer cell lectin like receptor C2 (KLRC2, CD159c, NKG2C); killer cell lectin like receptor C3 (KLRC3, NKG2E); killer cell lectin like receptor C4 (KLRC4, NKG2F);

35 killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail (KIR2DL1); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail

2 (KIR2DL2); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 3 (KIR2DL3); killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 1 (KIR3DL1); killer cell lectin like receptor D1 (KLRD1); and SLAM family member 7 (SLAMF7). In some embodiments, the methods entail co-administering one or more blockers or inhibitors of one or more T-cell inhibitory immune checkpoint proteins or receptors. In some embodiments, the methods entail co-administering one or more T-cell inhibitory immune checkpoint proteins or receptors selected from CD274 (CD274, PDL1, PD-L1); programmed cell death 1 ligand 2 (PDCD1LG2, PD-L2, CD273); programmed cell death 1 (PDCD1, PD1, PD-1); cytotoxic T-lymphocyte associated protein 4 (CTLA4, CD152); CD276 (B7H3); V-set domain containing T cell activation inhibitor 1 (VTCN1, B7H4); V-set immunoregulatory receptor (VSIR, B7H5, VISTA); immunoglobulin superfamily member 11 (IGSF11, VSIG3); TNFRSF14 (HVEM, CD270), TNFSF14 (HVEM); CD272 (B and T lymphocyte associated (BTLA)); PVR related immunoglobulin domain containing (PVRIG, CD112R); T cell immunoreceptor with Ig and ITIM domains (TIGIT); lymphocyte activating 3 (LAG3, CD223); hepatitis A virus cellular receptor 2 (HAVCR2, TIMD3, TIM3); galectin 9 (LGALS9); killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 1 (KIR, CD158E1); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 1 (KIR2DL1); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 2 (KIR2DL2); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 3 (KIR2DL3); and killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 1 (KIR3DL1). In some embodiments, the methods entail co-administering one or more agonists or activators of one or more T-cell stimulatory immune checkpoint proteins or receptors. In some embodiments, the methods entail co-administering one or more T-cell stimulatory immune checkpoint proteins or receptors selected from CD27, CD70; CD40, CD40LG; inducible T cell co-stimulator (ICOS, CD278); inducible T cell co-stimulator ligand (ICOSLG, B7H2); TNF receptor superfamily member 4 (TNFRSF4, OX40); TNF superfamily member 4 (TNFSF4, OX40L); TNFRSF9 (CD137), TNFSF9 (CD137L); TNFRSF18 (GITR), TNFSF18 (GITRL); CD80 (B7-1), CD28; nectin cell adhesion molecule 2 (NECTIN2, CD112); CD226 (DNAM-1); Poliovirus receptor (PVR) cell adhesion molecule (PVR, CD155). In some embodiments, the methods entail co-administering AGEN-2373 and/or AGEN-1223. In some embodiments, the methods entail co-administering one or more blockers or inhibitors of one or more NK-cell inhibitory immune checkpoint proteins or receptors. In some embodiments, the methods entail co-administering one or more NK-cell inhibitory immune checkpoint proteins or receptors selected from killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 1 (KIR, CD158E1); killer cell immunoglobulin like

receptor, two Ig domains and long cytoplasmic tail 1 (KIR2DL1); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 2 (KIR2DL2); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 3 (KIR2DL3); killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 1 (KIR3DL1);

5 killer cell lectin like receptor C1 (KLRC1, NKG2A, CD159A); and killer cell lectin like receptor D1 (KLRD1, CD94). In some embodiments, the methods entail co-administering one or more agonists or activators of one or more NK-cell stimulatory immune checkpoint proteins or receptors. In some embodiments, the methods entail co-administering one or more NK-cell stimulatory immune checkpoint proteins or receptors are selected from CD16, CD226 (DNAM-1);

10 1); killer cell lectin like receptor K1 (KLRK1, NKG2D, CD314); and SLAM family member 7 (SLAMF7). In some embodiments, the methods entail co-administering one or more proteinaceous inhibitors of PD-L1 (CD274), PD-1 (PDCD1) or CTLA4. In some embodiments, the methods entail co-administering one or more proteinaceous inhibitors of CTLA4 selected from ipilimumab, tremelimumab, BMS-986218, AGEN1181, AGEN1884, BMS-986249, MK-

15 1308, REGN-4659, ADU-1604, CS-1002, BCD-145, APL-509, JS-007, BA-3071, ONC-392, AGEN-2041, JHL-1155, KN-044, CG-0161, ATOR-1144, PBI-5D3H5, FPT-155 (CTLA4/PD-L1/CD28), PF-06936308 (PD-1/CTLA4), MGD-019 (PD-1/CTLA4), KN-046 (PD-1/CTLA4), MEDI-5752 (CTLA4/PD-1), XmAb-20717 (PD-1/CTLA4) and AK-104 (CTLA4/PD-1). In some embodiments, the methods entail co-administering one or more proteinaceous inhibitors of

20 PD-L1 (CD274) or PD-1 (PDCD1) selected from zimberelimab (AB122), pembrolizumab, nivolumab, cemiplimab, pidilizumab, AMP-224, MEDI0680 (AMP-514), spartalizumab, atezolizumab, avelumab, ASC22, durvalumab, BMS-936559, CK-301, PF-06801591, BGB-A317 (tislelizumab), GLS-010 (WBP-3055), AK-103 (HX-008), AK-105, CS-1003, HLX-10, MGA-012, BI-754091, AGEN-2034, JS-001 (toripalimab), JNJ-63723283, genolimzumab

25 (CBT-501), LZM-009, BCD-100, LY-3300054, SHR-1201, SHR-1210 (camrelizumab), Sym-021, ABBV-181, PD1-PIK, BAT-1306, (MSB0010718C), CX-072, CBT-502, TSR-042 (dostarlimab), MSB-2311, JTX-4014, BGB-A333, SHR-1316, CS-1001 (WBP-3155, KN-035, IBI-308 (sintilimab), HLX-20, KL-A167, STI-A1014, STI-A1015 (IMC-001), BCD-135, FAZ-053, TQB-2450, MDX1105-01, FPT-155 (CTLA4/PD-L1/CD28), PF-06936308 (PD-1/

30 CTLA4), MGD-013 (PD-1/LAG-3), FS-118 (LAG-3/PD-L1) MGD-019 (PD-1/CTLA4), KN-046 (PD-1/CTLA4), MEDI-5752 (CTLA4/PD-1), RO-7121661 (PD-1/TIM-3), XmAb-20717 (PD-1/CTLA4), AK-104 (CTLA4/PD-1), M7824 (PD-L1/TGF β -EC domain), CA-170 (PD-L1/VISTA), CDX-527 (CD27/PD-L1), LY-3415244 (TIM3/PDL1), and INBRX-105 (4-1BB/PDL1). In some embodiments, the methods entail co-administering one or more small

35 molecule inhibitors of CD274 (PDL1, PD-L1), programmed cell death 1 (PDCD1, PD1, PD-1)

or CTLA4. In some embodiments, the methods entail co-administering one or more small molecule inhibitors of CD274 or PDCD1 selected from GS-4224, GS-4416, INCB086550 and MAX10181. In some embodiments, the methods entail co-administering BPI-002 (a small molecule inhibitor of CTLA4). In some embodiments, the methods comprise co-administering to the subject one or more antiviral agents. In some embodiments, the methods comprise co-administering one or more antiviral agents selected from lamivudine (LAM), adefovir dipivoxil (ADV), entecavir (ETV), telbivudine (LdT), tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF or VEMSIDY®) and ledipasvir + sofosbuvir (HARVONI®). In some embodiments, the methods comprise co-administering to the subject one or more therapeutic agents selected from HBV antigen inhibitors (*e.g.*, HBV core antigen (HBcAg) inhibitors, HBV surface antigen (HBsAg) inhibitors, HBx inhibitors, HBV E antigen inhibitors), anti-HBV antigen antibodies, inhibitory nucleic acids targeting HBV (*e.g.*, antisense oligonucleotide, short interfering RNA (siRNA), DNA-directed RNA interference (ddRNAi)), gene editors targeting HBV (*e.g.*, CRISPR-Cas (*e.g.*, Cas9, Cas12, Cascade, Cas13), zinc finger nucleases, homing endonucleases, homing meganucleases (*e.g.*, ARCUS), synthetic nucleases, TALENs), covalently closed circular DNA (cccDNA) inhibitors and HBsAg secretion or assembly inhibitors and HBV viral entry inhibitors. In some embodiments, the method activates in the subject CD8⁺ T cells and/or CD4⁺ T cells targeting one or more HBV polypeptide epitopes. In some embodiments, the method elicits in the subject production of antibodies that bind one or more HBV polypeptides.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] Figure 1 illustrates the immunogenicity of HBsAg-expressing adenovirus vectors from genotypes (GT) A, B, C and D in DO mice. Five- to seven-week-old Diversity Outbred (DO) mice (n=8 per group) were injected intramuscularly with 1×10^8 viral particles (vp) of adenovirus encoding HBsAg consensus sequences of HBV genotypes (GT)-A, B, C, D (SEQ ID NOs: 1-4, respectively). On day 14 after injection, splenocytes were harvested and T cell responses were evaluated by interferon (IFN)- γ ELISPOT (BD mouse IFN- γ ELISPOT kit, catalog #551083). Each symbol corresponds to an individual mouse which was assessed for responses to overlapping peptide pools corresponding to GT-A, B, C, and D HBsAg.

[0020] Figure 2 illustrates schematics of each Pol-containing antigen design. Each Pol domain is indicated separately (TP, terminal protein; RT, Reverse Transcriptase; RNH, RNase H). Approximate location of the D to H mutation in the YMDD motif (SEQ ID NO: 97) in RT and of the E to H mutation in the AELL motif (SEQ ID NO: 98) in RNH are indicated below the RT and RNH domains. Designation of each construct is shown at left, and the amino acid size

range of the GT-A, B, C, and D constructs is shown at right. “YMHD” and “AHL” disclosed as SEQ ID NOS 99 and 100, respectively.

[0021] Figure 3 illustrates the immunogenicity of Core-Pol fusion protein-expressing

adenovirus vectors in C57BL/6 mice. Six- to eight-week-old C57BL/6 mice (n=5 per group)

were injected with 1×10^8 viral particles (vp) of adenovirus encoding core-Pol fusion variants of

SEQ ID NOS: 15-26. The genotype of each antigen is shown above each graph, while the

antigen designations are shown on the horizontal axis (Mut: core-Pol^{mut}, $\Delta 1$: core-Pol ^{$\Delta 1$} , $\Delta 3$:

core-Pol ^{$\Delta 3$}). On day 14 after injection, splenocytes were harvested and T cell responses

evaluated by IFN- γ ELISPOT (BD mouse IFN- γ ELISPOT kit, catalog #551083) using

overlapping peptide pools corresponding to GT-D core and Pol. Bars show stacked geometric

mean responses for each group. SFU, spot forming units.

[0022] Figures 4A-4B illustrate the immunogenicity of Core-Pol fusion protein-

expressing adenovirus vectors in DO mice. Five- to seven-week-old DO mice (n=8 per group)

were injected intramuscularly with 1×10^8 viral particles (vp) of adenovirus encoding GT-A core-

Pol ^{$\Delta 3$} or GT-B, C, or D core-Pol ^{$\Delta 1$} . On day 14 after injection, splenocytes were harvested and T

cell responses evaluated by IFN- γ ELISPOT (BD mouse IFN- γ ELISPOT kit, catalog #551083)

responses to overlapping peptide pools corresponding to GT-A and D core and Pol. Statistical

comparisons between responses to peptides of different genotypes within mice receiving the

same vaccine were assessed with Wilcoxon signed-rank tests. Statistical comparisons between

mice receiving different vaccines were assessed with Mann-Whitney tests. (A) Responses to Pol

peptides. (B) Responses to Core peptides. SFU, spot forming units.

[0023] Figure 5 illustrates the immunogenicity of Pol-expressing adenovirus vectors.

Six- to eight-week-old C57BL/6 mice (n=5 per group) were injected with 1×10^8 viral particles

(vp) of adenovirus expressing Pol antigen variants of SEQ ID NOS: 8, 12, 13, 14, or a full-

length, unmodified GT-D Pol sequence (GT-D Pol^{Ctrl}). On day 14 after injection, splenocytes

were harvested and T cell responses evaluated by IFN- γ ELISPOT (BD mouse IFN- γ ELISPOT

kit, catalog #551083) using overlapping peptide pools corresponding to GT-D Pol. SFU: spot

forming units.

[0024] Figure 6 illustrates the study design assessing the efficacy of HBV-expressing

Ad5 and vaccinia vectors in the AAV mouse model of CHB (AAV-HBV). Six- to eight-week-

old C57BL/6 mice were transduced with 10^{12} genome copies of AAV-HBV on day -35. Mice

were randomized to treatment groups based on serum HBsAg levels at day -7. Adenovirus type

5 priming vaccines expressing HBV antigens were administered intramuscularly (i.m.) in 50 μ l

on day 0, and vaccinia boost vaccines expressing the same HBV antigens were administered i.m. in 50 µl on day 32. From days 46-67, mice were given either anti-PD-1 (anti-CD279) monoclonal antibody RMP1-14 or isotype control mAb. Blood samples were collected for viral antigen testing on days -7, 14, 27, 46, 60, 67, and 88. Splenocytes were harvested on day 88 and assessed for IFN-γ ELISPOT.

[0025] Figure 7 illustrates the immunogenicity of Ad5 prime-vaccinia boost vaccination in AAV-HBV mice. Splenocytes were harvested on day 88 in the study shown in Figure 6. T cell responses to HBsAg and Pol were evaluated by IFN-γ ELISPOT (BD mouse IFN-γ ELISPOT kit, catalog #551083) using overlapping peptide pools corresponding to GT-D sAg and Pol. Dashed line indicates the highest signal in HBsAg ELISPOT observed in mice receiving control vaccine. mAb: monoclonal antibody administered. Iso: isotype control. αPD-1: anti-PD-1. Vax: indicates whether the vaccine contained HBV antigens or control (Ctrl) antigens. SFU, spot forming units.

[0026] Figure 8 illustrates the effects of HBV-expressing Ad5 prime-vaccinia boost vaccination in combination with PD-1 blockade in AAV-HBV mice. Serum HBeAg levels in the study shown in Figure 6 were determined by ELISA (International Immunodiagnostics) at the indicated timepoints. Dashed line indicates the lower limit of detection. Ad: adenovirus 5 vector. Vac: vaccinia vector. Ctrl: control antigen. Isotype: isotype control antibody. αPD-1: anti-mouse PD-1 antibody.

[0027] Figures 9A-9C illustrate an overview of the arenavirus vector platforms demonstrated in the examples provided herein. (A) Schematic of a phylogenetic tree of the arenavirus family (Arenaviridae). In the examples provided herein, Lymphocytic choriomeningitis mammarenavirus (LCMV)(NCBI:txid11623) from the Old World (OW) clade and Cali mammarenavirus (*a.k.a.*, Pichinde mammarenavirus or Pichinde arenavirus (PICV)) (NCBI:txid2169993) from the New World (NW) clade were selected for generation of HBV antigen encoding vectors. *See, e.g.*, Buchmeier *et al.*, 2001, “Arenaviridae: The Viruses and Their Replication,” Fields Virology Vol 2, 1635-1668. Arenavirus taxonomy is more recently reviewed in, *e.g.*, Radoshitzky, *et al.*, *Arch Virol.* (2015) 160(7):1851-74. Phylogenetic information for Arenaviridae is also available at the Virus Pathogen Resource website, located at vprbrc.org. (B) Schematic of replication-defective arenavirus vectors having a bi-segmented genome, described in WO2009083210, and (C) replication-attenuated arenavirus vectors having a tri-segmented genome, described in WO2016075250 and WO2017198726. Replication-defective arenavirus vectors having a bi-segmented genome, described in WO2009083210 and used in the examples provided herein, encode three of the four viral proteins (L, Z and NP) and

an open reading frame for insertion of a heterologous polynucleotide, *e.g.*, encoding an antigen. The replication-defective arenavirus vectors having a bi-segmented genome can only propagate when viral GP is delivered in trans. Replication-attenuated arenavirus vectors having a tri-segmented genome, described in WO2016075250 and WO2017198726, have an artificial
5 duplication of the genomic S-segment, encode all four viral proteins (L, Z, NP & GP) and have two open reading frames for insertion of one or two heterologous polynucleotides, *e.g.*, encoding one or two antigens.

[0028] Figure 10 illustrates the immunogenicity of Pol antigens in replication-incompetent lymphocytic choriomeningitis mammarenavirus (LCMV) vectors. Six- to eight-
10 week-old C57BL/6 mice (n=6 per group) were injected intravenously with 1×10^6 focus forming units (FFU) of replication-incompetent LCMV vectors expressing Pol antigen variants GT-D and GT-B Pol^{Δ1} (SEQ ID NOs: 6 and 8), Pol^{Δ3} (SEQ ID NOs: 10 and 12), and Pol³⁰⁰ (SEQ ID NOs: 13 and 14), or with media as a negative control. On day 7 after injection, splenocytes were harvested and T cell responses evaluated by IFN-γ ELISPOT (BD mouse IFN-γ ELISPOT kit,
15 catalog #551083) using Pol overlapping peptide pools corresponding to the immunization antigen genotype in each group. SFU, spot forming units.

[0029] Figure 11 illustrates the immunogenicity of Core-HBsAg fusion protein-expressing LCMV vectors in C57BL/6 mice. Six- to eight-week-old C57BL/6 mice (n=6 per group) were injected with 1×10^6 focus forming units (FFU) of replication-incompetent LCMV
20 vectors expressing core-HBsAg fusion variants of SEQ ID NOs: 38-41 or mock immunized as a negative control. On day 7 after injection, splenocytes were harvested and T cell responses evaluated by IFN-γ ELISPOT (BD mouse IFN-γ ELISPOT kit, catalog #551083) using core and HBsAg overlapping peptide pools corresponding to the immunization antigen genotype in each group. SFU, spot forming units.

[0030] Figure 12 illustrates the antibody response to HBsAg obtained in mice administered with core-sAg fusion protein-expressing replication-incompetent LCMV vectors. Six- to eight-week-old C57BL/6 (left) or Balb/c (right) mice (n=5 per group) were injected with 1×10^6 focus forming units (FFU) of replication-incompetent LCMV vectors expressing core-sAg fusion variants of SEQ ID NOs: 38-41 or with media as a negative control. On day 17 after
25 injection, serum was collected and tested for anti-HBsAg antibody by ELISA (International Immunodiagnostics). Dashed line indicates the lower limit of detection of 11 mIU/ml. *p<0.05 by Mann-Whitney test.
30

[0031] Figure 13 illustrates the effect of nucleotide sequence modification on T-cell immunogenicity of core-P2A-sAg fusion proteins. Six- to eight- week old C57BL/6 mice (n=6 per group) were injected with 1×10^6 focus forming units (FFU) of replication-incompetent LCMV vectors with GT-D core-P2A-sAg (SEQ ID NO:36) or GT-D iCore-P2A-sAg (SEQ ID NO: 37), or mock immunized as a negative control. On day 7 after injection, splenocytes were harvested and T cell responses evaluated by IFN- γ ELISPOT (BD mouse IFN- γ ELISPOT kit, catalog #551083) using core and sAg overlapping peptide pools. Statistical analyses were performed with Mann-Whitney Tests.

[0032] Figures 14A-14B illustrate the immunogenicity of prime/boost vaccination with replication-incompetent LCMV vectors (VV1) encoding GT-B/C Core-P2A-sAg or GT-D iCore-P2A-sAg (Fig 14A) and GT-B Pol $\Delta 3$ or GT-B Pol 300 (Fig 14B) in diversity outbred mice. Animals were administered with 2 doses of each vaccine at day 0 and day 28 as described in Table 9. Splenocytes were harvested at day 42 and T cell responses to HBV antigens were measured by IFN- γ ELISPOT using sAg, core and polymerase peptide pools from various viral genotypes as indicated. Data are expressed as background (no peptide)-subtracted values. Statistical analyses were performed with Mann-Whitney tests. ns: not statistically significant; * $p < 0.0332$.

[0033] Figures 15A-15B illustrate the breadth of HBV-specific T cell responses generated upon prime/boost vaccination with replication-incompetent LCMV (VV1) vectors encoding GT-D iCore-P2A-sAg (Fig 15A) or GT-B Pol 300 (Fig 15B) in diversity outbred mice. IFN- γ ELISPOT was performed using peptides from the same viral genotype (filled circles) or from a different viral genotype (open circles).

[0034] Figures 16A-16B illustrate the immunogenicity of prime/boost vaccination with replication-incompetent LCMV (VV1) vectors encoding GT-D iCore-P2A-sAg and GT-B Pol 300 when delivered either as single vectors or as a co-formulated mixture in C57BL/6 mice. Animals were administered with 2 doses of the vectors at day 0 and day 21 as described in Table 10. Splenocytes were harvested at day 28 and HBV-specific T cell responses were measured by IFN- γ ELISPOT using core (16A), sAg (16B) and Pol (16C) peptide pools.

[0035] Figures 17A-17F illustrate the immunogenicity of repeat vaccinations with replication-incompetent LCMV vectors encoding GT-D iCore-P2A-sAg and GT-B Pol 300 in cynomolgus macaques. A group of animals was also vaccinated with Ad5 and vaccinia vectors encoding the same HBV antigens. Animals were administered with the vectors as described Table 11. 17A: Group 1; 17B: Group 2; 17C: Group 3; 17D; Group 4; 17E: Group 5; 17F:

Group 6. T cell responses to HBV antigens were assessed by performing IFN- γ ELISPOT using sAg, core and Pol peptide pools at the indicated timepoints. Data are expressed as total HBV-specific T cell responses defined as the sum of IFN- γ ELISPOT values obtained after stimulation with sAg, core and polymerase peptide pools.

- 5 **[0036]** Figures 18A-18F illustrate the immunogenicity of repeat vaccinations with replication-incompetent LCMV vectors encoding GT-D iCore-P2A-sAg and GT-B Pol³⁰⁰ in cynomolgus macaques as described in Figure 17 and Table 11. Figures 18A-18F focus on IFN- γ ELISPOT obtained after stimulation with core peptide pools. 18A: Group 1; 18B: Group 2; 18C: Group 3; 18D: Group 4; 18E: Group 5; 18F: Group 6.
- 10 **[0037]** Figures 19A-19F illustrate the immunogenicity of repeat vaccinations with replication-incompetent LCMV vectors encoding GT-D iCore-P2A-sAg and GT-B Pol³⁰⁰ in cynomolgus macaques as described in Figure 17 and Table 11. Figures 19A-19F focus on IFN- γ ELISPOT obtained after stimulation with sAg peptide pools. 19A: Group 1; 19B: Group 2; 19C: Group 3; 19D: Group 4; 19E: Group 5; 19F: Group 6.
- 15 **[0038]** Figures 20A-20F illustrate the immunogenicity of repeat vaccinations with replication-incompetent LCMV vectors encoding GT-D iCore-P2A-sAg and GT-B Pol300 in cynomolgus macaques as described in Figure 17 and Table 11. Figures 20A-20F focus on IFN- γ ELISPOT obtained after stimulation with Pol peptide pools. 20A: Group 1; 20B: Group 2; 20C: Group 3; 20D: Group 4; 20E: Group 5; 20F: Group 6.
- 20 **[0039]** Figures 21A-21B illustrate the frequency of peripheral HBV-specific IFN- γ CD8⁺ T cells (A) and CD4⁺ T cells (B) at week 14 cynomolgus macaques from group 1, 2 and 6 as described in Table 11. Data are obtained from PBMCs harvested at week 14 and re-stimulated with HBV sAg, core and polymerase peptide pools. CD4⁺ and CD8⁺ T subsets were then analyzed for intracellular IFN- γ by flow cytometry.
- 25 **[0040]** Figures 22A-22C illustrate the antibody response to HBsAg in cynomolgus macaques from group 1 (22A), group 2 (22B) and group 6 (22C) as described in Table 11. Serum samples were collected at the indicated timepoints and quantified for anti-HBsAg antibody by ELISA. Dashed line indicates the lower limit of quantitation of the assay (5 mIU/mL).
- 30 **[0041]** Figure 23 illustrates the study design assessing the immunogenicity of replication-incompetent LCMV vectors encoding GT-D iCore-P2A-sAg and GT-B Pol³⁰⁰ (HBV vaccine) alone or in combination with the immunomodulators anti-PD1, anti-CTLA4, anti-CD137 and FLT3L-Fc fusion in the AAV-HBV mouse model. Six- to ten-week-old C57BL/6

mice were transduced with 10^{11} genome copies of AAV-HBV on day -35. Mice were randomized to treatment groups based on serum HBsAg levels at day -11. Replication-incompetent LCMV vectors encoding GT-D iCore-P2A-sAg and GT-B Pol300 were administered intravenously (i.v.) in 200 μ l on day 0, day 21 and day 42. Mice were given intraperitoneally 200 μ l of i) saline solution at day 0, 7, 14, 21, 28, 35, 42, 49 and 56; ii) anti-PD-1 monoclonal antibody RMP1-14 at day 42, 46, 49, 53, 56 and 60; iii) anti-CTLA-4 monoclonal antibody clone 9D9 at day 0, 4, 7, 11, 14, 18, 21, 25, 28, 32, 35, 39, 42, 46, 49 and 53; iv) anti-CD137 monoclonal antibody clone mAb8 (IgG2b) at day 0, 21 and 42; v) FLT3L-Fc fusion protein at day -7, 14 and 35. Asterisks depict doses of each immunomodulator. Splenocytes were harvested on day 105 and assessed for IFN- γ ELISPOT using sAg, core and Pol peptide pools. A group of C57BL/6 mice that did not receive the AAV-HBV but was administered the replication-incompetent LCMV vectors alone was used as a positive control for IFN- γ ELISPOT.

[0042] Figures 24A-24C illustrates the immunogenicity of repeat vaccinations with replication-incompetent LCMV vectors encoding GT-D iCore-P2A-sAg and GT-B Pol300 in AAV-HBV mice as described in Table 12 and Figure 23. Splenocytes were harvested on day 105 and assessed for IFN- γ ELISPOT using sAg (24A), core (24B) and polymerase (24C) peptide pools. Statistical analyses were performed with Mann-Whitney tests. ns: not statistically significant; * $p < 0.0332$, ** $p < 0.0021$, *** $p < 0.0002$, **** $p < 0.0001$.

[0043] Figure 25 illustrates the immunogenicity of prime-boost vaccination with replication-incompetent PICV (VV2) vectors encoding GT-B Pol³⁰⁰ ori or GT-B Pol³⁰⁰ dint in C57BL/6 mice. Animals were administered with 2 doses of vaccine at day 0 and day 21. Splenocytes were harvested at day 28 and HBV Polymerase-specific T cell responses were measured by IFN- γ ELISPOT using Pol peptide pools. Data are expressed as background (no peptide)-subtracted values. Statistical analyses were performed with Mann-Whitney tests. ** $p < 0.0021$.

[0044] Figures 26A-26C illustrate the immunogenicity of homologous and heterologous prime/boost vaccination with replication-incompetent LCMV (VV1) and PICV (VV2) vectors encoding GT-D iCore-P2A-sAg or GT-B Pol³⁰⁰ in C57BL/6 mice. Animals were administered with 2 doses of vector at day 0 and day 21 as described in Table 15. Splenocytes were harvested at day 28 and HBV-specific T cell responses were measured by IFN- γ ELISPOT using sAg (26A), core (26B) and polymerase (26C) peptide pools. Data are expressed as background (no peptide)-subtracted values.

[0045] Figure 27 illustrates the antibody response to HBsAg in C57BL/6 mice administered with replication-incompetent LCMV and PICV vectors encoding GT-D iCore-P2A-sAg using homologous (VV1/VV1) or heterologous (VV2/VV1) prime/boost vaccination at day 0 and day 21. Serum samples were collected at day 28 and quantified for anti-HBsAg antibody by ELISA. Dashed line indicates the lower limit of quantitation of the assay (20 mIU/mL). Statistical analyses were performed with Mann-Whitney tests. ** p<0.0021.

[0046] Figures 28A-28C illustrate the immunogenicity of homologous and heterologous prime/boost vaccination with replication-attenuated LCMV (TT1) and PICV (TT2) vectors encoding GT-D core-P2A-sAg and GT-B Pol300 in C57BL/6 mice. Animals were administered with 2 doses of the vectors at day 0 and day 21 as described in Table 16. Splenocytes were harvested at day 28 and HBV-specific T cell responses were measured by IFN- γ ELISPOT using sAg (28A), core (28B) and polymerase (28C) peptide pools.

[0047] Figure 29 illustrates the immunogenicity of homologous and heterologous prime/boost vaccination with replication-deficient LCMV (VV1) and PICV (VV2) vectors encoding GT-D core-P2A-sAg and GT-B Pol³⁰⁰ in cynomolgus macaques. Animals were administered with 2 doses of the vectors, one at week 0 and one at week 4. PBMCs were harvested at week 6 and HBV-specific T cell responses were measured by IFN- γ ELISPOT using sAg, core and polymerase peptide pools. Data are expressed as total HBV-specific T cell responses defined as the sum of IFN- γ ELISPOT values obtained after stimulation with sAg, core and polymerase peptide pools. The lower limit of quantitation (LLOQ) ELISPOT (dashed line) was defined as 200 IFN- γ + SFU/10⁶ PBMC. Statistical analysis was performed with Mann-Whitney test.

[0048] Figure 30 illustrates the immunogenicity of homologous and heterologous prime/boost vaccination with replication-deficient LCMV (VV1) and PICV (VV2) vectors encoding GT-D core-P2A-sAg and GT-B Pol³⁰⁰ administered every week in cynomolgus macaques. Animals were administered 4 doses of the vectors at week 0, 1, 2 and 3. PBMCs were harvested at week 4 and HBV-specific T cell responses were measured by IFN- γ ELISPOT using sAg, core and polymerase peptide pools. Data are expressed as total HBV-specific T cell responses defined as the sum of IFN- γ ELISPOT values obtained after stimulation with sAg, core and polymerase peptide pools. The lower limit of quantitation (LLOQ) ELISPOT (dashed line) was defined as 200 IFN- γ + SFU/10⁶ PBMC.

DETAILED DESCRIPTION

1. Introduction

[0049] Provided are polypeptides useful to elicit a protective immune response against one or more hepatitis B virus (HBV) antigens in a human. The immunogenic polypeptides described herein are capable of eliciting preventative and/or therapeutic immune responses in a human against one or more hepatitis B virus (HBV) antigens. Generally, the immunogenic polypeptides described herein contain highly conserved portions of HBV proteins in order to induce responses against epitopes that are identical in the vaccine antigen and in the infecting HBV present in the patient, while also excluding poorly conserved regions, thereby avoiding eliciting immunodominant T cell responses targeting epitopes that are not present in the patient's infecting HBV strain. The herein described immunogenic polypeptides furthermore induce both CD4+ and CD8+ T cell responses to facilitate infected cell elimination, and additionally anti-sAg antibody responses that facilitate sAg clearance, thereby reducing or eliminating spread of residual virus if sterilizing viral clearance is not completely achieved. Moreover, the herein described immunogenic polypeptides are demonstrated to be immunogenic when delivered using vaccine technologies capable of inducing the desired responses in humans, and stable in the delivery vectors through sufficient rounds of vector replication to enable commercial-scale vaccine manufacture. The immunogenic polypeptides can be used in various vector systems known to induce CD4+ and CD8+ T cell, and antibody responses in humans and other non-human primates. In certain embodiments, the immunogenic polypeptides are expressed from arenavirus vectors that can be repeatedly dosed without inducing anti-vector antibodies, thereby overcoming a limitation of many previous viral vector technologies and providing the possibility of enhancing therapeutic benefit with repeated dosing.

2. Polypeptides Useful to Promote Immune Response Against Hepatitis B Virus (HBV)

[0050] Provided are immunogenic polypeptides useful to promote, induce and/or elicit an immunogenic response against one or more hepatitis B virus (HBV) antigens. In various embodiments, the immunogenic polypeptides comprise variants and/or fragments of polypeptides encoded by an HBV polymerase (Pol) gene and fusion polypeptides having in sequential order, from the N-terminus to the C-terminus, a variant and/or fragment of a polypeptide encoded by an HBV core gene and a variant and/or fragment of a polypeptide encoded by the surface antigen (sAg) gene. The immunogenic polypeptides can contain amino acid sequences based on consensus or near-consensus sequences from HBV A, B, C or D genotypes, and combinations thereof. Generally, the immunogenic polypeptides described herein do not comprise sequences of HBV X protein (HBx), pre-core, pre-S1, pre-S2, or fragments thereof.

[0051] In various embodiments, immunogenic polypeptides described herein, and/or the polynucleotides encoding such polypeptides, are provided in isolated form. This means that such the polypeptide or polynucleotide is at least 50% w/w pure of interfering proteins, cellular and other contaminants arising from its production or purification but does not exclude the possibility that the agent is combined with an excess of pharmaceutical acceptable carrier(s) or other vehicle intended to facilitate its use. The term “isolated,” when applied to a polypeptide or polynucleotide, as described herein, denotes that the polypeptide or polynucleotide is essentially free of cellular components with which it is associated in the natural state. It can be, for example, in a homogeneous state and may be in either a dry or aqueous solution. Purity and homogeneity can be determined using known methods, *e.g.*, analytical chemistry techniques such as polyacrylamide gel electrophoresis, column chromatography, thin layer chromatography, or high-performance liquid chromatography (HPLC) analysis. A protein that is the predominant species present in a preparation is substantially purified. An “isolated” or “purified” polypeptide or polynucleotide is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or chemical precursors or other chemicals when chemically synthesized. In various embodiments, purified polypeptides and/or polynucleotides are at least 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% (w/w), separated from, purified of, or free of interfering proteins and contaminants from production or purification. Often an agent is the predominant macromolecular species remaining after its purification.

HBV Polymerase Polypeptide Variants

[0052] In various embodiments, provided are truncated and/or internal deletion mutant hepatitis B virus (HBV) polymerase polypeptides.

[0053] Wild-type HBV polymerase has four domains, arranged in tandem in a single polypeptide from N-terminus to C-terminus: the terminal protein (TP) domain conserved across the hepadnaviridae (amino acid residues 1 to 177), the Spacer region (amino acid residues 178 to 335), linking TP to the reverse transcriptase (RT) domain (amino acid residues 336 to 678; comprising NCBI conserved domain pfam00078 or cd01645) and the C-terminal RNase H (RH) domain (amino acid residues 679 to 832). *See, e.g.,* Lanford, *et al.*, *J. Virol.* (1999) 73(3): 1885–93; Vörös, *et al.*, *J Virol.* (2014) 88(5):2584-99 and Jones, *et al.*, *J Virol.* (2014) 88(3):1564-72. In the HBV polymerase variants described herein, all or part of the Spacer region has been deleted or removed. In the HBV polymerase truncation mutants, the entire TP domain has been deleted or removed.

[0054] Generally, the enzymatic domains, *i.e.*, the reverse transcriptase and RNase H domains, are inactivated in the HBV polymerase protein mutants described herein. In various embodiments, the reverse transcriptase domain does not comprise a YMDD motif (SEQ ID NO: 97). In some embodiments, the YMDD motif (SEQ ID NO: 97) in the reverse transcriptase domain is changed to YMHD (SEQ ID NO: 99). In some embodiments, the RNase H domain does not comprise an AELL motif (SEQ ID NO: 98). In some embodiments, the AELL motif (SEQ ID NO: 98) in the RNase H domain is changed to AHLL (SEQ ID NO: 100).

Truncated Polymerase Mutants

[0055] In some embodiments, the truncated HBV polymerase polypeptides comprise an inactivated reverse transcriptase domain and an inactivated RNase H, wherein the polypeptide does not comprise all of the terminal protein (TP) domain and does not comprise all or part of the Spacer domain (*i.e.*, the terminal protein (TP) domain and all or part of the Spacer domain is removed, excised or excluded). In the truncated HBV polymerase polypeptides described herein, all of the TP domain and all or part of the Spacer domain or region is deleted or removed. For example, in some embodiments, the N-terminal 300 amino acids of a native or wild-type HBV polymerase are deleted or removed from the truncated HBV polymerase polypeptides described herein. In various embodiments, the inactivated reverse transcriptase domain and the inactivated RNase H can be directly fused or operably linked or connected via a linker, as described herein. In some embodiments, the truncated HBV polymerase polypeptide is no longer than 600 amino acids in length, *e.g.*, no longer than 595, 590, 585, 580, 575, 570, 565, 560, 555, 550, 545, 540 or 535 amino acids in length. In some embodiments, the truncated HBV polymerase polypeptides comprise the C-terminal 528, 529, 530, 531, 532, 533, 534 or 535 amino acids of a native or wild-type HBV polymerase.

[0056] In some embodiments, the truncated HBV polymerase polypeptides comprise an amino acid sequence corresponding to amino acid residues 300-832, 301-832, 302-832, 303-832, 304-832, 305-832, 306-832, 307-832, 308-832, 309-832, 310-832, 311-832, 312-832, 313-832, 314-832, 315-832, 316-832, 317-832, 318-832, 319-832, 320-832, 325-832, 326-832, 327-832, 328-832, 329-832, 330-832, 331-832, 332-832, 333-832, 334-832, 335-832 or 336-832 of a native or wild-type HBV polymerase. As used herein, numbering of a given amino acid polymer or nucleic acid polymer “corresponds to”, is “corresponding to” or is “relative to” the numbering of a selected or reference amino acid polymer or nucleic acid polymer when the position of any given polymer component (*e.g.*, amino acid, nucleotide, also referred to generically as a “residue”) is designated by reference to the same or to an equivalent position (*e.g.*, based on an optimal alignment or a consensus sequence) in the selected amino acid or

nucleic acid polymer, rather than by the actual numerical position of the component in the given polymer. In various embodiments, the truncated HBV polymerase polypeptides comprise an amino acid sequence corresponding to amino acid residues 300-832. In such embodiments, the N-terminus corresponds to amino acid position 300 of the prototype genotype D pol protein.

- 5 The N-terminal 6 amino acid residues of this sequence is SARSQS (SEQ ID NO: 95) in the genotype D Pol antigen, and SSRSQS (SEQ ID NO: 96) in the genotype B Pol antigen.

Literature reports have indicated that this N-terminal start site allows for function of the RT domain (*see, e.g., Lanford, et al., supra*) and expression of the truncated protein *in vitro* (*see, e.g., Vörös, et al., supra*).

- 10 **[0057]** In some embodiments, the truncated HBV polymerase polypeptide is from HBV genotype B and comprises or consists of an amino acid sequence of SEQ ID NO: 13, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 13. In some
15 embodiments, the truncated HBV polymerase polypeptide is from HBV genotype B and does not comprise a polypeptide sequence (*i.e.*, the sequence is excluded, excised or removed; the sequence is not included) of SEQ ID NO: 50, or a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NO: 50.

- [0058]** In some embodiments, the truncated HBV polymerase polypeptide is from HBV genotype D and comprises or consists of an amino acid sequence of SEQ ID NO: 14, or a
20 sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 14. In some embodiments, the truncated HBV polymerase polypeptide is from HBV genotype D and does not comprise a polypeptide sequence of SEQ ID NO: 51, or a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NO: 51.

- 25 **[0059]** Modifications may be made in the structure of the polypeptides and polynucleotides encoding such polypeptides, described herein, and still obtain a functional molecule that encodes a variant or derivative polypeptide with desirable (*e.g.*, immunogenic) characteristics. When it is desired to alter the amino acid sequence of a polypeptide to create an equivalent, or even an improved, variant or portion of a polypeptide described herein, one
30 skilled in the art will typically change one or more of the codons of the encoding DNA sequence.

[0060] For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of its ability to bind other polypeptides (*e.g.*, antigens)

or cells. Since it is the binding capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence, and, of course, its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. It is thus contemplated that various changes may be made in the

5 polypeptide sequences of the disclosed polypeptides, or corresponding DNA sequences that encode such polypeptides without appreciable loss of their biological utility or activity.

[0061] A “substitution,” as used herein, denotes the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

[0062] In many instances, a polypeptide variant will contain one or more conservative
10 substitutions. A “conservative substitution” is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged.

[0063] As used herein, “identity” means the percentage of identical nucleotide or amino
15 acid residues at corresponding positions in two or more sequences when the sequences are aligned to maximize sequence matching, *i.e.*, taking into account gaps and insertions. Sequences are generally aligned for maximum correspondence over a designated region, *e.g.*, a region at least 20, 25, 30, 35, 40, 45, 50, 55, 60, 65 or more amino acids or nucleotides in length, and can be up to the full length of the reference polypeptide or polynucleotide sequence. For sequence
20 comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer program, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Otherwise, standard parameters can be used. The sequence comparison algorithm then calculates the percent sequence identity for the test
25 sequence(s) relative to the reference sequence, based on the designated program parameters.

[0064] When comparing polynucleotide and polypeptide sequences, two sequences are said to be “identical” if the sequence of nucleotides or amino acids in the two sequences is the same when aligned for maximum correspondence, as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window
30 to identify and compare local regions of sequence similarity. A “comparison window” as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, or over the full length of a sequence, in which a sequence may be compared to a

reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

[0065] Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins - Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J. (1990) Unified Approach to Alignment and Phylogenies pp. 626-645 Methods in Enzymology vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M. (1989) CABIOS 5: 151-153; Myers, E.W. and Muller W. (1988) CABIOS 4:11-17; Robinson, E.D. (1971) Comb. Theor 77: 105; Santou, N. Nes, M. (1987) Mol. Biol. Evol. 4:406-425; Sneath, P.H.A. and Sokal, R.R. (1973) Numerical Taxonomy - the Principles and Practice of Numerical Taxonomy, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J. (1983) Proc. Natl. Acad., Sci. USA 80:726-730.

[0066] Alternatively, optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman (1981) Add. APL. Math 2:482, by the identity alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for similarity methods of Pearson and Lipman (1988) Proc. Natl. Acad. Sci. USA 85: 2444, by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by inspection.

[0067] One example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al. (1977) Nucl. Acids Res. 25:3389-3402 and Altschul et al. (1990) J. Mol. Biol. 215:403-410, respectively. BLAST and BLAST 2.0 can be used, for example with the parameters described herein, to determine percent sequence identity for the polynucleotides and polypeptides described herein. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (blast.ncbi.nlm.nih.gov/Blast.cgi).

[0068] In one illustrative example, cumulative scores can be calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from

its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a word length (W) of 11, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1989) Proc. Natl. Acad. Sci. USA 89: 10915) alignments, (B) of 50, expectation (E) of 10, M=5, N=-4 and a comparison of both strands.

[0069] For amino acid sequences, a scoring matrix can be used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment.

[0070] In one approach, the “percentage of sequence identity” is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, *e.g.*, at least 50 positions, at least 100 positions, or over the full length of a reference sequence, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residues occur in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (*i.e.*, the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

[0071] A “polypeptide variant,” as the term is used herein, is a polypeptide that typically differs from a polypeptide specifically disclosed herein in one or more substitutions, deletions, additions and/or insertions. Such variants may be naturally occurring or may be synthetically generated, for example, by modifying one or more of the above polypeptide sequences described herein and evaluating one or more biological activities of the polypeptide as described herein and/or using any of a number of techniques well known in the art. The term “variant” may also refer to any naturally occurring or engineered molecule comprising one or more nucleotide or amino acid mutations.

[0072] Illustrative HBV polymerase truncation mutants for use in promoting, inducing or eliciting an immunogenic response, *e.g.*, against a polymerase antigen expressed by HBV, are provided in Table A. Illustrative N-terminal sequence segments deleted or removed from, and therefore not contained in, the HBV polymerase truncation mutants described herein are

5 provided in Table B.

Table A - <u>Pol³⁰⁰ mutants</u> - Motifs containing inactivating mutations are underlined (YMDD mutated to YMHD, AELL mutated to AHLL) .			
SEQ ID NO:	HBV geno-type	Length (# amino acids)	Polypeptide sequence
13	B	534	MSSRSQSQGPVLSCWWLQFRNSEPCSEYCLCHIVNLIEDWGPCTE HGEHRIRTPRTPARVTGGVFLVDKNPHNTTESRLVVDFSQFSRGN TRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAM PHLLVGSSGLSRYVARLSSNSRIINNQHRTMQNLHDSCSRNLYVS LMLLYKTYGRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAIC SVVRRAFPCHCLAFSYMHDVVLGAKSVQHLESLYAAVTNFLLSLGI HLNPHKTKRWGYSLNFMGYVIGSWGTLTQEHIVQKIKMCFRKLVP NRPIDWKVCQRIVGLLGFAAPFTQCGYPALMPYACIQAKQAFTE SPTYKAFLSKQYLHLYPVARQRPGLCQVFADATPTGWGLAIGHQR MRGAFVSPLP IHTAHLLAACFARSRS GAKLIGTDNSVLSRKYTS FPWLLGCAANWILRGTSFVYVPSALNPADDP SRGRLGLYRPLRL LYRPTTGRTSLYADSPSVP SHLPDRVHFASPLHVAWRPP
14	D	534	MSARSQSERPVFPCWWLQFRNSKPCSDYCLSHIVNLLEDWGPCA HGEHHIRIPRTPARVTGGVFLVDKNPHNTAESRLVVDFSQFSRGN YRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAM PHLLVGSSGLSRYVARLSSNSRIIFYQHGTMQNLHDSCSRNLYVS LMLLYQTFGRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAIC SVVRRAFPCHCLAFSYMHDVVLGAKSVQHLESFTAVTNFLLSLGI HLNPNKTKRWGYSLHFMGYVIGCYGSLPQDHI IQKIKECFRKLVP NRPIDWKVCQRIVGLLGFAAPFTQCGYPALMPYACIQSKQAFTE SPTYKAFLCKQYLNLYPVARQRPGLCQVFADATPTGWGLVMGHQR MRGTFKAPLP IHTAHLLAACFARSRS GANILGTNSVLSRKYTS FPWLLGCAANWILRGTSFVYVPSALNPADDP SRGRLGLYRPLRL PFRPTTGRTSLYADSPSVP SHLPDRVHFASPLHVAWRPP

Table B - N-terminal polypeptide sequence removed from Pol³⁰⁰ truncated mutants		
SEQ ID NO:	HBV geno-type	Polypeptide sequence
50	B	PLSYQHFRKLLLLDDEAGPLEEELPRLADEGLNRRVAEDLNLGNLNVSIPTWTHKV GNFTGLYSSTVPVFNPEWQTPSFPHIHLQEDI INRCQQYVGPLTVNEKRRLKLM PARFYPNLT KYLPLDKGIKPYYPEHVVNHYFQTRHYLHTLWKAGILYKRESTRSA SFCGSPYSWEQDLQHGRVLVFQTSKRHGDKSFCPQSPGILPRSSVGPCIQNQLRKS RLGPQPAQGQLAGRQQGGSGSIRARVHPSPWGTGVEPSGSGHIHNCASNSSSCL HQSAVRKAAYSHISTSKGHSSSGH AVELHHFPFS
51	D	PLSYQHFRLLLLDDEAGPLEEELPRLADEGLNRRVAEDLNLGNLNVSIPTWTHKV GNFTGLYSSTVPVFNPHWKTPSFPHIHLQEDI IKKCEQFVGPLTVNEKRRLQLIM PARFYPNVT KYLPLDKGIKPYYPEHLVNHYFQTRHYLHTLWKAGILYKRETTSHA

Table B – N-terminal polypeptide sequence removed from Pol³⁰⁰ truncated mutants		
SEQ ID NO:	HBV geno-type	Polypeptide sequence
		SFCGSPYSWEQELQHGAESFHQQSSGILSRPPVGSSSLQSKHRKSRLGLQSQQGHL ARRQQGRGWSIRAGIHPTARRPFGVEPSGSGHTANLASKSASCLYQSAVRKAAYP VVSTFKKHSSSGHAVELHNLPPN

[0073] In some embodiments, the truncated HBV polymerase polypeptide does not comprise an amino sequence or fragment thereof from another HBV protein. In some embodiments, truncated HBV polymerase polypeptide does not comprise an amino sequence or fragment thereof from an HBV protein selected from the group consisting of pre-core, core, X and envelope (*e.g.*, small, medium or large surface antigen (sAg)).

Internal Deletion Polymerase Mutants

[0074] Further provided are HBV polymerase internal deletion mutant polypeptides. In various embodiments, the HBV polymerase internal deletion mutant polypeptides comprise in sequential order, from the N-terminus to C-terminus, a terminal protein (TP) domain, an inactivated reverse transcriptase domain, an inactivated RNase H, wherein the mutant polypeptide does not comprise all or part of a Spacer domain (*i.e.*, all or part of the Spacer domain or region is deleted or removed). In various embodiments, the HBV polymerase deletion mutant polypeptide is no longer than 800 amino acids in length, *e.g.*, no longer than 795, 790, 785, 780, 775, 770, 765, 760, 755, 750, 745, 740, 735, 730, 725, 720, 715, 710 or 705 amino acids in length. In some embodiments, the HBV polymerase internal deletion mutant polypeptides comprise in sequential order, from the N-terminus to C-terminus, a terminal protein (TP) domain, and an amino acid sequence corresponding to amino acid residues 300-832, 301-832, 302-832, 303-832, 304-832, 305-832, 306-832, 307-832, 308-832, 309-832, 310-832, 311-832, 312-832, 313-832, 314-832, 315-832, 316-832, 317-832, 318-832, 319-832, 320-832, 325-832, 326-832, 327-832, 328-832, 329-832, 330-832, 331-832, 332-832, 333-832, 334-832, 335-832 or 336-832 of a native or wild-type HBV polymerase. In various embodiments, the terminal protein (TP) domain, the inactivated reverse transcriptase domain, and the inactivated RNase H independently can be directly fused or operably linked or connected via a linker, *e.g.*, as described herein, *e.g.*, as provided in Table J.

[0075] In some embodiments, the HBV polymerase internal deletion mutant polypeptide is from HBV genotype A and comprises or consists of an amino acid sequence of any one of SEQ ID NOs: 5 and 9, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one

of SEQ ID NOs: 5 and 9. In some embodiments, the HBV polymerase internal deletion mutant polypeptide is from HBV genotype A and does not comprise a polypeptide of SEQ ID NO: 42 or 46, or a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NO: 42 or 46.

5 **[0076]** In some embodiments, the HBV polymerase internal deletion mutant polypeptide is from HBV genotype B and comprises or consists of an amino acid sequence of any one of SEQ ID NOs: 6 and 10, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 6 and 10. In some embodiments, the HBV polymerase internal deletion mutant
10 polypeptide is from HBV genotype B and does not comprise a polypeptide of SEQ ID NO: 43 or 47, or a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NO: 43 or 47.

[0077] In some embodiments, the HBV polymerase internal deletion mutant polypeptide is from HBV genotype C and comprises or consists of an amino acid sequence of any one of
15 SEQ ID NOs: 8 and 11, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 8 and 11. In some embodiments, the HBV polymerase internal deletion mutant polypeptide is from HBV genotype C and does not comprise a polypeptide of SEQ ID NO: 44 or 48, or a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%
20 identical to any one of SEQ ID NO: 44 or 48.

[0078] In some embodiments, the HBV polymerase internal deletion mutant polypeptide is from HBV genotype D and comprises or consists of an amino acid sequence of any one of SEQ ID NOs: 9 and 12, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one
25 of SEQ ID NOs: 9 and 12. In some embodiments, the HBV polymerase internal deletion mutant polypeptide is from HBV genotype D and does not comprise a polypeptide of SEQ ID NO: 45 or 49, or a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NO: 45 or 49.

[0079] In some embodiments, the HBV polymerase internal deletion mutant polypeptide
30 does not comprise an amino sequence or fragment thereof from another HBV protein. In some embodiments, HBV polymerase internal deletion mutant polypeptide does not comprise an amino sequence or fragment thereof from an HBV protein selected from the group consisting of pre-core, core, X and envelope (*e.g.*, small, medium or large surface antigen (sAg)).

[0080] Illustrative HBV polymerase internal deletion mutants for use in promoting, inducing or eliciting an immunogenic response, *e.g.*, against a polymerase antigen expressed by HBV, are provided in Tables C and E. Illustrative internal amino acid sequence segments deleted or removed from, and therefore not contained in, the HBV polymerase internal deletion mutants described herein, *e.g.*, corresponding to all or part of an HBV polymerase Spacer region, are provided in Tables D and F.

