(19) DANMARK

(10) **DK/EP 4037708 T3**

(12) Oversættelse af europæisk patentskrift

Patent- og Varemærkestyrelsen

(51) Int.Cl.: ***A 61 K 39/29 (2006.01)*** ***A 61 P 31/20 (2006.01)*** ***C 12 N 15/86 (2006.01)***

(45) Oversættelsen bekendtgjort den: **2024-09-30**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2024-09-18**

(86) Europæisk ansøgning nr.: **20792808.6**

(86) Europæisk indleveringsdag: **2020-09-28**

(87) Den europæiske ansøgnings publiceringsdag: **2022-08-10**

(86) International ansøgning nr.: **US2020053060**

(87) Internationalt publikationsnr.: **WO2021067181**

(30) Prioritet: **2019-09-30 US 201962908494 P**

(84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**

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(54) Benævnelse: **HBV-vacciner og fremgangsmåder til at behandle HBV**

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DESCRIPTION

Description

BACKGROUND

[0001] 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-Bourgine, 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; Cavenaugh, 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 (Cavenaugh, *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.

[0002] 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; Cavenaugh, *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; Cavenaugh, *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.

[0003] WO2017/076988 discloses genetically modified arenaviral vectors suitable as vaccines for the prevention and treatment of Hepatitis B virus infections. Also disclosed are pharmaceutical compositions and methods for the treatment of Hepatitis B virus infections.

SUMMARY

[0004] The present invention is defined in the appended claims.

[0005] Disclosed herein are truncated hepatitis B virus (HBV) polymerase polypeptides, e.g., capable of inducing or eliciting an immune response in a human upon administration. The truncated HBV polymerase polypeptide may comprise an inactivated reverse transcriptase domain and an inactivated RNase H, and may, in some instances, not comprise all of the terminal protein (TP) domain and all or part of the Spacer domain. The polypeptide may be 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. 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). 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). The polypeptide may be from an HBV genotype A, B, C or D. The polypeptide may be 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 the polypeptide may be 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. 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] Also disclosed herein are HBV polymerase deletion mutant polypeptides. The HBV polymerase deletion mutant polypeptide may comprise 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. The polypeptide may be 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. The reverse transcriptase domain may not comprise a YMDD motif (SEQ ID NO: 97) and the RNase H domain may not comprise an AELL motif (SEQ ID NO: 98). The YMDD motif (SEQ ID NO: 97) in the reverse transcriptase domain may be mutated to YMHD (SEQ ID NO: 99) and wherein the AELL motif (SEQ ID NO: 98) in the RNase H domain may be mutated to AHLL (SEQ ID NO: 100). The polypeptide may be from an HBV genotype A, B, C or D. (a) The polypeptide may be 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 may be 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 may be 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 may be 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. The HBV polymerase deletion mutant polypeptide may comprise or consist 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. The HBV polymerase deletion mutant polypeptide may further comprise (*e.g.*, is a fusion protein including) an HBV core polypeptide. The HBV polymerase deletion mutant polypeptide may comprise 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. The HBV polymerase deletion mutant polypeptide may comprise or consist 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] Disclosed herein is an HBV core-sAg fusion protein. The core-sAg fusion protein may comprise in sequential order from the N-terminus to the C-terminus, an HBV core polypeptide and an HBV small surface antigen (sAg) polypeptide. The core polypeptide may be from an HBV genotype B or C and the sAg polypeptide is from an HBV genotype C. The core polypeptide may be from an HBV genotype D and the sAg polypeptide may be from an HBV genotype D. The core-sAg fusion protein may comprise: (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 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 to SEQ ID NO: 4. The core polypeptide may comprise 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. The sAg polypeptide may comprise 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. The sAg polypeptide may comprise 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. The sAg polypeptide may not comprise a pre-S1 polypeptide. The sAg polypeptide may not comprise a pre-S2 polypeptide. The sAg polypeptide may 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. The sAg polypeptide may not comprise both of an HBV pre-S1 polypeptide and an HBV pre-S2 polypeptide. The sAg polypeptide may 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. The core-sAg fusion protein may comprise a cleavable linker operably linked to and positioned between the HBV core polypeptide and the HBV sAg polypeptide. The cleavable linker may be a 2A cleavable peptide. The cleavable linker may be 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). The cleavable linker may be a porcine teschovirus-1 (P2A) linker. The cleavable linker may comprise or consist 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), APVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 57), QCTNYALLKLAGDVESNPGP (SEQ ID NO: 58), or EGRGSLLTCGDVEENPGP (SEQ ID NO: 59). The cleavable linker may comprise or consist 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 ATNFSLLKQAGDVEENPGP (SEQ ID NO: 56). The core-sAg fusion protein may comprise 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. The furin recognition/cleavage site may comprise or consist of an amino acid sequence selected from RAKR (SEQ ID NO: 60), REKR (SEQ ID NO: 61) and RRKR (SEQ ID NO: 62). The flexible linker may comprise a polyglycine or polyalanine sequence. The flexible linker may comprise or consist of a polyglycine or polyalanine sequence selected from AA, AAA, AAY, GG, GGG, GGSand GGGS (SEQ ID NO: 63). The core-sAg fusion protein may be 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. The core-sAg fusion protein may comprise or consist 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. The fusion polypeptide may comprise 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. The core-sAg fusion polypeptide may 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, the truncated HBV polymerase polypeptide, the HBV polymerase deletion mutant polypeptide, or the core-sAg fusion protein, as described herein, may further comprise an N-terminal signal peptide or leader sequence. The signal peptide or leader sequence may be 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. The signal peptide or leader sequence may be 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). The signal peptide or leader sequence may be 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. 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. The truncated HBV polymerase polypeptide, the HBV polymerase deletion mutant polypeptide, and/or the core-sAg fusion protein, as described herein, may be 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. The truncated HBV polymerase polypeptide, the HBV polymerase deletion mutant polypeptide, and/or the core-sAg fusion protein, as described herein, may be 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. The truncated HBV polymerase polypeptide, the HBV polymerase deletion mutant polypeptide, and/or the core-sAg fusion protein, as described herein, may be 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] Also disclosed herein are polynucleotides encoding the immunogenic HBV polypeptides, as described herein. For example, disclosed herein 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. The polynucleotide may comprise cDNA, mRNA, self-amplifying RNA (SAM), self-replicating RNA, or self-amplifying replicon RNA (RepRNA). The polynucleotide may comprise self-replicating or self-amplifying alphavirus replicons. The polynucleotide may comprise or consist 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] Also disclosed herein is a lipid nanoparticle (LNP) comprising one or more of the polynucleotides encoding an immunogenic HBV polypeptide, as described herein.

[0011] Also disclosed herein 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. The polynucleotide may be operably linked to and under the control of a constitutive promoter. The promoter may be selected from cytomegalovirus major immediate-early (CMV), the CMV enhancer fused to the chicken beta-actin promoter (CAG), human elongation factor-1α (HEF-1α), mouse cytomegalovirus (mouse CMV), Chinese hamster elongation factor-1α (CHEF-1α), and phosphoglycerate kinase (PGK).

[0012] Disclosed herein are vectors comprising one or more of the polynucleotides encoding an immunogenic HBV polypeptide, as described herein, or one or more expression cassettes comprising such polynucleotides. The vector may be a plasmid vector, a bacterial vector or a viral vector. The vector may be a viral vector. The viral vector may be a DNA virus or an RNA virus. The viral vector may be from a virus selected from adenovirus, adeno-associated virus, arenavirus, alphavirus, poxvirus, cytomegalovirus, rhabdovirus, vesicular stomatitis virus, flavivirus, maraba virus and vaccinia virus. The viral vector may be 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 the present invention, the viral vector is an arenavirus vector, and may be 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 viral vector is an arenavirus vector selected from Lymphocytic choriomeningitis mammarenavirus (LCMV) or Cali mammarenavirus (*a.k.a.,* Pichinde mammarenavirus or Pichinde arenavirus (PICV)). Disclosed herein, the viral vector may be a human adenovirus or a simian adenovirus (*e.g.*, a chimpanzee adenovirus, a gorilla adenovirus or a rhesus adenovirus). The viral vector may be an adenovirus vector 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), 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), 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). The viral vector may be replication-defective, replication-deficient, replication-attenuated or replication-competent. The viral vector may be a replication-defective arenavirus having a bi-segmented genome. The viral vector may be a replication-attenuated arenavirus having a tri-segmented genome.

[0013] Also disclosed herein are arenavirus vectors. The arenavirus vector may comprise 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:41and wherein the sAg polypeptide does not comprise an HBV pre-S1 polypeptide and/or an HBV pre-S2 polypeptide. The core polypeptide may comprise 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. The sAg polypeptide may comprise 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. The sAg polypeptide may comprise 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. The core-sAg fusion polypeptide may comprise 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. The polynucleotide may comprise or consist of a nucleic acid sequence of any one of SEQ ID NOs: 33-37, or that is at least 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. The polynucleotide may comprise or consist of a 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 SEQ ID NO: 37. The arenavirus vector may have 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. The truncated HBV polymerase may 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 NO: 51. The polynucleotide may comprise or consist 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. The arenavirus vector may be a Lymphocytic choriomeningitis mammarenavirus (LCMV) vector and the polynucleotide may comprise or consist 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. The arenavirus vector may be a Cali mammarenavirus *(a.k.a.,* Pichinde mammarenavirus or Pichinde arenavirus (PICV)) vector and the polynucleotide may comprise or consist 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] In another aspect of the invention, there is provided an arenavirus vector comprising a polynucleotide encoding a truncated HBV polymerase comprising or consisting of an amino acid sequence of any one of SEQ ID NOs: 13-14. Also disclosed herein is an arenavirus vector comprising a polynucleotide encoding a truncated HBV polymerase comprising or consisting of an amino acid 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. The truncated HBV polymerase may 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 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 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 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to 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 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 90. In some embodiments, the arenavirus vector is replication-defective, replication-deficient, or replication-incompetent.

[0015] In a further aspect of the invention, there are provided host cells comprising one or more vectors comprising the arenavirus vectors of the invention. Also disclosed herein are host cells comprising one or more polynucleotides encoding one or more immunogenic HBV polypeptides as disclosed herein, or one or more vectors comprising such polynucleotides. The one or more polynucleotides encoding one or more immunogenic HBV polypeptides, as described herein, may be not integrated into the host cell genome, *e.g.,* are episomal. The one or more polynucleotides may be integrated into the host cell genome. The host cell may be a mammalian cell, *e.g.,* a human cell. The host cell may be *in vitro* or *in vivo*.

[0016] Also disclosed herein are immunogenic compositions comprising one or more of the immunogenic HBV polypeptides, as described herein. The immunogenic composition may comprise 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 composition may comprise 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 composition may comprise one or more, *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 acceptable carrier. The immunogenic composition may comprise one or more polynucleotides in the form of DNA, cDNA, mRNA, or self-replicating RNA. In an aspect of the present invention, there is provided an immunogenic composition comprising one or more arenavirus vectors of the present invention. 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 of the invention; and (b) the second viral expression vector comprises a polynucleotide encoding the core-sAg fusion protein of the present invention. Also disclosed herein is an immunogenic composition which 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: 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. Also disclosed herein is an immunogenic composition which 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 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 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 polynucleotide encoding an HBV polymerase polypeptide mutant comprising or consisting of an amino acid sequence of 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 . 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. 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. Also disclosed herein, the sAg polypeptide may comprise 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. The core-sAg fusion polypeptide may comprise 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: 29 and 89-94 *e.g.,* SEQ ID NOs: 29, 89, 90 or 92, or a sequence that is at least 99% identical to any one of SEQ ID NOs: 29 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 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 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 29 or at least 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 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 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 37. As disclosed herein, the first viral expression vector and the second viral expression vector may be 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). 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 the immunogenic compositions of the invention, 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 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 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 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 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 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] Also disclosed herein are kits. The kit may comprise 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. The kit may comprise 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. The kit may comprise 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. The kit may comprise one or more, *e.g.,* two or more, unitary doses of one or more, *e.g.,* two or more, immunogenic compositions, as described herein. The one or more unitary doses in the kit may be in a single container. The one or more unitary doses in the kit may be in two or more separate containers. The kit may comprise one or more containers selected from vials, ampules and pre-loaded syringes. The kit may comprise 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. The one or more unitary doses can be the same or different. The kit may comprise 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. The kit may comprise 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. The kit may comprise 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. The kit may comprise 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. The core polypeptide may comprise 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. The sAg polypeptide may comprise 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. The core-sAg fusion polypeptide may comprise 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. The kit may comprise 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. The kit may 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. The kit may 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 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. The kit may comprise 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)). The kit may comprise 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). The kit may comprise (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)). The kit may comprise (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. The kit may comprise 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. The kit may comprise 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. The kit may further comprise one or more unitary doses of one or more additional therapeutic agents. The kit may further comprise one or more agonists or activators of one or more toll-like receptors (TLRs). The kit may further comprise 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. The kit may further comprise a TLR7 agonist selected from GS 9620 (vesatolimod), R848 (Resiquimod), DS-0509, LHC-165 and TMX-101 (imiquimod). The kit may further comprise a TLR8 agonist selected from GS-9688, R848 (Resiquimod) and NKTR-262 (dual TLR7/TLR8 agonist). The kit may further comprise one or more interleukin receptor agonists of an interleukin receptor selected from IL-2, IL-7, IL-12 and IL-15. The kit may further comprise one or more cytokines selected from IL-2, IL-7, IL-12, IL-15, and variants thereof. The kit may further comprise one or more innate immune activators. The kit may further comprise 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). The kit may further comprise one or more unitary doses of GS-3583 and/or GS-9992. The kit may further comprise 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. The kit may further comprise 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); 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). 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. The kit may further comprise 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 (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). The kit may further comprise one or more agonists or activators of one or more T-cell stimulatory immune checkpoint proteins or receptors. The kit may further comprise 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). The kit may further comprise one or more unitary doses of AGEN-2373 and/or AGEN-1223. The kit may further comprise one or more blockers or inhibitors of one or more NK-cell inhibitory immune checkpoint proteins or receptors. The kit may further comprise 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); killer cell lectin like receptor C1 (KLRC1, NKG2A, CD159A); and killer cell lectin like receptor D1 (KLRD1, CD94). The kit may further comprise one or more agonists or activators of one or more NK-cell stimulatory immune checkpoint proteins or receptors. The kit may further comprise 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). The kit may further comprise one or more proteinaceous inhibitors of PD-L1 (CD274), PD-1 (PDCD1) and/or CTLA4. The kit may further comprise 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-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). The kit may further comprise 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), 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). The kit may further comprise one or more small molecule inhibitors of CD274 (PDL1, PD-L1), programmed cell death 1 (PDCD1, PD1, PD-1) and/or CTLA4. The kit may further comprise one or more small molecule inhibitors of CD274 or PDCD1 selected from GS-4224, GS-4416, INCB086550 and MAX10181. The kit may further comprise the small molecule inhibitor of CTLA4, BPI-002. The kit may further comprise one or more one or more anti-viral agents. The kit may further comprise one or more antiviral agents selected from lamivudine (LAM), adefovir dipivoxil (ADV), entecavir (ETV), telbivudine (LdT), tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF or VEMLIDY®) and ledipasvir + sofosbuvir (HARVONI®). The kit may further comprise 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] Also disclosed herein are methods for eliciting an immune response to human hepatitis B virus (HBV) in a subject in need thereof. Also disclosed herein are methods of treating or preventing human hepatitis B virus (HBV) in a subject in need thereof. The methods may comprise administering to the subject a therapeutically effective amount of one or more immunogenic compositions, as described herein. The methods may 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. The methods may 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. The methods may 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. The methods may 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. The core polypeptide may comprise 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. The sAg polypeptide may comprise 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. The sAg polypeptide may comprise 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. The core-sAg fusion polypeptide may comprise 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. The methods may 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. The methods may 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 methods, the first viral expression vector and the second viral expression vector are from Arenaviridae. In some 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 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 methods, the first viral expression vector and the second viral expression vector are replication-defective or replication-deficient. In some methods, the first viral expression vector and the second viral expression vector are replication-attenuated. The methods may 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. The methods may 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) 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 methods, the subject is infected with HBV, is suspected of being infected with HBV, or is at risk of being infected with HBV. In some methods, the subject is asymptomatic. In some methods, the subject is chronically infected with HBV. In some methods, the subject is exhibiting or experiencing one or more symptoms selected from hepatic failure, hepatic cancer, hepatic fibrosis and hepatic cirrhosis. In some methods, the subject is acutely infected with HBV. In some 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 methods, the subject is co-infected with hepatitis D virus (HDV). In some methods, the composition is administered via a route selected from intravenous, intramuscular, intradermal, subcutaneous and mucosal (*e.g.* buccal, intranasal, intrarectal, intravaginal). The methods may 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, 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 methods, the one or more compositions are administered multiple times. The methods may 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). The methods may entail multiple administrations of the 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. The methods may 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 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 methods, the priming composition and the boosting composition can comprise the same immunogenic composition or can comprise different immunogenic compositions. In some methods, the priming composition and the boosting composition comprise the same one or more polypeptides and same viral expression vector. In some methods, the priming composition and the boosting composition comprise different polypeptides and/or different viral expression vectors. The methods may 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. The prime-boost regimen may comprise: (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. The methods may 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 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 boosting composition comprising one or more replication deficient

Pichinde mammarenavirus (PICV) viral expression vectors. The priming composition and the boosting composition may comprise an immunogenic composition as described herein. The subject may be not receiving antiviral therapy or antiviral therapy may be discontinued prior to administration of the one or more immunogenic compositions. In some methods, antiviral therapy is discontinued after one or more administrations of the one or more immunogenic compositions. The methods may further comprise administering to the subject one or more additional therapeutic agents, *e.g.* two, three, four, or more additional therapeutic agents. The methods may comprise co-administering one or more agonists or activators of one or more toll-like receptors (TLRs). The methods may comprise co-administereing 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 methods entail co-administering a TLR7 agonist selected from GS-9620 (vesatolimod), R848 (Resiquimod), DS-0509, LHC-165 and TMX-101 (imiquimod). The methods may entail co-administering a TLR8 agonist selected from GS-9688, R848 (Resiquimod) and NKTR-262 (dual TLR7/TLR8 agonist). The methods may entail co-administering one or more interleukin receptor agonists of an interleukin receptor selected from IL-2, IL-7, IL-12 and IL-15. The methods may entail co-administering one or more cytokines selected from IL-2, IL-7, IL-12, IL-15, and variants thereof. The methods may entail co-administering one or more innate immune activators. The methods may 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). The methods may entail co-administering GS-3583 and/or GS-9992. The methods may entail co-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. The methods may 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); 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). The methods may entail co-administering one or more blockers or inhibitors of one or more T-cell inhibitory immune checkpoint proteins or receptors. The methods may 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 (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). The methods may entail co-administering one or more agonists or activators of one or more T-cell stimulatory immune checkpoint proteins or receptors. The methods may 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). The methods may entail co-administering AGEN-2373 and/or AGEN-1223. The methods may entail co-administering one or more blockers or inhibitors of one or more NK-cell inhibitory immune checkpoint proteins or receptors. The methods may 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); 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); killer cell lectin like receptor K1 (KLRK1, NKG2D, CD314); and SLAM family member 7 (SLAMF7). The methods may entail co-administering one or more proteinaceous inhibitors of PD-L1 (CD274), PD-1 (PDCD1) or CTLA4. The methods may entail co-administering 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-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). The methods may entail co-administering one or more proteinaceous inhibitors of PD-L1 (CD274) or PD-1 (PDCD1) selected from zimberelimab (AB 122), 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). The methods may entail co-administering one or more small molecule inhibitors of CD274 (PDL1, PD-L1), programmed cell death 1 (PDCD1, PD1, PD-1) or CTLA4. The methods may entail co-administering one or more small molecule inhibitors of CD274 or PDCD1 selected from GS-4224, GS-4416, INCB086550 and MAX10181. The methods may entail co-administering BPI-002 (a small molecule inhibitor of CTLA4). The methods may comprise co-administering to the subject one or more antiviral agents. The methods may 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 VEMLIDY®) and ledipasvir + sofosbuvir (HARVONI®). The methods may 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. The method may activate in the subject CD8+ T cells and/or CD4+ T cells targeting one or more HBV polypeptide epitopesThe method may elicit 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.

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 "AHLL" disclosed as SEQ ID NOS 99 and 100, respectively.

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}, Δ1: core-Pol$^{\Delta1}$, Δ3: core-Pol$^{\Delta3}$). 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.

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$^{\Delta3}$ or GT-B, C, or D core-Pol$^{\Delta1}$. 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.

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.

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 µl 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.

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.

Figure 8 illustrates the effects of HBV-expressingAd5 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.

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 viprbrc.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 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.

Figure 10 illustrates the immunogenicity of Pol antigens in replication-incompetent lymphocytic choriomeningitis mammarenavirus (LCMV) vectors. Six- to eight-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$^{\Delta1}$ (SEQ ID NOs: 6 and 8), Pol$^{\Delta3}$ (SEQ ID NOs: 10 and 12), and Pol^{300} (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, catalog #551083) using Pol overlapping peptide pools corresponding to the immunization antigen genotype in each group. SFU, spot forming units.

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 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.

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 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.

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.

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$^{\Delta3}$ 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.

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).

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.

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 at 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.

Figures 18A-18F 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 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.

Figures 19A-19F 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 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.

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.

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.

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).

Figure 23 illustrates the study design assessing the immunogenicity of replication-incompetent LCMV vectors encoding GT-D iCore-P2A-sAg and GT-B Pol^{300} (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 µl on day 0, day 21 and day 42. Mice were given intraperitoneally 200 µl 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 administrated the replication-incompetent LCMV vectors alone was used as a positive control for IFN-γ ELISPOT.

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.

Figure 25 illustrates the immunogenicity of prime-boost vaccination with replication-incompetent PICV (VV2) vectors encoding GT-B Pol^{300} ori or GT-B Pol^{300} 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.

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 Pol^{300} 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.

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.

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.

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 Pol^{300} 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 at 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/10^6 PBMC. Statistical analysis was performed with Mann-Whitney test.

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 Pol^{300} 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 at 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/10^6 PBMC.

DETAILED DESCRIPTION

1. Introduction

[0020] Disclosed herein 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. 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)

[0021] Disclosed herein are immunogenic polypeptides useful to promote, induce and/or elicit an immunogenic response against one or more hepatitis B virus (HBV) antigens. The immunogenic polypeptides may 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-S 1, pre-S2, or fragments thereof.

[0022] The immunogenic polypeptides described herein, and/or the polynucleotides encoding such polypeptides, may be 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. The purified polypeptides and/or polynucleotides may be 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

[0023] Disclosed herein are truncated and/or internal deletion mutant hepatitis B virus (HBV) polymerase polypeptides.

[0024] 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 described herein, the entire TP domain has been deleted or removed.

[0025] Generally, the enzymatic domains, *i.e.,* the reverse transcriptase and RNase H domains, are inactivated in the HBV polymerase protein mutants described herein. The reverse transcriptase domain does not comprise a YMDD motif (SEQ ID NO: 97). The YMDD motif (SEQ ID NO: 97) in the reverse transcriptase domain is changed to YMHD (SEQ ID NO: 99). The RNase H domain does not comprise an AELL motif (SEQ ID NO: 98). The AELL motif (SEQ ID NO: 98) in the RNase H domain is changed to AHLL (SEQ ID NO: 100).

Truncated Polymerase Mutants

[0026] The truncated HBV polymerase polypeptides disclosed herein may 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, 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. 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. The truncated HBV polymerase polypeptide disclosed herein may be 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. The truncated HBV polymerase polypeptides may comprise the C-terminal 528, 529, 530, 531, 532, 533, 534 or 535 amino acids of a native or wild-type HBV polymerase.

[0027] The truncated HBV polymerase polypeptides disclosed herein may 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. The truncated HBV polymerase polypeptides disclosed herein may comprise an amino acid sequence corresponding to amino acid residues 300-832. In such polypeptides, the N-terminus corresponds to amino acid position 300 of the prototype genotype D pol protein. 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*).

[0028] The truncated HBV polymerase polypeptide disclosed herein may be from HBV genotype B and comprise or consist 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. The truncated HBV polymerase polypeptide disclosed herein may be 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.

[0029] The truncated HBV polymerase polypeptide disclosed herein may be from HBV genotype D and comprises or consists of an amino acid sequence of SEQ ID NO: 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 SEQ ID NO: 14. The truncated HBV polymerase polypeptide disclosed herein may be 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.

[0030] 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 skilled in the art will typically change one or more of the codons of the encoding DNA sequence.

[0031] 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 polypeptide sequences of the disclosed polypeptides, or corresponding DNA sequences that encode such polypeptides without appreciable loss of their biological utility or activity.

[0032] A "substitution," as used herein, denotes the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

[0033] In many instances, a polypeptide variant will contain one or more conservative 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.

[0034] As used herein, "identity" means the percentage of identical nucleotide or amino 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 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 sequence(s) relative to the reference sequence, based on the designated program parameters.

[0035] 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 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.

[0036] 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 Phylogenes 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.

[0037] 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.

[0038] 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).

[0039] 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.

[0040] 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.

[0041] 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.

[0042] 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.

[0043] 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 provided in Table B.

Table A - Pol^{300} mutants - Motifs containing inactivating mutations are underlined (YMDD mutated to YMHD, AELL mutated to AHLL).

SEQ ID NO:	HBV genotype	Length (# amino acids)	Polypeptide sequence
13	B	534	MSRSQSQGPVLSCWWLQFRNSEPCSEYCLCHIVNLIEDWGPCTE HGEHRIRIPRTPARVTGGVFLVDKNPHNTTESRLVVDFSQFSRGN YRVSWFKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAM PHLLVGSSGLSRYVARLSSNSRIINNQHRTMQNLHDSCSRNLYVS LMLLYKTYGRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAIC SVVRRAFPHCLAFSYMHDVVLGAKSVQHLESLYAAVTNFLLSLGI HLNPHKTKRWGYSLNFMGYVIGSWGTLPQEHIVQKIKMCFRKLPV NRPIDWKVCQRIVGLLGFAAPFTQCGYPALMPLYACIQAKQAFTF SPTYKAFLSKQYLNLYPVARQRPGLCQVFADATPTGWGLAIGHQR MRGAFVSPLPIHTAELLAACFARSRSGAKLIGTDNSVVLSRKYTS FPWLLGCAANWILRGTSFVYVPSALNPADDPSRGRLGLSRPLLRL LYRPTTGRTSLYADSPSVPSHLPDRVHFASPLHVAWRPP
14	D	534	MSARSQSERPVFPCWWLQFRNSKPCSDYCLSHIVNLLEDWGPCAE HGEHHIRIPRTPARVTGGVFLVDKNPHNTAESRLVVDFSQFSRGN YRVSWFKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAM PHLLVGSSGLSRYVARLSSNSRIFNYQHGTMQNLEDSCSRNLYVS LMLLYQTFGRKLHLYSHPIILGFRKIPMGVGLSPFLAQFTSAIC SVVRRAFPHCLAFSYKHDVVLGAKSVQHLESLFTAVTNFLLSLGI HLNPNKTKRWCYSLHFMGYVIGCYGSLPQDHIIQKIKECFRKLPV
			NRPIDWKVCQRIVGLLGFAAPFTQCGYPALMPLYACIQSKQAFTF SPCYKAFLCKQYLNLYPVARQRPGLCQVFADATPTGWGLVMGHQR KRGTFKAPLPIHTAELLAACFARSRSGANILGTDNSVVLSRKYTS FPWLLGCAANWILRGTSFVYVPSALNPADDPSRGRLGLYRPLLRL PFRPTTGRTSLYADSPSVPSHLPDRVHFASPLHVAWRPP

Table B - N-terminal polypeptide sequence removed from Pol^{300} truncated mutants

SEQ ID NO:	HBV genotype	Polypeptide sequence
50	B	PLSYQHFRKLLLLDDEAGPLEEELPRLADEGLNRRVAEDLNLGNLNVSIPWTHKV GNFTGLYSSTVPVFNPEWQTPSFPHIHLQEDIINRCQQYVGPLTVNEKRRLKLIM PARFYPNLTKYLPLDKGIKPYYPEHVVNHYFQTRHYLHTLWKAGILYKRETTRSA SFCGSPYSWEQDLQHGRLVFQTSKRHCDKSFCPQSPCILPRSSVCPCIQNQLRKS RLGPQPAQGLAGRQQGGSGSIRARVHPSPWGTVGVEPSGSGHIHNCASNSSSCL HQSAVRKAAYSHISTSKGHSSSGHAVELHFPPS
51	D	PLSYQHFRRLLLLDDEAGPLEEELPRLADEGLNRRVAEDLNLGNLNVSIPWTHKV GNFTGLYSSTVPVFNPHWKTPSFPNIHLHQDIIKKCEQFVGPLTVNEKRRLQLIM PARFYPNVTKYLPLDKGIKPYYPEHLVNHYFQTRHYLHTLWKAGILYKRETTHSA SFCGSPYSWEQELQHGAESFHQQSSGILSRPVGSSLQSKHRKSRLGLQSQQGHL ARRQQGRGWSTRAGTHPTARRPFGVEPSGSGHTANIASKSASCLYQSAVRKAAYP VVSTFKKHSSSGHAVELHNLPPN

[0044] The truncated HBV polymerase polypeptide disclosed herein may not comprise an amino sequence or fragment thereof from another HBV protein. The truncated HBV polymerase polypeptide disclosed herein may 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

[0045] Further disclosed herein are HBV polymerase internal deletion mutant polypeptides. The HBV polymerase internal deletion mutant polypeptides may 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). The HBV polymerase deletion mutant polypeptide may be 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. The HBV polymerase internal deletion mutant polypeptides may 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. 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.

[0046] The HBV polymerase internal deletion mutant polypeptide disclosed herein may be from HBV genotype A and comprise or consist 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. The HBV polymerase internal deletion mutant polypeptide disclosed herein may be 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.

[0047] The HBV polymerase internal deletion mutant polypeptide disclosed herein may be 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. The HBV polymerase internal deletion mutant polypeptide disclosed herein may be 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.

[0048] The HBV polymerase internal deletion mutant polypeptide disclosed herein may be from HBV genotype C and comprises or consists of an amino acid sequence of any

one of 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. The HBV polymerase internal deletion mutant polypeptide disclosed herein may be 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.

[0049] The HBV polymerase internal deletion mutant polypeptide disclosed herein may be 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 of SEQ ID NOs: 9 and 12. The HBV polymerase internal deletion mutant polypeptide disclosed herein may be 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.

[0050] The HBV polymerase internal deletion mutant polypeptide disclosed herein may not comprise an amino sequence or fragment thereof from another HBV protein. The HBV polymerase internal deletion mutant polypeptide may 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)).

[0051] 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

[0052] The truncated and internal deletion HBV polymerase polypeptide variants described herein may be fused to an HBV core polypeptide. The core polypeptide can be positioned either N-terminal or C-terminal to the HBV polymerase. Further disclosed 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. The core-Pol fusion polypeptide comprises the HBV polymerase deletion mutant polypeptide, described herein, may comprise 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.

[0053] The core-Pol fusion polypeptide may comprise or consist 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.

[0054] The HBV core - polymerase internal deletion mutant fusion protein may 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)).

[0055] 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[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 genotype	Length (# amino acids)	Polypeptide sequence
5	A	755	MPLSYQHFRKLLLLDDEAGPLEEELPRLADELLNRRVAEDLNLGNLNVSIPWTHKVGNFTGLYSSTVP IFNPEWQTPSFPKIHLHEDIINRCQQFVGPLTVNEKRRLKLIMPARFYPNLTKYLPLDKGIKPYYPDHVV NHYFQTRHYLHTLWKAGILYKRETTRSASFCGSPYSWEQELHHGRLVIKTSQRHGDEPFCSQPSGILSRS SVG***PE***FHSFPDSSARSQSQGPVFSCWWLQFRNTQTCSKYCLSHLVNLLEDWGPCAEHGEHHIRIPRTPAR VTGGVFLVDKNPHNTAESRLVVDFSQFSRGNTRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHIP LHPAAMPHLLVGSSGLSRYVARLSSNSRIINNQHGTLQNLEDSCSRQLYVSLMLLYKTYGRKLHLYSHPI ILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCLAFS***YMHD***VVLGAKSVQHLESLYTAVTNFLLSLG IHLNPNKTKRWGYSLNFMGYVIGSWGTLPQDHIVQKIKECFRKLPINRPIDWKVCQRIVGLLGFAAPFTQ CGYPALMPLYACIQAKQAFTFSPTYKAFLSKQYLNLYPVARQRPGLCQVFADATPTGWGLAIGHQRMRGT FVAPLPIHT***AHLL***AACFARSRSGAKLIGTDNSVVLSRKYTSFPWLLGCAANWILRGTSFVYVPSALNPAD DPSRGRLGLYRPLLRLPYRPTTGRTSLYAVSPSVPSHLPVRVHFASPLHVAWRPP
6	B	749	MPLSYQHFRKLLLLDDEAGPLEEELPRLADESLNRRVAEDLNLGNLNVSIPWTHKVGNFTGLYSSTVPVF NPEWQTPSFPHIHLQEDIINRCQQYVGPLTVNEKRRLKLIMPARFYPNLTKYLPLDKGIKPYYPEHVVNH YFQTRHYLHTLWKAGILYKRESTRSASFCGSPYSWEQDLQHGRLVFQTSKRHGDKSFCPQSPGILPR***SE***L HHFPSSSRSQSQGPVLSCWWLQFRNSEPCSEYCLCHIVNLIEDWGPCTEHGEHRIRTPRTPARVTGGVF LVDKNPFNTTESRLVVDFSQFSRGNTRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAM PHLLVGSSGLSRYVARLSSNSRIINKQHRTMQNLHDSCSRNLYVSLMLLYKTYGRKLHLYSHPIILGFRK IPMGVGLSPFLLAQFTSAICSVVRRAFPHCLAFS***YMHD***VVLGAKSVQHLESLYTAVTNFLLSLGIHLNPH KTKRWGYSLNFMGYVIGSWGTLPQEHIVQKIKECFRKLPVNRPIDWKVCQRIVGLLGFAAPFTQCGYPAL MPLYACIQAKQAFTFSPTYKAFISKQYLNLYPVARQRPGLCQVFADATPTGWGIAIGHQRMRGAFVSPLP IHTAHLLAACFARSRSGAKLIGTDNSVVLSRKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDPSRGR LGLYRPLLRLLYRPTTGRTSLYADSPSVPSHLPDRVHFASPLHVAWRPP
7	C	753	MPLSYQHFRKLLLLDDEAGPLEEELPRLADEDLNRRVAEDLNLGNLNVSIPWTHKVGNFTGLYSSTVPVF NPEWQTPSFPHIHLQEDIINRCQQYVGPLTVNEKRRLKLIMPARFYPNLTKYLPLDKGIKPYYPEHIVNH YFKTRHYLHTLWKAGILYKRETTRSASFCGSPYSWEQELQEGRLVFQTSTRHGDESFCSQSSGILSRSPV G***PE***LHNFPSSSARSQSEGPLLSCWWLQFRNSKPCSDYCLSFIVNLLEDWGPCTEHGEHNIRIPRTPARVT
			CGVFLVDKNPHKTTESRLVVDFSQFSRGSTHVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYELPLH PAAMPHLLVGSSGLSRYVARLSSTSRNINYQHGAMQDLHDSCSRNLYVSLLLLYKTFGRKLHLYSHPIIL GFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCLAFSYMHDVVLGAKSVQHLESLFTAVTNFLLSLGIH LNPNKTKRWGYSLNFMGYVIGSWGTLPQEHIVLKLKECFRKLPVNRPIDWKVCQRIVGLLGFAAPFTQCG YPALMPLYACIQAKQAFTFSPTYKAFLCKQYLNLYPVARQRSGLCQVFADATPTGWGLAVGHQRMRGTFV SPLPIHTAHLLAACFARSRSGAKLIGTDNSVVLSRKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDP SRGRLGLYRPLLRLPFRPTTGRTSLYAVSPSVPSHLPVRVHFASPLHVAWRPP
8	D	742	MPLSYQHFRKLLLLDDEAGPLEEELPRLADESLNRRVAEDLNLGNLNVSIPWTHKVGNFTGLYSSTVPVF NPAWKTPSFPNLHLHQDIIKKCEQFVGPLTVNEKRRLQLIMPARFYPNVTKYLPLDKGIKPYYPEHLVNH YFQTRHYLHTLWKAGILYKRETTHSASFCGSPYSWEQELQEGAESFHQQSSGILSRPPVG***SE***LHNLPPNS ARSQSERPVFPQWWLQFRNSKPCSDYCLSHIVNLLEDWGPCAEHGEHHIRIPRTPARVTGGVFLVDKNPH NTAESRLVVDFSQFSRGNYRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLEPAAMPHLLVGS SGLSRYVARLSSNSRIINYQQGTMQNLHDSCSRNLYVSLMLLYQTFGRKLHLYSHPIILGFRKIPMGVGL SPFLLAQFTSACSVVRRAFPHCLAFSYMHDVVLGAKSVQFLESLFTAVTNFLLSLGTHLNPNKTKRWGY SLHFMGYVIGSCGSLPQDHIIQKIKECFRKLPVNRPIDWKVCQRIVGLLGFAAPFTQCGYPALMPLYACI QSKQAFTFSPTYKAFLCKQYLNLYPVARQRPGLCQVFADATPTGWGLVMGHQRMRGTFKAPLPIHTAELL AACFARSRSGANILGTDNSVVLSRKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDPSRGRLGLYRPL LRLPFRPTTGRTSLYADSPSVPSHLPDRVHFASPLHVAWRPP

Table D - Internal Spacer polypeptide sequences removed from Pol$^{\Delta 1}$ mutants and Core-Pol$^{\Delta 1}$ fusion proteins

SEQ ID NO:	HBV genotype	Polypeptide sequence
42	A	CIRSQFKQSRLGLQPHQGPLATSQSGRSGSIRARVHSPTRRCFGVEPSGSGHIGHSASSSSSCLHQSAVRKAAYSHLSTSKRQSSSGHAV
43	B	SVGPCIQNQLRKSRLGPQPAQGQLAGRQQGGSGSIRARVHPSPWGTVGVEPSGSGHIHNCASNSSSCLHQSAVRKAAYSHISTSKGHSSSGHAV
44	C	CIRSQLKQSRLGLQPQQGSLARSKSGRSGSIRARVHPTTRQSFGVEPSGSGHIDNSASSASSCLHQSAVRKTAYSHLSTSKRQSSSGHAV
45	D	SLQSKHRKSRLGLQSQQGHLARRQQGRGWSIRAGIHPTARRPFGVEPSGSGHTANLASKSASCLYQSAVRKAAYPVVSTFKKHSSSGHAV

Table E - Pol$^{\Delta 3}$ 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 genotype	Length (# amino acids)	Polypeptide sequence
9	A	705	MPLSYQHFRKLLLLDDETEAGPLEEELPRLADEDLNRRVAEDLNLGNLNVSIPWTHKVGNFTGLYSSTVPIFNPEWQTPSFPKIHLHEDIANRCQQFVGPLTVNEKRRLRLIMPARFYPNSTKYLPLDKGIKPYYPDHVVNHYFQTRHYLHTLWKAGILYKRETTRSASFCGSPYSWEQELHH***GC***WWLQFRNTQPCSKYCLSHLVNLLEDWGPCDEHGEHHIRIPRTPARVTGGVFLVDKNPHNTAESRLVVDFSQFSRGTTRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHIPLHPAAMPHLLVGSSGLSRYVARLSSNSRIHNNQHGTLQNLHDSCSRQLYVSLMLLYKTYGRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCLAFSYMHDVVLGAKSVQHLESLYTAVTNFLLSLGIHLNPNKTKRWGYSLNFMGYVIGSWGTLPQDHIVQKIKHCFRKLPINRPIDWKVCQRIVGLLGFAAPFTQCGYPALMPLYACIQAKQAFTFSPTYKAFLSKQYLNLYPVARQRPGLCQVFADATPTGWGLAIGHQRMRGTFVAPLPIHTAHLLAACFARSRSGAKLIGTDNSVVLSRKYTSFPWLLGCTANWILRGTSFVYVPSALNPADDPSRGRLGLYRPLLRLPYRPTTGRTSLYAVSPSVPSHLPVRVHFASPLHVAWRPP
10	B	703	MPLSYQHFRKLLLLDDEACPLEEELPRLADEGLNRRVAEDLNLGNLNVSIPWTHKVGNFTGLYSSTVPVFNPEWQTPSFPHIHLQEDIINRCQQYVGPLTVNEKRRLKLIMPARFYPNLTKYLPLDKGIKPYYPEHVVNHYFQTRHYLHTLWKAGILYKRESTRSASFCGSPYSWEQDLQH***GC***WWLQFRNSEPCSEYCLCHIVNLLEDWGPCTEHGEHRIRTPRTPARVTGGVFLVDKNPHNTTESRLVVDFSQFSRGNTRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHIPLHPAAMPHLLVGSSGLSRYVARLSSNSRII
			NNQHRTMQNLHDSCSRNLYVSLMLLYKTYGRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCLAFSYMHDVVLGAKSVQHLESLYAAVTNFLLSLGIHLNPHKTKRWGYSLNFMGYVIGSWGTLPQEHIVQKIKMCFRKLPVNRPIDWKVCQRIVGLLGFAAPFTQCGYPALMPLYACIQAKQAFTFSPTYKAFLSKQYLHLYPVARQRPGLCQVFADATPTGWGLAIGHQRMRGAFVSPLPIHTAHLLAACFARSRSGAKLIGTDNSVVLSRKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDPSRGRLGLYRPLLRLLYRPTTGRTSLYADSPSVPSHLPDRVHFASPLHVAWRPP
11	C	703	MPLSYQHFRKLLLLDDEAGPLEEELPRLADEDLNRRVAEDLNLGNLNVSIPWTHKVGNFTGLYSSTVPVFNPEWQTPSFPHIHLQEDIINRCQQYVGPLTVNEKRRLKLIMPARFYPNLTKYLPLDKGIKPYYPEHTVNHYFKTRHYLHTLWKAGILYKRETTRSASFCGSPYSWEQELQH***GC***WWLQFRNSKPCSDYCLSHIVNLLEDWGPCTEHGEHNIRIPRTPARVTGGVFLVDKNPHNTTESRLVVDFSQFSRGSTHVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMPHLLVGSSGLSRYVARLSSTSRNINYQHGAMQDLHDSCSRNLYVSLLLLYKTFGRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCLAFSYMHDVVLGAKSVQHLESLFTAVTNFLLSLGIHLNPNKTKRWGYSLNFMGYVIGSWGTLPQEHIVLKIKQCFRKLPVNRPIDWKVCQRIVGLLGFAAPFTQCGYPALMPLYACIQAKQAFTFSPTYKAFLCKQYLNLYPVARQRSGLCQVFADATPTGWGLAVGHQRMRGTFVSPLPIHTAHLLAACFARSRSGAKLIGTDNSVVLSRKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDPSRGRLGLYRPLLRLPFRPTTGRTSLYAVSPSVPSHLPVRVHFASPLHVAWRPP
12	D	703	MPLSYQHFRRLLLLDDEAGPLEEELPRLADEGLNRRVAEDLNLGNLNVSIPWTHKVGNFTGLYSSTVPVFNPHWKTPSFPNIHLHQDIIKKCEQFVGPLTVNEKRRLQLIMPARFYPNVTKYLPLDKGIKPYYPEHLVNHYFQTRHYLHTLWKAGILYKRETTHSASFCGSPYSWEQELQH***GC***WWLQFRNSKPCSDYCLSHIVNLLEDWGPCAEHGEHHIRIPRTPARVTGGVFLVDKNPHNTAESRLVVDFSQFSRGNYRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMPHLLVGSSGLSRYVARLSSNSRIFNYQHGTMQNLHDSCSRNLYVSLMLLYQTFGRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCLAFSYMHDVVLGAKSVQHLESLFTAVTNFLLSLGIHLNPNKTKRWGYSLHFMGYVIGCYGSLPQDHIIQKIKECFRKLPVNRPIDWKVCQRIVGLLGFAAPFTQCGYPALMPLYACIQSKQAFTFSPTYKAFLCKQYLNLYPVARQRPGLCQVFADATPTGWGLVMGHQRMRGTFKAPLPIHTAHLLAACFARSRSGANILGTDNSVVLSRKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDPSRGRLGLYRPLLRLPFRPTTGRTSLYADSPSVPSHLPDRVHFASPLHVAWRPP

