Therefore, an aim of the present invention is to provide an improved vaccine for HBV infection.

In a first aspect, the present disclosure refers to a multi-HBV immunogen viral vector vaccine comprising:

a viral vector comprising an immunogen expression cassette, wherein the expression of a protein encoded by the expression cassette is arranged to be driven by a promoter, wherein the immunogen expression cassette encodes:

a) HBV Core;

b) a modified HBV polymerase (P_{mut}), wherein the modification is a mutation to wild-type HBV polymerase to substantially remove polymerase function;

c) HBV surface antigen (HbsAg); and

d) an intergenic sequence that is arranged to cause expression of at least the HBV surface antigen (HbsAg) as a separate protein from the HBV core and the modified HBV polymerase (P_{mut}),

wherein the intergenic sequence is downstream (3') of the sequences encoding the HBV core and the modified HBV polymerase (P_{mut}) and upstream (5') of the sequence encoding the HBV surface antigen (HbsAg).

The intergenic sequence may comprise a cleavage domain, an IRES (Internal Ribosomal Entry Site), a splicing signal, or a secondary promoter. In one example, the intergenic sequence comprises a cleavage domain comprising a sequence arranged to cause ribosome skipping. In another example, the intergenic sequence comprises a secondary promoter to promote expression of at least the surface antigen (HbsAg).

Induction of a strong, multi-antigen specific T cell response against different HBV proteins is thought to play a major role in viral clearance of a resolving chronic HBV infection. For induction of a multi-antigen T-cell response, it can be important to have maximal number of T-cell epitopes, which requires encoding full-length HBV proteins within a T-cell inducing HBV vaccine. Some of the current HBV vaccines under development do not encode full-length HBV antigens. The present disclosure vaccine can encode 3 full-length proteins of HBV (namely the Core, optionally with Pre-Core region, Polymerase and Surface proteins). In addition, the vaccine design utilizes an at least two-protein expression, for example using an F2A peptide cleavage strategy to

encode a separate surface protein, which in addition to the induction of T-cell response could also induce an antibody response that could possibly have a role in potentiating the therapeutic effect of T-cell vaccine, with possible help in clearance of the virus within a chronically infected individual. Inducing antibodies based on a mammalian system (compared to other systems, for e.g. Yeast) has a selective advantage of providing the mammalian-type glycosylation that could induce antibodies appropriate for the natural host.

The disclosure advantageously provides a single vaccine encoding full-length multiple-HBV antigens that could induce broad T-cell response and in-addition could also induce antibodies to the surface protein. The new HBV immunogen at least encompasses three full length HBV-antigens (namely the Core, Polymerase and Surface) and encoded them into a viral vector, such as the Chimpanzee adeno and MVA viral vectors. In addition, the HBV-polymerase protein in the immunogen is provided with mutations that abolish its function, which avoids its participation in HBV genome replication and improves safety for the vaccine.

By using a peptide cleavage strategy, for example by using a Furin-2A cleavage sequence, or a secondary promoter, the transgene cassette can generate at least two proteins, a fused core and polymerase protein and a separate surface protein, both of which generates a T-cell immune response. The encoded surface protein, in addition to the generation of T-cell immune response can also generate an antibody response.

Immunogen expression cassette

The skilled person will recognize that the cleavage domain may not lead to cleavage on all occasions, for example F2A based ribosomal skipping events may occur in a small proportion of expressions, such that a small proportion of proteins made will be a fusion of all the HBV proteins/antigens that would otherwise be separated by the cleavage. Therefore, in one example, the immunogen expression cassette may further encode a fusion protein comprising at least the HBV Core, modified HBV polymerase (Pmut) and HBV surface antigen.

In one example, the immunogen expression cassette further encodes HBV Pre-Core (PreC). In another example, the immunogen expression cassette further

encodes HBV PreS1, and/or a truncated form thereof. In another example, the immunogen expression cassette further encodes HBV PreS2.

In one example, the immunogen expression cassette further encodes HBV Pre-Core (PreC) and HBV PreS1, or a truncated form of HBV PreS1. In another example, the immunogen expression cassette further encodes HBV Pre-Core (PreC) and HBV PreS2. In one example, the immunogen expression cassette further encodes HBV Pre-Core (PreC) and HBV PreS1, and a truncated form of PreS1. In one example, the immunogen expression cassette further encodes HBV Pre-Core (PreC), HBV PreS1, and/or a truncated form thereof, and HBV PreS2.

In an example wherein the HBV Pre-core and Core are encoded, the immunogen expression cassette may be capable of expressing the HBV e-Antigen. The skilled person in the art will recognise that HBV e-Antigen comprises or consists of the 10 C-terminal amino acids of Pre-Core and the 149 N-terminal amino acids of the Core. For the expression of HBV e-Antigen, the Pre-Core and Core are expressed together and this whole expressed protein undergoes N-terminal and C-terminal cleavage.