Core-Polymerase Fusion Polypeptides

[0081] In various embodiments, the truncated and internal deletion HBV polymerase polypeptide variants described herein are fused to an HBV core polypeptide. The core polypeptide can be positioned either N-terminal or C-terminal to the HBV polymerase. Further provided are fusion polypeptides comprising in sequential order from the N-terminus to the C-terminus, an HBV core polypeptide and a truncated or internal deletion HBV polymerase polypeptide mutant, as described herein. In some embodiments, the core-Pol fusion polypeptide comprises the HBV polymerase deletion mutant polypeptide, described herein, comprises in sequential order from the N-terminus to the C-terminus, an HBV core polypeptide and an internal deletion HBV polymerase polypeptide mutant, as described herein.

[0082] In some embodiments, the core-Pol fusion polypeptide comprises or consists of an amino acid sequence of any one of SEQ ID NOs: 19-26, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 19-26.

[0083] In some embodiments, the HBV core – polymerase internal deletion mutant fusion protein does not comprise an amino sequence or fragment thereof from an HBV protein selected from the group consisting of X, pre-core, and envelope (*e.g.*, small, medium or large surface antigen (sAg)).

[0084] Illustrative core-polymerase fusion proteins for use in promoting, inducing or eliciting an immunogenic response, *e.g.*, against a core and/or polymerase antigen expressed by HBV, are provided in Table G.

Table C - Pol^{Δ1} mutants: Motifs containing inactivating mutations are underlined (YMDD mutated to YMHD, AELL mutated to AHLL). Amino acids in bold + underline + italic mark the site of deletion (last amino acid prior to the deleted region, and the first amino acid after the deleted region).

SEQ ID NO:	HBV geno-type	Length (# amino acids)	Polypeptide sequence
5	A	755	MPLSYQHFRKLLLLDDETEAGPLEEELPRLADEDLNRRVAEDLNLGNLNVSIPTWTHKVGNF TGLYSSTVP IFNPEWQTPSF PKIHLH EDIANRCQQFVGPLTVNEKRRLRLIMPARFYPNSTKYLP LDKG IKPYYPDHVVNH YFQ TRHYLHTLWKAGILYKRETT RSASFCGSPYSWEQELHH GRLVIKTSQRHGDEPFCSQPSGILSRSVGV <u>PE</u> FHSFPPSSARSQSQGPVFSCWWLQFRNTQPCSKYCLSHLVNLL EDWG PCDEHGEHHIRIPRTPARVTGGVFLVDKNPHNTAESRLVVDFSQFSRGITRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHIPLHPAAMP PHLLVGSSGLS RYVARLSSNSRIHNNQHGT LQNLH DCSRQLYVSLMMLLYKTYGRKLHLYSHP IILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFP HCLAFSYM HDVVLGA KS VQHLES LYTAVTNFLLSLGIHLNPNKTKRWGYSLNFMGYVIGSWGTL PQDHIVQKIKHCFRKL PINRPIDWKVCQ RIVGLLGFAAPFTQCGYPALMPLYACIQAKQAF TFSP TYKAFLSKQY LNLYPVARQRPGLCQVFADATPTGWGLAIGHQRM RGT FVAPLP IHTAHLLA ACFARSRS GAKLIGTDNSV LSRKYTSFPWLLGCTANWILRGTSFVYVPSALNPADDP SRGRLGLYRPLLRLPYRPTTGRTSLYAVSPSVP SHLPVRVHFASPLHVAWRPP
6	B	749	MPLSYQHFRKLLLLDDEAGPLEEELPRLADEGLNRRVAEDLNLGNLNVSIPTWTHKVGNF TGLYSSTVPVF NPEWQTPSFPHIHLQEDIINRCQQYVGPLTVNEKRRLKLIMPARFYPNLT KYLPLDKGI KPYYPEHVVNHYFQTRHYLHTLWKAGILYKRESTRSASFCGSPYSWEQDLQHGR LVFQTSKR HGDKSFCPQSPGILPR <u>SEL</u> HHFPPSSSR SQSQGPVLSCWWLQFRN SEPCSEYCLCHIVNLI EDWG PCTEHGEHRI RTPTPARVTGGVF LVDKNPHNTTESRLVVDFSQFSRGNTRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMP PHLLVGSSGLS RYVARLSSNSRIINNQHRTMQNLHDCSRNLYVSLMMLLYKTYGRKLHLYSHP IILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFP HCLAFSYM HDVVLGA KS VQHLES LYAAVTNFLLSLGIHLNPHKTKRWGYSLNFMGYVIGSWGTL PQEHIVQKIKMCFRKL PVNRPIDWKVCQ RIVGLLGFAAPFTQCGYPALMPLYACIQAKQAF TFSP TYKAFLSKQY LHLYPVARQRPGLCQVFADATPTGWGLAIGHQRM RGA FVSPLPIHTAHLLAACFARSRS GAKLIGTDNSV LSRKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDP SRGRLGLYRPLLRLPYRPTTGRTSLYADSPSVP SHLPDRVHFASPLHVAWRPP

Table C - Pol^{A1} mutants: Motifs containing inactivating mutations are underlined (YMDD mutated to YMHD, AELL mutated to AHLL). Amino acids in bold + underline + italic mark the site of deletion (last amino acid prior to the deleted region, and the first amino acid after the deleted region).

SEQ ID NO:	HBV geno-type	Length (# amino acids)	Polypeptide sequence
7	C	753	MPLSYQHFRKLLLLLDDEAGPLEEELPRLADEDLNRRVAEDNLGNLNVSIPTWTHKVGNETGLYSSTVPVF NPEWQTPSFPHIHLQEDIINRCQQYVGPLTVNEKRRLKLIMPARFYPNLT KYLPLDKGIKPYYPEHTVNH YFKTRHYLHTLWKAGILYKRETTTSASFCGSPYSWEQELQHGRLVFQTSTRHGDESFCSSQSSGILSRSPV <u>PE</u> LHNFPSSARSQSEGPLLSCWWLQFRNSKPCSDYCLSHIVNLLLEDWGPCTEHGEHNIRIPRTPARVT GGVFLVDKNPHNTTESRLVVD FSQFSRGSTHVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLH PAAMPHLLVGSSGLSRYVARLSSTSRNINYQHGMQDLHDSCSRNLYVSLLLLYKTFRKLHLYSHPIIL GFRKIPMGVGLSPFLLAQFTSAICSVVRRAFP HCLAFSYMHDVVLGAKSVQHLES LFTAVTNFLLSLGIH LNPNTKRWGYSLNFMGYVIGSWGTL PQEHIVLKIKQCFRKL P VNRPIDWKVCQRIVGLLGFAAPFTQCG YPALMPYACIQAKQAFTFSPTYKAFLCKQYLNLYPVARQRSGLCQVFADATPTGWGLAVGHQMRMGTFV SPLPIHTAHLLAACFARSRS GAKLIGTDNSVVLSRKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDP SRGRLGLYRPLLRLPFRPTTGRTSLYAVSPSVPSHLPVRVHFASPLHVAWRPP
8	D	742	MPLSYQHFRLLLLLDDEAGPLEEELPRLADEGLNRRVAEDNLGNLNVSIPTWTHKVGNETGLYSSTVPVF NPHWKTPSFPNIHLHQDI IKKCEQFVGPLTVNEKRRLQLIMPARFYPNVT KYLPLDKGIKPYYPEHLVNH YFQTRHYLHTLWKAGILYKRETTTSASFCGSPYSWEQELQHGAESFHQQSSGILSRPPVG <u>SE</u> LHNLPNS ARSQSERPVFPCWWLQFRNSKPCSDYCLSHIVNLLLEDWGPCA EHGEHHIRIPRTPARVTGGVFLVDKNPH NTAESRLVVD FSQFSRGNYRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLH PAAMPHLLVGS SGLSRYVARLSSNSRIFNYQHGT MQNLHDSCSRNLYVSLMLLYQTFRKLHLYSHPIILGFRKIPMGVGL SPFLLAQFTSAICSVVRRAFP HCLAFSYMHDVVLGAKSVQHLES LFTAVTNFLLSLGIHLNPNKTKRWGY SLHFMGYVIGCYGSLPQDHIIQKIKECFRKL P VNRPIDWKVCQRIVGLLGFAAPFTQCGYPALMPYACI QSKQAFTFSPTYKAFLCKQYLNLYPVARQRPGLCQVFADATPTGWGLVMGHQMRMGTFKAPLP IHTAHLL AACFARSRSGANILGT DNSVVLSRKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDP SRGRLGLYRPL LRLPFRPTTGRTSLYADSPSVPSHLPDRVHFASPLHVAWRPP

Table D – Internal Spacer polypeptide sequences removed from Pol ^{A1} mutants and Core-Pol ^{A1} fusion proteins		
SEQ ID NO:	HBV geno-type	Polypeptide sequence
42	A	CIRSQFKQSRLGLQPHQGPLATSQSGRSGSIRARVHSPTRRCFGVEPSGSGHIGHSSASSSSSCLHQSAVRKAAYSHLSTSKRQSSSGHAV
43	B	SVGPCIQNQLRKSRLLGPQPAQGGLAGRQQGGSGSIRARVHPSPWGTGVEPSGSGHHCASNSSSCLHQSAVRKAAYSHISTSKGHSSSGH AV
44	C	CIRSQKQSRLGLQPQQGSLARSKSGRSGSIRARVHPTTRQSFGEVPSGSGHIDNSASSASSSCLHQSAVRKTAYSHLSTSKRQSSSGHAV
45	D	SLQSKHRKSRLGLQSQQGHLARRQQGRGWSIRAGIHPTARRPFGVEPSGSGHTANLASKSASCLYQSAVRKAAYPVVSTFKKHSSSGHAV

Table E – Pol ^{A3} mutants – Motifs containing inactivating mutations are underlined (YMDD mutated to YMHD, AELL mutated to AHLI). Amino acids in bold + underline + italic mark the site of deletion (last amino acid prior to the deleted region, and the first amino acid after the deleted region).			
SEQ ID NO:	HBV geno-type	Length (# amino acids)	Polypeptide sequence
9	A	705	MPLSYQHFRKLLLLDDEAGPLEEELPRLADEDLNRRVAEDLNLGNLNVSIPTWKVGNFTGLYSSTVPIFNPEWQTPSFP KIHLEHDIANRCQQFVGPLTVNEKRRLRLIMPARFYPNSTKYLPLDKGIKPYYPDHVVNHYFQTRHYLHTLWKAGILYKRET TRSASFCSGPYSWEQELHH <u>GC</u> WWLQFRNTQPCSKYCLSHLVNLLDWDGPCDEHGEHHIRIPRTPARVTGGVFLVDKNPHNTA ESRLVVDFSQFSRGITRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHIPLHPAAMPHLLVGSSGLSRYVARLSSNSR IHNNQHGTQLNLHDSCSRQLYVSLMMLLYKTYGRKLHLYSHP IILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAPPHCLAFS <u>YMHD</u> VVLGAKSVQHLESLEYTAVTNFLLSLGIHLNPNKTKRWGYSLNFMGYVIGSWGTLPODHIVQIKHCFRKLPIINRPIDW KVCQRIVGLLGFAAPFTQCGYPALMPYACIQAKQAFTFSPITYAKFLSKQYLNLYPVARQRPGLCQVFADATPTGWGLAIGH QRMRGTFVAPLPIHTA <u>HLL</u> AACFARSRSAGKLIGTDNSVVLRSKYTSFPWLLGCTANWILRGTSFVYVPSALNPADDP SRGR LGLYRPLLRLPYRPTTGRTSLYAVSPSVPSHLPVRVHFASPLHVAWRPP
10	B	703	MPLSYQHFRKLLLLDDEAGPLEEELPRLADEGLNRRVAEDLNLGNLNVSIPTWKVGNFTGLYSSTVPIFNPEWQTPSFP HLQEDIINRCQQYVGPLTVNEKRRLKLIMPARFYPNLTLYLPLDKGIKPYYPEHVVNHYFQTRHYLHTLWKAGILYKRESTR SASFCSGPYSWEQDLQH <u>GC</u> WWLQFRNSEPCSEYCLCHIVNLIEDWGPCTEHGEHRIPTPRTPARVTGGVFLVDKNPHNTTES RLVVDFSQFSRGNTRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHPLHPAAMPHLLVGSSGLSRYVARLSSNSRII NNQHRTMQNLHDSCSRNLYVSLMMLLYKTYGRKLHLYSHP IILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAPPHCLAFS <u>YM</u>

Table E - <u>Pol^{A3} mutants</u> - Motifs containing inactivating mutations are underlined (YMDD mutated to YMHD, AELL mutated to AHLL). Amino acids in bold + underline + italic mark the site of deletion (last amino acid prior to the deleted region, and the first amino acid after the deleted region).			
SEQ ID NO:	HBV geno-type	Length (# amino acids)	Polypeptide sequence
			<u>HDVVLGAKSVQHLES</u> LYAAVTN <u>FLLSLGIHLNPNKTKRWG</u> YSLNFMGYVIGSWGTL <u>PQEHIVQKIKMCFRKL</u> PVNRPIDWKV CQRIVGLLGFAAPFTQCGYPALMPYACIQAKQAFTFSPTYKAFLSKQYLHLYPVARQRPGLCQVFADATPTGWGLAIGHQR MRGAFVSPPLIHTA <u>HL</u> LAACFARSRSKAKLIGTDNSVVLRSKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDP <u>SRGRLG</u> LYRPLLRLPFRPTTGRTSLYADSPSPVSHLPDRVHFASPLHVAWRPP
11	C	703	MPLSYQHFRLLLLDDEAGPLEEELPRLADEDLNRRVAEDLNLGNLNVSIPWTHKVGNTGLYSSTVPVFNP EWQTPSFPHI HLQEDIINRCQQYVGPLTVNEKRRLKLIMPARFYPNLT KYLPLDKGIKPYYPEHTVNH YFKTRHYLHTLWKAGILYKRETR SASF CGSPYSWEQELQH <u>GC</u> WWLQFRNSKPCSDYCLSHIVNLLEDWGPCTEHGEHNIRIPRTPARVTGGVFLVDKNPHNTTES RLVVDFSQFSRGSTHVS WPKF AVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMP HLLVGSSGLSRYVARLSSTSRNI NYQH GAMD LHDSCSRNLYVSLLLLYKT FGRKLHLYSHP IILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAPHPCLAFSY <u>M</u> <u>HDVVLGAKSVQHLES</u> LFTAVTN <u>FLLSLGIHLNPNKTKRWG</u> YSLNFMGYVIGSWGTL <u>PQEHIVL</u> KIKQCFRKL PVNRPIDWKV CQRIVGLLGFAAPFTQCGYPALMPYACIQAKQAFTFSPTYKAFLCKQYLNLYPVARQRSGLCQVFADATPTGWGLAVGHQR MRGTFVSPPLIHTA <u>HL</u> LAACFARSRSKAKLIGTDNSVVLRSKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDP <u>SRGRLG</u> LYRPLLRLPFRPTTGRTSLYAVSPSPVSHLPVRVHFASPLHVAWRPP
12	D	703	MPLSYQHFRLLLLDDEAGPLEEELPRLADEGLNRRVAEDLNLGNLNVSIPWTHKVGNTGLYSSTVPVFNP HWKTPSF PNI HLHQDI IKKCEQFVGPLTVNEKRRLQLIMPARFYPNVT KYLPLDKGIKPYYPEHLVNH YFQTRHYLHTLWKAGILYKRETH SASF CGSPYSWEQELQH <u>GC</u> WWLQFRNSKPCSDYCLSHIVNLLEDWGPCA EHGEHHIRIPRTPARVTGGVFLVDKNPHNTAES RLVVDFSQFSRGNYRVSWPKF AVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMP HLLVGSSGLSRYVARLSSNSRIF NYQH GTM QNLHDSCSRNLYVSLM LLYQT FGRKLHLYSHP IILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAPHPCLAFSY <u>M</u> <u>HDVVLGAKSVQHLES</u> LFTAVTN <u>FLLSLGIHLNPNKTKRWG</u> YSLHFMGYVIGCYGSLPQDHI IQKIKECFRKL PVNRPIDWKV CQRIVGLLGFAAPFTQCGYPALMPYACIQSKQAFTFSPTYKAFLCKQYLNLYPVARQRPGLCQVFADATPTGWGLVMGHQR MRGTFKAPLP IHTA <u>HL</u> LAACFARSRSGANILGTDNSVVLRSKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDP <u>SRGRLG</u> LYRPLLRLPFRPTTGRTSLYADSPSPVSHLPDRVHFASPLHVAWRPP

Table F – Internal Spacer polypeptide sequences removed from Pol^{Δ3} mutants and Core-Pol^{Δ3} fusion proteins		
SEQ ID NO:	HBV geno-type	Polypeptide sequence
46	A	RLVIKTSQRHGDEPFCSQPSGILSRSSVGPCIRSQFKQSRLGLQPHQGPLATSQSGRSGSIRARVHSPTRRCFGVEPSGSGHIGHSSASSSSS CLHQSAVRKAAYSHLSTSKRQSSSGHAVEFHSPFPSSARSQSQGPVFS
47	B	RLVFQTSKRHGDKSFQSPGILPRSSVGPCIQNLKRSRLGPQPAQGQLAGRQQGGSGSIRARVHPSPWGTGVEPSGSGHIHNCASNSSS CLHQSAVRKAAYSHISTSKGHSSSGHAVELHHFPSSSRQSQGPVLS
48	C	RLVFQTSTRHGDESFCSQSSGILSRSPVGPCIRSQLKQSRLGLQPQQGSLARSKSGRSGSIRARVHPTTRQSFQVEPSGSGHIDNSASSASS CLHQSAVRKTAYSHLSTSKRQSSSGHAVELHNFPPSSARSQSEGPLLS
49	D	AESFHQQSSGILSRPPVGSSLQSKHRKSRLGLQSQQGHARRQQGRGWSIRAGIHPTARRPFGVEPSGSGHTANLASKSASCLYQSAVRKAA YPVVSTFKKHSSSGHAVELHNLPPNSARSQSERPVPF

Table G - Core-Pol fusion proteins			
SEQ ID NO:	HBV geno-type	Length (# amino acids)	Polypeptide sequence
Core-Pol ^{mut} fusion proteins - Core sequences are indicated with bold + underline. Motifs containing inactivating mutations in Pol are underlined (YMDD mutated to YMHD, AELL mutated to AHLL).			
15	A	1030	<u>MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAILCWGELMTLATWVGNNLEDPASR</u> <u>DLVVNYVNTNMGLKIRQLLWFHISCLTFGRETVLEYLVSFQVWIRTPPAYRPPNAPILSTLPETTIVRRRDRGRSPRRRTPS</u> <u>PRRRRSQSPRRRSQSRESQC</u> MPLSYQHFRKLLLLLDDTEAGPLEEELPRLADEDLNRRVAEDLNLGNLNVSIPTWKVGNF TGLYSSTVPIFNPEWQTPSFPKIHLHEDIANRCQQFVGPLTVNEKRRLRLIMPARFYPNSTKYLPLDKGIKPYYPDHVVNHY FQTRHYLHTLWKAGILYKRETRRSASFCGSPYSWEQELHHGRLVIKTSQRHGDEPFCSQPSGILSRSSVGPCIRSQFKQSRL GLQPHQGPLATSQSGRSGSIRARVHSPTRRCFGVEPSGSGHIGHSASSSSCLHQSAVRKAAYSHLSTSKRQSSSGHAVEFH SFPSSARSQSQGPVFCWWLQFRNTQPCSKYCLSHLVNLLDWDGPCDEHGEHHIRIPRTPARVTGGVFLVDKNPHNTAESR LVVDFSQFSRGITRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHIPLHPAAMPHLLVGSSGLSRYVARLSSNSRIHN NQHGTLQNLHDSCSRQLYVSLMMLLYKTYGRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAPFHCLAFSYM HVDVVLGAKSVQHLESLEYTAVTNFLLSLGIHLNPNKTKRWGYSNFMGYVIGSWGTLPODHIVQKIKHCFRKLPIINRPIDWKVC QRIVGLLGFAAPFTQCGYPALMPYACIQAKQAFTFSPYKAFLSKQYLNLYPVARQRPGLCQVFADATPTGWGLAIGHQRM RGTFVAPLP IHTAHLLAACFARSRSAGKLIGTDNSVVLRSKYTSFPWLLGCTANWILRGTSFVYVPSALNPADDP SRGRLGL YRPLLRLPYRPTTGRTSLYAVSPSVP SHLPVRVHFASPLHVAWRPP
16	B	1026	<u>MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAILCWGELMNLATWVGSNLEDPASR</u> <u>ELVVSIVNVNMGLKIRQLLWFHISCLTFGRETVLEYLVSFQVWIRTPPAYRPPNAPILSTLPETTIVRRRGRSPRRRTPSR</u> <u>RRRSQSPRRRSQSRESQC</u> MPLSYQHFRKLLLLLDEAGPLEEELPRLADEGLNRRVAEDLNLGNLNVSIPTWKVGNFTGLY SSTVPVFNPEWQTPSFPPIHLQEDIINRCQQYVGPLTVNEKRRLKLIMPARFYPNLTLYLPLDKGIKPYYPEHVVNHYFQTR HYLHTLWKAGILYKRESTRSASFCGSPYSWEQDLQHGRLVFQTSKRHGDKSFCQSPGILPRSSVGPICIQNQLRKSRLGPQP AQGQLAGRQQGGSGSIRARVHPSPWGTGVEPSGSGHIHNCASNSSCLHQSAVRKAAYSHISTSKGHSSSGHVELHHFPP SSRSQSQGPVLSWWLQFRNSEPCSEYCLCHIVNLIEDWGPCTEHGEHRIRTPRTPARVTGGVFLVDKNPHNTTESRLVVD FSQFSRGNTRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHPLHPAAMPHLLVGSSGLSRYVARLSSNSRIINNQH TMQNLHDSCSRNLYVSLMMLLYKTYGRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAPFHCLAFSYMHDVVL GAKSVQHLESLEYAAVTNFLLSLGIHLNPHKTKRWGYSNFMGYVIGSWGTLQEHIVQKIKMCFRKLPIVNRPIDWKVCQRIV GLLGFAAPFTQCGYPALMPYACIQAKQAFTFSPYKAFLSKQYHLHYLPVARQRPGLCQVFADATPTGWGLAIGHQRM VSPLPIHTAHLLAACFARSRSAGKLIGTDNSVVLRSKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDP SRGRLGLYRPL LRLLYRPTTGRTSLYADSPSVP SHLPDRVHFASPLHVAWRPP
17	C	1026	<u>MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAILCWGELMNLATWVGSNLEDPASR</u> <u>ELVVSIVNVNMGLKIRQLLWFHISCLTFGRETVLEYLVSFQVWIRTPPAYRPPNAPILSTLPETTIVRRRGRSPRRRTPSR</u> <u>RRRSQSPRRRSQSRESQC</u> MPLSYQHFRKLLLLLDEAGPLEEELPRLADEDLNRRVAEDLNLGNLNVSIPTWKVGNFTGLY

Table G – Core-Pol fusion proteins			
SEQ ID NO:	HBV geno-type	Length (# amino acids)	Polypeptide sequence
			SSTVPVFNP EWQTPSFPHIHLQEDIINRCQQYVGPLTVNEKRRLKLIMPARFYPNLTKYLPLDKGIKPYYPEHTVNHYFKTR HYLHTLWKAGILYKRETTTRSASFCSGSPYSWEQELQHGRVLVFTSTRHGDESFCSSGILSRSPVGPICIRSQLKQSRLGLQP QQGSLARSKSGRSGSIRARVHPPTTRQSFVPEPSGSGHIDNSASSASSCLHQSAVRKTAYSHLSTSKRQSSSGHAVELHNFPP SSARSQSEGPLLSCWWLQFRNSKPCSDYCLSHIVNLLLEDWGPCTEHGEHNIRIPRTPARVTGGVFLVDKNPHNTTESRLVVD FSQFSRGSTHVSWPKFVAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLP LHPAAMP HLLVGSSGLSRYVARLSSTSRNINYQH AMQDLHDSCSRNLYVSLLLLYKTFRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRAFP HCLAFSYMHDVVL GAKSVQHLES LFTAVTNFLLSLGIHLNPNKTKRWGYS LNFMGYVIGSWGTL PQEHIVLKIKQCFRKL PVNRPIDW KVCQRIV GLLGFAAPFTQCGYPALMPLYACIQAKQAF TFSPTYKAFLCKQYLNLYPVARQRSGLCQVFADATPTGWGLAVGHQRM RGT VSPLPIHTAHL LAACFARSRS GAKLIGTDNSV VLSRKYSF P WLLGCAANWILRGTSFVYVPSALNPADDP SRGRLGLYRPL LRLPFRPTTGRTSLYAVSPSPVSHLPVRVHFASPLHVAWRPP
18	D	1015	<u>MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAILCWGELMNLATWVGVNLEDPASR</u> <u>DLVVSYVNTNMGLKFRQLLWFHISCLTFGRET VLEYLVSF G V WIRTPPAYRPPNAPILSTLPETT V VRRRGRSPRRRTPSPR</u> <u>RRRSQSPPRRRSQSRESQC</u> M PLSYQHFRLLLLLDDEAGPLEEELPRLADEGLNRRVAEDLNLGNLNV SIPWTHKVG NFTGLY SSTVPVFNP HWKTPSF PNIHLHQDI IKKCEQFVGPLTVNEKRRLQLIMPARFYPNVTKYLPLDKGIKPYYPEHLVNH YFQTR HYLHTLWKAGILYKRETT HSASFCSGSPYSWEQELQHGAESFHQQSSGILSRPPVGSS LQSKHRKSRLGLQSQQGHLARRQQG RGWSIRAGIHP TARRPFGVEPSGSGHTANLASKSASCLYQSAVRKAAYPVVSTFKKHSSSGHAVELHNLPPNSARSQSERPV FPCWWLQFRNSKPCSDYCLSHIVNLLLEDWGP CAEHGEHHIRIPRTPARVTGGVFLVDKNPHNTAESRLVVD FSQFSRGNYRV SWPKFVAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLP LHPAAMP HLLVGSSGLSRYVARLSSNSRIFNYQHGT M QNLHDSCSR NLYVSLMLLYQTFRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRAFP HCLAFSYMHDVVLGAKSVQHLES L FTAVTNFLLSLGIHLNPNKTKRWGYS LHFMGYVIGCYGSLPQDHIIQKIKECFRKL PVNRPIDW KVCQRIVGLLGFAAPFTQ CGYPALMPLYACIQSKQAF TFSPTYKAFLCKQYLNLYPVARQRPGLCQVFADATPTGWGLVMGHQRM RGT FKAPLP IHTAHL LAACFARSRS GANILGTDNSV VLSRKYSF P WLLGCAANWILRGTSFVYVPSALNPADDP SRGRLGLYRPL LRLPFRPTTGRT TSLYADSPSPVSHLPDRVHFASPLHVAWRPP
Core-Pol ^{A1} fusion proteins – Core sequences are indicated with bold + underline. Motifs containing inactivating mutations in Pol are underlined (YMDD mutated to YMHD, AELL mutated to AHLL). Amino acids in bold + italic mark the site of deletion (last amino acid prior to the deleted region, and the first amino acid after the deleted region).			
19	A	940	<u>MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAILCWGELMTLATWVGNNLEDPASR</u> <u>DLVVNYVNTNMGLKIRQLLWFHISCLTFGRET VLEYLVSF G V WIRTPPAYRPPNAPILSTLPETT V VRRRDRGRSPRRRTPS</u> <u>PRRRRSQSPPRRRSQSRESQC</u> M PLSYQHFRLLLLLDDETEAGPLEEELPRLADEDLNRRVAEDLNLGNLNV SIPWTHKVG N TGLYSSTVP I FNP EWQTPSF PKIHLHEDIANRCQQFVGPLTVNEKRRLRLIMPARFYPNSTKYLPPLDKGIKPYYPDHVVNH Y

Table G – Core-Pol fusion proteins			
SEQ ID NO:	HBV geno-type	Length (# amino acids)	Polypeptide sequence
			FQTRHYLHTLWKAGILYKRETTTSASFCGSPYSWEQELHHGRLVIKTSQRHGDEPFCSQPSGILSRSSVGP <u>PE</u> FHSFPSSAR SQQGPVFSCWWLQFRNTQPCSKYCLSHLVNLLLEDWGPCDEHGEHHIRIPRTPARVTGGVFLVDKNPHNTAESRLVVDFSQF SRGITRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHIPLHPAAMPHLLVGSSGLSRYVARLSSNSRIHNNQHGTQNL LHDSCSRQLYVSLMMLLYKTYGRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPCHCLAFSYMHDVVLGAKS VQHLESLEYTAVTNFLLSLGIHLNPNKTKRWGYSNFMGYVIGSWGTLPODHIVQKIKHCFRKLPIINRPIDWKVCQRIVGLLG FAAPFTQCGYPALMPLYACIQAKQAFTFSPTYKAFLSKQYLNLYPVARQRPGLCQVFADATPTGWGLAIGHQMRGTFVAPL PIHTAHLAALACFARSRSAGKLTGDNSSVLSRKYTSFPWLLGCTANWILRGTSFVYVPSALNPADDP SRGRLGLYRPLLRPL YRPTTGRTSLYAVSPSVP SHLPVRVHFASPLHVAWRPP
20	B	932	<u>MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAILCWGELMNLATWVGSNLEDPASR</u> <u>ELVVSYVNVNMGLKIRQLLWFHISCLTFGRETVLEYLVSFQVWIRTPPAYRPPNAPILSTLPETTIVRRRGRSPRRRTPSPR</u> <u>RRRSQSPRRRRSQSRESQCMPLSYQHFRKLLLLDDEAGPLEEELPRLADEGLNRRVAEDLNLGNLNVSIPTWKVGNFTGLY</u> SSTVPVFNPEWQTPSFPHIHLQEDIINRCQQYVGPLTVNEKRRLKLIMPARFYPNLTKYLP LDKGIKPYYPEHVVNHYFQTR HYLHTLWKAGILYKRETTTSASFCGSPYSWEQDLQHGRLVFQTSKRHGDKSFCQSPGILPR <u>SE</u> LHHFPSSSRSSQSGPVL SCWWLQFRNSEPCSEYCLCHIVNLIEDWGPCTEHGEHRIIRTPRTPARVTGGVFLVDKNPHNTTESRLVVDFSQFSRGNTRVS WPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLP LHPAAMPHLLVGSSGLSRYVARLSSNSRIINNQHRTMQNLHDSCSRN LYVSLMMLLYKTYGRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPCHCLAFSYMHDVVLGAKSVQHLESLEY AAVTNFLLSLGIHLNPNKTKRWGYSNFMGYVIGSWGTLPOEHIVQKIKMCFRKLPIINRPIDWKVCQRIVGLLGFAAPFTQC GYPALMPLYACIQAKQAFTFSPTYKAFLSKQYLNLYPVARQRPGLCQVFADATPTGWGLAIGHQMRGAFVSPLPIHTAHL AACFARSRSAGKLTGDNSSVLSRKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDP SRGRLGLYRPLLRLLYRPTTGRT SLYADSPSVP SHLPDRVHFASPLHVAWRPP
21	C	936	<u>MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAILCWGELMNLATWVGSNLEDPASR</u> <u>ELVVSYVNVNMGLKIRQLLWFHISCLTFGRETVLEYLVSFQVWIRTPPAYRPPNAPILSTLPETTIVRRRGRSPRRRTPSPR</u> <u>RRRSQSPRRRRSQSRESQCMPLSYQHFRKLLLLDDEAGPLEEELPRLADEGLNRRVAEDLNLGNLNVSIPTWKVGNFTGLY</u> SSTVPVFNPEWQTPSFPHIHLQEDIINRCQQYVGPLTVNEKRRLKLIMPARFYPNLTKYLP LDKGIKPYYPEHTVNHYFKTR HYLHTLWKAGILYKRETTTSASFCGSPYSWEQELQHGRLVFQTSRHDGDESFCSSGILSRSPVGP <u>PE</u> LHNFPPSSARSQSE GPLLSCWWLQFRNSKPCSDYCLSHIVNLLLEDWGPCTEHGEHNIRIPRTPARVTGGVFLVDKNPHNTTESRLVVDFSQFSRGS THVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLP LHPAAMPHLLVGSSGLSRYVARLSSTSRNINYQHGMQDLHDS CSRNLVVSLLLLYKTFGRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPCHCLAFSYMHDVVLGAKSVQHL ESLFTAVTNFLLSLGIHLNPNKTKRWGYSNFMGYVIGSWGTLPOEHIVLKIQCFCRKLPIINRPIDWKVCQRIVGLLGFAAP FTQCGYPALMPLYACIQAKQAFTFSPTYKAFLCKQYLNLYPVARQRSGLCQVFADATPTGWGLAVGHQMRGTFVVSPLPIHT

Table G – Core-Pol fusion proteins			
SEQ ID NO:	HBV geno-type	Length (# amino acids)	Polypeptide sequence
			AHLLAACFARSRSRGAKLIGTDNSVLSRKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDP SRGRLGLYRPLLRLPFRPT TGR TSLYAVSPSPVSHLPVRVHFASPLHVAWRPP
22	D	925	<u>MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAILCWGELMNLATWVGVNLEDPASR</u> <u>DLVVS YVNTNMGLKFRQLLWFHISCLTFGRET VLEYLV SFGVWIRTPPAYRPPNAPILSTLPETT VVRRRGRSPRRRTPSR</u> <u>RRRSQSPPRRRSQSRESQC</u> MPLSYQHFRRLLLLDDEAGPLEEELPRLADEGLNRRVAEDLNLGNLNV SIPWTHKVG NFTGLY SSTVPVFNPHWKTPSFPNIHLHQDI IKKCEQFVGPLTVNEKRRLQLIMPARFYPNVT KYLP LDKGIKPYYPEHLVNHYFQTR HYLHTLWKAGILYKRETHSASF CGSPYSWEQELQHGAESFHQQSSGILSRPPVG <u>SEL</u> HNLPNSARSQSERPVFPCWWLQF RNSKPCSDYCLSHIVNLLD WGP CAEHGEH HIRIPRTPARVTGGVFLVDKNPHNTAESRLVVD FSQFSRGNYRVSWPKFAVP NLQSLTNLLSSNLSWLSLDVSAAFYHLP LHPAAMP HLLVGSSGLSRYVARLSSNSRIFNYQHGT MQNLHDSCSRNLYVSLML LYQTFGRKLHLYSHPI ILGFRKIPMGVGLSPFLLAQFTSAICSVVRAAFPHCLAFSYMHDVVLGAKSVQHLES LFTAVTNFL LSLGIHLNPNKTKRWGYS LHFMGYVIGCYGSLPQDHIIQKIKECFRKL PVNRPIDWKVCQRIVGLLGFAAPFTQCGYPALMP LYACIQSKQAFTFSPTYKAFLCKQYLNLYPVARQRPGLCQVFADATPTGWGLVMGHQRMRGTFKAPLP IHTAHLLAACFARS RSGANILGTDNSVLSRKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDP SRGRLGLYRPLLRLPFRPTTGR TSLYADSP SVPSHLPDRVHFASPLHVAWRPP
Core-Pol ^{A3} fusion proteins – Core sequences are indicated with bold + underline. Motifs containing inactivating mutations in Pol are underlined (YMDD mutated to YMHD, AELL mutated to AHLL). Amino acids in bold + underline + italic mark the site of deletion (last amino acid prior to the deleted region, and the first amino acid after the deleted region).			
23	A	890	<u>MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAILCWGELMTLATWVGNNLEDPASR</u> <u>DLVVNYVNTNMGLKIRQLLWFHISCLTFGRET VLEYLV SFGVWIRTPPAYRPPNAPILSTLPETT VVRRRDRGRSPRRRTPS</u> <u>PRRRRSQSPPRRRSQSRESQC</u> MPLSYQHFRKLLLLDDETEAGPLEEELPRLADEDLNRRVAEDLNLGNLNV SIPWTHKVG N TGLYSSTVPIFNPEWQTPSFPKIHLHEDIANRCQQFVGPLTVNEKRRLRLIMPARFYPNSTKYLP LDKGIKPYYPDHV VNH FQTRHYLHTLWKAGILYKRETT RSASF CGSPYSWEQELHH <u>GC</u> WWLQFRNTQPCSKYCLSHLVNLLD WGPCDEHGEH HIRIP RTPARVTGGVFLVDKNPHNTAESRLVVD FSQFSRGITRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHIPLHPAAMP HLLVGSSGLSRYVARLSSNSRIHNNQHGT LQNLHDSCSRQLYVSLMLLYKTYGRKLHLYSHPI ILGFRKIPMGVGLSPFLLA QFTSAICSVVRAAFPHCLAFSYMHDVVLGAKSVQHLES LYTAVTNFLLSLGIHLNPNKTKRWGYS LNFMGYVIGSWGTL PQD HIVQKIKHCFRKLPI NRPIDWKVCQRIVGLLGFAAPFTQCGYPALMP LYACIQAKQAFTFSPTYKAFLSKQYLNLYPVARQR PGLCQVFADATPTGWGLAIGHQRMRGTFVAPLP IHTAHLLAACFARSRSRGAKLIGTDNSVLSRKYTSFPWLLGCTANWILR GTSFVYVPSALNPADDP SRGRLGLYRPLLRLPYRPTTGR TSLYAVSPSPVSHLPVRVHFASPLHVAWRPP
24	B	886	<u>MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAILCWGELMNLATWVGSNLEDPASR</u> <u>ELVVS YVNVNMGLKIRQLLWFHISCLTFGRET VLEYLV SFGVWIRTPPAYRPPNAPILSTLPETT VVRRRGRSPRRRTPSR</u>

Table G – Core-Pol fusion proteins			
SEQ ID NO:	HBV geno-type	Length (# amino acids)	Polypeptide sequence
			<u>RRRSQSPRRRRSQSRESQC</u>MPLSYQHFRKLLLLDDEAGPLEEELPRLADEGLNRRVAEDLNLGNLNVSIPTWTHKVGNFRTGLY SSTVPVFNPWEQTPSFPPIHLQEDIINRCQQYVGPLTVNEKRRLKLIMPARFYPNLT KYLPLDKGIKPYYPEHVVNHYFQTR HYLHTLWKAGILYKRESTRSASFCGSPYSWEQDLQH<u>GC</u>WWLQFRNSEPCSEYCLCHIVNLIEDWGPCTEHGEHRIRTPRTPA RVTGGVFLVDKNPHNTTESRLVVDFSQFSRGNTRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLP LHPAAMP HLLV GSSGLSRYVARLSSNSRIINNQHRTMQLNLDSCSRNLYVSLMLLYKTYGRKLHLYSHP IILGFRKIPMGVGLSPFLLAQFTS AICSVVRRAPPHCLAFSYMHDVVLGAKSVQHLESLEYAAVTNFLLSLGIHLNPHKTKRWGYSNFMGYVIGSWGTL PQEHIVQ KIKMCFRKL PVNRPIDWKVCQRIVGLLGFAAPFTQCGYPALMP LYACIQAKQAFTFSPTYKAF LSKQYLHLYPVARQRPGLC QVFADATPTGWGLAIGHQRMGAFVSPLPIHTAHLAACFARSRS GAKLIGTDNSVVL SRKYTSFPWLLGCAANWILRGTSF VYVPSALNPADDP SRGRLGLYRPLRLLYRPTTGRTSLYADSPSVP SHLPDRVHFASPLHVAWRPP
25	C	886	<u>MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAILCWGELMNLATWVGSNLEDPASR</u> <u>ELVVS YVNVMGLKIRQLLWFHISCLTFGRETVLEYLV SFGVWIRTPPAYRPPNAPILSTLPETT VVRRRGRSPRRRTPSPR</u> <u>RRRSQSPRRRRSQSRESQC</u>MPLSYQHFRKLLLLDDEAGPLEEELPRLADEGLNRRVAEDLNLGNLNVSIPTWTHKVGNFRTGLY SSTVPVFNPWEQTPSFPPIHLQEDIINRCQQYVGPLTVNEKRRLKLIMPARFYPNLT KYLPLDKGIKPYYPEHTVNHYFKTR HYLHTLWKAGILYKRETTSTRSASFCGSPYSWEQELQH<u>GC</u>WWLQFRNSKPCSDYCLSHIVNLLLEDWGPCTEHGEHNIRIPRTPA RVTGGVFLVDKNPHNTTESRLVVDFSQFSRGSTHVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLP LHPAAMP HLLV GSSGLSRYVARLSSTSRNINYQHGMQDLHDSCSRNLYVSLLLLYKTFGRKLHLYSHP IILGFRKIPMGVGLSPFLLAQFTS AICSVVRRAPPHCLAFSYMHDVVLGAKSVQHLESLEF TAVTNFLLSLGIHLNPNKTKRWGYSNFMGYVIGSWGTL PQEHIVL KIKQCFRKL PVNRPIDWKVCQRIVGLLGFAAPFTQCGYPALMP LYACIQAKQAFTFSPTYKAF LCKQYLNLYPVARQRSGLC QVFADATPTGWGLAVGHQRMGTFVSPLPIHTAHLAACFARSRS GAKLIGTDNSVVL SRKYTSFPWLLGCAANWILRGTSF VYVPSALNPADDP SRGRLGLYRPLRLPFRPTTGRTSLYAVSPSVP SHLPVRVHFASPLHVAWRPP
26	D	886	<u>MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAILCWGELMNLATWVGVNLEDPASR</u> <u>DLVVS YVNTNMGLKFRQLLWFHISCLTFGRETVLEYLV SFGVWIRTPPAYRPPNAPILSTLPETT VVRRRGRSPRRRTPSPR</u> <u>RRRSQSPRRRRSQSRESQC</u>MPLSYQHFRRLLLLLDDEAGPLEEELPRLADEGLNRRVAEDLNLGNLNVSIPTWTHKVGNFRTGLY SSTVPVFNPHWKTPSFPNIHLHQDI IKKCEQFVGPLTVNEKRRLQLIMPARFYPNVT KYLPLDKGIKPYYPEHLVNHYFQTR HYLHTLWKAGILYKRETTSTRSASFCGSPYSWEQELQH<u>GC</u>WWLQFRNSKPCSDYCLSHIVNLLLEDWGPCA EHGEHHIRIPRTPA RVTGGVFLVDKNPHNTAESRLVVDFSQFSRGNYRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLP LHPAAMP HLLV GSSGLSRYVARLSSNSRIFNYQHGT MQLNLDSCSRNLYVSLMLLYQTFGRKLHLYSHP IILGFRKIPMGVGLSPFLLAQFTS AICSVVRRAPPHCLAFSYMHDVVLGAKSVQHLESLEF TAVTNFLLSLGIHLNPNKTKRWGYSLHFMGYVIGCYGSLPQDHI IQ KIKECFRKL PVNRPIDWKVCQRIVGLLGFAAPFTQCGYPALMP LYACIQSKQAFTFSPTYKAF LCKQYLNLYPVARQRPGLC QVFADATPTGWGLVMGHQRMGTFKAPLP IHTAHLAACFARSRS GANILGTDNSVVL SRKYTSFPWLLGCAANWILRGTSF VYVPSALNPADDP SRGRLGLYRPLRLPFRPTTGRTSLYADSPSVP SHLPDRVHFASPLHVAWRPP

Core-sAg Fusion Proteins

[0085] Further provided are fusion proteins composed of an N-terminal portion comprising an HBV core polypeptide, or an immunogenic fragment thereof, and a C-terminal portion comprising an HBV small surface antigen, or an immunogenic fragment thereof. In various embodiments, the HBV core polypeptide or fragment thereof and the HBV small surface antigen (sAg), or fragment thereof, are directly fused or abutted. In some embodiments, the HBV core polypeptide or fragment thereof and the HBV small surface antigen, or fragment thereof, are connected via a linker.

HBV core polypeptide, or an immunogenic fragment thereof

10 [0086] In various embodiments, the HBV core polypeptide, or immunogenic fragment thereof, of the core-sAg fusion protein independently can be from an HBV genotype A, B/C or D. Illustrative HBV core polypeptide amino acid sequences that can be used in the herein described core-sAg fusion proteins are provided in Table H.

Table H - Illustrative HBV core polypeptide sequences		
SEQ ID NO:	HBV geno-type	Polypeptide sequence
64	A	MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALSPE HCSPHHTALRQAILCWGELMTLATWVGNNLEDPASRDLVVNYVNTN MGLKIRQLLWFHISCLTFGRETVLEYLVSFQVWIRTPPAYRPPNAP ILSTLPETTVVRRRDRGRSPRRRTPSPRRRRSQSPRRRRSQSRESQ C
65	B/C	MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALSPE HCSPHHTALRQAILCWGELMNLATWVGSNLEDPASRELVSYSVNVN MGLKIRQLLWFHISCLTFGRETVLEYLVSFQVWIRTPPAYRPPNAP ILSTLPETTVVRRRGRSPRRRTPSPRRRRSQSPRRRRSQSRESQC
66	D	MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALSPE HCSPHHTALRQAILCWGELMNLATWGVNLEDPASRDLVVSYSVNTN MGLKFRQLLWFHISCLTFGRETVLEYLVSFQVWIRTPPAYRPPNAP ILSTLPETTVVRRRGRSPRRRTPSPRRRRSQSPRRRRSQSRESQC

15 [0087] In some embodiments, the core polypeptide in the core-sAg fusion polypeptide comprises or consists of an amino acid sequence of any one of SEQ ID NOs: 64-66, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 64-66. In some embodiments, the core polypeptide comprises a serine (S) residue at the amino acid

position corresponding to position 12, and an asparagine (N) residue at the amino acid position corresponding to position 67, wherein the position numbers are with reference to SEQ ID NO:65 or SEQ ID NO:66.

HBV small surface antigen, or an immunogenic fragment thereof

5 **[0088]** In various embodiments, the HBV sAg polypeptide, or immunogenic fragment thereof, of the core-sAg fusion protein independently can be from an HBV genotype A, B, C or D. Illustrative HBV sAg polypeptide amino acid sequences that can be used in the herein described core-sAg fusion proteins are provided in Table 1, in Example 1 below.

10 **[0089]** In some embodiments, the sAg polypeptide in the core-sAg fusion polypeptide comprises or consists of an amino acid sequence of any one of SEQ ID NOs: 1-4, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 1-4, *e.g.*, comprising one or more of a serine (S) residue at the amino acid position corresponding to position 53, an isoleucine (I) residue at the amino acid position corresponding to position 68, a threonine (T)
15 residue at the amino acid position corresponding to position 125, a proline (P) residue at the amino acid position corresponding to position 127, an phenylalanine (F) residue at the amino acid position corresponding to position 161, a tyrosine (Y) residue at the amino acid position corresponding to position 200, a serine (S) residue at the amino acid position corresponding to position 210, and a leucine (L) residue at the amino acid position corresponding to position 213.

20 **[0090]** With respect to the core-sAg fusion proteins, the HBV core polypeptide and the HBV sAg polypeptide can be from the same or different HBV genotypes. In some embodiments, the core-sAg fusion protein comprises in sequential order, from the N-terminus to the C-terminus, an HBV core polypeptide and an HBV small surface antigen (sAg) polypeptide, wherein:

- 25 • the core polypeptide is from an HBV genotype A and the sAg polypeptide is from an HBV genotype A;
- the core polypeptide is from an HBV genotype B or C and the sAg polypeptide is from an HBV genotype B;
- the core polypeptide is from an HBV genotype B or C and the sAg polypeptide is from
30 an HBV genotype C;
- the core polypeptide is from an HBV genotype D and the sAg polypeptide is from an HBV genotype D;

- the core polypeptide is from an HBV genotype A and the sAg polypeptide is from an HBV genotype B;
- the core polypeptide is from an HBV genotype A and the sAg polypeptide is from an HBV genotype C;
- 5 • the core polypeptide is from an HBV genotype A and the sAg polypeptide is from an HBV genotype D;
- the core polypeptide is from an HBV genotype B or C and the sAg polypeptide is from an HBV genotype A;
- the core polypeptide is from an HBV genotype B or C and the sAg polypeptide is from an HBV genotype D;
- 10 • the core polypeptide is from an HBV genotype D and the sAg polypeptide is from an HBV genotype A;
- the core polypeptide is from an HBV genotype D and the sAg polypeptide is from an HBV genotype B; or
- 15 • the core polypeptide is from an HBV genotype D and the sAg polypeptide is from an HBV genotype C.

[0091] In some embodiments, the core-sAg fusion protein comprises in sequential order, from the N-terminus to the C-terminus, an HBV core polypeptide and an HBV small surface antigen (sAg) polypeptide, wherein:

- 20 • the core polypeptide is from an HBV genotype B or C and the sAg polypeptide is from an HBV genotype C; or
- the core polypeptide is from an HBV genotype D and the sAg polypeptide is from an HBV genotype D.

[0092] In some embodiments, the core-sAg fusion protein comprises in sequential order, from the N-terminus to the C-terminus, (i) an HBV core polypeptide comprising or consisting of an amino acid sequence of SEQ ID NO: 65, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 65; and (ii) an HBV small surface antigen (sAg) polypeptide comprising or consisting of an amino acid sequence of SEQ ID NO: 3, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 3.

[0093] In some embodiments, the core-sAg fusion protein comprises in sequential order, from the N-terminus to the C-terminus, (i) an HBV core polypeptide comprising or consisting of

an amino acid sequence of SEQ ID NO: 66, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 66; and (ii) an HBV small surface antigen (sAg) polypeptide comprising or consisting of an amino acid sequence of SEQ ID NO: 4, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 4.

[0094] In various embodiments, the core-sAg fusion proteins described herein comprise an HBV small surface antigen isoform but do not comprise an HBV medium surface antigen isoform or an HBV large surface antigen isoform. Accordingly, in some embodiments, the core-sAg fusion proteins described herein do not comprise an HBV pre-S1 polypeptide. In some embodiments, the core-sAg fusion proteins described herein do not comprise an HBV pre-S2 polypeptide. In some embodiments, the core-sAg fusion proteins described herein do not comprise both of an HBV pre-S1 polypeptide and an HBV pre-S2 polypeptide.

[0095] An illustrative HBV pre-S2 polypeptide not included in the herein described core-sAg fusion protein is provided below:

MQWNST **[A/T]** FHQ **[T/A]** LQDPRVR **[A/G]** LYFP **[A/G]** GGSS **[L/S]** G **[A/T]** **[V/I]** NPV **[L/P]** TT **[A/V]** S **[P/H]** **[L/I]** SSIF **[S/A]** RIGDP **[A/V]** **[L/M/P/T]** N (SEQ ID NO: 79) .

[0096] An illustrative HBV pre-S2 consensus polypeptide from HBV genotype A not included in the herein described core-sAg fusion protein is provided below:

MQWNSTAFHQALQDPRVRGLYFPAGGSSSGTVNPAPNIASHISSISARTGDPVTN (SEQ ID NO: 80) .

[0097] An illustrative HBV pre-S2 consensus polypeptide from HBV genotype B not included in the herein described core-sAg fusion protein is provided below:

MQWNSTTFHQTLQDPRVRALYFPAGGSSSGTVSPAQNTVSAISSILSKTGDPVPN (SEQ ID NO: 81) .

[0098] An illustrative HBV pre-S2 consensus polypeptide from HBV genotype C not included in the herein described core-sAg fusion protein is provided below:

MQWNSTTFHQALLDPRVRGLYFPAGGSSSGTVNPVPTTASPISSIFSRTGDPAPN (SEQ ID NO: 82) .

[0099] An illustrative HBV pre-S2 consensus polypeptide from HBV genotype D not included in the herein described core-sAg fusion protein is provided below:

MQWNSTTFHQTLQDPRVRGLYFPAGGSSSGTVNPVPTTASPISSIFSRIGDPALN (SEQ ID NO: 83) .

[0100] In some embodiments, the core-sAg fusion proteins described herein do not comprise an HBV pre-S2 polypeptide comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 79-83, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 79-83.

[0101] An illustrative HBV pre-S1-pre-S2 polypeptide not included in the herein described core-sAg fusion protein is provided below:

10 MGQNLSTSNPLGFFPDHQL **[D/A]** PAFRANT **[A/G/R]** NPDWDFNPKNKTWPDANKVGAGAFGL
GFTPPHGGLLGWSPQAQGI **[I/L]** QT **[L/V]** PANPPAS **[T/A]** NRQ **[S/T]** GRQPTPLSPPLR
[N/D] THPQAMQWNST **[A/T]** FHQ **[T/A]** LQDPRVR **[A/G]** LYFP **[A/G]** GGSS **[L/S]** G **[A/T]**
[V/I] NPV **[L/P]** TT **[A/V]** S **[P/H]** **[L/I]** SSIF **[S/A]** RIGDP **[A/V]** **[L/M/P/T]** N
(SEQ ID NO: 84) .