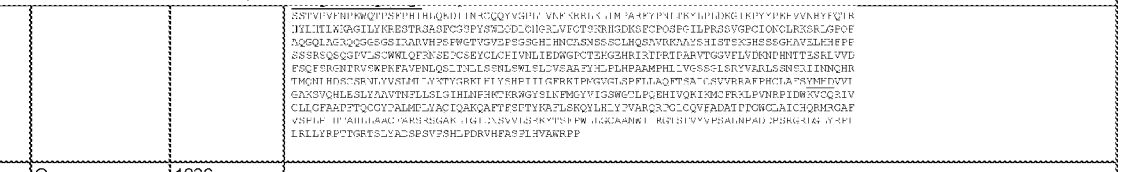
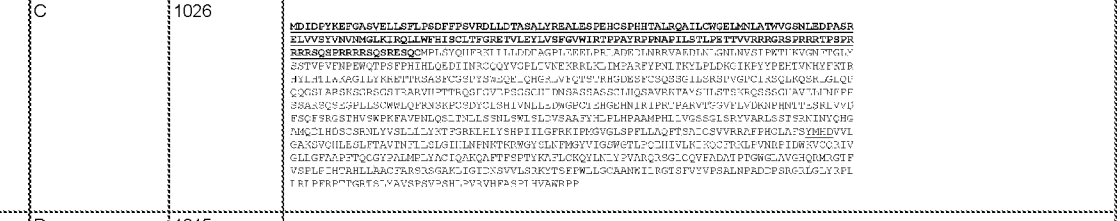
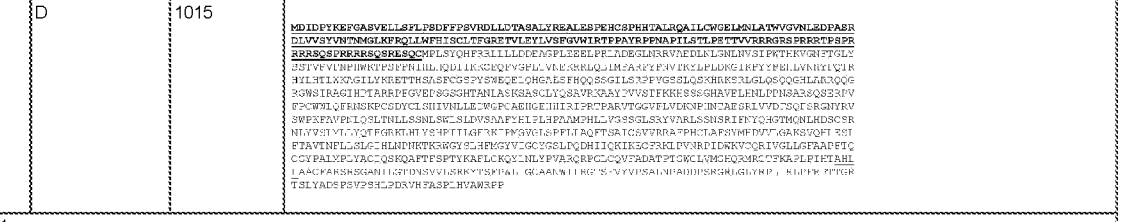
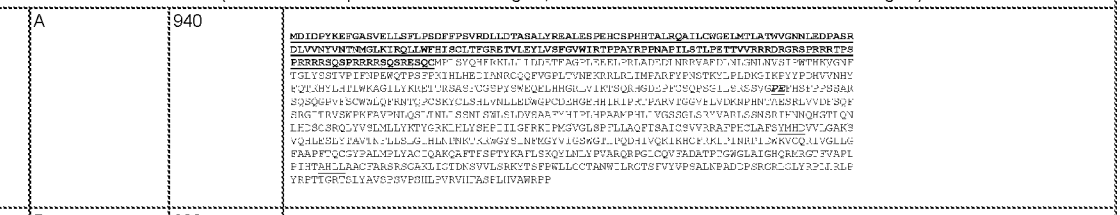
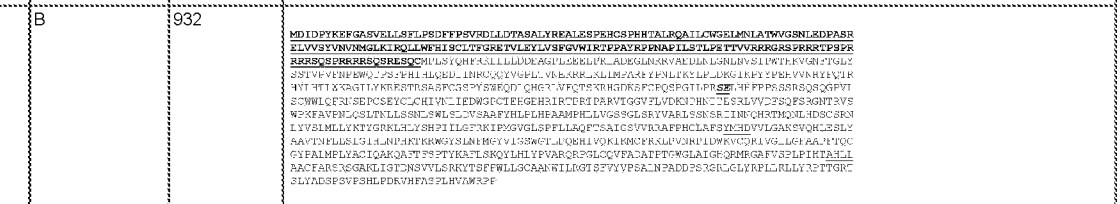
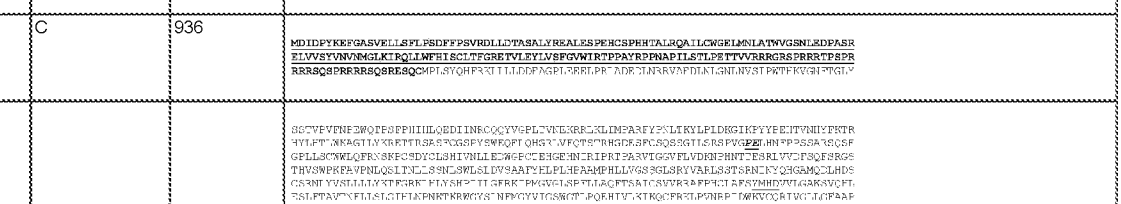
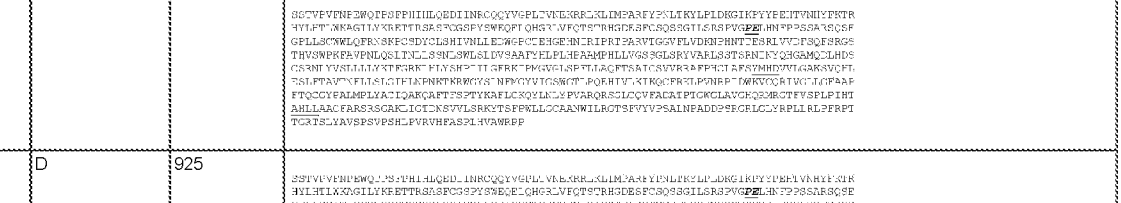
Table F - Internal Spacer polypeptide sequences removed from Pol$^{\Delta 3}$ mutants and Core-Pol$^{\Delta 3}$ fusion proteins

SEQ ID NO:	HBV genotype	Polypeptide sequence
46	A	RLVIKTSQRHGDEPFCSQPSGILSRSSVGPCIRSQFKQSRLGLQPHQGPLATSQSGRSGSIRARVHSPTRRCFGVEPSGSGHIGHSASSSSSCLHQSAVRKAAYSHLSTSKRQSSSGHAVEFHSFPPSSARSQSQGPVFS
47	B	RLVFQTSKRHGDKSFCPQSPGILPRSSVGPCIQNQLRKSRLGPQPAQGQLAGRQQGGSGSIRARVHPSPWGTVGVEPSGSGHIHNCASNSSSCLHQSAVRKAAYSHISTSKGHSSSGHAVELHHFPPSSSRSQSQGPVFS
48	C	RLVFQTSTRHGDESFCSQSSGILSRSPVGPCIRSQLKQSRLGLQPQQGSLARSKSGRSGSIRARVHPTTRQSFGVEPSGSGHIDNSASSASSCLHQSAVRKTAYSHLSTSKRQSSSGHAVELHNFPPSSARSQSEGPLLS
49	D	AESFHQQSSGILSRPPVGSSLQSKHRKSRLGLQSQQGHLARRQQGRGWSIRAGIHPTARRPFGVEPSGSGHTANLASKSASCLYQSAVRKAAYPVVSTFKKHSSSGHAVELHNLPPNSARSQSERPVFP

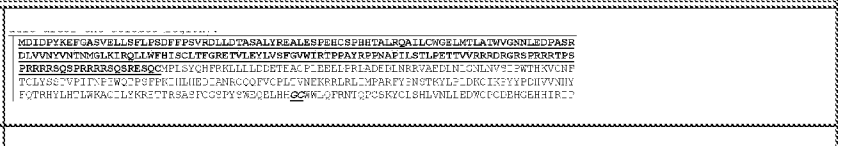
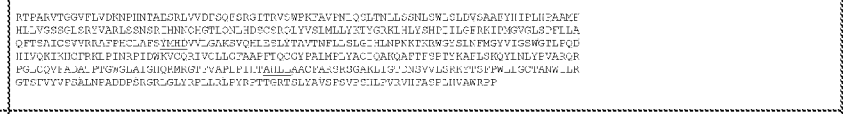
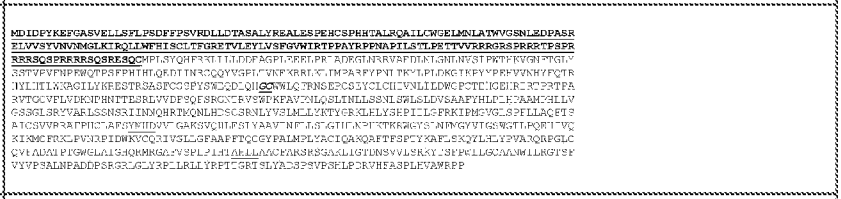
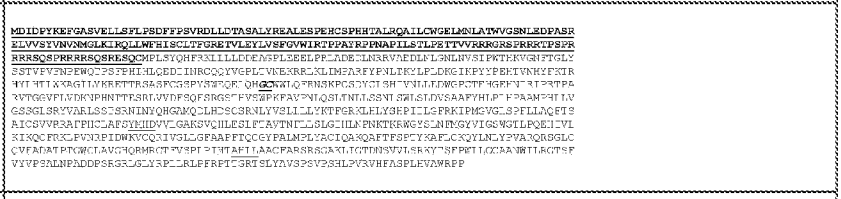
Table G - Core-Pol fusion proteins

SEQ ID NO:	HBV genotype	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	**MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAILCWGELMTLATWVGNNLEDPASRDLVVNYVNTNMGLKIRQLLWFHISCLTFGRETVLEYLVSFGVWIRTPPAYRPPNAPILSTLPETTVVRRRDRGRSPRRRTPSPRRRRSQSPRRRRSQSRESQC**MPLSYQHFRKLLLLDDETEAGPLEEELPRLADEDLNRRVAEDLNLGNLNVSIPWTHKVGNFTGLYSSTVPIFNPEWQTPSFPKIHLHEDIANRCQQFVGPLTVNEKRRLRLIMPARFYPNSTKYLPLDKGIKPYYPDHVVNHYFQTRHYLHTLWKAGILYKRETTRSASFCGSPYSWEQELHHGRLVIKTSQRHGDEPFCSQPSGILSRSSVGPCIRSQFKQSRLGLQPHQGPLATSQSGRSGSIRARVHSPTRRCFGVEPSGSGHIGHSASSSSSCLHQSAVRKAAYSHLSTSKRQSSSGHAVEFHSFPPSSARSQSQGPVFSCWWLQFRNTQPCSKYCLSHLVNLLEDWGPCDEHGEHHIRIPRTPARVTGGVFLVDKNPHNTAESRLVVDFSQFSRGITRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHIPLHPAAMPHLLVGSSGLSRYVARLSSNSRIHNNQHGTLQNLHDSCSRQLYVSLMLLYKTYGRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCLAFSYMHDVVLGAKSVQHLESLYTAVTNFLLSLGIHLNPNKTKRWGYSLNFMGYVIGSWGTLPQDHIVQKIKHCFRKLPINRPIDWKVCQRIVGLLGFAAPFTQCGYPALMPLYACIQAKQAFTFSPTYKAFLSKQYLNLYPVARQRPGLCQVFADATPTGWGLAIGHQRMRGTFVAPLPIHTAHLLAACFARSRSGAKLIGTDNSVVLSRKYTSFPWLLGCTANWILRGTSFVYVPSALNPADDPSRGRLGLYRPLLRLPYRPTTGRTSLYAVSPSVPSHLPVRVHFASPLHVAWRPP
16	B	1026	**MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAILCWGELMNLATWVGSNLEDPASRELVVSYVNVNMGLKIRQLLWFHISCLTFGRETVLEYLVSFGVWIRTPPAYRPPNAPILSTLPETTVVRRRGRSPRRRTPSPRRRRSQSPRRRRSQSRESQC**MPLSYQHFRKLLLLDDEAGPLEEELPRLADEGLNRRVAEDLNLGNLNVSIPWTHKVGNFTGLY

Table G - Core-Pol fusion proteins

SEQ ID NO:	HBV genotype	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).			
			
17	C	1026	
18	D	1015	
Core-Pol$^{\Delta 1}$ 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	
20	B	932	
21	C	936	
			
22	D	925	
Core-Pol$^{\Delta 3}$ 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			

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) .

23	A	890	
			
24	B	886	
25	C	886	
26	D	886	

Core-sAg Fusion Proteins

[0056] Further disclosed herein 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. The HBV core polypeptide or fragment thereof and the HBV small surface antigen (sAg), or fragment thereof, may be directly fused or abutted. The HBV core polypeptide or fragment thereof and the HBV small surface antigen, or fragment thereof, may be connected via a linker.

HBV core polypeptide, or an immunogenic fragment thereof

[0057] The HBV core polypeptide, or immunogenic fragment thereof, of the core-sAg fusion protein independently may 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 genotype	Polypeptide sequence
64	A	MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPE HCSPHHTALRQAILCWGELMTLATWVGNNLEDPASRDLVVNYVNTN MGLKIRQLLWFHISCLTFGRETVLEYLVSFGVWIRTPPAYRPPNAP ILSTLPETTVVRRRDRGRSPRRRTPSPRRRRSQSPRRRRSQSRESQ C
65	B/C	MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPE HCSPHHTALRQAILCWGELMNLATWVGSNLEDPASRELVVSYVNVN MGLKIRQLLWFHISCLTFGRETVLEYLVSFGVWIRTPPAYRPPNAP ILSTLPETTVVRRRGRSPRRRTPSPRRRRSQSPRRRRSQSRESQC
66	D	MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPE HCSPHHTALRQAILCWGELMNLATWVGVNLEDPASRDLVVSYVNTN MGLKFRQLLWFHISCLTFGRETVLEYLVSFGVWIRTPPAYRPPNAP ILSTLPETTVVRRRGRSPRRRTPSPRRRRSQSPRRRRSQSRESQC

[0058] The core polypeptide in the core-sAg fusion polypeptide may comprise or consist 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. The core polypeptide may comprise 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

[0059] The HBV sAg polypeptide, or immunogenic fragment thereof, of the core-sAg fusion protein independently may 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.

[0060] The sAg polypeptide in the core-sAg fusion polypeptide may comprise or consist 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) 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.

[0061] 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. The core-sAg fusion protein may comprise 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 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 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;
- 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;
- 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
- the core polypeptide is from an HBV genotype D and the sAg polypeptide is from an HBV genotype C.

[0062] The core-sAg fusion protein may comprise 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 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.

[0063] The core-sAg fusion protein may comprise 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.

[0064] The core-sAg fusion protein may comprise 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.

[0065] The core-sAg fusion proteins described herein may 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, the core-sAg fusion proteins described herein may not comprise an HBV pre-S1 polypeptide. The core-sAg fusion proteins described herein may not comprise an HBVpre-S2 polypeptide. The core-sAg fusion proteins described herein may not comprise both an HBV pre-S1 polypeptide and an HBV pre-S2 polypeptide.

[0066] 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).

[0067] 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) .

[0068] 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) .

[0069] 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) .

[0070] 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) .

[0071] The core-sAg fusion proteins described herein may 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.

[0072] An illustrative HBV pre-S1-pre-S2 polypeptide not included in the herein described core-sAg fusion protein is provided below:
MGQNLSTSNPLGFFPDHQL**[D/A]**PAFRANT**[A/G/R]**NPDWDFNPNKDTWPDANKVGAGAFGL
GFTPPHGGLLGWSPQAQGI**[I/L]**QT**[L/V]**PANPPPAS**[T/A]**NRQ**[S/T]**GRQPTPLSPPLR
[N/D]TEPQAMQWNST**[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).

[0073] 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:
MGGWSSKPRKGMGTNLSVPNPLGFFPDHQLDPAFGANSNNPDWDFNPIKDHWPAANQVGVGAFG
PGLTPPHGGILGWSPQAQGILTTVSTIPPPASTNRQSGRQPTPISPPLRDSHPQAMQWNSTAFH
QALQDPRVRGLYFPAGGSSSGTVNPAPNIASHISSISARTGDPVTN (SEQ ID NO: 85).

[0074] 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:
MGGWSSKPRKGMGTNLSVPNPLGFFPDHQLDPAFKANSENPDWDLNPHKDNWPDANKVGVGAFG
PGFTPPHGGLLGWSPQAQGLLTTVPAAPPPASTNRQSGRQPTPLSPPLRDTHPQAMQWNSTTFH
QTLQDPRVRALYFPAGGSSSGTVSPAQNTVSAISSILSKTGDPVPN (SEQ ID NO: 86).

[0075] 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:
MGGWSSKPRQGMGTNLSVPNPLGFFPDHQLDPAFGANSNNPDWDFNPNKDHWPEANQVGAGAFG
PGFTPPHGGLLGWSPQAQGILTTVPAAPPPASTNRQSGRQPTPISPPLRDSHPQAMQWNSTTFH
QALLDPRVRGLYFPAGGSSSGTVNPVPTTASPISSIFSRTGDPAPN (SEQ ID NO: 87).

[0076] 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
GWSPQAQGILQTLPANPPPASTNRQSGRQPTPLSPPLRNTHPQAMQWNSTTFHQTLQDPRVRGL
YFPAGGSSSGTVNPVPTTASPISSIFSRIGDPALN (SEQ ID NO: 88).

[0077] The core-sAg fusion proteins described herein may 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

[0078] 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. The one or more peptide linkers may be 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. The polyalanine linker may comprise or consist 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). A polyglycine linker may be used, *e.g.*, GG, GGG, GGS, GSG or GGGS (SEQ ID NO:63). The cleavable linker may be 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)). A furin recognition/cleavage sequence (*e.g.*, RAKR (SEQ ID NO: 60), REKR (SEQ ID NO: 61) and RRKR (SEQ ID NO: 62)) may be 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. The linker may comprise a porcine teschovirus-1 (P2A) linker. The 2A cleavable linker may comprise or consist 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), APVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 57), QCTNYALLKLAGDVESNPGP (SEQ ID NO: 58), or EGRGSLLTCGDVEENPGP (SEQ ID NO: 59). The 2A cleavable linker may comprise or consist 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 ATNFSLLKQAGDVEENPGP (SEQ ID NO: 56). As appropriate, a furin recognition/cleavage sequence can be positioned either at the N-terminus or the C-terminus of a 2A linker. The cleavable linker may comprise or consist of a furin 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)	GGS
	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

[0079] The core-sAg fusion protein disclosed herein may be 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.

[0080] The core-sAg fusion protein disclosed herein may 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.

[0081] The core-sAg fusion protein disclosed herein may comprise or consist 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. The fusion polypeptide may comprise 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.

[0082] 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 genotype	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	409	**MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAILCWGEL MNLATWVGSNLEDPASRELVVSYVNVNMGLKIRQLLWFHISCLTFGRETVLEYLVSFGVWIRTPP AYRPPNAPILSTLPETTVVRRRGRSPRRRTPSPRRRRSQSPRRRRSQSRESQC**MESTTSGFLGPL LVLQAGFFLLTRILTIPQSLDSWWTSLNFLGGAPTCPGQNSQSPTSNHSPTSCPPICPGYRWMCL RRFIIFLCILLLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGPCKTCTTPAQGISMFPSCCCTKP TDGNCTCIPIPSSWAFARFLWEWASVRFSWLSLLVPFVQWFVGLSPTVWLSVIWMMWYWGPSLYN ILSPFLPLLPIFFCLWVYI
39	Core: B/C	430	**MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAILCWGEL MNLATWVGSNLEDPASRELVVSYVNVNMGLKIRQLLWFHISCLTFGRETVLEYLVSFGVWIRTPP AYRPPNAPILSTLPETTVVRRRGRSPRRRTPSPRRRRSQSPRRRRSQSRESQC*GSG*ATNFSLLKQ AGDVEENPGPESTTSGFLGPLVLQAGFFLLTRILTIPQSLDSWWTSLNFLGGAPTCPGQNSQSP TSNHSPTSCPPICPGYRWMCLRRFIIFLCILLLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGPC KTCTTPAQGTSMFPSCCCTKPIDGNCTCIPIPSSWAFARFLWEWASVRFSWLSLLVPFVQWFVGL SPTVWLSVIWMMWYWGPSLYNILSPFLPLLPIFFCLWVYI
40	Core: D	409	**MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAILCWGEL MNLATWVGVNLEDPASRDLVVSYVNTNMGLKFRQLLWFHISCLTFGRETVLEYLVSFGVWIRTPP AYRPPNAPILSTLPETTVVRRRGRSPRRRTPSPRRRRSQSPRRRRSQSRESQC**MENITSGFLGPL LVLQAGFFLLTRILTIPQSLDSWWTSLNFLGGTTVCLGQNSQSPTSNHSPTSCPPICPGYRWMCL RRFIIFLFILLLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGPCRICTTPAQGISMYPSCCCTKP SDGNCTCIPIPSSWAFGKFLWEWASARFSWLSLLVPFVQWFVGLSPTVWLSVIWMMWYWGPSLYS ILSPFLPLLPIFFCLWVYI
41	Core: D	430	**MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAILCWGEL MNLATWVGVNLEDPASRDLVVSYVNTNMGLKFRQLLWFHISCLTFGRETVLEYLVSFGVWIRTPP AYRPPNAPILSTLPETTVVRRRGRSPRRRTPSPRRRRSQSPRRRRSQSRESQC*GSG*ATNFSLLKQ AGDVEENPGPENITSGFLGPLVLQAGFFLLTRILTIPQSLDSWWTSLNFLGGTTVCLGQNSQSP TSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGPC RICTTPAQGTSMYPSCCCTKPSDGNCTCIPIPSSWAFGKFLWEWASARFSWLSLLVPFVQWFVGL SPTVWLSVIWMMWYWGPSLYSILSPFLPLLPIFFCLWVYI

Signal or Leader Sequences

[0083] The immunogenic polypeptides described herein may comprise a signal sequence or signal peptide, *e.g.*, to direct intracellular trafficking of the polypeptide to a proteasomal or lysosomal compartment. The immunogenic polypeptide may comprise a signal sequence at the N-terminus and/or the C-terminus. The immunogenic polypeptide may comprise an N-terminal signal peptide or leader sequence. The signal peptide or leader sequence may be 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. The signal peptide or leader sequence may be 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). The signal peptide or leader sequence may be 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. The immunogenic polypeptide may comprise 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	MRKAAVSHWQQQSYLDSGIHSGATTTAPSLS
72	CCL7, MCP-3	MNPSAAVIFCLILLGLSGTQGILDMAQPVGINTSTTCCYRFI NKKIPKQRLESYRRTTSSHCPREAVIFKTKLDKEICADPTQK WVQDFMKHLDKKTQTPKLASAGA
73	ubiquitin	MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQR LIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG

TABLE L - illustrative signal sequences		
SEQ ID NO:	**source protein name**	**SEQUENCE**
74	calreticulin	MLLSVPLLLGLLGLAVA
75	VSV-G	MKCLLYLAFLFIGVNC
76	CXCL10, IP-10	MNQTAILICCLIFLTLSGIQG
77	LAMP-1 N-terminal	MAPRSARRPLLLLLLLLLLGLMHCASAAMFVVKNGNGTACIM ANFSAAFSVNYDTKSGPKNMTLDLPSDATVVLNRSSCGKENT
		SDPSLVIAFGRGHTLTLNFTRNATRYSVQLMSFVYNLSDTHL FPNASSKEIKTVESITDIRADIDKKYRCVSGTQVHMNNVTVT LHDATIQAYLSNSSFSRGETRCFQDRPSPTTAPPAPPSPSPS PVPKSPSVDKYNVSGTNGTCLLASMGLQLNLTYERKDNTTVT RLLNINPNKTSASGSCGAHLVTLELHSEGTTVLLFQFGMNAS SSRFFLQGIQLNTILPDARDPAFKAANGSLRALQATVGNSYK CNAEEHVRVTKAFSVNIFKVWVQAFKVEGGQFGSVEECLLDE NSLRDT
78	LAMP-1 C-terminal	GSEFTLIPIAVGGALAGLVIVLIAYLVGRKRSHAGYQTI

[0084] Further disclosed herein are methods for making the immunogenic polypeptides described herein. The methods may comprise constructing the immunogenic polypeptides using peptide synthesis. The methods may 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. 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

[0085] Disclosed herein 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. Disclosed 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. The polynucleotide may be a DNA, a cDNA, an mRNA, a self-amplifying RNA (SAM), a self-replicating RNA, or a self-amplifying replicon RNA (RepRNA). The polynucleotide may comprise or be 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.

[0086] 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. A nucleotide may refer 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 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 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 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.

[0087] A "substitution," as used herein, denotes the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

[0088] 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.

[0089] 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 described herein and/or using any of a number of techniques well known in the art.

[0090] The nucleic acid molecule disclosed herein may be 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, disclosed herein are polynucleotides encoding an immunogenic polypeptide, described herein, wherein the polynucleotides are 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.*, at kazusa.or.jp/codon/; and genscript.com/tools/codon-frequency-table.

[0091] The polynucleotide encoding an immunogenic polypeptide, as described herein, may have 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 consisting of SEQ ID NOs: 27-37 and 89-94, as provided in Table M.

[0092] As appropriate, the 3'-end of a polynucleotide encoding one or more of the immunogenic polypeptides described herein may comprise 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.

[0093] Further disclosed herein are expression cassettes, comprising a polynucleotide encoding an immunogenic polypeptide, as described herein, operably linked to one or more regulatory sequences. The polynucleotide may be operably linked to and under the control of a constitutive promoter. The promoter may be selected from

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

Table M - Polynucleotides encoding immunogenic polypeptides

SEQ ID NO:	HBV genotype	name	Polynucleotide sequence
27	B	$Pol^{\Delta 1}$	ATGCCCCTGAGCTACCAGCACTTCACCAAGCTGCTGCTCCTCCATGATGAGCCTGGCCCTCTGGAGGACCAGC TGCCCAGGCTGGCAGATGAGGCCTCAACAGGAGAGTGGCAGAGGACCTGAACCTGGGCAACCTGAATGTGAG CATCCCCTGGACCCACAAAGTGGGGAACTTCACTGGCCTCTACAGCAGCACAGTGCCAGTGTTCAACCCTGAG TGGCAGACCCCCTCCTTCCCCCACATCCACCTCCAGGAGGACATCACAACAGATGTCAGCAGTATGTGGGCC CTCTGACAGTCAATGAGAAGAGGAGGCTGAAGCTGATCATGCCTGCCAGGTTCTACCCCAACCTGACCAAGTA CCTCCCACTCGACAAGGCCATCAACCCATACTATCCTGACCAIGTCGTGAACCACTACTTTCAGACCACCCAC TACCTGCACACACTGTGGAAGGCCGGCATCCTGTACAAGAGGCAGAGCACCAGATCAGCCTCTTTCTGIGGCT CCCCCTACAGCTGGGAGCAGGATCICCAGCATGGCAGACCTGGIGTCCAGACCTCCAAGAGGCATGGGGACAA GTCCTTTTGCCCCAGAGCCCTGGCATCCTGCCCAGGAGCGAGCCCCACCACTTCCCCCCTCCTCCAGCAGA AGCCAGTCCCAGGSACCTGTGCCTGICCGGCIGGTGGCTCCAGITCAGGAACAGTGAGCCCTGCASTGAGTACT GTCIGTGTCACATTGTGAACCTGATIGAGGACTGGGGGCCCTGCACCGAGCATGGAGAGCACAGGATCAGAAC CCCCAGGACCCCAGCCAGAGTCACICCAGCTCCTTCCTGCICCACAACAACCCCCACAACACCACAGACAGC AGACTGGTGGTGGACTTCTCCCAGITTICAAGGGCCAACACCAGAGYGTCCTGGCCCAAGTTTGCAGTGCCCA ACCTCCAGAGCCTGACCAACCTGCTGTCCTCCAACCTGAGCTGGCTGTCCTGGATGTTGTCTGCTGCCTTC*A CCACCTGCCCCTGCACCCTGCAGCCATGCCTCACCTCCTGGTCGGCACCTCAGGCCTGAGCAGGTATGICGCC AGGCTGTCAAGCAACTCCAGAACATCAICAACAACCAGCACAGGACCATGCAGAACCTGCATSACTCTTGCAGCA GGAACCTGTATGTGAGCCTGATGCTGCTGTACAAGACCTATGGCAGGAAGCTGCACCTGTACTCCCACCCCAT CATCCTGGGTTTCAGGAAGATCCCCATGGGAGTGGGACTGTCCCCCTTCCTGCTGGCCCAGTTCACCTCTGCC ATCTGCTCTGTGGTGAGGAGAGCCTTCCCCCACTGCCTGGCCTTCTCCTACATGCATGATGTGGTGCTGGGGG CCAAGTCAGTCAGCAGCACCTGGAGTCTC*GTATCCTGCAGTCACCAACTTCCTGCTCAGCCTGGGCATCCACCT GAACCCCCACAAGACCAAGAGGTGGGGCTACTCTCTGAACTTCATGGGCTATGTGATAGGCAGCTGGGGCACC CTGCCACAGGAGCACATAGTGCAGAAGATCAAGATGTGCTTCAGGAAGCTGCCAGTGAACAGGCCATTGATT GGAAGGTGTGCAGAGATTGTGGGCCTGCTGGGCTTTGCAGCACCCTCAACAGAGTGTGCTACCCAGCCCT GATCCCCCTGTATGCCTGCATCCAGGCCAAGCAGGCCTTCACCTICCCCCCACTTACAAGGCCTTCCTGCCC AAGCAGTACCTGCACCTGTACCCCTGIGGCAAGGCAGAGCCCAGGCCCCTGCCAGGTGTTTGCAGATGCCACCC CCACAGGCTCGGGCCCTGGCATCCTGGCCACCAGAGCATGAGAGGGGCCTTCTGTGATCCCACTGCCAACAC AGCCCACCTGCTGGCAGCATGCTCTGCCAGTCCCAGGTCCGGIGCAAAGCTTATTGCCACTGACAACAGTGCG GTGCTGTCCAGAAAGTACACCAGCTTCCCCTGGCTGCTGGGATGCCTGCCAACTGGATTCTGAGGGGCACCA GCTTTGTCTATCTGCCCTCCGCACICAACCCTCCAGATCACCCCCTCCAGGGCCAGACTGGGGCTGTACACCC ACTGCTCAGACTGCTGTACAGCCCACCACIGGCAGAACCCCCCTGTACGCAGACAGCCCCTCAGTGCCCCT CACCTGCCAGACAGAGTGCACTCCGCCAGCCCCTGCATGTIGCCTGGAGGCCCCC
28	B	$Pol^{\Delta 3}$	ATGCCCCTGAGCTACCAGCACTTCACCAAGCTGCTGCTCCTCCATGATGAGCCTGGCCCTCTGGAGGACCAGC TGCCCAGGCTGGCAGATGAGGCCTCAACAGGAGAGTGGCAGAGGACCTGAACCTGGGCAACCTGAATGTGAG CATCCCCTGGACCCACAAAGTGGGGAACTTCACTGGCCTCTACAGCAGCACAGTGCCAGTGTTCAACCCTGAG TGGCAGACCCCCTCCTTCCCCCACATCCACCTCCAGGAGCACATCACAACAGATGTCAGCAGTATGTCCGCC CTCTGACAGTCAATGAGAAGAGGAGGCTGAAGCTGATCATGCCTGCCAGGTTCTACCCCAACCTGACCAAGTA CCTCCCACTGGACAAGGGCATCAAGCCATACTATCCTGAGCATGTGGTGAACCACTACTTTCAGACCAGGCAC TACCTGCACACACTGTGGAAGGCCGGCATCCTGTACAAGAGGGAGAGCACCAGATCAGCCTCTTTCTGTGGCT CCCCCTACAGCTGGGAGCAGGATCTCCAGCATGGCAGACCTGGTGGCTCCAGTTCAGGAACAGTGAGCCCTGCAG TGATACTGTCTGTGTCACATTGTGAACCTGATTGAGGACTGGGGGCCCCTGCACTAGCATGGAGAGCACAGG ATCAGAACCCCCAGGACCCCAGCCAGAGTGACTCGAGGTGTCTTCCTGGTGCACAACAACCCCCACAACACCA CAGAGAGCAGACTGGTGGTGGACTTCTCCCAGTTTCAAGGGCCAACACCAGAGTGTCCTGGCCCAAGTTGC AGTGCCCAACCTCCAGAGCCTGACCAACCTGCTGTCATCAAACCCGAGCTGGCTGTCCTGGATGTGTCTGCT GCCTTCTACCACCTGCCCCTGCACCCTGCAGCCATGCCTCACCTCCTCGTCGGCACCTCAGGCCTGAGCAGGT ATGTGGCCAGGCTGTCAAGCAACTCCAGAATCATCAACAACCAGCACAGGACCATGCAGAACCTGCATGACTC TGCAGCAGGAACCTGTATGTGAGCCTGATGCTGCTGTACAAGACCTATGGCAGGAAGCTGCACCTGTACTCCC ACCCCATCATCCTGGGTTTCAGGAACATCCCCATGGGAGTCCGACCTCCCCCTTCCTGCTGGCCCACTTCA CCTCTGCCATCTGCTCTGTGGTGAGGAGAGCCTTCCCCCACTGCCTGGCCTTCTCCTACATGCATGATGTGGT GCTGGGGGCCAAGTCAGTCAGCAGCACCTGGAGTCTCTGTATCCTGCAGTCACCAACTTCCTGCTCAGCCTGG ATCCACCTGAACCCCCACAAGACCAAGAGGTGGGGCTACTCTCTGAACTTCATGGGCTATGTGATAGGCAGCT GGGGCACCCTGCCACAGGAGCACATAGTGCAGAAGATCAAGATGTGCTTCAGGAAGCTGCCAGTGAACAGGCC CATTGATTGGAAGGTGTGCCAGAGGATTGTGGGCCTGCTGGGCTTTGCAGCACCCTTCACACAGTGTGCTAC CCACCTCTGATCCCCCTGTATCCCTCCATCCAGGCCAACCACCCCTCCACCTTCTCCCCCACTTACAACCCCT TCCTGCCCAAGCAGTACCTGCACCTGTACCCCTGTGGCAAGGCAGAGGCCAGGCCTCTGCCAGGTGTTTGCAGA TGCCACCCCACAGGCTGGGGCCTGGCCATTGGCCACCAGAGGATGAGAGGGGCCTTTGTGATCCCACTGCCA ACCACACAGCCCACCTGCTGCCAGCATGCTCTGCCAGTCCAGGTCTGCTCAAACCTGATTGCCACTGACAACA ACAGTGTGCTACCCAGCTTTCCCCTGGCTGCTGGGATGCCTGCCAACTGGATTCTGAGGGGCACCAGCTTTG GGCCACCAGCTTTGTCTATCTGCCCTCCGCACTGAACCCTCCAGATCACCCCCTCCAGGGGCAGACTGGGGCTG TACAGGCCACTGCTCAGACTGCCGTACAGGCCCACCACTGGCAGAACCCCCCTGTATGCAGACAGCCCCTCAG TGCCCCTCACCTGCCAGACAGAGTGCACTTCGCCAGCCCCCTGCATGTTGCCTGGAGGCCCCCC
29	B	Pol^{300}	ATGTCCAGCAGAAGCAGTCCCAGCCACCTCTGCTGTCCGCIGGTGCCTCCAGTTCAGGAACAGTGACCCCT GCACTGAGTACTGTCTGTGCCACAITGIGAACCTGATTGAGGACTGGGGGCCCTGCACTGAGCATGGAGAGCA CAGGATCAGAACCCCCAGGACCCCAGCCAGAGTGACTGGAGGTGTCCTCGTGGTGGACAACAACCCCCACAAC ACCACACAGACAGCACTGGTGCTGCACTTCICCCAGTTTCCAAGGGGCAACACCAGAGTGTCCTGGCCCAAGT TGCIGCCTTCTACCACCTGCCCCTGCACCCTGCAGCCATGCCTCACCTCCTCGTCGGCAGCTCAGGCCIGAGC AGGTATGTGGCCAGGCTGTCAAGCAACTCCAGAATCATCAACAACCAGCACAGGACCATGCAGAACCTCCATG ACTCTTGCAGCAGGAACCTGTATGTGAGCCTGATGCTGCTGTACAAGACCTATGGCAGGAAGCTGCACCTGTA CTCCCACCCCATCATCCTGGGTTTCAGGAAGATCCCCATGGGAGTGGGACTGTCCCCCTTCCTGCTGGCCCAG TTCACCTCTGCCATCTGCTCTGTGGTGAGGAGAGCCTTCCCCCACTGCCTGGCCTTCTCCTACATGCATGATG GGCCACCACTGAACCCCCACAAGACCAAGAGGTGGGGCTACTCTCTGAACTTCATGGGCTATGTGATAGGCAG AGCTGGGGCACCCTGCCACAGGAGCACATAGTGCAGAAGATCAAGATGTGCTTCAGGAAGCTGCCAGTGAACA GGCCCATTGATTGGAAGGTGTGCCAGAGGATTGTGGGCCTGCTGGGCTTTGCAGCACCCTTCACACAGTGTGG CTACCCAGCTGTGATCCCCCTCTATCCCTCCATCCAGGCCAACCACCCCTCCACCTTCTCCCCCACTTACAAG GCCTTCCTGTCCAAGCAGTACCTGCACCTGTACCCTGTGGCAAGGCAGAGGCCAGGCCTCTGCCAGGTGTTTG CAGATGCCACCCCCACAGGCTGGGGCCTGGCCATTGGCCACCAGAGGATGAGAGGGGCCTTTGTGATCCCACT GCCAACCACACAGCCCACCTCCTCCCCAGCATGCTTTGCCACCTCCACGTCTGGTGCAAAGCTGATTCCCACT GACAACAGTGTGGTGCTGTCCAGAAAGTACACCAGCTTCCCCTGGCTCCTGGGATGTGCTGCCAACTGGATTC TGAGGGGCACCAGCTTTGTCTATGTGCCCTCCGCACTGAACCCTGCAGATGACCCCTCCAGGGGCAGACTGGG GCTGTACAGGCCACTGCTCAGACTGCTGTACAGGCCCACCACTGGCAGAACCTCCCTGTATGTAGACAGCCCCT TCACTCCCCTCTCACCTGCCACACACAGTGCACTTTGCCAGCCCCCCCCATCTTGCCTGGAGGCCCCCC
89	B	Pol^{300} ori	ATGTCTCAAGATCCCAGAGTCAGGGCCCTGTACTTTCCCGCIGGTGCCTCCAGTTCAGGAACAGTGACCCT GCTGCAATACTGTCTGTGCCACATGCIGAACCTGATTGAGGACTGGGGGCCCTGTACCGAACATGGAGAGCA TCAGATCAGAACCCCCAGGACCCCAGCCAGAGTGACTGGAGGTGTCCTCGTGGTGGACAAAAATCCTCACAAT ACCACACAGAGTGTAGACTCGTGGTGGACTTCTCCCAATTTCCIAGGGGGAACACCCGTGTGTCTTGGCCAAAAT TCGCAGTCCCAAATCTCCAGTCACICACCAACGTGTTGTCCICCAACTTGTCCTGGTTATCGCTGGATGTGTCC TGCGGCGTTTTATCATCTTCCTCTGCATCCIGCTGCTATGCCICATCTCCTTGTTTGGTTCTTCTGGACIACCA AGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCATCAACAACCAGCACCGGRCCATGCAAAACCTGCACG
			ACTCCTGCTCAAGGAACCTCTATGTTTCCCICATGTTGCTGTACAAAACCTACGGACGGAAACTGCACITGTA TTCCCATCCCATCATCTTGGGCTTCGCAAAATTCCTATGGGAGTGGGCCTCAGTCCGTTTCTCTTGGCTCAG TTACTAGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCACTGTCTGGCTTTCAGTTATATGCAIGATG TGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTATGCCGCTGTTACCAATTTTCTTTTGICCTT GGGTATCATTTAAACCCTCACAAAACAAAAGATGGGGATATTCCCTAACTTCATGGGATATTGTAATTGGG ACTTGGGCCACATTGCCGCAGCAACATATTCCACAAAAACTCAAAACTCTGTTTTACGAAACTTCCTGTAAACC GGCCTATTGATTGGAAAGTATGTCAACGAATTGTGGGTCCTTIGGGGTTCGCCGCCCTTTCACGCAATGGG ACAICCTGCTTTAATGCCTTACATGCATGTACACAAGCAAAACAGGCCTTACTTTCTCGCCAACTTACAAG GCCTTCCTAAGTAACAGTATCCGCACCTTIACCCCGTTGCICGGCAACGGCCTGGTCTGTGCCAAGTGTTTG CTGACGCAACCCCCACTGGTTGGGGCTTGGCCATAGGCCATCAGCGCATGCGTGGAGCCTTCGTGTCTCCCCT GCCGATCCATACTGCGCATCTCCTGGCCGCTTGTTTGCTCCGCAGCAGGTCTGGGGCAAAACTCATCGGGACT GACAATTCTGTCGTGCTCTCCCGCAAGTATACATCCTTTCCATGGCTGCCTAGGCTGTGCTGCCAACTGGACCC TGCCCGGGACGTCCTTTGTTACGTCCCGTCGGGCCTGAATCCCGCGGACGACCCCTCCCGGGGCCGCTTGGG GCTCTACCCCCCCTTCTCCGCCTCCTTCTACCCACCCAGCTACCGGGCCCCACCTCTCTCTACGCCGACTCCCCC TCTGTGCCTTCTCATCTGCCGGACCGTGTGCACTTCGCTTCACCTCTGCACGTCGCATGGAGACCACCGT