In one example, the HBV Core and modified polymerase (Pmut) are arranged to be expressed as a fusion protein. In an example wherein the immunogen expression cassette encodes HBV Pre-core, the HBV Pre-core, HBV Core and modified polymerase (Pmut) are arranged to be expressed as a fusion protein.

In one example the immunogen expression cassette encodes at least two proteins, comprising a first fusion protein comprising at least the HBV Core and the modified HBV polymerase (Pmut), and a second protein comprising at least the HBV surface antigen (HbsAg). In another example the immunogen expression cassette encodes only two proteins, comprising a first fusion protein comprising at least the HBV Core and the modified HBV polymerase (Pmut), and a second protein comprising at least the HBV surface antigen (HbsAg).

In one example, the immunogen expression cassette does not encode HBV X protein.

In one example, the immunogen expression cassette may comprise a nucleic acid sequence encoding any one of:

SIi-HBV-CPmutS;

SIi-HBV-SCPmut;

HBV-CPmutS

SIi-HBV-CPmutPreS-S(sh);

SIi-HBV-CPmutPreS-TPA-S(sh);

SIi-HBV-PreS-Pmut-C;

MVA-SIi-HBV-PreS-Pmut-C-S(sh); or

MVA-SIi-HBV-PreS-Pmut-C-TPA-S(sh), as described herein.

In one example, the immunogen expression cassette comprises SEQ ID NO: 46 (SIi-HBV-CPmutS) or a variant thereof. A variant of SEQ ID NO: 46 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 46. The variant of SEQ ID NO: 46 may encode protein that substantially retains the immunogenicity of equivalent protein encoded by SEQ ID NO: 46.

In one example, the immunogen expression cassette comprises SEQ ID NO: 47 (SIi-HBV-SCPmut) or a variant thereof. A variant of SEQ ID NO: 47 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 47. The variant of SEQ ID NO: 47 may encode protein that substantially retains the immunogenicity of equivalent protein encoded by SEQ ID NO: 47.

In another example, the immunogen expression cassette comprises SEQ ID NO: 48 (SIi-HBV-CPmutPreS-S(sh)) or a variant thereof. A variant of SEQ ID NO: 48 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 48. The variant of SEQ ID NO: 48 may encode protein that substantially retains the immunogenicity of equivalent protein encoded by SEQ ID NO: 48.

In another example, the immunogen expression cassette comprises SEQ ID NO: 49 (SIi-HBV-CPmutPreS-TPA-S(sh)) or a variant thereof. A variant of SEQ ID NO: 49 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 49. The variant of SEQ ID NO: 49 may encode

protein that substantially retains the immunogenicity of equivalent protein encoded by SEQ ID NO: 49.

In one example, the immunogen expression cassette comprises SEQ ID NO: 59 (SIi-HBV-PreS-Pmut-C) or a variant thereof. A variant of SEQ ID NO: 59 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 59. The variant of SEQ ID NO: 59 may encode protein that substantially retains the immunogenicity of equivalent protein encoded by SEQ ID NO: 59.

In one example, the immunogen expression cassette comprises SEQ ID NO: 24 (MVA-SIi-HBV-PreS-Pmut-C-S(sh)) or a variant thereof. A variant of SEQ ID NO: 24 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 24. The variant of SEQ ID NO: 24 may encode protein that substantially retains the immunogenicity of equivalent protein encoded by SEQ ID NO: 24.

In one example, the immunogen expression cassette comprises SEQ ID NO: 27 (MVA-SIi-HBV-PreS-Pmut-C-TPA-S(sh)) or a variant thereof. A variant of SEQ ID NO: 27 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 27. The variant of SEQ ID NO: 27 may encode protein that substantially retains the immunogenicity of equivalent protein encoded by SEQ ID NO: 27. In one example, the immunogen expression cassette comprises SEQ ID NO: 58 (MVA-SIi-HBV-PreS-Pmut-C-TPA-S(sh)) or a variant thereof. A variant of SEQ ID NO: 58 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 58. The variant of SEQ ID NO: 58 may encode protein that substantially retains the immunogenicity of equivalent protein encoded by SEQ ID NO: 58.

Viral vector

The viral vector may comprise a virus. The immunogen expression cassette sequence of the disclosure may be cloned into any suitable viral vector that is known to elicit good immune response. Suitable viral vectors have been described in Dicks et al (Vaccine. 2015 Feb 25;33(9):1121-8. doi: 10.1016/j.vaccine.2015.01.042. Epub 2015 Jan 25), Antrobus et

al (Mol Ther. 2014 Mar;22(3):668-74. doi: 10.1038/mt.2013.284. Epub 2013 Dec 30.), and (Warimwe et al. (Virol J. 2013 Dec 5;10:349. doi: 10.1186/1743-422X-10-349), which are incorporated herein by reference.