15 **[0102]** An illustrative HBV pre-S1-pre-S2 consensus polypeptide from HBV genotype A not included in the herein described core-sAg fusion protein is provided below:

MGGWSSKPRKGMGTNLSVPNPLGFFPDHQLDPAFGANSNNPDWDFNP IKDHWPAAANQVGAGAFG
PGLTPPHGGILGWSPQAQGILTTVSTIPPPASTNRQSGRQPTPISPPLRDSHPQAMQWNSTAFH
QALQDPRVRGLYFPAGGSSSGTVNPAPNIASSHISARTGDPVTN (SEQ ID NO: 85) .

20 **[0103]** An illustrative HBV pre-S1-pre-S2 consensus polypeptide from HBV genotype B not included in the herein described core-sAg fusion protein is provided below:

MGGWSSKPRKGMGTNLSVPNPLGFFPDHQLDPAFKANSENPDWDLNPHKDNWPDANKVGAGAFG
PGFTPPHGGLLGWSPQAQGLLTTPVPAAPPPASTNRQSGRQPTPISPPLRDSHPQAMQWNSTTFH
QTLQDPRVRALYFPAGGSSSGTVSPAQNTVSAISSILSKTGDPVPN (SEQ ID NO: 86) .

25 **[0104]** An illustrative HBV pre-S1-pre-S2 consensus polypeptide from HBV genotype C not included in the herein described core-sAg fusion protein is provided below:

MGGWSSKPRQGMGTNLSVPNPLGFFPDHQLDPAFGANSNNPDWDFNPKNKDHWPANQVGAGAFG
PGFTPPHGGLLGWSPQAQGILTTVPAAPPPASTNRQSGRQPTPISPPLRDSHPQAMQWNSTTFH
QALLDPRVRGLYFPAGGSSSGTVNPVPTTASPISSIFSRIGDPAPN (SEQ ID NO: 87) .

30 **[0105]** An illustrative HBV pre-S1-pre-S2 consensus polypeptide from HBV genotype D not included in the herein described core-sAg fusion protein is provided below:

MGQNLSTSNPLGFFPDHQLDPAFRANTANPDWDFNPNKDTWPDANKVGAGAFGLGFTPPHGGLL
GWSPQAQGILQTL PANPPPASTNRQSGRQFTPLSPPLRNTHPQAMQWNSTTFHQTLQDPRVRGL
YFPAGGSSSGTVNPVPTTASPISSIFSRIGDPALN (SEQ ID NO: 88) .

[0106] In some embodiments, the core-sAg fusion proteins described herein do not
comprise an HBV pre-S1-pre-S2 polypeptide comprising or consisting of an amino acid
sequence of any one of SEQ ID NOs: 84-88, or a sequence that is at least 80%, 81%, 82%, 83%,
84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%
identical to any one of SEQ ID NOs: 84-88.

Optional Polypeptide Linker

[0107] As appropriate, the HBV core polypeptide and the HBV sAg polypeptide in the
core-sAg fusion protein can be directly abutted or fused, or can be joined, connected or linked
by one or more peptide linkers. In various embodiments, the one or more peptide linkers is
selected from one or more of a polyalanine linker, a polyglycine linker, a cleavable linker, a
flexible linker, a rigid linker, and combinations thereof, *e.g.*, within a linker or within a full-
length fusion polypeptide. Illustrative fusion protein linkers that can be used in the present
fusion polypeptides to connect the HBV core polypeptide and the HBV sAg polypeptide are
described, *e.g.*, in Chen, *et al.*, *Adv Drug Deliv Rev.* (2013) 65(10): 1357–1369. In some
embodiments, the polyalanine linker comprises or consists of 2 or 3 contiguous alanine residues,
e.g. AA, AAA, AAY or AAX, wherein X is any amino acid (*e.g.*, A, C, D, E, F, G, H, I, K, L,
M, N, P, Q, R, S, T, V, Y). In some embodiments, a polyglycine linker is used, *e.g.*, GG, GGG,
GGG, GSG or GGGS (SEQ ID NO:63). In some embodiments, the cleavable linker is selected
from a 2A cleavable peptide. Illustrative 2A cleavable peptides that can be used to connect the
HBV core polypeptide and the HBV sAg polypeptide are described, *e.g.*, in Donnelly, *et al.*,
J. Gen. Virol (2001), 82, 1027–1041 and Chng, *et al.*, *mAbs* (2015) 7:2, 403-412. Illustrative
2A cleavable peptides that can be used to link the HBV core polypeptide and the HBV sAg
polypeptide include without limitation 2A cleavage sequences (*e.g.*, foot-and-mouth disease
virus (F2A), equine rhinitis A virus (E2A), porcine teschovirus-1 (P2A) and Thosea asigna virus
(T2A)), optionally in combination with a furin recognition/cleavage sequences (*e.g.* RAKR
(SEQ ID NO: 60), REKR (SEQ ID NO: 61) and RRKR (SEQ ID NO: 62)). In certain
embodiments, a furin recognition/cleavage sequence (*e.g.*, RAKR (SEQ ID NO: 60), REKR
(SEQ ID NO: 61) and RRKR (SEQ ID NO: 62)) is combined or fused with a 2A cleavable
peptide (*e.g.*, foot-and-mouth disease virus (F2A), equine rhinitis A virus (E2A), porcine
teschovirus-1 (P2A) and Thosea asigna virus (T2A)) in a single linker. *See, e.g.*, Chng, *et al.*,
mAbs (2015) 7:2, 403-412. In some embodiments, the linker comprises a porcine teschovirus-1

(P2A) linker. In various embodiments, the 2A cleavable linker comprises or consists of an amino acid sequence of ATNFSLLKQAGDVEENPGP (SEQ ID NO: 56),

APVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 57), QCTNYALLKLAGDVESNPGP (SEQ ID NO: 58), or EGRGSLLTCGDVEENPGP (SEQ ID NO: 59), or an amino acid

5 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or at least 99% identical to ATNFSLLKQAGDVEENPGP (SEQ ID NO: 56),

APVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 57), QCTNYALLKLAGDVESNPGP (SEQ ID NO: 58), or EGRGSLLTCGDVEENPGP (SEQ ID NO: 59). In various embodiments,

the 2A cleavable linker comprises or consists of an amino acid sequence of

10 ATNFSLLKQAGDVEENPGP (SEQ ID NO: 56), or an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or at least 99% identical to

ATNFSLLKQAGDVEENPGP (SEQ ID NO: 56). As appropriate, in certain embodiments, a furin recognition/cleavage sequence can be positioned either at the N-terminus or the C-terminus of a 2A linker. In some embodiments, the cleavable linker comprises or consists of a furin

15 recognition/cleavage site selected from RAKR (SEQ ID NO: 60), REKR (SEQ ID NO: 61) and RRKR (SEQ ID NO: 62). Illustrative linkers that can be used to link or connect the HBV core polypeptide and the HBV sAg polypeptide are provided in Table J.

TABLE J – illustrative linkers for connecting HBV core and HBV sAg polypeptides in the core-sAg fusion protein		
SEQ ID NO:	NAME	SEQUENCE
	poly-alanine (2)	AA
	poly-alanine (3)	AAA
	poly-alanine-Tyr	AAY
	poly-alanine-XXX	AAX (X=any amino acid)
	poly-glycine (2)	GG
	poly-glycine (3)	GGG
	poly-glycine/serine (3)	GGG
	poly-glycine/serine (3)	GSG
63	Gly3Ser	GGGS
60	furin recognition site	RAKR
61	furin recognition site	REKR
62	furin recognition site	RRKR
56	P2A	ATNFSLLKQAGDVEENPGP
57	F2A	APVKQTLNFDLLKLAGDVESNPGP
58	E2A	QCTNYALLKLAGDVESNPGP
59	T2A	EGRGSLLTCGDVEENPGP

[0108] In some embodiments, the core-sAg fusion protein is no longer than 450 amino acids in length, *e.g.*, no longer than 445, 440, 435, 430, 425, 420, 415 or 410 amino acids in length.

[0109] In some embodiments, the core-sAg fusion protein does not comprise an amino sequence or fragment thereof from an HBV protein selected from the group consisting of X, pre-core, pre-S1, pre-S2 and polymerase.

[0110] In some embodiments, the core-sAg fusion protein comprises or consists of an amino acid sequence of any one of SEQ ID NOs: 38-41, *e.g.*, SEQ ID NO: 41, or a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 38-41, SEQ ID NO: 41. In some embodiments, the fusion polypeptide comprises one or more of a serine (S) residue at the amino acid position corresponding to position 12, an asparagine (N) residue at the amino acid position corresponding to position 67, a valine (V) residue at the amino acid position corresponding to position 74, a phenylalanine (F) residue at the amino acid position corresponding to position 97, a threonine (T) residue at the amino acid position corresponding to position 249, a threonine (T) residue at the amino acid position corresponding to position 250, a serine (S) residue at the amino acid position corresponding to position 317, a serine (S) residue at the amino acid position corresponding to position 318, an arginine (R) residue at the amino acid position corresponding to position 326, a tyrosine (Y) residue at the amino acid position corresponding to position 338, a glycine (G) residue at the amino acid position corresponding to position 363, and an alanine (A) residue at the amino acid position corresponding to position 372, wherein the position numbers are with reference to SEQ ID NO:41.

[0111] Illustrative core-sAg fusion proteins, *e.g.*, for use in promoting, inducing or eliciting an immunogenic response, *e.g.*, against core and/or small surface antigens expressed by HBV, are provided in Table K.

Table K - Core-sAg fusion proteins			
SEQ ID NO:	HBV geno-type	Length (# amino acids)	Polypeptide sequence
Core-sAg fusion proteins - Core sequences are indicated with bold + underline. Flexible GSG linker indicated by italics. Cleavable P2A linker indicated by underlining.			
38	Core: B/C sAg: C	409	<u>MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAILCWGEL</u> <u>MNLATWVGSNLEDPASRELVSIVNVNMGLKIRQLLWFHISCLTFGRETVLEYLVSFQVWIRTPP</u> <u>AYRPPNAPILSTLPETTVVRRRGRSPRRRTPSPRRRRSQSPRRRRSQSRESQC</u> <u>MESTTSGFLGPL</u> LVLQAGFFLLTRILTIPQSLDSWWTSLNFLGGAPTCPGQNSQSPTSNSHPTSCPPICPGYRWMCL RRFIIIFLCILLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGPKCTCTTPAQGTSMFPSCCCTKP TDGNCTCIPISSWAFARFLWEWASVRFSWLSLLVPFVQWFVGLSPTVWLSVIWMMWYWGPSLYN ILSPFLPLLPIFFCLWVYI
39	Core: B/C sAg: C	430	<u>MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAILCWGEL</u> <u>MNLATWVGSNLEDPASRELVSIVNVNMGLKIRQLLWFHISCLTFGRETVLEYLVSFQVWIRTPP</u> <u>AYRPPNAPILSTLPETTVVRRRGRSPRRRTPSPRRRRSQSPRRRRSQSRESQC</u> <u>GSGATNFSLLKQ</u> AGDVEENPGPESTTSGFLGPLLVLQAGFFLLTRILTIPQSLDSWWTSLNFLGGAPTCPGQNSQSP TSNSHPTSCPPICPGYRWMCLRRFIIIFLCILLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGPC KTCTTPAQGTSMFPSCCCTKPTDGNCTCIPISSWAFARFLWEWASVRFSWLSLLVPFVQWFVGL SPTVWLSVIWMMWYWGPSLYNILSPFLPLLPIFFCLWVYI
40	Core: D sAg: D	409	<u>MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAILCWGEL</u> <u>MNLATWVGVNLEDPASRDLVSIVNTNMGLKFRQLLWFHISCLTFGRETVLEYLVSFQVWIRTPP</u> <u>AYRPPNAPILSTLPETTVVRRRGRSPRRRTPSPRRRRSQSPRRRRSQSRESQC</u> <u>MENITSGFLGPL</u> LVLQAGFFLLTRILTIPQSLDSWWTSLNFLGGTTVCLGQNSQSPTSNSHPTSCPPICPGYRWMCL RRFIIIFLFILLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGPCRTCTTPAQGTSMYPSCCCTKP SDGNCTCIPISSWAFGKFLWEWASARFSWLSLLVPFVQWFVGLSPTVWLSVIWMMWYWGPSLYS ILSPFLPLLPIFFCLWVYI
41	Core: D	430	<u>MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAILCWGEL</u> <u>MNLATWVGVNLEDPASRDLVSIVNTNMGLKFRQLLWFHISCLTFGRETVLEYLVSFQVWIRTPP</u>

Table K - Core-sAg fusion proteins			
SEQ ID NO:	HBV geno-type	Length (# amino acids)	Polypeptide sequence
	sAg: D		<u>AYRPPNAPILSTLPETTVVRRRGRSPRRRTSPRRRRSQSPRRRRSQSRESQC</u> GSGATNFSLLKQ AGDVEENPGPENITSGFLGPLLVLQAGFFLLTRILTIPQSLDSWWTSLNFLGGTTVCLGQNSQSP TSNHSP TSCPPICPGYRWMCLRRFIIFLFILLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGPC RTCTTPAQGTSMYPSCCCTKPSDGNCTCIPIPSSWAFGKFLWEWASARFSWLSLLVPFVQWFVGL SPTVWLSVIWMMWYWGPSLYSILSPFLPLLPIFFCLWVYI

Signal or Leader Sequences

[0112] In various embodiments, the immunogenic polypeptides described herein comprise a signal sequence or signal peptide, *e.g.*, to direct intracellular trafficking of the polypeptide to a proteasomal or lysosomal compartment. In various embodiments, the immunogenic polypeptide comprises a signal sequence at the N-terminus and/or the C-terminus. In some embodiments, the immunogenic polypeptide comprises an N-terminal signal peptide or leader sequence. In various embodiments, the signal peptide or leader sequence is from a source protein selected from a serum protein, a cytokine, a chemokine, a chaperone protein, an invariant protein, and a protein that directs proteins to the lysosomal compartment. In some embodiments, the signal peptide or leader sequence is from a source protein selected from colony stimulating factor 2 (CSF2, GM-CSF), tissue type plasminogen activator (PLAT, t-PA), C-C motif chemokine ligand 7 (CCL7, MCP-3), C-X-C motif chemokine ligand 10 (CXCL10, IP-10), catenin beta 1 (CTNNB1), CD74 (p33; DHLAG; HLADG; Ia-GAMMA, invariant chain), serum albumin (ALB), polyubiquitin B/C (UBB/UBC), calreticulin (CALR), vesicular stomatitis virus G protein (VSV-G), lysosomal associated membrane protein 1 (LAMP-1) and lysosomal associated membrane protein 2 (LAMP-2). In various embodiments, the signal peptide or leader sequence is selected from an amino acid sequence of any one of SEQ ID NOs: 67-76, or a sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to any one of SEQ ID NOs: 67-76. In certain embodiments, the immunogenic polypeptide comprises N-terminal and C-terminal signal sequences from LAMP-1, *e.g.*, SEQ ID NOs: 77 and 78, respectively. Illustrative signal sequences that can be used in the present immunogenic polypeptides are provided in Table L.

TABLE L - illustrative signal sequences		
SEQ ID NO:	source protein name	SEQUENCE
67	CSF2, GM-CSF	MWLQSLLLLGTVACSISV
68	PLAT, t-PA	MDAMKRGLCCVLLLCGAVFVSAR
69	CD74	MHRRRSRSCREDQKPV
70	albumin	KWVTFISLLFLFSSAYS
71	β -catenin	MRKAAVSHWQQQSYLDSGIHSGATTTAPSL
72	CCL7, MCP-3	MNPSAAVIFCLILLGLSGTQGILDMAQPVGINTSTTCCYRFI NKKIPKQRLESYRRTTSSHCPREAVIFKTKLDKEICADPTQK WVQDFMKHLDKKTQTPKLASAGA
73	ubiquitin	MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQR LIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLGG
74	calreticulin	MLLSVPLLLGLLGLAVA

TABLE L - illustrative signal sequences		
SEQ ID NO:	source protein name	SEQUENCE
75	VSV-G	MKCLLYLAFLFIGVNC
76	CXCL10, IP-10	MNQTAILICCLIFLTLSGIQG
77	LAMP-1 N-terminal	MAPRSARRPLLLLLLLLLLLLLGLMHCSAAMFMVKNNGTACIM ANFSAAFSVNYDTKSGPKNMTLDLPSDATVVLNRSSCGKENT SDPSLVIAFGRGHTLTNLNFTRNATRYSVQLMSFVYNLSDTHL FPNASSKEIKTVESITDIRADIDKKYRCVSGTQVHMNNVTVT LHDATIQAYLSNSSFSRGETRCEQDRPSPTTAPPAPPSPSPS PVPKSPSVDKYNVSGTNGTCLLASMGLQLNLTYERKDNTTVT RLLNINPNKTSASGSCGAHLVTLELHSEGTTVLLFQFGMNAS SSRFFLQGIQLNTILPDARDPAFKAANGSLRALQATVGNSYK CNAEEHVVRVTKAFSVNIFKVWVQAFKVEGGQFGSVEECLLDE NSLEDI
78	LAMP-1 C-terminal	GSEFTLIPIAVGGALAGLVIVLIAAYLVGRKRSHAGYQTI

[0113] Further provided are methods for making the immunogenic polypeptides described herein. In some implementations, the methods comprise constructing the immunogenic polypeptides using peptide synthesis. In some implementations, the methods comprise constructing, using synthetic or recombinant DNA technology, polynucleotides encoding each of the polypeptides of the bivalent antigen and expressing the polypeptides from an expression vector. In some implementations, the methods may further comprise inserting the polynucleotides into one or more vectors and expressing the encoded polypeptides in a cell. This can be done employing known recombinant techniques.

3. Polynucleotides Encoding Immunogenic Polypeptides

[0114] Provided are polynucleotides encoding the immunogenic polypeptides, described herein, vectors comprising such polynucleotides, and host cells (*e.g.*, human cells, mammalian cells, yeast cells, plant cells, insect cells, bacterial cells, *e.g.*, *E. coli*) comprising such polynucleotides or expression vectors. Provided herein are polynucleotides comprising nucleotide sequence(s) encoding any of the immunogenic polypeptides provided herein, as well as expression cassettes and vector(s) comprising such polynucleotide sequences, *e.g.*, expression vectors for their efficient expression in host cells, *e.g.*, mammalian cells. In various embodiments, the polynucleotide is a DNA, a cDNA, an mRNA, a self-amplifying RNA (SAM), a self-replicating RNA, or a self-amplifying replicon RNA (RepRNA). In some embodiments, the polynucleotide comprises or is expressed from an alphavirus self-replicating or self-amplifying replicon RNA (RepRNA). Self-replicating RNA and self-amplifying replicon RNA

as modes of vaccine delivery are described, *e.g.*, by Tews, *et al.*, *Methods Mol Biol.* (2017) 1499:15-35; Démoulins, *et al.*, *Methods Mol Biol.* (2017) 1499:37-75; Englezou, *et al.*, *Mol Ther Nucleic Acids.* (2018) 12:118-134; McCollough, *et al.*, *Vaccines* (Basel). (2014) 2(4):735-54; and McCollough, *et al.*, *Mol Ther Nucleic Acids.* (2014) 3:e173.

5 **[0115]** The terms “polynucleotide” and “nucleic acid molecule” interchangeably refer to a polymeric form of nucleotides and includes both sense and anti-sense strands of RNA, cDNA, genomic DNA, and synthetic forms and mixed polymers of the above. As used herein, the term nucleic acid molecule may be interchangeable with the term polynucleotide. In some
10 embodiments, a nucleotide refers to a ribonucleotide, deoxynucleotide or a modified form of either type of nucleotide, and combinations thereof. The terms also include without limitation, single- and double-stranded forms of DNA. In addition, a polynucleotide, *e.g.*, a cDNA or mRNA, may include either or both naturally occurring and modified nucleotides linked together by naturally occurring and/or non-naturally occurring nucleotide linkages. The nucleic acid molecules may be modified chemically or biochemically or may contain non-natural or
15 derivatized nucleotide bases, as will be readily appreciated by those of skill in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analogue, internucleotide modifications such as uncharged linkages (*e.g.*, methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, etc.), charged linkages (*e.g.*, phosphorothioates, phosphorodithioates, etc.), pendent
20 moieties (*e.g.*, polypeptides), intercalators (*e.g.*, acridine, psoralen, etc.), chelators, alkylators, and modified linkages (*e.g.*, alpha anomeric nucleic acids, etc.). The above term is also intended to include any topological conformation, including single-stranded, double-stranded, partially duplexed, triplex, hairpinned, circular and padlocked conformations. A reference to a nucleic acid sequence encompasses its complement unless otherwise specified. Thus, a reference to a
25 nucleic acid molecule having a particular sequence should be understood to encompass its complementary strand, with its complementary sequence. The term also includes codon-biased polynucleotides for improved expression in a desired viral expression vector or host cell.

[0116] A “substitution,” as used herein, denotes the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

30 **[0117]** An “isolated” nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location. “Isolated nucleic acid encoding an

immunogenic polypeptide” refers to one or more nucleic acid molecules encoding such immunogenic polypeptides, including such nucleic acid molecule(s) in a single vector or multiple separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

5 **[0118]** A “polynucleotide variant,” as the term is used herein, is a polynucleotide that typically differs from a polynucleotide specifically disclosed herein in one or more substitutions, deletions, additions and/or insertions. Such variants may be naturally occurring or may be synthetically generated, for example, by modifying one or more of the polynucleotide sequences described herein and evaluating one or more biological activities of the encoded polypeptide as
10 described herein and/or using any of a number of techniques well known in the art.

[0119] In some embodiments, the nucleic acid molecule is codon-biased to enhance expression in a desired host cell, *e.g.*, in human cells, mammalian cells, yeast cells, plant cells, insect cells, or bacterial cells, *e.g.*, *E. coli* cells. Accordingly, provided are polynucleotides encoding an immunogenic polypeptide, described herein, wherein the polynucleotides are
15 codon-biased, comprise replacement heterologous signal sequences, and/or have mRNA instability elements eliminated. Methods to generate codon-biased nucleic acids can be carried out by adapting the methods described in, *e.g.*, U.S. Patent Nos. 5,965,726; 6,174,666; 6,291,664; 6,414,132; and 6,794,498. Preferred codon usage for expression of the immunogenic polypeptides from desired viral expression vectors and/or in desired host cells is provided, *e.g.*,
20 at kazusa.or.jp/codon/; and genscript.com/tools/codon-frequency-table.

[0120] In some embodiments, the polynucleotide encoding an immunogenic polypeptide, as described herein, has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical, or 100% identical to a nucleic acid sequence selected from the group
25 consisting of SEQ ID NOs: 27-37 and 89-94, as provided in Table M.

[0121] As appropriate, in certain embodiments, the 3'-end of a polynucleotide encoding one or more of the immunogenic polypeptides described herein comprises one or multiple tandem stop codons, *e.g.*, two or more tandem TAG (“amber”), TAA (“ochre”) or TGA (“opal” or “umber”) stop codons. The multiple tandem stop codons can be the same or different.

30 **[0122]** Further provided are expression cassettes, comprising a polynucleotide encoding an immunogenic polypeptide, as described herein, operably linked to one or more regulatory sequences. In some embodiments, the polynucleotide is operably linked to and under the control of a constitutive promoter. In some embodiments, the promoter is selected from

cytomegalovirus major immediate-early (CMV), the CMV enhancer fused to the chicken beta-actin promoter (CAG), human elongation factor-1 α (HEF-1 α), mouse cytomegalovirus (mouse CMV), Chinese hamster elongation factor-1 α (CHEF-1 α), and phosphoglycerate kinase (PGK).

Table M - Polynucleotides encoding immunogenic polypeptides			
SEQ ID NO:	HBV geno-type	name	Polynucleotide sequence
27	B	Pol ^{Δ1}	ATGCCCCCTGAGCTACCAGCACTTCAGGAAGCTGCTGCTGCTGGATGATGAGGCTGGCCCTCTGGAGGAGGAGC TGCCCAGGCTGGCAGATGAGGGCCTCAACAGGAGAGTGGCAGAGGACCTGAACCTGGGCAACCTGAATGTGAG CATCCCCCTGGACCCACAAAGTGGGGAACCTTCACTGGCCTCTACAGCAGCACAGTGCCAGTGTTCAACCCTGAG TGGCAGACCCCCCTCCTTCCCCCACATCCACCTCCAGGAGGACATCATCAACAGATGTCAGCAGTATGTGGGCC CTCTGACAGTCAATGAGAAGAGGAGGCTGAAGCTGATCATGCCTGCCAGGTTCTACCCCCAACCTGACCAAGTA CCTCCCCTGAGACAAGGGCATCAAGCCATACTATCCTGAGCATGTGGTGAACCACTACTTTTTCAGACCAGGCAC TACCTGCACACACTGTGGAAGGCTGGCATCCTGTACAAGAGGGAGAGCACCAGATCAGCCTCTTTTCTGTGGCT CCCCCTACAGCTGGGAGCAGGATCTCCAGCATGGCAGACTGGTGTTCCAGACCTCCAAGAGGCATGGGGACAA GTCCTTTTGGCCCCAGAGCCCTGGCATCCTGCCAGGAGCGAGCTCCACCACTTCCCCCCCCTCCTCCAGCAGA AGCCAGTCCCAGGGACCTGTGCTGTCTGCTGGTGGCTCCAGTTCAGGAACAGTGAGCCCTGCAGTGAGTACT GTCTGTGTCACATTGTGAACCTGATTGAGGACTGGGGGCCCTGCACTGAGCATGGAGAGCACAGGATCAGAAC CCCCAGGACCCCAGCCAGAGTGACTGGAGGTGTGTTTCTGGTGGACAAGAACCCCCACAACACCACAGAGAGC AGACTGGTGGTGGACTTCTCCCAGTTTTTCAAGGGGCAACACCAGAGTGTCTGGCCCCAAGTTTGCAGTGCCCCA ACCTCCAGAGCCTGACCAACCTGCTGTTCATCAACCTGAGCTGGCTGTCCCTGGATGTGTCTGCTGCCTTCTA CCACCTGCCCCCTGCACCCTGCAGCCATGCCTCACCTCCTGGTGGGCAGCTCAGGCCTGAGCAGGTATGTGGCC AGGCTGTCAAGCAACTCCAGAATCATCAACAACCAGCACAGGACCATGCAGAACCTGCATGACTCTTGAGCA GGAACCTGTATGTGAGCCTGATGCTGCTGTACAAGACCTATGGCAGGAAGCTGCACCTGTACTCCCACCCCAT CATCCTGGGTTTTAGGAAGATCCCCATGGGAGTGGGACTGTCCCCCTTCTGCTGGCCCAGTTCACCTCTGCC ATCTGCTCTGTGGTGGAGAGGCCTTCCCCCACTGCCTGGCCTTCTCCTACATGCATGATGTGGTGTGGGGG CCAAGTCAGTGACGACCTGGAGTCTCTGTATGCTGCAGTACCAACTTCTGCTCAGCCTGGGCATCCACCT GAACCCCCACAAGACCAAGAGGTGGGGCTACTCTCTGAACCTTTCATGGGCTATGTGATAGGCAGCTGGGGCACC CTGCCACAGGAGCACATAGTGCAGAAGATCAAGATGTGCTTCAGGAAGCTGCCAGTGAACAGGCCCCATTGATT GGAAGGTGTGCCAGAGGATTGTGGGCCTGCTGGGCTTTGCAGCACCCCTTACACAGTGTGGCTACCCAGCTCT GATGCCCCCTGTATGCCTGCATCCAGGCCAAGCAGGCCTTACCTTCTCCCCCACTTACAAGGCCTTCTGTCC AAGCAGTACCTGCACCTGTACCCTGTGGCAAGGCAGAGGCCAGGCCTCTGCCAGGTGTTTGCAGATGCCACCC CCACAGGCTGGGGCCTGGCCATTGGCCACCAGAGGATGAGAGGGGCCTTTGTGAGCCCACTGCCAATCCACAC AGCCCACCTGCTGGCAGCATGCTTTGCCAGGTCCAGGTCTGGTGCAAAGCTGATTGGCACTGACAACAGTGTG GTGCTGTCCAGAAAGTACACCAGCTTCCCCTGGCTGCTGGGATGTGCTGCCAACTGGATTCTGAGGGGCACCA

Table M - Polynucleotides encoding immunogenic polypeptides			
SEQ ID NO:	HBV geno-type	name	Polynucleotide sequence
			GCTTTGTCTATGTGCCCTCTGCACTGAACCCTGCAGATGACCCCTCCAGGGGCAGACTGGGGCTGTACAGGCC ACTGCTCAGACTGCTGTACAGGCCCACTTGGCAGAACCTCCCTGTATGCAGACAGCCCCCTCAGTGCCCTCT CACCTGCCAGACAGAGTGCACCTTTGCCAGCCCCCTGCATGTTGCCTGGAGGCCCCC
28	B	Pol ^{Δ3}	ATGCCCCCTGAGCTACCAGCACTTCAGGAAGCTGCTGCTGCTGGATGATGAGGCTGGCCCTCTGGAGGAGGAGC TGCCCAGGCTGGCAGATGAGGGCCTCAACAGGAGAGTGGCAGAGGACCTGAACCTGGGCAACCTGAATGTGAG CATCCCCCTGGACCCACAAAGTGGGGAACCTTCACTGGCCTCTACAGCAGCACAGTGCCAGTGTTCAACCCTGAG TGGCAGACCCCCCTCCTTCCCCCACATCCACCTCCAGGAGGACATCATCAACAGATGTCAGCAGTATGTGGGCC CTCTGACAGTCAATGAGAAGAGGAGGCTGAAGCTGATCATGCCTGCCAGGTTCTACCCCAACCTGACCAAGTA CCTCCCACTGGACAAGGGCATCAAGCCATACTATCCTGAGCATGTGGTGAACCACTACTTTTCAGACCAGGCAC TACCTGCACACACTGTGGAAGGCTGGCATCCTGTACAAGAGGGAGAGCACCAGATCAGCCTCTTTCTGTGGCT CCCCCTACAGCTGGGAGCAGGATCTCCAGCATGGCTGCTGGTGGCTCCAGTTCAGGAACAGTGAGCCCTGCAG TGAGTACTGTCTGTGTACATTGTGAACCTGATTGAGGACTGGGGGCCCTGCACTGAGCATGGAGAGCACAGG ATCAGAACCCCCAGGACCCCAGCCAGAGTGACTGGAGGTGTGTTCTGGTGGACAAGAACCCCCACAACACCA CAGAGAGCAGACTGGTGGTGGACTTCTCCCAGTTTTCAAGGGGCAACACCAGAGTGTCTGGCCCAAGTTTGC AGTGCCCAACCTCCAGAGCCTGACCAACCTGCTGTATCAACCTGAGCTGGCTGTCCCTGGATGTGTCTGCT GCCTTCTACCACTGCCCCCTGCACCCTGCAGCCATGCCTCACCTCCTGGTGGGCAGCTCAGGCCTGAGCAGGT ATGTGGCCAGGCTGTCAAGCAACTCCAGAATCATCAACAACCAGCACAGGACCATGCAGAACCTGCATGACTC TTGCAGCAGGAACCTGTATGTGAGCCTGATGCTGCTGTACAAGACCTATGGCAGGAAGCTGCACCTGTACTCC CACCCCATCATCCTGGGTTTTAGGAAGATCCCCATGGGAGTGGGACTGTCCCCCTTCTGCTGGCCCAAGTTCA CCTCTGCCATCTGCTCTGTGGTGAGGAGAGCCTTCCCCCACTGCCTGGCCTTCTCCTACATGCATGATGTGGT GCTGGGGGCCAAGTCAGTGCAGCACCTGGAGTCTCTGTATGCTGCAGTCACCAACTTCCTGCTCAGCCTGGGC ATCCACCTGAACCCCCACAAGACCAAGAGGTGGGGCTACTCTCTGAACTTCATGGGCTATGTGATAGGCAGCT GGGGCACCTGCCACAGGAGCACATAGTGCAGAAGATCAAGATGTGCTTCAGGAAGCTGCCAGTGAACAGGCC CATTGATTGGAAGGTGTGCCAGAGGATTGTGGCCTGCTGGGCTTTGCAGCACCTTCACACAGTGTGGCTAC CCAGCTCTGATGCCCTGTATGCCTGCATCCAGGCCAAGCAGGCCTTCACCTTCTCCCCCACTTACAAGGCCT TCCTGTCCAAGCAGTACCTGCACCTGTACCCTGTGGCAAGGCAGAGGCCAGGCCTCTGCCAGGTGTTTGCAGA TGCCACCCCCACAGGCTGGGGCCTGGCCATTGGCCACCAGAGGATGAGAGGGGCCCTTTGTGAGCCCACTGCCA ATCCACACAGCCCACCTGCTGGCAGCATGCTTTGCCAGGTCCAGGTCTGGTGCAAAGCTGATTGGCACTGACA ACAGTGTGGTGTGTCCAGAAAGTACACCAGCTTCCCCCTGGCTGCTGGGATGTGCTGCCAACTGGATTCTGAG GGGCACCAGCTTTGTCTATGTGCCCTCTGCACTGAACCCTGCAGATGACCCCTCCAGGGGCAGACTGGGGCTG

Table M - Polynucleotides encoding immunogenic polypeptides			
SEQ ID NO:	HBV geno-type	name	Polynucleotide sequence
			TACAGGCCACTGCTCAGACTGCTGTACAGGCCACCCTGGCAGAACCTCCCTGTATGCAGACAGCCCCTCAGTGCCCTCTCACCTGCCAGACAGAGTGCACCTTGCCAGCCCCCTGCATGTTGCCTGGAGGCCCCC
29	B	Pol ³⁰⁰	ATGTCCAGCAGAAGCCAGTCCCAGGGACCTGTGCTGTCCTGCTGGTGGCTCCAGTTCAGGAACAGTGAGCCCTGCAGTGAGTACTGTCTGTGTACATTGTGAACCTGATTGAGGACTGGGGGCCCTGCACTGAGCATGGAGAGCACAGGATCAGAACCCCCAGGACCCCAGCCAGAGTGACTGGAGGTGTGTTCCCTGGTGGACAAGAACCCCCACAACACCACAGAGAGCAGACTGGTGGTGGACTTCTCCAGTTTTCAAGGGGCAACACCAGAGTGTCTGGCCCAAGTTTGCAGTGCCCAACCTCCAGAGCCTGACCAACCTGCTGTGCATCAAACCTGAGCTGGCTGTCCCTGGATGTGCTGCTGCCTTCTACCACCTGCCCCCTGCACCCTGCAGCCATGCCTCACCTCCTGGTGGGCAGCTCAGGCCTGAGCAGGTATGTGGCCAGGCTGTCAAGCAACTCCAGAATCATCAACAACCAGCACAGGACCATGCAGAACCTGCATGACTCTTGCAGCAGGAACCTGTATGTGAGCCTGATGCTGCTGTACAAGACCTATGGCAGGAAGCTGCACCTGTACTCCCACCCCATCATCCTGGGTTTTAGGAAGATCCCCATGGGAGTGGGACTGTCCCCCTTCTGCTGGCCCAGTTCACCTCTGCCATCTGCTCTGTGGTGAGGAGAGCCTTCCCCCACTGCCTGGCCTTCTCCTACATGCATGATGTGGTGCTGGGGGCCAAGTCAGTGCAGCACCTGGAGTCTCTGTATGCTGCAGTCACCAACTTCTGCTCAGCCTGGGCATCCACCTGAACCCCCACAAGACCAAGAGGTGGGGCTACTCTCTGAACCTCATGGGCTATGTGATAGGCAGCTGGGGCACCTGCCACAGGAGCACATAGTGCAGAAAGATCAAGATGTGCTTCAGGAAGCTGCCAGTGAACAGGCCATTGATTGGAAGGTGTGCCAGAGGATTGTGGGCCTGCTGGGCTTTGCAGCACCTTACACAGTGTGGCTACCCAGCTCTGATGCCCCTGTATGCCTGCATCCAGGCCAAGCAGGCCTTACCTTCTCCCCCACTTACAAGGCCTTCTGTCCAAGCAGTACCTGCACCTGTACCCTGTGGCAAGGCAGAGGCCAGGCCTCTGCCAGGTGTTTGCAGATGCCACCCCCACAGGCTGGGGCCTGGCCATTGGCCACCAGAGGATGAGAGGGGCCTTTGTGAGCCCCTGCCAATCCACACAGCCCACCTGCTGGCAGCATGCTTTGCCAGGTCCAGGTCTGGTGCAAAGCTGATTGGCACTGACAACAGTGTGGTGCTGTCCAGAAAGTACACCAGCTTCCCCTGGCTGCTGGGATGTGCTGCCAACTGGATTCTGAGGGGCACCAGCTTTGTCTATGTGCCCTCTGCACTGAACCTGCAGATGACCCCTCCAGGGGCAGACTGGGCTGTACAGGCCACTGCTCAGACTGCTGTACAGGCCACCCTGGCAGAACCTCCCTGTATGCAGACAGCCCCTCAGTGCCCTCTCACCTGCCAGACAGAGTGCACCTTGCCAGCCCCCTGCATGTTGCCTGGAGGCCCCC
89	B	Pol ³⁰⁰ ori	ATGTCTTCAAGATCCCAGAGTCAGGGCCCTGTACTTTCTGCTGGTGGCTCCAGTTCAGGAACAGTGAGCCCTGCTCCGAATACTGTCTCTGCCATATCGTCAATCTTATCGAAGACTGGGGACCCTGTACCGAACATGGAGAACA TCGCATCAGGACTCCTAGGACCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTGACAAAAATCCTCACAATACCACAGAGTCTAGACTCGTGGTGGACTTCTCTCAATTTTCTAGGGGGAACACCCGTGTGTCTTGGCCAAAATTCGCAGTCCCAAATCTCCAGTCACTACCAACCTGTTGTCTCCAATTTGTCTGGTTATCGCTGGATGTGCTGCGGCGTTTTATCATCTTCTCTGCATCCTGCTGCTATGCCTCATCTTCTTGTGGTTCTTCTGGACTATCA

Table M - Polynucleotides encoding immunogenic polypeptides			
SEQ ID NO:	HBV geno-type	name	Polynucleotide sequence
			AGGTATGTTGCCCGTTTGTCTCTAATTCCAGGATCATCAACAACCAGCACCGGACCATGCAAAACCTGCACG ACTCCTGCTCAAGGAACCTCTATGTTTCCCTCATGTTGCTGTACAAAACCTACGGACGGAAACTGCACTTGTA TTCCCATCCCATCATCTTGGGCTTTTCGCAAAATTCCTATGGGAGTGGGCCTCAGTCCGTTTCTCTTGGCTCAG TTTACTAGTGCCATTTGTTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTCTGGCTTTTCAGTTATATGCATGATG TGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTTATGCCGCTGTTACCAATTTTCTTTTGTCTTT GGGTATACATTTAAACCCTCACAAAACAAAAGATGGGGATATTCCCTTAACTTCATGGGATATGTAATTGGG AGTTGGGGCACATTGCCGCAGGAACATATTGTACAAAAAATCAAATGTGTTTTAGGAAACTTCCTGTAAACC GGCCTATTGATTGGAAAGTATGTCAACGAATTGTGGGTCTTTTGGGGTTTGCCGCCCTTTTCACGCAATGTGG ATATCCTGCTTTAATGCCTTTATATGCATGTATACAAGCAAAACAGGCTTTTACTTTCTCGCCAACCTTACAAG GCCTTCCTAAGTAAACAGTATCTGCACCTTTACCCCGTTGCTCGGCAACGGCCTGGTCTGTGCCAAGTGTGTTG CTGACGCAACCCCCACTGGTTGGGGCTTGGCCATAGGCCATCAGCGCATGCGTGGAGCCTTCGTGTCTCCTCT GCCGATCCATACTGCGCATCTCCTGGCCGCTTGTTTTGCTCGCAGCAGGTCTGGGGCAAAACTCATCGGGACT GACAATTCTGTCTGCTCTCCCGCAAGTATACATCCTTTCCATGGCTGCTAGGCTGTGCTGCCAACTGGATCC TGCGCGGGACGTCTTTGTTTACGTCCCGTCGGCGCTGAATCCCGCGGACGACCCCTCCCGGGGCCGCTTGGG GCTCTACCGCCCGCTTCTCCGCTTGTTGTACCGACCGACTACGGGGCGCACCTCTCTCTACGCGGACTCCCCG TCTGTGCCTTCTCATCTGCCGGACCGTGTGCACTTCGCTTCACCTCTGCACGTCGCATGGAGACCACCGT
90	B	Pol ³⁰⁰ dint	ATGTCATCCAGATCCCAGAGTCAGGGCCCTGTCCTTTCTGTTGGTGGCTCCAGTTCAGGAACAGTGAGCCCT GTTCTGAGTACTGTCTCTGCCACATTGTCAATCTGATTGAGGACTGGGGCCCCTGCACAGAGCATGGTGAACA CAGGATCAGGACTCCCAGGACCCCTGCCAGGGTGACTGGTGGGGTTTTCTTGTGACAAAAATCCTCACAAC ACCACAGAGTCAAGGCTTGTGGTGGACTTCTCTCAATTTTCAAGGGGGAACACAAGGGTGTCTTGGCCCAAAT TTGCAGTCCCAAATCTCCAGTCTCTGACCAACCTGTTGTCCTCCAATTTGTCCTGGTTGTCTCTGGATGTCTC TGCTGCCTTTTATCATCTTCCCTCTCCATCCTGCTGCCATGCCTCATCTTCTTGTGTTGGTTCTTCTGGCCTCTCT AGGTATGTTGCCAGATTGTCCTCCAATTCAGGATCATCAACAACCAGCACAGGACCATGCAAAACCTGCATG ACTCCTGCTCCAGAAACCTCTATGTTTCTCTCATGTTGCTGTACAAAACCTATGGCAGGAAACTGCATTTGTA TTCCCATCCCATCATCTTGGGCTTCAGGAAAATTCCTATGGGAGTGGGCCTCAGTCCCTTCTCTTGGCTCAG TTCACCAAGTGCCATTTGTTCTGTTGTGTCAGGAGGGCTTTCCCCCACTGTCTTGCTTTTCAGTTACATGCATGATG TGGTCTTGGGGGCCAAGTCTGTCCAACATCTTGAGTCACCTTTATGCTGCTGTGACCAACTTTCTTTTGTCTTT GGGCATCCATTTGAACCCTCACAAAACAAAAGATGGGGCTATTCCCTCAATTTTCATGGGCTATGTCAATTGGG AGTTGGGGCACTTTGCCCCAGGAACACATTGTGCAAAAAATCAAGATGTGTTTCAGGAAACTTCCTGTGAACA GGCCAATTGACTGGAAAGTCTGTGAGAGAATTGTGGGTCTTTTGGGGTTTGCAGCTCCTTTACCCAATGTGG

Table M - Polynucleotides encoding immunogenic polypeptides			
SEQ ID NO:	HBV geno-type	name	Polynucleotide sequence
			CTATCCTGCTTTGATGCCCTTGTATGCCTGCATCCAGGCCAAACAGGCTTTCACCTTTCTCCCCACTTACAAG GCCTTCCTCAGCAAACAGTATCTCCACCTTTACCCTGTTGCAAGGCAGAGGCCTGGTCTGTGCCAAGTGTTTG CTGATGCAACCCCCACTGGTTGGGGCTTGGCCATTGGCCATCAGAGAATGAGAGGTGCCTTTGTGTCTCCTCT CCCCATCCACACTGCTCATCTCCTGGCAGCTTGCTTTGCAAGGAGCAGGTCTGGAGCCAACTCATAGGGACT GACAATTCTGTGGTGCTCTCCAGAAAGTACACCTCCTTTTCTTGGCTGCTGGGCTGTGCAGCCAACTGGATCC TGAGGGGGACTTCCTTTGTTTATGTCCCCTCTGCCCTGAATCCTGCAGATGACCCCTCCAGGGGCAGGTTGGG GCTCTACAGACCCCTTCTCAGGTTGTTGTACAGACCAACAACAGGGAGGACCTCTCTCTATGCAGATTCCCCC TCTGTTTCTTCTCATCTTCCAGACAGAGTGCACCTTTGCTTCTCCTCTGCATGTGGCTTGGAGACCTCCC
91	B	Pol ³⁰⁰ huCo low GC	ATGTCTAGCAGAAGCCAGTCCCAGGGACCTGTGCTGTCTTGTGGTGGCTTCAGTTTCGGAATAGCGAGCCAT GTAGCGAGTATTGCCTGTGTACATCGTGAATCTGATTGAGGATTGGGGACCATGCACAGAGCACGGAGAGCA CCGGATCAGAACCCTTAGGACACCAGCCCGGTGACAGGAGGCGTGTTCCTGGTGGATAAGAACCCCCATAAT ACAACAGAGAGCAGACTGGTGGTGGATTTTTCTCAGTTTTCTCGGGGCAATACAAGAGTGTCTGGCCAAAGT TTGCCGTGCCCAATCTCCAGAGCCTGACAAACCTGCTGTCTTCTAATCTGAGCTGGCTGTCCCTGGACGTGTC CGCCGCCTTTTACCACCTGCCACTGCACCCTGCCGCCATGCCCCACCTGCTGGTGGGCAGCTCCGGACTGAGC AGATACGTGGCAAGGCTGTCTAGCAATTCTAGAATTATTAATAATCAGCACAGAACAATGCAGAATCTGCATG ATTCTTGTAGCAGGAATCTGTACGTGAGCCTGATGCTGCTGTATAAGACATATGGACGCAAGCTGCACCTGTA TTCTCACCTATTATTCTGGGCTTCCGGAAGATCCCTATGGGCGTGGGACTGTCCCCATTCTGCTGGCCCAG TTTACCTCCGCCATCTGCTCTGTGGTGGGAGAGCCTTCCCACATTGTCTGGCCTTTTCTTACATGCACGATG TGGTGCTGGGCGCCAAATCCGTGCAGCACCTGGAGTCTCTGTATGCCGCCGTGACAAACTTCCTGCTGAGCCT GGGCATCCACCTGAATCCACATAAGACAAAGCGGTGGGGCTATTCTCTGAATTTTATGGGCTATGTGATCGGC AGCTGGGGAACCCTGCCACAGGAGCACATTGTGCAGAAGATCAAGATGTGCTTTCGCAAGCTGCCCCGTGAATC GGCCTATCGATTGGAAGGTGTGCCAGAGGATCGTGGGACTGCTGGGATTTCGAGCACCCTTTACCCAGTGCGG CTACCCAGCCCTGATGCCACTGTATGCCTGTATCCAGGCCAAACAGGCCTTCACCTTTTCCCCTACATATAAG GCTTTTCTGTCTAAGCAGTACCTGCATCTGTATCCAGTGGCAAGGCAGAGGCCAGGACTGTGCCAGGTGTTTG CAGATGCAACACCAACAGGATGGGGACTGGCAATCGGACACCAGAGGATGAGAGGAGCCTTCGTGAGCCCACT GCCAATTCACACCGCCCACCTGCTGGCAGCATGCTTTGCAAGGTCCCGCTCTGGAGCAAAGCTGATTGGCACC GATAACAGCGTGGTGCTGTCCAGAAAATACACCAGCTTCCCCTGGCTGCTGGGATGTGCAGCAAATTGGATTTC TGAGGGGACACAGCTTCGTGTATGTGCCTTCCGCCCTGAATCCTGCCGATGATCCATCTCGAGGCAGACTGGG ACTGTATAGGCCACTGCTGAGACTGCTGTATAGGCCTACCACAGGCAGAACATCCCTGTATGCCGACAGCCCA TCCGTGCCCTCTCACCTGCCAGATAGAGTGCATTTTCGCAAGCCCCTGCATGTGGCATGGAGGCCACCC

Table M - Polynucleotides encoding immunogenic polypeptides			
SEQ ID NO:	HBV geno-type	name	Polynucleotide sequence
92	B	Pol ³⁰⁰ ori_d el CpG	ATGTCTTCAAGATCCCAGAGTCAGGGCCCTGTACTTTCTGCTGGTGGCTCCAGTTCAGGAACAGTGAGCCCT GCTCTGAATACTGTCTCTGCCATATTGTCAATCTTATAGAAGACTGGGGACCCTGTACTGAACATGGAGAACA TAGGATCAGGACTCCTAGGACCCCTGCTAGAGTTACAGGGGGGTTTTCTTGTGACAAAAATCCTCACAAT ACCACAGAGTCTAGACTTGTGGTGGACTTCTCTCAATTTTCTAGGGGGAACACCAGGGTGTCTTGGCCAAAAT TTGCAGTCCCAAATCTCCAGTCACTACCAACCTGTTGTCCTCCAATTTGTCCTGGTTATCCCTGGATGTGTC TGCAGCCTTTTTATCATCTTCCCTCTGCATCCTGCTGCTATGCCTCATCTTCTTGTGTTGGTTCTTCTGGACTATCA AGGTATGTTGCCAGGTTGTCCTCTAATTCAGGATCATCAACAACCAGCACAGGACCATGCAAAACCTGCATG ACTCCTGCTCAAGGAACCTCTATGTTTCCCTCATGTTGCTGTACAAAACCTATGGAAGGAAACTGCATTGTA TTCCCATCCCATCATCTTGGGCTTTAGAAAAATTCCTATGGGAGTGGGCCTCAGTCCCTTTCTCTTGGCTCAG TTTACTAGTGCCATTTGTTTCAAGTGGTTAGAAAGGGCTTTCCCCCACTGTCTGGCTTTTCAAGTTATATGCATGATG TGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTTATGCTGCTGTTACCAATTTTCTTTTGTCTTT GGGTATACATTTAAACCCTCACAAAACAAAAGATGGGGATATTCCCTTAACTTCATGGGATATGTAATTGGG AGTTGGGGCACATTGCCTCAGGAACATATTGTACAAAAAATCAAAATGTGTTTTAGGAAACTTCCTGTAAACA GGCCTATTGATTGGAAGTATGTCAAAGAATTGTGGGTCTTTTGGGGTTTGCAGCCCCCTTTCACCCAATGTGG ATATCCTGCTTTAATGCCTTTATATGCATGTATACAAGCAAAACAGGCTTTTACTTTCTCCCCAACTTACAAG GCCTTCCTAAGTAAACAGTATCTGCACCTTTACCCTGTTGCTAGGCAAAGGCCTGGTCTGTGCCAAGTGTG CTGATGCAACCCCCACTGGTTGGGGCTTGGCCATAGGCCATCAGAGGATGAGGGGAGCCTTTGTGTCTCCTCT GCCTATCCATACTGCCCATCTCCTGGCAGCTTGTGTTTGTAGGAGCAGGTCTGGGGCAAACTCATTGGGACT GACAATTCTGTTGTGCTCTCCAGAAAGTATACATCCTTTCCATGGCTGCTAGGCTGTGCTGCCAACTGGATCC TGAGGGGGACATCCTTTGTTTATGTCCCTTCAGCACTGAATCCTGCTGATGACCCCTCCAGGGGCAGATTGGG GCTCTACAGGCCCTTCTCAGGTTGTTGTACAGACCCACTACTGGGAGAACCTCTCTCTATGCAGACTCCCC TCTGTGCCTTCTCATCTGCCTGACAGGGTGCACCTTTGCTTCACCTCTGCATGTTGCATGGAGACCACCT
93	B	Pol ³⁰⁰ IDT	ATGAGTTCCCGATCACAGAGTCAGGGGGCCCGTCCTTTTCATGTTGGTGGCTTCAGTTTCGAAACTCCGAGCCAT GTTCTGAGTATTGTCTCTGCCACATTGTGAATCTTATTGAAGACTGGGGCCCCTGCACCGAGCACGGCGAGCA CCGAATACGGACACCTCGAACGCCAGCAAGAGTGACGGGCGGAGTGTTCCCTCGTCGACAAGAATCCACACAAC ACGACGGAGAGTAGATTGGTCGTTGATTTTCAAGAGGCAATACACGAGTTTCTTGGCCGAAAT TCGCCGTACCGAATCTGCAATCCTTGACAAATTTGCTTAGTTCTAATTTGTCTTGGCTTTCTCTCGATGTTTC CGCCGCTTTCTATCACTTGCCCCTTCACCCAGCCGCGATGCCGCATCTCTTGGTGGGCAGCTCTGGACTTAGT AGATACGTAGCTAGACTCAGTTCTAACTCACGGATAATAAATAACCAACATCGCACTATGCAGAACCTGCATG ATTCTTGTTCCCGGAACCTTGATGTCTCCTTGATGTTGTTGTATAAAACTTATGGGCGAAAGCTTCATCTGTA