Table M - Polynucleotides encoding immunogenic polypeptides			
SEQ ID NO:	HBV genotype	name	Polynucleotide sequence
90	B	Pol^{300} dint	ATGTCATCCAGATCCCAGAGTCAGGGCCCTGTCCTTTCCTGTTGGTGGCTCCAGTTCAGGAACAGTGAGCCCT GTTCTGAGTACTGTCTCTGCCACATTGTCAATCTGATTGAGGACTGGGGCCCCTGCACAGAGCATGGTGAACA CAGGATCAGGACTCCCAGGACCCCTGCCAGGGTGACTGGTGGGGTTTTCCTTGTTGACAAAAATCCTCACAAC ACCACAGAGTCAAGGCTTGTGGTGGACTTCTCTCAATTTTCAAGGGGAACACAAGGGTGTCTTGGCCCAAAT TTGCAGTCCCAAATCTCCAGTCTCTGACCAACCTGTTGTCCTCCAATTTGTCCTGGTTGTCTCTGGATGTCTC TGCTGCCTTTTATCATCTTCCTCTCCATCCTGCTGCCATGCCTCATCTTCTTGTTGGTTCTTCTGGCCTCTCT AGGTATGTTGCCAGATTGTCCTCCAATTCCAGGATCATCAACAACCAGCACAGGACCATGCAAAACCTGCATG ACTCCTGCTCCAGAAACCTCTATGTTTCTCTCATGTTGCTGTACAAAACCTATGGCAGGAAACTGCATTTGTA TTCCCATCCCATCATCTTGGGCTTTCAGGAAAATTCCCATGGGAGTGGGCCTCAGTCCCTTCCTCTTGGCTCAG TTCACCAGTGCCATTTGTTCTGTTGTCAGGAGGGCTTTCCCCCACTGTCTTGCTTTCAGTTACATGCATGATG TGGTCTTGGGGGCCAAGTCTGTCCAACATCTTGAGTCACTTTATGCTGCTGTGACCAACTTTCTTTTGTCTTT GGGCATCCATTTGAACCCTCACAAAACCAAAAGATGGGGCTATTCCCTCAATTTCATGGGCTATGTCATTGGG AGTTGGGGCACTTTGCCCCAGGAACACATTGTGCAAAAAATCAAGATGTGTTTCAGGAAACTTCCTGTGAACA GGCCAATTGACTGGAAAGTCTGTCAGAGAATTGTGGGTCTTTTGGGGTTTGCAGCTCCTTTCACCCAATGTGG
			CTATCCTGCTTTGATGCCCTTGTATGCCTGCATCCAGGCCAAACAGGCTTTCACTTTCTCCCCCACTTACAAG GCCTTCCTCAGCAAACAGTATCTCCACCTTTACCCTGTTGCAAGGCAGAGGCCTGGTCTGTGCCAAGTGTTTG CTGATGCAACCCCCACTGGTTGGGGCTTGGCCATTGGCCATCAGAGAATGAGAGGTGCCTTTGTGTCTCCTCT CCCCATCCACACTGCTCATCTCCTGGCAGCTTGCTTTGCAAGGAGCAGGTCTGGAGCCAAACTCATAGGGACT GACAATTCTGTGGTGCTCTCCAGAAAGTACACCTCCTTTCCTTGGCTGCTGGGCTGTGCAGCCAACTGGATCC TGAGGGGGACTTCCTTTGTTTATGTCCCCTCTGCCCTGAATCCTGCAGATGACCCCTCCAGGGGCAGGTTGGG GCTCTACAGACCCCTTCTCAGGTTGTTGTACAGACCAACAACAGGGAGGACCTCTCTCTATGCAGATTCCCCC TCTGTTCCTTCTCATCTTCCAGACAGAGTGCACTTTGCTTCTCCTCTGCATGTGGCTTGGAGACCTCCC
91	B	Pol^{300} huCo low GC	ATGTCTAGCCAGAAGCCAGTCCCAGCCACCTCTGCTGTCTTGTTGGTGCCTTCAGTTTCGGAATAGCCACCCAT GTAGCGAGTATTGCCTGTGTCACATCGTGAATCTGATTGAGGATTGGGGACCATGCACAGAGCACGGAGAGCA CCGGATCAGAACCCCTAGGACACCAGCCCGCGTGACAGGAGGCGTGTTCCTGGTGGATAAGAACCCCCATAAT ACAACAGAGAGCAGACTGGTGGTGGATTTTTCTCAGTTTTCTCGGGGCAATACAAGAGTGTCCTGGCCAAAGT TTGCCGTGCCCAATCTCCAGAGCCTGACAAACCTGCTGTCTTCTAATCTGAGCTGGCTGTCCCTGGACGTGTC CGCCGCCTTTTACCACCTGCCACTCCACCCTGCCCCCATGCCCCACCTGCTCGTGGGCAGCTCCGGACTCAGC AGATACGTGGCAAGGCTGTCTAGCAATTCTAGAATTATTAATAATCAGCACAGAACAATGCAGAATCTGCATG ATTCTTGTAGCAGGAATCTGTACGTGAGCCTGATGCTGCTGTATAAGACATATGGACGCAAGCTGCACCTGTA TTCTCACCCTATTATTCTGGGCTTCCGGAAGATCCCTATGGGCGTGGGACTGTCCCCATTCCTGCTGGCCCAG TTTACCTCCGCCATCTGCTCTCTGCTCCGGAGAGCCTTCCCACATTGTCTCGCCCTTTTCTTACATGCACCATG TGGTGCTGGGCGCCAAATCCGTGCAGCACCTGGAGTCTCTGTATGCCGCCGTGACAAACTTCCTGCTGAGCCT GGGCATCCACCTGAATCCACATAAGACAAAGCGGTGGGGCTATTCTCTGAATTTTATGGGCTATGTGATCGGC AGCTGGGGAACCCTGCCACAGGAGCACATTGTGCAGAAGATCAAGATGTGCTTTCGCAAGCTGCCCGTGAATC GCCCTATCGATTGGAAGCTGTCCCACAGGATCGTCGGACTGCTGGGATTCGCAGCACCCTTTACCCAGTCCCG CTACCCAGCCCTGATGCCACTGTATGCCTGTATCCAGGCCAAACAGGCCTTCACCTTTTCCCCTACATATAAG GCTTTTCTGTCTAAGCAGTACCTGCATCTGTATCCAGTGGCAAGGCAGAGGCCAGGACTGTGCCAGGTGTTTG CAGATGCAACACCAACAGGATGGGGACTGGCAATCGGACACCAGAGGATGAGAGGAGCCTTCGTGAGCCCACT GCCAATTCACACCGCCCACCTGCTGGCAGCATGCTTTGCAAGGTCCCGCTCTGGAGCAAAGCTGATTGGCACC GATAACAGCGTGGTGCTGTCCAGAAAATACACCAGCTTCCCCTGGCTGCTGGGATGTGCAGCAAATTGGATTC TGAGGGGCACCAGCTTCGTGTATGTGCCTTCCGCCCTGAATCCTGCCGATGATCCATCTCGAGGCAGACTGGG
			ACTGTATAGGCCACTGCTGAGACTGCTGTATAGGCCTACCACAGGCAGAACATCCCTGTATGCCGACAGCCCA TCCGTGCCCTCTCACCTGCCAGATAGAGTGCATTTCGCAAGCCCACTGCATGTGGCATGGAGGCCACCC
92	B	Pol^{300} ori_d el CpG	ATGTCTTCAAGATCCCAGAGTCAGCCCCCTCTACTTTCCTGCTGGTGCCTCCAGTTCAGGAACAGTGACCCCT GCTCTGAATACTGTCTCTGCCATATTGTCAATCTTATAGAAGACTGGGGACCCTGTACTGAACATGGAGAACA TAGGATCAGGACTCCTAGGACCCCTGCTAGAGTTACAGGGGGGTTTTTCTTGTTGACAAAAATCCTCACAAT ACCACAGAGTCTAGACTTGTGCTGCACTTCTCTCAATTTTCTAGGGGCAACACCAGGCTGTCTTGGCCAAAAT TTGCAGTCCCAAATCTCCAGTCACTCACCAACCTGTTGTCCTCCAATTTGTCCTGGTTATCCCTGGATGTGTC TGCAGCCTTTTATCATCTTCCTCTCCATCCTGCTCCTATGCCTCATCTTCTTGTTGGTTCTTCTGGACTATCA AGGTATGTTGCCAGGTTGTCCTCTAATTCCAGGATCATCAACAACCAGCACAGGACCATGCAAAACCTGCATG ACTCCTGCTCAAGGAACCTCTATGTTTCCCTCATGTTGCTGTACAAAACCTATGGAAGGAAACTGCACTTGTA TTCCCATCCCATCATCTTGGGCTTTAGAAAAATTCCTATGGGAGTGGGCCTCAGTCCCTTTCTCTTGGCTCAG TTTACTAGTGCCATTTGTTCAGTGGTTAGAAGGGCTTTCCCCCACTGTCTGGCTTTCAGTTATATGCATGATG TGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTTATGCTGCTGTTACCAATTTTCTTTTGTCTTT GGGTATACATTTAAACCCTCACAAAACAAAAAGATGGGGATATTCCCTTAACTTCATGGGATATGTAATTGGG AGTTGGGGCACATTGCCTCAGGAACATATTGTACAAAAAATCAAAATGTGTTTAGGAAACTTCCTGTAAACA GGCCTATTCATTGGAAAGTATCTCAAAGAATTGTCGGTCTTTTGGGGTTTGCAGCCCCTTTCACCCAATCTGG ATATCCTGCTTTAATGCCTTTATATGCATGTATACAAGCAAAACAGGCTTTTACTTTCTCCCCAACTTACAAG GCCTTCCTAAGTAAACAGTATCTGCACCTTTACCCTGTTGCTAGGCAAAGGCCTGGTCTGTGCCAAGTGTTTG CTGATGCAACCCCCACTGGTTGGGCCTTGGCCATAGGCCATCAGAGGATGACGGGAGCCTTTGTGTCTCCTCT GCCTATCCATACTGCCCATCTCCTCCCAGCTTGTTTTGCTACCAGCACGTCTGGGCAAAACTCATTGCCACT GACAATTCTGTTGTGCTCTCCAGAAAGTATACATCCTTTCCATGGCTGCTAGGCTGTGCTGCCAACTGGATCC TGAGGGGACATCCTTTGTTTATGTCCCTTCAGCACTGAATCCTGCTGATGACCCCTCCAGGGGCAGATTGGG GCTCTACAGGCCCCTTCTCAGCTTCTTGTACAGACCCACTACTGGCACAACCTCTCTCTATGCAGACTCCCCC TCTGTGCCTTCTCATCTGCCTGACAGGGTGCACTTTGCTTCACCTCTGCATGTTGCATGGAGACCACCT
93	B	Pol^{300} IDT	ATGAGTTCCCGATCACAGAGTCAGGGGCCCGTCCTTTCATGTTGGTGGCTTCAGTTTCGAAACTCCGAGCCAT GTTCTGAGTATTGTCTCTGCCACATTCTGAATCTTATTCAACACTGGCGCCCCTGCACCGAGCACGGCCAGCA CCGAATACGGACACCTCGAACGCCAGCAAGAGTGACGGGCGGAGTGTTCCTCGTCGACAAGAATCCACACAAC ACGACGGAGAGTAGATTGGTCGTTGATTTCAGTCAATTTTCAAGAGGCAATACACGAGTTTCTTGGCCGAAAT TCCCCGTACCCAATCTGCAATCCTTCACAAATTTCCTTAGTTCTAATTTGTCTTGGCTTTCTCTCGATCTTTC
			CGCCGCTTTCTATCACTTGCCCCTTCACCCAGCCGCGATGCCGCATCTCTTGGTGGGCAGCTCTGGACTTAGT AGATACGTAGCTAGACTCAGTTCTAACTCACGGATAATAAATAACCAACATCGCACTATGCAGAACCTGCATG ATTCTTGTTCCCGGAACTTGTATGTCTCCTTGATGTTGTTGTATAAAACTTATGGGCGAAAGCTTCATCTGTA TAGCCATCCGATTATATTGGGTTTTAGGAAAATTCCTATGGGTGTTGGCTTGAGCCCTTTTCTGCTGGCGCAA TTTACTTCAGCTATCTGCTCAGTAGTACGCCGGGCGTTTCCCCATTGTCTTGCTTTCTCATACATGCATGATG TAGTACTTGGGGCCAAGTCTGTACAACACCTTGAGAGTTTGTATGCCGCCGTAACTAATTTCCTTCTCTCTCT CGGGATCCATCTTAACCCTCACAAAACGAAGAGGTGGGGTTATTCTCTGAATTTCATGGGATATGTTATCGGG TCTTGGGGAACGCTGCCTCAGGAACACATCGTCCAGAAAATCAAGATGTGTTTCAGAAAGTTGCCAGTGAACA GACCGATAGATTGGAAGGTTTGCCAAAGAATTGTTGGCTTGTTGGGATTCGCAGCCCCATTCACACAGTGCGG GTATCCGGCTTTGATGCCCCTTTATGCTTGTATCCAGGCAAAACAGGCATTCACCTTTTCACCGACTTACAAA GCATTTCTTTCTAAGCAGTATCTCCATCTTTACCCTGTCGCTCGACACCGGCCGGGGCTTTGCCAGGTTTTCG CAGACGCAACCCCAACTGGTTGGGGTCTTGCGATCGGCCACCAGAGGATGCGCGGTGCATTCGTGTCCCCGCT CCCAATCCATACGGCCCACTTGCTGGCGGCGTGCTTCGCTCGAAGTAGAAGCGGGGCTAAATTGATCGGCACG GACAATTCAGTCGTGTTGTCACGCAAATATACCTCCTTTCCCTGGTTGCTCGGTTGCGCAGCAAACTGGATAC TTCGGGGAACTAGTTTCGTTTATGTGCCCTCTGCTCTCAACCCCGCCGACGATCCTTCACGAGGGAGGCTGGG TCTTTACCGCCCATTGCTCAGGCTGCTTTACCGGCCTACCACTGGGAGAACAAGCTTGTACGCCGACAGCCCG AGCGTCCCGTCTCATCTGCCCGACAGAGTTCACTTTGCGAGTCCATTGCACGTCGCTTGGCGCCCGCCG
94	B	Pol^{300} _IDT_ CpGde 1	ATGAGTTCCAGATCACAGAGTCAGGGGCCTGTCCTTTCATGTTGGTGGCTTCAGTTTAGAAACTCAGAGCCAT GTTCTGAGTATTGTCTCTGCCACATTCTGAATCTTATTCAACACTGGCGCCCCTGCACAGAGCATGGACAGCA CAGAATAAGGACACCTAGAACCCCAGCAAGAGTGACAGGTGGAGTGTTCCTGGTAGACAAGAATCCACACAAC ACAACTGAGAGTAGATTGGTGGTTGATTTCAGTCAATTTTCAAGAGGCAATACAAGAGTTTCTTGGCCAAAAT TTGCTGTACCCAATCTGCAATCCTTGACAAATTTGCTTAGTTCTAATTTGTCTTGGCTTTCTCTAGATGTTTC TGCAGCTTTCTATCACTTGCCCCTTCACCCAGCAGCTATGCCTCATCTCTTGGTGGGCAGCTCTGGACTTAGT AGATATGTAGCTAGACTCAGTTCTAACTCAAGGATAATAAATAACCAACATAGGACTATGCAGAACCTGCATG ATTCTTGTTCCAGGAACTTGTATGTCTCCTTGATGTTGTTGTATAAAACTTATGGGAGAAAGCTTCATCTGTA TAGCCATCCTATTATATTGGGTTTTAGGAAAATTCCTATGGGTGTTGGCTTGAGCCCTTTTCTGCTGGCCCAA TTTACTTCAGCTATCTGCTCAGTAGTAAGGAGGGCCTTTCCCCATTGTCTTGCTTTCTCATACATGCATGATG TAGTACTTGGGGCCAAGTCTGTACAACACCTTGAGAGTTTGTATGCAGCAGTAACTAATTTCCTTCTCTCTCT

Table M - Polynucleotides encoding immunogenic polypeptides

SEQ ID NO:	HBV genotype	name	Polynucleotide sequence
			TGGGATCCATCTTAACCCTCACAAAACCAAGAGGTGGGGCTAITCTCTGAATTTCATGGGATATGTTAIAGGG
			TCTTGGGGAACCCTGCCTCAGGAACACATTGCCCAGAAAATCAAGAACGCTGTTCAGAAAGTTGCCAGTGAACA GACCAATAGATTCGAAGGTTICCCAAAGAATCGTTGGCTCGTIGCGATTCGCAGCCCATTCACACAGICCGG GCAICCTGCTTTGATGCCCCTTCAIGCTTGIATCCAGGCAAAACAGGCATTCACCTTTTCACCCACTTACAAA GCAITCCTTCTAAGCAGTATCCCCATCTTIACCCTGTGGCIAGACAGAGGCCAGGGCTTTGCCAGGTIITTG CAGATGCAACCCCAACTGGTIGGGGICTTGCAATIGGCCACCAGAGGATGACAGGTGCATTTGTGTCCCCACT CCCAACCCATACTCCCACCTGCTGGCAGCIGCTTTGCTAGAAGTAGAAGTGGGGCTAAATTGATTGGCACA GACAACTCAGTTGTTGTCAAGGAAATATACCTCCTTTCCCIGGTTGCTTGGTTGTGCASCAAACTGGATAC TTAGGGGAACTAGTTTTGTTTATGIGCCCTCTGCTCTCAACCCTGCAGALGATCCTTCAAGAGGAGGCTGGG TCTITACAGGCCATTGCTCAGGCCGCTTACAGGCCTACCACIGGGAGCAACAAGCTTGTATGCAGACAGCCCC AGTGTCCCCTCTCATCTGCCTGCACAGAGTTCACTTTGCAAGICCATTGCATGTTGCTTGGAGACCTCCA
30	D	$Pol^{\Delta 1}$	ACGCCCTGAGCTACCAACACTTCACCAGACTGCTGCTCCTCCATGATGAGCCAGCCCCTTGTGGAGGACCAGC TGCCCAGGCTGGCAGATGAGGGCCIGAACAGGAGGGTGGCTGAGGACCTGAACCTGGGCAACCTGAATGTGAG CATCCCTTGGACCCACAAAGTGGGCAACTTCACAGGCCTGTACAGCAGCACTGTGCCTGTGTTCAACCCCCAC TGGAAGACACCCAGCTTCCCCAACATCCACCTGCACCACGACATCATCAAGAAGTGTGAGCAGTTTGTCCCCC CCCTGACAGTCAATGAGAAGACGACCCTCCAGCTCATCATCCCAGCCAGGTTCTACCCCAATGTGACCAAGTA CCTCCCCCTGGACAAGGGCATCAAGCCTTACTATCCAGAGCACCTGGTGAACCACTACTTCCAGACCAGACAC TACCTGCACACACTGTGCAAGCCACCCATGCCGTACAACAGCCAGACCACACACAGTGCCTCCTTCTGICGCA GCCCCTACTCCTGCGACCAGGACCICCAACATGGAGCTCAGICCTTCCACCAGCAGTCCAGTGGCATCCTGAC CAGCCCCCCTGTGGGCAGGAGCCTCAACATGGAGCTCAGTCCTTCCACCAGCAGTCCAGTGGCATCCTGAC TGCTGGAGGACTGGGGCCCCTGCGCAGAGCATGGGGAGCACCACATCAGAATCCCCAGGACCCCTGCCAGGGT GACAGGAGAGGGTSTTCCTGGTGGACAAGAACCCCCACAACACTGCAGAGTCCAGGCTGGTSGTGGACTICCCC CAGTTCAGCAGGGSCAACTACAGAGICCCTGGCCAAAGCTTGCTGTGCCCAACCTCCAGAGCCTGACAAACC TGCTGAGCAGCAACCTGTCCTCGCICTCCCTGGATGTGAGTCCAGCCTTCTATCAGCCTGCCCCTGCACCCAGC AGCCATGCCAGACCTGGTGGTGGGCTCCAGTGGCCTGTCCAGGTATGTGGCCAGGCTCTCCTCCAACTCCAGG ATCITCAACTATCAGCATGGCACCATCGCAGAACCTGCATGACAGCTGCCCCAGGAACCTGTATGTGTCCCTGA TGCTGCTCTATCAGACCTTTGGCAGGAAGCTGCACCTGTACAGCCACCCCATCATCCTGGGGTTCAGGAAGAT CCCCATGGGTGTGGGCCTGCCCCCITCCTGCTGGCCCAGTICACCAGTGCCATCTGCTCAGTGGTGAGGAGG
			GCCITCCCACACTGCCTGGCCTCCICTCACATGCATGATGTGGTCCTGGGTGCCAAGTCTGTGCAGCACCTGG AGACCCTGTTCACAGCTGTGACAAACTTICICCTGAGCCCGGGCATCCACCTGAACCCCAACAAGACCAAGAG GCGGGGTTATTCACTGCACCTCATGGGCTAIGTGATTGGCTGCTATGGCCCICTGCCACAGGACCACATCATC CAGAAGATCAAGGAGTGCTCCAGAAAGCTGCCAGTGAACAGGCCAATTGACTGGAAGGTGTGCCAGAGGACTG TGGGCCTGCTGGGCTTTGCAGCCCCCTCACCCAGTGTGGCIACCCTGCCCTGATGCCCCTGTATGCCIGCAT CCACAGCAAGCAGGCCTTCACCTTIGCCCCACTIACAAGGCCTCCGTGTGCAASCAGTACCTGAACCIGCAC CCTGTGGCCAGGCAGAGACCTGGGCIGTGCCAGGTGTTIGCAGATGCCACCCCCACAGGATGGGGACTGCCA TGCCACACCAGACGATCAGCCCCACCTCCAAGGCACCCCCGCCCATCCACACAGCCCACCTGCTGCCTCCCTG CTTIGCCAGGAGCAGGAGTGGCGCCAACATCCTGCGCGCACAGACAACCCTGTGTGTGCTGAGCAGGAAGTACACA TCCITCCCCTGGCTGCTGGGATGTGCAGCCAACTGGATCCTGAGGGGCACCAGCTTTGTGTATGTGCCCTCTG CCCICAACCCTGCAGATCACCCAACCACGCCCAGCCTCCGACTGCACAGCCCACTGCTCAGACTGCCCITCAC GCCCACCACTGGCAGGACCAGCCCTATAGCTGACTCCCCATCTGTGCCCTCCCACCTGCCTGACAGAGTGCAC TTTGCCTCCCCACTGCATGTGGCCTGGAGGCCCCCA
31	D	$Pol^{\Delta 3}$	ATGCCCCTGAGCTACCAACACTTCACCAGACTGCTGCTCCTCCATGATGAGCCAGCCCCTCTGGAGGACCAGC TGCCCAGGCTGGCAGATGAGGGCCTGAACAGGAGGGTGGCTGAGGACCTGAACCTGGGCAACCTGAATGTGAG CATCCCTTGGACCCACAAAGTGGGCAACTTCACAGGCCTGTACAGCAGCACTGTGCCTGTGTTCAACCCCCAC TGGAAGACACCCAGCTTCCCCAACATCCACCTGCACCACGACATCATCAAGAAGTGTGAGCAGTTTGTCGGCC CCCTCACAGTCAATGAGAAGACGACCCTCCAGCTCATCATCCCAGCCAGGTTCTACCCCAATGTGACCAAGTA CCTCCCCCTGGACAAGGGCATCAACCCTTACTATCCAGAGCACCTGGTGAACCACTACTTCCAGACCAGACAC TACCTGCACACACTGTGGAAGCCAGGCATCCTGTACAAGAGGGAGACCACACACAGTGCCTCCTTCTGTGGCA GCCCCTACTCCTGCGACCAGGAGCICCAACATGGATGCTGGTCCCTCCAGTTCAGCAACAGCAACCCCICCTC AGACTACTGCCTGAGCCACATTGTGAACCTGCTGGAGGACCGGGGCCCCCTGTGCAGAGCATGGGGAGCACCAC ACCAGAATCCCAGGACCCCTGCCAGGGTGACAGGAGGGGTGTTCCTGGTGGACAAGAACCCCCACAACACTG CAGAGTCCAGGCTGGTGGTGGGACTICTCCCAGTTCAGCAGGGGCAACTACAGAGTCTCCTGGCCAAAGTTGC TGTGCCCAACCTCCAGAGCCTGACAAACCTGCTGAGCAGCAACCTGCCCTGGCTCTCCCTGGATGTGAGTGCA GCCTTCTATCACCTGCCCCTGCACCCAGCAGCCATGCCACACCTGCTGTGGCTCCAGTGGCCTGTCCAGGT ACTGGGCCAGGCTCTCCTCCAACCCCAGGAICCTCAACTATCAGCATGGCACCATGCAGAACCTGCATGACAG CAGCTCCAGGAACCTGTATGTGTCCCTGATGCTGCTCTATCAGACCTTTGGCAGGAAGCTGCACCTGTACAGC CACCCCATCATCCTGGGGTTCAGGAAGATCCCCATGGGTGTGGGCCTGCCCCCTTCCTGCTGGCCCAGTTCA
			CCACTGCCATCTGCTCAGTGGTGAGGAGCCAGTGTTCCCTTGCTGGTGGCTCCAGTTCAGGAACAGCAAGCCCT GCTCAGACTACTGCCTGAGCCACATTGTGAACCTGCTGGAGGACCGGGGCCCCTGTGCAGAGCATGGGGAGCA CCACAACAGAATCCCCAGGACCCCTGCCAGGGTGACAGGAGGGGTGTTCCTGGTGGACAAGAACCCCCACAAC ACTGCAGAGTCCAGGCTGGTGGTGGGACTTCTCCCAGTTCAGCAGGGGCAACTACAGAGTCTCCTGGCCAAAGT TGCTGTGCCCAACCTCCAGAGCCTGACAAACCTGCTGAGCAGCAACCTGCCCTGGCTCTCCCTGGATGTGAGC TGCAGCCTTCTATCACCTGCCCCTGCACCCAGCAGCCATGCCAGACCTGGTGGTGGGCTCCAGTGGCCTGTCC AGGTATGTGGCCAGGCTCTCCTCCAACCCCAGGATCTTCAACTATCAGCATGGCACCATGCAGAACCTGCATG ACAGCTGCTCCAGGAACCTGTATGTGTCCCTGATGCTGCTCTATCAGACCTTTGGCAGGAAGCTGCACCTGTA CAGCCACCCCATCATCCTGGGGTTCAGGAAGATCCCCATGGGTGTGGGCCTGTCCCCCTTCCTGCTGGCCCAG TTCACCAGTGCCATCTGCTCAGTGGTGAGGAGGGCCTTCCCACACTGCCTGCCCTCTCTTACATGCATGATG TGGTCCTGGGTGCCAAGTCTGTGCAGCACCTGGAGAGCCTGTTCACACCTGTGACAAACTTTCTCCTGACCCT GGGCATCCACCTGAACCCCAACAAGACCAAGAGGTGGGCTTATTCACTGCACCTCATGGGCTATGTGATTGGC TGCTATGGCTCTCTGCCACAGGACCACATCATCCAGAAGATCAAGGAGTGCTTCAGAAAGCTGCCAGTGAACA GGCCAATTGACTGGAAGGTGTGCCAGAGGATCTGGGCCCTGCTGGGCTTTGCAGCCCCCTTCACCCAGTGTGC CCACCTGCCTGACAGAGTGCACTTTGCCTCCCCACTCCATCTGCCTGGAGGCCCCCA
			CAGATGCCACCCCCACAGGATGGGGACTGGTCATGGGACACCAGAGGATGACGGGCACCTTCAAGGCACCCCT GCCCATCCACACAGCCCACCTGCTGGCTGCCTTTGCCAGGAGCAGGAGTGGGGCCAACATCCTGGGCACA GACAACTCTGTGGTGCTGAGCAGGAAGTACACATCCTTCCCCTGGCTGCCTGGATGTGCAGCCAACTGGATCC TGAGGGGCACCAGCTTTGTGTATGTGCCCTCTGCCCTCAACCCTGCAGATGATCCAAGCAGGGGCAGGCTGGG ACTCTACAGGCCACTGCTCAGACTCCCCTTCAGGCCCACCACTGCCACGACCAGCCTGTATGCTGACTCCCCA TCTCTGCCCTCCCACCTGCCTCACACAGTGCACTTTGCCTCCCCACTCCATCTGCCTGGAGGCCCCCA
33	B/C	Core-sAg	ATGCACATTGACCCCTACAAAGCAGTTTGGGCCCACTGTCGACCTGCCCCTTTTCTGCCATCTGACTTCTCCC CCAGTGTGAGGGACCTGCTGGACACTGCCTCAGCACTGTACAGAGAGGCCCTGGAGAGCCCAGAGCACTGCCC CCCCACCACACAGCCCTGAGGCAGGCCATCCCTGCTGGGGGAGGCTGATGAACCTGGCCACCTGGGIGGGC TCCAACCTGGAGGACCCTGCCTCAAGGAGCTGGTGGTCAGCTATGTCAATGTGAACATGGGCCTCAAGATCA GGCAGCTGCTGTGGTTCCACATCTCCTGCCTGACCTTTGGCAGGGAGACAGTCCTGGAGTACCTGGTGAGC TT

Table M - Polynucleotides encoding immunogenic polypeptides

SEQ ID NO:	HBV genotype	name	Polynucleotide sequence
			TGGGGTGTGGATCAGGACCCCCCCTGCCTACAGGCCCCCAAIGCTCCCAICCTGTCCACCCTGCCAGAGACC ACTTTGGTCAGGAGAAGGGGCAGGICCCCCAGGAGGAGAACCCCCTCTCCCAGGAGGAGGAAGCCAGTCCC CCACCAGGACGAGCGAGCCAGACCACACAGTCCCACTGCATCGCAGACCACCACATCAGCCTTCCTGGCCCCCCT GCTGGTGCTCCAGGCAGGCTCCTTCTGCTGACCAGGATTCIGACCATCCCCCAGTCCCTGGACAGCTGGTGG ACCICCCTGAATTTTCTGGGGGGGGCCCCTACCTGTCCTGGCCAGAACTCTCAGTCTCCCACCTCGAAICACT CACCAACCAGCTGTCCCCCCATCTGTCCTTGCCTACAGGTGGATGTGCCTGAGGAGATTCATCATCTTCCTGTG CATCCTGCTGCTGTTGCCTGAICCTCTGCTTGGTGCTGCTGGACTACCAGGGTATGTTGCCAGTGTGCCCTCCC ATCCCAGGCAGCTCCACCACATCCACAGGACCTTGCAAGACATGCACCACCACCAGCCCAGGGCACCAGCATGT TCCCCTCCTGCTGTTGCACCAAGCCAACAGATGGCAACTGCACATCGCATTCCCATCCCCTCCAGCTGGCCCTT TGCCAGGTTTCTGTGGCAGTGCCCCACTGTCAGATTTTCCTCCCTGCTCCTTCTGGTCCCTTTTGTGCACTGG TTTGTGGGCCTGTCCCCTACAGCGIGCCTGAGTGTCATCCGGATGATGTGGTACTGGGGCCCCTCCTGTATCA ACATCCTCTCTCCCTTTCTGCCCCIGCIGCCAATCTTCTTTIGCCTGTGGGIGTACATC
34	B/C	Core-P2A-sAg	ACGGACATTGACCCCTACAAGGAGTTTGGGGCCAGTGTGGAGCTGCTGCTTTTCTGCCATCTGACTTCTCCC CCAGTGTGAGGAACCTGCTGGACACIGCCTCAGCACTGTACAGAGAGGCCCTGGAGAGCCCAGAGCACIGCTC CCCCCACCACACAGCCCTGAGGCAGCCATCCTCTGCTGGGGGAGCTGATCAACCTGGCCACCTGGGIGGGC TCCAACCTGGAGGACCCTGCCTCAAGGGAGCCTGGTGGTCAGCIATGCCAATCTGAACATGGGCCTCAAGATCA GGCAGCTGCTGTGGTTCCACATCCCCTGCCTGACCTTTGGCAGGGAGACTGTGCTGGAGTACCTGGTGAGCTT CCGTGCCTGATCTTTCTGCTGCTGCTGGACTACCAGGGCATGCTGCCACTGTGCCCTCTCATCCCAGGCA GCTCCACCACATCCACAGGACCCTGCAAGACATGCACCACCACCAGCCCAGGGCACCAGCATGTTCCCCTCCTG CTGTTGCACCAAGCCATCAGATGGCAACTGCACATCGCATTCCCATCCCCTCCAGCTGGCCCTTTGCCAGGCT TCTCCCTTACAGTGTGGCTGAGTGTCATCTGGATGATGTGGTACTGGGGCCCCTCCTGTATCAACATCCTCTC TCCCTTTCTGCCTCTGCTGCCAATCTTCTTTIGCCTGTGGGIGTACATC
			ACGGACATTGACCCCTACAAGGAGTTTGGGGCCAGTGTCGACCTGCTCCTCCTTCCTGCCCTCAGACTTCTCCC CCAGTGTGAGGGACCTGCTGGACACAGCCTCCGCCTCTACAGAGAGGCCCTGGAGAGCCCAGAGCATIGCTC CCCCCACCACACAGCACTGAGCCACCCCATCCCTGCTCGCCCGAGCTCATCAACCTGCCCACCTGGGICGGT GTCCAACCTGGAGGACCCAGCTTCCAGGGATCCGGTGGTCAGCIATGCGAACACAAACATGGGCCTCAAGTCCA GGCAGCTGCTCTGGTTCCACATCCCCTGCCIGACCTTTGGCAGGGAGACTGTGCTGGAGTACCTGGTGAGCTT TGGAGTGTGGATCAGGACCCCACCIGCCTACAGGCCCCCAAIGCCCCCATCCTGTCCACCCTGCCTGAGACC ACAGTGGTGAGGAGGAGGGGAGGICCCCCAGAAGGAGGACCCCTTCTCCCAGGAGGAGGAGGAGTCAGTCTC CCAGGAGGAGGAGGCAGACCAGAGTCCCAGTGTACGGAGAACACCACCTGTGGCTTTCTGGGACCCCCT GCTGGTGCTCCAGGCAGGCTTTTCCCTGCTGACCAGGATCCIGACCATCCCCCAGTCCCTGGACAGCTGGTGG ACATCCTGAATTTTCTTGGGGGCACCACTCCGTGCCTGGGACAGAACTCCCAGTCTCCCACCTCCAACCACA GCCCAACATCCTGTCCCCCATCTGCCCAGGCTACAGGTGGATGTGCCTGAGGAGTTCATCATCTTCCTGTT CATCCTGCTGCTGTTGCCTGATCCTCTGCTTGGTGCTGCTGGACTATCAGGGCATGTTGCCAGTGTGCCCACTC ATCCCAGGCAGCTCCACCACAAGCACAGGACCTTGCAAGACATGCACCACCACCAGCCCAGGGCACTTCCATGTT ACCCATCTTGGTGTTGCACCAAGCCATCTGATGGCAATTGCACCTGCATCCCCATCCCCTCAAGCTGGCCCTT TGCCAAGTTCCTGTGGCAGTGCCCCAACTGCCAGATTCTCTCCCCCTGACCCTCCTGGTCCCTTTTGTGCACTGG TTTGTGGGCCTGAGCCCCACTGCGIGGCCTGTCCGTGATCCGGATGATGTGGTACTGGGGCCCCTCCTGTATT CAATCCTGAGCCCTTTTCTGCCACIGCIGCCAATCTTCTTTIGTCTGTGGGIGTACATC
36	D/D	Core-P2A-sAg	ACGGACATTGACCCCTACAAGCAGTTTGGGCCCACTGTCGACCTGCTCCTCCTTCCTGCCCTCAGACTTCTCCC CCAGTGTGAGGGACCTGCTTGACACACCCTCCGCCCTCTACAGAGAGCCCCTGGAGAGCCCAGAGCATTCCTC CCCCCACCACACAGCACTGAGCCACCCCATCCCTGCTCGCCCGAGCTCATCAACCTGCCCACCTGGGICGGT GTCCAACCTGGAGGACCCAGCTTCCAGGGATCCGGTGGTCAGCIATGCGAACACAAACATGGGCCTCAAGTCCA GGCAGCTGCTCTGGTTCCACATCCCCTGCCIGACCTTTGGCAGGGAGACTGTGCTGGAGTACCTGGTGAGCTT TGGAGTGTGGATCAGGACCCCACCIGCCTACAGGCCCCCAAIGCCCCCATCCTGTCCACCCTGCCTGAGACC ACAGTGGTGAGGAGGAGGGGAGGTCCCCCAGAAGGAGGACCCCTTCTCCCAGGAGGAGGAGGAGTCAGTCTC CCAGAGGAGGAGGAGCCAGAGCACAGAGTCCCAGTGTGGCAGTGGGCAACCAACTTCAGCCTCCTGAAACA GGCAGGGGATGTGGAGGAAAACCCAGGCCCCGAGPACATCACCTCTGGCTTTCTGGACCCCCTGCTGGTGCTC CAGGCAGGCTTTTTCCTGCTGACCAGGATCCTGACCATCCCTCAGAGCCTGGACTCCTGGTGGACATCTCTGA ACTTTCTTGGGGGCAICCACTGTGTGCCTGGGACAGAACTCCCAGTCTCCCACCTCTCAACCACAGCCCAACA CCGTCCCCCCATGTGCCCAGGCTACAGGTGGATGTGCCTGACCAGGTTCATCATCTTCCTGTTCATCCTCCTG CTGTTGCCTGATCTTTCTGCTGCTGCTGGACTATCAGGGCATGCTCCCACTGTGCCCATCTGATCCCACGCA GCTCCACCACAAGCACAGGACCTTCCAAGGAGACCTGGTGGTCAGCIATGCCAATCTGAACATGGGCCTCAAGATCA CAGTTGCACCAAGCCATCTGATGGCAATTGCACCTGCATCCCCATCCCCTCAAGCTGGCCCTTTGCCAGGCTT TGACCCCCACTGTGTGGCTGTCCGIGATCTGGATGATGTGGTACTGGGGCCCCTCCTGTATTCAATCCTGAG CCCTTTTCTGCCACTGCTGCCAICITCTTTGTCTGTGGGIGTACATC
37	D/D	icore -P2A-sAg	ATGGACATTGACCCCTACAAGGAGTTTGGGGCCAGTGTCGAGCTGCTCCTCTTTCTGCCATCTGACTTCTCCC CCAGTGTGAGGGACCTGCTGGACACIGCCTCAGCACTGTACAGAGAGGCCCTGGAGAGCCCAGAGCACIGCTC CCCCCACCACACAGCCCTGACCCACCCCATCCCTCGCTCGCCCGAGCTGATCAACCTGCCCACCTGGGICGGC GTCCAACCTGGAGGACCCTGCCTCAAGGGACCCGGTGGTCAGCIATGCCAATCAGGACATGGGCCTCAAGTCCA GGCAGCTGCTGTGGTTCCACATCCCCTGCCIGACCTTTGGCAGGGAGACAGTCCTGGAGTACCTGGTGAGCTT TGGGGTGTGGATCAGGACCCCCCCIGCCTACAGGCCCCCAAIGCTCCCATCCTGTCCACCCTGCCAGAGACC ACTGTGGTCAGGAGAAGGGGCAGGICCCCCAGGAGGAGAACCCCCTCTCCCAGGAGGAGGAGGAAGCCAGTCCC CCAGAGGAGGAGGAGCCAGAGCACAGAGAGTCCCAGTGCGGCAGTGGGGCAACCAACTTCAGCCTCCTGAAACA GGCAGCGCATGTGCAGCAAAACCCACCCCCCCCAGAACATCACATCAGCCTCCTGGCCCCCCTGCTGCTGCTCC CAGCCAGGCTTCTTTCTGCTGACCACCATTCTGACCATCCCCCAGTCCCTGGACAGCTGGTGGACCTCCCCGA ACTTTCTGGGGGGGACCACTGTCCCTGTGCCCACAACTCTCAGTCTCCCCACCTCGAATCACTCACCAACCAG
			CTGTCCCCCCATCTGTCCTGGCTACAGGTGGATGTGCCTGAGGAGATTCATCATCTTCCTGTTCATCCTGCTG CTGTGCCTGATCTTTCTGCTGGTGCCTGCTGGACTACCAGGGCATGCTGCCAGTGTGCCCTCTCATCCCAGGCA GCTCCACCACACCCACAGGACCTTCCACGACATGCACCACCACCAGCCCAGGGCACCAGCATGTACCCCTCCTG CTGTTGCACCAAGCCATCAGATGGCAACTGCACATCGCATTCCCATCCCCTCCAGCTGGGGCTTTGGCAAGTTT CCGTGGGAGTGGGCCAGICCCAGTTTTCCTGGCTGTCTCTTCTGGTGCCCTCTGTGCAGTGCTTTGTCCGCC TCTCCCTTACAGTGTGGCTGAGTGTCATCTGGATGATGTGGTACTGGGGCCCCTCCTGTATCAGCATCCTCTC TCCCTTTCTGCCTCTGCTGCCAATCTTCTTTTGCCTGTGGGTGTACATC

4. Vectors and Host Cells

[0094] Further disclosed herein 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. A vector may comprise 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.

[0095] 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.

[0096] 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.

[0097] 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. The vectors may 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.

[0098] The vector that is used may be pcDNA™3.1+ (ThermoFisher, MA).

[0099] The vector may be a 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). The viral vector may be 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. The viral vector may be 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.*, Coxsackievirus, 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.

[0100] In the present invention, the viral expression vector is an arenavirus vector, and may be selected from Lymphocytic choriomeningitis mammarenavirus (LCMV) (NCBI:txid11623), Cali 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 (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 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.

[0101] The viral expression vector disclosed herein may be an adenovirus vector, *e.g.*, from a human adenovirus or a simian adenovirus (e.g., a chimpanzee adenovirus, a gorilla adenovirus or a rhesus monkey adenovirus). The adenovirus vector may be 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), Ch Ad 11 (AdC11), 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), 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 27:2426-2441Guo, 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.

[0102] The viral expression vector may be 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. The viral expression vector may be 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. The viral expression vector may be 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.

[0103] Further provided are host cells comprising one or more vectors comprising the arenavirus vectors of the invention. Also disclosed herein 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. The host cell may be a prokaryotic cell, for example, *E. coli*. The host cell may be 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*.

[0104] 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.

[0105] 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. The host cells may be 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. 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. The host cells may express the Coxsackievirus and adenovirus receptor (CAR), *e.g.*, MDCK, Caco-2 or Calu-3 host cells. The polynucleotides may integrate into the genome of the host cell.

5. Pharmaceutical Compositions / Immunogenic Compositions

[0106] Dislcosed herein 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 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 domestic animals.

[0107] Generally, the pharmaceutical compositions described herein are immunogenic. The pharmaceutical composition may comprise 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., 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.

[0108] Various pharmaceutically acceptable diluents, carriers, and excipients, and techniques for the preparation and use of pharmaceutical compositions will be known to those of 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), "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.

[0109] The polynucleotides or vectors may be formulated into lipid nanoparticles. For example, 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 of between about 10 to about 1000 nanometers, and which comprise a solid lipid core matrix that can solubilize lipophilic molecules. The lipid core may be stabilized by surfactants (e.g., emulsifiers), and can comprise one or more of triglycerides (e.g., tristearin), diglycerides (e.g., glycerol bahenate), monoglycerides (e.g., glycerol monostearate), fatty acids (*e.g.,* stearic acid), steroids (*e.g.,* cholesterol), and waxes (*e.g.,* cetyl palmitate), including 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. A self-replicating or self-amplifying RNA molecule encoding one or more of the immunogenic HBV polypeptides described herein may be formulated or condensed into polyethylenimine (PEI)-polyplex delivery vehicles, *e.g.,* as described in Démoulins, et al., Nanomedicine. (2016) Apr;12(3):711-722 and Démoulins, et al., J Control Release. (2017) Nov 28;266:256-271, which can be nanoparticulate.

[0110] Where the immunogenic HBV polypeptides are expressed from a 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. 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. The viral expression vectors may be delivered via microneedle-mediated delivery, *e.g.,* as described in Zaric, et al., Expert Opin Drug Deliv. (2017) Oct;14(10):1177-1187.

[0111] Each carrier, diluent or excipient may be "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.

[0112] The arenavirus vector (*e.g.,* a LCMV or Pichinde mammarenavirus vector (PICV)) described herein may be 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.

[0113] 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, intraarterial, 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. The pharmaceutical compositions may be formulated for parenteral, *e.g.,* intravenous, subcutaneous, or oral administration. The pharmaceutical compositions may be formulated for mucosal, *e.g.,* buccal, intranasal, intrarectal and/or intravaginal administration.

[0114] The pharmaceutical compositions disclosed herein may be sterile. The pharmaceutical composition disclosed herein may have 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. The pharmaceutical composition disclosed herein may have an osmolarity in the range of 240-260 or 250-330 mOsmol/L. The pharmaceutical composition disclosed herein may be isotonic or near isotonic.

[0115] The pharmaceutical compositions disclosed herein may be liquids or solids. The pharmaceutical composition disclosed herein may comprise an aqueous solution or suspension. The pharmaceutical composition disclosed herein may be lyophilized or a frozen liquid.

[0116] The pharmaceutical composition disclosed herein may further comprise 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.

[0117] The pharmaceutical composition disclosed herein may 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 Freunds'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.

[0118] The pharmaceutical compositions or immunogenic compositions disclosed herein may 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. The pharmaceutical composition may comprise 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.

[0119] The immunogenic composition may comprise 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%, 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.

[0120] The immunogenic composition may comprise 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: 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.

[0121] The immunogenic composition may 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 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.

[0122] With respect to the core-sAg fusion polypeptide in the immunogenic composition, 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. 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. The sAg polypeptide may comprise 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. The core-sAg fusion polypeptide may comprise 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.

[0123] The immunogenic composition disclosed herein may 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 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.

[0124] The immunogenic composition disclosed herein may 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 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.

[0125] The immunogenic composition disclosed herein may comprise 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.

[0126] The immunogenic composition disclosed herein may comprise 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.

[0127] 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 be measured in units of plaque forming units (PFU), focus forming units (FFU), infectious units (IU), or viral particles (vp).

[0128] In various embodiments, the one or more polynucleotides are DNA, cDNA, mRNA, or self-replicating RNA.

[0129] 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.

[0130] 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.

[0131] As disclosed herein, the first viral expression vector and the second viral expression vector in the immunogenic composition may be 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). 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, 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)).

[0132] In the immunogenic compositions of the invention,, 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)).

[0133] 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.

6. Methods of Treatment

[0134] Further disclosed herein are methods for eliciting a protective 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 disclosed herein are methods of inhibiting HBV replication in an infected individual. Further disclosed herein are methods for reducing the viral load associated with HBV infection. The methods disclosed herein may comprise administering to the subject an effective amount of an immunogenic composition, as described herein. The "subject" or the "individual" may be a human, a woodchuck, a Peking duck, a mouse or a non-human primate (*e.g.,* a chimpanzee).

[0135] "Treatment" or "treat" or "treating" as used herein refers to an approach for 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 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 the disease, increasing the quality of life, and/or prolonging survival.

[0136] "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 can, in effect, encompass prevention, in that the individual does not develop the disease or condition.

[0137] "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, the term "preventing HBV infection" may refer 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.

[0138] "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.

[0139] The administered immunogenic composition disclosed herein may comprise 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.

[0140] The administered immunogenic composition disclosed herein may comprise 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.

[0141] Also disclosed herein, the administered immunogenic composition may comprise 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.

[0142] With respect to the core-sAg fusion polypeptide in the administered immunogenic composition, 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. 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. 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. 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.

[0143] Also disclosed herein, the administered immunogenic composition may comprise 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.

[0144] Also disclosed herein, the administered immunogenic composition may comprise 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.

[0145] Also disclosed herein, the administered immunogenic composition may comprise 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 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.

[0146] Also disclosed herein, the first viral expression vector and the second viral expression vector in the administered immunogenic composition independently may be 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), as described above and herein. 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, both the first viral expression vector and the second viral expression vector in the administered immunogenic composition are from Adenoviridae, Arenaviridae, or Poxviridae (*e.g.* Vaccinia virus, *e.g.* modified vaccinia Ankara (MVA)).

[0147] In the administered immunogenic compositions of the invention, 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 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)).

[0148] 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.

[0149] Also disclosed herein, the administered immunogenic composition may comprise 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.

[0150] Also disclosed herein, the administered immunogenic composition may comprise 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.

[0151] Also disclosed herein, the subject may be infected with HBV, may be suspected of being infected with HBV, or may be 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). The subject may be 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, the subject may be acutely infected with HBV. The subject may be co-infected with hepatitis D virus (HDV).

[0152] The subject may be asymptomatic. The subject may be 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.

[0153] 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).

[0154] 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. The methods may 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).

[0155] Also disclosed herein, the methods comprise a prime-boost regimen. The prime-boost regimen may entail 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. The administrations of the priming composition and the one or more boosting compositions may be 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.

[0156] Also disclosed herein, 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 may 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. 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 may be 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.

[0157] As appropriate or desired, the priming composition and the boosting composition can comprise the same immunogenic composition or different immunogenic compositions. The priming composition and the boosting composition may comprise the same one or more polypeptides and same expression vector (*e.g.*, viral expression vector). The priming composition and the boosting composition may comprise different polypeptides and/or different expression vectors (*e.g.*, viral expression vectors). For example, the priming composition and the boosting composition may 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). The priming composition and the boosting composition may comprise different immunogenic polypeptides and the same expression vector (*e.g.*, viral expression vector).

[0158] Also disclosed herein, the methods may 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. The prime-boost regimen may comprise:

1. 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;
2. 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;
3. 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;
4. 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;
5. 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;
6. 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;
7. 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;
8. 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;
9. 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;
10. 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;
11. 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;
12. 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;
13. 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;
14. 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;
15. 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;
16. 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

17. 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.

[0159] Also disclosed herein, the methods may 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. The prime-boost regimen may comprise:

1. 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;
2. 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;
3. c) 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; or
4. d) 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.

[0160] Also disclosed herein, the priming composition and the boosting composition may comprise an immunogenic composition as described herein.

[0161] Also disclosed herein, the subject may not receiving antiviral therapy or antiviral therapy is discontinued prior to administration of the one or more immunogenic compositions. The antiviral therapy may be discontinued after one or more administrations of the compositions.

[0162] Also disclosed herein, the treatment methods may activate in the subject CD8+ T cells targeting one or more HBV polypeptide epitopes. The treatment methods may elicit in the subject production of antibodies that bind one or more HBV polypeptides.

7. Combination Therapies

[0163] Also disclosed herein, 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, two, three, four or more additional therapeutic agents. 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 two additional therapeutic agents. 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 three additional therapeutic agents. 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 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 be selected from different classes of therapeutic agents.

[0164] "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 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, a unit dose of an immunogenic composition of the present disclosure may be administered first, followed within seconds or minutes by administration of a unit dose of one or more additional therapeutic agents. Alternatively, a unit dose of one or more additional therapeutic agents may be administered first, followed by administration of a unit dose of an immunogenic composition of the present disclosure within seconds or minutes. A unit dose of the immunogenic polypeptides, polynucleotides encoding such polypeptides, vectors, LNPs and immunogenic compositions comprising such polypeptides or polynucleotides, as described herein, may be 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. A unit dose of one or more additional therapeutic agents may be 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.

[0165] 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.

[0166] Also disclosed herein, 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 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.

[0167] Also disclosed herein, 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, 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.

[0168] Also disclosed herein, 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 DARPins®, 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.

[0169] Also disclosed herein, 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, 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 NOD 1, 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.

HBV Inhibiting Antiviral Drugs

[0170] Also disclosed herein, 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 antiviral agents. The one or more antiviral agents may be 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

[0171] 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, gentiopicrin (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, picroside, 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).

[0172] 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-IFNα; adefovir and PEG-IFNα; and INO-1800 (INO-9112 and RG7944).

HBV Vaccines

[0173] Also disclosed herein, 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 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 (Hansenual 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, Comvax, 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.

[0174] Examples of HBV therapeutic vaccines that can be combined or co-administered (*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, 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-SIi-HBV-CPmut-TPA-Ssh prime and MVA-SIi-HBV-CPmut-TPA-Ssh boost), Lm HBV and BM32 (Tulaeva, et al., EBioMedicine (2020) 102953). HBV Arenavirus vaccines are described, *e.g.*, in WO2017076988 and WO2017198726.

HBV DNA Polymerase Inhibitors

[0175] Also disclosed herein, 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 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 exalidex, besifovir, entecavir (BARACLUDE®), entecavir maleate, telbivudine (TYZEKA®), filocilovir, pradefovir, 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

[0176] Also disclosed herein, 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 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

[0177] Also disclosed herein, 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 agonists or stimulators of a toll-like receptor (TLR). 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 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 TLR10 (NCBI Gene ID: 81793), TLR11, TLR12 and TLR13.

[0178] Examples of TLR3 agonists that can be combined or co-administered include rintatolimod, poly-ICLC, RIBOXXON®, Apoxxim, RIBOXXIM®, IPH-33, MCT-465, MCT-475 and ND-1. 1.

[0179] Examples of TLR4 agonists that can be combined or co-administered include G-100, and GSK-1795091.

[0180] 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-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), US20140275167 (Novira Therapeutics), and US20130251673 (Novira Therapeutics).

[0181] Example dual TLR7/TLR8 agonists that can be combined or co-administered is NKTR-262, telratolimod and BDB-001.

[0182] 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, 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 (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.), US20180065938 (Gilead Sciences, Inc.), and US20180086755 (Gilead Sciences, Inc.).

[0183] 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, tilsotolimod and PUL-042.

[0184] 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), 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), 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 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), WO2017038909 (Takeda Pharmaceutical), WO2015095780 (University of Kansas), WO2015023958 (University of Kansas).

[0185] Also disclosed herein, 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 a TLR7, TLR8 or TLR9 agonist.

Interferon Alpha Receptor Ligands

[0186] Also disclosed herein, 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 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, Algeron, Alfarona, Ingaron (interferon gamma), rSIFN-co (recombinant super 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 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).

Hyaluronidase Inhibitors

[0187] Also disclosed herein, 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 hyaluronidase inhibitors. Examples of hyaluronidase inhibitors that can be combined or co-administered include astodrimer.

Hepatitis B Surface Antigen (HBsAg) Inhibitors

[0188] Also disclosed herein, 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 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.

Cyclophilin Inhibitors

[0189] Also disclosed herein, 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 cyclophilin inhibitors. Examples of cyclophilin inhibitors that can be combined or co-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

[0190] Also disclosed herein, 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 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

[0191] Also disclosed herein, 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 inhibitory nucleic acids (*e.g.,* antisense oligonucleotide, short interfering RNA (siRNA), DNA-directed RNA interference (ddRNAi)) specifically targeting an HBV polynucleotideThe HBV polynucleotide may encode an HBV protein (*i.e.,* is in a coding region within the HBV genome).

Antisense Oligonucleotide Targeting Viral mRNA

[0192] Also disclosed herein, 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 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)

[0193] Also disclosed herein, 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 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)

[0194] Also disclosed herein, 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 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.

Endonuclease Modulators

[0195] Also disclosed herein, 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 endonuclease modulators. Examples of endonuclease modulators that can be combined or co-administered include PGN-514.

Ribonucleotide Reductase Inhibitors

[0196] Also disclosed herein, 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 ribonucleotide reductase inhibitors. Examples of inhibitors of ribonucleotide reductase that can be combined or co-administered include Trimidox.

Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

[0197] Also disclosed herein, 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 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).

HBV Replication Inhibitors

[0198] Also disclosed herein, 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 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

[0199] Also disclosed herein, 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 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

[0200] Also disclosed herein, 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 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

[0201] Also disclosed herein, 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 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 lenvervimab (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 Kornyeyev, et al., J Virol. 2019 Jul 30;93(16). pii: e00248-19.

[0202] 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).

[0203] Examples of fully human monoclonal HBV antibodies that can be combined or co-administered include HBC-34.

[0204] 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

[0205] Also disclosed herein, 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 CCR2 chemokine antagonists. Examples of CCR2 chemokine antagonists that can be combined or co-administered include propagermanium.

Thymosin Agonists

[0206] Also disclosed herein, 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 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)

[0207] Also disclosed herein, 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 interleukin receptor agonists of an interleukin receptor selected from IL-2, IL-7, IL-12 and IL-15. 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 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

[0208] Also disclosed herein, 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 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.

[0209] 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).

[0210] 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

[0211] Also disclosed herein, 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 innate immune activators. The one or more innate immune activators may comprise 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), nucleotide binding oligomerization domain containing 2 (NOD2). The methods may entail co-administering GS-3583 and/or GS-9992. The methods may entail combining or co-administering a FLT3 agonist, *e.g.*, GS-3583 or CDX-301.

STING agonists, RIG-I and NOD2 modulators

[0212] Also disclosed herein, 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 a stimulator of interferon response cGAMP interactor 1 (STING or STING1; NCBI Gene ID: 340061) agonist. The STING/STING1 agonist or activator may be 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 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), WO2018100558 (Takeda), WO2018067423 (Merck), WO2018060323 (Boehringer).

[0213] Also disclosed herein, 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 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.

[0214] Also disclosed herein, 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 a nucleotide binding oligomerization domain containing 2 (NOD2; NCBI Gene ID: 64127) agonist, such as inarigivir soproxil (SB-9200; GS-9992), and IR-103.

Phosphatidylinositol 3-kinase (PI3K) Inhibitors

[0215] Also disclosed herein, 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 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). The PI3K inhibitor may be 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), BYI,719 (alpelisib), 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), omipalisib (GSK2126458, GSK458), OXY111A, panulisib (P7170, AK151761), PA799, perifosine (KRX-0401), Pilaralisib (SAR245408; XI,147), 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, voxtalisib (SAR245409), VS-5584, WX-037, X-339, X-414, XI,499, XI,756, 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).

Immune Checkpoint Modulators

[0216] Also disclosed herein, 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 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. The immune checkpoint proteins or receptors may regulate T cell responses (*e.g.*, reviewed in Xu, et al., J Exp Clin Cancer Res. (2018) 37:110). The immune checkpoint proteins or receptors may 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).

[0217] 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, TRAELR2; NCBI Gene ID: 8795); TNFRSF10 (TRAIL; NCBI Gene ID: 8743); TNFRSF14 (HVEM, CD270; NCBI Gene ID: 8764); TNFSF14 (HVEML; 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 (IPH2102BMS-986015), IPH-4102); and killer cell lectin like receptor D1 (KLRD1; NCBI Gene ID: 3824).

[0218] Also disclosed herein, 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 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). The agents, as described herein, may be 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.

[0219] Also disclosed herein, 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 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).

[0220] Also disclosed herein, 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 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)

[0221] Also disclosed herein, 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 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)

[0222] Also disclosed herein, 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 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/TGFβ-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).

[0223] 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), 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 (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), 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 Discovery Technologies Ltd), WO2012168944 (Aurigene Discovery Technologies Ltd), WO2015036927 (Aurigene Discovery Technologies Ltd), WO2015044900 (Aurigene Discovery Technologies Ltd), WO2018026971 (Arising International).

[0224] Also disclosed herein, 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 proteinaceous (*e.g.*, antibody or fragment thereof, or antibody mimetic) inhibitors of PD-L1 (CD274), PD-1 (PDCD1) or CTLA4. The one or more immune checkpoint inhibitors may comprise a small organic molecule inhibitor of PD-L1 (CD274), PD-1 (PDCD1) or CTLA4. The small molecule inhibitor of CD274 or PDCD1 may be 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). The small molecule inhibitor of CTLA4 may comprise BPI-002.

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

[0225] Also disclosed herein, 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 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).

TNF Receptor Superfamily (TNFRSF) Member Agonists or Activators

[0226] Also disclosed herein, 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 agonists of one or more TNF receptor superfamily (TNFRSF) members, *e.g.*, an agonist of one or more of TNFRSF 1A (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), TNFRSF 11A (CD265, RANK, NCBI Gene ID: 8792), TNFRSF 11B (NCBI Gene ID: 4982), TNFRSF 12A (CD266, NCBI Gene ID: 51330), TNFRSF 13B (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).

[0227] 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.

[0228] Example anti-TNFRSF5 (CD40) antibodies that can be combined or co-administered include without limitation RG7876, SEA-CD40, APX-005M and ABBV-428.

[0229] Also disclosed herein, the anti-TNFRSF7 (CD27) antibody varlilumab (CDX-1127) may be combined or co-administered.

[0230] 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.

[0231] Example anti-TNFRSF18 (GITR) antibodies that can be combined or co-administered include without limitation, MEDI1873, FPA-154, INCAGN-1876, TRX-518, BMS-986156, MK-1248, GWN-323, and those described in WO2017096179, WO2017096276, WO2017096189, and WO2018089628. Also disclosed herein, an antibody, or fragment thereof, co-targeting TNFRSF4 (OX40) and TNFRSF18 (GITR) may be co-administered. Such antibodies are described, *e.g.*, in WO2017096179 and WO2018089628.

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

[0232] Also disclosed herein, 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 inhibitors of indoleamine 2,3-dioxygenase 1 (IDO1; NCBI Gene ID: 3620). Examples 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, Inc.).

LAG-3 and TIM-3 inhibitors

[0233] Also disclosed herein, 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 an anti-TIM-3 antibody, such as TSR-022, LY-3321367, MBG-453, INCAGN-2390. Also disclosed herein, 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 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)

[0234] Also disclosed herein, 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 an inhibitor of apoptosis proteins family protein (IAP). Examples of IAP inhibitors include APG-1387.

Bruton's Tyrosine Kinase (BTK) Inhibitors

[0235] Also disclosed herein, 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 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

[0236] Also disclosed herein, 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 an inhibitor of a lysine demethylase (KDM). Examples of KDM5 inhibitors that can be combined or co-admnistered 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).

[0237] 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

[0238] Also disclosed herein, 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 an arginase inhibitor. Examples of Arginase inhibitors include CB-1158, C-201, and resminostat.

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

[0239] Also disclosed herein, 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 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.,* 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 FcyR (which mediates antibody-dependent cell cytotoxicity), SLAM family receptors (*e.g.,* 2B4, SLAM6 and SLAM7), killer cell immunoglobulin-like receptors (KIR) (KIR-2DS and KIR-3DS), DNAM-1 and CD137 (41BB). As appropriate, the 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

[0240] Also disclosed herein, 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 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 described in Henry, et al., Eur J Pharm Sci. (2019) 136:104958.

Gene Therapy and Cell Therapy

[0241] Also disclosed herein, 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 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.

Gene Editors

[0242] 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, PreSI, PreS2, S, P* or *SP* gene refers to (1) reducing or eliminating *PreC, C, X, PreSI, 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, PreSI, 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).

[0243] 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

[0244] 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. The antigen-binding domain may be a domain disclosed herein. The antigen-binding domain may be other than a domain disclosed herein. The antigen may be HBsAg (i.e. HbsAg-CART). The immune effector cell is a T-cell or an NK cell. The T-cell may be 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.

TCR-T cell therapy

[0245] 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.

[0246] TCR-T cell therapy includes T-Cells expressing HBV surface antigen (HBsAg)-specific TCR, such as IMC-I109V.

[0247] TCR-T cell therapy includes TCR-T therapy directed to treatment of HBV, such as LTCR-H2-1.

[0248] Also disclosed herein, 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 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 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 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).

[0249] Also disclosed herein, 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 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 DARPins®, 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.

[0250] Also disclosed herein, 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 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).

[0251] Also disclosed herein, 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 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), 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), 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 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 (Quanticel), US20140171432, US20140213591 (Quanticel), US20160039808 (Quanticel), US20140275084 (Quanticel), WO2014164708 (Quanticel), US9186337B2 (Oryzon Genomics), and other drugs for treating HBV, and combinations thereof.

[0252] Also disclosed herein, 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 5-30 mg tenofovir alafenamide fumarate, tenofovir alafenamide hemifumarate, or tenofovir alafenamide. 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 5-10; 5-15; 5-20; 5-25; 25-30; 20-30; 15-30; or 10-30 mg tenofovir alafenamide fumarate, tenofovir alafenamide hemifumarate, or tenofovir alafenamide. 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 10 mg tenofovir alafenamide fumarate, tenofovir alafenamide hemifumarate, or tenofovir alafenamide. 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 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.

[0253] Also disclosed herein, 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 100-400 mg tenofovir disoproxil fumarate, tenofovir disoproxil hemifumarate, or tenofovir disoproxil. An agent disclosed herein, or a pharmaceutically acceptable salt thereof, may be 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. An agent disclosed herein, or a pharmaceutically acceptable salt thereof, may be combined with 300 mg tenofovir disoproxil fumarate, tenofovir disoproxil hemifumarate, or tenofovir disoproxil. An agent disclosed herein, or a pharmaceutically acceptable salt thereof, may be combined with 250 mg tenofovir disoproxil fumarate, tenofovir disoproxil hemifumarate, or tenofovir disoproxil. An agent herein, or a pharmaceutically acceptable salt thereof, may be 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

[0254] Further disclosed herein 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. The kit may comprise 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.

[0255] As appropriate or desired, the one or more unitary doses can be in a single container or in two or more separate containers. The one or more containers may be selected from the group consisting of vials, ampules and pre-loaded syringes.

[0256] Also disclosed herein, the one or more containers may comprise the one or more polypeptides, one or more polynucleotides, one or more vectors or one or more immunogenic compositions in an aqueous solution. The one or more containers may comprise the one or more polypeptides, one or more polynucleotides, one or more vectors or one or more immunogenic compositions as a lyophilized preparation.

[0257] As appropriate or desired, the one or more unitary doses can be the same or different. The kit may comprise 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.

[0258] Also disclosed herein, the kit may comprise 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.

[0259] The kit may comprise 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: 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.

[0260] The kit may 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 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.

[0261] With respect to the core-sAg fusion polypeptide in the kit (e.g., expressable from a vector; in an immunogenic composition), the core polypeptide may comprise 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. The sAg polypeptide may comprise 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. The sAg polypeptide may comprise 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. The core-sAg fusion polypeptide may comprise 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.

[0262] Also disclosed herein, the kit may 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 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.

[0263] Also disclosed herein, the kit may 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 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.

[0264] Also disclosed herein, the kit may comprise: (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).

[0265] Also disclosed herein, the kit may comprise: (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)).

[0266] Also disclosed herein, the kit may comprise: (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.

[0267] Also disclosed herein, the kit maycomprise: (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)).

[0268] Also disclosed herein, the kit may comprise 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.

[0269] Also disclosed herein, the kit may comprise 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.

[0270] Also disclosed herein, the kit may comprise one or more unitary doses of one or more additional therapeutic agents.

[0271] For example, the kit may comprise one or more agonists or 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. The TLR7 agonist may be selected from the group consisting of GS 9620 (vesatolimod), R848 (Resiquimod), DS-0509, LHC-165 and TMX-101 (imiquimod), and/or wherein the TLR8 agonist is selected from the group consisting of GS-9688, R848 (Resiquimod) and NKTR-262 (dual TLR7/TLR8 agonist).

[0272] Also disclosed herein, the kit may comprise one or more interleukin receptor agonists of an interleukin receptor selected from IL-2, IL-7, IL-12 and IL-15. The kit may comprise one or more cytokines selected from the group consisting of IL-2, IL-7, IL-12, IL-15, and variants thereof.

[0273] Also disclosed herein, the kit may comprise one or more innate immune activators. The one or more innate immune activators may comprise 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), nucleotide binding oligomerization domain containing 2 (NOD2). The kit may comprise one or more unitary doses of GS-3583 and/or GS-9992.

[0274] Also disclosed herein, the kit may comprise 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. The one or more immune checkpoint proteins or receptors may be 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).

[0275] Also disclosed herein, the kit may comprise one or more blockers or inhibitors of one or more T-cell inhibitory immune checkpoint proteins or receptors. Tthe blockers or inhibitors of one or more T-cell inhibitory immune checkpoint proteins or receptors may be 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 (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).

[0276] Also disclosed herein, the kit may comprise one or more agonists or activators of one or more T-cell stimulatory immune checkpoint proteins or receptors. The agonists or activators of one or more T-cell stimulatory immune checkpoint proteins or receptors may be 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); Poliovirus receptor (PVR) cell adhesion molecule (PVR, CD155). The kit may comprise one or more unitary doses of AGEN-2373 and/or AGEN-1223.

[0277] Also disclosed herein, the kit may comprise one or more blockers or inhibitors of one or more NK-cell inhibitory immune checkpoint proteins or receptors. The NK-cell inhibitory immune checkpoint proteins or receptors may be 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).

[0278] Also disclosed herein, the kit may comprise one or more agonists or activators of one or more NK-cell stimulatory immune checkpoint proteins or receptors. The NK-cell stimulatory immune checkpoint proteins or receptors may be selected from CD16, CD226 (DNAM-1); killer cell lectin like receptor K1 (KLRK1, NKG2D, CD314); and SLAM family member 7 (SLAMF7).

[0279] In the kit disclosed herein, the one or more immune checkpoint inhibitors may comprise a proteinaceous inhibitor of PD-L1 (CD274), PD-1 (PDCD1) or CTLA4. The proteinaceous inhibitor of CTLA4 may be 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). The proteinaceous inhibitor of PD-L1 (CD274) or PD-1 (PDCD1) may be 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). The one or more immune checkpoint inhibitors may comprise a small molecule inhibitor of CD274 (PDL1, PD-L1), programmed cell death 1 (PDCD1, PD1, PD-1) or CTLA4. The small molecule inhibitor of CD274 or PDCD1 may be selected from the group consisting of GS-4224, GS-4416, INCB086550 and MAX10181. The small molecule inhibitor of CTLA4 may comprise BPI-002.

[0280] Also disclosed herein, the kit may comprise 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 VEMLIDY®) and ledipasvir + sofosbuvir (HARVONI®). The kit may comprise 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.

[0281] 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

[0282] 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

[0283] 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.

[0284] *Selection of near-consensus, naturally occurring HBVsAg 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	MENITSGFLGPLLVLQAGFFLLTRILTIPQSLDSWWTSLNFLGGTPVC LGQNSQSPTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLV LLDYQGMLPVCPLIPGSTTTSTGPCKTCTIPAQGNSMFPSCCCTKPTD GNCTCIPIPSSWAFAKYLWEWASVRFSWLSLLVPFVQWFVGLSPTVWL SVIWMMWYWGPSLYNILSPFIPLLPIFFCLWVYI
2	B	MESTISGFLGPLLVLQAGFFLLTRILTIPQSLDSWWTSLNFLGGAPTC PGQNLQSPTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLV
		LLDYQGMLPVCPLIPGSSTTSTGPCRTCTTPAQGTSMFPSCCCTKPTD GNCTCIPIPSSWAFAKYLWEWASVRFSWLSLLVPFVQWFVGLSPTVWL SVIWMMWYWGPSLYNILSPFMPLLPIFFCLWVYI
3	C	MESTISGFLGPLLVLQAGFFLLTRILTIPQSLDSWWTSLNFLGGAPTC PGQNSQSPTSNHSPTSCPPICPGYRWMCLRRFIIFLCILLLCLIFLLV LLDYQGMLPVCPLIPGSSTTSTGPCKTCTTPAQGTSMFPSCCCTKPTD GNCTCIPIPSSWAFARFLWEWASVRFSWLSLLVPFVQWFVGLSPTVWL SVIWMMWYWGPSLYNILSPFLPLLPIFFCLWVYI

Table 1: Naturally-occurring, near-consensus sAg polypeptide sequences		
SEQ ID NO:	**HBV genotype**	**Polypeptide sequence**
4	D	MENITSGFLGPLLVLQAGFFLLTRILTIPQSLDSWWTSLNFLGGTTVC LGQNSQSPTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLV LLDYQGMLPVCPLIPGSSTTSTGPCRTCTTPAQGTSMYPSCCCTKPSD GNCTCIPIPSSWAFGKFLWEWASARFSWLSLLVPFVQWFVGLSPTVWL SVIWMMWYWGPSLYSILSPFLPLLPIFFCLWVYI

Methods

[0285] 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

[0286] 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

[0287] In this example, we identified near-consensus, naturally occurring sequences of 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.

[0288] *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 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. 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 genotype.

[0289] 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, 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 genotype**	**Polypeptide sequence - Motifs containing inactivating mutations in Pol are underlined (YMDD mutated to YMHD, AELL mutated to AHLL)**
52	A	MPLSYQHFRKLLLLDETEAGPLEEELPRLADEDLNRRVAEDLNLGNLNV SIPWTHKVGNFTGLYSSTVPIFNPEWQTPSFPKIHLHEDIANRCQQFVGP
		LTVNEKRRLRLIMPARFYPNSTKYLPLDKGIKPYYPDHVVNHYFQTRHYL HTLWKAGILYKRETTRSASFCGSPYSWEQELHHGRLVIKTSQRHGDEPFC SQPSGILSRSSVGPCIRSQFKQSRLGLQPHQGPLATSQSGRSGSIRARVH SPTRRCFGVEPSGSGHIGHSASSSSSCLHQSAVRKAAYSHLSTSKRQSSS GHAVEFHSFPPSSARSQSQGPVFSCWWLQFRNTQPCSKYCLSHLVNLLED WGPCDEHGEHHIRIPRTPARVTGGVFLVDKNPHNTAESRLVVDFSQFSRG ITRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHIPLHPAAMPHLL VGSSGLSRYVARLSSNSRIHNNQHGTLQNLHDSCSRQLYVSLMLLYKTYG RKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCLAFS YMHDVVLGAKSVQHLESLYTAVTNFLLSLGIHLNPNKTKRWGYSLNFMGY VIGSWGTLPQDHIVQKIKHCFRKLPINRPIDWKVCQRIVGLLGFAAPFTQ CGYPALMPLYACIQAKQAFTFSPTYKAFLSKQYLNLYPVARQRPGLCQVF ADATPTGWGLAIGHQRMRGTFVAPLPIHTAHLLAACFARSRSGAKLIGTD NSVVLSRKYTSFPWLLGCTANWILRGTSFVYVPSALNPADDPSRGRLGLY RPLLRLPYRPTTGRTSLYAVSPSVPSHLPVRVHFASPLHVAWRPP
53	B	MPLSYQHFRKLLLLDEAGPLEEELPRLADEGLNRRVAEDLNLGNLNVSI PWTHKVGNFTGLYSSTVPVFNPEWQTPSFPHIHLQEDIINRCQQYVGPLT VNEKRRLKLIMPARFYPNLTKYLPLDKGIKPYYPRHVVNHYFQTRHYLHT LWKAGILYKRESTRSASFCGSPYSWEQDLQHGRLVFQTSKRHGDKSFCPQ SPGILPRSSVGPCIQNQLRKSRLGPQPAQGQLARGRQQGGSGSIRARVHPS PWGTVGVEPSGSGHIHNCASNSSSCLFQSAVRKAAYSHISTSKGHSSSGH AVELHHFPPSSSRSQSQGPVLSCWWLQFRNSEPCSEYCLCHIVNLIEDWG PCTEHGEHRIRTPRTPARVTGGVFLVDKNPHNTTESRLVVDFSQFSRGNT RVSWPKFAVPNTQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMPHLLVG SSGLSRYVARLSSNSRIINNQHRTMQNLHDSCSRNLYVSLMLLYKTYGRK LHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPECLAFSYM HDVVLGAKSVQHLESLYAAVTNFLLSLGIHLNPHKTKRWGYSLNFMGYVI GSWGTLPQEHIVQKIKMCFRKLPVNRPIDWKVCQRIVGLLGFAAPFTQCG YPALMPLYACIQAKQAFTFSPTYKAFLSKQYLHLYPVARQRPGLCQVFAD ATPTGWGLAIGHQRMRGAFVSPLPIHTAHLLAACFARSRSGAKLIGTDNS

Table 2 - Pol^{mut} polypeptide sequence

SEQ ID NO:	HBV genotype	Polypeptide sequence - Motifs containing inactivating mutations in Pol are underlined (YMDD mutated to YMHD, AELL mutated to AHLL)
		[illegible] VVLSRKYTSFPWTLGCAANWILRGTSFVYVPSALNPADDPSRGRLGLYRP LLRLLYRPTTGRTSLYADSPSVPSHLPDRVHFASPLHVAWRPP
54	C	MPLSYQHFRKLLLLDDEAGPLEEELPRLADEDLNRRVAEDLNLGNLNVST PWTHKVGNFTGLYSSTVPVFNPEWQTPSFPHIHLQEDIINRCQQYVGPLT VNEKRRLKLIMPARFYPNLTKYLPLDKGIKPYYPEHTVNHYFKTRHYLHT LWKAGILYKRETTRSASFCGSPYSWEQELQHGRLVFQTSTRHGDESFCSQ SSGILSRSPVGPCIRSQLKQSRLGLQPQQGSLARSKSGRSGSIEARVHPT TRQSFGVEPSGSGHIIDNSASSASSCLFQSAVRKTAYSHLSTSKRQSSSGH AVELHNFPPSSARSQSEGPLLSCVWLQFRNSKPCSDYCLSHIVNLLEDWG PCTEHGEHNIRIPRTPARVTGGVFLVDKNPHNTTESRLVVDFSQFSRGST HVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMPHLLVG SSGLSRYVARLSSTSRNINYQHGAMQDLHDSCSRNLYVSLLLLYKTFGRK LHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCLAFSYM HDVVLGAKSVQHLESLFTAVTNFLLSLGIHLNPNKTKRWGYSLNFMGYVI
		GSWGTLPQEHIVLKIKQCFRKLPVNRPIDWKVCQRIVGLLGFAAPFTQCG YPALMPLYACIQAKQAFTFSPTYKAFLCKQYLNLYPVARQRSGLCQVFAD ATPTGWGLAVGHQRMRGTFVSPLPIHTAHLLAACFARSRSGAKLIGTDNS VVLSRKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDPSRGRLGLYRP LLRLPFRPTTGRTSLYAVSPSVPSHLPVRVHFASPLHVAWRPP
55	D	GSWGTLPQEHIVLKIKQCFRKLPVNRPIDWKVCQRIVGLLGFAAPFTQCG YPALMPLYACIQAKQAFTFSPTYKAFLCKQYLNLYPVARQRSGLCQVFAD ATPTGWGLAVGHQRMRGTFVSPLPIHTAHLLAACFARSRSGAKLIGTDNS VVLSRKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDPSRGRLGLYRP LLRLPFRPTTGRTSLYAVSPSVPSHLPVRVHFASPLHVAWRPP MPLSYQHFRRLLLLDDEAGPLEEELPRLADEGLNRRVAEDLNLGNLNVSI PWTHKVGNFTGLYSSTVPVFNPHWKTPSFPNIHLHQDIIKKCEQFVGPLT VNEKRRLQLIMPARFYPNVTKYLPLDKGIKPYYPEHLVNHYFQTRHYLHT LWKAGILYKRETTHSASFCGSPYSWEQELQHGAESFHQQSSGILSRPPVG SSLQSKHRKSRLGLQSQQGHLARRQQGRGWSIRAGIHPTARRPFGVEPSG SGHTANLASKSASCLYQSAVKKAAYPVVSTFKKHSSSGHAVELHNLPPNS ARSQSERPVFPCWWLQFRNSKPCSDYCLSHIVNLLEDWGPCAEHGEHHIR IPRTPARVTGGVFLVDKNPHNTAESRLVVDFSQFSRGNYRVSWPKFAVPN LQSLTNLLSSNLSWLSLDVSAAFYILPLHPAAMPILLVGSSGLSRYVARL SSNSRTFNYQHGTMQNLHDSCSRNLYVSLMLLYQTFGRKLHLYSHPIILG FRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCLAFSYMHDVVLGAKSVQ HLESLFTAVTNFLLSLGTHLNPNKTKRWGYSLHFMGYVTGCYGSLPQDHI IQKLKECFRKLPVNRPIDWKVCQRIVGLLGFAAPFTQCGYPALMPLYACI QSKQAFTFSPTYKAFLCKQYLNLYPVARQRPGLCQVFADATPTGWGLVMG HQRMRGTFKAPLPIHTAHLLAACFARSRSGANILGTDNSVVLSRKYTSFP WLLGCAANWILRGTSFVYVPSALNPADDPSRGRLGLYRPLLRLPFRPTTG RTSLYADSPSVPSHLPDRVHFASPLHVAWRPP

[0290] 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 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 region. In one set of sequences, designated Pol$^{\Delta 1}$, 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$^{\Delta 3}$, the entire poorly conserved region was identified by sequence alignment and deleted. Core-Pol fusions were generated by fusing the near-consensus core sequences to the Pol^{mut}, Pol$^{\Delta 1}$ and Pol$^{\Delta 3}$ 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 Pol^{300}. The Pol^{300} 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$^{\Delta 1}$
9-12 for Genotype A-D, respectively	Pol$^{\Delta 3}$
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$^{\Delta 1}$
23-26 for Genotype A-D, respectively	Core-Pol$^{\Delta 3}$

Methods

[0291] 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

[0292] All antigen genotypes showed little change in immunogenicity between core-Pol^{mut} and core-Pol$^{\Delta 1}$. GT-A antigen had an increased response to core-Pol$^{\Delta 3}$ vs core-Pol^{mut} and core-Pol$^{\Delta 1}$, whereas GT-B, C, and D all demonstrated reduced immunogenicity with core-Pol$^{\Delta 3}$.

[0293] 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$^{\Delta 3}$ or GT-B, C, or D core-Pol$^{\Delta 1}$, 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$^{\Delta 1}$ 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$^{\Delta 1}$ were statistically significantly higher than responses to GT-A core-Pol$^{\Delta 3}$ using GT-D peptides, and to GT-C core-Pol$^{\Delta 1}$ using both peptide genotypes. The geometric mean Pol ELISPOT responses to GT-D core-Pol$^{\Delta 1}$ were numerically lower than GT-B core-Pol$^{\Delta 1}$, 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$^{\Delta 1}$ yielding the overall best results, although for core no comparisons between antigen genotypes reached statistical significance.

Example 3

Identification of Smaller Immunogenic Pol Antigens

[0294] Different viral vector systems have differing limits on the maximum size of encoded antigens.