Table M - Polynucleotides encoding immunogenic polypeptides			
SEQ ID NO:	HBV geno-type	name	Polynucleotide sequence
			TAGCCATCCGATTATATTGGGTTTTAGGAAAATTCCTATGGGTGTTGGCTTGAGCCCTTTTCTGCTGGCGCAA TTTACTTCAGCTATCTGCTCAGTAGTACGCCGGGCGTTTCCCCATTGCTTTGCTTTCTCATAACATGCATGATG TAGTACTTGGGGCCAAGTCTGTACAACACCTTGAGAGTTTGTATGCCGCCGTAACATAATTTCTTCTCTCTCT CGGGATCCATCTTAACCCTCACAAAACGAAGAGGTGGGGTTATTCTCTGAATTTTCATGGGATATGTTATCGGG TCTTGGGGAACGCTGCCTCAGGAACACATCGTCCAGAAAATCAAGATGTGTTTCAGAAAGTTGCCAGTGAACA GACCGATAGATTGGAAGGTTTGCCAAAGAATTGTTGGCTTGTTGGGATTTCGCAGCCCCATTACACAGTGC GG GTATCCGGCTTTGATGCCCCTTTATGCTTGTATCCAGGCAAAACAGGCATTACCTTTTACCGACTTACAAA GCATTTCTTTCTAAGCAGTATCTCCATCTTTACCCTGTCGCTCGACAGCGGCCGGGGCTTTGCCAGGTTTTCG CAGACGCAACCCCAACTGGTTGGGGTCTTGCGATCGGCCACCAGAGGATGCGCGGTGCATTTCGTGTCCCCGCT CCCAATCCATACGGCCCCACTTGCTGGCGGCGTGCTTCGCTCGAAGTAGAAGCGGGGCTAAATTGATCGGCACG GACAATTCAGTCGTGTTGTCACGCAAATATACCTCCTTTCCCTGGTTGCTCGGTTGCGCAGCAAACCTGGATAC TTCGGGGAAGTAACTAGTTTTCGTTTATGTGCCCTCTGCTCTCAACCCCGCCGACGATCCTTCACGAGGGAGGCTGGG TCTTTACCGCCCATTGCTCAGGCTGCTTTACCGGCCTACCACTGGGAGAACAAGCTTGTACGCCGACAGCCCCG AGCGTCCCGTCTCATCTGCCCCGACAGAGTTCACTTTGCGAGTCCATTGCACGTCGCTTGGCGCCCGCCG
94	B	Pol ³⁰⁰ _IDT_ CpGde 1	ATGAGTTCCAGATCACAGAGTCAGGGGCCTGTCTTTTCATGTTGGTGGCTTCAGTTTAGAACTCAGAGCCAT GTTCTGAGTATTGTCTCTGCCACATTGTGAATCTTATTGAAGACTGGGGCCCCCTGCACAGAGCATGGAGAGCA CAGAATAAGGACACCTAGAACCCAGCAAGAGTGACAGGTGGAGTGTTCCCTGGTAGACAAGAATCCACACAAC ACAACTGAGAGTAGATTGGTGGTTGATTTTCAGTCAATTTTCAAGAGGCAATACAAGAGTTTCTTGGCCAAAAT TTGCTGTACCCAATCTGCAATCCTTGACAAATTTGCTTAGTTCTAATTTGTCTTGGCTTTCTCTAGATGTTTC TGCAGCTTTCTATCACTTGCCCCCTTACCCAGCAGCTATGCCTCATCTCTTGGTGGGCAGCTCTGGACTTAGT AGATATGTAGCTAGACTCAGTTCTAACTCAAGGATAATAAATAACCAACATAGGACTATGCAGAACCTGCATG ATTCTTGTTCAGGAACCTTGATGTCTCCTTGATGTTGTTGTATAAACTTATGGGAGAAAGCTTCATCTGTA TAGCCATCCTATTATATTGGGTTTTAGGAAAATTCCTATGGGTGTTGGCTTGAGCCCTTTTCTGCTGGCCCAA TTTACTTCAGCTATCTGCTCAGTAGTAAGGAGGGCCTTTCCCCATTGCTTTGCTTTCTCATAACATGCATGATG TAGTACTTGGGGCCAAGTCTGTACAACACCTTGAGAGTTTGTATGCAGCAGTAACATAATTTCTTCTCTCTCT TGGGATCCATCTTAACCCTCACAAAACCAAGAGGTGGGGTTATTCTCTGAATTTTCATGGGATATGTTATAGGG TCTTGGGGAACCCTGCCTCAGGAACACATTGTCCAGAAAATCAAGATGTGTTTCAGAAAGTTGCCAGTGAACA GACCAATAGATTGGAAGGTTTGCCAAAGAATTGTTGGCTTGTTGGGATTTGCAGCCCCATTACACAGTGTGG GTATCCTGCTTTGATGCCCCTTTATGCTTGTATCCAGGCAAAACAGGCATTACCTTTTACCCACTTACAAA GCATTTCTTTCTAAGCAGTATCTCCATCTTTACCCTGTGGCTAGACAGAGGCCAGGGCTTTGCCAGGTTTTTG

Table M - Polynucleotides encoding immunogenic polypeptides			
SEQ ID NO:	HBV geno-type	name	Polynucleotide sequence
			CAGATGCAACCCCAACTGGTTGGGGTCTTGCAATTGGCCACCAGAGGATGAGAGGTGCATTTGTGTCCCCACT CCCAATCCATACTGCCCACCTTGCTGGCAGCTTGCTTTGCTAGAAAGTAGAAGTGGGGCTAAATTGATTGGCACA GACAATTCAGTTGTGTTGTCAAGGAAATATAACCTCCTTTCCCTGGTTGCTTGTTGTGCAGCAAACCTGGATAC TTAGGGGAAGTAGTTTTTGTATGTGCCCTCTGCTCTCAACCCTGCAGATGATCCTTCAAGAGGGAGGCTGGG TCTTTACAGGCCATTGCTCAGGCTGCTTTACAGGCCTACCACTGGGAGAACAAGCTTGTATGCAGACAGCCCC AGTGTCCCCTCTCATCTGCCTGACAGAGTTCACTTTGCAAGTCCATTGCATGTTGCTTGGAGACCTCCA
30	D	Pol ^{Δ1}	ATGCCCCCTGAGCTACCAACACTTCAGGAGACTGCTGCTGCTGGATGATGAGGCAGGCCCTCTGGAGGAGGAGC TGCCCAGGCTGGCAGATGAGGGCCTGAACAGGAGGGTGGCTGAGGACCTGAACCTGGGCAACCTGAATGTGAG CATCCCTTGGACCCACAAAGTGGGCAACTTCACAGGCCTGTACAGCAGCACTGTGCCTGTGTTCAACCCCCAC TGGAAGACACCCAGCTTCCCCAACATCCACCTGCACCAGGACATCATCAAGAAGTGTGAGCAGTTTGTGGGCC CCCTGACAGTCAATGAGAAGAGGAGGCTCCAGCTGATCATGCCAGCCAGGTTCTACCCCAATGTGACCAAGTA CCTCCCCCTGGACAAGGGCATCAAGCCTTACTATCCAGAGCACCTGGTGAACCACTACTTCCAGACCAGACAC TACCTGCACACACTGTGGAAGGCAGGCATCCTGTACAAGAGGGAGACCACACACAGTGCCTCCTTCTGTGGCA GCCCCTACTCCTGGGAGCAGGAGCTGCAACATGGAGCTGAGTCCTTCCACCAGCAGTCCAGTGGCATCCTGAG CAGGCCCCCTGTGGGCAGCGAGCTGCACAACCTGCCCCCAACTCTGCCAGATCCCAGTCTGAGAGGCCAGTG TTCCCTTGCTGGTGGCTCCAGTTCAGGAACAGCAAGCCCTGCTCAGACTACTGCCTGAGCCACATTGTGAACC TGCTGGAGGACTGGGGCCCCCTGTGCAGAGCATGGGGAGCACCACATCAGAATCCCCAGGACCCCTGCCAGGGT GACAGGAGGGGTGTTCCCTGGTGGACAAGAACCCCCACAACACTGCAGAGTCCAGGCTGGTGGTGGACTTCTCC CAGTTCAGCAGGGGGCAACTACAGAGTCTCCTGGCCAAAGTTTGCTGTGCCCAACCTCCAGAGCCTGACAAACC TGCTGAGCAGCAACCTGTCCTGGCTCTCCCTGGATGTGAGTGCAGCCTTCTATCACCTGCCCCCTGCACCCAGC AGCCATGCCACACCTGCTGGTGGGCTCCAGTGGCCTGTCCAGGTATGTGGCCAGGCTCTCCTCCAACCTCCAGG ATCTTCAACTATCAGCATGGCACCATGCAGAACCTGCATGACAGCTGCTCCAGGAACCTGTATGTGTCCCTGA TGCTGCTCTATCAGACCTTTTGGCAGGAAGCTGCACCTGTACAGCCACCCCATCATCCTGGGGTTCAGGAAGAT CCCCATGGGTGTGGGCCTGTCCCCCTTCTGCTGGCCAGTTTACCAGTGCCATCTGCTCAGTGGTGAGGAGG GCCTTCCCACACTGCCTGGCCTTCTCTTACATGCATGATGTGGTCCCTGGGTGCCAAGTCTGTGCAGCACCTGG AGAGCCTGTTACAGCTGTGACAAACTTTCTCCTGAGCCTGGGCATCCACCTGAACCCCAACAAGACCAAGAG GTGGGGTTATTCACTGCACCTTCATGGGCTATGTGATTGGCTGCTATGGCTCTCTGCCACAGGACCACATCATC CAGAAGATCAAGGAGTGCTTCAGAAAGCTGCCAGTGAACAGGCCAATTGACTGGAAGGTGTGCCAGAGGATTG TGGGCCTGCTGGGCTTTGCAGCCCCCTTACCCAGTGTGGCTACCCTGCCCTGATGCCCTGTATGCCTGCAT CCAGAGCAAGCAGGCCTTCACCTTTTCCCCACTTACAAGGCCTTCCCTGTGCAAGCAGTACCTGAACCTGTAC

Table M - Polynucleotides encoding immunogenic polypeptides			
SEQ ID NO:	HBV geno-type	name	Polynucleotide sequence
			CCTGTGGCCAGGCAGAGACCTGGGCTGTGCCAGGTGTTTGCAGATGCCACCCCCACAGGATGGGGACTGGTCA TGGGACACCAGAGGATGAGGGGCACCTTCAAGGCACCCCTGCCCATCCACACAGCCCACCTGCTGGCTGCCTG CTTTGCCAGGAGCAGGAGTGGGGCCAACATCCTGGGCACAGACAACCTCTGTGGTGCTGAGCAGGAAGTACACA TCCTTCCCCTGGCTGCTGGGATGTGCAGCCAACCTGGATCCTGAGGGGGCACCAGCTTTGTGTATGTGCCCTCTG CCCTCAACCCTGCAGATGATCCAAGCAGGGGCAGGCTGGGACTGTACAGGGCCACTGCTCAGACTGCCCTTCAG GCCCACCACTGGCAGGACCAGCCTGTATGCTGACTCCCCATCTGTGCCCTCCCACCTGCCTGACAGAGTGCAC TTTGCCTCCCCACTGCATGTGGCCTGGAGGCCCCCA
31	D	Pol ^{Δ3}	ATGCCCCCTGAGCTACCAACACTTCAGGAGACTGCTGCTGCTGGATGATGAGGCAGGCCCTCTGGAGGAGGAGC TGCCCAGGCTGGCAGATGAGGGCCTGAACAGGAGGGTGGCTGAGGACCTGAACCTGGGCAACCTGAATGTGAG CATCCCTTGGACCCACAAAGTGGGCAACTTCACAGGCCTGTACAGCAGCACTGTGCCTGTGTTCAACCCCCAC TGGAAGACACCCAGCTTCCCCAACATCCACCTGCACCAGGACATCATCAAGAAGTGTGAGCAGTTTGTGGGCC CCCTGACAGTCAATGAGAAGAGGAGGCTCCAGCTGATCATGCCAGCCAGGTTCTACCCCCAATGTGACCAAGTA CCTCCCCCTGGACAAGGGCATCAAGCCTTACTATCCAGAGCACCTGGTGAACCACTACTTCCAGACCAGACAC TACCTGCACACACTGTGGAAGGCAGGCATCCTGTACAAGAGGGAGACCACACACAGTGCCTCCTTCTGTGGCA GCCCCTACTCCTGGGAGCAGGAGCTGCAACATGGATGCTGGTGGCTCCAGTTCAGGAACAGCAAGCCCTGCTC AGACTACTGCCTGAGCCACATTGTGAACCTGCTGGAGGACTGGGGCCCCCTGTGCAGAGCATGGGGAGCACCAC ATCAGAATCCCCAGGACCCCTGCCAGGGTGACAGGAGGGGTGTTTCTGGTGGACAAGAACCCCCACAACACTG CAGAGTCCAGGCTGGTGGTGGACTTCTCCCAGTTCAGCAGGGGCAACTACAGAGTCTCCTGGCCAAAGTTTGC TGTGCCCCAACCTCCAGAGCCTGACAAACCTGCTGAGCAGCAACCTGTCCTGGCTCTCCCTGGATGTGAGTGCA GCCTTCTATCACCTGCCCCTGCACCCAGCAGCCATGCCACACCTGCTGGTGGGCTCCAGTGGCCTGTCCAGGT ATGTGGCCAGGCTCTCCTCCAACCTCCAGGATCTTCAACTATCAGCATGGCACCATGCAGAACCTGCATGACAG CTGCTCCAGGAACCTGTATGTGTCCCTGATGCTGCTCTATCAGACCTTTGGCAGGAAGCTGCACCTGTACAGC CACCCCATCATCCTGGGGTTTCAGGAAGATCCCCATGGGTGTGGGCCTGTCCCCCTTCCCTGCTGGCCCAGTTCA CCAGTGCCATCTGCTCAGTGGTGAGGAGGGCCTTCCCACACTGCCTGGCCTTCTCTTACATGCATGATGTGGT CCTGGGTGCCAAGTCTGTGCAGCACCTGGAGAGCCTGTTACAGCTGTGACAAACTTTCTCCTGAGCCTGGGC ATCCACCTGAACCCCAACAAGACCAAGAGGTGGGGTTATTCACTGCACTTCATGGGCTATGTGATTGGCTGCT ATGGCTCTCTGCCACAGGACCACATCATCCAGAAGATCAAGGAGTGCTTCAGAAAGCTGCCAGTGAACAGGCC AATTGACTGGAAGGTGTGCCAGAGGATTGTGGGCCTGCTGGGCTTTGCAGCCCCCTTCACCCAGTGTGGCTAC CCTGCCCTGATGCCCCTGTATGCCTGCATCCAGAGCAAGCAGGCCTTCACCTTTTCCCCCACTTACAAGGCCT TCCTGTGCAAGCAGTACCTGAACCTGTACCCTGTGGCCAGGCAGAGACCTGGGCTGTGCCAGGTGTTTGCAGA

Table M - Polynucleotides encoding immunogenic polypeptides			
SEQ ID NO:	HBV geno-type	name	Polynucleotide sequence
			TGCCACCCCCACAGGATGGGGACTGGTCATGGGACACCAGAGGATGAGGGGCACCTTCAAGGCACCCCTGCCC ATCCACACAGCCCACCTGCTGGCTGCCTGCTTTGCCAGGAGCAGGAGTGGGGCCAACATCCTGGGCACAGACA ACTCTGTGGTGTCTGAGCAGGAAGTACACATCCTTCCCCCTGGCTGCTGGGATGTGCAGCCAACTGGATCCTGAG GGGCACCAGCTTTGTGTATGTGCCCTCTGCCCTCAACCCTGCAGATGATCCAAGCAGGGGCAGGCTGGGACTG TACAGGCCACTGCTCAGACTGCCCTTCAGGCCACCCTGGCAGGACCAGCCTGTATGCTGACTCCCCATCTG TGCCCTCCACCTGCCTGACAGAGTGCACCTTGCCTCCCCACTGCATGTGGCCTGGAGGCCCCCA
32	D	Pol ³⁰⁰	ATGTCTGCCAGATCCCAGTCTGAGAGGCCAGTGTTCCTTGCTGGTGGCTCCAGTTCAGGAACAGCAAGCCCT GCTCAGACTACTGCCTGAGCCACATTGTGAACCTGCTGGAGGACTGGGGCCCCCTGTGCAGAGCATGGGGAGCA CCACATCAGAATCCCCAGGACCCCTGCCAGGGTGACAGGAGGGGTGTTCCCTGGTGGACAAGAACCCCCACAAC ACTGCAGAGTCCAGGCTGGTGGTGGACTTCTCCCAGTTCAGCAGGGGCAACTACAGAGTCTCCTGGCCAAAGT TTGCTGTGCCCAACCTCCAGAGCCTGACAAACCTGCTGAGCAGCAACCTGTCTGGCTCTCCCTGGATGTGAG TGCAGCCTTCTATCACCTGCCCCCTGCACCCAGCAGCCATGCCACACCTGCTGGTGGGCTCCAGTGGCCTGTCC AGGTATGTGGCCAGGCTCTCCTCCAACCTCCAGGATCTTCAACTATCAGCATGGCACCATGCAGAACCTGCATG ACAGCTGCTCCAGGAACCTGTATGTGTCCCTGATGCTGCTCTATCAGACCTTTGGCAGGAAGCTGCACCTGTA CAGCCACCCCATCATCCTGGGGTTTCCAGGAAGATCCCCATGGGTGTGGGCCTGTCCCCCTTCTGCTGGCCAG TTCACCAAGTGCCATCTGCTCAGTGGTGAGGAGGGCCTTCCACACTGCCTGGCCTTCTCTTACATGCATGATG TGGTCCCTGGGTGCCAAGTCTGTGCAGCACCTGGAGAGCCTGTTACAGCTGTGACAACTTTCTCCTGAGCCT GGGCATCCACCTGAACCCCAACAAGACCAAGAGGTGGGGTTATTCACTGCACTTCATGGGCTATGTGATTGGC TGCTATGGCTCTCTGCCACAGGACCACATCATCCAGAAGATCAAGGAGTGCTTCAGAAAGCTGCCAGTGAACA GGCCAATTGACTGGAAGGTGTGCCAGAGGATTGTGGGCCTGCTGGGCTTTGCAGCCCCCTTACCCAGTGTGG CTACCCTGCCCTGATGCCCTGTATGCCTGCATCCAGAGCAAGCAGGCCTTACCTTTTCCCCCACTTACAAG GCCTTCTCTGTGCAAGCAGTACCTGAACCTGTACCCTGTGGCCAGGCAGAGACCTGGGCTGTGCCAGGTGTTTG CAGATGCCACCCCCACAGGATGGGGACTGGTCATGGGACACCAGAGGATGAGGGGCACCTTCAAGGCACCCCT GCCATCCACACAGCCCACCTGCTGGCTGCCTGCTTTGCCAGGAGCAGGAGTGGGGCCAACATCCTGGGCACA GACAACTCTGTGGTGTCTGAGCAGGAAGTACACATCCTTCCCCCTGGCTGCTGGGATGTGCAGCCAACTGGATCC TGAGGGGCACCAGCTTTGTGTATGTGCCCTCTGCCCTCAACCCTGCAGATGATCCAAGCAGGGGCAGGCTGGG ACTGTACAGGCCACTGCTCAGACTGCCCTTCAGGCCACCCTGGCAGGACCAGCCTGTATGCTGACTCCCCA TCTGTGCCCTCCACCTGCCTGACAGAGTGCACCTTGCCTCCCCACTGCATGTGGCCTGGAGGCCCCCA
33	B/C	Core-sAg	ATGGACATTGACCCCTACAAGGAGTTTGGGGCCAGTGTGGAGCTGCTGTCTTTTCTGCCATCTGACTTCTTCC CCAGTGTGAGGGACCTGCTGGACACTGCCTCAGCACTGTACAGAGAGGCCCTGGAGAGCCAGAGCACTGCTC

Table M - Polynucleotides encoding immunogenic polypeptides			
SEQ ID NO:	HBV geno-type	name	Polynucleotide sequence
			CCCCACACACAGCCCTGAGGCAGGCCATCCTCTGCTGGGGGGAGCTGATGAACCTGGCCACCTGGGTGGGC TCCAACCTGGAGGACCCTGCCTCAAGGGAGCTGGTGGTCAGCTATGTCAATGTGAACATGGGCCTCAAGATCA GGCAGCTGCTGTGGTTCCACATCTCCTGCCTGACCTTTGGCAGGGAGACAGTCCTGGAGTACCTGGTGAGCTT TGGGGTGTGGATCAGGACCCCCCTGCCTACAGGCCCCCAATGCTCCCATCCTGTCCACCCTGCCAGAGACC ACTGTGGTCAGGAGAAGGGGCAGGTCCCCCAGGAGGAGAACCCCCTCTCCCAGGAGGAGGAGAAGCCAGTCCC CCAGGAGGAGGAGGAGCCAGAGCAGAGAGTCTCAGTGCATGGAGAGCACCACATCAGGCTTCCTGGGCCCCCT GCTGGTGCTCCAGGCAGGCTTCTTTCTGCTGACCAGGATTCTGACCATCCCCAGTCCCTGGACAGCTGGTGG ACCTCCCTGAATTTTCTGGGGGGGGCCCCCTACCTGTCTGGCCAGAACTCTCAGTCTCCCACCTCGAATCACT CACCAACCAGCTGTCCCCCATCTGTCTGGCTACAGGTGGATGTGCCTGAGGAGATTTCATCATCTTCCTGTG CATCCTGCTGCTGTGCCTGATCTTTCTGCTGGTGTGCTGGACTACCAGGGCATGCTGCCAGTGTGCCCTCTC ATCCCAGGCAGCTCCACCACATCCACAGGACCTTGCAAGACATGCACCACACCAGCCAGGGCACCAGCATGT TCCCCTCCTGCTGTTGCACCAAGCCAACAGATGGCAACTGCACATGCATTCCCATCCCCTCCAGCTGGGCCTT TGCCAGGTTTCTGTGGGAGTGGGCCAGTGTGAGATTTTCTGGCTGTCTCTTCTGGTGCCCTTTGTGCAGTGG TTTGTGGGCCTGTCCCCTACAGTGTGGCTGAGTGTCTGATGATGTGGTACTGGGGCCCCCTCCCTGTACA ACATCCTCTCTCCCTTTCTGCCTCTGCTGCCAATCTTCTTTTGCCTGTGGGTGTACATC
34	B/C	Core-P2A-sAg	ATGGACATTGACCCCTACAAGGAGTTTGGGGCCAGTGTGGAGCTGCTGTCTTTTCTGCCATCTGACTTCTTCC CCAGTGTGAGGGACCTGCTGGACACTGCCTCAGCACTGTACAGAGAGGCCCTGGAGAGCCCAGAGCACTGCTC CCCCACACACAGCCCTGAGGCAGGCCATCCTCTGCTGGGGGGAGCTGATGAACCTGGCCACCTGGGTGGGC TCCAACCTGGAGGACCCTGCCTCAAGGGAGCTGGTGGTCAGCTATGTCAATGTGAACATGGGCCTCAAGATCA GGCAGCTGCTGTGGTTCCACATCTCCTGCCTGACCTTTGGCAGGGAGACAGTCCTGGAGTACCTGGTGAGCTT TGGGGTGTGGATCAGGACCCCCCTGCCTACAGGCCCCCAATGCTCCCATCCTGTCCACCCTGCCAGAGACC ACTGTGGTCAGGAGAAGGGGCAGGTCCCCCAGGAGGAGAACCCCCTCTCCCAGGAGGAGGAGAAGCCAGTCCC CCAGGAGGAGGAGGAGCCAGAGCAGAGAGTCTCAGTGCAGGAGTGGGGCAACCAACTTCAGCCTCCTGAAACA GGCAGGGGATGTGGAGGAAAACCCAGGCCCCGAGAGCACCACATCAGGCTTCCTGGGCCCCCTGCTGGTGCTC CAGGCAGGCTTCTTTCTGCTGACCAGGATTCTGACCATCCCCAGTCCCTGGACAGCTGGTGGACCTCCCTGA ATTTTCTGGGGGGGGCCCCCTACCTGTCTGGCCAGAACTCTCAGTCTCCCACCTCGAATCACTCACCAACCAG CTGTCCCCCATCTGTCTGGCTACAGGTGGATGTGCCTGAGGAGATTTCATCATCTTCCTGTGCATCCTGCTG CTGTGCCTGATCTTTCTGCTGGTGTGCTGGACTACCAGGGCATGCTGCCAGTGTGCCCTCTCATCCCAGGCA GCTCCACCACATCCACAGGACCTTGCAAGACATGCACCACACCAGCCAGGGCACCAGCATGTTCCCCTCCTG CTGTTGCACCAAGCCAACAGATGGCAACTGCACATGCATTCCCATCCCCTCCAGCTGGGCCTTTGCCAGGTTT

Table M - Polynucleotides encoding immunogenic polypeptides			
SEQ ID NO:	HBV geno-type	name	Polynucleotide sequence
			CTGTGGGAGTGGGCCAGTGTGAGATTTTCCTGGCTGTCTCTTCTGGTGCCCTTTGTGCAGTGGTTTGTGGGCC TGTCCTTACAGTGTGGCTGAGTGTATCTGGATGATGTGGTACTGGGGCCCCCTCCCTGTACAACATCCTCTC TCCCTTTCTGCCTCTGCTGCCAATCTTCTTTTGCCTGTGGGTGTACATC
35	D/D	Core-sAg	ATGGACATTGACCCCTACAAGGAGTTTGGGGCCAGTGTGGAGCTGCTCTCCTTCCTGCCCTCAGACTTCTTTC CCAGTGTGAGGGACCTGCTTGACACAGCCTCTGCCCTCTACAGAGAGGCCCTGGAGAGCCCAGAGCATTGCTC CCCCACCACACAGCACTGAGGCAGGCCATCCTGTGCTGGGGGGAGCTCATGAACCTGGCCACCTGGGTGGGT GTCAACCTGGAGGACCCAGCTTCCAGGGATCTGGTGGTCAGCTATGTGAACACAAACATGGGCCTCAAGTTCA GGCAGCTGCTCTGGTTCCACATCTCCTGCCTGACCTTTGGCAGGGAGACTGTGCTGGAGTACCTGGTGAGCTT TGGAGTGTGGATCAGGACCCACCTGCCTACAGGCCCCCAATGCCCCCATCCTGTCCACCCTGCCTGAGACC ACAGTGGTGAGGAGGAGGGGGAGGTCCCCCAGAAGGAGGACCCCTTCTCCCAGGAGGAGGAGGAGTCACTCTC CCAGGAGGAGGAGGAGCCAGAGCAGAGAGTCCAGTGTATGGAGAACATCACCTCTGGCTTTCTGGGACCCCT GCTGGTGCTCCAGGCAGGCTTTTTCTGCTGACCAGGATCCTGACCATCCCTCAGAGCCTGGACTCCTGGTGG ACATCTCTGAATTTTCTTGGGGGCACCACTGTGTGCCTGGGACAGAACTCCCAGTCTCCACCTCCAACCACA GCCCCAACATCCTGTCCCCCATCTGCCAGGCTACAGGTGGATGTGCCTGAGGAGGTTTCATCATCTTCTGT CATCCTGCTGCTGTGCCTGATCTTCTGCTGGTGCTCCTGGACTATCAGGGCATGCTGCCAGTGTGCCCACTG ATCCCAGGCAGCTCCACCACAAGCACAGGACCTTGACAGACATGCACCACACCTGCCCAGGGCACTTCCATGT ACCCATCTTGCTGTTGCACCAAGCCATCTGATGGCAATTGCACCTGCATCCCCATCCCCTCAAGCTGGGCCTT TGGCAAGTTCCTGTGGGAGTGGGCAAGTGCCAGATTCTCTTGGCTGAGCCTGCTGGTCCCTTTTGTGCAGTGG TTTGTGGGCCTGAGCCCCACTGTGTGGCTGTCTGTGATCTGGATGATGTGGTACTGGGGCCCCCTCCCTGTATT CAATCCTGAGCCCTTTTCTGCCACTGCTGCCCATCTTCTTTTGTCTGTGGGTGTACATC
36	D/D	Core-P2A-sAg	ATGGACATTGACCCCTACAAGGAGTTTGGGGCCAGTGTGGAGCTGCTCTCCTTCCTGCCCTCAGACTTCTTTC CCAGTGTGAGGGACCTGCTTGACACAGCCTCTGCCCTCTACAGAGAGGCCCTGGAGAGCCCAGAGCATTGCTC CCCCACCACACAGCACTGAGGCAGGCCATCCTGTGCTGGGGGGAGCTCATGAACCTGGCCACCTGGGTGGGT GTCAACCTGGAGGACCCAGCTTCCAGGGATCTGGTGGTCAGCTATGTGAACACAAACATGGGCCTCAAGTTCA GGCAGCTGCTCTGGTTCCACATCTCCTGCCTGACCTTTGGCAGGGAGACTGTGCTGGAGTACCTGGTGAGCTT TGGAGTGTGGATCAGGACCCACCTGCCTACAGGCCCCCAATGCCCCCATCCTGTCCACCCTGCCTGAGACC ACAGTGGTGAGGAGGAGGGGGAGGTCCCCCAGAAGGAGGACCCCTTCTCCCAGGAGGAGGAGGAGTCACTCTC CCAGGAGGAGGAGGAGCCAGAGCAGAGAGTCCAGTGTGGCAGTGGGGCAACCAACTTCAGCCTCCTGAAACA GGCAGGGGATGTGGAGGAAAACCCAGGCCCCGAGAACATCACCTCTGGCTTTCTGGGACCCCTGCTGGTGCTC CAGGCAGGCTTTTTCTGCTGACCAGGATCCTGACCATCCCTCAGAGCCTGGACTCCTGGTGGACATCTCTGA

Table M - Polynucleotides encoding immunogenic polypeptides			
SEQ ID NO:	HBV geno-type	name	Polynucleotide sequence
			ATTTTCTTGGGGGCACCACTGTGTGCCTGGGACAGAACTCCCAGTCTCCACCTCCAACCACAGCCCAACATC CTGTCCCCCATCTGCCCAGGCTACAGGTGGATGTGCCTGAGGAGGTTTCATCATCTTCCTGTTTCATCCTGCTG CTGTGCCTGATCTTTCTGCTGGTGCTCCTGGACTATCAGGGCATGCTGCCAGTGTGCCACTGATCCCAGGCA GCTCCACCACAAGCACAGGACCTTGCAGGACATGCACCACACCTGCCAGGGCACTTCCATGTACCCATCTTG CTGTTGCACCAAGCCATCTGATGGCAATTGCACCTGCATCCCCATCCCCTCAAGCTGGGCCTTTGGCAAGTTC CTGTGGGAGTGGGCAAGTGCCAGATTCTCTTGGCTGAGCCTGCTGGTCCCTTTTGTGCAGTGGTTTGTGGGCC TGAGCCCCACTGTGTGGCTGTCTGTGATCTGGATGATGTGGTACTGGGGCCCCTCCCTGTATTCAATCCTGAG CCCTTTTCTGCCACTGCTGCCCATCTTCTTTTGTCTGTGGGTGTACATC
37	D/D	iCore-P2A-sAg	ATGGACATTGACCCCTACAAGGAGTTTGGGGCCAGTGTGGAGCTGCTGTCTTTTCTGCCATCTGACTTCTTCC CCAGTGTGAGGGACCTGCTGGACACTGCCTCAGCACTGTACAGAGAGGCCCTGGAGAGCCCAGAGCACTGCTC CCCCCACCACACAGCCCTGAGGCAGGCCATCCTCTGCTGGGGGGAGCTGATGAACCTGGCCACCTGGGTGGGC GTCAACCTGGAGGACCTGCCTCAAGGGACCTGGTGGTCAGCTATGTCAATACGAACATGGGCCTCAAGTTCA GGCAGCTGCTGTGGTTCCACATCTCCTGCCTGACCTTTGGCAGGGAGACAGTCCTGGAGTACCTGGTGAGCTT TGGGGTGTGGATCAGGACCCCCCTGCCTACAGGCCCCCAATGCTCCCATCCTGTCCACCCTGCCAGAGACC ACTGTGGTCAGGAGAAGGGGCAGGTCCCCCAGGAGGAGAACCCCCTCTCCCAGGAGGAGGAGAAGCCAGTCCC CCAGGAGGAGGAGGAGCCAGAGCAGAGAGTCTCAGTGCGGCAGTGGGGCAACCAACTTCAGCCTCCTGAAACA GGCAGGGGATGTGGAGGAAAACCCAGGCCCCGAGAACATCACATCAGGCTTCCTGGGCCCCCTGCTGGTGCTC CAGGCAGGCTTCTTTCTGCTGACCAGGATTCTGACCATCCCCCAGTCCCTGGACAGCTGGTGGACCTCCCTGA ATTTTCTGGGGGGGACCACTGTCTGTCTTGGCCAGAACTCTCAGTCTCCACCTCGAATCACTCACCAACCAG CTGTCCCCCATCTGTCTGGCTACAGGTGGATGTGCCTGAGGAGATTTCATCATCTTCCTGTTTCATCCTGCTG CTGTGCCTGATCTTTCTGCTGGTGCTGCTGGACTACCAGGGCATGCTGCCAGTGTGCCCTCTCATCCCAGGCA GCTCCACCACATCCACAGGACCTTGCAGGACATGCACCACACCAGCCCAGGGCACCAGCATGTACCCCTCCTG CTGTTGCACCAAGCCATCAGATGGCAACTGCACATGCATTCCCATCCCCTCCAGCTGGGCCTTTGGCAAGTTT CTGTGGGAGTGGGCCAGTGCGAGATTTTCTGGCTGTCTCTTCTGGTGGCCTTTGTGCAGTGGTTTGTGGGCC TGTCCTTACAGTGTGGCTGAGTGTATCTGGATGATGTGGTACTGGGGCCCCTCCCTGTACAGCATCCTCTC TCCCTTTCTGCCTCTGCTGCCAATCTTCTTTTGCCTGTGGGTGTACATC

4. Vectors and Host Cells

[0123] Further provided are vectors comprising one or more polynucleotides encoding one or more of the immunogenic polypeptides, described herein, or an expression cassette comprising such polynucleotides. A vector can be of any type, for example, a recombinant vector such as an expression vector. Vectors include without limitation, plasmids, cosmids, bacterial artificial chromosomes (BAC) and yeast artificial chromosomes (YAC) and vectors derived from bacteriophages or plant or animal (including human) viruses. Vectors can comprise an origin of replication recognized by the proposed host cell and in the case of expression vectors, promoter and other regulatory regions recognized by the host cell. In additional embodiments, a vector comprises one or more polynucleotides encoding one or more immunogenic polypeptides of the disclosure operably linked to a promoter and optionally additional regulatory elements. Certain vectors are capable of autonomous replication in a host into which they are introduced (*e.g.*, vectors having a bacterial origin of replication can replicate in bacteria). Other vectors can be integrated into the genome of a host upon introduction into the host, and thereby are replicated along with the host genome. Vectors include without limitation, those suitable for recombinant production of the immunogenic polypeptides disclosed herein.

[0124] The term “vector,” as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Some vectors are suitable for delivering the nucleic acid molecule or polynucleotide of the present application. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as expression vectors.

[0125] The term “operably linked” refers to two or more nucleic acid sequence or polypeptide sequence elements that are usually physically linked and are in a functional relationship with each other. For instance, in the context of nucleic acid sequence elements, a promoter is operably linked to a coding sequence if the promoter is able to initiate or regulate the transcription or expression of a coding sequence, in which case, the coding sequence should be understood as being “under the control of” the promoter.

[0126] The choice of the vector is dependent on the recombinant procedures followed and the host used. Introduction of vectors into host cells can be effected by *inter alia* calcium

phosphate transfection, DEAE-dextran-mediated transfection, lipofectamine transfection, electroporation, virus infection, or via administration to a subject, as described herein. Vectors may be autonomously replicating or may replicate together with the chromosome into which they have been integrated. In certain embodiments, the vectors contain one or more selection markers. The choice of the markers may depend on the host cells of choice. These include without limitation, kanamycin, neomycin, puromycin, hygromycin, zeocin, thymidine kinase gene from Herpes simplex virus (HSV-TK), and dihydrofolate reductase gene from mouse (dhfr). Vectors comprising one or more nucleic acid molecules encoding the immunogenic polypeptides described herein, operably linked to one or more nucleic acid molecules encoding proteins or peptides that can be used to isolate the immunogenic polypeptides, are also covered by the disclosure. These proteins or peptides include without limitation, glutathione-S-transferase, maltose binding protein, metal-binding polyhistidine, green fluorescent protein, luciferase and beta-galactosidase.

[0127] In other embodiments, the vector that is used is pcDNA™3.1+ (ThermoFisher, MA).

[0128] In some embodiments, the vector is viral vector. As appropriate, the viral vector can be a DNA virus or a RNA virus, including a self-replicating RNA virus. Self-replicating RNA viruses include Alphaviruses, and are described, *e.g.*, in Lundstrom, *Molecules*. (2018) 23(12). pii: E3310 (PMID: 30551668); and Ljungberg, *et al.*, *Expert Rev Vaccines*. (2015) 14(2):177-94). In various embodiments, the viral vector is from a virus selected from the group consisting of adenovirus, adeno-associated virus, arenavirus, alphavirus, self-replicating alphavirus, poxvirus, cytomegalovirus, rhabdovirus, vesicular stomatitis virus, flavivirus, maraba virus and vaccinia virus. In some embodiments, the viral vector is from a viral family selected from the group consisting of: Adenoviridae (*e.g.*, Adenovirus, adeno-associated virus), Arenaviridae (*e.g.*, lymphocytic choriomeningitis mammarenavirus, Cali mammarenavirus (*a.k.a.*, Pichinde mammarenavirus (PICV)), Poxviridae (*e.g.*, Vaccinia virus), Herpesviridae (*e.g.*, Cytomegalovirus, Herpesvirus, *e.g.*, HSV-1), Parvoviridae (*e.g.*, Parvovirus H1), Poxviridae (*e.g.* Vaccinia virus, *e.g.* modified vaccinia Ankara (MVA)), Flaviviridae (*e.g.* Yellow fever virus), Reoviridae (*e.g.*, Reovirus), Retroviridae (*e.g.*, Lentivirus), Picornaviridae (*e.g.*, Cocksackievirus, Seneca Valley Virus, Poliovirus), Paramyxoviridae (*e.g.*, Measles virus, Newcastle disease virus (NDV)), Rhabdoviridae (*e.g.*, Vesiculovirus, including Maraba vesiculovirus and Vesicular stomatitis virus (VSV)), Togaviridae (*e.g.*, Alphavirus, *e.g.*, self-replicating Alphavirus; Sindbis virus), Enteroviridae (*e.g.*, Echovirus). Illustrative modified

vaccinia viral vectors of use for expressing the present immunogenic polypeptides are described, *e.g.*, in WO 2019/134049.

[0129] In some embodiments, the viral expression vector is an arenavirus vector selected from Lymphocytic choriomeningitis mammarenavirus (LCMV)(NCBI:txid11623), Cali

5 mammarenavirus (*a.k.a.*, Pichinde mammarenavirus or Pichinde arenavirus) (NCBI:txid2169993), Guanarito virus (GTOV) (NCBI:txid45219), Argentinian mammarenavirus (*a.k.a.*, Junin virus (JUNV))(NCBI:txid2169991), Lassa virus (LASV)(NCBI:txid11620), Lujo virus (LUJV)(NCBI:txid649188), Machupo virus (MACV)(NCBI:txid11628), Brazilian mammarenavirus (*a.k.a.*, Sabia virus
10 (SABV))(NCBI:txid2169992), and Whitewater Arroyo virus (WWAV)(NCBI:txid46919). In some embodiments, the viral expression vector is an arenavirus vector selected from Lymphocytic choriomeningitis mammarenavirus (LCMV) or Cali mammarenavirus (*a.k.a.*, Pichinde mammarenavirus or Pichinde arenavirus (PICV)). Illustrative arenavirus vectors that can be used as delivery and expression vehicles for the herein described immunogenic
15 polypeptides are described, *e.g.*, in WO 2009/083210; WO 2015/183895; WO 2016/075250; WO 2017/198726; and U.S. Patent Nos. 9,943,585 and 10,342,861, which are hereby incorporated herein by reference in their entireties for all purposes.

[0130] In some embodiments, the viral expression vector is an adenovirus vector, *e.g.*, from a human adenovirus or a simian adenovirus (*e.g.*, a chimpanzee adenovirus, a gorilla

20 adenovirus or a rhesus monkey adenovirus). In various embodiments, the adenovirus vector is selected from adenovirus serotype 5 (Ad5), adenovirus serotype 26 (Ad26), adenovirus serotype 34 (Ad34), adenovirus serotype 35 (Ad35), adenovirus serotype 48 (Ad48), chimpanzee adenovirus (*e.g.* ChAdOx1, ChAdOx2, ChAd3 (AdC3), ChAd5 (AdC5), ChAd6 (AdC6), ChAd7 (AdC7), ChAd8 (AdC8), ChAd9 (AdC9), ChAd10 (AdC10), ChAd11 (AdC11),
25 ChAd17 (AdC17), ChAd16 (AdC16), ChAd19 (AdC19), ChAd20 (AdC20), ChAd22 (AdC22), ChAd24 (AdC24), ChAdY25, ChAd26 (AdC26), ChAd28 (AdC28), ChAd30 (AdC30), ChAd31 (AdC31), ChAd37 (AdC37), ChAd38 (AdC38), ChAd43 (AdC43), ChAd44 (AdC44), ChAd55 (AdC55), ChAd63 (AdC63), ChAdV63, ChAd68 (AdC68), ChAd73 (AdC73), ChAd82 (AdC82), ChAd83 (AdC83), ChAd143 (AdC143), ChAd144 (AdC144), ChAd145 (AdC145),
30 ChAd147 (AdC147)), gorilla adenovirus (*e.g.* GC44, GC45, GC46) and rhesus adenovirus (*e.g.*, RhAd51, RhAd52, RhAd53, RhAd54, RhAd55, RhAd56, RhAd57, RhAd58, RhAd59, RhAd60, RhAd61, RhAd62, RhAd63, RhAd64, RhAd65, RhAd66). Illustrative Chimpanzee, Gorilla and Rhesus monkey adenovirus vectors that can be used as delivery and expression vehicles for the herein described immunogenic polypeptides are described, *e.g.*, in WO2012/172277

(ChAdOx1), WO2017/221031 (ChAdOx2), WO2019/076880; WO2019/076877; Andrabi *et al.*, (2019) *Cell Reports* 27:2426–2441 Guo, *et al.*, *Hum Vaccin Immunother.* (2018) 14(7):1679-1685; Abbink, *et al.*, *J Virol.* (2015) 89(3):1512-22; and Abbink, *et al.*, *J Virol.* (2018) 92(6). pii: e01924-17.

5 **[0131]** In various embodiments, the viral expression vector is incapable of replication (*i.e.*, replication-defective or replication-deficient), has reduced or diminished capacity for replication, *e.g.*, in comparison to a wild-type viral vector (*i.e.*, replication-attenuated) or is replication competent. In various embodiments, the viral expression vector is a replication-defective or replication-deficient arenavirus vector having a bi-segmented genome, *e.g.*, as described in WO 2009/083210 and WO 2017/076988. In various embodiments, the viral expression vector is a replication-attenuated arenavirus vector having a tri-segmented genome, *e.g.*, as described in WO 2016/075250, WO 2017/076988 and WO 2017/198726.

15 **[0132]** Further provided are host cells comprising one or more polynucleotides encoding one or more of the immunogenic polypeptides or one or more vectors expressing the immunogenic polypeptides, as described herein. Any of a variety of host cells can be used. In one embodiment, a host cell is a prokaryotic cell, for example, *E. coli*. In another embodiment, a host cell is a eukaryotic cell, for example, a yeast cell, a plant cell, an insect cell, a mammalian cell, such as a Chinese Hamster Ovary (CHO)-based or CHO-origin cell line (*e.g.*, CHO-S, CHO DG44, ExpiCHO™, CHOZN® ZFN-modified GS-/- CHO cell line, CHO-K1, CHO-K1a), COS cells, BHK cells, NSO cells or Bowes melanoma cells. Examples of human host cells are, *inter alia*, HeLa, 911, AT1080, A549 and HEK293 (*e.g.*, HEK293E, HEK293F, HEK293H, HEK293T, Expi293™). In addition, the immunogenic polypeptides can be expressed in a yeast cell such as *Pichia* (see, *e.g.*, Powers *et al.*, *J Immunol Methods.* 251:123-35 (2001)), *Hanseula*, or *Saccharomyces*.

25 **[0133]** The terms “host cell,” “host cell line,” and “host cell culture” are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include “transformants” and “transformed cells,” which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

30 **[0134]** As appropriate, the host cells can be stably or transiently transfected with one or more polynucleotides encoding one or more immunogenic polypeptides, as described herein. As

appropriate, the host cells can be infected with one or more vectors expressing one or more immunogenic polypeptides, as described herein. In some embodiments, the host cells are capable of being infected with and propagating one or more replication-attenuated or replication competent vectors expressing one or more immunogenic polypeptides, as described herein.

5 Illustrative cells useful for infecting with and/or propagating viral vectors include without limitation BHK-21, A549, Vero and HEK293 (*e.g.*, HEK293E, HEK293F, HEK293H, HEK293T, Expi293™) cells. In certain embodiments, the host cells express the Coxsackievirus and adenovirus receptor (CAR), *e.g.*, MDCK, Caco-2 or Calu-3 host cells. In certain embodiments, the polynucleotides integrate into the genome of the host cell.

10 **5. Pharmaceutical Compositions / Immunogenic Compositions**

[0135] Provided are pharmaceutical compositions or immunogenic compositions comprising one or more of the immunogenic HBV polypeptides, as described herein, or a polynucleotide encoding one or more of the immunogenic HBV polypeptides, as described herein, or a viral expression vector comprising one or more of such polynucleotides, and a
15 pharmaceutically acceptable diluent, carrier or excipient. "Pharmaceutically acceptable excipient" includes without limitation any adjuvant, carrier, excipient, glidant, sweetening agent, diluent, preservative, dye/colorant, flavor enhancer, surfactant, wetting agent, dispersing agent, suspending agent, stabilizer, isotonic agent, solvent, or emulsifier which has been approved by the United States Food and Drug Administration as being acceptable for use in humans or
20 domestic animals.

[0136] Generally, the pharmaceutical compositions described herein are immunogenic. In certain embodiments, the pharmaceutical composition comprises a therapeutically effective amount of the one or more (*e.g.*, two or more, three or more) immunogenic HBV polypeptides, or one or more (*e.g.*, two or more, three or more) polynucleotides encoding one or more (*e.g.*,
25 two or more, three or more) of the immunogenic HBV polypeptides, or one or more (*e.g.*, two or more, three or more) viral expression vectors containing one or more (*e.g.*, two or more, three or more) of the polynucleotides encoding one or more of the immunogenic HBV polypeptides.

[0137] Various pharmaceutically acceptable diluents, carriers, and excipients, and techniques for the preparation and use of pharmaceutical compositions will be known to those of
30 skill in the art in light of the present disclosure. Illustrative pharmaceutical compositions and pharmaceutically acceptable diluents, carriers, and excipients are also described in, *e.g.*, Loyd V. Allen Jr (Editor), "Remington: The Science and Practice of Pharmacy," 22nd Edition, 2012, Pharmaceutical Press; Brunton, Knollman and Hilal-Dandan, "Goodman and Gilman's The

Pharmacological Basis of Therapeutics,” 13th Edition, 2017, McGraw-Hill Education / Medical; McNally and Hastedt (Editors), “Protein Formulation and Delivery, 2nd Edition, 2007, CRC Press; Banga, “Therapeutic Peptides and Proteins: Formulation, Processing, and Delivery Systems,” 3rd Edition, 2015, CRC Press; Lars Hovgaard, Frokjaer and van de Weert (Editors),
 5 “Pharmaceutical Formulation Development of Peptides and Proteins,” 2nd Edition, 2012, CRC Press; Carpenter and Manning (Editors), “Rational Design of Stable Protein Formulations: Theory and Practice,” 2002, Springer (Pharmaceutical Biotechnology (Book 13)); Meyer (Editor), “Therapeutic Protein Drug Products: Practical Approaches to Formulation in the Laboratory, Manufacturing, and the Clinic, 2012, Woodhead Publishing.

10 **[0138]** In certain embodiments, the polynucleotides or vectors are formulated into lipid nanoparticles. For example, in some embodiments where the immunogenic HBV polypeptides are expressed from self-replicating or self-amplifying RNA molecules, the self-replicating or self-amplifying RNA can be formulated into lipid nanoparticles (LNPs). As used herein, the term “lipid nanoparticle” refers to one or more spherical nanoparticles with an average diameter
 15 of between about 10 to about 1000 nanometers, and which comprise a solid lipid core matrix that can solubilize lipophilic molecules. In certain embodiments, the lipid core is stabilized by surfactants (*e.g.*, emulsifiers), and can comprise one or more of triglycerides (*e.g.*, tristearin), diglycerides (*e.g.*, glycerol behenate), monoglycerides (*e.g.*, glycerol monostearate), fatty acids (*e.g.*, stearic acid), steroids (*e.g.*, cholesterol), and waxes (*e.g.*, cetyl palmitate), including
 20 combinations thereof. Lipid nanoparticles are described, for example, in Petrilli et al., Curr Pharm Biotechnol. 15:847-55, 2014; and U.S. Patent Nos. 6,217,912; 6,881,421; 7,402,573; 7,404,969; 7,550,441; 7,727,969; 8,003,621; 8,691,750; 8,871,509; 9,017,726; 9,173,853; 9,220,779; 9,227,917; and 9,278,130, each of which is incorporated by reference in its entirety. In one embodiment, a self-replicating or self-amplifying RNA molecule encoding one or more
 25 of the immunogenic HBV polypeptides described herein is formulated or condensed into polyethylenimine (PEI)-polyplex delivery vehicles, *e.g.*, as described in Démoulin, *et al.*, *Nanomedicine*. (2016) Apr;12(3):711-722 and Démoulin, *et al.*, *J Control Release*. (2017) Nov 28;266:256-271, which can be nanoparticulate.

[0139] In embodiments where the immunogenic HBV polypeptides are expressed from a
 30 viral expression vector, the viral expression vector can be formulated for the desired route of administration, *e.g.*, as an isotonic pharmaceutically acceptable aqueous solution or suspension suitable for intravenous, intramuscular, subcutaneous or intradermal administration. In some embodiments, the viral expression vector can be formulated for mucosal, *e.g.*, buccal, intranasal, intravaginal or intra-rectal delivery. Illustrative formulations for viral expression vectors that

can be used in the herein described pharmaceutical compositions and methods are described, *e.g.*, in Manfredsson and Benskey, *editors*, “Viral Vectors for Gene Therapy: Methods and Protocols (Methods in Molecular Biology),” 2019, Book 1937 in Methods in Molecular Biology Series, Humana Press; WO 2017/013169 (formulation of Adenoviral vectors in an aqueous mixture or freeze dried composition in the presence of amorphous sugar and low salt concentration); and Kumru, *et al.*, *J Pharm Sci.* (2018) Nov;107(11):2764-2774 (aqueous formulations buffered in Tris and containing proline, lactose, and mannitol as stabilizing additives). Formulation of arenavirus vectors is described, *e.g.*, in WO 2009/083210; WO 2016075250 and WO 2017/198726. In certain embodiments, the viral expression vectors are delivered via microneedle-mediated delivery, *e.g.*, as described in Zaric, *et al.*, *Expert Opin Drug Deliv.* (2017) Oct;14(10):1177-1187.