Methods

[0295] 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$^{\Delta 1}$, Pol$^{\Delta 3}$, and Pol^{300}, and GT-B Pol^{300}, 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

[0296] 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

[0297] 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

[0298] 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 TranslMed. (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$^{\Delta 1}$. AAV-HBV mice were vaccinated with Ad5 prime and vaccinia boost vectors encoding GT-C sAg and GT-B core-Pol$^{\Delta 1}$ 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$^{\Delta 1}$ GT-B	Vac-sAg GT-C Vac-core-pol$^{\Delta 1}$ 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$^{\Delta 1}$ GT-B	Vac-sAg GT-C Vac-core-pol$^{\Delta 1}$ GT-B	α-PD-1
5	12	N	Ad-sAg GT-C Ad-core-pol$^{\Delta 1}$ GT-B	Vac-sAg GT-C Vac-core-pol$^{\Delta 1}$ GT-B	None
Ad: Adenovirus 5 vector. Vac: vaccinia vector. β-gal: beta-galactosidase. GFP: green fluorescent protein.					

Results

[0299] 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*). 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 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.

[0300] To evaluate any antiviral effects of the HBV-specific T cells induced by 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 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 ntigens in Arenavirus Vectors

[0301] 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).

[0302] Arenavirus vectors can accommodate antigens of approximately 500-800 amino acids per open reading frame. Therefore, we tested GT-D and GT-B $Pol^{\Delta 1}$ (SEQ ID NOs: 6 and 8), $Pol^{\Delta 3}$ (SEQ ID NOs: 10 and 12), and Pol^{300} (SEQ ID NOs: 13 and 14) for immunogenicity in replication-incompetent LCMV vectors. C57BL/6 mice were immunized intravenously with 10^6 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 Pol^{300} induced robust T cell responses, while GT-D $Pol^{\Delta 1}$ and $Pol^{\Delta 3}$ 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

[0303] 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*-$Pol^{\Delta 1}$	P1
GT-B	VV1-$Pol^{\Delta 3}$	P1
GT-B	VV1-Pol^{300}	P5
GT-D	VV1-$Pol^{\Delta 1}$	P1
GT-D	VV1-$Pol^{\Delta 3}$	P1
GT-D	VV1-Pol^{300}	P2
*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

[0304] 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 $Pol^{\Delta 1}$
28	10	GT-B $Pol^{\Delta 3}$
29	13	GT-B Pol^{300}
30	8	GT-D $Pol^{\Delta 1}$

Sequences vector antigens and antigen-encoding genes used in LCMV vectors		
Polynucleotide SEQ ID NO:	**Polypeptide SEQ ID NO:**	**Polypeptide**
31	12	GT-D Pol$^{\Delta 3}$
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

[0305] 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.

[0306] 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

[0307] 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	VV 1-iCore-P2A-sAg	P6
*VV1 refers to replication-incompetent LCMV vectors. "P#" indicates the number of passages (*e.g.*, P1 equals 1 passage).		

[0308] 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).

[0309] 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

[0310] 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

[0311] 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**
1	8	Mock	Mock	42	-
2	8	W1-GT-B/C Core-P2A-sAg	W1-GT-B/C Core-P2A-sAg	42	10^6 FFU
3	8	W1-GT-D iCore-P2A-sAg	W1-GT-D iCore-P2A-sAg	42	10^6 FFU

Study Groups in Immunogenicity Study					
Group	**N**	**Prime vector - Day 0**	**Boost vector - Day 28**	**Harvest Day**	**Dose /vector**
4	8	W1-GT-B $Pol^{\Delta 3}$	W1-GT-B $Pol^{\Delta 3}$	42	10^6 FFU
5	8	W1-GT-B Pol^{300}	W1-GT-B Pol^{300}	42	10^6 FFU

Results

[0312] 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 Pol^{300} induced a numerically superior T cell response specific to pol antigens than the vector encoding GT-B $Pol^{\Delta 3}$ (Fig. 14B). Thus, the vectors encoding for GT-D iCore-P2A-sAg and GT-B Pol^{300} are more immunogenic than the vectors encoding for GT-B/C Core-P2A-sAg and GT-B $Pol^{\Delta 3}$ in outbred mice.

[0313] 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 Pol^{300} 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 Pol^{300} 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

[0314] 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

[0315] 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	W1-GT-D iCore_P2A_sAg	W1-GT-D iCore_P2A_sAg	28	10^6 FFU
3	5	Single vector	W1-GT-B Pol^{300}	W1-GT-B Pol^{300}	28	10^6 FFU
4	5	Co-formulated	W1-GT-D iCore_P2A_sAg + VV1-GT-B Pol^{300}	W1-GT-D iCore_P2A_sAg + VV1-GT-B Pol^{300}	28	10^6 FFU

Results

[0316] Consistent with data described above, vectors encoding GT-D iCore-P2A-sAg and GT-B Pol^{300} 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 Pol^{300} does not interfere with their immunogenicity in C57BL/6 mice.

Example 11

Immunogenicity of Replication-Incompetent LCMV vectors in Cynomolgus Macaques.

[0317] We evaluated the immunogenicity of the replication-incompetent LCMV (VV1) vectors GT-D iCore-P2A-sAg and GT-B Pol^{300} vectors in cynomolgus macaques. Ad5 and vaccinia vectors encoding for the core, sAg, and Pol^{300} antigens were also tested.

Methods

[0318] 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 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	W1-GT-D iCore-P2A-sAg + W1-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

Study Groups in Immunogenicity Study					
Group	**N**	**Vaccine**	**Dose**	**Route**	**Immunization schedule (week)**
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)	10^{11} vp/vector	i.m.	0 (Ad5), 4 (Ad5), 8 (Vac), 12 (Vac)
		2.Vaccinia GT-D core-sAg + Vaccinia GT-B Pol^{300} (days 8 and 12)	10^8 PFU/vector		

Results

[0319] 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 Pol^{300} 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 Pol^{300} 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/10^6 PBMC in animals administered the full human dose (10^8 FFU, Group 2), and approximately 2-fold lower at the lower dose 5×10^6 FFU (group 1) indicating dose-responsiveness.

[0320] 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.

[0321] 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

[0322] 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

[0323] 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	W1-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 909 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

[0324] 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.

[0325] 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

[0326] 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).

[0327] In contrast, when the same GT-B Pol^{300} 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 Pol^{300} antigen using different nucleotide sequences, designated VV2-Pol300_IDT_CpGdel (SEQ ID NO: 94), Pol^{300} ori (SEQ ID NO: 89), and Pol^{300} 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 Pol^{300} transgenes in VV2 vectors were evident between the different nucleotide sequences despite encoding the identical polypeptide antigen, with Pol^{300} dint, Pol^{300} ori, and Pol^{300} 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).			

[0328] Next, to assess potential differences in immunogenicity between vectors carrying the Pol^{300} dint and Pol^{300} ori transgenes, C57BL/6 mice were immunized twice at day 0 and day 21 with replication-incompetent PICV (VV2) vectors encoding GT-B Pol^{300} ori or GT-B Pol^{300} dint. HBV-specific T cell responses were then measured from splenocytes by IFN-γ ELISPOT using Pol peptide pools. Surprisingly, VV2-GT-B Pol^{300} dint induced a much stronger T cell response than VV2-GT-B Pol^{300} ori despite encoding identical amino acid sequences (Fig. 25). Thus, VV2-GT-B Pol^{300} dint is more immunogenic than VV2-GT-B Pol^{300} 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

[0329] We evaluated the immunogenicity of replication-incompetent LCMV (VV1) and PICV (VV2) vectors encoding GT-D iCore-P2A-sAg and GT-B Pol^{300} 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

[0330] C57BL/6 mice were immunized twice with replication-incompetent LCMV and PICV vectors encoding GT-D iCore-P2A-sAg and GT-B Pol^{300} 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	W1-GT-D iCore-P2A-sAg	W1-GT-D iCore-P2A-sAg	28	10^6 FFU
3	5	Heterologous Prime/Boost	W2-GT-D iCore-P2A-sAg	W1-GT-D iCore-P2A-sAg	28	10^6 FFU
4	5	Homologous Prime/Boost	W1-GT-B Pol^{300}	W1-GT-B Pol^{300}	28	10^6 FFU

Study Groups in Immunogenicity Study						
Group	**N**	**Immunization Regimen**	**Prime vector Day 0**	**Boost vector Day 21**	**Harvest Day**	**Dose /vector**
5	5	Heterologous Prime/Boost	W2-GT-B Pol^{300} dint	W1-GT-B Pol^{300}	28	10^6 FFU

Results

[0331] Administration of the replication-incompetent LCMV vector (VV1) encoding GT-D iCore-P2A-sAg or encoding GT-B Pol^{300} 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

[0332] 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 Pol^{300}) 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 Pol^{300} inserted into segment 2 into the LCMV backbone (TT1-GT-D core-P2A-sAg/GT-B Pol^{300}), ii) GT-D core-P2A-sAg inserted into segment 1 and GT-B Pol^{300} inserted into segment 2 into the PICV backbone (TT2-GT-D core-P2A-sAg/GT-B Pol^{300}), iii) GT-D core-P2A-sAg inserted into segment 2 and GT-B Pol^{300} inserted into segment 1 into the LCMV backbone (TT1-GT-B Pol^{300}/GT-D core-P2A-sAg) and iv) GT-D core-P2A-sAg inserted into segment 2 and GT-B Pol^{300} inserted into segment 1 into the PICV backbone (TT2-GT-B Pol^{300}/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

[0333] C57BL/6 mice were immunized twice with replication-attenuated LCMV and PICV vectors encoding GT-D Core-P2A-sAg and GT-B Pol^{300} 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	TT1-8T-8 Pol^{300}/ GT-D core-P2A-sAg	TT1-8T-8 Pol^{300}/ GT-D core-P2A-sAg	28	$5x10^4$
6	5	TT2-8T-8 Pol^{300}/ GT-D core-P2A-sAg	TT2-8T-8 Pol^{300}/ GT-D core-P2A-sAg	28	$5x10^4$
7	5	TT2-8T-8 Pol^{300}/GT-D core-P2A-sAg	TT1-8T-8 Pol^{300}/GT-D core-P2A-sAg	28	$5x10^4$

Results

[0334] 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.

Example 16

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

[0335] 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 cynomolgus macaques.

Methods

[0336] Cynomolgus macaques (n=5) were immunized with VV2 vectors (5×10^6 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

[0337] 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 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

[0338] 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

[0339] Cynomolgus macaques were immunized as described in Table 17. HBV-specific 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	W1	W1	10^8 FFU	0 (W1)	Week 4
					1 (W1)	
					2 (W1)	
					3 (W1)	
2	5	W2	W1	10^8 FFU	0 (W2)	Week 4
					1 (W1)	
					2 (W2)	
					3 (W1)	

Results

[0340] 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).

[0341] 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 within the scope of the appended claims.

[0342] Any references in the description to methods of treatment refer to the compounds, pharmaceutical compositions and medicaments of the present invention for use in a method for treatment of the human (or animal) body by therapy.

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Patentkrav

1. Arenavirusvektor, omfattende en polynukleotid, der koder for en HBV-kerne-sAg-fusionspolypeptid, der omfatter eller består af en aminosyresekvens af en hvilket som helst af SEQ ID Nos: 38-41, hvor HBV-kerne-sAg-fusionspolypeptidet ikke er længere end 450 aminosyrer i længde, og hvor sAg-polypeptidet ikke omfatter et HBV-præ-S1-polypeptid og/eller et HBV-præ-S2-polypeptid.

2. Arenavirusvektor ifølge krav 1, hvor polynukleotidet, der koder for HBV-kerne-sAg-fusionspolypeptidet, omfatter eller består af aminosyresekvensen af SEQ ID NO: 41.

3. Arenavirusvektor ifølge krav 1 eller 2, hvor polynukleotidet omfatter eller består af en nukleinsyresekvens af en hvilken som helst af SEQ ID NOs: 33-37, eller det er mindst 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% eller 99% identisk med den fulde længde af en hvilken som helst af SEQ ID NO: 33-37, fortrinsvis hvor polynukleotidet omfatter eller består af en nukleinsyresekvens med SEQ ID NO: 37, eller det er mindst 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% eller 99% identisk med den fulde længde af SEQ ID NO: 37.

4. Arenavirusvektor omfattende et polynukleotid, der koder for en trunkeret HBV-polymerase, som består af en aminosyresekvens af en hvilken som helst af SEQ ID NO: 13-14.

5. Arenavirusvektor ifølge krav 4, hvor polynukleotidet omfatter eller består af en nukleinsyresekvens af en hvilken som helst af SEQ ID NO: 29 og 89-94, eller en nukleinsyresekvens, der er mindst 99% identisk med den fulde længde af enhver en af SEQ ID NO: 29 og 89-94.

6. Arenavirusvektor ifølge krav 4 eller 5, hvor arenavirusvektoren er en vektor af den lymfocytiske choriomeningitis-mammarenavirus (LCMV), og polynukleotidet omfatter eller består af en nukleinsyresekvens af SEQ ID NO: 29, eller det er mindst 85%, 86% , 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% eller 99% identisk med den fulde længde af SEQ ID NO: 29 .

7. Arenavirusvektor ifølge et hvilket som helst af kravene 4 til 6, hvor arenavirusvektoren er en Cali mammarenavirusvektor, og polynukleotidet omfatter eller består af en nukleinsyresekvens af SEQ ID NO: 90, eller det er mindst 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% eller 99% identisk med den fulde længde af SEQ ID NO: 90.

8. Arenavirusvektor ifølge et hvilket som helst af kravene 4 til 7, hvor arenavirusvektoren er replikationsdefekt, replikationsmangelfuld eller replikationsinkompetent.

9. Værtscelle omfattende en eller flere arenavirusvektorer ifølge et hvilket som helst af kravene 1 til 8.

10. Immunogen sammensætning omfattende en eller flere arenavirusvektorer ifølge et hvilket som helst af kravene 1 til 8; og en farmaceutisk acceptabel bærer.

11. Immunogen sammensætning ifølge krav 10, omfattende en første arenavirusvektor og en anden arenavirusvektor, hvor:
(a) den første arenavirusvektor omfatter et polynukleotid, der koder for en trunkeret HBV-polymerase bestående af en aminosyresekvens af en hvilken som helst af SEQ ID NO: 13-14; og

(b) den anden arenavirusvektor omfatter et polynukleotid, der koder for et HBV-kerne-sAg-fusionspolypeptid, der omfatter eller består af en aminosyresekvens af en hvilken som helst af SEQ ID NOs: 38-41, hvor fusionsproteinet ikke er længere end 450 aminosyrer i længde, og hvor sAg-polypeptidet ikke omfatter et HBV-præ-S1-polypeptid og/eller et HBV-præ-S2-polypeptid.

12. Immunogen sammensætning ifølge krav 11, hvor den første arenavirusvektor og den anden arenavirusvektor er uafhængigt udvalgt blandt lymfocytisk choriomeningitis mammarenavirus (LCMV), Cali mammarenavirus, Guanarito virus (GTOV), Junin virus (JUNV), Lassa virus (LASV), Lujo virus (LUJV), Machupo virus (MACV), Sabia virus (SABV) og Whitewater Arroyo virus (WWAV).

13. Immunogen sammensætning ifølge et hvilket som helst af kravene 11 til 12, omfattende en første LCMV-arenavirus-ekspressionsvektor og en anden LCMV-arenavirus-ekspressionsvektor, hvor:
a) den første LCMV-arenavirus-ekspressionsvektor omfatter et polynukleotid, der omfatter eller består af en nukleinsyresekvens med SEQ ID NO: 29, eller en sekvens, der er mindst 85%, 86%, 87%, 88%, 89%, 90% 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% eller 99% identisk med SEQ ID NO: 29; og
b) den anden LCMV-arenavirus-ekspressionsvektor omfatter et polynukleotid, der omfatter eller består af en nukleinsyresekvens med SEQ ID NO: 37 eller en sekvens, der er mindst 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% eller 99% identisk med en hvilken som helst af SEQ ID NO: 37.

14. Immunogen sammensætning ifølge et hvilket som helst af kravene 11 til 12, omfattende en første LCMV-arenavirus-ekspressionsvektor og en anden LCMV-arenavirus-ekspressionsvektor, hvor:
a) den første LCMV-arenavirus-ekspressionsvektor omfatter et polynukleotid, der omfatter eller består af en nukleinsyresekvens med SEQ ID NO: 90, eller

en sekvens, der er mindst 88%, 89%, 90%, 91%, 92%, 93% 94%, 95%, 96%, 97%, 98% eller 99% identisk med SEQ ID NO: 90; og

b) den anden LCMV-arenavirus-ekspressionsvektor omfatter et polynukleotid, der omfatter eller består af en nukleinsyresekvens med SEQ ID NO: 37 eller en sekvens, der er mindst 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% eller 99% identisk med en hvilken som helst af SEQ ID NO: 37.

15. Immunogen sammensætning ifølge et hvilket som helst af kravene 11 til 14, hvor den første arenavirusvektor og den anden arenavirusvektor er tilvejebragt i et forhold i området fra 1:10 til 10:1.

16. Immunogen sammensætning ifølge et hvilket som helst af kravene 11 til 15, omfattende i området på ca.10^3 til omkring 10^{12} virale fokusdannende enheder (ffu) eller plakdannende enheder (pfu) eller infektiøse enheder (iu) eller virale partikler (vp) pr. milliliter af hver af den første arenavirusvektor og den anden arenavirusvektor.

17. Immunogen sammensætning ifølge et hvilket som helst af kravene 10 til 16, formuleret til indgivelse via en vej valgt fra gruppen bestående af intravenøs, intramuskulær, intradermal, subkutan og mucosal, eventuelt bukkal, intranasal, intrarektal eller intravaginal.

18. Immunogen sammensætning ifølge et hvilket som helst af kravene 10 til 17, formuleret som en væske, eventuelt som en vandig opløsning eller suspension.

19. Immunogen sammensætning ifølge et hvilket som helst af kravene 10 til 18, hvor sammensætningen er lyofiliseret.

20. Immunogen sammensætning ifølge et hvilket som helst af kravene 10 til 19 til anvendelse til at fremkalde et beskyttende immunrespons på humant hepatitis B-virus (HBV) eller til behandling eller forebyggelse af humant hepatitis B-virus (HBV).

DRAWINGS

Drawing

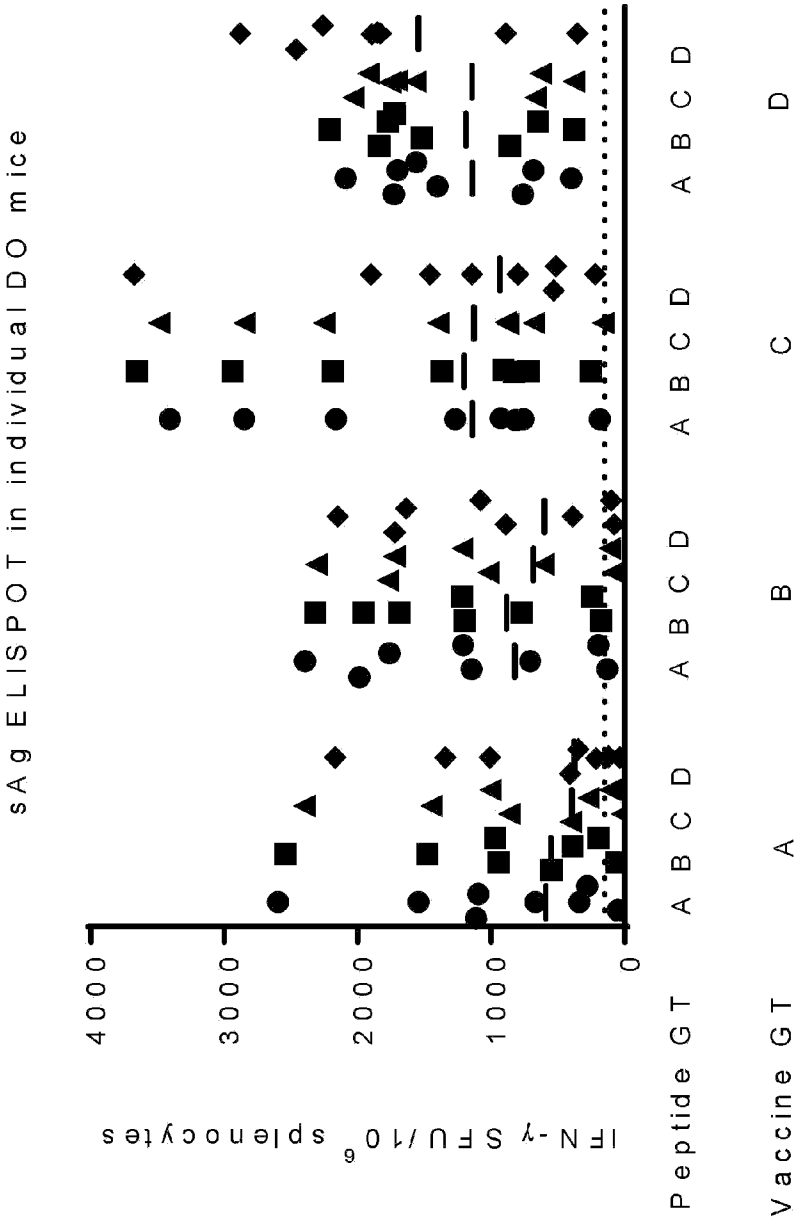
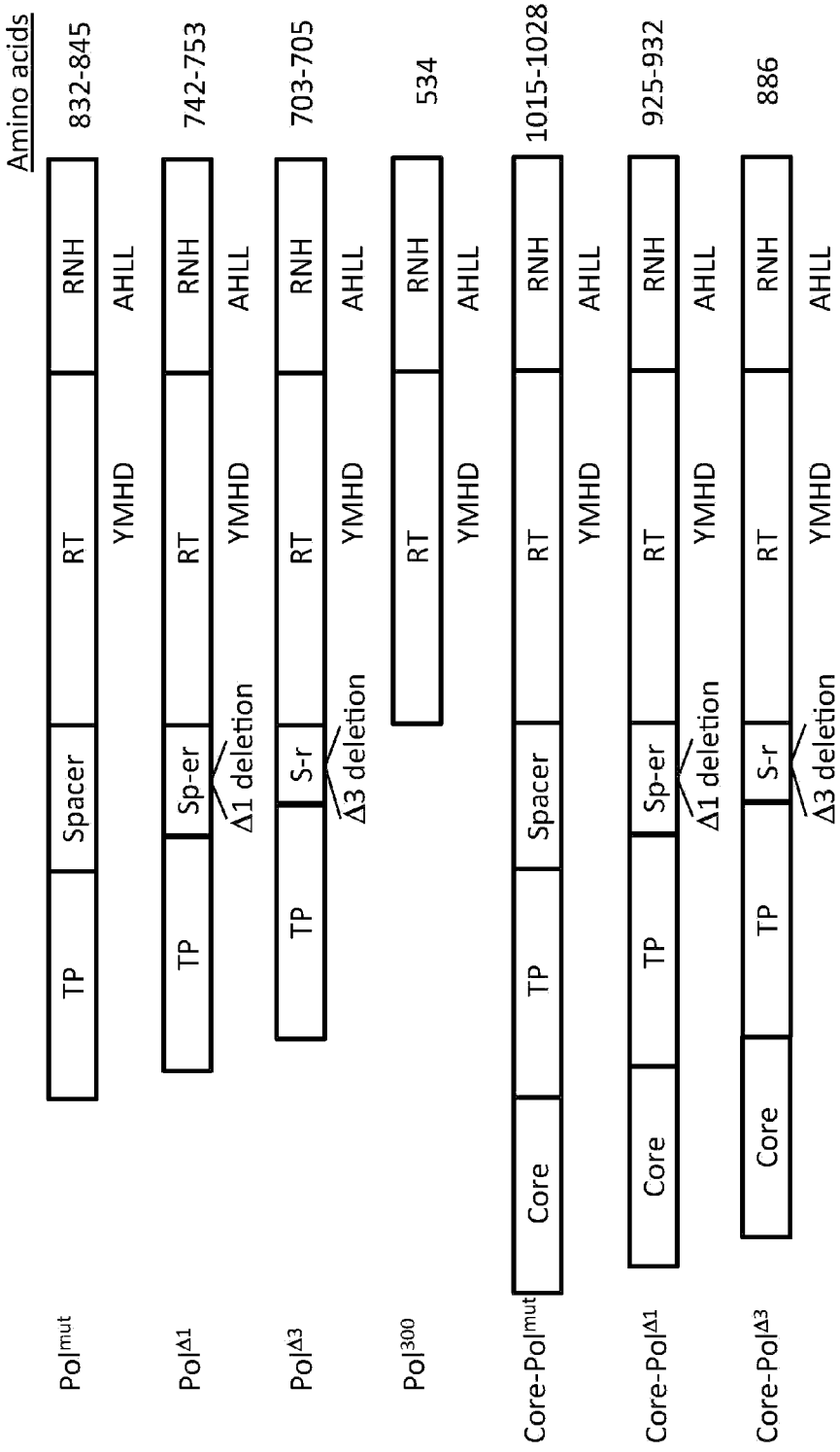


Fig. 1


Amino acids
Pol^mut
TP
Spacer
RT
RNH
YMHD
AHLL
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TP
Sp-er
RT
RNH
Δ1 deletion
YMHD
AHLL
742-753
Pol^Δ3
TP
S-r
RT
RNH
Δ3 deletion
YMHD
AHLL
703-705
Pol^300
RT
RNH
YMHD
AHLL
534
Core-Pol^mut
Core
TP
Spacer
RT
RNH
YMHD
AHLL
1015-1028
Core-Pol^Δ1
Core
TP
Sp-er
RT
RNH
Δ1 deletion
YMHD
AHLL
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S-r
RT
RNH
Δ3 deletion
YMHD
AHLL
886

Fig. 2

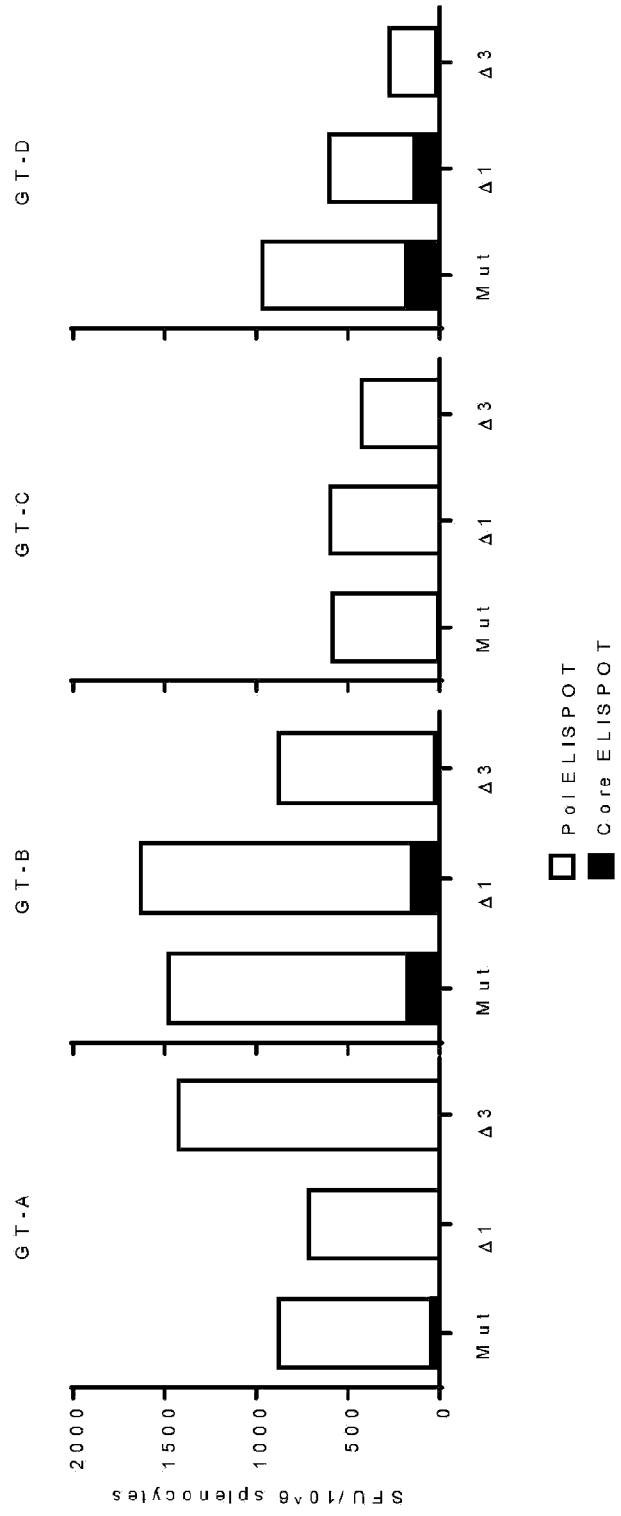

GT-A
GT-B
GT-C
GT-D
Mut
Δ1
Δ3
0
500
1000
1500
2000
SFU/10^6 splenocytes
Pol ELISPOT
Core ELISPOT

Fig. 3

Fig. 4A

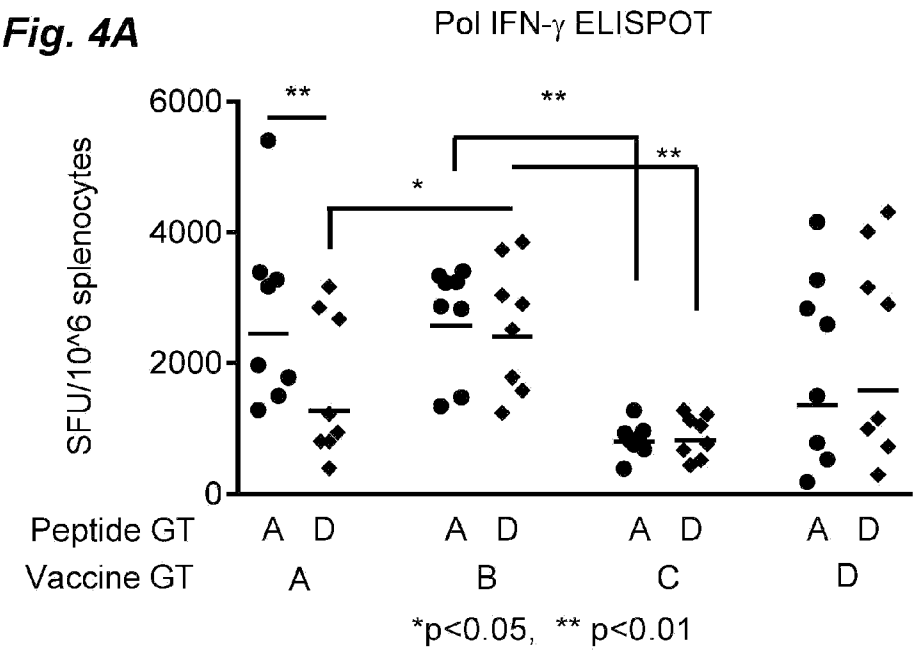
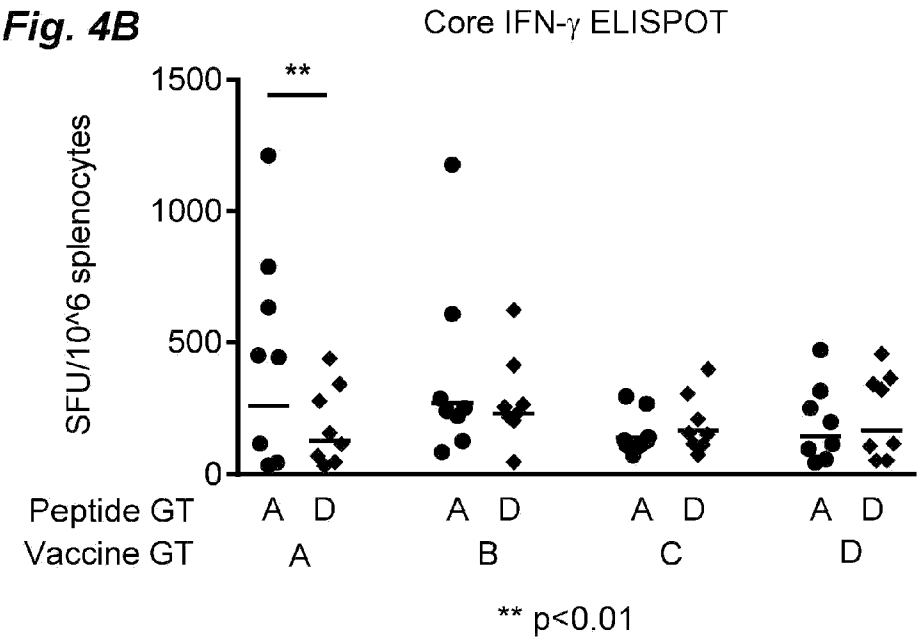


Fig. 4B



Figs. 4A-4B

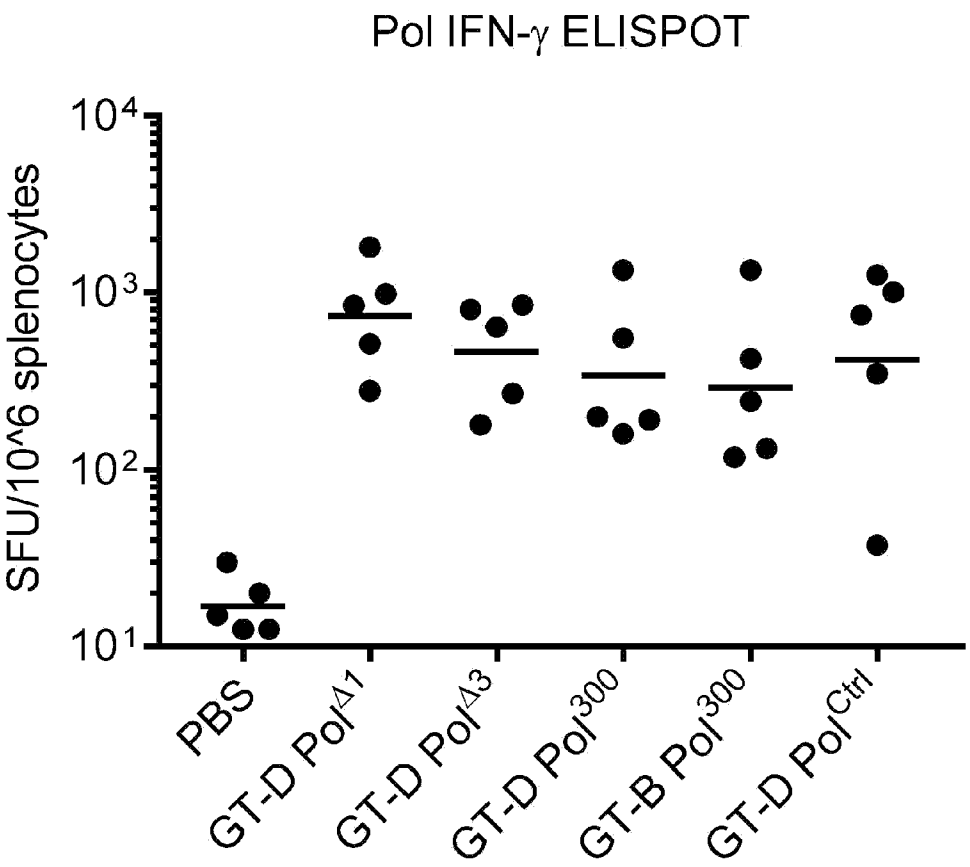

Pol IFN-γ ELISPOT
SFU/10^6 splenocytes
10^4
10^3
10^2
10^1
PBS
GT-D Pol^Δ1
GT-D Pol^Δ3
GT-D Pol^300
GT-B Pol^300
GT-D Pol^Ctrl

Fig. 5

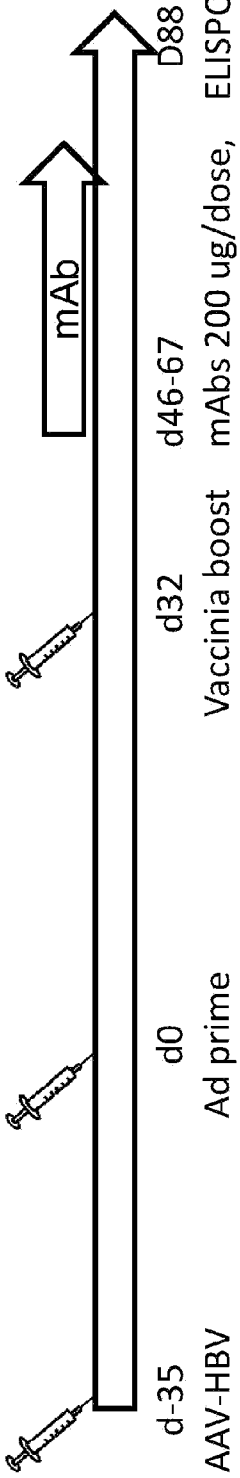

mAb
d-35
AAV-HBV
d0
Ad prime
d32
Vaccinia boost
d46-67
mAbs 200 ug/dose,
3x per week
D88
ELISPOT

Fig. 6

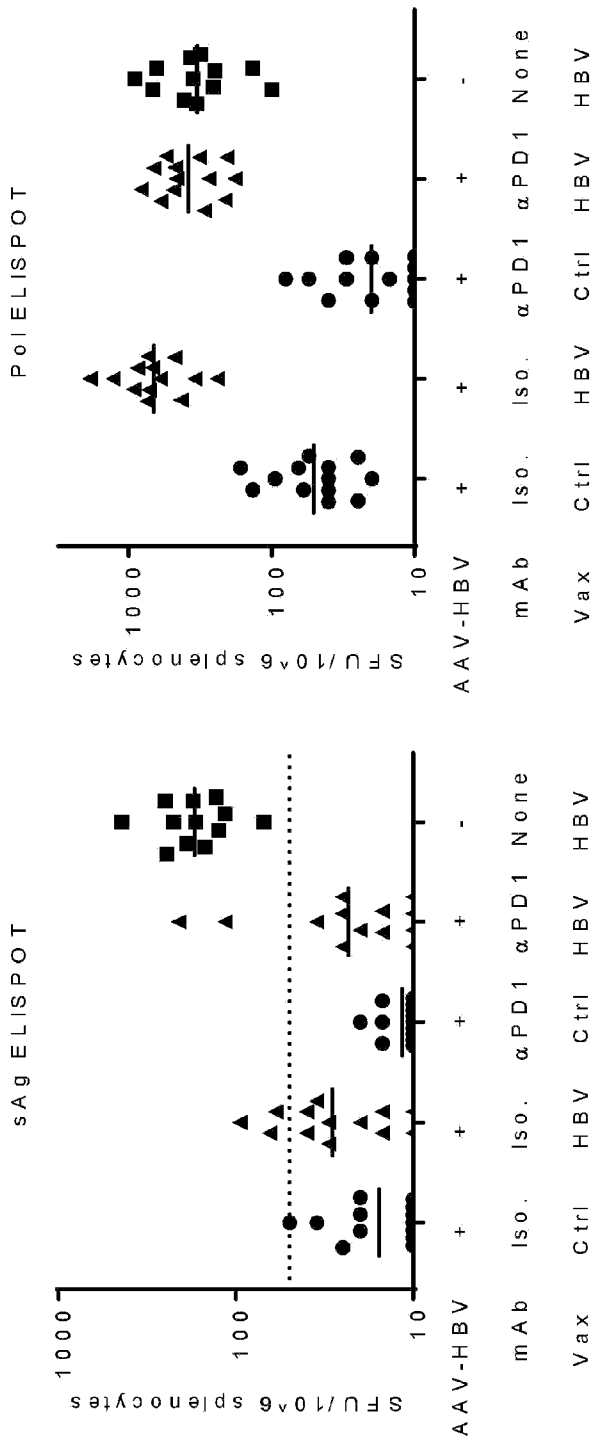

sAg ELISPOT
Pol ELISPOT
SFU/10^6 splenocytes
1000
100
10
AAV-HBV + + + -
mAb Iso. αPD1 None
Vax Ctrl HBV HBV

Fig. 7

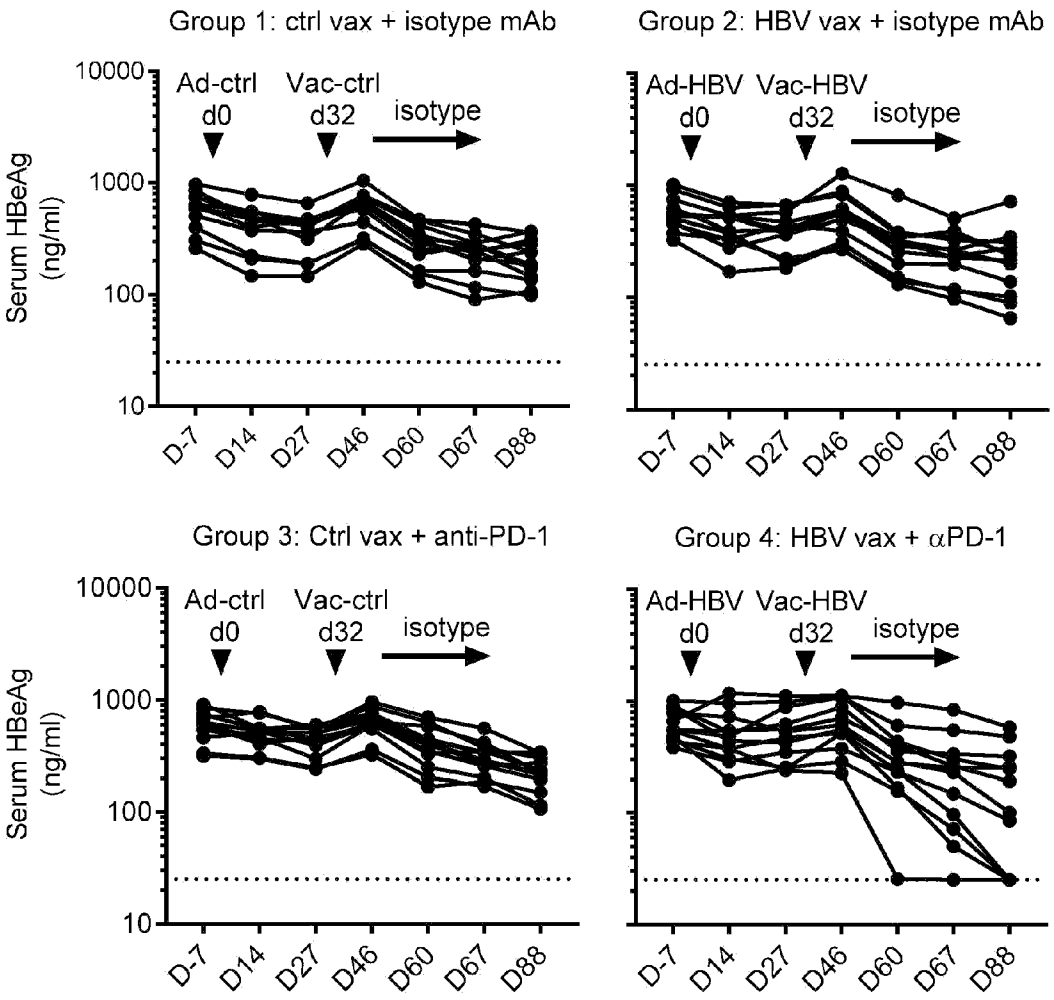

Group 1: ctrl vax + isotype mAb
Group 2: HBV vax + isotype mAb
Group 3: Ctrl vax + anti-PD-1
Group 4: HBV vax + αPD-1
Ad-ctrl
Vac-ctrl
Ad-HBV
Vac-HBV
d0
d32
isotype
Serum HBeAg (ng/ml)
10000
1000
100
10
D-7
D14
D27
D46
D60
D67
D88

Fig. 8

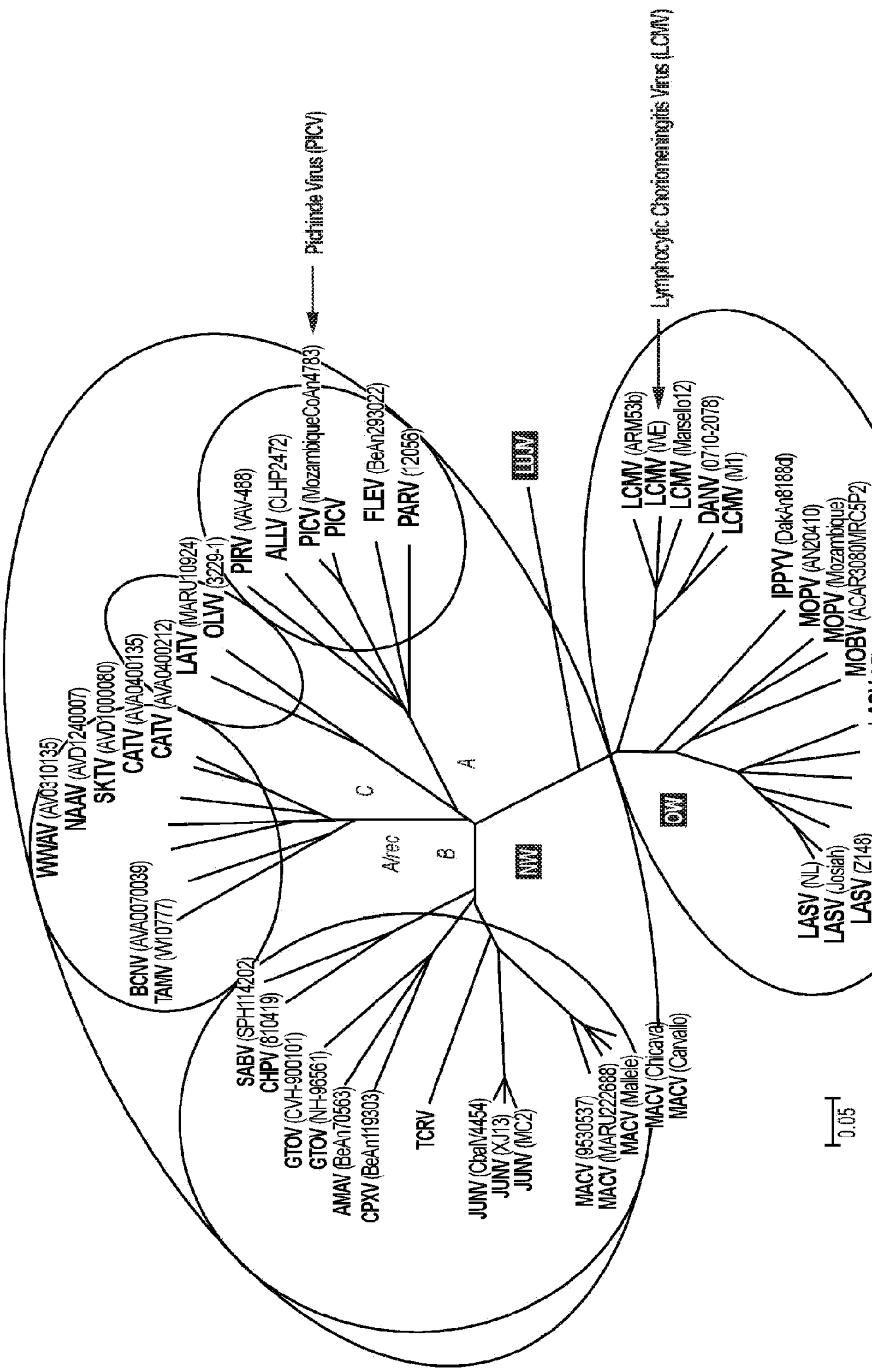

Pichinde Virus (PICV)
Lymphocytic Choriomeningitis Virus (LCMV)
WWAV (AV0310135)
NAAV (AVD1240007)
SKTV (AVD1000080)
CATV (AVA0400135)
CATV (AVA0400212)
LATV (MARU10924)
OLVV (3229-1)
PIRV (VAV-488)
ALLV (CLHP2472)
PICV (MozambiqueCoAn4783)
PICV
FLEV (BeAn293022)
PARV (12056)
LUJV
LCMV (ARM53b)
LCMV (WE)
LCMV (Marsello12)
DANV (0710-2078)
LCMV (M1)
IPPYV (DakAn8188d)
MOPV (AN20410)
MOPV (Mozambique)
MOBV (ACAR3080MRC5P2)
LASV (LP)
LASV (803213)
LASV (NL)
LASV (Josiah)
LASV (Z148)
LASV (AV)
LASV (Weller)
OW
NW
A
B
C
Afrec
BCNV (AVA0070039)
TAMV (W10777)
SABV (SPH114202)
CHPV (810419)
GTOV (CVH-960101)
GTOV (INH-95561)
AMAV (BeAn70563)
CPXV (BeAn119303)
TCRV
JUNV (CbalV4454)
JUNV (XJ13)
JUNV (MC2)
MACV (9530537)
MACV (MARU222688)
MACV (Mallele)
MACV (Chicava)
MACV (Carvallo)
0.05

Fig. 9A

Fig. 9B

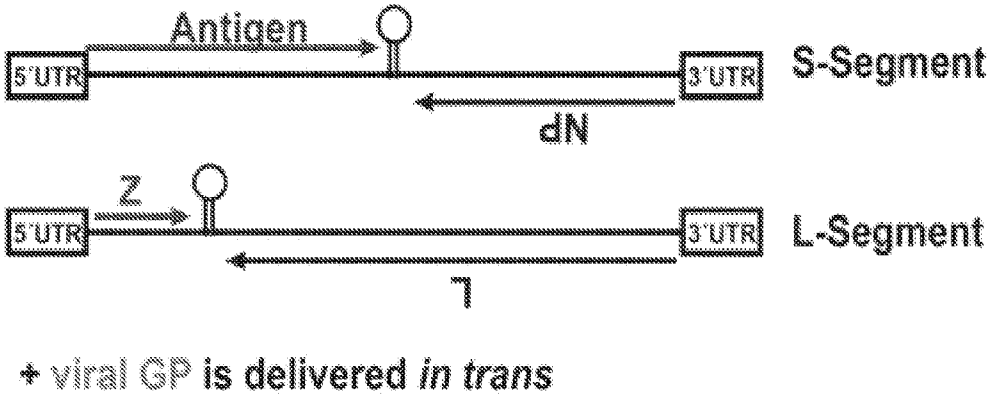
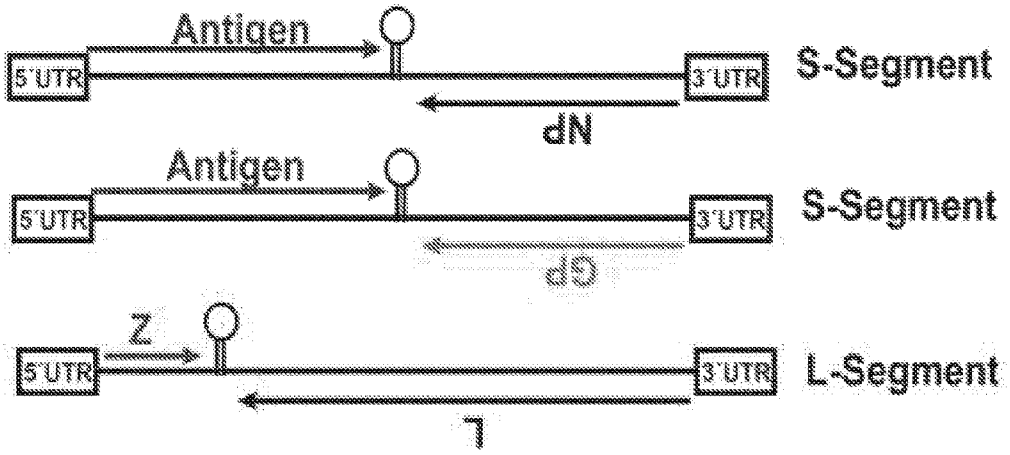


Fig. 9C



Figs. 9B-9C

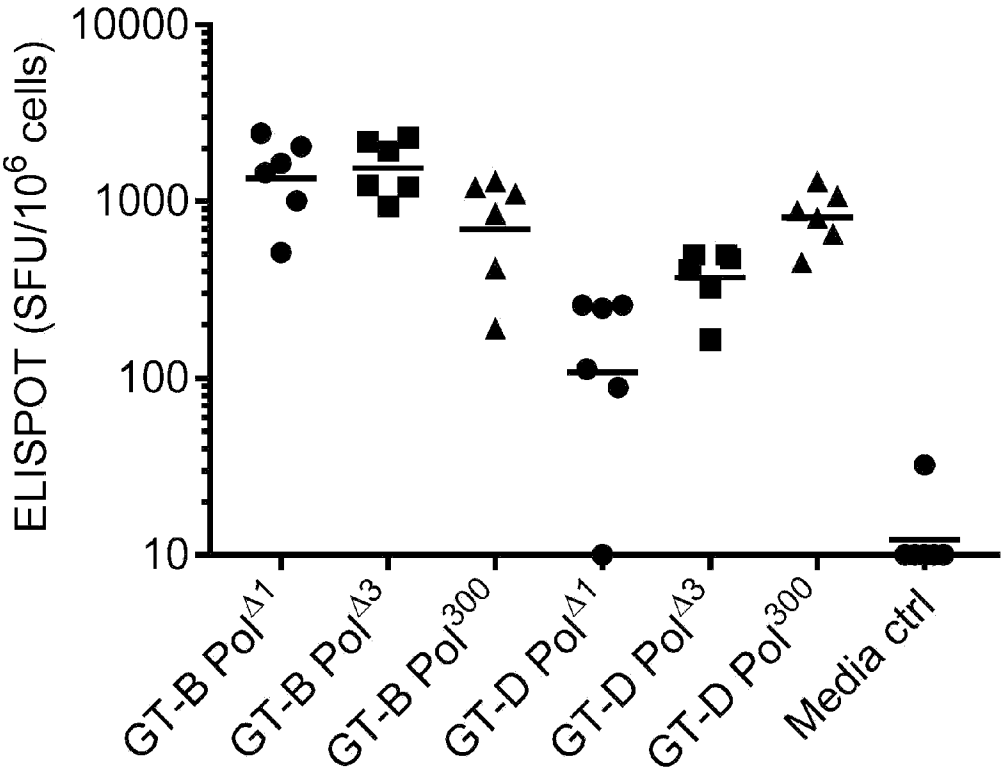

10000
1000
100
10
ELISPOT (SFU/10^6 cells)
GT-B Pol^Δ1
GT-B Pol^Δ3
GT-B Pol^300
GT-D Pol^Δ1
GT-D Pol^Δ3
GT-D Pol^300
Media ctrl

Fig. 10

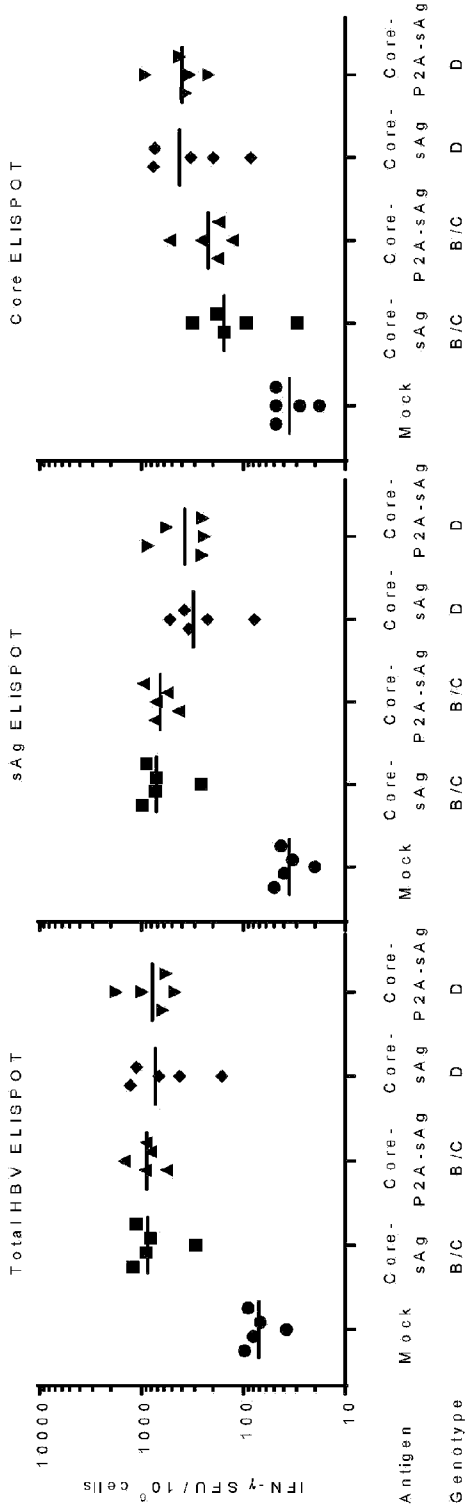

Total HBV ELISPOT
sAg ELISPOT
Core ELISPOT
IFN-γ SFU / 10^5 cells
10000
1000
100
10
Antigen
Mock
Core-sAg P2A-sAg
Core-sAg
Core-P2A-sAg
Genotype
B/C
D

Fig. 11

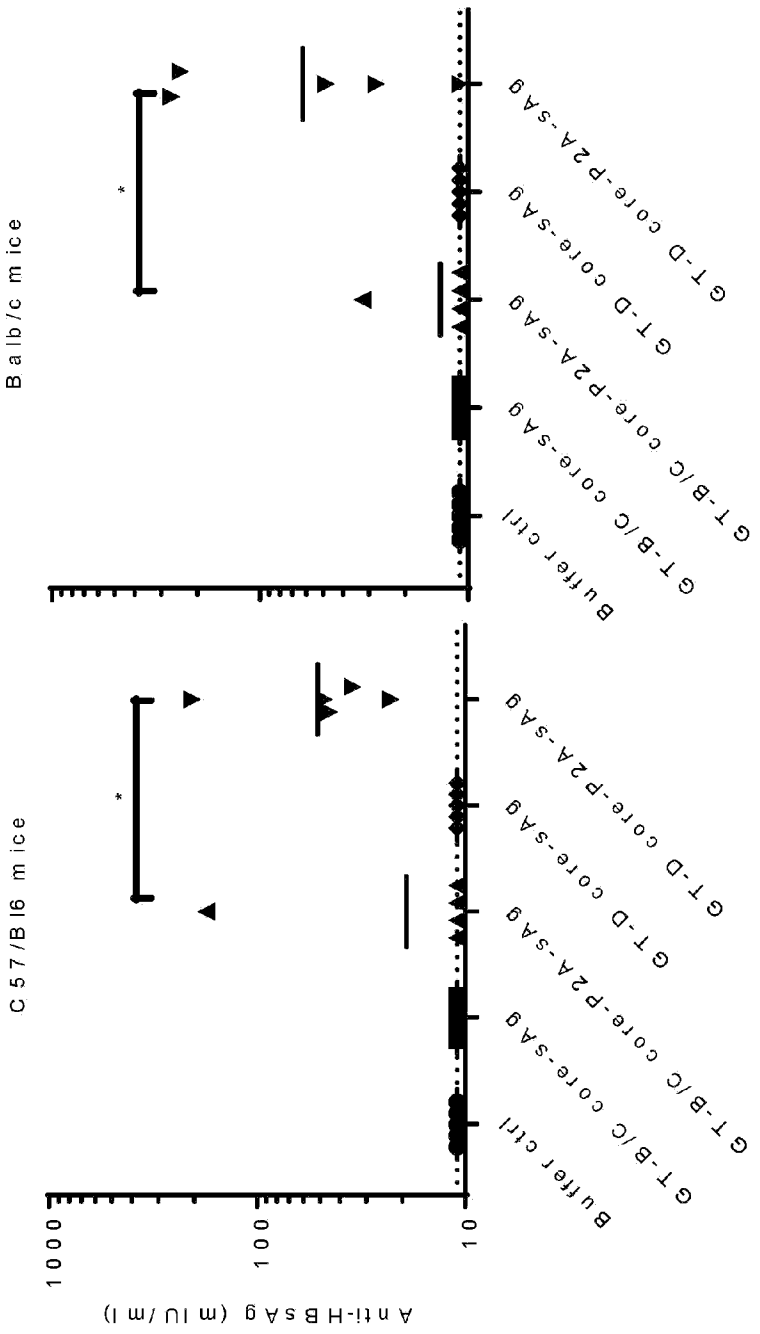

Anti-HBsAg (mIU/ml)
1000
100
10
C57/Bl6 mice
Balb/c mice
*
*
Buffer ctrl
GT-B/C core-sAg
GT-B/C core-P2A-sAg
GT-D core-sAg
GT-D core-P2A-sAg

Fig. 12

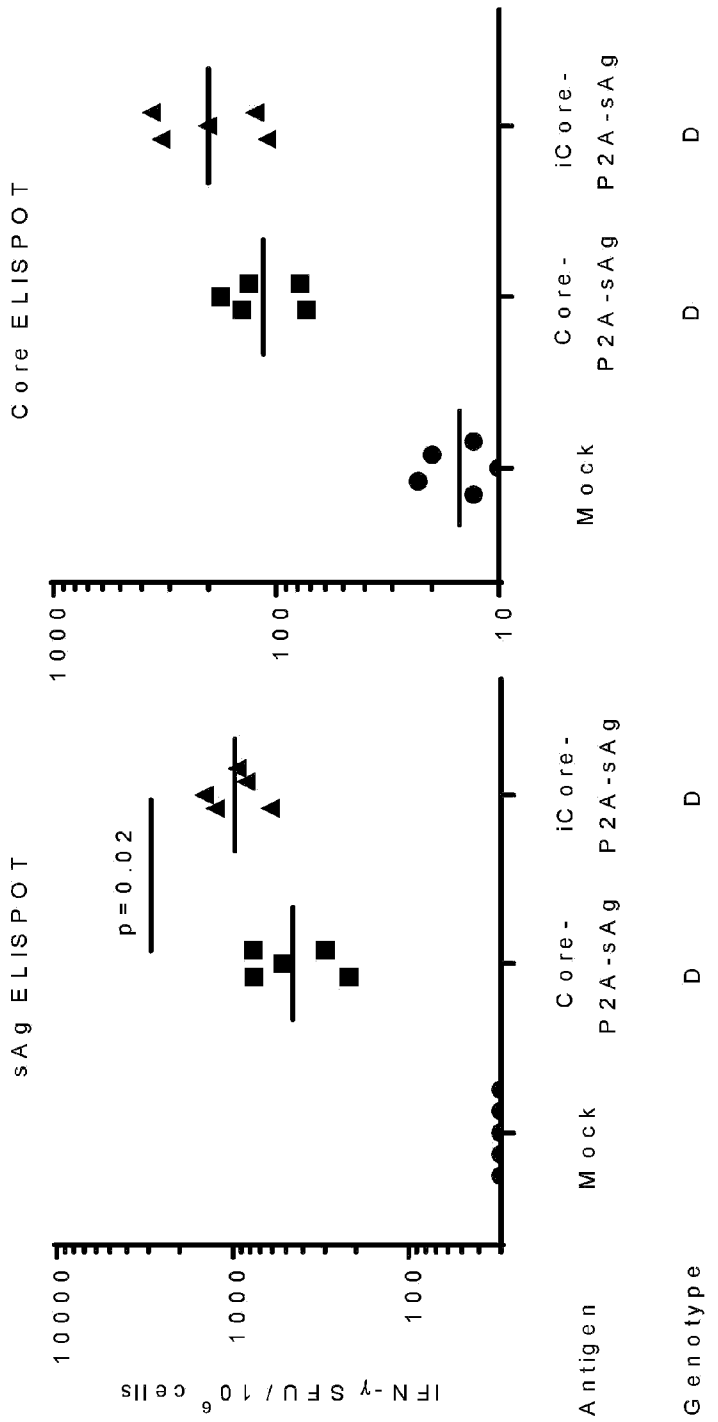

sAg ELISPOT
Core ELISPOT
IFN-γ SFU / 10^6 cells
10000
1000
100
10
p = 0.02
Mock
Core-P2A-sAg
iCore-P2A-sAg
Antigen
Genotype
D

Fig. 13

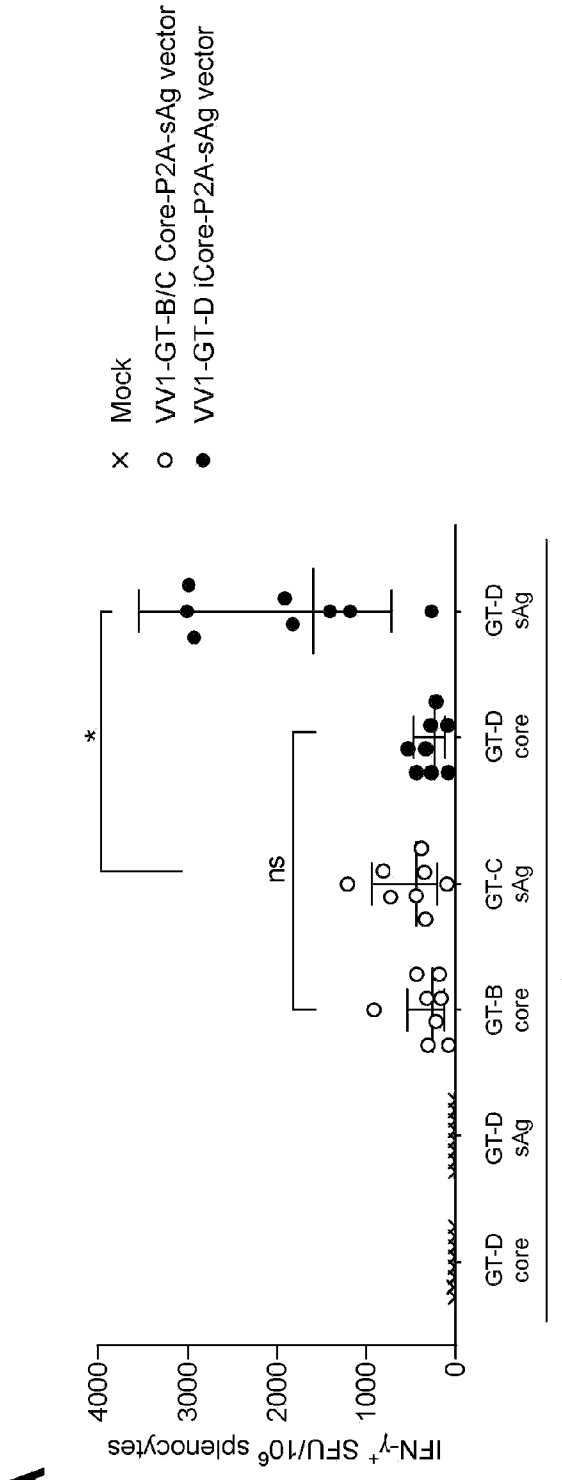

A
IFN-γ+ SFU/10^6 splenocytes
4000
3000
2000
1000
0
Mock
VV1-GT-B/C Core-P2A-sAg vector
VV1-GT-D iCore-P2A-sAg vector
*
ns
GT-D core
GT-D sAg
GT-B core
GT-C sAg
GT-D core
GT-D sAg
Peptide pool

Fig. 14A

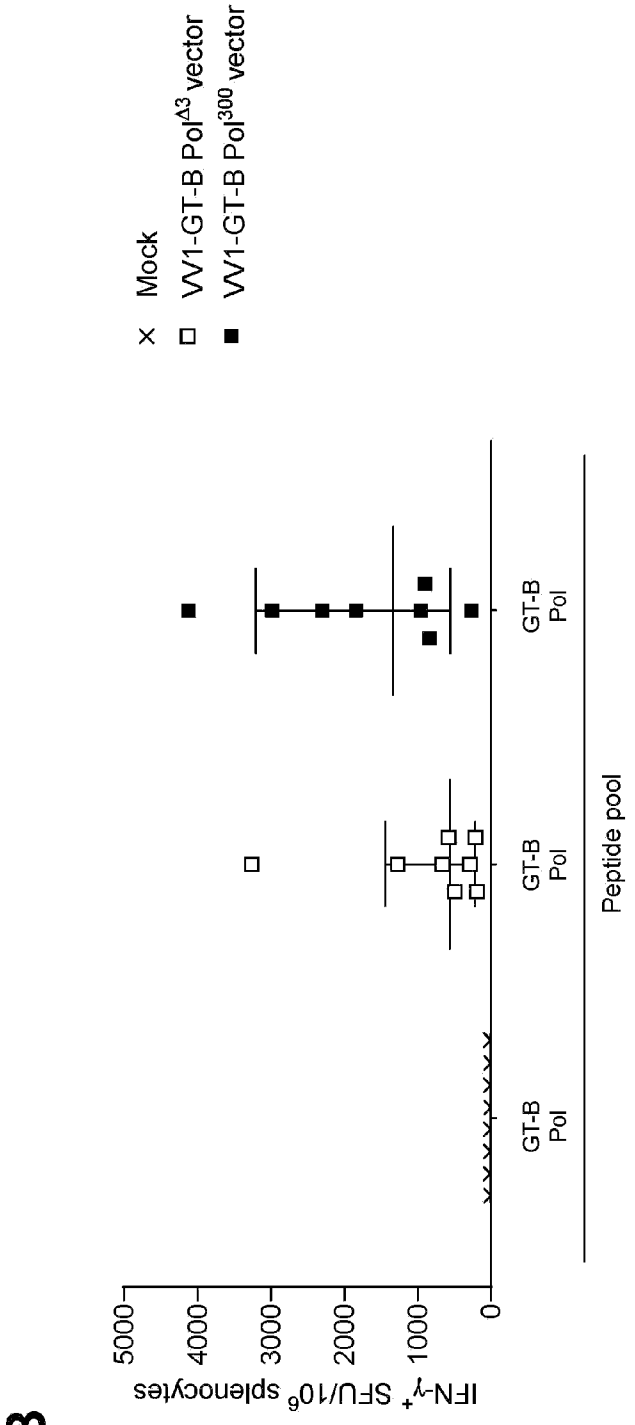

B
Mock
VV1-GT-B Pol$^{\Delta 3}$ vector
VV1-GT-B Pol^{300} vector
IFN-γ$^+$ SFU/10^6 splenocytes
5000
4000
3000
2000
1000
0
GT-B Pol
GT-B Pol
GT-B Pol
Peptide pool

Fig. 14B

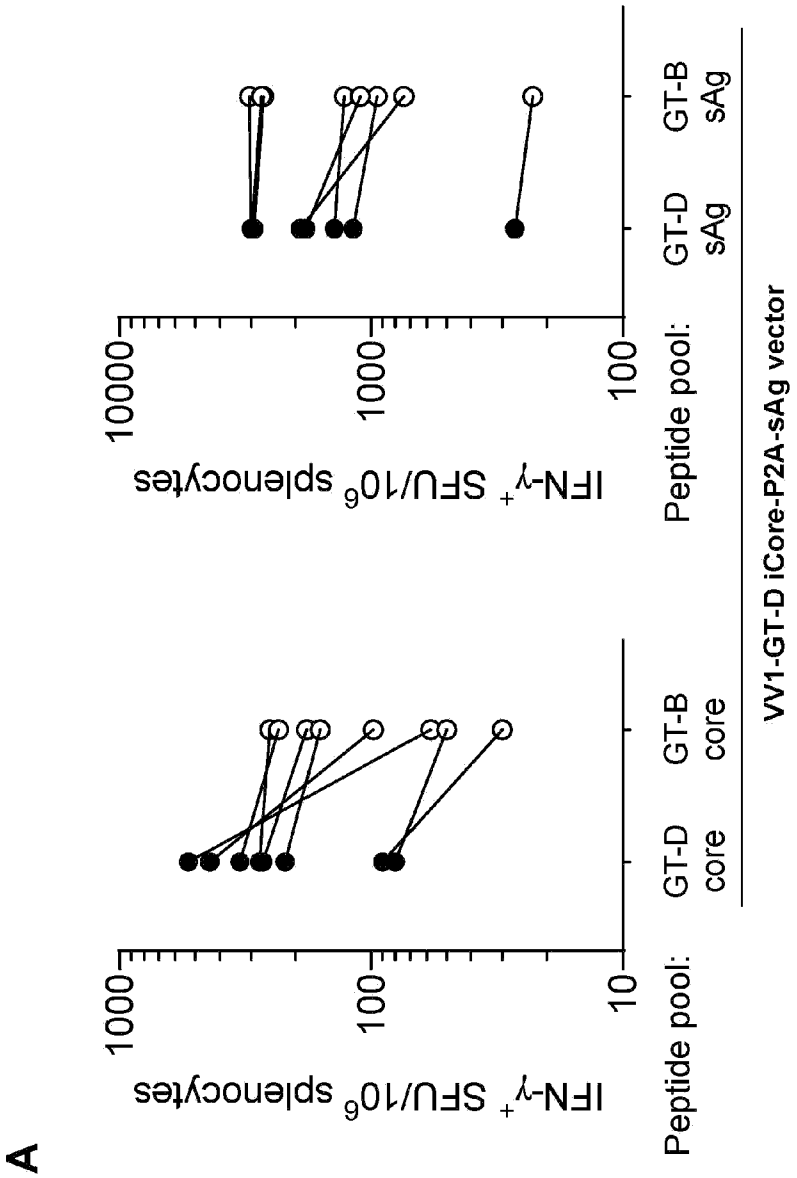

A
IFN-γ+ SFU/10^6 splenocytes
10000
1000
100
10
Peptide pool:
GT-D core
GT-B core
GT-D sAg
GT-B sAg
VV1-GT-D iCore-P2A-sAg vector

Fig. 15A

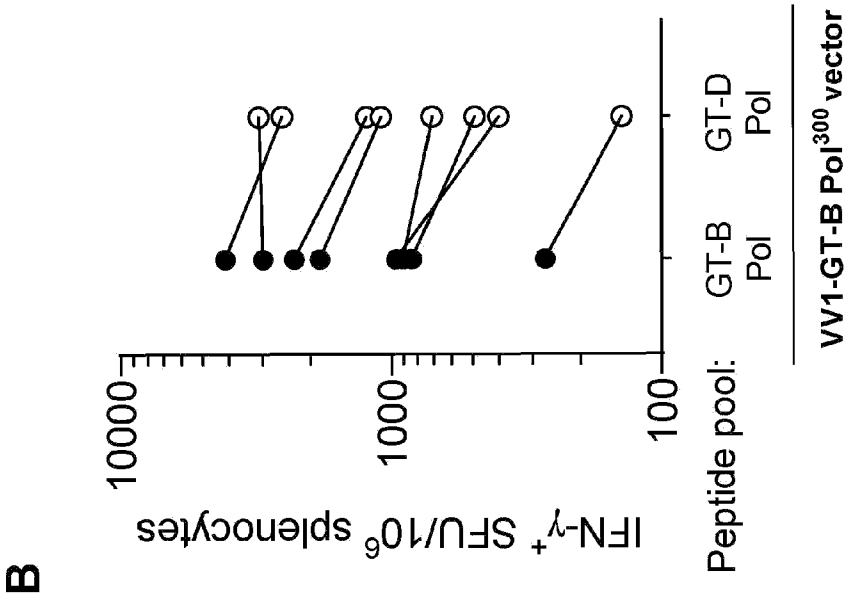

B
IFN-γ+ SFU/10^6 splenocytes
10000
1000
100
Peptide pool:
GT-B Pol
GT-D Pol
VV1-GT-B Pol300 vector

Fig. 15B

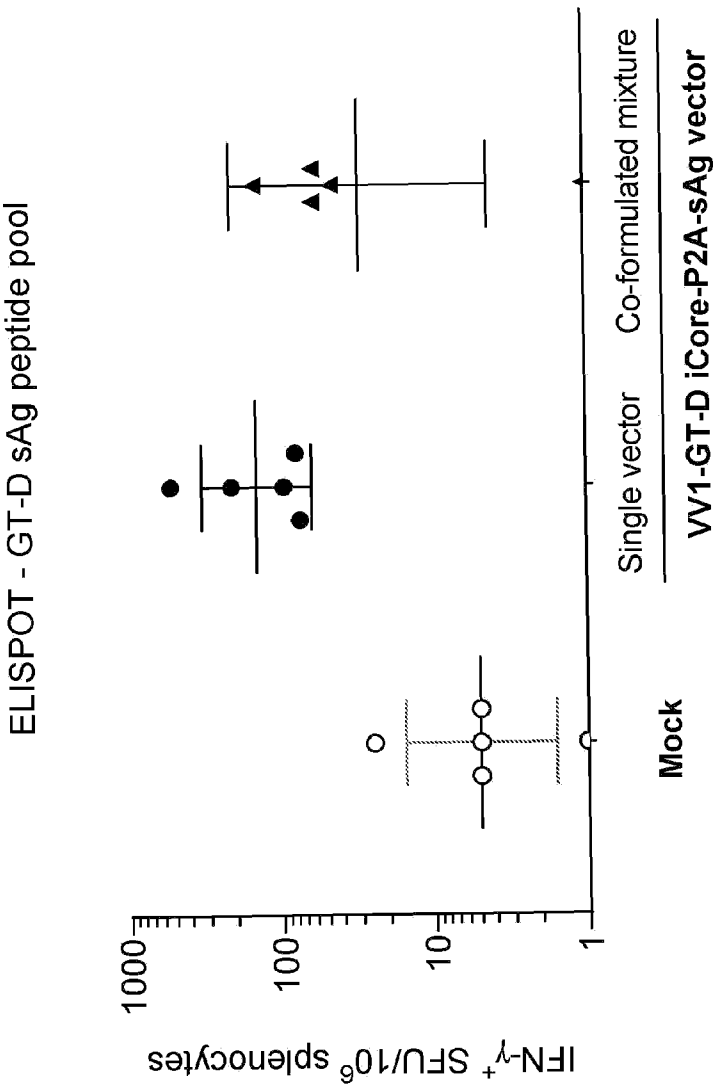

ELISPOT - GT-D sAg peptide pool
IFN-γ+ SFU/10^6 splenocytes
1000
100
10
1
Mock
Single vector
Co-formulated mixture
VV1-GT-D iCore-P2A-sAg vector

Fig. 16A

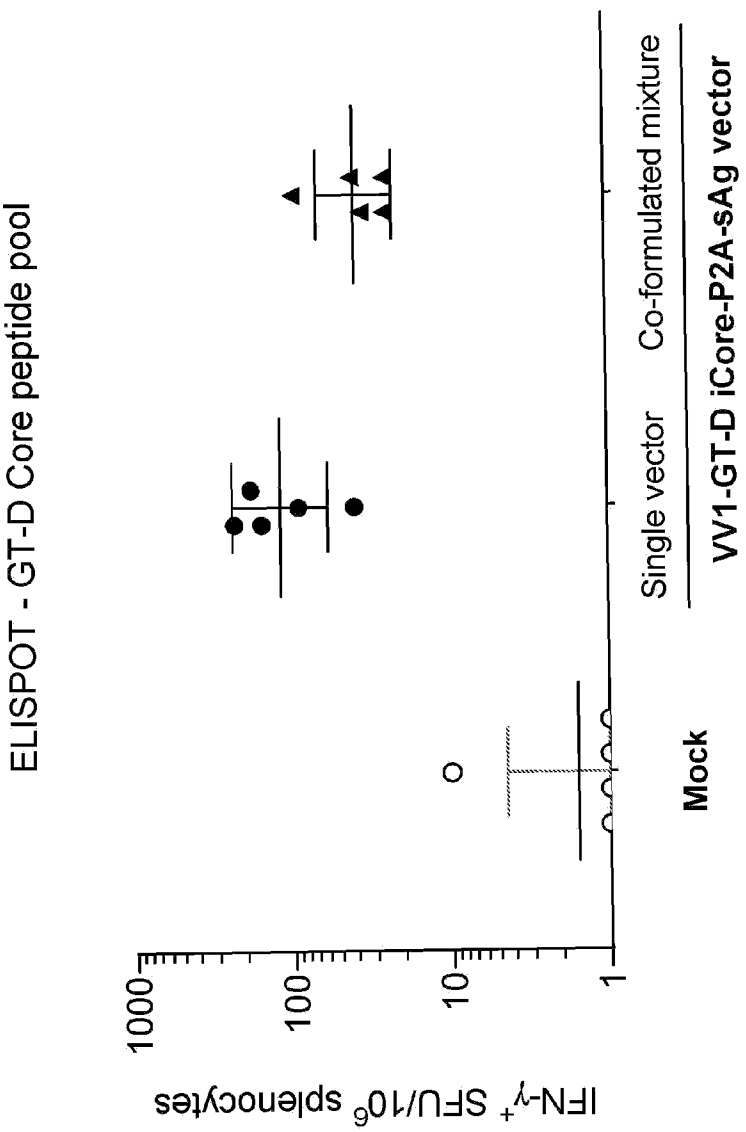

ELISPOT - GT-D Core peptide pool
IFN-γ+ SFU/10^6 splenocytes
1000
100
10
1
Mock
Single vector
Co-formulated mixture
VV1-GT-D iCore-P2A-sAg vector

Fig. 16B

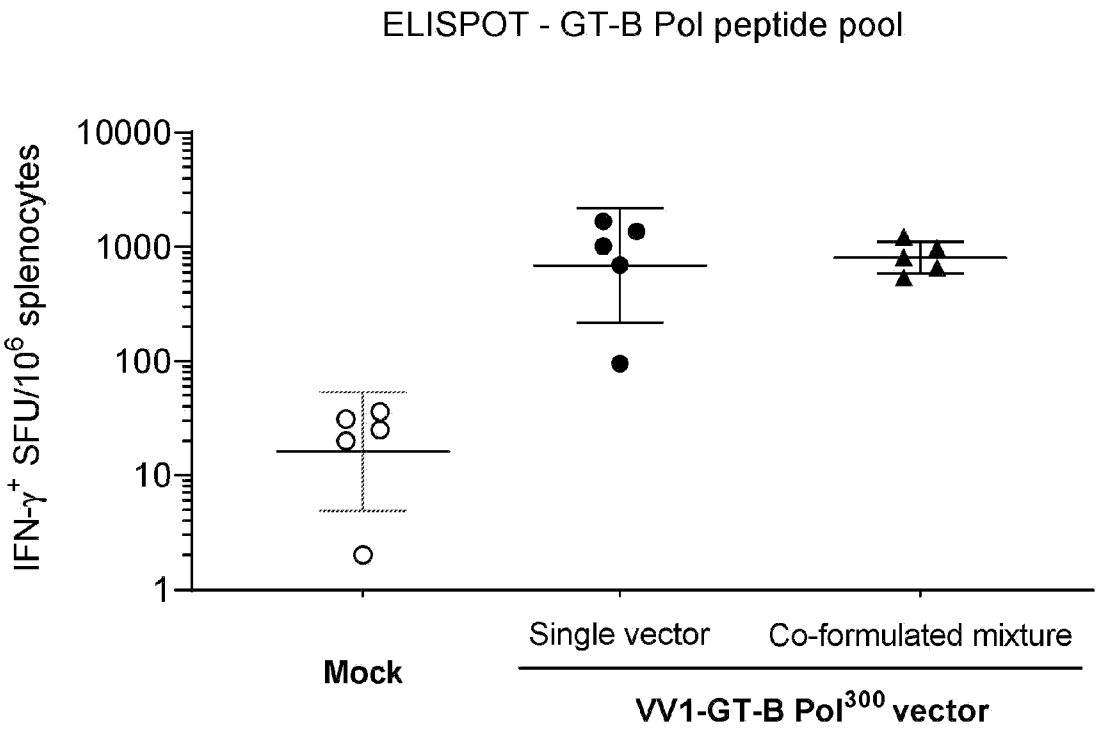


Fig. 16C

Fig. 17A

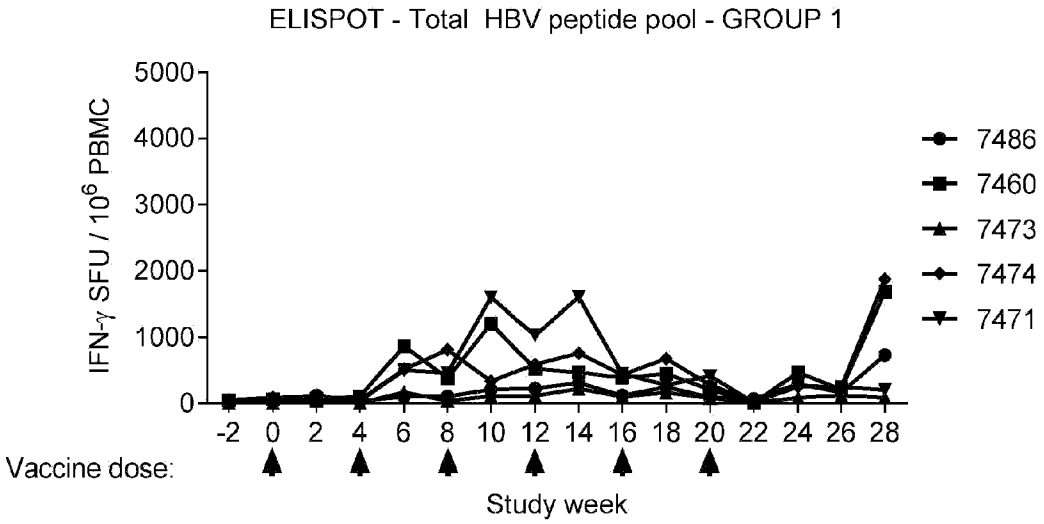
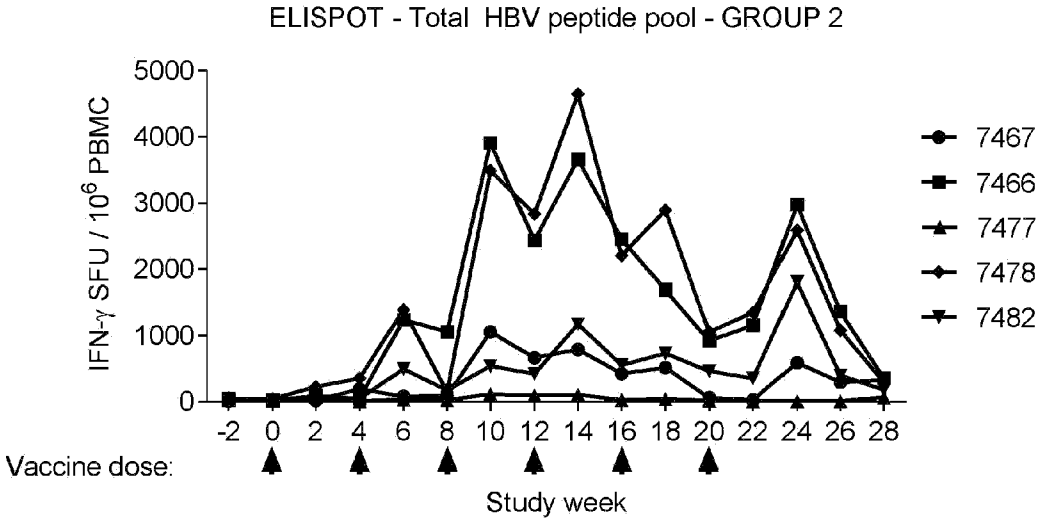