[0140] In some embodiments, each carrier, diluent or excipient is “acceptable” in the sense of being compatible with the other ingredients of the pharmaceutical composition and not injurious to the subject. Often, the pharmaceutically acceptable carrier is an aqueous pH-buffered solution. Some examples of materials which can serve as pharmaceutically-acceptable carriers, diluents or excipients include: water; buffers, *e.g.*, a buffer having a pKa in the range of about 6.0 to about 8.0, *e.g.*, a physiologically acceptable buffer, *e.g.*, selected from phosphate, carbonate, bicarbonate, citrate, maleate, glycine-glycine, HEPES, HEPPSO, HEPPS, imidazole, BICINE, TRICINE, Tris, and BIS-Tris; sugars, such as lactose, trehalose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Hank’s solution, Ringer’s solution; ethyl alcohol; phosphate buffer solutions; amino acids (*e.g.*, charged amino acids, including without limitation, aspartate, asparagine, glutamate, glutamine, histidine, arginine, lysine); and other non-toxic compatible substances employed in pharmaceutical formulations. Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

[0141] In one particular formulation, an arenavirus vector (*e.g.*, a LCMV or Pichinde mammarynavirus vector (PICV)) described herein is formulated in an isotonic aqueous solution

comprising a biologically compatible buffer having a pKa in the range of about 6.0 to about 8.0 (e.g., HEPES and NaCl), at a neutral or near-neutral pH and a non-ionic surfactant (e.g., PLURONIC® F68 (a.k.a., poloxamer 188)). In one particular formulation, an arenavirus vector (e.g., a LCMV or Pichinde mammarenavirus vector) described herein is formulated in an isotonic aqueous solution comprising HEPES buffer at pH 7.4, NaCl, and PLURONIC® F68 (a.k.a., poloxamer 188). Schleiss, *et al.* (*Clin Vaccine Immunol.* 2017 Jan 5;24(1):e00300-16) describes an LCMV formulating LCMV vectors in a diluent of 25 mM HEPES, 150 mM NaCl, 0.01% PLURONIC® F68; pH 7.4), which can be used to formulate the herein described arenavirus vectors. A final concentration of 10% sorbitol was added before freezing below -60°C.

[0142] The formulation of and delivery methods of pharmaceutical compositions will generally be adapted according to the site and the disease to be treated. Exemplary formulations include without limitation, those suitable for parenteral administration, e.g., intravenous, intra-arterial, intramuscular, or subcutaneous administration, including formulations encapsulated in micelles, liposomes or drug-release capsules (active agents incorporated within a biocompatible coating designed for slow-release); ingestible formulations; formulations for topical use, such as creams, ointments and gels; and other formulations such as inhalants, aerosols and sprays. In some embodiments, the pharmaceutical compositions are formulated for parenteral, e.g., intravenous, subcutaneous, or oral administration. In some embodiments, the pharmaceutical compositions are formulated for mucosal, e.g., buccal, intranasal, intrarectal and/or intravaginal administration.

[0143] In certain embodiments, pharmaceutical compositions are sterile. In certain embodiments, the pharmaceutical composition has a pH in the range of 4.5 to 8.5, 4.5 to 6.5, 6.5 to 8.5, 6.0 to 8.0, 6.5 to 8.5, or a pH of about 5.0, about 5.5, about 6.0, about 6.5, about 6.6, about 6.7, about 6.8, about 6.9, about 7.0, about 7.1, about 7.2, about 7.3, about 7.5, about 8.0 or about 8.5. In one embodiment, the pharmaceutical composition has an osmolarity in the range of 240-260 or 250-330 mOsmol/L. In certain embodiments, the pharmaceutical composition is isotonic or near isotonic.

[0144] In some embodiments, the pharmaceutical compositions are liquids or solids. In some embodiments, the pharmaceutical composition comprises an aqueous solution or suspension. In some embodiments, the pharmaceutical composition is lyophilized or is a frozen liquid.

[0145] In some embodiments, the pharmaceutical composition further comprises one or more additional therapeutic agents, *e.g.*, a second therapeutic agent, or second and third therapeutic agents, for use in combination therapies, as described herein.

[0146] In certain embodiments, the pharmaceutical composition further comprises an adjuvant. Illustrative adjuvants that can be co-formulated or co-administered with the herein described immunogenic HBV polypeptides, polynucleotides encoding such immunogenic HBV polypeptides and vectors expressing such immunogenic HBV polypeptides include without limitation cytokines, chemokines, immune co-stimulatory molecules, toll-like receptor agonists or inhibitors of immune suppressive pathways, as described herein, and in Li, *et al.*, *Curr Issues Mol Biol.* (2017) 22:17-40. Other adjuvants that can be co-formulated or co-administered with the herein described immunogenic HBV polypeptides, polynucleotides encoding such immunogenic HBV polypeptides and vectors expressing such immunogenic HBV polypeptides include without limitation mineral salts (*e.g.*, aluminum salts (*e.g.*, alum), calcium phosphate, incomplete Freund's adjuvant), lipid particles (*e.g.*, MF59, cochleates, virus-like particles), microparticles (*e.g.*, virosomes, polylactic acid (PLA), poly[lactide-coglycolide] (PLG)), immune potentiators (*e.g.*, dsRNA:Poly(I:C), Poly-IC:LC, Monophosphoryl lipid A (MPL), LPS, Flagellin, Imidazoquinolines: imiquimod (R837), resiquimod (848), CpG oligodeoxynucleotides (ODN), Muramyl dipeptide (MDP), Saponins (QS-21)), and mucosal adjuvants (*e.g.*, Cholera toxin (CT), Heat-labile enterotoxin (LTK3 and LTR72), Chitosan). Adjuvants that can be co-formulated or co-administered with the herein described immunogenic HBV polypeptides, polynucleotides encoding such immunogenic HBV polypeptides and vectors expressing such immunogenic HBV polypeptides are summarized in Apostólico, *et al.*, *J Immunol Res.* (2016) 2016:1459394.

[0147] In some embodiments, the pharmaceutical compositions or immunogenic compositions comprise mixtures of two or more immunogenic HBV polypeptides, two or more polynucleotides encoding such immunogenic HBV polypeptides, or two or more vectors expressing such immunogenic HBV polypeptides. In some embodiments, the pharmaceutical composition comprises two or more immunogenic HBV polypeptides, two or more polynucleotides encoding such immunogenic HBV polypeptides, or two or more vectors expressing such immunogenic HBV polypeptides.

[0148] In various embodiments, the immunogenic composition comprises one or more polynucleotides encoding, or one or more vectors capable of expressing, two immunogenic polypeptides, the immunogenic polypeptides comprising: (a) an HBV polymerase polypeptide mutant comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 5-14, or

a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 5-14; and

(b) an HBV core-sAg fusion protein comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 38-41, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%,

5 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 38-41.

[0149] In various embodiments, the immunogenic composition comprises one or more polynucleotides encoding, or one or more vectors capable of expressing, two immunogenic polypeptides, the immunogenic polypeptides comprising: (a) an HBV polymerase polypeptide

10 mutant comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 13-14, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 13-14; and (b) an HBV core-sAg fusion protein comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 38-41, or a sequence that is at least 80%, 81%, 82%, 83%, 84%,
15 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 38-41.

[0150] In various embodiments, the immunogenic composition comprises one or more polynucleotides encoding, or one or more vectors capable of expressing, two immunogenic polypeptides, the immunogenic polypeptides comprising: (a) an HBV polymerase polypeptide

20 mutant comprising or consisting of an amino acid sequence of SEQ ID NO: 13, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 13; and (b) an HBV core-sAg fusion protein comprising or consisting of an amino acid sequence of SEQ ID NO: 41, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%,
25 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 41.

[0151] With respect to the core-sAg fusion polypeptide in the immunogenic composition, in some embodiments, the core polypeptide comprises a serine (S) residue at the amino acid position corresponding to position 12, and an asparagine (N) residue at the amino acid position corresponding to position 67, wherein the position numbers are with reference to
30 SEQ ID NO:65 or SEQ ID NO:66. In some embodiments, the sAg polypeptide comprises an isoleucine (I) residue at the amino acid position corresponding to position 68, wherein the position numbers are with reference to SEQ ID NO:3 or SEQ ID NO:4. In some embodiments, the sAg polypeptide comprises one or more of a serine (S) residue at the amino acid position corresponding to position 53, an isoleucine (I) residue at the amino acid position corresponding

to position 68, a threonine (T) residue at the amino acid position corresponding to position 125, a proline (P) residue at the amino acid position corresponding to position 127, an phenylalanine (F) residue at the amino acid position corresponding to position 161, a tyrosine (Y) residue at the amino acid position corresponding to position 200, a serine (S) residue at the amino acid position corresponding to position 210, and a leucine (L) residue at the amino acid position corresponding to position 213, wherein the position numbers are with reference to SEQ ID NO:3 or SEQ ID NO:4. In some embodiments, the core-sAg fusion polypeptide comprises one or more of a serine (S) residue at the amino acid position corresponding to position 12, an asparagine (N) residue at the amino acid position corresponding to position 67, a valine (V) residue at the amino acid position corresponding to position 74, a phenylalanine (F) residue at the amino acid position corresponding to position 97, a threonine (T) residue at the amino acid position corresponding to position 249, a threonine (T) residue at the amino acid position corresponding to position 250, a serine (S) residue at the amino acid position corresponding to position 317, a serine (S) residue at the amino acid position corresponding to position 318, an arginine (R) residue at the amino acid position corresponding to position 326, a tyrosine (Y) residue at the amino acid position corresponding to position 338, a glycine (G) residue at the amino acid position corresponding to position 363, and an alanine (A) residue at the amino acid position corresponding to position 372, wherein the position numbers are with reference to SEQ ID NO:41.

[0152] In some embodiments, the immunogenic composition comprises a first viral expression vector and a second viral expression vector, wherein: (a) the first viral expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of any one of SEQ ID NOs: 27-32 and 89-94, *e.g.*, SEQ ID NOs: 29, 89, 90 and 92, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 27-32 and 89-94, *e.g.*, SEQ ID NOs: 29, 89, 90 and 92; and (b) the second viral expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of any one of SEQ ID NOs: 33-37 or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 33-37.

[0153] In some embodiments, the immunogenic composition comprises a first viral expression vector and a second viral expression vector, wherein: (a) the first viral expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 29 or 90, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%,

88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NOs: 29, 89, 90 or 92; and (b) the second viral expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 37 or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NO: 37.

[0154] In some embodiments, the immunogenic composition comprises a first LCMV arenavirus expression vector and a second LCMV arenavirus expression vector, wherein: (a) the first LCMV arenavirus expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 29, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 29; and (b) the second LCMV arenavirus expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 37 or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NO: 37.

[0155] In some embodiments, the immunogenic composition comprises a first Pichinde arenavirus expression vector and a second Pichinde arenavirus expression vector, wherein: (a) the first Pichinde arenavirus expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 90, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 90; and (b) the second Pichinde arenavirus expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 37 or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NO: 37.

[0156] As appropriate or desired, the HBV polymerase polypeptide mutant and the HBV core-sAg fusion protein can be provided in the immunogenic composition in a ratio in the range of from 1:10 to 10:1, *e.g.*, in the range of 1:9 to 9:1, 1:8 to 8:1, 1:7 to 7:1, 1:6 to 6:1, 1:5 to 5:1, 1:4 to 4:1, 1:3 to 3:1, 1:2 to 2:1 or 1:1. In various embodiments, ratios can be measured by measured in units of plaque forming units (PFU), focus forming units (FFU), infectious units (IU), or viral particles (vp).

[0157] In various embodiments, the one or more polynucleotides are DNA, cDNA, mRNA, or self-replicating RNA.

[0158] In some embodiments, the immunogenic composition comprises a first viral expression vector and a second viral expression vector, wherein: (a) the first viral expression vector comprises a polynucleotide encoding a truncated HBV polymerase polypeptide or an HBV polymerase deletion mutant polypeptide, as described herein; and (b) the second viral expression vector comprises a polynucleotide encoding the core-sAg fusion protein, as described herein. As appropriate or desired, the first viral expression vector and the second viral expression vector can be provided in a ratio in the range of from 1:10 to 10:1, *e.g.*, in the range of 1:9 to 9:1, 1:8 to 8:1, 1:7 to 7:1, 1:6 to 6:1, 1:5 to 5:1, 1:4 to 4:1, 1:3 to 3:1, 1:2 to 2:1 or 1:1.

[0159] In some embodiments, the immunogenic composition comprise in the range of about 10^3 to about 10^{12} viral focus forming units (FFU) or plaque forming units (PFU) or infectious units (IU) or viral particles (vp), *e.g.* from about 10^4 to about 10^7 viral FFU or PFU, *e.g.* from about 10^3 to about 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} or 10^{12} viral FFU or PFU or IU or vp per milliliter, of each of the first viral expression vector and the second viral expression vector.

[0160] In various embodiments, the first viral expression vector and the second viral expression vector in the immunogenic composition independently are from a taxonomic family selected from Adenoviridae, Arenaviridae, Herpesviridae (*e.g.* Cytomegalovirus), Poxviridae (*e.g.* Vaccinia virus, *e.g.* modified vaccinia Ankara (MVA)), Flaviviridae (*e.g.* Yellow fever virus), Rhabdoviridae (*e.g.* Vesiculovirus, *e.g.* Maraba vesiculovirus), Togaviridae (*e.g.*, Alphavirus). In various embodiments, the first viral expression vector and the second viral expression vector can be from the same taxonomic family or from different taxonomic families. For example, in some embodiments, both the first viral expression vector and the second viral expression vector in the immunogenic composition are from Adenoviridae, Arenaviridae, or Poxviridae (*e.g.* Vaccinia virus, *e.g.* modified vaccinia Ankara (MVA)).

[0161] In some embodiments, the first viral expression vector and the second viral expression vector in the immunogenic composition are from Arenaviridae. In some embodiments, the first viral expression vector and the second viral expression vector are from an arenavirus vector selected from Lymphocytic choriomeningitis mammarenavirus (LCMV), Cali mammarenavirus (*a.k.a.*, Pichinde mammarenavirus or Pichinde arenavirus (PICV)), Guanarito virus (GTOV), Junin virus (JUNV), Lassa virus (LASV), Lujo virus (LUJV), Machupo virus (MACV), Sabia virus (SABV), and Whitewater Arroyo virus (WWAV). In some embodiments, the first viral expression vector and the second viral expression vector are from an arenavirus vector selected from Lymphocytic choriomeningitis mammarenavirus (LCMV) or Cali mammarenavirus (*a.k.a.*, Pichinde mammarenavirus or Pichinde arenavirus (PICV)).

[0162] In various embodiments, the first viral expression vector and the second viral expression vector in the immunogenic composition are replication-defective or replication-deficient. In some embodiments, the first viral expression vector and the second viral expression vector in the immunogenic composition are replication-attenuated.

5 6. Methods of Treatment

[0163] Further provided are methods for eliciting an immune response to human hepatitis B virus (HBV) in a subject in need thereof. Also provided are methods of treating or preventing human hepatitis B virus (HBV) in a subject in need thereof. Also provided are methods of inhibiting HBV replication in an infected individual. Further provided are methods
10 for reducing the viral load associated with HBV infection. In various embodiments, the methods comprise administering to the subject an effective amount of an immunogenic composition, as described herein. In various embodiments, the “subject” or the “individual” is a human, a woodchuck, a Peking duck, a mouse or a non-human primate (*e.g.*, a chimpanzee).

[0164] “Treatment” or “treat” or “treating” as used herein refers to an approach for
15 obtaining beneficial or desired results. For purposes of the present disclosure, beneficial or desired results include, but are not limited to, alleviation of a symptom and/or diminishment of the extent of a symptom, delaying of progression and/or preventing a worsening of a symptom associated with a disease or condition. “Treatment” or “treating” can include one or more of the following: a) inhibiting the disease or condition (*e.g.*, decreasing one or more symptoms
20 resulting from the disease or condition, and/or diminishing the extent of the disease or condition); b) slowing or arresting the development of one or more symptoms associated with the disease or condition (*e.g.*, stabilizing the disease or condition, delaying the worsening or progression of the disease or condition); and c) relieving the disease or condition, *e.g.*, causing the regression of clinical symptoms, ameliorating the disease state, delaying the progression of
25 the disease, increasing the quality of life, and/or prolonging survival.

[0165] “Delaying” as used herein refers to development of a disease or condition means to defer, hinder, slow, retard, stabilize and/or postpone development of the disease or condition. This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay
30 can, in effect, encompass prevention, in that the individual does not develop the disease or condition.

[0166] “Prevent” or “prevention” or “preventing” as used herein refers to a regimen that protects against the onset of the disease or disorder such that the clinical symptoms of the

disease do not develop. Thus, “prevention” relates to administration of a therapy (*e.g.*, administration of a therapeutic substance) to a subject before signs of the disease are detectable in the subject (*e.g.*, administration of a therapeutic substance to a subject in the absence of detectable infectious agent (*e.g.*, virus) in the subject). The subject may be an individual at risk of developing the disease or disorder, such as an individual who has one or more risk factors known to be associated with development or onset of the disease or disorder. Thus, in certain embodiments, the term “preventing HBV infection” refers to administering to a subject who does not have a detectable HBV infection an anti-HBV therapeutic substance. It is understood that the subject for anti-HBV preventative therapy may be an individual at risk of contracting the HBV virus. It is also understood that prevention does not require a 100% success rate. In some instances, prevention may be understood as a reduction of the risk of infection, but not a complete elimination the occurrence of an infection.

[0167] “Therapeutically effective amount” or “effective amount” as used herein refers to an amount that is effective to elicit the desired biological or medical response, including the amount of an immunogenic composition that, when administered to a subject for treating a disease, is sufficient to effect such treatment for the disease. The effective amount will vary depending on the immunogenic composition, the disease, and its severity and the age, weight, etc., of the subject to be treated. The effective amount can include a range of amounts. An effective amount may be in one or more doses, *i.e.*, a single dose or multiple doses may be required to achieve the desired treatment endpoint. An effective amount may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable or beneficial result may be or is achieved. Suitable doses of any co-administered compounds may optionally be lowered due to the combined action (*e.g.*, additive or synergistic effects) of the compounds.

[0168] In various embodiments, the administered immunogenic composition comprises a mixture comprising a first viral expression vector and a second viral expression vector, wherein: (a) the first viral expression vector comprises a polynucleotide encoding the truncated HBV polymerase polypeptide, as described herein, or the HBV polymerase deletion mutant polypeptide as described herein; and (b) the second viral expression vector comprises a polynucleotide encoding the core-sAg fusion protein, as described herein.

[0169] In various embodiments, the administered immunogenic composition comprises a mixture comprising a first viral expression vector and a second viral expression vector, wherein: (a) the first viral expression vector comprises a polynucleotide encoding an HBV polymerase

polypeptide mutant comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 5-14, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 5-14; and (b) the second viral expression vector comprises a polynucleotide encoding the core-sAg fusion protein comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 38-41, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 38-41. Such an immunogenic composition can be administered in a priming composition and/or in a boosting composition.

[0170] In various embodiments, the administered immunogenic composition comprises a first viral expression vector and a second viral expression vector, wherein: (a) the first viral expression vector comprises a polynucleotide encoding an HBV polymerase polypeptide mutant comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 13-14, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 13-14; and (b) the second viral expression vector comprises a polynucleotide encoding the core-sAg fusion protein comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 38-41, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 38-41. Such an immunogenic composition can be administered in a priming composition and/or in a boosting composition.

[0171] With respect to the core-sAg fusion polypeptide in the administered immunogenic composition, in some embodiments, the core polypeptide comprises a serine (S) residue at the amino acid position corresponding to position 12, and an asparagine (N) residue at the amino acid position corresponding to position 67, wherein the position numbers are with reference to SEQ ID NO:65 or SEQ ID NO:66. In some embodiments, the sAg polypeptide comprises an isoleucine (I) residue at the amino acid position corresponding to position 68, wherein the position numbers are with reference to SEQ ID NO:3 or SEQ ID NO:4. In some embodiments, the sAg polypeptide comprises one or more of a serine (S) residue at the amino acid position corresponding to position 53, an isoleucine (I) residue at the amino acid position corresponding to position 68, a threonine (T) residue at the amino acid position corresponding to position 125, a proline (P) residue at the amino acid position corresponding to position 127, an phenylalanine (F) residue at the amino acid position corresponding to position 161, a tyrosine (Y) residue at the amino acid position corresponding to position 200, a serine (S) residue at the

amino acid position corresponding to position 210, and a leucine (L) residue at the amino acid position corresponding to position 213, wherein the position numbers are with reference to SEQ ID NO:3 or SEQ ID NO:4. In some embodiments, the core-sAg fusion polypeptide comprises one or more of a serine (S) residue at the amino acid position corresponding to position 12, an asparagine (N) residue at the amino acid position corresponding to position 67, a valine (V) residue at the amino acid position corresponding to position 74, a phenylalanine (F) residue at the amino acid position corresponding to position 97, a threonine (T) residue at the amino acid position corresponding to position 249, a threonine (T) residue at the amino acid position corresponding to position 250, a serine (S) residue at the amino acid position corresponding to position 317, a serine (S) residue at the amino acid position corresponding to position 318, an arginine (R) residue at the amino acid position corresponding to position 326, a tyrosine (Y) residue at the amino acid position corresponding to position 338, a glycine (G) residue at the amino acid position corresponding to position 363, and an alanine (A) residue at the amino acid position corresponding to position 372, wherein the position numbers are with reference to SEQ ID NO:41.

[0172] In various embodiments, the administered immunogenic composition comprises a mixture comprising a first viral expression vector and a second viral expression vector, wherein: (a) the first viral expression vector comprises a polynucleotide encoding an HBV polymerase polypeptide mutant comprising or consisting of an amino acid sequence of SEQ ID NO: 13, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 13; and (b) the second viral expression vector comprises a polynucleotide encoding the core-sAg fusion protein comprising or consisting of an amino acid sequence of SEQ ID NO: 41, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 41. Such an immunogenic composition can be administered in a priming composition and/or in a boosting composition.

[0173] In various embodiments, the administered immunogenic composition comprises a mixture comprising a first viral expression vector and a second viral expression vector, wherein: (a) the first viral expression vector comprises a polynucleotide comprising or consisting of a nucleic sequence of any one of SEQ ID NOs: 27-32 and 89-94, *e.g.*, SEQ ID NOs: 29, 89, 90 and 92, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 27-32 and 89-94, *e.g.*, SEQ ID NOs: 29, 89, 90 and 92; and (b) the second viral expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of any

one of SEQ ID NOs: 33-37, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 33-37. Such an immunogenic composition can be administered in a priming composition and/or in a boosting composition.

5 **[0174]** In various embodiments, the administered immunogenic composition comprises a mixture comprising a first viral expression vector and a second viral expression vector, wherein: (a) the first viral expression vector comprises a polynucleotide comprising or consisting of a nucleic sequence of SEQ ID NOs: 29, 89, 90 or 92, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NOs: 29, 89, 90 or 92; and (b) the second viral expression
10 vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 37, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 37. Such an immunogenic composition can be administered in a priming composition and/or in a
15 boosting composition.

[0175] In various embodiments, the first viral expression vector and the second viral expression vector in the administered immunogenic composition independently are from a taxonomic family selected from Adenoviridae, Arenaviridae, Herpesviridae (*e.g.* Cytomegalovirus), Poxviridae (*e.g.* Vaccinia virus, *e.g.* modified vaccinia Ankara (MVA)),
20 Flaviviridae (*e.g.* Yellow fever virus), Rhabdoviridae (*e.g.* Vesiculovirus, *e.g.* Maraba vesiculovirus), Togaviridae (*e.g.*, Alphavirus), as described above and herein. In various embodiments, the first viral expression vector and the second viral expression vector can be from the same taxonomic family or from different taxonomic families. For example, in some embodiments, both the first viral expression vector and the second viral expression vector in the
25 administered immunogenic composition are from Adenoviridae, Arenaviridae, or Poxviridae (*e.g.* Vaccinia virus, *e.g.* modified vaccinia Ankara (MVA)).

[0176] In some embodiments, the first viral expression vector and the second viral expression vector are from Arenaviridae. In some embodiments, the first viral expression vector and the second viral expression vector in the administered immunogenic composition are from
30 an arenavirus vector selected from Lymphocytic choriomeningitis mammarenavirus (LCMV), Cali mammarenavirus (*a.k.a.*, Pichinde mammarenavirus or Pichinde arenavirus (PICV)), Guanarito virus (GTOV), Junin virus (JUNV), Lassa virus (LASV), Lujo virus (LUJV), Machupo virus (MACV), Sabia virus (SABV), and Whitewater Arroyo virus (WWAV). In some embodiments, the first viral expression vector and the second viral expression vector in the

administered immunogenic composition are from an arenavirus vector selected from Lymphocytic choriomeningitis mammarenavirus (LCMV) or Cali mammarenavirus (*a.k.a.*, Pichinde mammarenavirus or Pichinde arenavirus (PICV)).

[0177] In various embodiments, the first viral expression vector and the second viral expression vector in the administered immunogenic composition are replication-defective or replication-deficient. In some embodiments, the first viral expression vector and the second viral expression vector in the administered immunogenic composition are replication-attenuated.

[0178] In various embodiments, the administered immunogenic composition comprises a mixture comprising a first LCMV arenavirus expression vector and a second LCMV arenavirus expression vector, wherein: (a) the first LCMV arenavirus expression vector comprises a polynucleotide comprising or consisting of a nucleic sequence of SEQ ID NO: 29, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 29; and (b) the second LCMV arenavirus expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 37, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 37. Such an immunogenic composition can be administered in a priming composition and/or in a boosting composition.

[0179] In various embodiments, the administered immunogenic composition comprises a mixture comprising a first Pichinde arenavirus expression vector and a second Pichinde arenavirus expression vector, wherein: (a) the first Pichinde arenavirus expression vector comprises a polynucleotide comprising or consisting of a nucleic sequence of SEQ ID NO: 90, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 90; and (b) the second Pichinde arenavirus expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 37, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 37. Such an immunogenic composition can be administered in a priming composition and/or in a boosting composition.

[0180] In various embodiments, the subject is infected with HBV, is suspected of being infected with HBV, or is at risk of being infected with HBV. “At risk individual” as used herein refers to an individual who is at risk of developing a condition to be treated. An individual “at risk” may or may not have detectable disease or condition, and may or may not have displayed

detectable disease prior to the treatment of methods described herein. “At risk” denotes that an individual has one or more so-called risk factors, which are measurable parameters that correlate with development of a disease or condition and are known in the art. An individual having one or more of these risk factors has a higher probability of developing the disease or condition than an individual without these risk factor(s). In various embodiments, the subject is chronically infected with HBV, *e.g.*, infected with HBV for longer than 6 months. Typically, the individual is suffering from a chronic hepatitis B infection, although it is within the scope of the present disclosure to treat people who are acutely infected with HBV. Accordingly, in some embodiments, the subject is acutely infected with HBV. In some embodiments, the subject is co-infected with hepatitis D virus (HDV).

[0181] In various embodiments, the subject may be asymptomatic. In some embodiments, the subject is experiencing or exhibiting symptoms associated with HBV infection. Symptoms of HBV can include, *e.g.*, jaundice, visible webs of swollen blood vessels in the skin, dark-colored (*e.g.*, orange or brown) urine, light-colored feces, fever, persistent fatigue, malaise, abdominal pain, abdominal fluid, loss of appetite, nausea, and vomiting. Chronic infection with HBV can lead to one or more symptoms including, *e.g.*, hepatic failure, hepatic cancer, hepatic fibrosis and hepatic cirrhosis. One or more administrations of the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, can prevent, delay, alleviate, mitigate, inhibit, reverse or eliminate one or more symptoms associated with or caused by HBV infection.

[0182] In some embodiments, the immunogenic composition is administered via a route selected from intravenous, intramuscular, intradermal, subcutaneous and mucosal (*e.g.* buccal, intranasal, intrarectal, intravaginal).

[0183] In some embodiments, the administered dose of the immunogenic composition comprises in the range of about 10^3 to about 10^{12} viral focus forming units (FFU) or plaque forming units (PFU) or infectious units (IU) or viral particles (vp), *e.g.*, from about 10^4 to about 10^7 viral FFU or PFU, *e.g.*, from about 10^3 to about 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} or 10^{12} viral FFU or PFU or IU or vp per milliliter, of each of the first viral expression vector and the second viral expression vector. In some embodiments, the methods entail administering intravenously or intramuscularly from about 10^6 to about 10^8 viral FFU or PFU or IU or vp per administration every other week (Q2W) or monthly (Q4W).

[0184] In various embodiments, the methods comprise a prime-boost regimen. In some embodiments, the prime-boost regimen entails administering a priming composition at a first time point and administering one or more boosting compositions at one or more subsequent time points. As appropriate, the methods can entail repeating the prime-boost regimen one or more iterations. In various embodiments, the administrations of the priming composition and the one or more boosting compositions are spaced at least 1 week and up to at least 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months or 6 months apart. As appropriate, the dosage or dosing frequency of the immunogenic composition may be adjusted over the course of the treatment, based on the judgment of the administering physician. As appropriate, a subject can be treated with multiple administrations over a time period of at least about 2 weeks to 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 19 months, 20 months, 21 months, 22 months, 23 months, 24 months, or longer, or until sAg is no longer detectable in the serum or plasma of the subject.

[0185] In some embodiments, after one or more administrations of the one or more immunogenic polypeptides, as described herein, or one or more polynucleotides encoding one or more immunogenic polypeptides, as described herein, or one or more vectors expressing one or more immunogenic polypeptides, as described herein, optionally with one or more additional therapeutic agents, described herein, the subject does not exhibit symptoms of HBV in the absence of antiviral treatment for at least 6 months, at least 1 year, at least 2 years, at least 3 years, or more. In some embodiments, after one or more administrations of the one or more immunogenic polypeptides, as described herein, or one or more polynucleotides encoding one or more immunogenic polypeptides, as described herein, or one or more vectors expressing one or more immunogenic polypeptides, as described herein, optionally with one or more additional therapeutic agents, described herein, sAg is no longer detectable in the serum or plasma of the subject, in the absence of antiviral treatment for at least 6 months, *e.g.*, at least 1 year, at least 2 years, at least 3 years, or more.

[0186] As appropriate or desired, the priming composition and the boosting composition can comprise the same immunogenic composition or different immunogenic compositions. In various embodiments, the priming composition and the boosting composition comprise the same one or more polypeptides and same expression vector (*e.g.*, viral expression vector). In some embodiments, the priming composition and the boosting composition comprise different polypeptides and/or different expression vectors (*e.g.*, viral expression vectors). For example, in some embodiments, the priming composition and the boosting composition comprise the same

one or more polypeptides and different expression vectors (*e.g.*, viral vectors from different virus species within a taxonomic family, viral vectors from different taxonomic families, viral vectors with different replication competencies). In some embodiments, the priming composition and the boosting composition comprise different immunogenic polypeptides and the same expression vector (*e.g.*, viral expression vector).

[0187] In some embodiments, the methods comprise priming with a priming composition comprising one or more viral expression vectors, and boosting with a boosting composition comprising one or more viral expression vectors. In some embodiments, the prime-boost regimen comprises:

a) Priming with a priming composition comprising one or more viral expression vectors and boosting with a boosting composition comprising one or more polynucleotides, wherein the one or more polynucleotides comprise DNA, cDNA, mRNA or self-replicating RNA;

b) Priming with a priming composition comprising one or more polynucleotides, wherein the one or more polynucleotides comprise DNA, cDNA, mRNA or self-replicating RNA, and boosting with a boosting composition comprising one or more viral expression vectors;

c) Priming with a priming composition comprising one or more viral expression vectors, and boosting with a boosting composition comprising one or more viral expression vectors, wherein the one or more viral expression vectors in the priming composition and the one or more viral expression vectors in the boosting composition are from identical, related or unrelated taxonomical families;

d) Priming with a priming composition comprising one or more replication-deficient viral expression vectors and boosting with a boosting composition comprising one or more replication-deficient viral expression vectors, wherein the one or more replication-deficient viral expression vectors in the priming composition and the one or more replication-deficient viral expression vectors in the boosting composition are from identical, related or unrelated taxonomical families;

e) Priming with a priming composition comprising one or more replication-attenuated viral expression vectors and boosting with a boosting composition comprising one or more replication-attenuated viral expression vectors, wherein the one or more replication-attenuated viral expression vectors in the priming composition and the one or more replication-attenuated viral expression vectors in the boosting composition are from identical, related or unrelated taxonomical families;

- f) Priming with a priming composition comprising one or more replication-deficient viral expression vectors and boosting with a boosting composition comprising one or more replication-attenuated viral expression vectors;
- 5 g) Priming with a priming composition comprising one or more replication-attenuated viral expression vectors and boosting with a boosting composition comprising one or more replication-deficient viral expression vectors;
- 10 h) Priming with a priming composition comprising one or more Lymphocytic choriomeningitis mammarenavirus (LCMV) viral expression vectors and boosting with a boosting composition comprising one or more Pichinde mammarenavirus (PICV) viral expression vectors;
- i) Priming with a priming composition comprising one or more Pichinde mammarenavirus (PICV) viral expression vectors and boosting with a boosting composition comprising one or more Lymphocytic choriomeningitis mammarenavirus (LCMV) viral expression vectors;
- 15 j) Priming with a priming composition comprising one or more replication deficient Pichinde mammarenavirus (PICV) viral expression vectors and boosting with a boosting composition comprising one or more replication deficient Lymphocytic choriomeningitis mammarenavirus (LCMV) viral expression vectors;
- 20 k) Priming with a priming composition comprising one or more replication deficient Lymphocytic choriomeningitis mammarenavirus (LCMV) viral expression vectors and boosting with a boosting composition comprising one or more replication deficient Pichinde mammarenavirus (PICV) viral expression vectors;
- 25 l) Priming with a priming composition comprising one or more arenavirus viral expression vectors and boosting with a boosting composition comprising one or more adenovirus viral expression vectors;
- m) Priming with a priming composition comprising one or more adenovirus viral expression vectors and boosting with boosting composition comprising one or more arenavirus viral expression vectors;
- 30 n) Priming with a priming composition comprising one or more poxvirus viral expression vectors and boosting with a boosting composition comprising one or more arenavirus viral expression vectors;
- o) Priming with a priming composition comprising one or more arenavirus viral expression vectors and boosting with boosting composition comprising one or more poxvirus viral expression vectors;

p) Priming with a priming composition comprising one or more poxvirus viral expression vectors and boosting with a boosting composition comprising one or more adenovirus viral expression vectors; or

5 q) Priming with a priming composition comprising one or more adenovirus viral expression vectors and boosting with boosting composition comprising one or more poxvirus viral expression vectors.

[0188] In some embodiments, the methods comprise priming with a priming composition comprising one or more viral expression vectors, and boosting with a boosting composition comprising one or more viral expression vectors. In some embodiments, the prime-
10 boost regimen comprises:

a) Priming with a priming composition comprising one or more Lymphocytic choriomeningitis mammarenavirus (LCMV) viral expression vectors and boosting with a boosting composition comprising one or more Pichinde mammarenavirus (PICV) viral expression vectors;

15 b) Priming with a priming composition comprising one or more Pichinde mammarenavirus (PICV) viral expression vectors and boosting with a boosting composition comprising one or more Lymphocytic choriomeningitis mammarenavirus (LCMV) viral expression vectors;

c) Priming with a priming composition comprising one or more replication deficient
20 Pichinde mammarenavirus (PICV) viral expression vectors and boosting with a boosting composition comprising one or more replication deficient Lymphocytic choriomeningitis mammarenavirus (LCMV) viral expression vectors; or

d) Priming with a priming composition comprising one or more replication deficient Lymphocytic choriomeningitis mammarenavirus (LCMV) viral expression vectors and boosting
25 with a boosting composition comprising one or more replication deficient Pichinde mammarenavirus (PICV) viral expression vectors.

[0189] In various embodiments, the priming composition and the boosting composition comprise an immunogenic composition as described herein.

[0190] In some embodiments, the subject is not receiving antiviral therapy or antiviral
30 therapy is discontinued prior to administration of the one or more immunogenic compositions. In some embodiments, the antiviral therapy is discontinued after one or more administrations of the compositions.

[0191] In some embodiments, the treatment methods activate in the subject CD8+ T cells targeting one or more HBV polypeptide epitopes. In some embodiments, the treatment methods elicit in the subject production of antibodies that bind one or more HBV polypeptides.

7. Combination Therapies

5 [0192] In certain embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one, two, three, four or more additional therapeutic agents. In certain embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and
10 immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with two additional therapeutic agents. In certain embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with three additional therapeutic agents. In
15 certain embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with four additional therapeutic agents. The one, two, three, four or more additional therapeutic agents can be different therapeutic agents selected from the same class of therapeutic agents, and/or they can
20 be selected from different classes of therapeutic agents.

[0193] “Co-administration” as used herein refers to administration of unit dosages of the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, before or after administration of unit dosages of one or more additional therapeutic
25 agents. For example, administration of the immunogenic composition disclosed herein within seconds, minutes, or hours of the administration of one or more additional therapeutic agents. For example, in some embodiments, a unit dose of an immunogenic composition of the present disclosure is administered first, followed within seconds or minutes by administration of a unit dose of one or more additional therapeutic agents. Alternatively, in other embodiments, a unit
30 dose of one or more additional therapeutic agents is administered first, followed by administration of a unit dose of an immunogenic composition of the present disclosure within seconds or minutes. In some embodiments, a unit dose of the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, is administered first,

followed, after a period of hours (*e.g.*, 1-12 hours), by administration of a unit dose of one or more additional therapeutic agents. In other embodiments, a unit dose of one or more additional therapeutic agents is administered first, followed, after a period of hours (*e.g.*, 1-12 hours), by administration of a unit dose of the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein.

[0194] Co-administration of the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, with one or more additional therapeutic agents generally refers to simultaneous or sequential administration of an immunogenic composition disclosed herein and one or more additional therapeutic agents, such that therapeutically effective amounts of each agent are present in the body of the patient.

[0195] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more additional therapeutic agents as described herein, the components of the composition are administered as a simultaneous or sequential regimen. When administered sequentially, the combination may be administered in two or more administrations.

[0196] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one, two, three, four or more additional therapeutic agents selected from HBV combination drugs, HBV vaccines, HBV DNA polymerase inhibitors, immunomodulators, toll-like receptor (TLR) modulators, interferon alpha receptor ligands, hyaluronidase inhibitors, HBV antigen inhibitors (*e.g.*, HBV core antigen (HBcAg) inhibitors, HBV surface antigen (HBsAg) inhibitors, HBx inhibitors, HBV E antigen inhibitors), anti-HBV antigen antibodies, inhibitory nucleic acids targeting HBV (*e.g.*, antisense oligonucleotide, short interfering RNA (siRNA), DNA-directed RNA interference (ddRNAi)), HBsAg secretion or assembly inhibitors, HBV viral entry inhibitors, immune checkpoint inhibitor, cytotoxic T-lymphocyte-associated protein 4 (CTLA4) inhibitors, cyclophilin inhibitors, endonuclease modulators, ribonucleotide reductase inhibitors, covalently closed circular DNA (cccDNA) inhibitors, farnesoid X receptor (FXR) agonists, STING agonists, anti-HBV antibodies, CCR2 chemokine antagonists, thymosin agonists, cytokines, nucleoprotein modulators, retinoic acid-inducible gene 1 stimulators, NOD2 stimulators, phosphatidylinositol 3-kinase (PI3K) inhibitors, indoleamine-2, 3-dioxygenase

(IDO) pathway inhibitors, ZCCHC14 inhibitors, inducers of tertiary lymphoid aggregates, nucleic acid polymers (*e.g.*, NAPs and STOPS), PD-1 inhibitors, PD-L1 inhibitors, recombinant thymosin alpha-1, Bruton's tyrosine kinase (BTK) inhibitors, lysine demethylase (KDM) inhibitors, HBV replication inhibitors, arginase inhibitors, gene therapy and cell therapy, gene editors, cellular therapy, TCR-T cell therapy, and other HBV drugs.

[0197] In certain embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, may be combined or co-administered with one or more of a chemotherapeutic agent, an immunomodulator, an immunotherapeutic agent, a therapeutic antibody, a therapeutic vaccine, a bispecific antibody and "antibody-like" therapeutic protein (such as DARPin[®], anti-pMHC TCR-like antibodies, DARTs[®], Duobodies[®], Bites[®], XmAbs[®], TandAbs[®], Fab derivatives), an antibody-drug conjugate (ADC), gene modifiers or gene editors targeting HBV (*e.g.*, CRISPR-Cas (*e.g.*, Cas9, Cas12, Cascade, Cas13), zinc finger nucleases, homing endonucleases, homing meganucleases (*e.g.*, ARCUS), synthetic nucleases, TALENs), cell therapies (*e.g.*, T-cells, NK cells, macrophages having a chimeric antigen receptor (CAR)), and TCR-T (an engineered T cell receptor) or any combination thereof.

[0198] In certain embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one, two, three, four or more additional therapeutic agents, *e.g.*, as 3-dioxygenase (IDO) inhibitors, apolipoprotein A1 modulator, arginase inhibitors, B- and T-lymphocyte attenuator inhibitors, Bruton's tyrosine kinase (BTK) inhibitors, CCR2 chemokine antagonist, CD137 inhibitors, CD160 inhibitors, CD305 inhibitors, CD4 agonist and modulator, compounds targeting hepatitis B core antigen (HBcAg), core protein allosteric modulators, covalently closed circular DNA (cccDNA) inhibitors, cyclophilin inhibitors, cytotoxic T-lymphocyte-associated protein 4 (CTLA4) inhibitors, DNA polymerase inhibitor, endonuclease modulators, epigenetic modifiers, farnesoid X receptor (FXR) agonists, HBV DNA polymerase inhibitors, HBV replication inhibitors, HBV RNase inhibitors, HBV viral entry inhibitors, HBx inhibitors, Hepatitis B large envelope protein modulator, Hepatitis B large envelope protein stimulator, Hepatitis B structural protein modulator, hepatitis B surface antigen (HBsAg) inhibitors, hepatitis B surface antigen (HBsAg) secretion or assembly inhibitors, hepatitis B virus E antigen inhibitors, hepatitis B virus replication inhibitors, Hepatitis virus structural protein inhibitor, HIV-1 reverse transcriptase inhibitor, Hyaluronidase inhibitor, inhibitor of apoptosis proteins family proteins (IAPs) inhibitors, IL-2 agonist, IL-7 agonist, immunomodulators, indoleamine-2 inhibitors,

inhibitors of ribonucleotide reductase, Interleukin-2 ligand, ipi4 inhibitors, lysine demethylase inhibitors, histone demethylase inhibitors, KDM1 inhibitors, KDM5 inhibitors, killer cell lectin-like receptor subfamily G member 1 inhibitors, lymphocyte-activation gene 3 inhibitors, lymphotoxin beta receptor activators, modulators of Axl, modulators of B7-H3, modulators of B7-H4, modulators of CD160, modulators of CD161, modulators of CD27, modulators of CD47, modulators of CD70, modulators of GITR, modulators of HEVEM, modulators of ICOS, modulators of Mer, modulators of NKG2A, modulators of NKG2D, modulators of OX40, modulators of SIRPalpha, modulators of TIGIT, modulators of Tim-4, modulators of Tyro, Na+taurocholate cotransporting polypeptide (NTCP) inhibitors, natural killer cell receptor 2B4 inhibitors, NOD2 gene stimulator, Nucleoprotein inhibitor, nucleoprotein modulators, OX-40 receptor agonist, PD-1 inhibitors, PD-L1 inhibitors, peptidylprolyl isomerase inhibitor, phosphatidylinositol-3 kinase (PI3K) inhibitors, Retinoic acid-inducible gene 1 stimulator, Reverse transcriptase inhibitor, Ribonuclease inhibitor, RNA DNA polymerase inhibitor, SLC10A1 gene inhibitor, SMAC mimetics, Src tyrosine kinase inhibitor, stimulator of interferon gene (STING) agonists, stimulators of NOD1, T cell surface glycoprotein CD28 inhibitor, T-cell surface glycoprotein CD8 modulator, Thymosin agonist, Thymosin alpha 1 ligand, Tim-3 inhibitors, TLR-3 agonists, TLR-7 agonists, TLR-9 agonists, TLR9 agonists or gene stimulator, toll-like receptor (TLR) modulators, viral ribonucleotide reductase inhibitors, and combinations thereof.

20 HBV Inhibiting Antiviral Drugs

[0199] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more antiviral agents. In some embodiments, the one or more antiviral agents are selected from the group consisting of lamivudine (LAM), adefovir dipivoxil (ADV), entecavir (ETV), telbivudine (LdT), tenofovir disoproxil fumarate (TDF), tenofovir disoproxil fumarate and emtricitabine (TRUVADA®), tenofovir alafenamide (TAF or VEMLIDY®) and ledipasvir and sofosbuvir (HARVONI®).

Other HBV Drugs

30 **[0200]** Examples of other drugs for the treatment of HBV that can be combined or co-administered include alpha-hydroxytropolones, amdoxovir, antroquinonol, beta-hydroxycytosine nucleosides, ARB-199, CCC-0975, ccc-R08, elvucitabine, ezetimibe, cyclosporin A, gentiopiricin (gentiopicroside), HH-003, hepalatide, JNJ-56136379, nitazoxanide,

birinapant, NJK14047, NOV-205 (molixan, BAM-205), oligotide, mivotilate, feron, GST-HG-131, levamisole, Ka Shu Ning, alloferon, WS-007, Y-101 (Ti Fen Tai), rSIFN-co, PEG-IIFNm, KW-3, BP-Inter-014, oleanolic acid, HepB-nRNA, cTP-5 (rTP-5), HSK-II-2, HEISCO-106-1, HEISCO-106, Hepbarna, IBPB-006IA, Hepuyinfen, DasKloster 0014-01, ISA-204, Jiangantai (Ganxikang), MIV-210, OB-AI-004, PF-06, picoside, DasKloster-0039, hepulantai, IMB-2613, NCO-48 Fumarate, XTYW-001, SFA-001, TCM-800B, reduced glutathione, RO-6864018, ENOB-HB-01, RG-7834, QL-007sofosbuvir, ledipasvir, UB-551, PA-1010, HPN-BV1, STSG-0002, and ZH-2N, and the compounds disclosed in US20150210682, (Roche), US 2016/0122344 (Roche), WO2015173164, WO2016023877, US2015252057A (Roche), WO16128335A1 (Roche), WO16120186A1 (Roche), US2016237090A (Roche), WO16107833A1 (Roche), WO16107832A1 (Roche), US2016176899A (Roche), WO16102438A1 (Roche), WO16012470A1 (Roche), US2016220586A (Roche), and US2015031687A (Roche).

[0201] Examples of combination drugs for the treatment of HBV that can be combined or co-administered include tenofovir disoproxil fumarate and emtricitabine (TRUVADA®), ledipasvir and sofosbuvir (HARVONI®); ABX-203 (NASVAC), lamivudine and PEG-IFN α ; adefovir and PEG-IFN α ; and INO-1800 (INO-9112 and RG7944).

HBV Vaccines

[0202] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more HBV vaccines. HBV vaccines that can be combined or co-administered (*e.g.*, in a prime-boost prevention regimen) include both prophylactic and therapeutic vaccines. Examples of HBV prophylactic vaccines include Vaxelis, Hexaxim, Heplisav, Mosquirix, DTwP-HBV vaccine, Bio-Hep-B, D/T/P/HBV/M (LBVP-0101; LBVW-0101), DTwP-HepB-Hib-IPV vaccine, Heberpenta L, DTwP-HepB-Hib, V-419, CVI-HBV-001, Tetrabhay, hepatitis B prophylactic vaccine (Advax Super D), Hepatrol-07, GSK-223192A, ENGERIX B®, recombinant hepatitis B vaccine (intramuscular, Kangtai Biological Products), recombinant hepatitis B vaccine (Hansenal polymorpha yeast, intramuscular, Hualan Biological Engineering), recombinant hepatitis B surface antigen vaccine, Bimmugen, CARG-101, Euforavac, Eutravac, anrix-DTaP-IPV-Hep B, HBAI-20, Infanrix-DTaP-IPV-Hep B-Hib, Pentabio Vaksin DTP-HB-Hib, Comvac 4, Twinrix, Euvax-B, Tritanrix HB, Infanrix Hep B, Comvac, DTP-Hib-HBV vaccine, DTP-HBV vaccine, Yi Tai, Heberbiovac HB, Trivac HB, GerVax, DTwP-Hep B-Hib vaccine, Bilive, Hepavax-Gene, SUPERVAX, Comvac5, Shanvac-

B, Hebsulin, Recombivax HB, Revac B mcf, Revac B+, Fendrix, DTwP-HepB-Hib, DNA-001, Shan5, Shan6, rhHBsAG vaccine, HBI pentavalent vaccine, LBVD, Infanrix HeXa, YS-HBV-001, IR-101H, TVAX-008, and DTaP-rHB-Hib vaccine.

[0203] Examples of HBV therapeutic vaccines that can be combined or co-administered

5 (e.g., in a prime-boost treatment regimen) include HBsAG-HBIG complex, ARB-1598, Bio-Hep-B, abi-HB (intravenous), ABX-203 (NASVAC), Tetrabhay, GX-110E, GS-4774, peptide vaccine (epsilonPA-44), Hepatrol-07, NASVAC (NASTERAP), IMP-321, BEVAC, Revac B mcf, Revac B+, MGN-1333, KW-2, CVI-HBV-002, AltraHepB, VGX-6200, FP-02, FP-02.2 (HepTcell), NU-500, HBVax, im/TriGrid/antigen vaccine, Mega-CD40L-adjuvanted vaccine, 10 HepB-v, RG7944 (INO-1800), recombinant VLP-based therapeutic vaccine (HBV infection, VLP Biotech), AdTG-17909, AdTG-17910 AdTG-18202, ChronVac-B, TG-1050, VVX-001, GSK-3528869A (ChAd155-hli-HBV + MVA-HBV +Hbc-HBs/AS01B-4), VBI-2601, VTP-300 (ChAdOx1-Sli-HBV-CPmut-TPA-Ssh prime and MVA-Sli-HBV-CPmut-TPA-Ssh boost), Lm HBV and BM32 (Tulaeva, *et al.*, EBioMedicine (2020) 102953). HBV Arenavirus vaccines are 15 described, e.g., in WO2017076988 and WO2017198726.

HBV DNA Polymerase Inhibitors

[0204] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one 20 or more polymerase inhibitors. Examples of HBV DNA polymerase inhibitors that can be combined or co-administered include adefovir (HEPSERA®), emtricitabine (EMTRIVA®), tenofovir disoproxil fumarate (VIREAD®), tenofovir alafenamide, tenofovir, tenofovir disoproxil, tenofovir alafenamide fumarate, tenofovir alafenamide hemifumarate, tenofovir dipivoxil, tenofovir dipivoxil fumarate, tenofovir octadecyloxyethyl ester, CMX-157, tenofovir 25 exalidex, besifovir, entecavir (BARACLUDE®), entecavir maleate, telbivudine (TYZEKA®), filocilovir, pradevovir, clevudine, ribavirin, lamivudine (EPIVIR-HBV®), phosphazide, famciclovir, fusolin, metacavir, SNC-019754, FMCA, AGX-1009, AR-II-04-26, HIP-1302, tenofovir disoproxil aspartate, tenofovir disoproxil orotate, AiB-001, and HS-10234.

Immunomodulators

30 **[0205]** In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more immunomodulators (e.g., an immune checkpoint inhibitor, a tumor necrosis factor

(TNF) receptor superfamily (TNFRSF) agonist, an immune stimulator, *e.g.*, a TLR agonist). Examples of immunomodulators that can be combined or co-administered include rintatolimod, imidol hydrochloride, ingaron, dermaVir, plaquenil (hydroxychloroquine), proleukin, hydroxyurea, mycophenolate mofetil (MPA) and its ester derivative mycophenolate mofetil (MMF), JNJ-440, WF-10, AB-452, ribavirin, IL-12, INO-9112, polymer polyethyleneimine (PEI), Gepon, VGV-1, MOR-22, CRV-431, JNJ-0535, TG-1050, ABI-H2158, BMS-936559, GS-9688, RO-7011785 and corresponding prodrug RO-702053, RG-7854, RO-6871765, AIC-649, and IR-103.

Toll-Like Receptor (TLR) Agonists

10 **[0206]** In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more agonists or stimulators of a toll-like receptor (TLR). In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and
15 immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with an agonist of a TLR, *e.g.*, an agonist of TLR1 (NCBI Gene ID: 7096), TLR2 (NCBI Gene ID: 7097), TLR3 (NCBI Gene ID: 7098), TLR4 (NCBI Gene ID: 7099), TLR5 (NCBI Gene ID: 7100), TLR6 (NCBI Gene ID: 10333), TLR7 (NCBI Gene ID: 51284), TLR8 (NCBI Gene ID: 51311), TLR9 (NCBI Gene ID: 54106), and/or
20 TLR10 (NCBI Gene ID: 81793), TLR11, TLR12 and TLR13.

[0207] Examples of TLR3 agonists that can be combined or co-administered include rintatolimod, poly-ICLC, RIBOXXON®, Apoxsim, RIBOXXIM®, IPH-33, MCT-465, MCT-475 and ND-1.1.

[0208] Examples of TLR4 agonists that can be combined or co-administered include G-
25 100, and GSK-1795091.