Fig. 17B



Figs. 17A-B

Fig. 17C

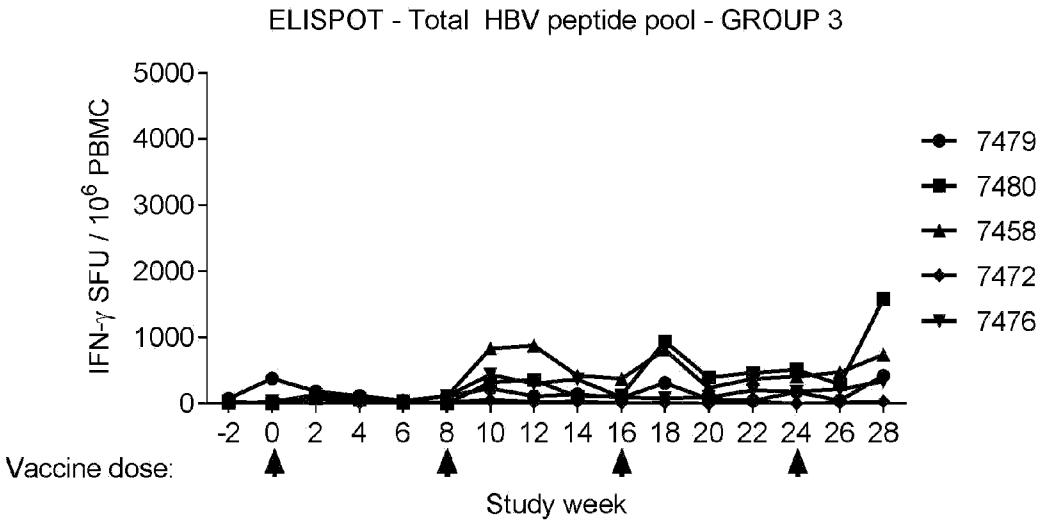
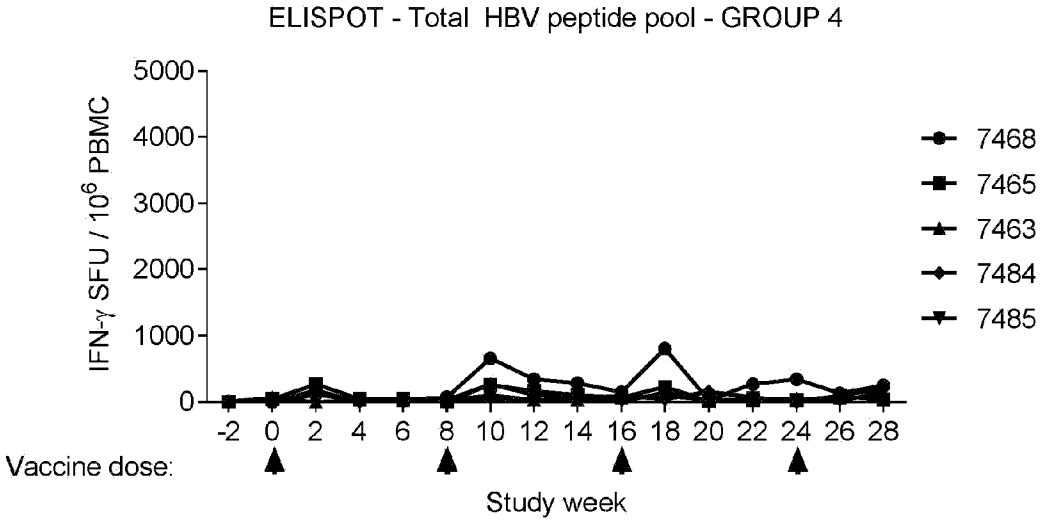


Fig. 17D



Figs. 17C-D

Fig. 17E

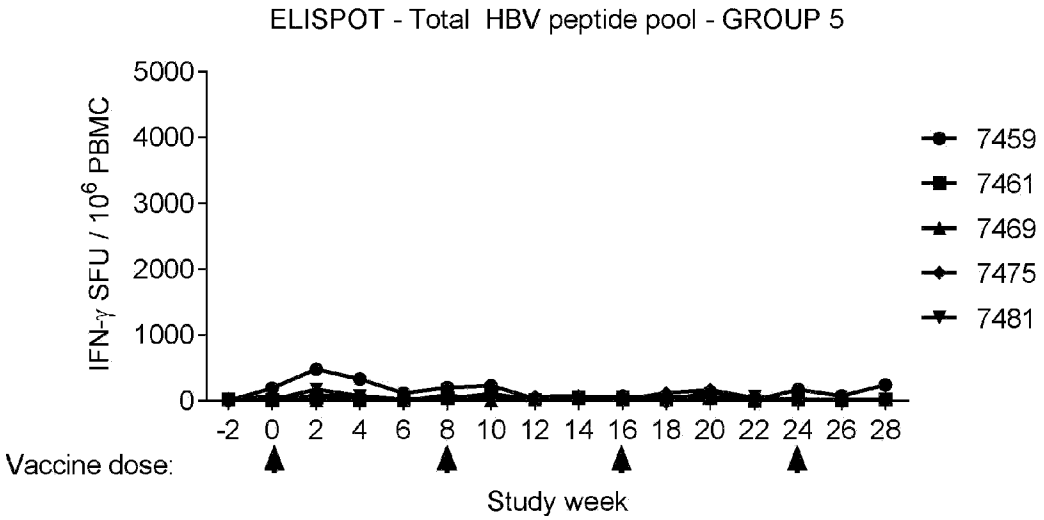
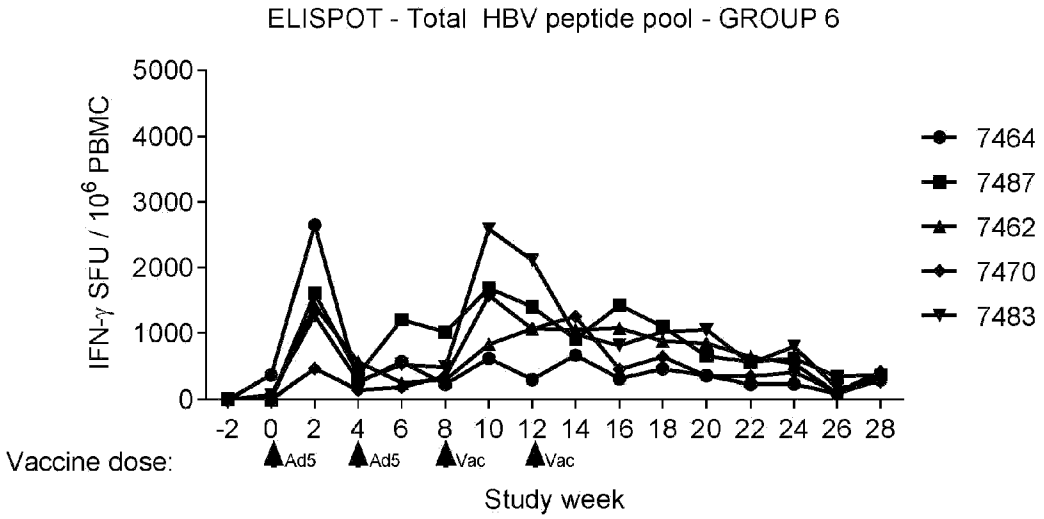


Fig. 17F



Figs. 17E-F

Fig. 18A

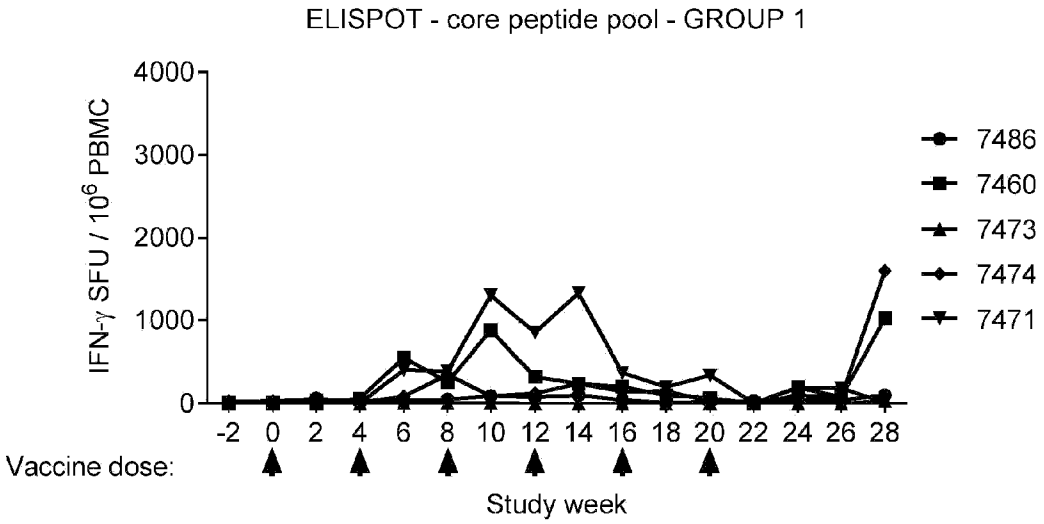
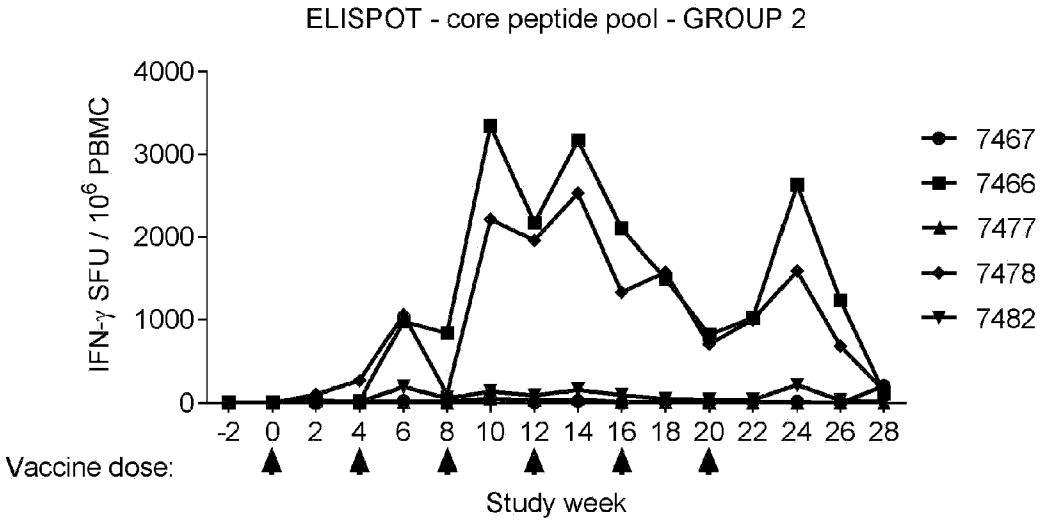


Fig. 18B



Figs. 18A-B

Fig. 18C

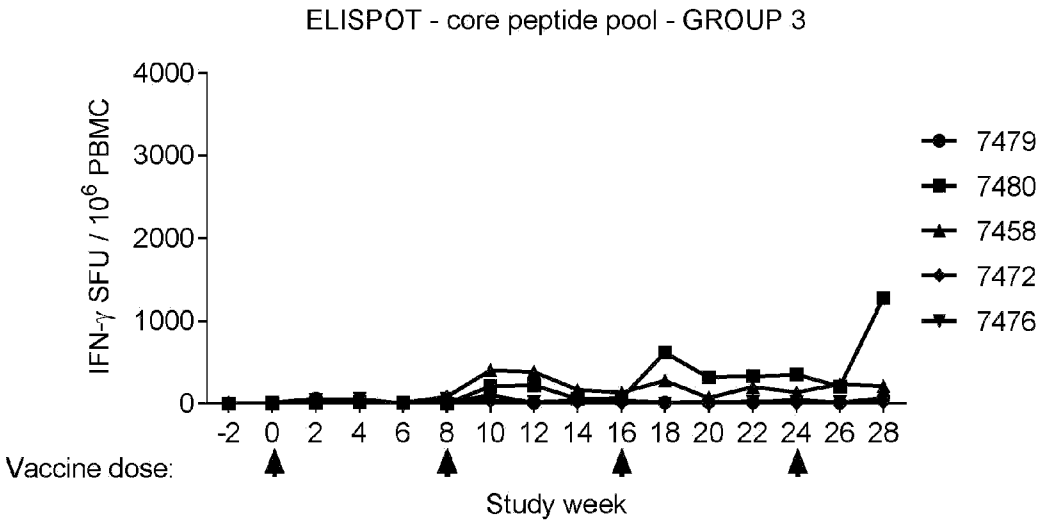
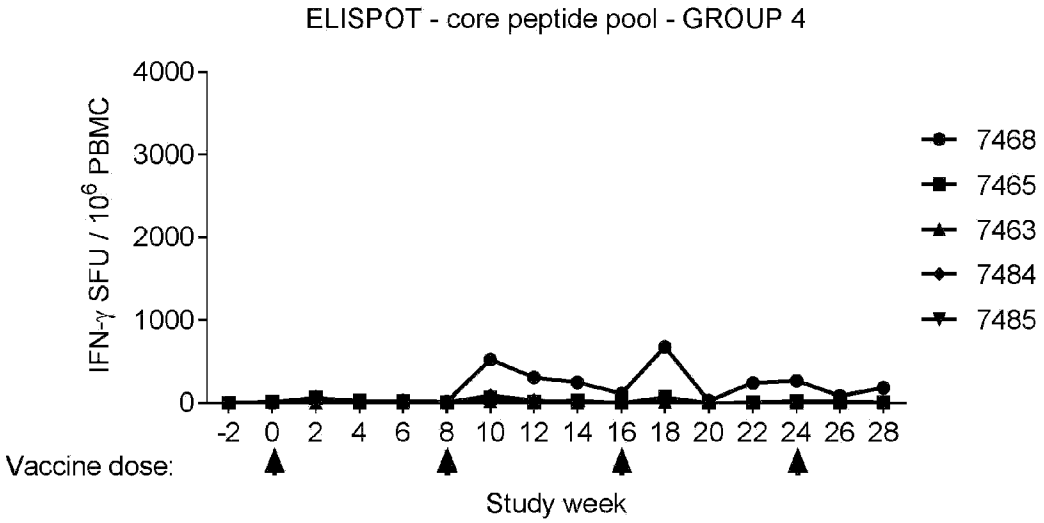


Fig. 18D



Figs. 18C-D

Fig. 18E

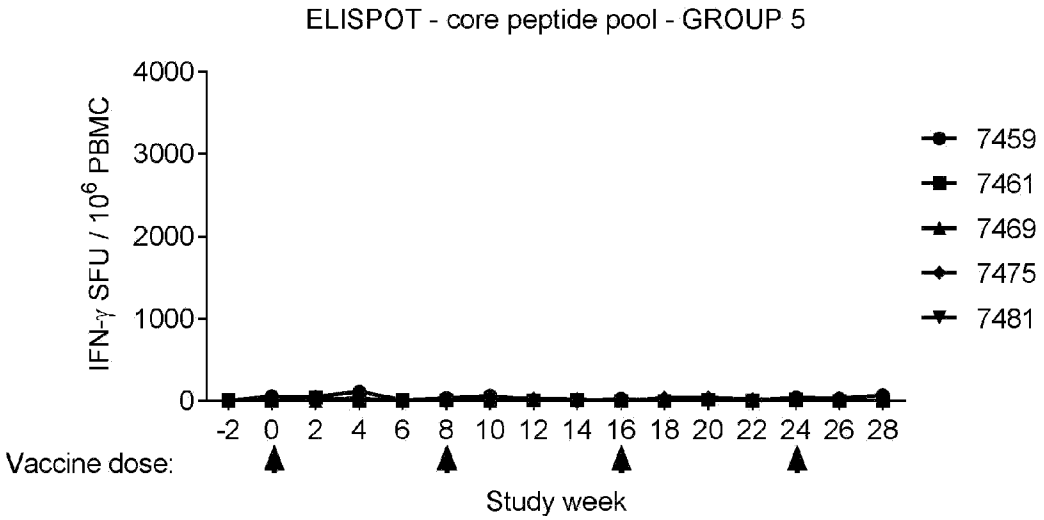
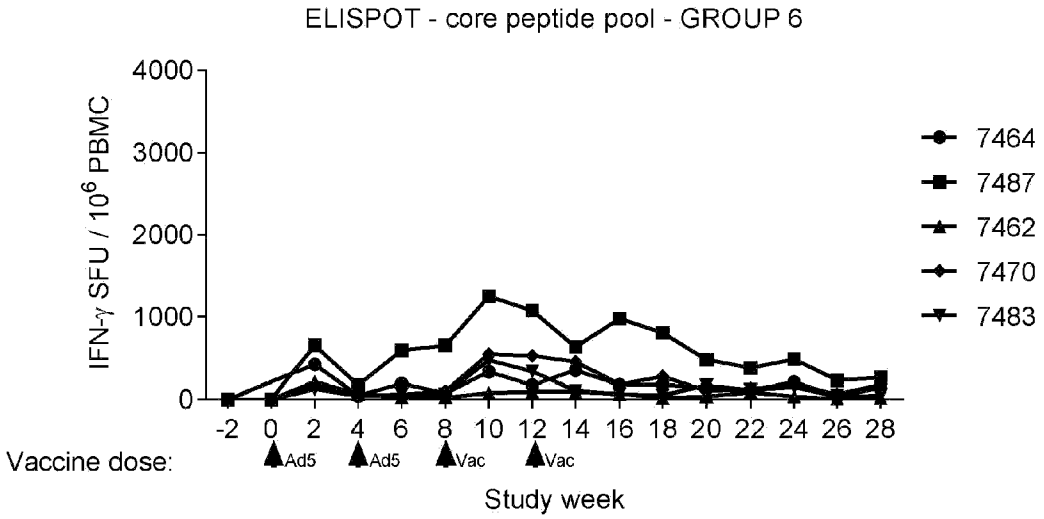


Fig. 18F



Figs. 18E-F

Fig. 19A

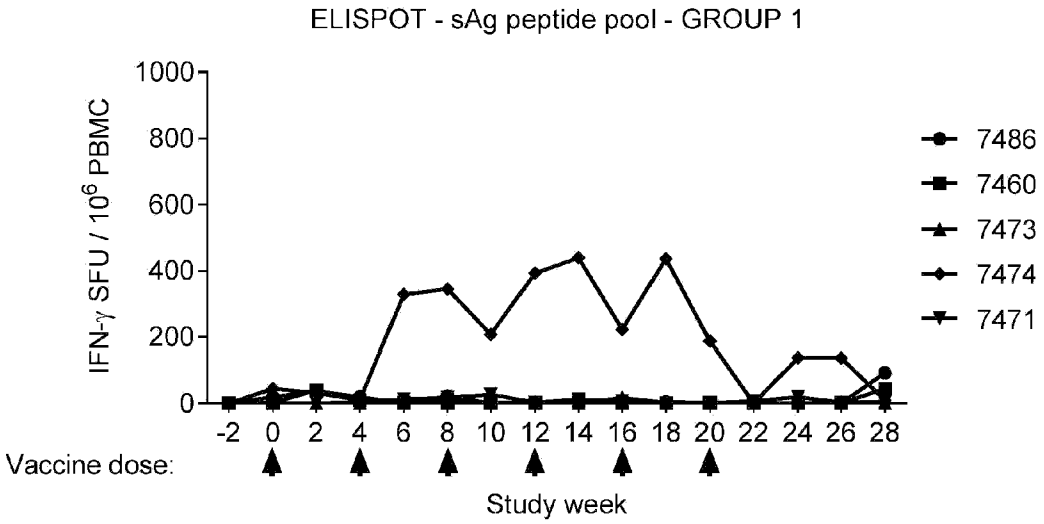
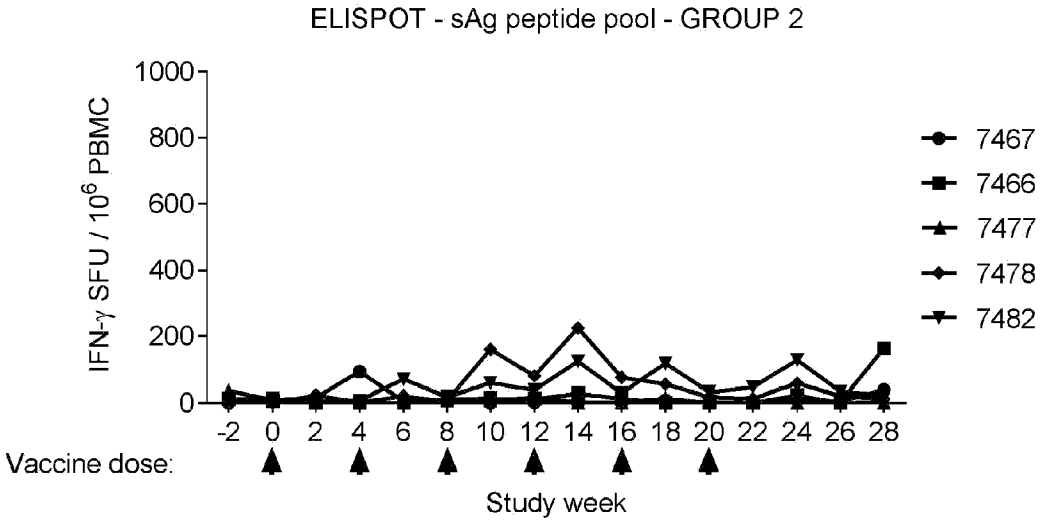


Fig. 19B



Figs. 19A-B

Fig. 19C

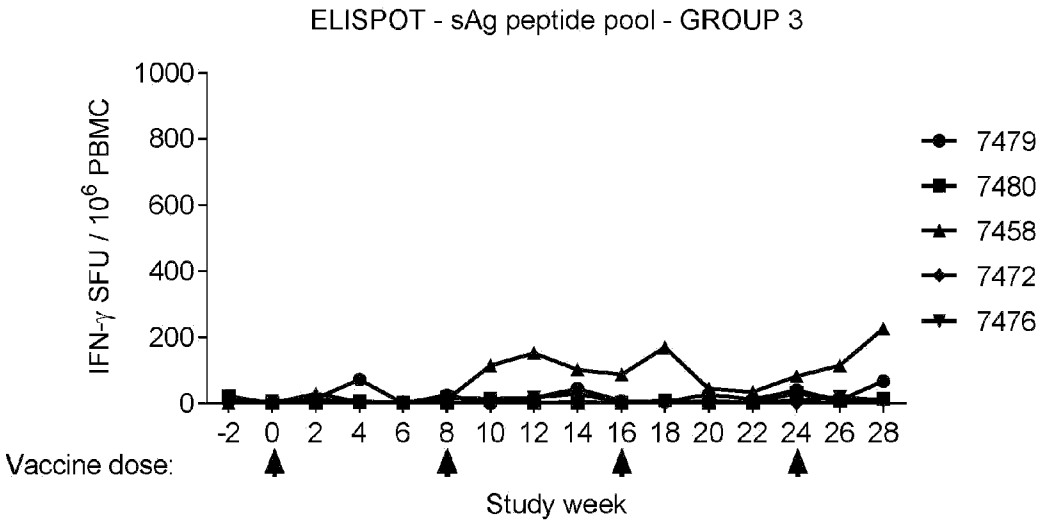
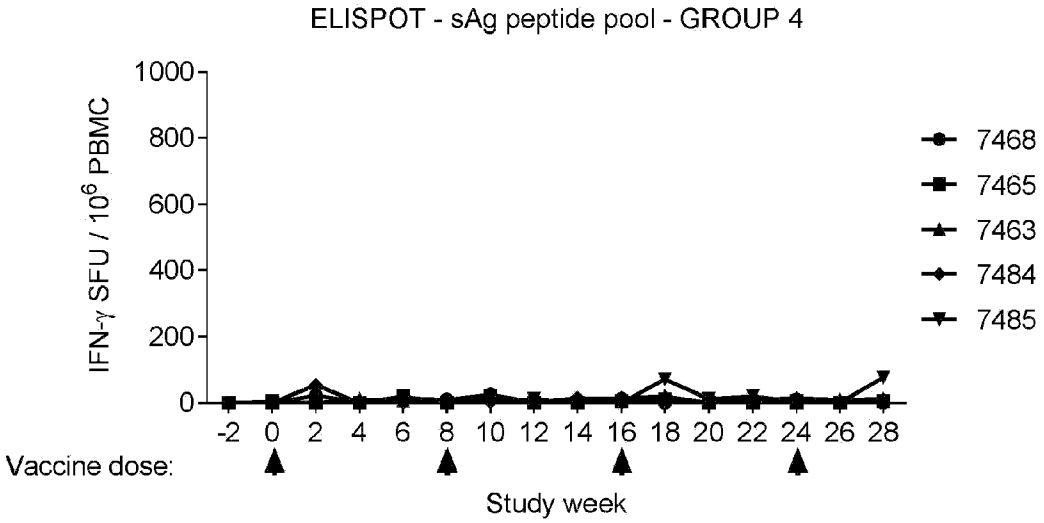


Fig. 19D



Figs. 19C-D

Fig. 19E

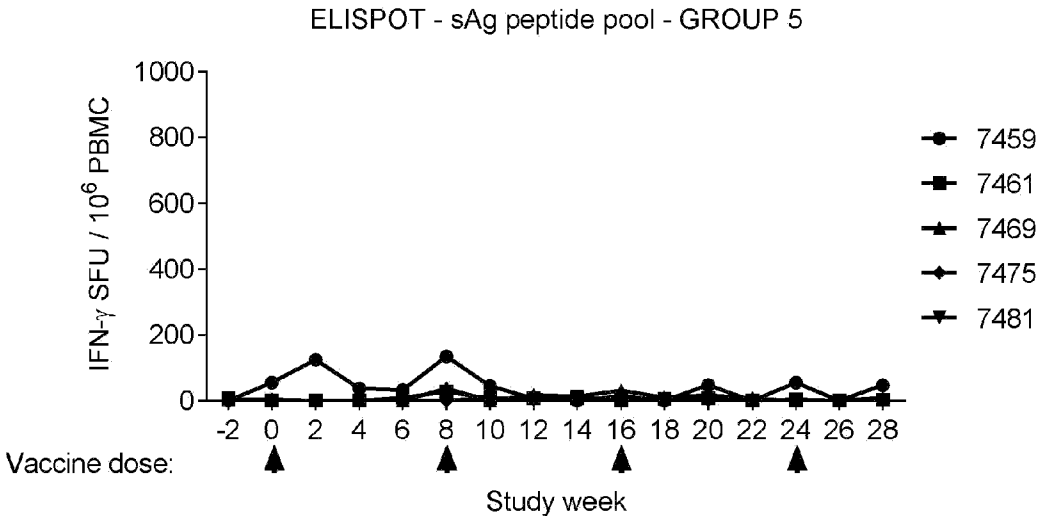
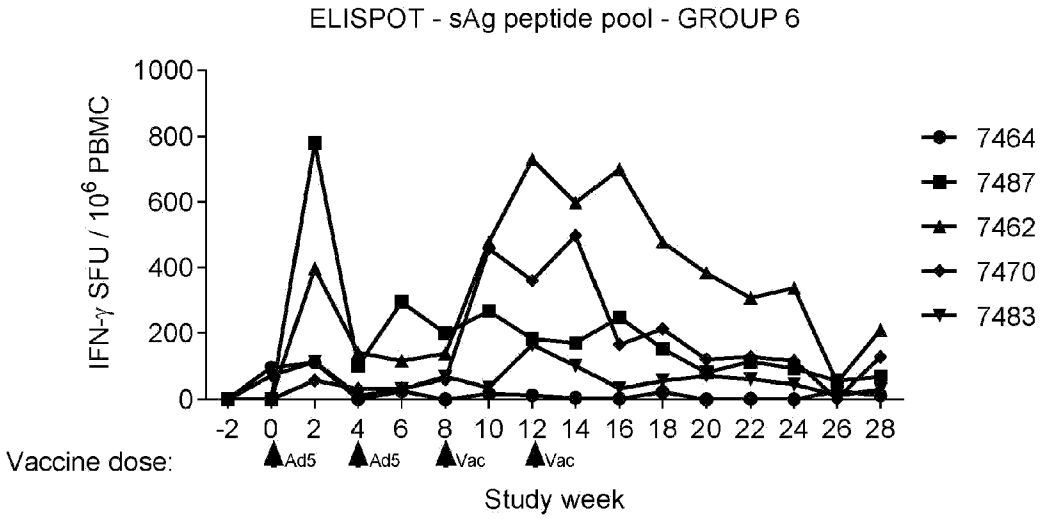


Fig. 19F



Figs. 19E-F

Fig. 20A

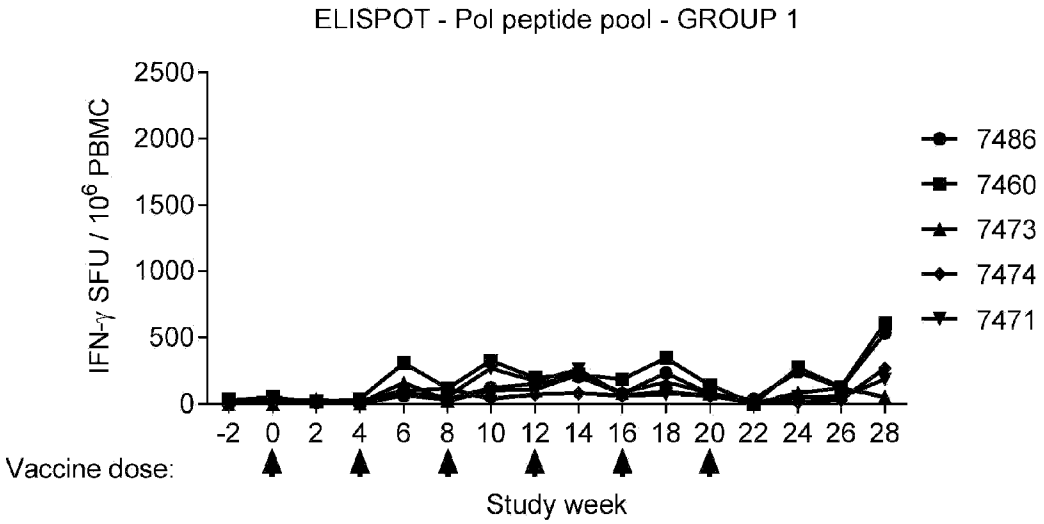
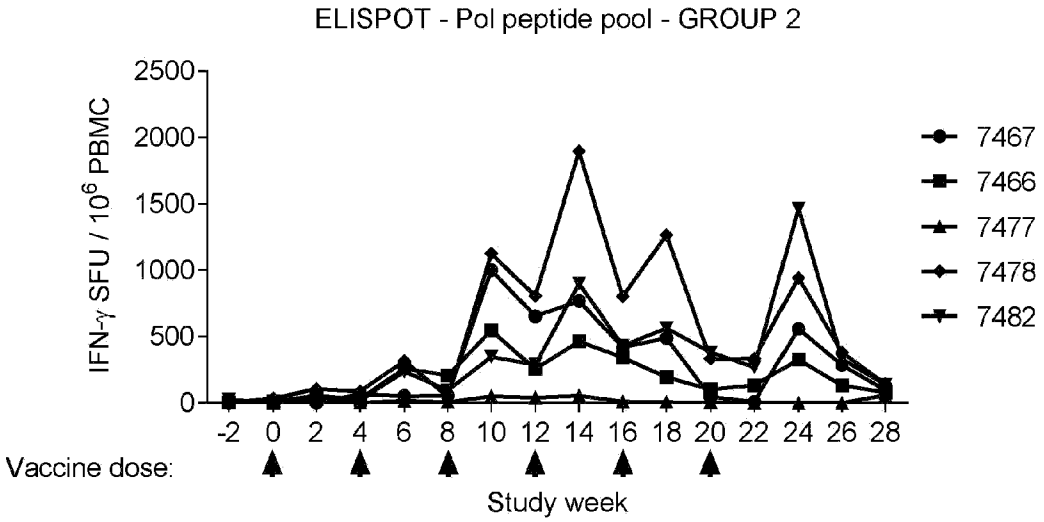


Fig. 20B



Figs. 20A-B

Fig. 20C

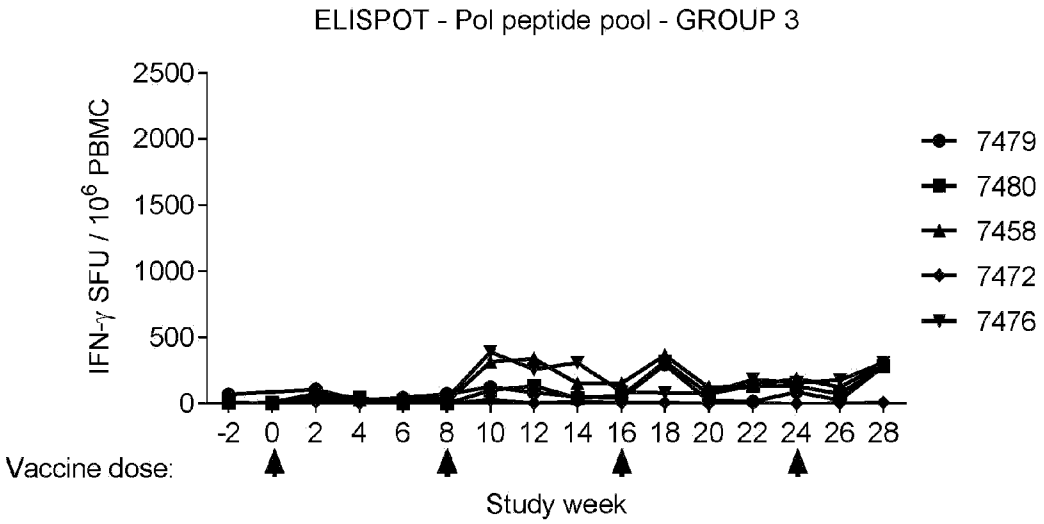
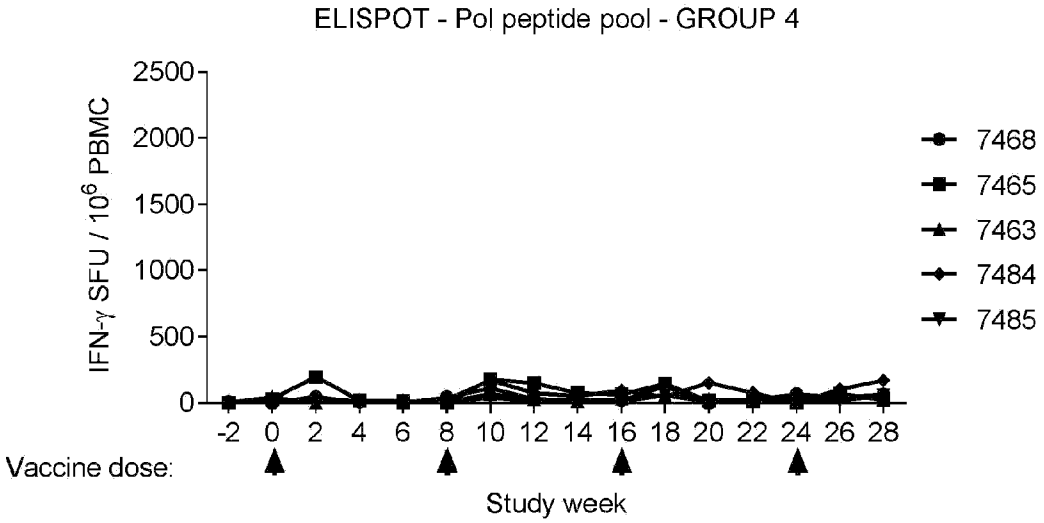


Fig. 20D



Figs. 20C-D

Fig. 20E

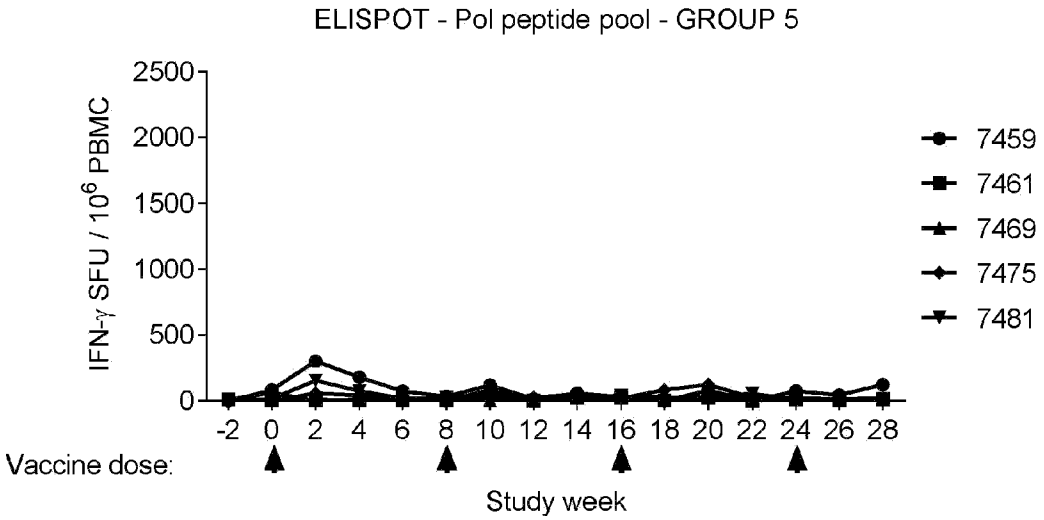
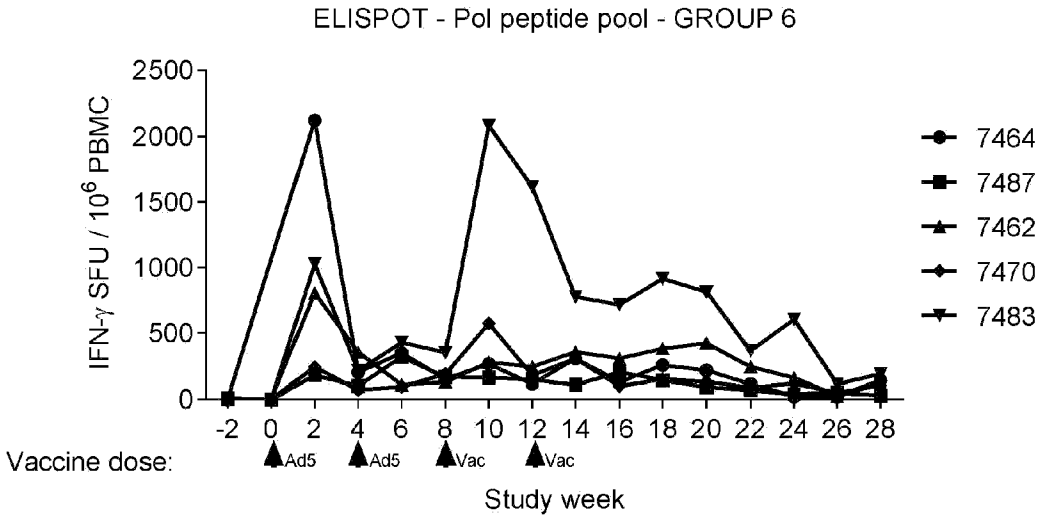


Fig. 20F



Figs. 20E-F

Fig. 21A

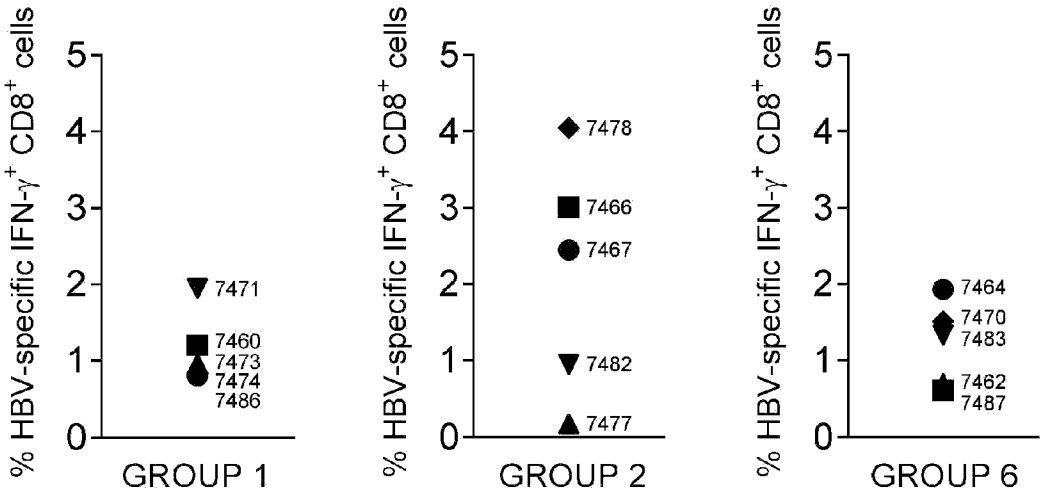
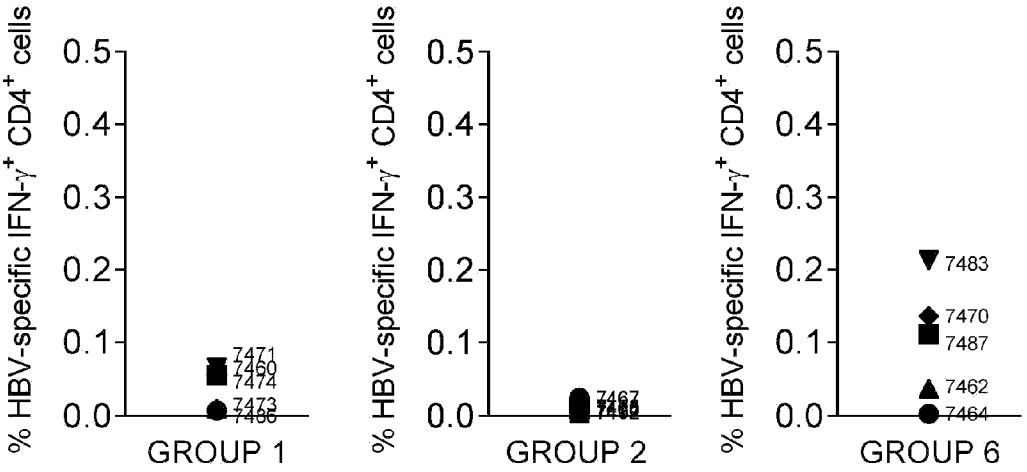


Fig. 21B



Figs. 21A-B

Fig. 22A

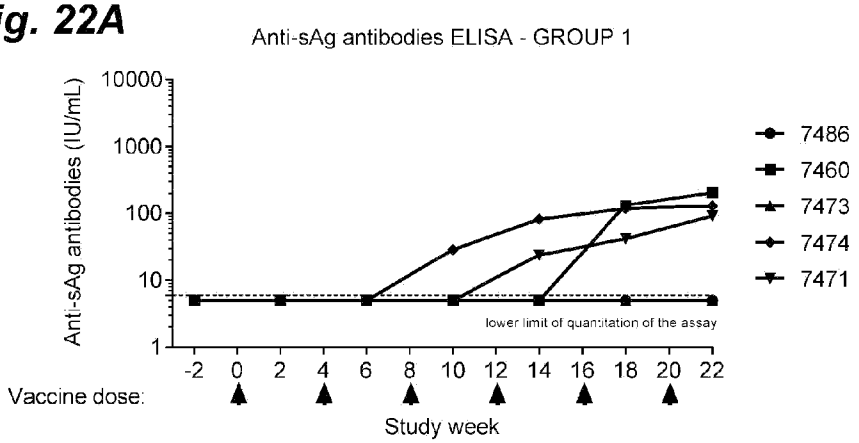


Fig. 22B

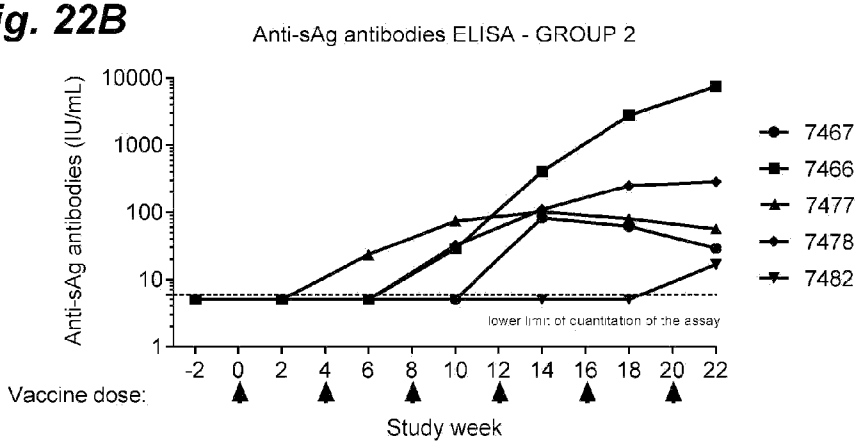
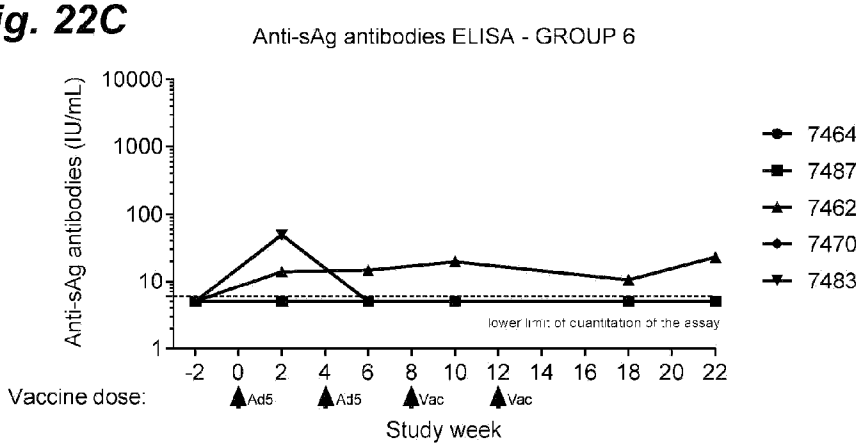


Fig. 22C



Figs. 22A-C

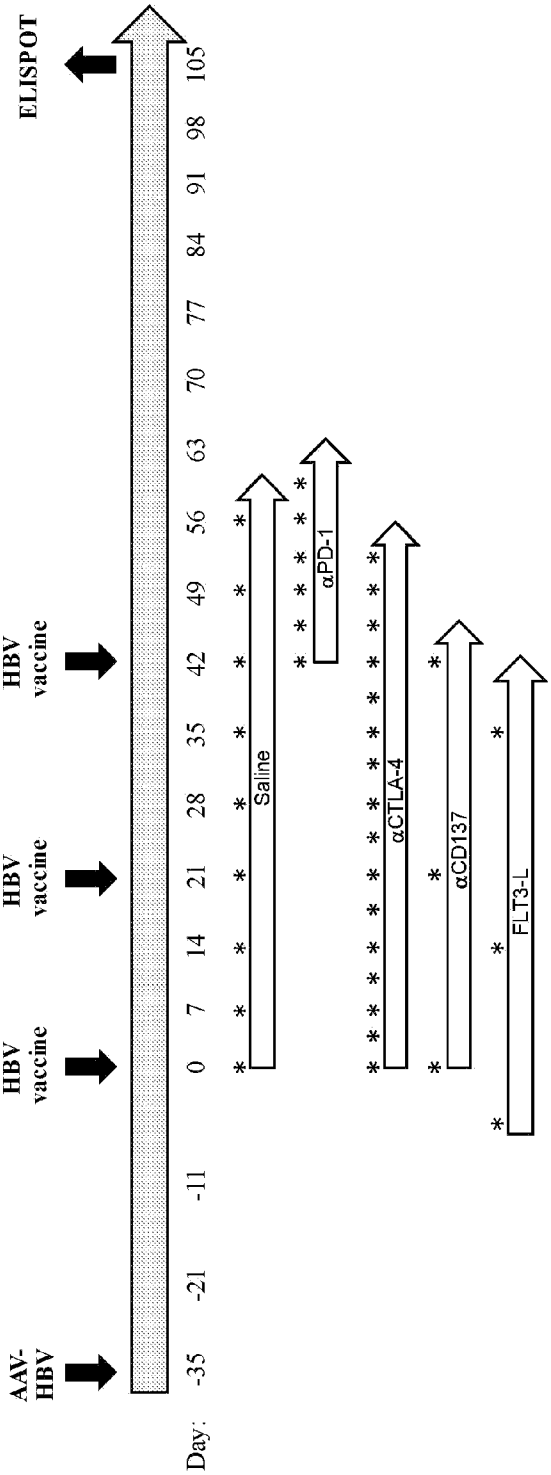

AAV-HBV
HBV vaccine
HBV vaccine
HBV vaccine
ELISPOT
Day:
-35
-21
-11
0
7
14
21
28
35
42
49
56
63
70
77
84
91
98
105
Saline
αPD-1
αCTLA-4
αCD137
FLT3-L

Fig. 23

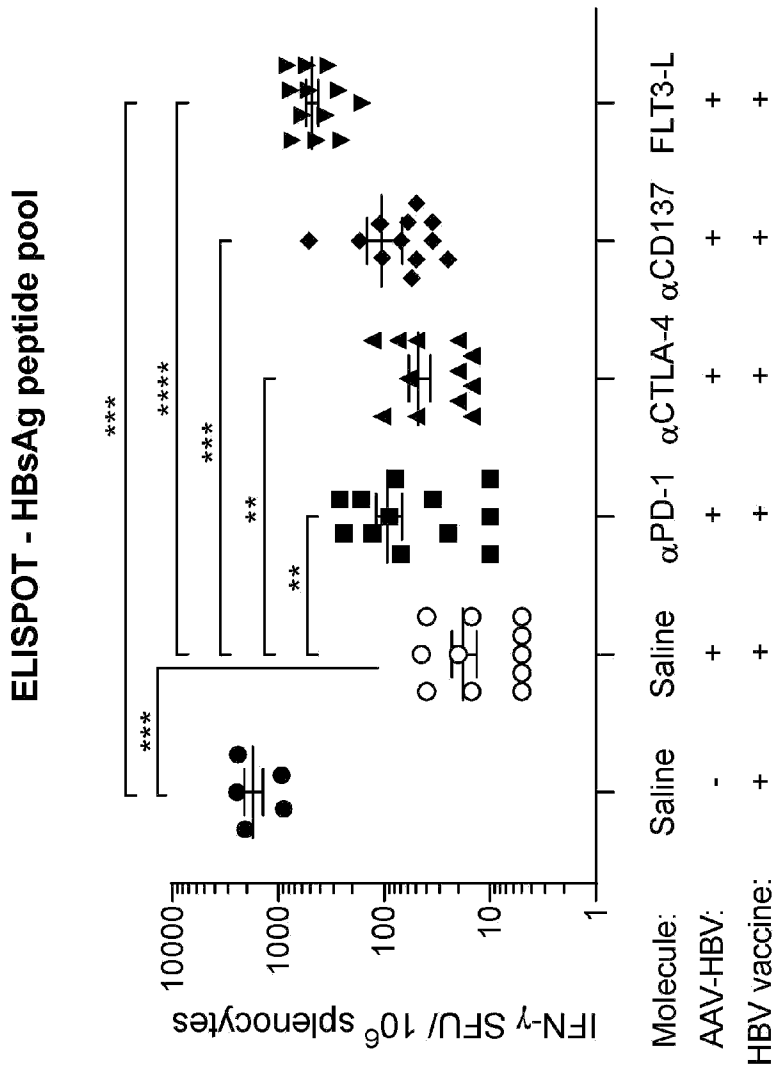

ELISPOT - HBsAg peptide pool
IFN-γ SFU/ 10^6 splenocytes
10000
1000
100
10
1
***
***
****
***
**
**
Molecule: Saline Saline αPD-1 αCTLA-4 αCD137 FLT3-L
AAV-HBV: - + + + + +
HBV vaccine: + + + + + +

Fig. 24A

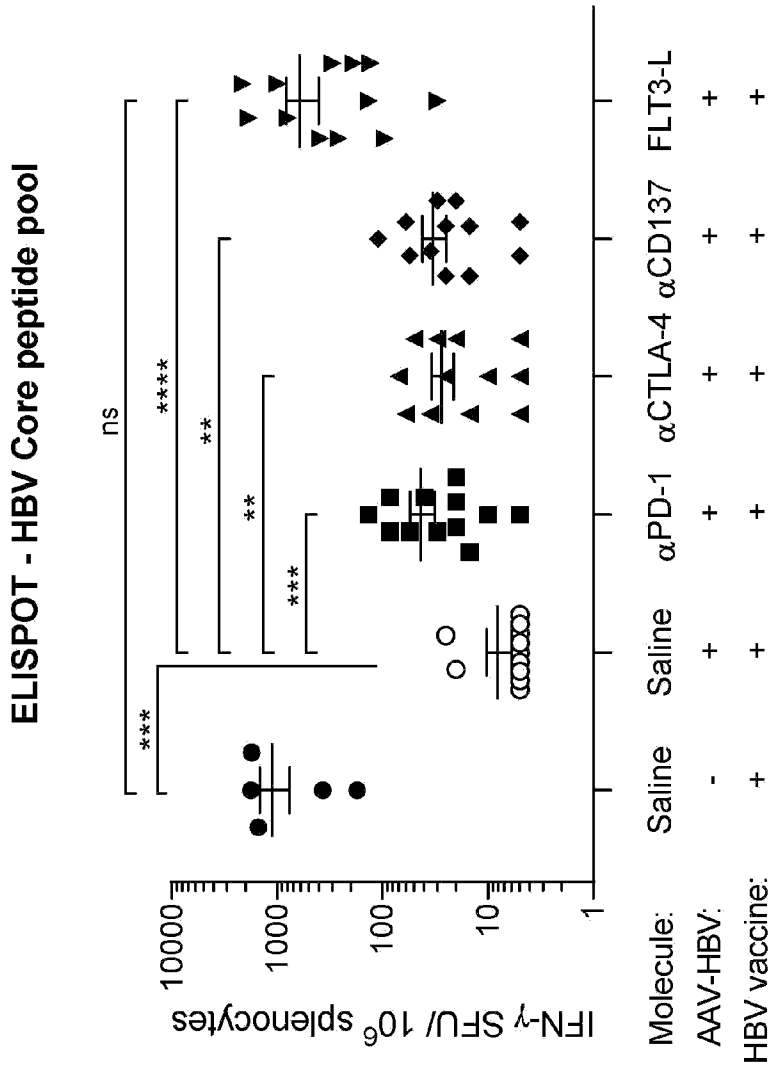

ELISPOT - HBV Core peptide pool
IFN-γ SFU/ 10^6 splenocytes
10000
1000
100
10
1
ns
***
****
**
**
***
Molecule: Saline Saline αPD-1 αCTLA-4 αCD137 FLT3-L
AAV-HBV: - + + + + +
HBV vaccine: + + + + + +

Fig. 24B

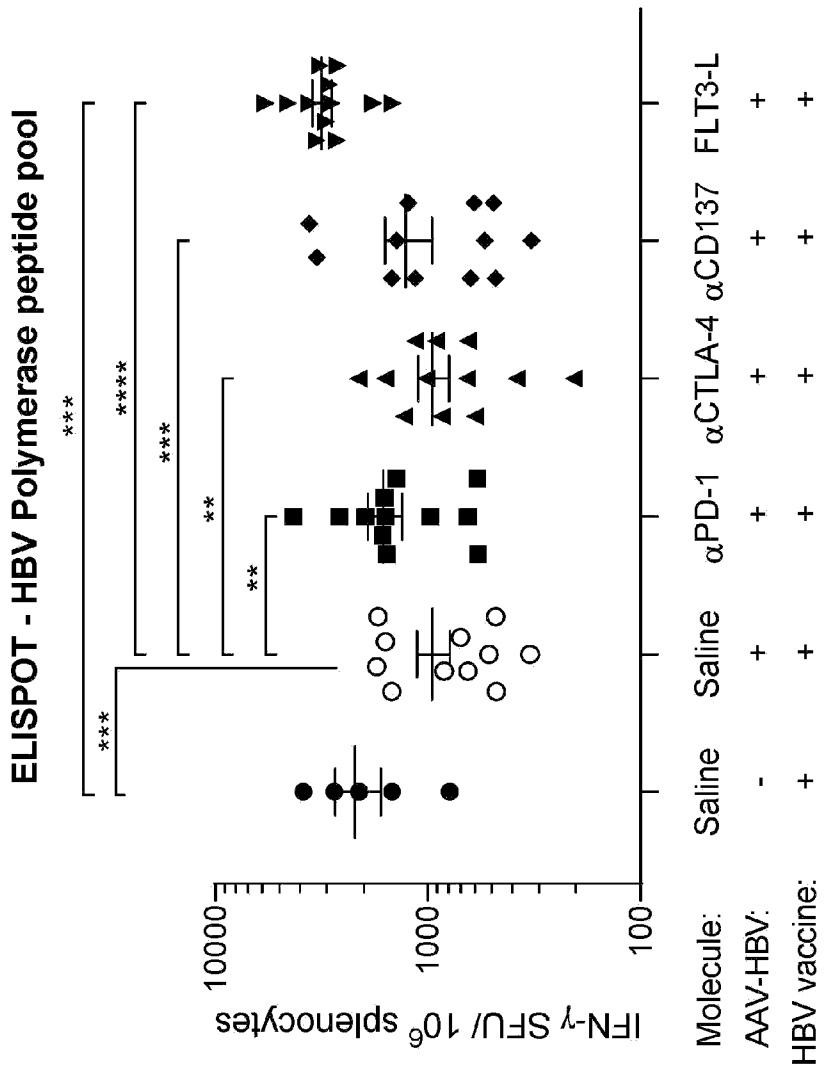

ELISPOT - HBV Polymerase peptide pool
IFN-γ SFU/ 10^6 splenocytes
10000
1000
100
***
***
****
***
**
**
Molecule: Saline Saline αPD-1 αCTLA-4 αCD137 FLT3-L
AAV-HBV: - + + + + +
HBV vaccine: + + + + + +

Fig. 24C

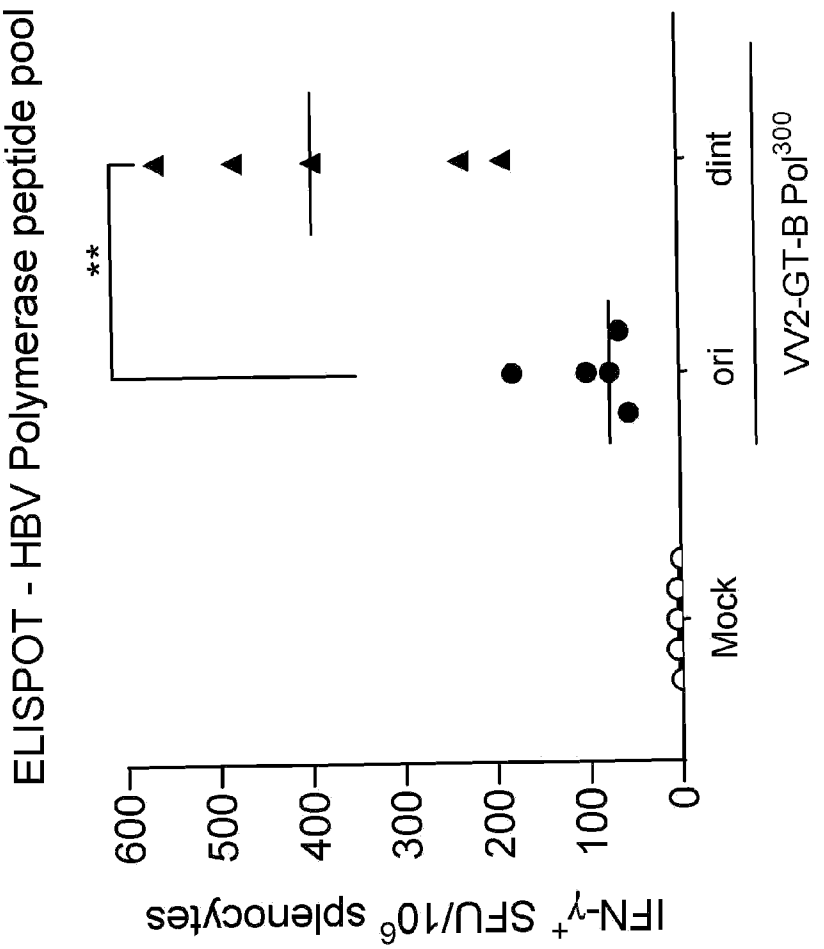

ELISPOT - HBV Polymerase peptide pool
IFN-γ+ SFU/10^6 splenocytes
600
500
400
300
200
100
0
**
Mock
ori
dint
VV2-GT-B Pol300

Fig. 25

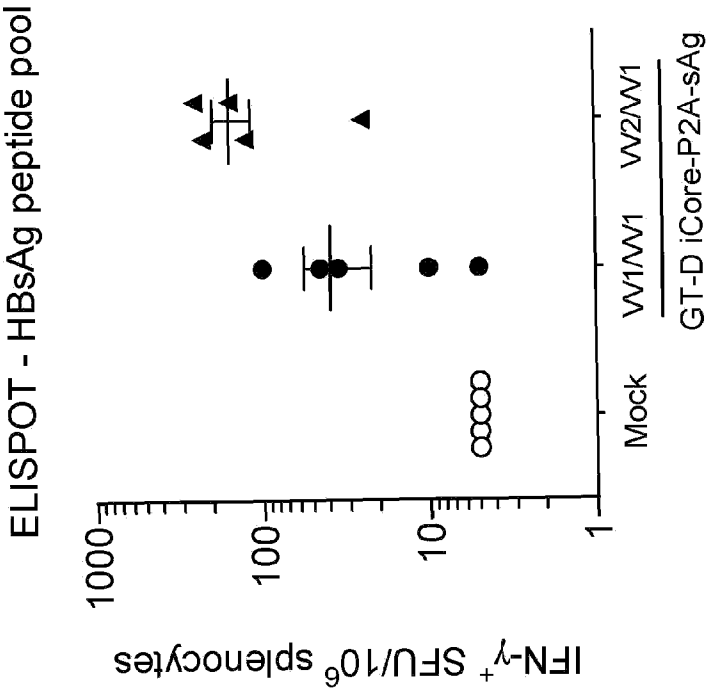

ELISPOT - HBsAg peptide pool
IFN-γ+ SFU/10^6 splenocytes
1000
100
10
1
Mock
W1/W1
W2/W1
GT-D iCore-P2A-sAg

Fig. 26A

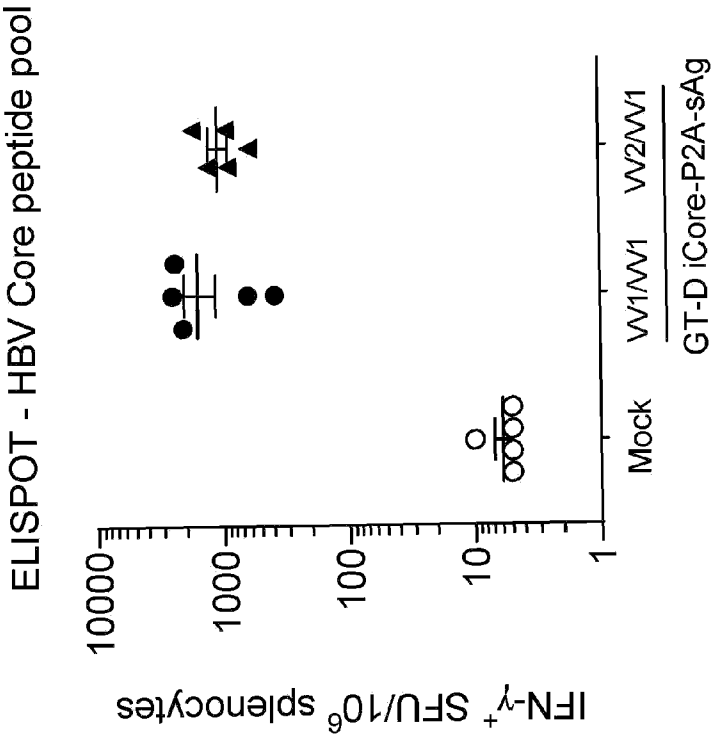

ELISPOT - HBV Core peptide pool
IFN-γ+ SFU/10^6 splenocytes
10000
1000
100
10
1
Mock
VV1/VV1
VV2/VV1
GT-D iCore-P2A-sAg

Fig. 26B

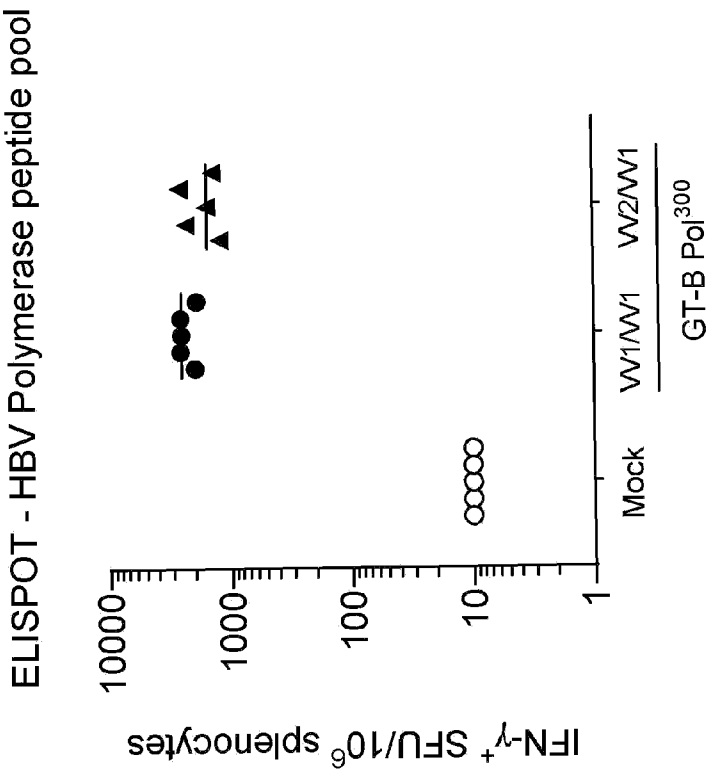

ELISPOT - HBV Polymerase peptide pool
IFN-γ+ SFU/10^6 splenocytes
10000
1000
100
10
1
Mock
VV1/VV1
VV2/VV1
GT-B Pol300

Fig. 26C

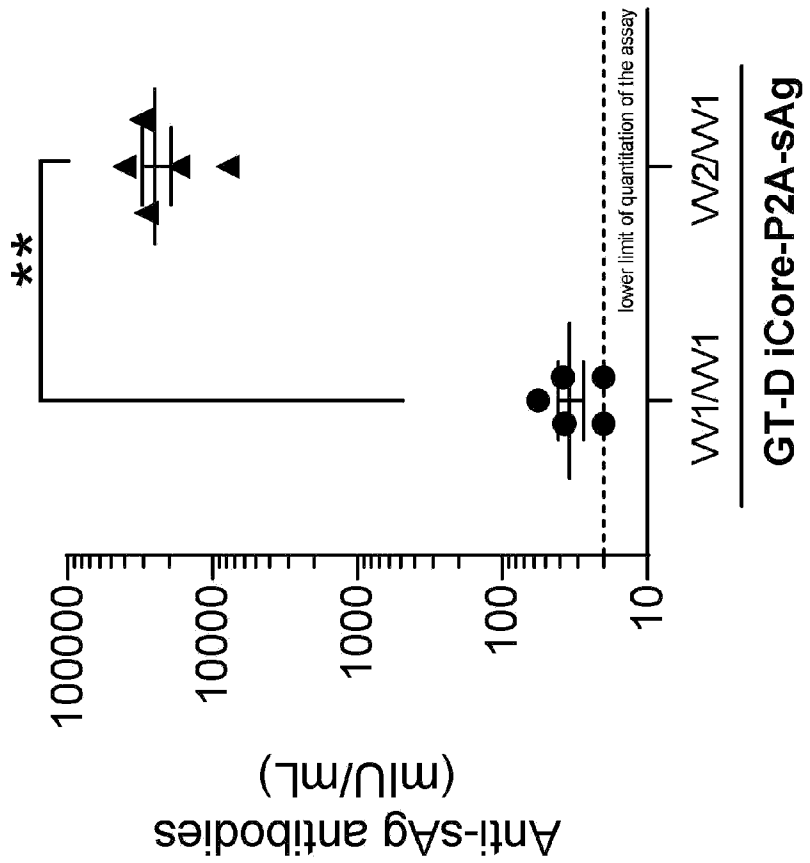

Anti-sAg antibodies
(mIU/mL)
100000
10000
1000
100
10
lower limit of quantitation of the assay
**
W1/W1
W2/W1
GT-D iCore-P2A-sAg

Fig. 27

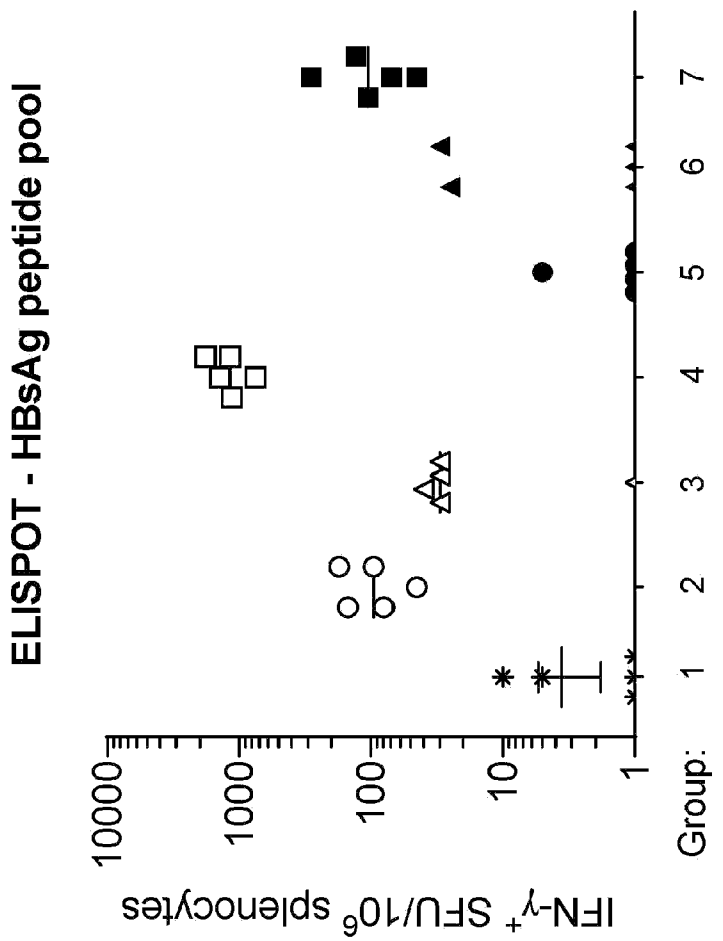

ELISPOT - HBsAg peptide pool
IFN-γ+ SFU/10^6 splenocytes
10000
1000
100
10
1
Group:
1
2
3
4
5
6
7

Fig. 28A

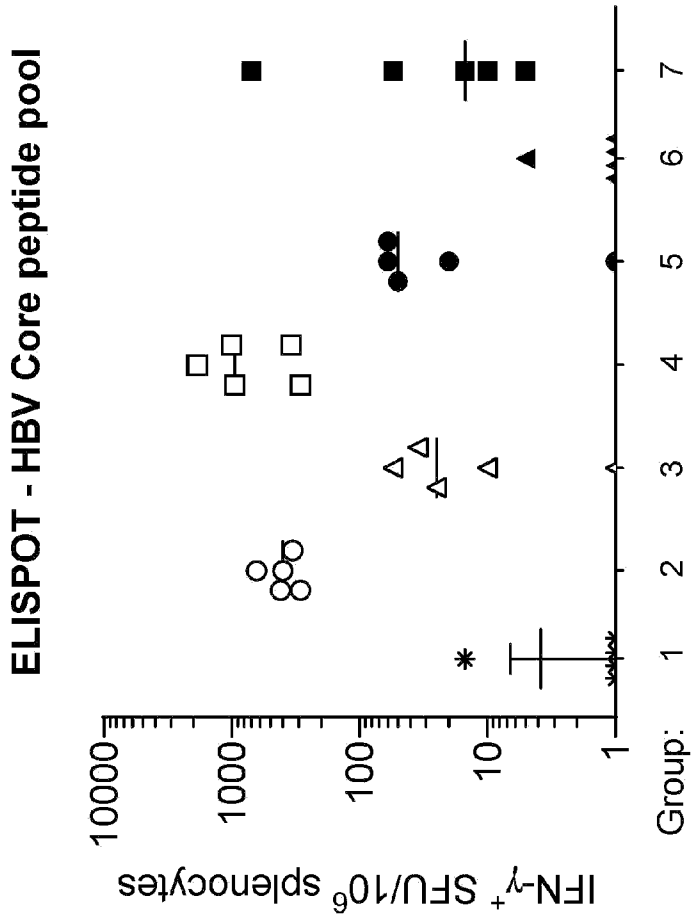

ELISPOT - HBV Core peptide pool
IFN-γ+ SFU/10^6 splenocytes
10000
1000
100
10
1
Group:
1
2
3
4
5
6
7

Fig. 28B

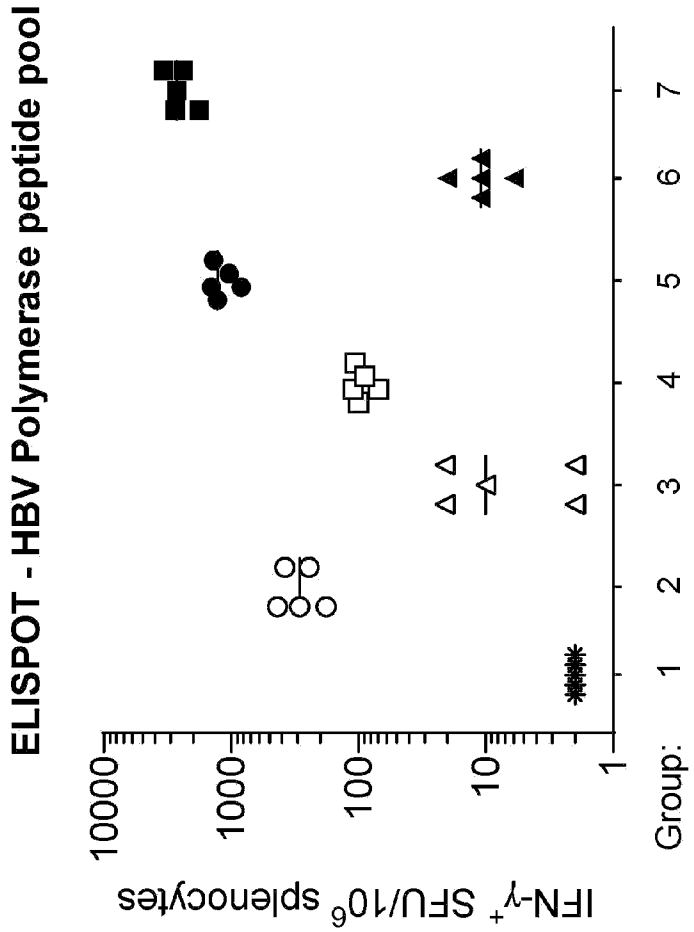

ELISPOT - HBV Polymerase peptide pool
IFN-γ+ SFU/10^6 splenocytes
10000
1000
100
10
1
Group:
1
2
3
4
5
6
7

Fig. 28C

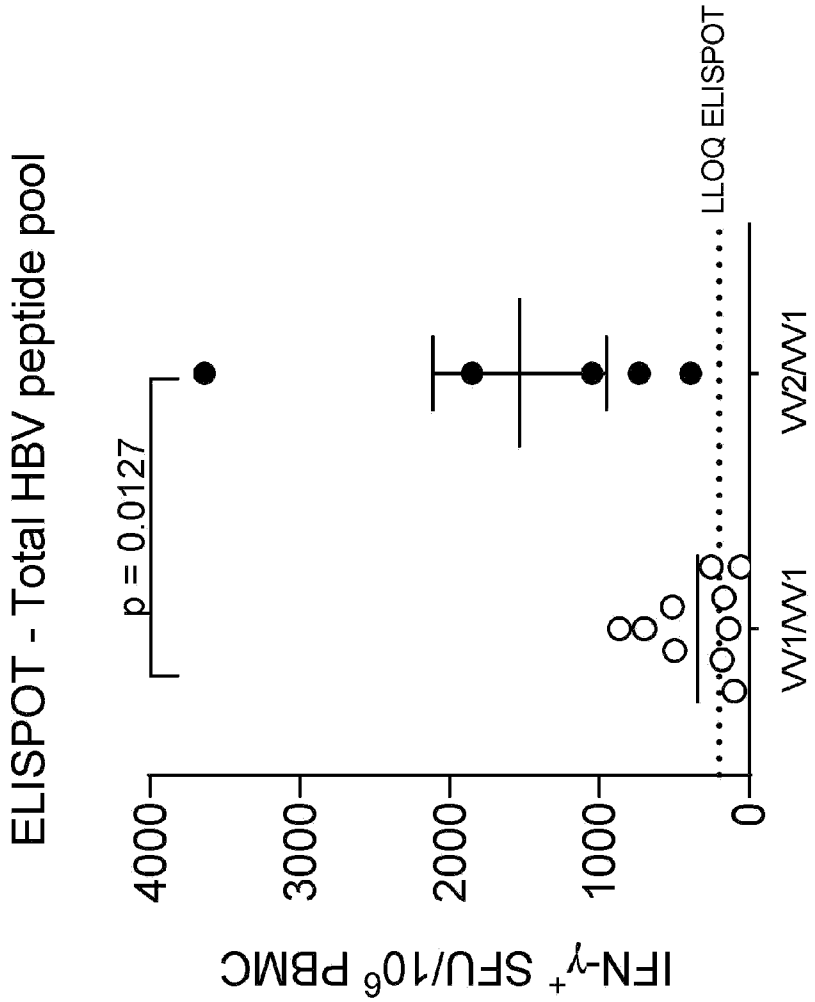

ELISPOT - Total HBV peptide pool
IFN-γ+ SFU/10^6 PBMC
4000
3000
2000
1000
0
p = 0.0127
LLOQ ELISPOT
W1/W1
W2/W1

Fig. 29

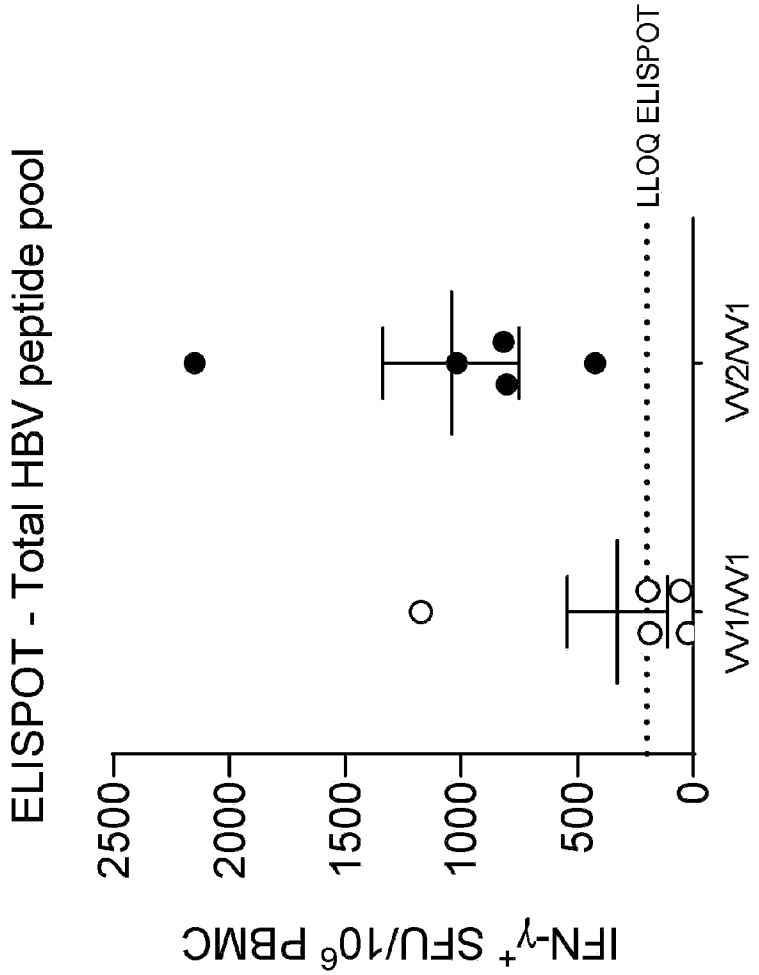

ELISPOT - Total HBV peptide pool
IFN-γ+ SFU/10^6 PBMC
2500
2000
1500
1000
500
0
LLOQ ELISPOT
W1/W1
W2/W1

Fig. 30

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.