[0209] Example TLR7 agonists that can be combined or co-administered include without limitation AL-034, DSP-0509, GS-9620 (vesatolimod), LHC-165, TMX-101 (imiquimod), GSK-2245035, resiquimod, DSR-6434, DSP-3025, IMO-4200, MCT-465, telratolimod (MEDI-9197), 3M-051, SB-9922, 3M-052, Limtop, TMX-30X, TMX-202, RG-7863, RG-7854, RG-
30 7795, RO-7011785 and corresponding prodrug RO-702053, and the compounds disclosed in US20100143301 (Gilead Sciences), US20110098248 (Gilead Sciences), and US20090047249 (Gilead Sciences), US20140045849 (Janssen), US20140073642 (Janssen), WO2014/056953 (Janssen), WO2014/076221 (Janssen), WO2014/128189 (Janssen), US20140350031 (Janssen),

WO2014/023813 (Janssen), US20080234251 (Array Biopharma), US20080306050 (Array Biopharma), US20100029585 (Ventirx Pharma), US20110092485 (Ventirx Pharma), US20110118235 (Ventirx Pharma), US20120082658 (Ventirx Pharma), US20120219615 (Ventirx Pharma), US20140066432 (Ventirx Pharma), US20140088085 (Ventirx Pharma),
5 US20140275167 (Novira Therapeutics), and US20130251673 (Novira Therapeutics).

[0210] Example dual TLR7/TLR8 agonists that can be combined or co-administered is NKTR-262, telratolimod and BDB-001.

[0211] Example TLR8 agonists that can be co-administered include without limitation E-6887, IMO-4200, IMO-8400, IMO-9200, MCT-465, telratolimod (MEDI-9197), motolimod,
10 resiquimod, selgantolimod (GS-9688), HRS-9950, VTX-1463, VTX-763, 3M-051, 3M-052, SBT6050, and the compounds disclosed in US2016289229 (Gilead Sciences), US20140045849 (Janssen), US20140073642 (Janssen), WO2014/056953 (Janssen), WO2014/076221 (Janssen), WO2014/128189 (Janssen), US20140350031 (Janssen), WO2014/023813 (Janssen), US20080234251 (Array Biopharma), US20080306050 (Array Biopharma), US20100029585
15 (Ventirx Pharma), US20110092485 (Ventirx Pharma), US20110118235 (Ventirx Pharma), US20120082658 (Ventirx Pharma), US20120219615 (Ventirx Pharma), US20140066432 (Ventirx Pharma), US20140088085 (Ventirx Pharma), US20140275167 (Novira Therapeutics), and US20130251673 (Novira Therapeutics), US Patent No. 9670205 (Gilead Sciences, Inc.), US20160289229 (Gilead Sciences, Inc.), WO2017/048727 (Gilead Sciences, Inc.),
20 US20180065938 (Gilead Sciences, Inc.), and US20180086755 (Gilead Sciences, Inc.).

[0212] Example TLR9 agonists that can be combined or co-administered include without limitation AST-008, cobitolimod, CMP-001, IMO-2055, IMO-2125, S-540956, litenimod, MGN-1601, BB-001, BB-006, IMO-3100, IMO-8400, IR-103, IMO-9200, agatolimod, DIMS-9054, DV-1079, DV-1179, AZD-1419, lefitolimod (MGN-1703), CYT-003, CYT-003-QbG10,
25 tilсотolimod and PUL-042.

[0213] Additional examples of TLR7, TLR8 and TLR9 modulators that can be combined or co-administered include the compounds disclosed in WO2017047769 (Teika Seiyaku), WO2015014815 (Janssen), WO2018045150 (Gilead Sciences Inc), WO2018045144 (Gilead Sciences Inc), WO2015162075 (Roche), WO2017034986 (University of Kansas),
30 WO2018095426 (Jiangsu Hengrui Medicine Co Ltd), WO2016091698 (Roche), WO2016075661 (GlaxoSmithKline Biologicals), WO2016180743 (Roche), WO2018089695 (Dynavax Technologies), WO2016055553 (Roche), WO2015168279 (Novartis), WO2016107536 (Medshine Discovery), WO2018086593 (Livo (Shanghai) Pharmaceutical), WO2017106607

(Merck), WO2017061532 (Sumitomo Dainippon Pharma), WO2016023511 (Chia Tai Tianqing Pharmaceutical), WO2017076346 (Chia Tai Tianqing Pharmaceutical), WO2017046112 (Roche), WO2018078149 (Roche), WO2017040233 (3M Co), WO2016141092 (Gilead Sciences), WO2018049089 (BristolMyers Squibb), WO2015057655 (Eisai Co Ltd),
5 WO2017001307 (Roche), WO2018005586 (BristolMyers Squibb), WO201704023 (3M Co), WO2017163264 (Council of Scientific and Industrial Research (India)), WO2018046460 (GlaxoSmithKline Biologicals), WO2018047081 (Novartis), WO2016142250 (Roche), WO2015168269 (Novartis), WO201804163 (Roche), WO2018038877 (3M Co), WO2015057659 (Eisai Co Ltd), WO2017202704 (Roche), WO2018026620 (BristolMyers
10 Squibb), WO2016029077 (Janus Biotherapeutics), WO201803143 (Merck), WO2016096778 (Roche), WO2017190669 (Shanghai De Novo Pharmatech), US09884866 (University of Minnesota), WO2017219931 (Sichuan KelunBiotech Biopharmaceutical), WO2018002319 (Janssen Sciences), WO2017216054 (Roche), WO2017202703 (Roche), WO2017184735 (IFM Therapeutics), WO2017184746 (IFM Therapeutics), WO2015088045 (Takeda Pharmaceutical),
15 WO2017038909 (Takeda Pharmaceutical), WO2015095780 (University of Kansas), WO2015023958 (University of Kansas).

[0214] In certain embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with a
20 TLR7, TLR8 or TLR9 agonist.

Interferon Alpha Receptor Ligands

[0215] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one
25 or more interferon alpha receptor ligands. Examples of interferon alpha receptor ligands that can be combined or co-administered include interferon alpha-2b (INTRON A®), pegylated interferon alpha-2a (PEGASYS®), PEGylated interferon alpha-1b, interferon alpha 1b (HAPGEN®), Veldona, Infradure, Roferon-A, YPEG-interferon alfa-2a (YPEG-rhIFNalpha-2a), P-1101, Algeon, Alfarona, Ingaron (interferon gamma), rSIFN-co (recombinant super
30 compound interferon), Ypeginterferon alfa-2b (YPEG-rhIFNalpha-2b), MOR-22, peginterferon alfa-2b (PEG-INTRON®), Bioferon, Novaferon, Inmutag (Inferon), MULTIFERON®, interferon alfa-n1 (HUMOFERON®), interferon beta-1a (AVONEX®), Shaferon, interferon alfa-2b (Axxo), Alfaferone, interferon alfa-2b (BioGeneric Pharma), interferon-alpha 2 (CJ), Laferonum, VIPEG, BLAUFERON-A, BLAUFERON-B, Intermax Alpha, Realdiron, Lanstion,

Pegaferon, PDferon-B, interferon alfa-2b (IFN, Laboratorios Bioprofarma), alfainterferona 2b, Kalferon, Pegnano, Feronsure, PegiHep, interferon alfa 2b (Zydus-Cadila), interferon alfa 2a, Optipeg A, Realfa 2B, Reliferon, interferon alfa-2b (Amega), interferon alfa-2b (Virchow), ropeginterferon alfa-2b, rHSA-IFN alpha-2a (recombinant human serum albumin intereferon
5 alpha 2a fusion protein), PEG-IFN-alpha, rHSA-IFN alpha 2b, recombinant human interferon alpha-(1b, 2a, 2b), peginterferon alfa-2b (Amega), peginterferon alfa-2a, Reaferon-EC, Proquiferon, Uniferon, Urifron, interferon alfa-2b (Changchun Institute of Biological Products), Anterferon, Shanferon, Layfferon, Shang Sheng Lei Tai, INTEFEN, SINOGEN, Fukangtai, Pegstat, rHSA-IFN alpha-2b, SFR-9216, and Interapo (Interapa).

10 Hyaluronidase Inhibitors

[0216] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more hyaluronidase inhibitors. Examples of hyaluronidase inhibitors that can be combined or
15 co-administered include astodrimer.

Hepatitis B Surface Antigen (HBsAg) Inhibitors

[0217] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one
20 or more HBsAg inhibitors. Examples of HBsAg inhibitors that can be combined or co-administered include AK-074, HBF-0259, GP-605, PBHBV-001, PBHBV-2-15, PBHBV-2-1, REP-9AC, REP-9C, REP-9, REP-2139, REP-2139-Ca, REP-2055, REP-2163, REP-2165, REP-2053, REP-2031 and REP-006, and REP-9AC'. Examples of HBsAg secretion inhibitors that can be combined or co-administered include BM601, GST-HG-131, AB-452 and ALG-010093.

25 Cyclophilin Inhibitors

[0218] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more cyclophilin inhibitors. Examples of cyclophilin inhibitors that can be combined or co-
30 administered include CPI-431-32, EDP-494, OCB-030, SCY-635, NVP-015, NVP-018, NVP-019, STG-175, and the compounds disclosed in US8513184 (Gilead Sciences), US20140030221 (Gilead Sciences), US20130344030 (Gilead Sciences), and US20130344029 (Gilead Sciences).

HBV Viral Entry Inhibitors

[0219] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more HBV viral entry inhibitors. Examples of HBV viral entry inhibitors that can be combined or co-administered include bulevirtide (Hepcludex; Myrcludex B).

Inhibitory Nucleic Acids

[0220] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more inhibitory nucleic acids (*e.g.*, antisense oligonucleotide, short interfering RNA (siRNA), DNA-directed RNA interference (ddRNAi)) specifically targeting an HBV polynucleotide. In some embodiments, the HBV polynucleotide encodes and HBV protein (*i.e.*, is in a coding region within the HBV genome).

Antisense Oligonucleotide Targeting Viral mRNA

[0221] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more antisense oligonucleotides. Examples of antisense oligonucleotide targeting viral mRNA that can be combined or co-administered include ISIS-HBVRx, IONIS-HBVRx, IONIS-HBV-LRx, IONIS-GSK6-LRx, GSK-3389404, BNC-1701 and RG-6004.

Short Interfering RNAs (siRNA)

[0222] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more siRNAs specifically targeting an HBV polynucleotide. Examples of siRNA specifically targeting an HBV polynucleotide that can be combine or co-administered include TKM-HBV (TKM-HepB), ALN-HBV, SR-008, HepB-nRNA, ARC-520, ARC-521, ARB-1740, ARB-1467, AB-729, DCR-HBVS, RG-6084 (PD-L1), RG-6217, ALN-HBV-02, JNJ-3989 (ARO-HBV), STSG-0002, LUNAR-HBV and DCR-HBVS (DCR-S219).

DNA-Directed RNA Interference (ddRNAi)

[0223] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such

polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more ddRNAi specifically targeting an HBV polynucleotide. Examples of ddRNAi specifically targeting an HBV polynucleotide that can be combined or co-administered include BB-HB-331.

5 Endonuclease Modulators

[0224] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more endonuclease modulators. Examples of endonuclease modulators that can be combined
10 or co-administered include PGN-514.

Ribonucleotide Reductase Inhibitors

[0225] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one
15 or more ribonucleotide reductase inhibitors. Examples of inhibitors of ribonucleotide reductase that can be combined or co-administered include Trimidox.

Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

[0226] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such
20 polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more NNRTIs. Examples of NNRTIs that can be combined or co-administered include the compounds disclosed in WO2018118826 (Merck), WO2018080903 (Merck), WO2018119013 (Merck), WO2017100108 (Idenix), WO2017027434 (Merck), WO2017007701 (Merck), WO2008005555 (Gilead).

25 HBV Replication Inhibitors

[0227] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one
30 or more HBV replication inhibitors. Examples of HBV replication inhibitors that can be combined or co-administered include GP-31502, isothiafludine, IQP-HBV, RM-5038, and Xingantie.

Covalently Closed Circular DNA (cccDNA) Inhibitors

[0228] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more cccDNA inhibitors. Examples of cccDNA inhibitors that can be combined or co-administered include BSBI-25, ccc-R08, and CHR-101.

Farnesoid X Receptor (FXR) Agonists

[0229] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more FXR agonists. Examples of FXR agonists that can be combined or co-administered include EYP-001, cilofexor (GS-9674), EDP-305, MET-409, Tropifexor, AKN-083, RDX-023, BWD-100, LMB-763, INV-3, NTX-023-1, EP-024297 and GS-8670.

Anti-HBV Antibodies

[0230] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more antibodies that specifically binds to an HBV antigen, including an HBV peptide presented in a major histocompatibility molecule (MHC) molecule (pMHC). Examples of HBV antibodies targeting the surface antigens of the hepatitis B virus that can be combined or co-administered include lenvovimab (GC-1102), XTL-17, XTL-19, KN-003, IV Hepabulin SN, and fully human monoclonal antibody therapy (hepatitis B virus infection, Humabs BioMed). Antibodies targeting HBV X protein (HBx) that can be combined or co-administered are described, *e.g.*, in Korniyev, *et al.*, *J Virol.* 2019 Jul 30;93(16). pii: e00248-19.

[0231] Examples of HBV antibodies, including monoclonal antibodies and polyclonal antibodies, that can be combined or co-administered include Zutectra, Shang Sheng Gan Di, Uman Big (Hepatitis B Hyperimmune), Omri-Hep-B, Nabi-HB, Hepatect CP, HepaGam B, igantibe, Niuliva, CT-P24, hepatitis B immunoglobulin (intravenous, pH4, HBV infection, Shanghai RAAS Blood Products), and Fovepta (BT-088).

[0232] Examples of fully human monoclonal HBV antibodies that can be combined or co-administered include HBC-34.

[0233] Antibodies against HBV viral peptide/major histocompatibility complex (MHC) class I (pMHC) complexes that can be combined or co-administered are described, *e.g.*, in Sastry, *et al.*, *J Virol.* 2011 Mar;85(5):1935-42 and in WO2011062562.

CCR2 Chemokine Antagonists

[0234] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more CCR2 chemokine antagonists. Examples of CCR2 chemokine antagonists that can be combined or co-administered include propagermanium.

Thymosin Agonists

[0235] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more thymosin agonists, *e.g.*, a recombinant thymosin alpha-1. Examples of thymosin agonists that can be combined or co-administered include Thymalfasin, and recombinant thymosin alpha 1 (GeneScience). Examples of recombinant thymosin alpha-1 include NL-004 and PEGylated thymosin alpha-1.

Interleukin Receptor Agonists (*e.g.*, Cytokines)

[0236] In some embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more interleukin receptor agonists of an interleukin receptor selected from IL-2, IL-7, IL-12 and IL-15. In some embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more cytokines selected from the group consisting of IL-2, IL-7, IL-12, IL-15, IL-21, IL-24, and variants thereof. Examples of IL-2 receptor agonists that can be combined or co-administered include proleukin (aldesleukin, IL-2); celmoleukin; pegylated IL-2 (*e.g.*, NKTR-214); modified variants of IL-2 (*e.g.*, THOR-707), bempegaldesleukin, AIC-284, ALKS-4230, CUI-101 and Neo-2/15. Examples of IL-15 receptor agonists that can be combined or co-administered include ALT-803, NKTR-255, and hetIL-15, interleukin-15/Fc fusion protein, AM-0015, NIZ-985, SO-C101, IL-15 Synthorin (pegylated IL-15), P-22339, and an IL-15 -PD-1 fusion protein N-809. Examples of IL-7 receptor agonists that can be combined or co-administered include CYT-107.

Nucleoprotein Modulators

[0237] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more nucleoprotein modulators. Nucleoprotein modulators may be either HBV core or capsid protein inhibitors. Examples of nucleoprotein modulators that can be combined or co-administered include GS-4882, AB-423, AB-836, AT-130, ALG-001075, ALG-001024, ALG-000184, EDP-514, GLS4, NVR-1221, NVR-3778, AL-3778, BAY 41-4109, morphothiadine mesilate, ARB-168786, ARB-880, ARB-1820, GST-HG-141, JNJ-379, JNJ-632, RG-7907, GST-HG-141, HEC-72702, KL-060332, AB-506, ABI-H0731, ABI-H3733, JNJ-440, AK-0605, HRS-5091, VNRX-9945, ABI-H2158, CB-HBV-001, AK-0605, SOC-10, SOC-11 and DVR-23.

[0238] Examples of capsid inhibitors that can be combined or co-administered include ALG-000184, ABI-H0731, NVR 3-778, and compounds disclosed in US2018161307 (Gilead Sciences), US20140275167 (Novira Therapeutics), US20130251673 (Novira Therapeutics), US20140343032 (Roche), WO2014037480 (Roche), US20130267517 (Roche), WO2014131847 (Janssen), WO2014033176 (Janssen), WO2014033170 (Janssen), WO2014033167 (Janssen), WO2015/059212 (Janssen), WO2015118057 (Janssen), WO2015011281 (Janssen), WO2014184365 (Janssen), WO2014184350 (Janssen), WO2014161888 (Janssen), WO2013096744 (Novira), US20150225355 (Novira), US20140178337 (Novira), US20150315159 (Novira), US20150197533 (Novira), US20150274652 (Novira), US20150259324, (Novira), US20150132258 (Novira), US9181288 (Novira), WO2014184350 (Janssen), WO2013144129 (Roche), WO2017198744 (Roche), US 20170334882 (Novira), US20170334898 (Roche), WO2017202798 (Roche), WO2017214395 (Enanta), WO2018001944 (Roche), WO2018001952 (Roche), WO2018005881 (Novira), WO2018005883 (Novira), WO2018011100 (Roche), WO2018011160 (Roche), WO2018011162 (Roche), WO2018011163 (Roche), WO2018036941 (Roche), WO2018043747 (Kyoto Univ), US20180065929 (Janssen), WO2016168619 (Indiana University), WO2016195982 (The Penn State Foundation), WO2017001655 (Janssen), WO2017048950 (Assembly Biosciences), WO2017048954 (Assembly Biosciences), WO2017048962 (Assembly Biosciences), US20170121328 (Novira), US20170121329 (Novira).

[0239] Examples of transcript inhibitors that can be combined or co-administered include compounds disclosed in WO2017013046 (Roche), WO2017016960 (Roche), WO2017017042 (Roche), WO2017017043 (Roche), WO2017061466 (Toyoma chemicals), WO2016177655 (Roche), WO2016161268 (Enanta). WO2017001853 (Redex Pharma),

WO2017211791 (Roche), WO2017216685 (Novartis), WO2017216686 (Novartis), WO2018019297 (Ginkgo Pharma), WO2018022282 (Newave Pharma), US20180030053 (Novartis), WO2018045911 (Zhejiang Pharma).

Innate Immune Activators

5 **[0240]** In some embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more innate immune activators. In various embodiments, the one or more innate immune activators comprises an agonist of a receptor selected from the group consisting of fms related tyrosine
10 kinase 3 (FLT3), stimulator of interferon genes (STING) receptor, DExD/H-box helicase 58 (DDX58; *a.k.a.*, RIG-I), nucleotide binding oligomerization domain containing 2 (NOD2). In some embodiments, the methods entail co-administering GS-3583 and/or GS-9992. In some embodiments, the methods entail combining or co-administering a FLT3 agonist, *e.g.*, GS-3583 or CDX-301.

15 STING agonists, RIG-I and NOD2 modulators

[0241] In some embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with a stimulator of interferon response cGAMP interactor 1 (STING or STING1; NCBI Gene ID: 340061) agonist.
20 In some embodiments, the STING/STING1 agonist or activator is selected from the group consisting of ADU-S100 (MIW-815), SB-11285, MK-1454, SR-8291, AdVCA0848, STINGVAX, GSK-532, SYN-STING, MSA-1, SR-8291, 5,6-dimethylxanthenone-4-acetic acid (DMXAA), cyclic-GAMP (cGAMP) and cyclic-di-AMP. Examples of STING agonists that can be combined or co-administered include the compounds disclosed in WO 2018065360 (Biolog
25 Life Science Institute Forschungslabor und Biochemica-Vertrieb GmbH, Germany), WO 2018009466 (Aduro Biotech), WO 2017186711 (InvivoGen), WO 2017161349 (Immune Sensor), WO 2017106740 (Aduro Biotech), US 20170158724 (Glaxo Smithkline), WO 2017075477 (Aduro Biotech), US 20170044206 (Merck), WO 2014179760 (University of California), WO2018098203 (Janssen), WO2018118665 (Merck), WO2018118664 (Merck),
30 WO2018100558 (Takeda), WO2018067423 (Merck), WO2018060323 (Boehringer).

[0242] In some embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with a DExD/H-box

helicase 58 (DDX58; *a.k.a.*, retinoic acid-inducible gene 1 (RIG-I), RIG1, RIGI, RLR-1, SGMRT2; NCBI Gene ID: 23586). Illustrative RIG-I agonists that can be combined or co-administered include inarigivir soproxil (SB-9200; GS-9992); SB-40, SB-44, ORI-7246, ORI-9350, ORI-7537, ORI-9020, ORI-9198, ORI-7170, and RGT-100.

- 5 **[0243]** In some embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with a nucleotide binding oligomerization domain containing 2 (NOD2; NCBI Gene ID: 64127) agonist, such as inarigivir soproxil (SB-9200; GS-9992), and IR-103.

10 Phosphatidylinositol 3-kinase (PI3K) Inhibitors

- [0244]** In some embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with an inhibitor of a phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit, *e.g.*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA, CLAPO, CLOVE, CWS5, MCAP, MCM, MCMTC, PI3K, PI3K-alpha, p110-alpha; NCBI Gene ID: 5290); phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit beta (PIK3CB, P110BETA, PI3K, PI3KBETA, PIK3C1; NCBI Gene ID: 5291); phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma (PIK3CG, PI3CG, PI3K, PI3Kgamma, PIK3, p110gamma, p120-PI3K; Gene ID: 5494); and/or phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta (PIK3CD, APDS, IMD14, P110DELTA, PI3K, p110D, NCBI Gene ID: 5293). In some embodiments, the PI3K inhibitor is a pan-PI3K inhibitor. Examples of PI3K inhibitors include without limitation, ACP-319, AEZA-129, AMG-319, AS252424, AZD8186, BAY 1082439, BEZ235, bimiralisib (PQR309), buparlisib (BKM120), BYL719 (alpelisib),
- 15 carboxyamidotriazole orotate (CTO), CH5132799, CLR-457, CLR-1401, copanlisib (BAY 80-6946), DS-7423, duvelisib (IPI-145), fimepinostat (CUDC-907), gedatolisib (PF-05212384), GDC-0032, GDC-0084 (RG7666), GDC-0077, pictilisib (GDC-0941), GDC-0980, GSK2636771, GSK2269577, idelalisib (Zydelig®), INCB040093, INCB50465, IPI-443, IPI-549, KAR4141, LY294002, LY3023414, NERLYNX® (neratinib), nemiralisib (GSK2269557),
- 20 omipalisib (GSK2126458, GSK458), OXY111A, panulisib (P7170, AK151761), PA799, perifosine (KRX-0401), Pilaralisib (SAR245408; XL147), puquitinib mesylate (XC-302), SAR260301, seletalisib (UCB-5857), serabelisib (INK-1117, MLN-1117, TAK-117), SF1126, sonolisib (PX-866), RG7604, rigosertib sodium (ON-01910 sodium), RP5090, tenalisib (RP6530), RV-1729, SRX3177, taselisib, TG100115, umbralisib (TGR-1202), TGX221,
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voxtalisib (SAR245409), VS-5584, WX-037, X-339, X-414, XL499, XL756, wortmannin, ZSTK474, and the compounds described in WO 2005/113556 (ICOS), WO 2013/052699 (Gilead Calistoga), WO 2013/116562 (Gilead Calistoga), WO 2014/100765 (Gilead Calistoga), WO 2014/100767 (Gilead Calistoga), and WO 2014/201409 (Gilead Sciences).

5 Immune Checkpoint Modulators

[0245] In some embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more blockers or inhibitors of inhibitory immune checkpoint proteins or receptors and/or with one or more stimulators, activators or agonists of one or more stimulatory immune checkpoint proteins or receptors. Blockade or inhibition of inhibitory immune checkpoints can positively regulate T-cell or NK cell activation and prevent immune escape of infected cells. Activation or stimulation of stimulatory immune check points can augment the effect of immune checkpoint inhibitors in infective therapeutics. In various embodiments, the immune checkpoint proteins or receptors regulate T cell responses (*e.g.*, reviewed in Xu, et al., J Exp Clin Cancer Res. (2018) 37:110). In various embodiments, the immune checkpoint proteins or receptors regulate NK cell responses (*e.g.*, reviewed in Davis, et al., Semin Immunol. (2017) 31:64–75 and Chiossone, et al., Nat Rev Immunol. (2018) 18(11):671-688).

[0246] Examples of immune checkpoint proteins or receptors include without limitation CD27 (NCBI Gene ID: 939); CD70 (NCBI Gene ID: 970); CD40 (NCBI Gene ID: 958); CD40LG (NCBI Gene ID: 959); CD47 (NCBI Gene ID: 961); CD48 (SLAMF2; NCBI Gene ID: 962); transmembrane and immunoglobulin domain containing 2 (TMIGD2, CD28H; NCBI Gene ID: 126259); CD84 (LY9B, SLAMF5; NCBI Gene ID: 8832); CD96 (NCBI Gene ID: 10225); CD160 (NCBI Gene ID: 11126); MS4A1 (CD20; NCBI Gene ID: 931); CD244 (SLAMF4; NCBI Gene ID: 51744); CD276 (B7H3; NCBI Gene ID: 80381); V-set domain containing T cell activation inhibitor 1 (VTCN1, B7H4; NCBI Gene ID: 79679); V-set immunoregulatory receptor (VSIR, B7H5, VISTA; NCBI Gene ID: 64115); immunoglobulin superfamily member 11 (IGSF11, VSIG3; NCBI Gene ID: 152404); natural killer cell cytotoxicity receptor 3 ligand 1 (NCR3LG1, B7H6; NCBI Gene ID: 374383); HERV-H LTR-associating 2 (HHLA2, B7H7; NCBI Gene ID: 11148); inducible T cell co-stimulator (ICOS, CD278; NCBI Gene ID: 29851); inducible T cell co-stimulator ligand (ICOSLG, B7H2; NCBI Gene ID: 23308); TNF receptor superfamily member 4 (TNFRSF4, OX40; NCBI Gene ID: 7293); TNF superfamily member 4 (TNFSF4, OX40L; NCBI Gene ID: 7292); TNFRSF8 (CD30; NCBI Gene ID: 943); TNFSF8 (CD30L; NCBI Gene ID: 944); TNFRSF10A (CD261,

DR4, TRAILR1; NCBI Gene ID: 8797); TNFRSF9 (CD137; NCBI Gene ID: 3604); TNFSF9 (CD137L; NCBI Gene ID: 8744); TNFRSF10B (CD262, DR5, TRAILR2; NCBI Gene ID: 8795); TNFRSF10 (TRAIL; NCBI Gene ID: 8743); TNFRSF14 (HVEM, CD270; NCBI Gene ID: 8764); TNFSF14 (HVEM; NCBI Gene ID: 8740); CD272 (B and T lymphocyte associated (BTLA); NCBI Gene ID: 151888); TNFRSF17 (BCMA, CD269; NCBI Gene ID: 608); TNFSF13B (BAFF; NCBI Gene ID: 10673); TNFRSF18 (GITR; NCBI Gene ID: 8784); TNFSF18 (GITRL; NCBI Gene ID: 8995); MHC class I polypeptide-related sequence A (MICA; NCBI Gene ID: 100507436); MHC class I polypeptide-related sequence B (MICB; NCBI Gene ID: 4277); CD274 (CD274, PDL1, PD-L1; NCBI Gene ID: 29126); programmed cell death 1 (PDCD1, PD1, PD-1; NCBI Gene ID: 5133); cytotoxic T-lymphocyte associated protein 4 (CTLA4, CD152; NCBI Gene ID: 1493); CD80 (B7-1; NCBI Gene ID: 941); CD28 (NCBI Gene ID: 940); nectin cell adhesion molecule 2 (NECTIN2, CD112; NCBI Gene ID: 5819); CD226 (DNAM-1; NCBI Gene ID: 10666); Poliovirus receptor (PVR) cell adhesion molecule (PVR, CD155; NCBI Gene ID: 5817); PVR related immunoglobulin domain containing (PVRIG, CD112R; NCBI Gene ID: 79037); T cell immunoreceptor with Ig and ITIM domains (TIGIT; NCBI Gene ID: 201633); T cell immunoglobulin and mucin domain containing 4 (TIMD4; TIM4; NCBI Gene ID: 91937); hepatitis A virus cellular receptor 2 (HAVCR2, TIMD3, TIM3; NCBI Gene ID: 84868); galectin 9 (LGALS9; NCBI Gene ID: 3965); lymphocyte activating 3 (LAG3, CD223; NCBI Gene ID: 3902); signaling lymphocytic activation molecule family member 1 (SLAMF1, SLAM, CD150; NCBI Gene ID: 6504); lymphocyte antigen 9 (LY9, CD229, SLAMF3; NCBI Gene ID: 4063); SLAM family member 6 (SLAMF6, CD352; NCBI Gene ID: 114836); SLAM family member 7 (SLAMF7, CD319; NCBI Gene ID: 57823); UL16 binding protein 1 (ULBP1; NCBI Gene ID: 80329); UL16 binding protein 2 (ULBP2; NCBI Gene ID: 80328); UL16 binding protein 3 (ULBP3; NCBI Gene ID: 79465); retinoic acid early transcript 1E (RAET1E; ULBP4; NCBI Gene ID: 135250); retinoic acid early transcript 1G (RAET1G; ULBP5; NCBI Gene ID: 353091); retinoic acid early transcript 1L (RAET1L; ULBP6; NCBI Gene ID: 154064); killer cell lectin like receptor C1 (KLRC1, NKG2A, CD159A; NCBI Gene ID: 3821); killer cell lectin like receptor K1 (KLRK1, NKG2D, CD314; NCBI Gene ID: 22914); killer cell lectin like receptor C2 (KLRC2, CD159c, NKG2C; NCBI Gene ID: 3822); killer cell lectin like receptor C3 (KLRC3, NKG2E; NCBI Gene ID: 3823); killer cell lectin like receptor C4 (KLRC4, NKG2F; NCBI Gene ID: 8302); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 1 (KIR2DL1; NCBI Gene ID: 3802); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 2 (KIR2DL2; NCBI Gene ID: 3803); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 3 (KIR2DL3; NCBI Gene ID: 3804); killer

cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 1 (KIR3DL1, KIR, CD158E1; NCBI Gene ID: 3811) (*e.g.*, Lirilumab (IPH2102/BMS-986015), IPH-4102); and killer cell lectin like receptor D1 (KLRD1; NCBI Gene ID: 3824).

[0247] In some embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more blockers or inhibitors of one or more T-cell inhibitory immune checkpoint proteins or receptors. Illustrative T-cell inhibitory immune checkpoint proteins or receptors include without limitation CD274 (CD274, PDL1, PD-L1); programmed cell death 1 ligand 2 (PDCD1LG2, PD-L2, CD273); programmed cell death 1 (PDCD1, PD1, PD-1); cytotoxic T-lymphocyte associated protein 4 (CTLA4, CD152); CD276 (B7H3); V-set domain containing T cell activation inhibitor 1 (VTCN1, B7H4); V-set immunoregulatory receptor (VSIR, B7H5, VISTA); immunoglobulin superfamily member 11 (IGSF11, VSIG3); TNFRSF14 (HVEM, CD270), TNFSF14 (HVEML); CD272 (B and T lymphocyte associated (BTLA)); PVR related immunoglobulin domain containing (PVRIG, CD112R); T cell immunoreceptor with Ig and ITIM domains (TIGIT); lymphocyte activating 3 (LAG3, CD223); hepatitis A virus cellular receptor 2 (HAVCR2, TIMD3, TIM3); galectin 9 (LGALS9); killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 1 (KIR, CD158E1); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 1 (KIR2DL1); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 2 (KIR2DL2); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 3 (KIR2DL3); and killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 1 (KIR3DL1). In various embodiments, the agents, as described herein, are combined with one or more agonist or activators of one or more T-cell stimulatory immune checkpoint proteins or receptors. Illustrative T-cell stimulatory immune checkpoint proteins or receptors include without limitation CD27, CD70; CD40, CD40LG; inducible T cell co-stimulator (ICOS, CD278); inducible T cell co-stimulator ligand (ICOSLG, B7H2); TNF receptor superfamily member 4 (TNFRSF4, OX40); TNF superfamily member 4 (TNFSF4, OX40L); TNFRSF9 (CD137), TNFSF9 (CD137L); TNFRSF18 (GITR), TNFSF18 (GITRL); CD80 (B7-1), CD28; nectin cell adhesion molecule 2 (NECTIN2, CD112); CD226 (DNAM-1); CD244 (2B4, SLAMF4), Poliovirus receptor (PVR) cell adhesion molecule (PVR, CD155). See, *e.g.*, Xu, et al., J Exp Clin Cancer Res. (2018) 37:110.

[0248] In some embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides

or polynucleotides, as described herein, are combined or co-administered with one or more blockers or inhibitors of one or more NK-cell inhibitory immune checkpoint proteins or receptors. Illustrative NK-cell inhibitory immune checkpoint proteins or receptors include without limitation killer cell immunoglobulin like receptor, three Ig domains and long

cytoplasmic tail 1 (KIR, CD158E1); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 1 (KIR2DL1); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 2 (KIR2DL2); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 3 (KIR2DL3); killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 1 (KIR3DL1); killer cell lectin like receptor C1 (KLRC1, NKG2A, CD159A); and killer cell lectin like receptor D1 (KLRD1, CD94).

[0249] In some embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more agonists or activators of one or more NK-cell stimulatory immune checkpoint proteins or receptors. Illustrative NK-cell stimulatory immune checkpoint proteins or receptors include without limitation CD16, CD226 (DNAM-1); CD244 (2B4, SLAMF4); killer cell lectin like receptor K1 (KLRK1, NKG2D, CD314); SLAM family member 7 (SLAMF7). See, *e.g.*, Davis, et al., *Semin Immunol.* (2017) 31:64–75; Fang, et al., *Semin Immunol.* (2017) 31:37-54; and Chiossone, et al., *Nat Rev Immunol.* (2018) 18(11):671-688.

Inhibitors of Cytotoxic T-Lymphocyte-Associated Protein 4 (CTLA4)

[0250] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more Inhibitors of cytotoxic T-lymphocyte-associated protein 4 (CTLA4) (CD152; NCBI Gene ID: 1493). Examples of inhibitors of CTLA4 that can be co-administered include without limitation ipilimumab, tremelimumab, BMS-986218, AGEN1181, AGEN1884, AGEN2041, BMS-986249, MK-1308, REGN-4659, ADU-1604, CS-1002, BCD-145, APL-509, JS-007, BA-3071, ONC-392, JHL-1155, KN-044, CG-0161, ATOR-1144, PBI-5D3H5, BPI-002, belatacept, PSI-001, PRS-010, JHL-1155, as well as multi-specific inhibitors FPT-155 (CTLA4/PD-L1/CD28), PF-06936308 (PD-1/ CTLA4), MGD-019 (PD-1/CTLA4), KN-046 (PD-1/CTLA4), MEDI-5752 (CTLA4/PD-1), XmAb-20717 (PD-1/CTLA4), and AK-104 (CTLA4/PD-1).

Inhibitors of PD-L1 (CD274) or PD-1 (PDCD1; CD279)

[0251] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more inhibitors of programmed cell death 1 ligand 1 (PD-L1; CD274; NCBI Gene ID: 29126) or programmed cell death 1 (PD-1; PDCD1; CD279; NCBI Gene ID: 5133). Examples of inhibitors of PD-L1 (CD274) or PD-1 (PDCD1) that can be combined or co-administered include without limitation zimberelimab (AB122), pembrolizumab, nivolumab, cemiplimab, pidilizumab, AMP-224, MEDI0680 (AMP-514), spartalizumab, atezolizumab, avelumab (MSB0010718C), ASC22, durvalumab, ALN-PDL, BMS-936559, CK-301, PF-06801591, BGB-108, BGB-A317 (tislelizumab), GLS-010 (WBP-3055), AK-103 (HX-008), GB-226, AK-105, CS-1003, HLX-10, MGA-012, BI-754091, PDR-001, AGEN-2034, JS-001 (toripalimab), JNJ-63723283, genolimzumab (CBT-501), LZM-009, BCD-100, LY-3300054, SHR-1201, SHR-1210 (camrelizumab), Sym-021, ABBV-181, PD1-PIK, BAT-1306, RO-6084 (PD-L1 antisense oligonucleotide), STI-1110, GX-P2, RG-7446, mDX-400, CX-072, CBT-502, TSR-042 (dostarlimab), MSB-2311, JTX-4014, BGB-A333, SHR-1316, CS-1001 (WBP-3155), MEDI-0680, envafolimab (KN-035), KD-033, KY-1003, IBI-308 (sintilimab), HLX-20, KL-A167, STI-A1014, STI-A1015 (IMC-001), BCD-135, FAZ-053, TQB-2450, MDX1105-01, MSB-0010718C, GS-4224, GS-4416, INCB086550, MAX10181, as well as multi-specific inhibitors FPT-155 (CTLA4/PD-L1/CD28), PF-06936308 (PD-1/CTLA4), MGD-013 (PD-1/LAG-3), FS-118 (LAG-3/PD-L1) MGD-019 (PD-1/CTLA4), KN-046 (PD-1/CTLA4), MEDI-5752 (CTLA4/PD-1), RO-7121661 (PD-1/TIM-3), XmAb-20717 (PD-1/CTLA4), AK-104 (CTLA4/PD-1), M7824 (PD-L1/TGF β -EC domain), CA-170 (PD-L1/VISTA), CDX-527 (CD27/PD-L1), LY-3415244 (TIM3/PDL1), GNS-1480 (Epidermal growth factor receptor antagonist; Programmed cell death ligand 1 inhibitor), M-7824 (PD-L1/TGF- β bifunctional fusion protein), and INBRX-105 (4-1BB/PDL1).

[0252] Examples of PD-1 inhibitors that can be combined or co-administered further include the compounds disclosed in WO2017112730 (Incyte Corp), WO2017087777 (Incyte Corp), WO2017017624, WO2014151634 (BristolMyers Squibb Co), WO201317322 (BristolMyers Squibb Co), WO2018119286 (Incyte Corp), WO2018119266 (Incyte Corp), WO2018119263 (Incyte Corp), WO2018119236 (Incyte Corp), WO2018119221 (Incyte Corp), WO2018118848 (BristolMyers Squibb Co), WO20161266460 (BristolMyers Squibb Co), WO2017087678 (BristolMyers Squibb Co), WO2016149351 (BristolMyers Squibb Co), WO2015033299 (Aurigene Discovery Technologies Ltd), WO2015179615 (Eisai Co Ltd; Eisai Research Institute), WO2017066227 (BristolMyers Squibb Co), WO2016142886 (Aurigene

Discovery Technologies Ltd), WO2016142852(Aurigene Discovery Technologies Ltd),
WO2016142835 (Aurigene Discovery Technologies Ltd; Individual), WO2016142833
(Aurigene Discovery Technologies Ltd), WO2018085750 (BristolMyers Squibb Co),
WO2015033303 (Aurigene Discovery Technologies Ltd), WO2017205464 (Incyte Corp),
5 WO2016019232 (3M Co; Individual; Texas A&M University System), WO2015160641
(BristolMyers Squibb Co), WO2017079669 (Incyte Corp), WO2015033301 (Aurigene
Discovery Technologies Ltd), WO2015034820 (BristolMyers Squibb Co), WO2018073754
(Aurigene Discovery Technologies Ltd), WO2016077518 (BristolMyers Squibb Co),
WO2016057624 (BristolMyers Squibb Co), WO2018044783 (Incyte Corp), WO2016100608
10 (BristolMyers Squibb Co), WO2016100285 (BristolMyers Squibb Co), WO2016039749
(BristolMyers Squibb Co), WO2015019284 (Cambridge Enterprise Ltd), WO2016142894
(Aurigene Discovery Technologies Ltd), WO2015134605 (BristolMyers Squibb Co),
WO2018051255 (Aurigene Discovery Technologies Ltd), WO2018051254 (Aurigene Discovery
Technologies Ltd), WO2017222976 (Incyte Corp), WO2017070089 (Incyte Corp),
15 WO2018044963 (BristolMyers Squibb Co), WO2013144704 (Aurigene Discovery
Technologies Ltd), WO2018013789 (Incyte Corp), WO2017176608 (BristolMyers Squibb Co),
WO2018009505 (BristolMyers Squibb Co), WO2011161699 (Aurigene Discovery
Technologies Ltd), WO2015119944 (Incyte Corp; Merck Sharp & Dohme Corp),
WO2017192961 (Incyte Corp), WO2017106634 (Incyte Corp), WO2013132317 (Aurigene
20 Discovery Technologies Ltd), WO2012168944 (Aurigene Discovery Technologies Ltd),
WO2015036927 (Aurigene Discovery Technologies Ltd), WO2015044900 (Aurigene Discovery
Technologies Ltd), WO2018026971 (Arising International).

[0253] In various embodiments, the immunogenic polypeptides, polynucleotides
encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such
25 polypeptides or polynucleotides, as described herein, are combined or co-administered with one
or more proteinaceous (*e.g.*, antibody or fragment thereof, or antibody mimetic) inhibitors of
PD-L1 (CD274), PD-1 (PDCD1) or CTLA4. In some embodiments, the one or more immune
checkpoint inhibitors comprises a small organic molecule inhibitor of PD-L1 (CD274), PD-1
(PDCD1) or CTLA4. In some embodiments, the small molecule inhibitor of CD274 or PDCD1
30 is selected from the group consisting of GS-4224, GS-4416, INCB086550 and MAX10181.
Additional examples of small molecule PD-L1 inhibitors include those disclosed in U.S.
Publication No. US2018305315 (Gilead Sciences), US2020017471 (Gilead Sciences) and
US2019270727 (Gilead Sciences). In some embodiments, the small molecule inhibitor of
CTLA4 comprises BPI-002.

Inhibitors of T cell immunoreceptor with Ig and ITIM domains (TIGIT)

[0254] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more inhibitors of T cell immunoreceptor with Ig and ITIM domains (TIGIT) (NCBI Gene ID: 201633). Example anti-TIGIT antibodies, that can be combined or co-administered include etigilimab, BMS-986207, tiragolumab (*a.k.a.*, MTIG-7192A; RG-6058; RO 7092284), AGEN1307, AGEN1327, AGEN1777, COM-902, IBI-939, AB154, MG1131 and EOS884448 (EOS-448).

10 TNF Receptor Superfamily (TNFRSF) Member Agonists or Activators

[0255] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more agonists of one or more TNF receptor superfamily (TNFRSF) members, *e.g.*, an agonist of one or more of TNFRSF1A (NCBI Gene ID: 7132), TNFRSF1B (NCBI Gene ID: 7133), TNFRSF4 (OX40, CD134; NCBI Gene ID: 7293), TNFRSF5 (CD40; NCBI Gene ID: 958), TNFRSF6 (FAS, NCBI Gene ID: 355), TNFRSF7 (CD27, NCBI Gene ID: 939), TNFRSF8 (CD30, NCBI Gene ID: 943), TNFRSF9 (4-1BB, CD137, NCBI Gene ID: 3604), TNFRSF10A (CD261, DR4, TRAILR1, NCBI Gene ID: 8797), TNFRSF10B (CD262, DR5, TRAILR2, NCBI Gene ID: 8795), TNFRSF10C (CD263, TRAILR3, NCBI Gene ID: 8794), TNFRSF10D (CD264, TRAILR4, NCBI Gene ID: 8793), TNFRSF11A (CD265, RANK, NCBI Gene ID: 8792), TNFRSF11B (NCBI Gene ID: 4982), TNFRSF12A (CD266, NCBI Gene ID: 51330), TNFRSF13B (CD267, NCBI Gene ID: 23495), TNFRSF13C (CD268, NCBI Gene ID: 115650), TNFRSF16 (NGFR, CD271, NCBI Gene ID: 4804), TNFRSF17 (BCMA, CD269, NCBI Gene ID: 608), TNFRSF18 (GITR, CD357, NCBI Gene ID: 8784), TNFRSF19 (NCBI Gene ID: 55504), TNFRSF21 (CD358, DR6, NCBI Gene ID: 27242), and TNFRSF25 (DR3, NCBI Gene ID: 8718).

[0256] Example anti-TNFRSF4 (OX40) antibodies that can be combined or co-administered include without limitation, MEDI6469, MEDI6383, MEDI0562 (tavolixizumab), MOXR0916, PF-04518600, RG-7888, GSK-3174998, INCAGN1949, BMS-986178, GBR-8383, ABBV-368, and those described in WO2016179517, WO2017096179, WO2017096182, WO2017096281, and WO2018089628.

[0257] Example anti-TNFRSF5 (CD40) antibodies that can be combined or co-administered include without limitation RG7876, SEA-CD40, APX-005M and ABBV-428.

[0258] In some embodiments, the anti-TNFRSF7 (CD27) antibody varlilumab (CDX-1127) is combined or co-administered.

5 [0259] Example anti-TNFRSF9 (4-1BB, CD137) antibodies that can be combined or co-administered include without limitation urelumab, utomilumab (PF-05082566), AGEN-2373 and ADG-106.

[0260] Example anti-TNFRSF18 (GITR) antibodies that can be combined or co-administered include without limitation, MEDI1873, FPA-154, INCAGN-1876, TRX-518,
10 BMS-986156, MK-1248, GWN-323, and those described in WO2017096179, WO2017096276, WO2017096189, and WO2018089628. In some embodiments, an antibody, or fragment thereof, co-targeting TNFRSF4 (OX40) and TNFRSF18 (GITR) is co-administered. Such antibodies are described, *e.g.*, in WO2017096179 and WO2018089628.

Indoleamine-pyrrole-2,3-dioxygenase (IDO1) inhibitors

15 [0261] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with one or more inhibitors of indoleamine 2,3-dioxygenase 1 (IDO1; NCBI Gene ID: 3620). Examples
20 of IDO1 inhibitors that can be combined or co-administered include without limitation, BLV-0801, epacadostat, resminostat, F-001287, GBV-1012, GBV-1028, GDC-0919, indoximod, NKTR-218, NLG-919-based vaccine, PF-06840003, pyranonaphthoquinone derivatives (SN-35837), SBLK-200802, BMS-986205, and shIDO-ST, EOS-200271, KHK-2455, LY-3381916, and the compounds disclosed in US20100015178 (Incyte), US2016137652 (Flexus Biosciences, Inc.), WO2014073738 (Flexus Biosciences, Inc.), and WO2015188085 (Flexus Biosciences,
25 Inc.).

LAG-3 and TIM-3 inhibitors

[0262] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with an
30 anti-TIM-3 antibody, such as TSR-022, LY-3321367, MBG-453, INCAGN-2390. In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with an anti-LAG-3 (Lymphocyte-

activation) antibody, such as relatlimab (ONO-4482), LAG-525, MK-4280, REGN-3767, INCAGN2385.

Inhibitors of apoptosis proteins family proteins (IAPs)

[0263] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with an inhibitor of apoptosis proteins family protein (IAP). Examples of IAP inhibitors include APG-1387.

Bruton's Tyrosine Kinase (BTK) Inhibitors

[0264] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with an inhibitor of Bruton tyrosine kinase (BTK, AGMX1, AT, ATK, BPK, IGHD3, IMD1, PSCTK1, XLA; NCBI Gene ID: 695). Examples of BTK inhibitors include without limitation, (S)-6-amino-9-(1-(but-2-ynoyl)pyrrolidin-3-yl)-7-(4-phenoxyphenyl)-7H-purin-8(9H)-one, ABBV-105, acalabrutinib (ACP-196), AC-058, AC-0025, ARQ-531, BMS-986142, dasatinib, ibrutinib (PCI-32765, CRA-032765), GDC-0853, PRN-1008, SNS-062, BGB-3111, CB988, HM71224, KBP-7536, M-2951 (evobrutinib), M7583, tirabrutinib (ONO-4059), ML-319, MSC-2364447, PRN-1008, RDX-022, RG-7845, spebrutinib (CC-292), TAK-020, TAS-5315, TP-0158, TP-4207, vecabrutinib (SNS-062), ARQ-531, SHR-1459, DTRMWXHS-12, and the compounds disclosed in US20140330015 (Ono Pharmaceutical), US20130079327 (Ono Pharmaceutical), and US20130217880 (Ono Pharmaceutical).

Lysine Demethylase (KDM) Inhibitors

[0265] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with an inhibitor of a lysine demethylase (KDM). Examples of KDM5 inhibitors that can be combined or co-administered include the compounds disclosed in WO2016057924 (Genentech/Constellation Pharmaceuticals), US20140275092 (Genentech/Constellation Pharmaceuticals), US20140371195 (Epitherapeutics), US20140371214 (Epitherapeutics), US20160102096 (Epitherapeutics), US20140194469 (Quanticel), US20140171432, US20140213591 (Quanticel), US20160039808 (Quanticel), US20140275084 (Quanticel), and WO2014164708 (Quanticel).

[0266] Examples of KDM1 inhibitors that can be combined or co-administered include the compounds disclosed in US9186337B2 (Oryzon Genomics), GSK-2879552, RG-6016, and ORY-2001.

Arginase inhibitors

- 5 **[0267]** In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with an arginase inhibitor. Examples of Arginase inhibitors include CB-1158, C-201, and resminostat.

Bi- and Tri-Specific Natural Killer (NK)-Cell Engagers

- 10 **[0268]** In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with a bi-specific NK-cell engager (BiKE) or a tri-specific NK-cell engager (TriKE) (*e.g.*, not having an Fc) or bi-specific antibody (*e.g.*, having an Fc) against an NK cell activating receptor, *e.g.*,
15 CD16A, C-type lectin receptors (CD94/NKG2C, NKG2D, NKG2E/H and NKG2F), natural cytotoxicity receptors (NKp30, NKp44 and NKp46), killer cell C-type lectin-like receptor (NKp65, NKp80), Fc receptor FcγR (which mediates antibody-dependent cell cytotoxicity), SLAM family receptors (*e.g.*, 2B4, SLAMF6 and SLAMF7), killer cell immunoglobulin-like receptors (KIR) (KIR-2DS and KIR-3DS), DNAM-1 and CD137 (41BB). As appropriate, the
20 anti-CD16 binding bi-specific molecules may or may not have an Fc. Illustrative bi-specific NK-cell engagers that can be co-administered target CD16 and one or more HBV-associated antigens as described herein. BiKEs and TriKEs are described, *e.g.*, in Felices, et al., *Methods Mol Biol.* (2016) 1441:333–346; Fang, et al., *Semin Immunol.* (2017) 31:37-54.

Long Acting Treatments

- 25 **[0269]** In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with a long acting treatment. Long acting entecavir (subcutaneous depot), long acting tenofovir (TFD and TAF) implants (devices) or subcutaneous depot. An example of long acting entecavir is
30 described in Henry, *et al.*, *Eur J Pharm Sci.* (2019) 136:104958.

Gene Therapy and Cell Therapy

[0270] In various embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with a gene or cell therapy regimen. Gene therapy and cell therapy include without limitation the genetic modification to silence a gene; genetic approaches to directly kill the infected cells; the infusion of immune cells designed to replace most of the patient's own immune system to enhance the immune response to infected cells, or activate the patient's own immune system to kill infected cells, or find and kill the infected cells; genetic approaches to modify cellular activity to further alter endogenous immune responsiveness against the infection.

10 Gene Editors

[0271] The genome editing system can be selected from the group consisting of: a CRISPR/Cas9 system, a zinc finger nuclease system, a TALEN system, a homing endonucleases system, and a meganuclease system (*e.g.*, an ARCUS system); *e.g.*, cccDNA elimination via targeted cleavage, and altering one or more of the hepatitis B virus (HBV) viral genes. Altering (*e.g.*, knocking out and/or knocking down) the *PreC*, *C*, *X*, *PreS1*, *PreS2*, *S*, *P* or *SP* gene refers to (1) reducing or eliminating *PreC*, *C*, *X*, *PreS1*, *PreS2*, *S*, *P* or *SP* gene expression, (2) interfering with Precore, Core, X protein, Long surface protein, middle surface protein, S protein (also known as HBs antigen and HBsAg), polymerase protein, and/or Hepatitis B spliced protein function (HBe, HBc, HBx, PreS1, PreS2, S, Pol, and/or HBSP or (3) reducing or eliminating the intracellular, serum and/or intraparenchymal levels of HBe, HBc, HBx, LHBs, MHBs, SHBs, Pol, and/or HBSP proteins. Knockdown of one or more of the *PreC*, *C*, *X*, *PreS1*, *PreS2*, *S*, *P* and/or *SP* gene(s) is performed by targeting the gene(s) within HBV cccDNA and/or integrated HBV DNA. Additional examples genome editing systems include, but are not limited to those disclosed in US2019284543 (Gilead Sciences), and US2019338263 (Gilead Sciences).

[0272] Examples of gene therapy, such as liver targeted anti-HBV gene therapy (using ARCUS technology), or using CRISPR/Cas9 gene editing technology, or EBT-106 (LNP-delivered CRISPR/CasX nuclease).

CAR-T cell therapy

[0273] CAR-T cell therapy includes a population of immune effector cells engineered to express a chimeric antigen receptor (CAR), wherein the CAR includes an HBV antigen-binding domain. In certain embodiments, the antigen-binding domain is a domain disclosed herein. In 5 certain embodiments, the antigen-binding domain is other than a domain disclosed herein. In certain embodiments, the antigen is HBsAg (i.e. HbsAg- CART). The immune effector cell is a T-cell or an NK cell. In certain embodiments, the T-cell is a CD4+ T-cell, a CD8+ T-cell, a NK cell or a combination thereof. Cells can be autologous or allogeneic. An example of a CART directed to HBV is described in Kruse, *et al.*, *Cytotherapy*. (2018) 20(5):697-705.

10 TCR-T cell therapy

[0274] TCR-T cell therapy includes T cells expressing HBV-specific T cell receptors. TCR-T cells are engineered to target HBV derived peptides presented on the surface of virus-infected cells. An example of a TCR directed to HBV is described in Wisskirchen, *et al.*, *J Clin Invest*. (2019) 129(7):2932-2945.

15 [0275] TCR-T cell therapy includes T-Cells expressing HBV surface antigen (HBsAg)-specific TCR, such as IMC-I109V.

[0276] TCR-T cell therapy includes TCR-T therapy directed to treatment of HBV, such as LTCR-H2-1.

[0277] In another specific embodiment, the immunogenic polypeptides, polynucleotides 20 encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with an HBV DNA polymerase inhibitor, one or two additional therapeutic agents selected from the group consisting of immunomodulators, TLR modulators, HBsAg inhibitors, HBsAg secretion or assembly inhibitors, HBV therapeutic vaccines, HBV antibodies including HBV antibodies 25 targeting the surface antigens of the hepatitis B virus and bispecific antibodies and “antibody-like” therapeutic proteins (such as DARTs®, DUOBODIES®, BITES®, XmAbs®, TandAbs®, Fab derivatives, or TCR-like antibodies), cyclophilin inhibitors, stimulators of retinoic acid-inducible gene 1, stimulators of RIG-I like receptors, PD-1 inhibitors, PD-L1 inhibitors, Arginase inhibitors, PI3K inhibitors, IDO inhibitors, and stimulators of NOD2, and one or two 30 additional therapeutic agents selected from the group consisting of HBV viral entry inhibitors, NTCP inhibitors, HBx inhibitors, cccDNA inhibitors, HBV antibodies targeting the surface antigens of the hepatitis B virus, siRNA, miRNA gene therapy agents, sshRNAs, KDM5 inhibitors, and nucleoprotein modulators (HBV core or capsid protein modulators).

[0278] In another specific embodiment, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with at least a second additional therapeutic agent selected from the group consisting of: HBV DNA polymerase inhibitors, immunomodulator, TLR modulators, HBsAg inhibitors, HBV therapeutic vaccines, HBV antibodies including HBV antibodies targeting the surface antigens of the hepatitis B virus and bispecific antibodies and “antibody-like” therapeutic proteins (such as DARPs®, anti-pMHC TCR-like antibodies, DARTs®, DUOBODIES®, BITES®, XmAbs®, TandAbs®, Fab derivatives, or TCR-like antibodies), cyclophilin inhibitors, stimulators of retinoic acid-inducible gene 1, stimulators of RIG-I like receptors, PD-1 inhibitors, PD-L1 inhibitors, Arginase inhibitors, PI3K inhibitors, IDO inhibitors, and stimulators of NOD2.

[0279] In another specific embodiment, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with at least a second additional therapeutic agent selected from the group consisting of: HBV DNA polymerase inhibitors, HBV viral entry inhibitors, NTCP inhibitors, HBx inhibitors, cccDNA inhibitors, HBV antibodies targeting the surface antigens of the hepatitis B virus, siRNA, miRNA gene therapy agents, sshRNAs, KDM5 inhibitors, and nucleoprotein modulators (HBV core or capsid protein inhibitors).

[0280] In a particular embodiment the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with compounds such as those disclosed in U.S. Publication No. 2010/0143301 (Gilead Sciences), U.S. Publication No. 2011/0098248 (Gilead Sciences), U.S. Publication No. 2009/0047249 (Gilead Sciences), U.S. Patent No. 8722054 (Gilead Sciences), U.S. Publication No. 2014/0045849 (Janssen), U.S. Publication No. 2014/0073642 (Janssen), WO2014/056953 (Janssen), WO2014/076221 (Janssen), WO2014/128189 (Janssen), U.S. Publication No. 2014/0350031 (Janssen), WO2014/023813 (Janssen), U.S. Publication No. 2008/0234251 (Array Biopharma), U.S. Publication No. 2008/0306050 (Array Biopharma), U.S. Publication No. 2010/0029585 (Ventirx Pharma), U.S. Publication No. 2011/0092485 (Ventirx Pharma), US2011/0118235 (Ventirx Pharma), U.S. Publication No. 2012/0082658 (Ventirx Pharma), U.S. Publication No. 2012/0219615 (Ventirx Pharma), U.S. Publication No. 2014/0066432 (Ventirx Pharma), U.S. Publication No. 2014/0088085 (Ventirx Pharma), U.S. Publication No. 2014/0275167 (Novira Therapeutics), U.S. Publication No. 2013/0251673 (Novira

Therapeutics), U.S. Patent No. 8513184 (Gilead Sciences), U.S. Publication No. 2014/0030221 (Gilead Sciences), U.S. Publication No. 2013/0344030 (Gilead Sciences), U.S. Publication No. 2013/0344029 (Gilead Sciences), US20140275167 (Novira Therapeutics), US20130251673 (Novira Therapeutics), U.S. Publication No. 2014/0343032 (Roche), WO2014037480 (Roche),
5 U.S. Publication No. 2013/0267517 (Roche), WO2014131847 (Janssen), WO2014033176 (Janssen), WO2014033170 (Janssen), WO2014033167 (Janssen), WO2015/059212 (Janssen), WO2015118057 (Janssen), WO2015011281 (Janssen), WO2014184365 (Janssen), WO2014184350 (Janssen), WO2014161888 (Janssen), WO2013096744 (Novira), US20150225355 (Novira), US20140178337 (Novira), US20150315159 (Novira),
10 US20150197533 (Novira), US20150274652 (Novira), US20150259324, (Novira), US20150132258 (Novira), US9181288 (Novira), WO2014184350 (Janssen), WO2013144129 (Roche), US20100015178 (Incyte), US2016137652 (Flexus Biosciences, Inc.), WO2014073738 (Flexus Biosciences, Inc.), WO2015188085 (Flexus Biosciences, Inc.), U.S. Publication No. 2014/0330015 (Ono Pharmaceutical), U.S. Publication No. 2013/0079327 (Ono
15 Pharmaceutical), U.S. Publication No. 2013/0217880 (Ono pharmaceutical), WO2016057924 (Genentech/Constellation Pharmaceuticals), US20140275092 (Genentech/Constellation Pharmaceuticals), US20140371195 (Epitherapeutics) and US20140371214 (Epitherapeutics). , US20160102096 (Epitherapeutics), US20140194469 (Quantice), US20140171432, US20140213591 (Quantice), US20160039808 (Quantice), US20140275084 (Quantice),
20 WO2014164708 (Quantice), US9186337B2 (Oryzon Genomics), and other drugs for treating HBV, and combinations thereof.

[0281] In certain embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with 5-30
25 mg tenofovir alafenamide fumarate, tenofovir alafenamide hemifumarate, or tenofovir alafenamide. In certain embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with 5-10; 5-15; 5-20; 5-25; 25-30; 20-30; 15-30; or 10-30 mg tenofovir alafenamide fumarate, tenofovir alafenamide
30 hemifumarate, or tenofovir alafenamide. In certain embodiments, the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with 10 mg tenofovir alafenamide fumarate, tenofovir alafenamide hemifumarate, or tenofovir alafenamide. In certain embodiments, the immunogenic

polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with 25 mg tenofovir alafenamide fumarate, tenofovir alafenamide hemifumarate, or tenofovir alafenamide. An agent as disclosed herein may be combined with the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, in any dosage amount of the compound (*e.g.*, from 50 mg to 500 mg of compound) the same as if each combination of dosages were specifically and individually listed.

[0282] In certain embodiments, the immunogenic polypeptides, polynucleotides

encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, are combined or co-administered with 100-400 mg tenofovir disoproxil fumarate, tenofovir disoproxil hemifumarate, or tenofovir disoproxil. In certain embodiments, an agent disclosed herein, or a pharmaceutically acceptable salt thereof, is combined with 100-150; 100-200, 100-250; 100-300; 100-350; 150-200; 150-250; 150-300; 150-350; 150-400; 200-250; 200-300; 200-350; 200-400; 250-350; 250-400; 350-400 or 300-400 mg tenofovir disoproxil fumarate, tenofovir disoproxil hemifumarate, or tenofovir disoproxil. In certain embodiments, an agent disclosed herein, or a pharmaceutically acceptable salt thereof, is combined with 300 mg tenofovir disoproxil fumarate, tenofovir disoproxil hemifumarate, or tenofovir disoproxil. In certain embodiments, an agent disclosed herein, or a pharmaceutically acceptable salt thereof, is combined with 250 mg tenofovir disoproxil fumarate, tenofovir disoproxil hemifumarate, or tenofovir disoproxil. In certain embodiments, an agent herein, or a pharmaceutically acceptable salt thereof, is combined with 150 mg tenofovir disoproxil fumarate, tenofovir disoproxil hemifumarate, or tenofovir disoproxil. An agent as disclosed herein may be combined with the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, in any dosage amount of the compound (*e.g.*, from 50 mg to 500 mg of compound) the same as if each combination of dosages were specifically and individually listed.

8. Kits

[0283] Further provided is a kit comprising one or more unitary doses of one or more of the truncated HBV polymerase polypeptide, one or more of the HBV polymerase deletion mutant polypeptide, one or more of the core-sAg fusion protein, one or more polynucleotides, one or more vectors, or one or more immunogenic compositions, as described herein. In some embodiments, the kit comprises one or more unitary doses of two or more of the truncated HBV polymerase polypeptide, the HBV polymerase deletion mutant polypeptide, the core-sAg fusion protein, the polynucleotides, the vectors, or the immunogenic compositions, described herein.

[0284] In various embodiments, as appropriate or desired, the one or more unitary doses can be in a single container or in two or more separate containers. In various embodiments, the one or more containers can be selected from the group consisting of vials, ampules and pre-loaded syringes.

[0285] In some embodiments, the one or more containers comprise the one or more polypeptides, one or more polynucleotides, one or more vectors or one or more immunogenic compositions in an aqueous solution. In some embodiments, the one or more containers comprise the one or more polypeptides, one or more polynucleotides, one or more vectors or one or more immunogenic compositions as a lyophilized preparation.

[0286] As appropriate or desired, the one or more unitary doses can be the same or different. In some embodiments, the kit comprises one or more unitary doses of one or more viral vectors capable of expressing the immunogenic polypeptides. In kits comprising viral vectors, the unitary doses can be in the range of about 10^3 to about 10^{12} viral focus forming units (FFU) or plaque forming units (PFU) or infectious units (IU) or viral particles (vp), *e.g.* from about 10^4 to about 10^7 viral FFU or PFU, *e.g.* from about 10^3 to about 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} or 10^{12} viral FFU or PFU or IU or vp.

[0287] In various embodiments, the kit comprises one or more polynucleotides encoding, or one or more vectors capable of expressing, or an immunogenic composition comprising, two immunogenic polypeptides, the immunogenic polypeptides comprising: (a) an HBV polymerase polypeptide mutant comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 5-14, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 5-14; and (b) an HBV core-sAg fusion protein comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 38-41, or a sequence that is at least 80%, 81%,

82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 38-41.

[0288] In various embodiments, the kit comprises one or more polynucleotides encoding, or one or more vectors capable of expressing, or an immunogenic composition

5 comprising, two immunogenic polypeptides, the immunogenic polypeptides comprising: (a) an HBV polymerase polypeptide mutant comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 13-14, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 13-14; and (b) an HBV core-sAg fusion protein comprising or
10 consisting of an amino acid sequence of any one of SEQ ID NOs: 38-41, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 38-41.

[0289] In various embodiments, the kit comprises one or more polynucleotides encoding, or one or more vectors capable of expressing, or an immunogenic composition

15 comprising, two immunogenic polypeptides, the immunogenic polypeptides comprising: (a) an HBV polymerase polypeptide mutant comprising or consisting of an amino acid sequence of SEQ ID NO: 13, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 13; and (b) an HBV core-sAg fusion protein comprising or consisting of an amino acid sequence
20 of SEQ ID NO: 41, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 41.

[0290] With respect to the core-sAg fusion polypeptide in the kit (*e.g.*, expressable from a vector; in an immunogenic composition), in some embodiments, the core polypeptide

25 comprises a serine (S) residue at the amino acid position corresponding to position 12, and an asparagine (N) residue at the amino acid position corresponding to position 67, wherein the position numbers are with reference to SEQ ID NO:65 or SEQ ID NO:66. In some embodiments, the sAg polypeptide comprises an isoleucine (I) residue at the amino acid position corresponding to position 68, wherein the position numbers are with reference to SEQ ID NO:3
30 or SEQ ID NO:4. In some embodiments, the sAg polypeptide comprises one or more of a serine (S) residue at the amino acid position corresponding to position 53, an isoleucine (I) residue at the amino acid position corresponding to position 68, a threonine (T) residue at the amino acid position corresponding to position 125, a proline (P) residue at the amino acid position corresponding to position 127, an phenylalanine (F) residue at the amino acid position

corresponding to position 161, a tyrosine (Y) residue at the amino acid position corresponding to position 200, a serine (S) residue at the amino acid position corresponding to position 210, and a leucine (L) residue at the amino acid position corresponding to position 213, wherein the position numbers are with reference to SEQ ID NO:3 or SEQ ID NO:4. In some embodiments, the core-sAg fusion polypeptide comprises one or more of a serine (S) residue at the amino acid position corresponding to position 12, an asparagine (N) residue at the amino acid position corresponding to position 67, a valine (V) residue at the amino acid position corresponding to position 74, a phenylalanine (F) residue at the amino acid position corresponding to position 97, a threonine (T) residue at the amino acid position corresponding to position 249, a threonine (T) residue at the amino acid position corresponding to position 250, a serine (S) residue at the amino acid position corresponding to position 317, a serine (S) residue at the amino acid position corresponding to position 318, an arginine (R) residue at the amino acid position corresponding to position 326, a tyrosine (Y) residue at the amino acid position corresponding to position 338, a glycine (G) residue at the amino acid position corresponding to position 363, and an alanine (A) residue at the amino acid position corresponding to position 372, wherein the position numbers are with reference to SEQ ID NO:41.

[0291] In some embodiments, the kit comprises a first viral expression vector and a second viral expression vector, wherein: (a) the first viral expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of any one of SEQ ID NOs: 27-32 and 89-94, *e.g.*, SEQ ID NOs: 29, 89, 90 and 92, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 27-32 and 89-94, *e.g.*, SEQ ID NOs: 29, 89, 90 and 92; and (b) the second viral expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of any one of SEQ ID NOs: 33-37, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 33-37.

[0292] In some embodiments, the kit comprises a first viral expression vector and a second viral expression vector, wherein: (a) the first viral expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NOs: 29, 89, 90 or 92, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NOs: 29, 89, 90 or 92; and (b) the second viral expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 37, or a sequence that is at least 80%,

81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 37.

[0293] In some embodiments, the kit comprises: (a) one or more unitary doses of an immunogenic composition as described above and herein, wherein the first and second viral expression vectors comprise a replication-deficient or replication-defective Cali mammarenavirus (*a.k.a.*, Pichinde mammarenavirus or Pichinde arenavirus (PICV)); and (b) one or more unitary doses of an immunogenic composition as described above and herein, wherein the first and second viral expression vectors comprise a replication-deficient or replication-defective Lymphocytic choriomeningitis mammarenavirus (LCMV).

[0294] In some embodiments, the kit comprises: (a) one or more unitary doses of an immunogenic composition as described above and herein, wherein the first and second viral expression vectors are from Adenoviridae; and (b) one or more unitary doses of an immunogenic composition as described above and herein, wherein the first and second viral expression vectors are from Poxviridae (*e.g.*, Vaccinia virus, *e.g.*, modified vaccinia Ankara (MVA)).

[0295] In some embodiments, the kit comprises: (a) one or more unitary doses of an immunogenic composition as described above and herein, wherein the first and second viral expression vectors are from Arenaviridae; and (b) one or more unitary doses of an immunogenic composition as described above and herein, wherein the first and second viral expression vectors are from Adenoviridae.

[0296] In some embodiments, the kit comprises: (a) one or more unitary doses of an immunogenic composition as described above and herein, wherein the first and second viral expression vectors are from Arenaviridae; and (b) one or more unitary doses of an immunogenic composition as described above and herein, wherein the first and second viral expression vectors are from Poxviridae (*e.g.*, Vaccinia virus, *e.g.*, modified vaccinia Ankara (MVA)).

[0297] In some embodiments, the kit comprises a first LCMV arenavirus expression vector and a second LCMV arenavirus expression vector, wherein: (a) the first LCMV arenavirus expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 29, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 29; and (b) the second LCMV arenavirus expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 37, or a

sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 37.

[0298] In some embodiments, the kit comprises a first Pichinde arenavirus expression vector and a second Pichinde arenavirus expression vector, wherein: (a) the first Pichinde

5 arenavirus expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 90, or a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 90; and (b) the second Pichinde arenavirus expression vector comprises a polynucleotide comprising or consisting of a nucleic acid sequence of SEQ ID NO: 37, or a
10 sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 37.

[0299] In various embodiments, the kit comprises one or more unitary doses of one or more additional therapeutic agents.

[0300] For example, in some embodiments, the kit comprises one or more agonists or
15 activators of one or more toll-like receptors (TLRs). In various embodiments, the TLR agonist or activator is selected from the group consisting of a TLR2 agonist, a TLR3 agonist, a TLR4 agonist, a TLR5 agonist, a TLR7 agonist, a TLR8 agonist and a TLR9 agonist. In some embodiments, the TLR7 agonist is selected from the group consisting of GS 9620 (vesatolimod), R848 (Resiquimod), DS-0509, LHC-165 and TMX-101 (imiquimod), and/or wherein the TLR8
20 agonist is selected from the group consisting of GS-9688, R848 (Resiquimod) and NKTR-262 (dual TLR7/TLR8 agonist).

[0301] In some embodiments, the kit comprises one or more interleukin receptor agonists of an interleukin receptor selected from IL-2, IL-7, IL-12 and IL-15. In some
25 embodiments, the kit comprises one or more cytokines selected from the group consisting of IL-2, IL-7, IL-12, IL-15, and variants thereof.

[0302] In some embodiments, the kit comprises one or more innate immune activators. In various embodiments, the one or more innate immune activators comprises an agonist of a receptor selected from the group consisting of fms related tyrosine kinase 3 (FLT3), stimulator of interferon genes (STING) receptor, DExD/H-box helicase 58 (DDX58; *a.k.a.*, RIG-I),
30 nucleotide binding oligomerization domain containing 2 (NOD2). In some embodiments, the kit comprises one or more unitary doses of GS-3583 and/or GS-9992.

[0303] In some embodiments, the kit comprises one or more antagonists or inhibitors of an inhibitory immune checkpoint protein or receptor and/or one or more activators or agonists of

a stimulatory immune checkpoint protein or receptor. In various embodiments, the one or more immune checkpoint proteins or receptors are selected from the group consisting of: CD27, CD70; CD40, CD40LG; CD47, CD48 (SLAMF2), transmembrane and immunoglobulin domain containing 2 (TMIGD2, CD28H), CD84 (LY9B, SLAMF5), CD96, CD160, MS4A1 (CD20), CD244 (SLAMF4); CD276 (B7H3); V-set domain containing T cell activation inhibitor 1 (VTCN1, B7H4); V-set immunoregulatory receptor (VSIR, B7H5, VISTA); immunoglobulin superfamily member 11 (IGSF11, VSIG3); natural killer cell cytotoxicity receptor 3 ligand 1 (NCR3LG1, B7H6); HERV-H LTR-associating 2 (HHLA2, B7H7); inducible T cell co-stimulator (ICOS, CD278); inducible T cell co-stimulator ligand (ICOSLG, B7H2); TNF receptor superfamily member 4 (TNFRSF4, OX40); TNF superfamily member 4 (TNFSF4, OX40L); TNFRSF8 (CD30), TNFSF8 (CD30L); TNFRSF10A (CD261, DR4, TRAILR1), TNFRSF9 (CD137), TNFSF9 (CD137L); TNFRSF10B (CD262, DR5, TRAILR2), TNFRSF10 (TRAIL); TNFRSF14 (HVEM, CD270), TNFSF14 (HVEML); CD272 (B and T lymphocyte associated (BTLA)); TNFRSF17 (BCMA, CD269), TNFSF13B (BAFF); TNFRSF18 (GITR), TNFSF18 (GITRL); MHC class I polypeptide-related sequence A (MICA); MHC class I polypeptide-related sequence B (MICB); CD274 (CD274, PDL1, PD-L1); programmed cell death 1 (PDCD1, PD1, PD-1); cytotoxic T-lymphocyte associated protein 4 (CTLA4, CD152); CD80 (B7-1), CD28; nectin cell adhesion molecule 2 (NECTIN2, CD112); CD226 (DNAM-1); Poliovirus receptor (PVR) cell adhesion molecule (PVR, CD155); PVR related immunoglobulin domain containing (PVRIG, CD112R); T cell immunoreceptor with Ig and ITIM domains (TIGIT); T cell immunoglobulin and mucin domain containing 4 (TIMD4; TIM4); hepatitis A virus cellular receptor 2 (HAVCR2, TIMD3, TIM3); galectin 9 (LGALS9); lymphocyte activating 3 (LAG3, CD223); signaling lymphocytic activation molecule family member 1 (SLAMF1, SLAM, CD150); lymphocyte antigen 9 (LY9, CD229, SLAMF3); SLAM family member 6 (SLAMF6, CD352); SLAM family member 7 (SLAMF7, CD319); UL16 binding protein 1 (ULBP1); UL16 binding protein 2 (ULBP2); UL16 binding protein 3 (ULBP3); retinoic acid early transcript 1E (RAET1E; ULBP4); retinoic acid early transcript 1G (RAET1G; ULBP5); retinoic acid early transcript 1L (RAET1L; ULBP6); lymphocyte activating 3 (CD223); killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 1 (KIR, CD158E1); killer cell lectin like receptor C1 (KLRC1, NKG2A, CD159A); killer cell lectin like receptor K1 (KLRK1, NKG2D, CD314); killer cell lectin like receptor C2 (KLRC2, CD159c, NKG2C); killer cell lectin like receptor C3 (KLRC3, NKG2E); killer cell lectin like receptor C4 (KLRC4, NKG2F); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 1 (KIR2DL1); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 2 (KIR2DL2); killer cell immunoglobulin like receptor, two Ig

domains and long cytoplasmic tail 3 (KIR2DL3); killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 1 (KIR3DL1); killer cell lectin like receptor D1 (KLRD1); and SLAM family member 7 (SLAMF7).

[0304] In some embodiments, the kit comprises one or more blockers or inhibitors of

5 one or more T-cell inhibitory immune checkpoint proteins or receptors. In various
embodiments, the blockers or inhibitors of one or more T-cell inhibitory immune checkpoint
proteins or receptors are selected from the group consisting of CD274 (CD274, PDL1, PD-L1);
programmed cell death 1 ligand 2 (PDCD1LG2, PD-L2, CD273); programmed cell death 1
(PDCD1, PD1, PD-1); cytotoxic T-lymphocyte associated protein 4 (CTLA4, CD152); CD276
10 (B7H3); V-set domain containing T cell activation inhibitor 1 (VTCN1, B7H4); V-set
immunoregulatory receptor (VSIR, B7H5, VISTA); immunoglobulin superfamily member 11
(IGSF11, VSIG3); TNFRSF14 (HVEM, CD270), TNFSF14 (HVEM); CD272 (B and T
lymphocyte associated (BTLA)); PVR related immunoglobulin domain containing (PVRIG,
CD112R); T cell immunoreceptor with Ig and ITIM domains (TIGIT); lymphocyte activating 3
15 (LAG3, CD223); hepatitis A virus cellular receptor 2 (HAVCR2, TIMD3, TIM3); galectin 9
(LGALS9); killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail
1 (KIR, CD158E1); killer cell immunoglobulin like receptor, two Ig domains and long
cytoplasmic tail 1 (KIR2DL1); killer cell immunoglobulin like receptor, two Ig domains and
long cytoplasmic tail 2 (KIR2DL2); killer cell immunoglobulin like receptor, two Ig domains
20 and long cytoplasmic tail 3 (KIR2DL3); and killer cell immunoglobulin like receptor, three Ig
domains and long cytoplasmic tail 1 (KIR3DL1).

[0305] In some embodiments, the kit comprises one or more agonists or activators of one

or more T-cell stimulatory immune checkpoint proteins or receptors. In various embodiments,
the agonists or activators of one or more T-cell stimulatory immune checkpoint proteins or
25 receptors are selected from the group consisting of CD27, CD70; CD40, CD40LG; inducible T
cell co-stimulator (ICOS, CD278); inducible T cell co-stimulator ligand (ICOSLG, B7H2); TNF
receptor superfamily member 4 (TNFRSF4, OX40); TNF superfamily member 4 (TNFSF4,
OX40L); TNFRSF9 (CD137), TNFSF9 (CD137L); TNFRSF18 (GITR), TNFSF18 (GITRL);
CD80 (B7-1), CD28; nectin cell adhesion molecule 2 (NECTIN2, CD112); CD226 (DNAM-1);
30 Poliovirus receptor (PVR) cell adhesion molecule (PVR, CD155). In some embodiments, the kit
comprises one or more unitary doses of AGEN-2373 and/or AGEN-1223.

[0306] In some embodiments, the kit comprises one or more blockers or inhibitors of

one or more NK-cell inhibitory immune checkpoint proteins or receptors. In various
embodiments, the NK-cell inhibitory immune checkpoint proteins or receptors are selected from

the group consisting of killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 1 (KIR, CD158E1); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 1 (KIR2DL1); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 2 (KIR2DL2); killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 3 (KIR2DL3); killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 1 (KIR3DL1); killer cell lectin like receptor C1 (KLRC1, NKG2A, CD159A); and killer cell lectin like receptor D1 (KLRD1, CD94).

[0307] In some embodiments, the kit comprises one or more agonists or activators of one or more NK-cell stimulatory immune checkpoint proteins or receptors. In various embodiments, the NK-cell stimulatory immune checkpoint proteins or receptors are selected from CD16, CD226 (DNAM-1); killer cell lectin like receptor K1 (KLRK1, NKG2D, CD314); and SLAM family member 7 (SLAMF7).

[0308] In various embodiments of the kits, the one or more immune checkpoint inhibitors comprises a proteinaceous inhibitor of PD-L1 (CD274), PD-1 (PDCD1) or CTLA4.

In some embodiments, the proteinaceous inhibitor of CTLA4 is selected from the group consisting of ipilimumab, tremelimumab, BMS-986218, AGEN1181, AGEN1884, BMS-986249, MK-1308, REGN-4659, ADU-1604, CS-1002, BCD-145, APL-509, JS-007, BA-3071, ONC-392, AGEN-2041, JHL-1155, KN-044, CG-0161, ATOR-1144, PBI-5D3H5, FPT-155 (CTLA4/PD-L1/CD28), PF-06936308 (PD-1/CTLA4), MGD-019 (PD-1/CTLA4), KN-046 (PD-1/CTLA4), MEDI-5752 (CTLA4/PD-1), XmAb-20717 (PD-1/CTLA4) and AK-104 (CTLA4/PD-1). In some embodiments, the proteinaceous inhibitor of PD-L1 (CD274) or PD-1 (PDCD1) is selected from the group consisting of zimberelimab (AB122), pembrolizumab, nivolumab, cemiplimab, pidilizumab, AMP-224, MEDI0680 (AMP-514), spartalizumab, atezolizumab, avelumab, ASC22, durvalumab, BMS-936559, CK-301, PF-06801591, BGB-A317 (tislelizumab), GLS-010 (WBP-3055), AK-103 (HX-008), AK-105, CS-1003, HLX-10, MGA-012, BI-754091, AGEN-2034, JS-001 (toripalimab), JNJ-63723283, genolimzumab (CBT-501), LZM-009, BCD-100, LY-3300054, SHR-1201, SHR-1210 (camrelizumab), Sym-021, ABBV-181, PD1-PIK, BAT-1306, (MSB0010718C), CX-072, CBT-502, TSR-042 (dostarlimab), MSB-2311, JTX-4014, BGB-A333, SHR-1316, CS-1001 (WBP-3155, KN-035, IBI-308 (sintilimab), HLX-20, KL-A167, STI-A1014, STI-A1015 (IMC-001), BCD-135, FAZ-053, TQB-2450, MDX1105-01, FPT-155 (CTLA4/PD-L1/CD28), PF-06936308 (PD-1/CTLA4), MGD-013 (PD-1/LAG-3), FS-118 (LAG-3/PD-L1) MGD-019 (PD-1/CTLA4), KN-046 (PD-1/CTLA4), MEDI-5752 (CTLA4/PD-1), RO-7121661 (PD-1/TIM-3), XmAb-20717 (PD-1/CTLA4), AK-104 (CTLA4/PD-1), M7824 (PD-L1/TGFβ-EC domain), CA-170 (PD-

L1/VISTA), CDX-527 (CD27/PD-L1), LY-3415244 (TIM3/PDL1), and INBRX-105 (4-1BB/PDL1). In some embodiments, the one or more immune checkpoint inhibitors comprises a small molecule inhibitor of CD274 (PDL1, PD-L1), programmed cell death 1 (PDCD1, PD1, PD-1) or CTLA4. In some embodiments, the small molecule inhibitor of CD274 or PDCD1 is selected from the group consisting of GS-4224, GS-4416, INCB086550 and MAX10181. In some embodiments, the small molecule inhibitor of CTLA4 comprises BPI-002.

[0309] In various embodiments, the kit comprises one or more anti-viral agents.

Illustrative anti-viral agents that can be in the kit include lamivudine (LAM), adefovir dipivoxil (ADV), entecavir (ETV), telbivudine (LdT), tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF or VEMSIDY®) and ledipasvir + sofosbuvir (HARVONI®). In some embodiments, the kit comprises one or more therapeutic agents selected from the group consisting of HBV antigen inhibitors (*e.g.*, HBV core antigen (HBcAg) inhibitors, HBV surface antigen (HBsAg) inhibitors, HBx inhibitors, HBV E antigen inhibitors), anti-HBV antigen antibodies, inhibitory nucleic acids targeting HBV (*e.g.*, antisense oligonucleotide, short interfering RNA (siRNA), DNA-directed RNA interference (ddRNAi)), gene editors targeting HBV (*e.g.*, CRISPR-Cas (*e.g.*, Cas9, Cas12, Cascade, Cas13), zinc finger nucleases, homing endonucleases, homing meganucleases (*e.g.*, ARCUS), synthetic nucleases, TALENs), covalently closed circular DNA (cccDNA) inhibitors and HBsAg secretion or assembly inhibitors and HBV viral entry inhibitors.

[0310] Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

EXAMPLES

[0311] The following examples are offered to illustrate, but not to limit the claimed invention.

Example 1

Identification of HBV sAg Sequences that Induce Robust, Genotype Cross-Reactive T Cell Responses

[0312] In this example, we identified near-consensus, naturally occurring sequences of HBV sAg in genotypes A, B, C, and D, generated adenovirus type 5 vectors encoding each antigen, and tested the magnitude and genotype cross-reactivity of the T cells induced by each of these vectors in outbred mice.

[0313] *Selection of near-consensus, naturally occurring HBV sAg sequences.* In selecting the specific amino acid sequence of an HBV sAg to be used for therapeutic vaccination, we sought an sAg sequence that was both efficiently expressed and processed for antigen presentation, while also inducing T cell responses that react broadly across a range of HBV genotypes. Although consensus sequences or mosaic antigens can be designed to attempt to improve T cell genotype reactivity, such sequences do not occur in nature and have a risk of being inefficiently expressed or poorly processed into T-cell epitopes. Consequently, we identified near-consensus, naturally occurring HBV sAg sequences from genotypes (GT) A, B, C and D. Using a database of sAg sequences from 14207 individuals infected with these HBV genotypes, we constructed consensus sequences for each genotype, then identified the naturally occurring sAg sequence closest to the consensus for each genotype. The naturally occurring, near-consensus sAg sequences for HBV genotypes A, B, C and D are provided in Table 1 as SEQ ID NOs: 1-4, respectively.

Table 1: Naturally-occurring, near-consensus sAg polypeptide sequences		
SEQ ID NO:	HBV genotype	Polypeptide sequence
1	A	MENITSGFLGPLLVLQAGFLLTRILTIPQSLDSWWTSNLFLGGTPVCLGQNSQSPTSNHSPTSCPPICPGYRWMCLRRFIIIFLFIILLCLIFLLVLLDYQGMLPVCPLIPGSTTTSTGPCKTCTTPAQGNSMFPSCCCTKPTDGNCTCIPIPSSWAFAYLWEWASVRFWSLSLLVPFVQWFVGLSPTVWLSVIWMMWYWGPSLYNILSPFIPLLP IFFCLWVYI
2	B	MESTTSGFLGPLLVLQAGFLLTRILTIPQSLDSWWTSNLFLGGAPTC PGQNLQSPTSNHSPTSCPPICPGYRWMCLRRFIIIFLFIILLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGPCRTCTTPAQGTSMFPSCCCTKPTD

Table 1: Naturally-occurring, near-consensus sAg polypeptide sequences		
SEQ ID NO:	HBV genotype	Polypeptide sequence
		GNCTCIPIPSSWAFACYLWEWASVRFSWLSLLVPFVQWFVGLSPTVWL SVIWMWYWGPSLYNILSPFPLLP IFFCLWVYI
3	C	MESTTSGFLGPLLVLQAGFFLLTRILTIPQSLDSWWTSLNFLGGAPTC PGQNSQSPTSNHSPTSCPPICPGYRWMCLRRFIIIFLCILLCLIFLLV LLDYQGMLPVCPLIPGSSTTSTGPCKTCTTPAQGTSMFPSCCCTKPTD GNCTCIPIPSSWAFARFLWEWASVRFSWLSLLVPFVQWFVGLSPTVWL SVIWMWYWGPSLYNILSPFLPLLP IFFCLWVYI
4	D	MENITSGFLGPLLVLQAGFFLLTRILTIPQSLDSWWTSLNFLGGTTVC LGQNSQSPTSNHSPTSCPPICPGYRWMCLRRFIIIFLFILLCLIFLLV LLDYQGMLPVCPLIPGSSTTSTGPCRTCTTPAQGTSMYPSCCCTKPSD GNCTCIPIPSSWAFGKFLWEWASARFSWLSLLVPFVQWFVGLSPTVWL SVIWMWYWGPSLYSILSPFLPLLP IFFCLWVYI

Methods

[0314] To evaluate the immunogenicity of each antigen and assess the genotype cross-reactivity of induced T cells across a broad range of epitopes *in vivo*, Diversity Outbred mice (DO mice) from Jackson Laboratories were used for vaccination. DO mice were developed by random outcross matings of 160 Collaborative Cross recombinant inbred mouse lines, and the colony is maintained by continued random matings that avoid crosses between siblings. The DO parental lines, the Collaborative Cross strains, were developed by crossing eight unique and genetically diverse inbred mouse strains (A/J, C57BL/6J, 129S1/SvImJ, NOD/ShiLtJ, NZO/HILtJ, CAST/EiJ, PWK/PhJ, and WSB/EiJ). Therefore, DO mice capture the diversity of epitope selection and magnitude of T cell responses present in a highly genetically diverse population.

Results

[0315] All four naturally occurring, near-consensus sequences of HBV sAg were robustly immunogenic in DO mice (Fig. 1). Induced T cells reacted to GT-A, B, C, and D HBV sAg peptides with approximately equal magnitude, demonstrating excellent genotype cross-reactivity of the T cell response. Geometric mean response magnitude was largest for GT-C and GT-D sAg.

Example 2

Identification of HBV Core and Pol Sequences that Induce Robust, Genotype Cross-Reactive T Cell Responses

[0316] In this example, we identified near-consensus, naturally occurring sequences of
5 HBV core and HBV polymerase (Pol) in genotypes A, B, C, and D, generated Adenovirus type
5 expression vectors encoding Pol antigens or core-Pol fusion proteins, and tested the magnitude
and genotype cross-reactivity of the T cells induced in inbred and outbred animals.

[0317] *Selection of near-consensus, naturally occurring HBV core and Pol sequences.*

In selecting the specific amino acid sequence of an HBV core and Pol antigens to be used for
10 therapeutic vaccination, we sought core and Pol sequences that were both efficiently expressed
and processed for antigen presentation, while also inducing T cell responses that react broadly
across a range of HBV genotypes. Although consensus sequences or mosaic antigens can be
designed to attempt to improve T cell genotype reactivity, such sequences do not occur in nature
and have a risk of being inefficiently expressed or poorly processed into T cell epitopes.

15 Consequently, we identified near-consensus, naturally occurring HBV core and Pol sequences
from genotypes A, B, C and D. Using a database of core sequences from 5528 individuals
infected with HBV genotypes A-D, and Pol sequences from 4713 individuals infected with HBV
genotypes A-D, we constructed consensus sequences for core and Pol for each genotype, then
identified the naturally occurring core and Pol sequences closest to the consensus for each
20 genotype.

[0318] GT-A, B, C, and D Pol sequences were then modified to improve antigen
performance. The enzymatic activity of polymerases can induce toxicity when overexpressed,
so the enzymatic activity of the reverse transcriptase (RT) and RNase H (RNH) domains was
ablated by mutations in the catalytic domains. The YMDD motif in RT was mutated to YMHD,
25 and the AELL motif in RNH was mutated to AHLL (Radziwill, *et al.*, *J Virol.* (1990) 64(2):613-
20). The resulting Pol sequences are referred to as Pol^{mut}. The Pol^{mut} sequences for HBV
genotypes A, B, C and D are provided in Table 2 as SEQ ID NOs: 52-55, respectively.

Table 2 - Pol ^{mut} polypeptide sequence		
SEQ ID NO:	HBV geno- type	Polypeptide sequence - Motifs containing inactivating mutations in Pol are underlined (YMDD mutated to YMHD, AELL mutated to AHLL)
52	A	MPLSYQHFRKLLLLLDDETEAGPLEEELPRLADEDLNRRVAEDLNLGNLNV SIPWTHKVGNFETGLYSSTVPIFNPEWQTPSFPKIHLLHEDIANRCQQFVGP

Table 2 – Pol ^{mut} polypeptide sequence		
SEQ ID NO:	HBV geno-type	Polypeptide sequence – Motifs containing inactivating mutations in Pol are underlined (YMDD mutated to YMHD, AELL mutated to AHLL)
		LTVNEKRRLRLIMPARFYPNSTKYLPLDKGIKPYYPDHVVNHYFQTRHYL HTLWKAGILYKRETTTRSASFSGSPYSWEQELHHGRLVIKTSQRHGDEPFC SQPSGILSRSSVGPCIRSQFKQSRGLQPHQGPLATSQSGRSGSIRARVH SPTRRCFGVEPSGSGHIGHSASSSSSCLHQSAVRKAAYSHLSTSKRQSSS GHAVEFHSFPPSSARSQSQGPVFSWWLQFRNTQPCSKYCLSHLVNLLD WGPCDEHGEHHIRIPRTPARVTGGVFLVDKNPHNTAESRLVVDVSQFSRG ITRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHIPLHPAAMPHLL VGSSGLSRYVARLSSNSRIHNNQHGTQLQNLHDSCSRQLYVSLMMLLYKTYG RKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCCLAFS <u>YMHD</u> VVLGAKSVQHLESLEYTAVTNFLLSLGIHLNPNKTKRWGYSLNFMGY VIGSWGTLTPQDHIVQKIKHCFRKLPINRPIDWKVCQRIVGLLGFAAPFTQ CGYPALMPLYACIQAKQAFTFSPTYKAFLSKQYLNLYPVARQRPGLCQVF ADATPTGWGLAIGHQRMRTFVAPLP IHTAHLLAACFARSRSAGKLIIGTD NSVVLSRKYTSFPWLLGCTANWILRGTSFVYVPSALNPADDP SRGRLGLY RPLLRLPYRPTTGRTSLYAVSPSVPSHLPVRVHFASPLHVAWRPP
53	B	MPLSYQHFRKLLLLLDDEAGPLEEELPRLADEGLNRRVAEDLNLGNLNVSI PWTHKVGNTGLYSSTVPVFNPEWQTPSFPHIHLQEDIINRCQQYVGPLT VNEKRRLKLIMPARFYPNLT KYLPLDKGIKPYYPEHVVNHYFQTRHYLHT LWKAGILYKRETTTRSASFSGSPYSWEQDLQHGRVLVFQTSKRHGDKSFCPO SPGILPRSSVGPCIQNLRLKSRGLGPQAQGLAGRQQGGSGSIRARVHPS PWGTVGVEPSGSGHIHNCASNSSSCLHQSAVRKAAYSHISTSKGHSSSGH AVELHHFPPSSSRQSQGPVLSWWLQFRNSEPCSEYCLCHIVNLIEDWG PCTEHGEHRIRTPRTPARVTGGVFLVDKNPHNTTESRLVVDVSQFSRGNT RVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLP LHPAAMPHLLVG SSGLSRYVARLSSNSRIINNQHRTMQNLHDSCSRNLYVSLMMLLYKTYGRK LHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCCLAFS <u>SYM</u> <u>HDV</u> VVLGAKSVQHLESLEYAAVTNFLLSLGIHLNPNKTKRWGYSLNFMGYVI GSWGTLTPQEHIVQKIKMCFRKLPVNRPIDWKVCQRIVGLLGFAAPFTQCG YPALMPLYACIQAKQAFTFSPTYKAFLSKQYLHLYPVARQRPGLCQVFAD ATPTGWGLAIGHQRMRTAFVSPLP IHTAHLLAACFARSRSAGKLIIGTDNS VVLSRKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDP SRGRLGLYRP LLRLLYRPTTGRTSLYADSPSVPSHLPDRVHFASPLHVAWRPP
54	C	MPLSYQHFRKLLLLLDDEAGPLEEELPRLADEGLNRRVAEDLNLGNLNVSI PWTHKVGNTGLYSSTVPVFNPEWQTPSFPHIHLQEDIINRCQQYVGPLT VNEKRRLKLIMPARFYPNLT KYLPLDKGIKPYYPEHTVNHYFKTRHYLHT LWKAGILYKRETTTRSASFSGSPYSWEQELQHGRVLVFQTSRHRGDESFCQ SSGILSRSPVGPCIRSQLKQSRGLQPPQGSLSARSKSGRSGSIRARVHPT TRQSFVGVEPSGSGHIDNSASSASSCLHQSAVRKTAYSHLSTSKRQSSSGH AVELHNFPPSSARSQSEGPLLSCWWLQFRNSKPCSDYCLSHIVNLLDWDG PCTEHGEHNIRIPRTPARVTGGVFLVDKNPHNTTESRLVVDVSQFSRGST HVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLP LHPAAMPHLLVG SSGLSRYVARLSSSTRNINYQHGMQDLHDSCSRNLYVSLLLLYKTFGRK LHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCCLAFS <u>SYM</u> <u>HDV</u> VVLGAKSVQHLESLEYTAVTNFLLSLGIHLNPNKTKRWGYSLNFMGYVI GSWGTLTPQEHIVLKIKQCFRKLPVNRPIDWKVCQRIVGLLGFAAPFTQCG YPALMPLYACIQAKQAFTFSPTYKAFLCKQYLNLYPVARQRSGLCQVFAD

Table 2 – Pol ^{mut} polypeptide sequence		
SEQ ID NO:	HBV geno-type	Polypeptide sequence – Motifs containing inactivating mutations in Pol are underlined (YMDD mutated to YMHD, AELL mutated to AHLL)
		ATPTGWGLAVGHQRMRTFVSPLPIHTAHLAACFARSRSAGKLTGTDNS VVLRSKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDP SRGRLGLYRP LLRLPFRPTTGRTSLYAVSPSVPSHLPVRVHFASPLHVAWRPP
55	D	MPLSYQHFRLLLLLDDEAGPLEEELPRLADEGLNRRVAEDLNLGNLNVSI PWTHKVGNTGLYSSTVPVFNPHWKTPSFPNIHLHQDIKKCEQFVGPLT VNEKRRLQLIMPARFYPNVTKYLP LDKGIKPYYPEHLVNHYFQTRHYLHT LWKAGILYKRETTTHSASFCGSPYSWEQELQHGAESFHQQSSGILSRPPVG SSLQSKHRKSRLGLQSQQGHLLARRQQGRGWSIRAGIHPTARRPFGVEPSG SGHTANLASKSASCLYQSAVRKAAYPVVSTFKKHSSSGHAVELNHLPNS ARSQSERPVFPCWWLQFRNSKPCSDYCLSHIVNLLEDWGPCAEGHEHHIR IPRTPARVTGGVFLVDKNPHNTAESRLVVDFSQFSRGNYRVSWPKFAVNP LQSLTNLLSSNLWSLSDVSAAFYHLP LHPAAMP HLLVGSSGLSRVVARL SSNSRIFNYQHGTMQNLHDSRNLVSLMLLYQTFRKLHLYSHP I ILG FRKIPMGVGLSPFLLAQFTSAICSVVRRAPFHCLAFSYMHADVVLGAKSVQ HLESLEFTAVTNFLLSLGIHLNPNKTKRWGYSLHFMGYVIGCYGSLPQDHI IQKIKECFRKLFPVNRPIDWKVCQRIVGLLGFAAPFTQCGYPALMPLYACI QSKQAFTEFPTYKAFLCKQYLNLYPVARQRPGLCQVFADATPTGWGLVMG HQRMRTGTFKAPLP IHTAHLAACFARSRSAGANILGTDNSVVLRSKYTSFP WLLGCAANWILRGTSFVYVPSALNPADDP SRGRLGLYRP LLRLPFRPTTG RTSLYADSPSVPSHLPDRVHFASPLHVAWRPP

[0319] Pol^{mut} sequences were then further modified to remove amino acid regions that are poorly conserved among HBV strains and genotypes, to generate Pol sequences of varying length to accommodate viral vectors with differing constraints on encoded antigen size, and to

5 create core-Pol fusions in order to encode two antigens with a single open reading frame. Pol consists of four functional domains, Terminal Protein (TP), Spacer, RT, and RNH. Of these three, TP, RT, and RNH are highly conserved amongst HBV strains and genotypes and so are likely to induce strain- and genotype- cross-reactive T cells, whereas the Spacer domain is highly variable. We generated GT-A, B, C, and D Pol sequences with deletions in the Spacer

10 region. In one set of sequences, designated Pol^{Δ1}, the deletion was based on a previously reported deletion mutant that retains enzymatic function *in vitro*, indicating that the deletion is not disruptive to the expression, structure and folding of the remaining protein (Radziwill, *et al.*, *J Virol.* (1990) 64(2):613-20). In a second set of vectors designated Pol^{Δ3}, the entire poorly conserved region was identified by sequence alignment and deleted. Core-Pol fusions were

15 generated by fusing the near-consensus core sequences to the Pol^{mut}, Pol^{Δ1} and Pol^{Δ3} sequences for GT-A, B, C, and D. Lastly, to accommodate viral vectors with smaller packaging limits, we constructed shorter versions of each near-consensus inactivated Pol sequence, designated as

Pol³⁰⁰. The Pol³⁰⁰ variants have large N-terminal deletions in which the entire TP and most of the Spacer domain is removed, but the RT and RNaseH domains are maintained (Lanford *et al.*, *J Virol.* (1999);73(3):1885-93). A listing of Pol-containing antigen sequences tested in adenovirus or arenavirus vectors is shown in Table 3 and Fig 2. Sequences of the amino acids removed from each Pol deletion constructs are provided in SEQ ID NOs: 42-51.

Table 3. Sequences of Pol-containing antigens	
Polypeptide SEQ ID NOs	Polypeptide
5-8 for Genotype A-D, respectively	Pol ^{Δ1}
9-12 for Genotype A-D, respectively	Pol ^{Δ3}
13-14 for Genotype B and D, respectively	Pol ³⁰⁰
15-18 for Genotype A-D, respectively	Core-Pol ^{mut}
19-22 for Genotype A-D, respectively	Core-Pol ^{Δ1}
23-26 for Genotype A-D, respectively	Core-Pol ^{Δ3}

Methods

[0320] The immunogenicity of each GT-A, B, C, and D core-Pol fusion construct was initially tested in C57BL/6 mice for induction of T cell responses reactive with GT-D core and Pol peptide pools, to identify the variant within each genotype inducing the largest immunogenic response (Fig 3). In all genotypes, a robust Pol response was detected but core responses were weaker or absent. The weak or absent core responses likely resulted from the fact that C57BL/6 mice are known to only respond to a single peptide from GT-D HBV core, namely, MGLKFRQL (Chiale, *et al.*, *Antiviral Res.* 2019 Aug;168:156-167). Responses to this peptide in C57BL/6 mice are often weak or absent, and the peptide has an alternate sequence in the GT-A, B, and C core sequences of MGLKIRQL.

Results

[0321] All antigen genotypes showed little change in immunogenicity between core-Pol^{mut} and core-Pol^{Δ1}. GT-A antigen had an increased response to core-Pol^{Δ3} vs core-Pol^{mut} and core-Pol^{Δ1}, whereas GT-B, C, and D all demonstrated reduced immunogenicity with core-Pol^{Δ3}.

[0322] T cell responses in inbred mouse strains are not ideal for comparing antigen immunogenicity across different genotypes because responses can be dominated by one or a few epitopes, which could vary in sequence among the antigens. To better compare the immunogenicity of core-Pol antigens across genotypes, immunogenicity was tested in DO mice to capture responses across a wide range of epitopes. DO mice were immunized with GT-A

core-Pol^{Δ3} or GT-B, C, or D core-Pol^{Δ1}, and T cell responses were assessed for IFN-γ ELISPOT response using GT-A and GT-D peptide pools (Fig 4). GT-B core-Pol^{Δ1} gave the best overall responses to Pol, with equally robust ELISPOT responses to GT-A and GT-D peptide pools (Fig 4A). Pol responses to GT-B core-Pol^{Δ1} were statistically significantly higher than responses to GT-A core-Pol^{Δ3} using GT-D peptides, and to GT-C core-Pol^{Δ1} using both peptide genotypes. The geometric mean Pol ELISPOT responses to GT-D core-Pol^{Δ1} were numerically lower than GT-B core-Pol^{Δ1}, but the difference was not statistically significant. Responses to core were clearly detectable in the DO mice for all four antigen genotypes (Fig 4B). The pattern of core responses was similar to the Pol responses with GT-B core-Pol^{Δ1} yielding the overall best results, although for core no comparisons between antigen genotypes reached statistical significance.

Example 3

Identification of Smaller Immunogenic Pol Antigens

[0323] Different viral vector systems have differing limits on the maximum size of encoded antigens.

Methods

[0324] To identify additional Pol variants that are smaller in size, and thus could be used in a wider range of vector systems, we evaluated the immunogenicity of Pol variants expressed without fusion to core. C57BL/6 mice were immunized with Adenovirus type 5 vectors encoding GT-D Pol^{Δ1}, Pol^{Δ3}, and Pol³⁰⁰, and GT-B Pol³⁰⁰, and compared to a control vector encoding a full-length, unmodified GT-D Polymerase (GT-D Pol^{Ctrl}) and mock vaccination with phosphate buffered saline (PBS) as a negative control. IFN-γ ELISPOT responses were measured 14 days after immunization with GT-D Pol peptide pools (Fig. 5).

Results

[0325] All tested Pol antigen designs were immunogenic, with no statistically significant differences between groups.

Example 4

Efficacy of Vaccination with Near-Consensus Antigens

in Combination with Anti-PD-1 in Adeno-Associated Virus (AAV)-HBV Mice

[0326] We used an Adeno-Associated Virus (AAV)-HBV model (Dion, *et al.*, *J Virol.* (2013) 87(10):5554-63; and Yang, *et al.*, *Cell Mol Immunol.* (2014) 11(1):71-8) to determine if

our near-consensus antigen designs could have antiviral effects in a model of chronic HBV infection.

Methods

[0327] In this model, C57BL/6 mice were transduced with AAV vectors encoding a 1.2x length GT-D HBV genome, resulting in persistent HBV protein and virion production in hepatocytes, accompanied by antigenemia and viremia in serum. Heterologous viral vector prime-boost regimens consisting of an adenovirus (Ad) prime and poxvirus boost have yielded strong T cell responses in humans (*see, e.g., Barnes, et al., Sci Transl Med.* (2012) 4(115):115ra1; Ewer, *et al., N Engl J Med.* (2016) 374(17):1635-46; Ewer, *et al. Nat Commun.* (2013) 4:2836; Green, *et al., Sci Transl Med.* (2015) 7(300):300ra126; Swadling, *et al., Sci Transl Med.* (2014) 6(261):261ra153), so we generated vaccinia vectors based on the Western Reserve strain (NCBI:txid696871) expressing GT-C sAg and GT-B core-Pol^{Δ1}. AAV-HBV mice were vaccinated with Ad5 prime and vaccinia boost vectors encoding GT-C sAg and GT-B core-Pol^{Δ1} or irrelevant control antigens beta-galactosidase and green fluorescent protein. Mice were further treated with either anti-mouse PD-1 monoclonal antibody or an isotype control antibody after the boost vaccination. A diagram of the AAV-HBV efficacy study is shown in Figure 6, and treatment groups are shown in Table 4. A control group received HBV vaccine but no AAV-HBV to determine if vaccine responses were reduced in the presence of persistent HBV.

Table 4
Study Groups in AAV-HBV Efficacy Study

Group	N	AAV-HBV	Prime	Boost	Antibody
1	12	Y	Ad-β-gal	Vac-GFP	Isotype ctrl
2	12	Y	Ad-sAg GT-C Ad-core-pol ^{Δ1} GT-B	Vac-sAg GT-C Vac-core-pol ^{Δ1} GT-B	Isotype ctrl
3	12	Y	Ad-β-gal	Vac-GFP	α-PD-1
4	12	Y	Ad-sAg GT-C Ad-core-pol ^{Δ1} GT-B	Vac-sAg GT-C Vac-core-pol ^{Δ1} GT-B	α-PD-1
5	12	N	Ad-sAg GT-C Ad-core-pol ^{Δ1} GT-B	Vac-sAg GT-C Vac-core-pol ^{Δ1} GT-B	None

Ad: Adenovirus 5 vector. Vac: vaccinia vector. β -gal: beta-galactosidase. GFP: green fluorescent protein.

Results

5 [0328] Figure 7 shows the IFN- γ ELISPOT responses in each group. Note that responses were evaluated using GT-D peptide pools matched to the HBV strain in the AAV-HBV vector, so T cell responses are detected only if they react with the virus present in the AAV-HBV mice. Responses to core were tested but none were detected in any group, consistent with the poor immunogenicity of core in C57BL/6 mice (Chiale, *et al.*, *supra*).
10 Robust Pol ELISPOT responses were detected in all groups receiving Ad prime and vaccinia boost vectors encoding HBV antigens. Pol ELISPOT magnitude was similar in AAV-HBV mice and in control mice that did not receive AAV-HBV, indicating that the AAV-HBV does not result in T-cell tolerance to Pol. In contrast, ELISPOT responses to sAg were greatly reduced in AAV-HBV mice compared to control mice, demonstrating that AAV-HBV induces T
15 cell tolerance to sAg. Nevertheless, in mice that received AAV-HBV and Adenovirus prime-vaccinia boost HBV vaccine, 2-3 mice per group demonstrated sAg ELISPOT responses above those detected in control-vaccinated mice. ELISPOT response magnitudes were not changed by anti-PD-1 treatment.

[0329] To evaluate any antiviral effects of the HBV-specific T cells induced by
20 vaccination, we measured serum e antigen (HBeAg). Serum HBeAg is a better marker of T-cell mediated antiviral efficacy than serum HBsAg, since the latter may be reduced by the action of anti-HBsAg antibodies induced by vaccination. Neither HBV vaccine alone nor anti-PD-1 alone caused any reduction in serum HBeAg compared to mice receiving control vaccine and isotype control antibody. However, the combination of HBV vaccine + anti-PD-1 resulted in loss of
25 detectable HBeAg in serum in 4 of 12 mice (Fig. 8). These data demonstrate that vaccination with viral vectors encoding our improved antigen sequences contributed to HBV clearance as part of a combination therapy strategy.

Example 5

Immunogenicity of Pol antigens in Arenavirus Vectors

30 [0330] We further improved our HBV antigen designs for use in arenavirus vectors. Unlike adenovirus vectors and most other viral vector systems, arenavirus vectors can be repeatedly administered without inducing neutralizing anti-vector antibodies. Additionally, arenavirus vectors can be produced in several variants differing in the source virus used to generate the vector, *e.g.*, replication-incompetent with a two-segment (*i.e.*, bi-segmented)

genome (Flatz, *et al.*, *Nat Med.* (2010) 16(3):339-45), or replication-attenuated with a three-segment (*i.e.*, tri-segmented) genome (Kallert, *et al.*, *Nat Commun.* (2017) 8:15327) (Figure 9).

Certain HBV antigens were expressed in tri-segmented replication-attenuated or bi-segmented replication-defective arenavirus platforms with either a Lymphocytic choriomeningitis

mammarenavirus (LCMV) or Cali mammarenavirus (*a.k.a.*, Pichinde mammarenavirus or Pichinde arenavirus (PICV)) vector backbone. Replication-defective arenavirus vectors used are described in WO 2009/083210. Replication-attenuated arenavirus vectors used are described in WO 2016075250 (LCMV) and WO 2017/198726 (Pichinde).

[0331] Arenavirus vectors can accommodate antigens of approximately 500-800 amino acids per open reading frame. Therefore, we tested GT-D and GT-B Pol^{Δ1} (SEQ ID NOs: 6 and 8), Pol^{Δ3} (SEQ ID NOs: 10 and 12), and Pol³⁰⁰ (SEQ ID NOs: 13 and 14) for immunogenicity in replication-incompetent LCMV vectors. C57BL/6 mice were immunized intravenously with 10⁶ focus forming units (FFU) of replication-incompetent LCMV vectors and IFN-γ ELISPOT responses were measured at day 7 post-immunization. All GT-B antigens and GT-D Pol³⁰⁰ induced robust T cell responses, while GT-D Pol^{Δ1} and Pol^{Δ3} elicited reduced ELISPOT responses compared to the other antigen designs (Fig 10).

Example 6

Identification of Genetically Stable Replication-Incompetent LCMV Vectors Encoding Immunogenic Pol Antigens

[0332] The stability of various immunogenic Pol transgenes within replication-incompetent LCMV vectors (VV1) was evaluated by polymerase chain reaction (PCR) after serial passaging of vector containing supernatant. Genetic stability was defined by the major band showing at the correct size of the full-length transgene (TG). Results are shown in Table 6.

Table 6

Overview Table for Assessment of Genetic Stability of Pol Transgenes

Genotype	Vector	Stable TG insertion until
GT-B	VV1*-Pol ^{Δ1}	P1
GT-B	VV1-Pol ^{Δ3}	P1
GT-B	VV1-Pol ³⁰⁰	P5
GT-D	VV1-Pol ^{Δ1}	P1
GT-D	VV1-Pol ^{Δ3}	P1

GT-D	VV1-Pol ³⁰⁰	P2
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*VV1 refers to replication-incompetent LCMV vectors.

“P#” indicates the number of passages (*e.g.*, P1 equals 1 passage).

Example 7

Immunogenicity of Core-sAg Fusion Proteins in

Replication-Incompetent LCMV vectors

[0333] Having identified stable, immunogenic Arenavirus vectors encoding HBV Pol, we additionally tested a series of core-sAg fusion proteins for immunogenicity in replication-incompetent LCMV vectors. Core-sAg fusions were generated by fusing near-consensus GT-B core and GT-C sAg, or GT-D core and GT-D sAg, with core at the N-terminus and sAg at the C-terminus. Direct fusions are expected to elicit T cell responses, but may not induce anti-sAg antibodies since the fusion protein will not secrete sAg. Therefore, additional antigen designs were tested with the core and sAg separated by a GSG linker followed by a 2A translational skip site derived from Porcine teschovirus-1 (P2A) (Kim, *et al.*, *PLoS ONE*. (2011) 6: e18556). This orientation will yield a 21 amino acid extension on the C-terminus of core, while enabling normal sAg secretion to elicit antibody responses. Sequence identification numbers for the amino acid sequences of antigens tested in Arenavirus vectors, and the nucleotide sequences used to encode antigens in Arenavirus vectors, is shown in Table 7.

Table 7

**Sequences vector antigens and antigen-encoding genes used
in LCMV vectors**

Polynucleotide SEQ ID NO:	Polypeptide SEQ ID NO:	Polypeptide
27	6	GT-B Pol ^{Δ1}
28	10	GT-B Pol ^{Δ3}
29	13	GT-B Pol ³⁰⁰
30	8	GT-D Pol ^{Δ1}
31	12	GT-D Pol ^{Δ3}
32	14	GT-D Pol ³⁰⁰
33	38	GT-B/C core-sAg
34	39	GT-B/C core-P2A-sAg
35	40	GT-D core-sAg
36	41	GT-D core-P2A-sAg
37	41	GT-D iCore-P2A-sAg

[0334] Replication-incompetent LCMV vectors encoding core-sAg variants were tested for immunogenicity by immunizing C57BL/6 mice (Fig 11). The total HBV-specific IFN- γ ELISPOT responses were indistinguishable for all tested vectors, and inclusion of a P2A site had no impact on ELISPOT responses for either GT-B/C or GT-D antigens. Responses to both core and sAg were observed for all tested vectors. Detection of core responses was notable, as core T cell responses tend to be weak and difficult to detect in this mouse strain (Chiale, *et al.*, *supra*). Similar results were seen in Balb/c mice immunized with the same vectors.

[0335] Antibody responses develop more slowly than T-cell responses after replication-incompetent LCMV vector vaccination, so an additional set of C57BL/6 mice was immunized and antibody responses were measured at day 17 post-immunization (Fig 12). As expected, direct core-sAg fusions did not elicit anti-sAg antibody responses. Among the P2A-containing constructs, only the GT-D core-P2A-sAg vector consistently induced anti-sAg antibodies, while anti-sAg antibodies were observed in only one of five mice immunized with GT-B/C core-P2A-sAg. This result was unexpected, since Western Blots showed efficient separation of core and sAg in both the GT-D and GT-B/C core-P2A-sAg vectors. To confirm that the difference in anti-sAg antibody responses was not an artifact of the mouse strain selected, the same experiment was run in Balb/c mice. Results in the Balb/c mice were similar to the results in C57BL/6 mice: anti-sAg antibodies were detected in 4 of 5 Balb/c mice immunized with GT-D core-P2A-sAg, but only 1 of 5 mice immunized with GT-B/C core-P2A-sAg (Figure 12).

Example 8

Identification of Genetically Stable Replication-Incompetent LCMV Vectors Encoding Immunogenic Core-sAg Fusion Proteins

[0336] The stability of various immunogenic core-sAg fusion transgenes within replication-incompetent LCMV vectors (VV1) was evaluated by PCR after serial passaging of vector containing supernatant. Genetic stability was defined by the major band showing at the correct size of the full-length transgene (TG). Results are shown in Table 8.

Table 8

Overview Table for Assessment of Genetic Stability of Core-sAg Transgenes

Genotype	Vector	Stable TG insertion until
GT-B/C	VV1-Core-sAg	P6

GT-B/C	VV1-Core-P2A-sAg	P7
GT-D	VV1-Core-sAg	P4
GT-D	VV1-Core-P2A-sAg	P2
GT-D	VV1-iCore-P2A-sAg	P6

*VV1 refers to replication-incompetent LCMV vectors.

“P#” indicates the number of passages (*e.g.*, P1 equals 1 passage).

[0337] GT-D core-P2A sAg induced robust T cell responses and the highest anti-sAg antibody responses of the tested core-sAg fusion designs, but did not have favorable genetic stability in this analysis. However, the modified transgene GT-D iCore-P2A-sAg (polynucleotide SEQ ID NO:37, encoding polypeptide SEQ ID NO:41) showed improved genetic stability in a replication-incompetent LCMV vector (Table 8).

[0338] To confirm that the modified transgene did not impair T-cell immunogenicity of GT-D iCore-P2A-sAg, C57BL/6 mice were immunized using replication-incompetent LCMV vectors with the GT-D core-P2A-sAg and GT-D iCore-P2A-sAg designs, or mock immunized, and T cell responses were measured 7 days later by IFN- γ ELISPOT (Fig. 13). sAg ELISPOT responses were significantly higher with GT-D iCore-P2A-sAg, and core ELISPOT responses were numerically higher as well. Thus, the modified transgene of GT-D iCore-P2A-sAg resulted in both improved genetic stability and improved immunogenicity.

Example 9

Immunogenicity of Replication-Incompetent LCMV vectors in Outbred Mice

[0339] The immunogenicity of the replication-incompetent LCMV (VV1) vectors encoding various HBV antigens were evaluated in Diversity Outbred (DO) mice. These mice have more diverse MHC alleles than inbred C57BL/6 mice, so are better for evaluating genotype cross-reactivity of the T cell responses induced by vaccination.

Methods

[0340] DO mice were immunized twice at day 0 and day 28 with replication-incompetent LCMV vectors as indicated in Table 9. HBV-specific T cell responses were measured at day 42 by IFN- γ ELISPOT using splenocytes.

Table 9

Study Groups in Immunogenicity Study

Group	N	Prime vector - Day 0	Boost vector - Day 28	Harvest Day	Dose /vector
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1	8	Mock	Mock	42	-
2	8	VV1-GT-B/C Core-P2A-sAg	VV1-GT-B/C Core-P2A-sAg	42	10 ⁶ FFU
3	8	VV1-GT-D iCore-P2A-sAg	VV1-GT-D iCore-P2A-sAg	42	10 ⁶ FFU
4	8	VV1-GT-B Pol ^{Δ3}	VV1-GT-B Pol ^{Δ3}	42	10 ⁶ FFU
5	8	VV1-GT-B Pol ³⁰⁰	VV1-GT-B Pol ³⁰⁰	42	10 ⁶ FFU

Results

[0341] Replication-incompetent LCMV vectors encoding GT-B/C Core-P2A-sAg and GT-D iCore-P2A-sAg induced comparable T cell responses specific for their respective core antigen (Fig. 14A). The vector encoding GT-D iCore-P2A-sAg induced a higher frequency of T cells specific for its respective sAg antigen when compared to the vector encoding GT-B/C Core-P2A-sAg (Fig. 14A). The vector encoding GT-B Pol³⁰⁰ induced a numerically superior T cell response specific to pol antigens than the vector encoding GT-B Pol^{Δ3} (Fig. 14B). Thus, the vectors encoding for GT-D iCore-P2A-sAg and GT-B Pol³⁰⁰ are more immunogenic than the vectors encoding for GT-B/C Core-P2A-sAg and GT-B Pol^{Δ3} in outbred mice.

[0342] In addition to inducing T cells responses specific to their cognate antigens (*i.e.*, GT-D core, GT-D sAg, GT-B Pol antigens), the GT-D iCore-sAg and GT-B Pol³⁰⁰ vectors were also able to generate T cells responses specific for antigens obtained from different viral genotypes of HBV (*i.e.*, GT-B core, GT-B sAg, GT-D Pol antigens) (Fig. 15A and 15B). Thus, the vectors coding for GT-D iCore-sAg and GT-B Pol³⁰⁰ produce T cells which are cross-reactive for different genotypes of HBV.

Example 10

Immunogenicity of Replication-Incompetent LCMV Vectors Administered as Single Vector or Co-Formulated in C57BL/6 Mice

[0343] Replication-incompetent LCMV vectors encoding GT-D iCore-P2A-sAg and GT-B Pol300 are immunogenic in mice. We next compared their immunogenicity of both vectors when delivered either as single vectors or as a co-formulated mixture in C57BL/6 mice.

Methods

[0344] C57BL/6 mice were immunized twice at day 0 and day 21 with replication-incompetent LCMV vectors as indicated in Table 10. HBV-specific T cell responses were measured at day 28 by IFN-γ ELISPOT using splenocytes.

Table 10
Study Groups in Immunogenicity Study

Group	N	Vector Format	Prime vector D0	Boost vector D21	Harvest Day	Dose /vector
1	5	-	Mock	Mock	28	10 ⁶ FFU
2	5	Single vector	VV1-GT-D iCore_P2A_sAg	VV1-GT-D iCore_P2A_sAg	28	10 ⁶ FFU
3	5	Single vector	VV1-GT-B Pol ³⁰⁰	VV1-GT-B Pol ³⁰⁰	28	10 ⁶ FFU
4	5	Co-formulated	VV1-GT-D iCore_P2A_sAg + VV1-GT-B Pol ³⁰⁰	VV1-GT-D iCore_P2A_sAg + VV1-GT-B Pol ³⁰⁰	28	10 ⁶ FFU

Results

- 5 **[0345]** Consistent with data described above, vectors encoding GT-D iCore-P2A-sAg and GT-B Pol³⁰⁰ induced T cells responses specific for sAg, core and Pol when administered as single vectors (Figures 16A-16C). Administration of the same vectors as a co-formulated mixture induced comparable T cell responses (Figures 16A-C). Thus, co-formulation of the LCMV vectors encoding GT-D iCore-P2A-sAg and GT-B Pol³⁰⁰ does not interfere with their
- 10 immunogenicity in C57BL/6 mice.

Example 11

Immunogenicity of Replication-Incompetent LCMV vectors in Cynomolgus Macaques.

- [0346]** We evaluated the immunogenicity of the replication-incompetent LCMV (VV1) vectors GT-D iCore-P2A-sAg and GT-B Pol³⁰⁰ vectors in cynomolgus macaques. Ad5 and
- 15 vaccinia vectors encoding for the core, sAg, and Pol³⁰⁰ antigens were also tested.

Methods

- [0347]** Cynomolgus macaques were immunized using different routes, different doses and different immunization schedules as indicated in Table 11. HBV-specific T cell responses were measured using PBMC every 2 weeks by IFN-γ ELISPOT. Intracellular cytokine staining
- 20 was also performed on CD4+ and CD8+ T cells at week 14 by flow cytometry. Anti-sAg antibody responses were quantified every 4 weeks by ELISA.

Table 11

Study Groups in Immunogenicity Study

Group	N	Vaccine	Dose	Route	Immunization schedule (week)
1	5	VV1-GT-D iCore-P2A-sAg + VV1-GT-B Pol ³⁰⁰	5x10 ⁶ FFU/vector	i.m.	Every 4 weeks: 0, 4, 8, 12, 16, 20
2	5		10 ⁸ FFU/vector	i.m.	Every 4 weeks: 0, 4, 8, 12, 16, 20
3	5		5x10 ⁶ FFU/vector	i.m.	Every 8 weeks: 0, 8, 16, 24
4	5		10 ⁸ FFU/vector	i.m.	Every 8 weeks: 0, 8, 16, 24
5	5		10 ⁸ FFU/vector	i.v.	Every 8 weeks: 0, 8, 16, 24
6	5	1. Ad5-GT-D core-sAg + Ad5-GT-B Pol ³⁰⁰ (days 0 and 5) 2. Vaccinia GT-D core-sAg + Vaccinia GT-B Pol ³⁰⁰ (days 8 and 12)	10 ¹¹ vp/vector 10 ⁸ PFU/vector	i.m.	0 (Ad5), 4 (Ad5), 8 (Vac), 12 (Vac)

Results

[0348] Total HBV-specific T cell responses (defined as the sum of core, sAg and polymerase-specific responses shown in Figures 18A-18F to 20A-20F) to the VV1 GT-D iCore-P2A-sAg and GT-B Pol³⁰⁰ vectors were highest when administered via the intramuscular route (i.m.) and every 4 weeks (groups 1 and 2) (Figures 17A-B). Ad5 and vaccinia vectors encoding for the same antigens also induced comparable T cell responses. HBV-specific immune responses were detected after the first dose of VV1 GT-D iCore-P2A-sAg and GT-B Pol³⁰⁰ vectors, and doses two through four induced progressive increases in the HBV-specific ELISPOT magnitude. The fifth and sixth doses did not further increase responses, indicating that the peak response to our vectors was reached after the fourth dose. The geometric mean response by week 14 was 1206 SFU/10⁶ PBMC in animals administered the full human dose (10⁸ FFU, Group 2), and approximately 2-fold lower at the lower dose 5x10⁶ FFU (group 1) indicating dose-responsiveness.

[0349] To quantify the contribution of CD4+ and CD8+ T cells to the total T cell response, PBMCs from animals from group 1, 2 and 6 were analyzed by intracellular cytokine staining (ICS) on study week 14, when T cell responses were the highest. Both groups 1 and 2

had increased levels of IFN- γ + CD8+ T cells in response to stimulation with HBV peptides. The background-corrected frequency of these cells ranged from 0.8 % to 1.9 % in Group 1 and from 0.2% to 4% in Group 2 (Fig 21A). In contrast, IFN- γ + CD4+ T cells specific for HBV were detectable but at less than 0.1% of total CD4+ T cells (Fig 21B). Thus, the T cell response induced by our vectors in non-human primates is predominantly composed of CD8+ T cells.

[0350] Anti-HBsAg antibodies were also induced by dosing with our vectors. Anti-sAg responses increased with dose level and with repeated administration of the vectors (Fig. 22).

Example 12

Immunogenicity of Replication-Incompetent LCMV Vectors in Combination with Immunomodulators in C57BL/6 Mice

[0351] We evaluated the immunogenicity of replication-incompetent LCMV (VV1) vectors GT-D iCore-P2A-sAg and GT-B Pol300 alone or in combination with various immunomodulators (anti-PD-1, anti-CTLA-4 and anti-CD137 and FLT3 ligand) in the AAV-HBV mouse model.

Methods

[0352] AAV-HBV C57BL/6 mice were administered 3 doses of VV1-GT-D iCore-P2A-sAg and GT-B Pol300 vectors at day 0, day 21 and day 42. Mice were also treated with saline, anti-mouse inhibitory PD-1 antibody, anti-mouse inhibitory CTLA-4 antibody, anti-mouse stimulatory CD137 antibody and mouse FLT3-L as indicated in Table 12 and Fig 23. A control group of mice received the HBV vaccine alone but no AAV-HBV to determine how the immunogenicity of the HBV vaccine was affected in the context of chronic HBV. HBV-specific T cell responses were measured at day 105 post first vaccination by IFN- γ ELISPOT using splenocytes. Data are expressed after subtraction of background signal in no-peptide control wells. Serum levels of HBeAg were measured at day -11 and day 105 by ELISA.

Table 12
Study Groups in AAV-HBV Immunogenicity Study

Group	N	AAV-HBV	HBV Vaccine	Immuno-modulator	Molecule and Dose
1	11	Yes	VV1-GT-D iCore-P2A-sAg + VV1-GT-B Pol ³⁰⁰	Vehicle	Saline
2	12	Yes		α -PD-1	Clone RMP1-14 8 mg/kg/dose
3	12	Yes		α -CTLA4	Clone 9D9 10 mg/kg/dose
4	12	Yes		α -CD137	Clone mAb8 2.5 mg/kg/dose
5	12	Yes		FLT3L	Murine FLT3L-Fc 1 mg/kg/dose
6	5	No		Vehicle	Saline

Results

[0353] Robust IFN- γ ELISPOT responses were observed for all 3 HBV antigens in mice in the absence of persistent HBV (Fig 24). The IFN- γ ELISPOT responses obtained from AAV-HBV mice that received the HBV vaccine alone were reduced but still present, demonstrating that VV1 GT-D iCore-P2A-sAg and GT-B Pol300 were immunogenic even in the context of an immune system tolerized to HBV. Combined administration of VV1 GT-D iCore-P2A-sAg and GT-B Pol300 with anti-PD-1, anti-CTLA-4 or anti-CD137 antibodies further improved the HBV-specific IFN- γ ELISPOT responses to core and sAg, while combination of VV1 GT-D iCore-P2A-sAg and GT-B Pol300 with FLT3-L gave the highest ELISPOT magnitude for all 3 HBV antigens.

[0354] In addition, administration of VV1 GT-D iCore-P2A-sAg and GT-B Pol300 reduced the serum levels of HBeAg in those AAV-HBV mice as measured at baseline day -11 and at day 105 (Table 13). Importantly, combined administration of VV1 GT-D iCore-P2A-sAg and GT-B Pol300 vectors with anti-PD-1, anti-CTLA-4, anti-CD137 antibodies or FTL3-L further reduced the serum levels of HBeAg (Table 13). Thus, VV1 GT-D iCore-P2A-sAg and GT-B Pol300 vectors show antiviral efficacy in the AAV-HBV mouse model which can be enhanced in combination with some immunomodulators.

Table 13
Overview Table of Serum HBeAg Levels in AAV-HBV Mice

Group	Serum HBeAg Level (geometric mean, ng/mL)		Animals with serum HBeAg <100 ng/mL at day 105
	Day -11	Day 105	
HBV vaccine + saline	868	528	0/11
HBV vaccine + α -PD-1	879	337	3/12
HBV vaccine + α -CTLA4	661	341	2/12
HBV vaccine + α -CD137	1069	500	1/12
HBV vaccine + FLT3L-Fc	773	315	3/12

Example 13

Identification of Replication-Incompetent Pichinde (PICV) Vectors

Encoding Immunogenic Nucleotide-optimized HBV Antigens

[0355] We generated replication-incompetent PICV (VV2) vectors encoding the GT-D core-P2A-sAg antigen (SEQ ID NO: 41) and the GT-B Pol300 antigen (SEQ ID NO: 13), initially using the same nucleotide sequences identified to be stable and immunogenic in the

replication-incompetent LCMV (VV1) vectors. The stability of the iCore-P2A-sAg transgene in VV2 vectors (SEQ ID NO: 37) was evaluated by PCR after serial passaging of vector containing supernatant and found to be sufficiently stable for manufacture (Table 13). Genetic stability was defined by the major band showing at the correct size of the full-length transgene (TG).

5 [0356] In contrast, when the same GT-B Pol³⁰⁰ transgene used in the VV1 vectors (SEQ ID NO: 29) was used in VV2 vectors, the transgene rapidly became unstable during serial passage (Table 14). To identify VV2 vectors with sufficient genetic stability for manufacturing, we generated three additional VV2 vectors encoding the same GT-B Pol³⁰⁰ antigen using different nucleotide sequences, designated VV2-Pol300_IDT_CpGdel (SEQ ID NO: 94), Pol³⁰⁰ 10 ori (SEQ ID NO: 89), and Pol³⁰⁰ dint (SEQ ID NO: 90), Pol300 huCo low GC (SEQ ID NO: 91), and Pol300 oridel CpG (SEQ ID NO: 92). Each vector was evaluated for transgene stability by PCR after serial passaging of vector containing supernatant, with genetic stability defined by the major band showing at the correct size of the full-length transgene (TG). Results are shown in Table 14. Surprisingly, major differences in the stability of Pol³⁰⁰ transgenes in VV2 vectors 15 were evident between the different nucleotide sequences despite encoding the identical polypeptide antigen, with Pol³⁰⁰ dint, Pol³⁰⁰ ori, and Pol³⁰⁰ oridel CpG polynucleotide sequences demonstrating the greatest stability, *e.g.*, at least through five passages.

Table 14
Overview Table for Assessment of Genetic Stability of
VV2-Core-P2A-sAg and Pol300 Transgenes

Genotype	Nucleic acid SEQ ID NO:	Vector	Stable TG insertion until
GT-D	37	VV2-iCore-P2A-sAg	P5
GT-B	29	VV2-Pol ³⁰⁰	P1
GT-B	94	VV2-Pol300_IDT_CpGdel	P3
GT-B	91	VV2-Pol ³⁰⁰ huCo lowGC	P4
GT-B	89	VV2-Pol ³⁰⁰ ori	P5
GT-B	92	VV2-Pol ³⁰⁰ oridel CpG	P5
GT-B	90	VV2-Pol ³⁰⁰ dint	P5

*VV2 refers to replication-incompetent PICV vectors.

“P#” indicates the number of passages (*e.g.*, P1 equals 1 passage).

[0357] Next, to assess potential differences in immunogenicity between vectors carrying the Pol³⁰⁰ dint and Pol³⁰⁰ ori transgenes, C57BL/6 mice were immunized twice at day 0 and day 21 with replication-incompetent PICV (VV2) vectors encoding GT-B Pol³⁰⁰ ori or GT-B Pol³⁰⁰ dint. HBV-specific T cell responses were then measured from splenocytes by IFN- γ ELISPOT using Pol peptide pools. Surprisingly, VV2-GT-B Pol³⁰⁰ dint induced a much stronger T cell response than VV2-GT-B Pol³⁰⁰ ori despite encoding identical amino acid sequences (Fig. 25). Thus, VV2-GT-B Pol³⁰⁰ dint is more immunogenic than VV2-GT-B Pol³⁰⁰ ori in C57BL/6 mice.

Example 14

Immunogenicity of Replication-Incompetent LCMV and PICV Arenavirus Vectors Using Homologous or Heterologous Prime-Boost Immunization Regimens in C57BL/6 Mice

[0358] We evaluated the immunogenicity of replication-incompetent LCMV (VV1) and PICV (VV2) vectors encoding GT-D iCore-P2A-sAg and GT-B Pol³⁰⁰ using homologous prime/boost (VV1 vector followed by VV1 vector) or heterologous prime-boost (VV2 vector followed by VV1 vector) immunization regimens in C57BL/6 mice.

Methods

[0359] C57BL/6 mice were immunized twice with replication-incompetent LCMV and PICV vectors encoding GT-D iCore-P2A-sAg and GT-B Pol³⁰⁰ as indicated in Table 15. HBV-specific T cell responses were measured at day 28 by IFN- γ ELISPOT using splenocytes. Anti-sAg antibody responses were quantified at day 28 by ELISA.

Table 15
Study Groups in Immunogenicity Study

Group	N	Immunization Regimen	Prime vector Day 0	Boost vector Day 21	Harvest Day	Dose /vector
1	5	-	Mock	Mock	28	10 ⁶ FFU
2	5	Homologous Prime/Boost	VV1-GT-D iCore-P2A-sAg	VV1-GT-D iCore-P2A-sAg	28	10 ⁶ FFU
3	5	Heterologous Prime/Boost	VV2-GT-D iCore-P2A-sAg	VV1-GT-D iCore-P2A-sAg	28	10 ⁶ FFU
4	5	Homologous Prime/Boost	VV1-GT-B Pol ³⁰⁰	VV1-GT-B Pol ³⁰⁰	28	10 ⁶ FFU
5	5	Heterologous Prime/Boost	VV2-GT-B Pol ³⁰⁰ dint	VV1-GT-B Pol ³⁰⁰	28	10 ⁶ FFU

Results

5 [0360] Administration of the replication-incompetent LCMV vector (VV1) encoding GT-D iCore-P2A-sAg or encoding GT-B Pol³⁰⁰ using a homologous prime/boost regimen (VV1/VV1) induced robust T cell responses in C57BL/6 mice (Figures 26A-C). Administration of the replication-incompetent PICV vector (VV2) followed by the administration of VV1 (heterologous prime-boost regimen VV2/VV1) yielded greater sAg-specific T cell response (Fig. 26A) and similar core and Pol-specific T cell responses (Figs. 26B-26C) compared to the VV1/VV1 regimen. Furthermore, while administration of the replication-incompetent LCMV vector using a homologous prime/boost regimen (VV1/VV1) inconsistently induced anti-sAg antibodies at low levels, immunization using the heterologous prime/boost regimen (VV2/VV1) unexpectedly led to robust and consistent induction of anti-sAg antibodies in all animals and an approximately 1000-fold increase in the average anti-sAg antibody titer (Fig. 27).

Example 15

Immunogenicity of Replication-Attenuated LCMV and PICV Arenavirus Vectors Using Homologous or Heterologous Prime-Boost Immunization Regimens in C57BL/6 Mice

20 [0361] In addition to the replication-incompetent arenavirus vectors LCMV (VV1) and PICV (VV2), replication-competent but attenuated vectors LCMV (TT1) and PICV (TT2) encoding HBV antigens can also be engineered. Unlike VV1 and VV2 vectors, TT1 and TT2 vectors contain three genomic segments allowing genomic space to insert the two HBV antigens

(the fusion protein GT-D core-P2A-sAg and the protein GT-B Pol³⁰⁰) into the same vector.

Because each antigen can be inserted into two different genomic segments, vectors covering the different combinations of insertion within both arenavirus vectors were generated as follows: i)

GT-D core-P2A-sAg inserted into segment 1 and GT-B Pol³⁰⁰ inserted into segment 2 into the

5 LCMV backbone (TT1-GT-D core-P2A-sAg/GT-B Pol³⁰⁰), ii) GT-D core-P2A-sAg inserted

into segment 1 and GT-B Pol³⁰⁰ inserted into segment 2 into the PICV backbone (TT2-GT-D

core-P2A-sAg/GT-B Pol³⁰⁰), iii) GT-D core-P2A-sAg inserted into segment 2 and GT-B Pol³⁰⁰

inserted into segment 1 into the LCMV backbone (TT1-GT-B Pol³⁰⁰/GT-D core-P2A-sAg) and

iv) GT-D core-P2A-sAg inserted into segment 2 and GT-B Pol³⁰⁰ inserted into segment 1 into

10 the PICV backbone (TT2-GT-B Pol³⁰⁰/GT-D core-P2A-sAg). We next evaluated the

immunogenicity of these 4 vectors using homologous or heterologous prime-boost

immunization regimens in C57BL/6 mice.

Methods

[0362] C57BL/6 mice were immunized twice with replication-attenuated LCMV and

15 PICV vectors encoding GT-D Core-P2A-sAg and GT-B Pol³⁰⁰ as indicated in Table 16. HBV-

specific T cell responses were measured at day 28 by IFN- γ ELISPOT using splenocytes.

Table 16
Study Groups in Immunogenicity Study

Group	N	Prime vector Day 0	Boost vector Day 21	Harvest Day	Dose/vector (RCV/FFU)
1	5	Mock	Mock	28	-
2	5	TT1-GT-D core-P2A-sAg/ GT-B Pol ³⁰⁰	TT1-GT-D core-P2A-sAg/ GT-B Pol ³⁰⁰	28	5x10 ⁴
3	5	TT2-GT-D core-P2A-sAg/ GT-B Pol ³⁰⁰	TT2-GT-D core-P2A-sAg/ GT-B Pol ³⁰⁰	28	5x10 ⁴
4	5	TT2-GT-D core-P2A-sAg/ GT-B Pol ³⁰⁰	TT1-GT-D core-P2A-sAg/ GT-B Pol ³⁰⁰	28	5x10 ⁴
5	5	TT1-GT-B Pol ³⁰⁰ / GT-D core-P2A-sAg	TT1-GT-B Pol ³⁰⁰ / GT-D core-P2A-sAg	28	5x10 ⁴
6	5	TT2-GT-B Pol ³⁰⁰ / GT-D core-P2A-sAg	TT2-GT-B Pol ³⁰⁰ / GT-D core-P2A-sAg	28	5x10 ⁴
7	5	TT2-GT-B Pol ³⁰⁰ / GT-D core-P2A-sAg	TT1-GT-B Pol ³⁰⁰ / GT-D core-P2A-sAg	28	5x10 ⁴

Results

[0363] Administration of all replication-competent vectors resulted in robust T cells responses specific for the 3 HBV antigens sAg, core and Pol (Figures 28A-28C). Thus, TT1 and TT2 vectors expressing HBV antigens are strongly immunogenic in C57BL/6 mice.

5

Example 16

Immunogenicity of Replication-Incompetent LCMV and PICV Arenavirus Vectors using Homologous or Heterologous Prime-Boost Immunization Regimens in Cynomolgus Macaques

[0364] We evaluated the immunogenicity of replication-incompetent LCMV (VV1) and
10 PICV (VV2) vectors encoding GT-D iCore-P2A-sAg and GT-B Pol300 using homologous prime/boost (VV1 vector followed by VV1 vector) or heterologous prime-boost (VV2 vector followed by VV1 vector) immunization regimens in cynomolgus macaques.

Methods

[0365] Cynomolgus macaques (n=5) were immunized with VV2 vectors (5×10^6
15 FFU/vector) at week 0 and then immunized with VV1 vectors (5×10^6 FFU/vector) at week 4, and HBV-specific T cell responses were measured using PBMC by IFN- γ ELISPOT at week 6. Data were compared to ELISPOTs from 10 cynomolgus macaques immunized with VV1 vectors only (5×10^6 FFU/vector) at both week 0 and week 4 (homologous prime boost regimen).

Results

20 [0366] Administration of the replication-incompetent LCMV vectors (VV1) encoding GT-D iCore-P2A-sAg and GT-B Pol300 using a homologous prime/boost regimen (VV1/VV1) induced HBV-specific T cell responses in 5 out of 10 cynomolgus macaques (Fig. 29). In contrast, administration of the replication-incompetent PICV vector (VV2) followed by VV1 (heterologous prime/boost regimen VV2/VV1) yielded statistically greater HBV-specific T cell
25 responses in all 5 animals compared to the VV1/VV1 homologous prime boot regimen (Fig. 29).

Example 17

Immunogenicity of Replication-Incompetent LCMV and PICV Arenavirus Vectors using Homologous or Heterologous Prime-Boost Immunization Regimens with 1-week Dosing Intervals in Cynomolgus Macaques

30 [0367] We evaluated the immunogenicity of replication-incompetent LCMV (VV1) and PICV (VV2) vectors encoding GT-D iCore-P2A-sAg and GT-B Pol300 using homologous prime/boost (VV1 vector followed by VV1 vector) or heterologous prime-boost (VV2 vector

followed by VV1 vector) immunization regimens administered with a 1-week dosing interval in cynomolgus macaques.

Methods

[0368] Cynomolgus macaques were immunized as described in Table 17. HBV-specific

5 T cell responses were measured using PBMC by IFN- γ ELISPOT at week 4.

Table 17
Study Groups in Immunogenicity Study

Group	N	Vaccine Prime	Vaccine Boost	Dose / vector	Immunization schedule (week)	ELISPOT analysis
1	5	VV1	VV1	10 ⁸ FFU	0 (VV1) 1 (VV1) 2 (VV1) 3 (VV1)	Week 4
2	5	VV2	VV1	10 ⁸ FFU	0 (VV2) 1 (VV1) 2 (VV2) 3 (VV1)	Week 4

Results

10 **[0369]** Administration of the replication-incompetent PICV vector (VV2) followed by VV1 (heterologous prime/boost regimen VV2/VV1) yielded greater HBV-specific T cell responses compared to vaccination with VV1 vector alone (Fig. 30).

[0370] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

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CLAIMS

What is claimed is:

1. A polynucleotide encoding a truncated hepatitis B virus (HBV) polymerase polypeptide, the polypeptide comprising an inactivated reverse transcriptase domain and an inactivated RNase H, wherein the polypeptide is no longer than 600 amino acids in length and does not comprise all of the terminal protein (TP) domain and does not comprise all or part of the Spacer domain, wherein the polypeptide comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 13-14.
2. The polynucleotide of claim 1, comprising or consisting of the nucleic acid sequence of any one of SEQ ID NOs: 29 and 89-94, or the nucleic acid sequence that is at least 99% identical to the full length of any one of SEQ ID NOs: 29 and 89-94.
3. An expression cassette, comprising a polynucleotide of claim 1 operably linked to one or more regulatory sequences.
4. A vector comprising one or more polynucleotides of claim 1.
5. The vector of claim 4, wherein the vector is a viral vector.
6. The vector of claim 5, wherein the viral vector is an arenavirus vector selected from Lymphocytic choriomeningitis mammarenavirus (LCMV), Pichinde mammarenavirus (PICV), Guanarito virus (GTOV), Junin virus (JUNV), Lassa virus (LASV), Lujo virus (LUJV), Machupo virus (MACV), Sabia virus (SABV), and Whitewater Arroyo virus (WWAV).
7. The vector of claim 6, wherein the viral vector is an arenavirus vector selected from Lymphocytic choriomeningitis mammarenavirus (LCMV) or Pichinde mammarenavirus (PICV).
8. The vector of claim 5, wherein the viral vector is a replication-defective arenavirus having a bi-segmented genome.

9. An isolated host cell comprising the vector of claim 5.

10. A kit comprising one or more unitary doses of the vector of claim 5.

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11. A polynucleotide encoding a fusion protein comprising or consisting of the amino acid sequence of SEQ ID NO: 41, or the amino acid sequence that is at least 94%, 95%, 96%, 97%, 98% or 99% identical to the full length of SEQ ID NO: 41, the fusion protein comprising in sequential order from the N-terminus to the C-terminus, an HBV core polypeptide and an HBV small surface antigen (sAg) polypeptide, wherein the fusion protein is no longer than 450 amino acids in length, does not comprise an HBV pre-S1 polypeptide and/or an HBV pre-S2 polypeptide, and wherein the core polypeptide is from an HBV genotype D and the sAg polypeptide is from an HBV genotype D, wherein the polynucleotide comprises or consists of the nucleic acid sequence of SEQ ID NO: 37, or the nucleic acid sequence that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the full length of SEQ ID NO: 37.

12. A truncated hepatitis B virus (HBV) polymerase polypeptide comprising an inactivated reverse transcriptase domain and an inactivated RNase H, wherein the polypeptide is no longer than 600 amino acids in length and does not comprise all of the terminal protein (TP) domain and does not comprise all or part of the Spacer domain, wherein the polypeptide comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 13-14.

13. A fusion protein comprising in sequential order from the N-terminus to the C-terminus, an HBV core polypeptide and an HBV small surface antigen (sAg) polypeptide, wherein the fusion protein is no longer than 450 amino acids in length, does not comprise an HBV pre-S1 polypeptide and/or an HBV pre-S2 polypeptide, and wherein: the core polypeptide is from an HBV genotype D and the sAg polypeptide is from an HBV genotype D, wherein the fusion protein comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 40-41, or the amino acid sequence that is at least 97%, 98% or 99% identical to the full length of any one of SEQ ID NOs: 40-41.

14. A polynucleotide encoding the core-sAg fusion protein of claim 13.

15. An expression cassette, comprising the polynucleotide of claim 14 operably linked to one or more regulatory sequences.

16. A vector comprising one or more polynucleotides of claim 14.

17. The vector of claim 16, wherein the vector is a viral vector.

18. The vector of claim 17, wherein the viral vector is an arenavirus vector selected from Lymphocytic choriomeningitis mammarenavirus (LCMV), Pichinde mammarenavirus (PICV), Guanarito virus (GTOV), Junin virus (JUNV), Lassa virus (LASV), Lujo virus (LUJV), Machupo virus (MACV), Sabia virus (SABV), and Whitewater Arroyo virus (WWAV).

19. The vector of claim 18, wherein the viral vector is an arenavirus vector selected from Lymphocytic choriomeningitis mammarenavirus (LCMV) or Pichinde mammarenavirus (PICV).

20. An arenavirus vector comprising a polynucleotide encoding an HBV core-sAg fusion polypeptide comprising or consisting of the amino acid sequence of any one of SEQ ID NOs: 40-41, or the amino acid sequence that is at least 97%, 98% or 99% identical to the full length of any one of SEQ ID NOs: 40-41, and wherein the sAg polypeptide is no longer than 450 amino acids in length and does not comprise an HBV pre-S1 polypeptide and/or an HBV pre-S2 polypeptide.

21. An arenavirus vector comprising a polynucleotide encoding an HBV core-sAg fusion polypeptide comprising or consisting of the amino acid sequence of any one of SEQ ID NO: 41, or the amino acid sequence that is at least 94%, 95%, 96%, 97%, 98% or 99% identical to the full length of any one of SEQ ID NOs: 41, and wherein the sAg polypeptide does not comprise an HBV pre-S1 polypeptide and/or an HBV pre-S2 polypeptide.

22. The arenavirus vector of claim 20, wherein the polynucleotide comprises or consists of the nucleic acid sequence of any one of SEQ ID NOs: 35-37, or that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the full length of any one of SEQ ID NOs: 35-37.

23. The arenavirus vector of claim 20, wherein the polynucleotide comprises or consists of the nucleic acid sequence of SEQ ID NO: 37, or that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the full length of SEQ ID NO: 37.

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24. The arenavirus vector of claim 20, wherein the vector has a bisegmented genome and further comprises a polynucleotide encoding a truncated HBV polymerase comprising or consisting of the amino acid sequence of any one of SEQ ID NOs: 13-14 and wherein the truncated HBV polymerase is no longer than 600 amino acids in length and does not
10 comprise all of an HBV polymerase terminal protein (TP) domain and does not comprise all or part of an HBV polymerase Spacer domain.

25. The arenavirus vector of claim 24, wherein the polynucleotide comprises or consists of the nucleic acid sequence of any one of SEQ ID NOs: 29 and 89-94, or that is at least
15 99% identical to the full length of any one of SEQ ID NOs: 29 and 89-94.

26. The arenavirus vector of claim 24, wherein the arenavirus vector is a Lymphocytic choriomeningitis mammarenavirus (LCMV) vector and the polynucleotide comprises or consists of the nucleic acid sequence of SEQ ID NO: 29, or that is at least 85%,
20 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the full length of SEQ ID NO: 29.

27. The arenavirus vector of claim 24, wherein the arenavirus vector is a Pichinde mammarenavirus (PICV) vector and the polynucleotide comprises or consists of the nucleic acid
25 sequence of SEQ ID NO: 90, or that is at least 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the full length of SEQ ID NO: 90.

28. An arenavirus vector comprising a polynucleotide encoding a truncated HBV polymerase comprising or consisting of the amino acid sequence of any one of SEQ ID NOs:
30 13-14, and wherein the truncated HBV polymerase is no longer than 600 amino acids in length and does not comprise all of an HBV polymerase terminal protein (TP) domain and does not comprise all or part of an HBV polymerase Spacer domain.

29. The arenavirus vector of claim 28, wherein the polynucleotide comprises or consists of the nucleic acid sequence of any one of SEQ ID NOs: 29 and 89-94, or that is at least 99% identical to the full length of any one of SEQ ID NOs: 29 and 89-94.

5 30. The arenavirus vector of claim 28, wherein the arenavirus vector is a Lymphocytic choriomeningitis mammarenavirus (LCMV) vector and the polynucleotide comprises or consists of the nucleic acid sequence of SEQ ID NO: 29, or the nucleic acid sequence that is at least 99% identical to the full length of any one of SEQ ID NO: 29.

10 31. The arenavirus vector of claim 28, wherein the arenavirus vector is a Pichinde mammarenavirus (PICV) vector and the polynucleotide comprises or consists of the nucleic acid sequence of SEQ ID NO: 90, or the nucleic acid sequence that is at least 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the full length of SEQ ID NO: 90.

15 32. The arenavirus vector of claim 20, wherein the arenavirus vector is replication-defective, replication-deficient, or replication-incompetent.

33. An isolated host cell comprising one or more vectors of claim 20.

20 34. A kit comprising one or more unitary doses of one or more vectors of claim 20.

35. An immunogenic composition comprising a first viral expression vector and a second viral expression vector, wherein: the first viral expression vector comprises a
25 polynucleotide encoding a truncated HBV polymerase polypeptide mutant, wherein the polypeptide is no longer than 600 amino acids in length, does not comprise all of the terminal protein (TP) domain and does not comprise all or part of the Spacer domain, wherein the polypeptide comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 13-14; and the second viral expression vector comprises a polynucleotide encoding a core-sAg
30 fusion protein comprising in sequential order from the N-terminus to the C terminus, an HBV core polypeptide and an HBV small surface antigen (sAg) polypeptide, wherein the fusion protein is no longer than 450 amino acids in length, and wherein the core polypeptide is from an HBV genotype D and the sAg polypeptide is from an HBV genotype D, wherein the fusion protein comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 40-41, or

the amino acid sequence that is at least 97%, 98% or 99% identical to the full length of any one of SEQ ID NOs: 40-41.

36. The immunogenic composition of claim 35, comprising a first viral expression vector and a second viral expression vector, wherein: the first viral expression vector comprises a polynucleotide encoding an HBV polymerase polypeptide mutant comprising or consisting of the amino acid sequence of SEQ ID NO: 13; and the second viral expression vector comprises a polynucleotide encoding the core-sAg fusion protein comprising or consisting of the amino acid sequence of SEQ ID NO: 41, or the amino acid sequence that is at least 97%, 98% or 99% identical to the full length of SEQ ID NO: 41.

37. The immunogenic composition of claim 35, comprising a first viral expression vector and a second viral expression vector, wherein: a) the first viral expression vector comprises a polynucleotide comprising or consisting of the nucleic acid sequence of any one of SEQ ID NOs: 29, 32, 89, 90, 91, 92, 93 and 94, or the nucleic acid sequence that is at least 99% identical to the full length of any one of SEQ ID NOs: 29, 32, 89, 90, 91, 92, 93 and 94; b) the second viral expression vector comprises a polynucleotide comprising or consisting of the nucleic acid sequence of any one of SEQ ID NOs: 33-37 or the nucleic acid sequence that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the full length of any one of SEQ ID NOs: 33-37.

38. The immunogenic composition of claim 35, comprising a first viral expression vector and a second viral expression vector, wherein: a) the first viral expression vector comprises a polynucleotide comprising or consisting of the nucleic acid sequence of SEQ ID NOs: 29, 89, 90 or 92, or the nucleic acid sequence that is at least 99% identical to the full length of SEQ ID NOs: 29, 89, 90 or 92; and b) the second viral expression vector comprises a polynucleotide comprising or consisting of the nucleic acid sequence of SEQ ID NO: 37 or the nucleic acid sequence that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the full length of any one of SEQ ID NO: 37.

39. The immunogenic composition of claim 35, wherein the first viral expression vector and the second viral expression vector are independently from a taxonomic family selected from Adenoviridae, Arenaviridae, Herpesviridae, Poxviridae, Flaviviridae, Rhabdoviridae, and Togaviridae.

40. The immunogenic composition of claim 35, wherein the first viral expression vector and the second viral expression vector are from the same taxonomic family.

5 41. The immunogenic composition of claim 40, wherein the first viral expression vector and the second viral expression vector are from Arenaviridae.

42. The immunogenic composition of claim 40, wherein the first viral expression vector and the second viral expression vector are independently from an arenavirus vector selected from Lymphocytic choriomeningitis mammarenavirus (LCMV), Pichinde
10 mammarenavirus (PICV), Guanarito virus (GTOV), Junin virus (JUNV), Lassa virus (LASV), Lujo virus (LUJV), Machupo virus (MACV), Sabia virus (SABV), and Whitewater Arroyo virus (WWAV).

43. The immunogenic composition of claim 40, wherein the first viral expression
15 vector and the second viral expression vector are from an arenavirus vector selected from Lymphocytic choriomeningitis mammarenavirus (LCMV) or Pichinde mammarenavirus (PICV).

44. The immunogenic composition of claim 40, wherein the first viral expression
20 vector and the second viral expression vector are replication-defective or replication-deficient.

45. The immunogenic composition of claim 40, wherein the first viral expression vector and the second viral expression vector are replication-attenuated.

25 46. The immunogenic composition of claim 35, wherein the first viral expression vector and the second viral expression vector are from different taxonomic families.

47. The immunogenic composition of claim 35, wherein the first viral expression vector and the second viral expression vector are provided in a ratio in the range of from 1:10 to
30 10:1.

48. The immunogenic composition of claim 35, comprising in the range of about 10^3 to about 10^{12} viral focus forming units (ffu) or plaque forming units (pfu) or infectious units (iu) or viral particles (vp) per milliliter of each of the first viral expression vector and the second
35 viral expression vector.

49. The immunogenic composition of claim 35, further comprising one or more of an adjuvant, a detergent, a micelle-forming agent, and an oil.

5 50. The immunogenic composition of claim 35, formulated for administration via a route selected from the group consisting of intravenous, intramuscular, intradermal, subcutaneous and mucosal.

10 51. The immunogenic composition of claim 35, formulated as a liquid.

52. The immunogenic composition of claim 35, wherein the composition is lyophilized.

15 53. A kit comprising one or more unitary doses of the immunogenic composition of claim 35.