The viral vector may be an attenuated viral vector. The viral vector may comprise an adenovirus, such as a human or simian adenovirus. In one example, the viral vector comprises an adenovirus, such as a group E simian adenovirus, when used in a prime vaccine of a prime boost regime. The viral vector may comprise a group E simian adenovirus. The viral vector may comprise ChAdOx1 (a group E simian adenovirus, like the AdCh63 vector used safely in malaria trials) or ChAdOx2. The skilled person will be familiar with ChAdOx1 based viral vectors, for example from patent publication WO2012172277, which is herein incorporated by reference. The viral vector may comprise AdCh63. The viral vector may comprise AdC3 or AdH6. In one example, the viral vector is a human serotype. In another example, the viral vector comprises Modified Vaccinia Ankara (MVA). The viral vector may comprise MVA when used as a vaccine boost in a prime boost regime. In one example, the viral vector comprises an adenovirus, such as a group E simian adenovirus, when used in a prime vaccine of a prime boost regime, and may comprise MVA when used as a vaccine boost in a prime boost regime. The skilled person will be familiar with MVA based viral vectors, for example from US patent publication US9273327, which is herein incorporated by reference.

MVA advantageously allows expression of more than one protein using different pox viral promoters.

In an alternative example, the viral vector may comprise Adeno-associated virus (AAV) or *Lentivirus*. In another example, the viral vector may comprise any of Vaccinia virus, fowlpox virus or canarypox virus (e.g. members of *Poxviridae* and the genus *Avipoxvirus*), or New York attenuated vaccinia virus (Tartaglia *et al*. Virology. 1992 May;188(1):217-32, which is herein incorporated by reference). In another example, the viral vector may comprise any of Herpes simplex virus, Human Cytomegalo virus, Measles virus (MeV), Sendai virus (SeV), *Flavivirus* (e.g. Yellow Fever Virus – 17D), or *alphavirus* vectors, such as Sindibis virus (SINV), Venezuelan equine encephalitis virus, or Semliki forest virus.

In one example, the viral vector may comprise nucleic acid comprising the sequence of SEQ ID NO: 39 and 40 (ChAdOx1) or a variant thereof. A variant of SEQ ID NO: 39 and 40 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 39 and 40. The variant of SEQ ID NO: 39 and 40 may encode a viral vector that substantially retains the function of the viral vector of SEQ ID NO: 39 and 40 (ChAdOx1).

In one example, the viral vector may comprise nucleic acid comprising the sequence of SEQ ID NO: 41 and 42 (ChAdOx2) or a variant thereof. A variant of SEQ ID NO: 41 and 42 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 41 and 42. The variant of SEQ ID NO: 41 and 42 may encode a viral vector that substantially retains the function of the viral vector of SEQ ID NO: 41 and 42 (ChAdOx2).

In one example, the viral vector may comprise nucleic acid comprising the sequence of SEQ ID NO: 44 and 45 (MVA) or a variant thereof. A variant of SEQ ID NO: 44 and 45 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 44 and 45. The variant of SEQ ID NO: 44 and 45 may encode a viral vector that substantially retains the function of the viral vector of SEQ ID NO: 44 and 45 (MVA).

In one example, the viral vector encodes any one of:
SIi-HBV-CPmutS;
SIi-HBV-SCPmut;
SIi-HBV-CPmutPreS-S(sh);
SIi-HBV-CPmutPreS-TPA-S(sh);
MVA-SIi-HBV-PreS-Pmut-C-S(sh); or
MVA-SIi-HBV-PreS-Pmut-C-TPA-S(sh), as described herein.

In one example, the viral vector encodes SEQ ID NO: 3 (SIi-HBV-CPmutS) or a variant thereof. A variant of SEQ ID NO: 3 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 3. The variant of SEQ ID NO: 3 may substantially retain the immunogenicity of SEQ ID NO: 3.

In one example, the viral vector encodes SEQ ID NO: 11 (SIi-HBV-SCPmut) or a variant thereof. A variant of SEQ ID NO: 11 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 11. The variant of SEQ ID NO: 11 may substantially retain the immunogenicity of SEQ ID NO: 11.

In one example, the viral vector encodes SEQ ID NO: 13 (SIi-HBV-CPmutPreS-S(sh)) or a variant thereof. A variant of SEQ ID NO: 13 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 13. The variant of SEQ ID NO: 13 may substantially retain the immunogenicity of SEQ ID NO: 13.

In one example, the viral vector encodes SEQ ID NO: 25 (SIi-HBV-CPmutPreS-TPA-S(sh)) or a variant thereof. A variant of SEQ ID NO: 25 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 25. The variant of SEQ ID NO: 25 may substantially retain the immunogenicity of SEQ ID NO: 25.

In one example, the viral vector encodes SEQ ID NO: 23 (MVA-SIi-HBV-PreS-Pmut-C-S(sh)) or a variant thereof. A variant of SEQ ID NO: 23 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 23. The variant of SEQ ID NO: 23 may substantially retain the immunogenicity of SEQ ID NO: 23.

In one example, the viral vector encodes SEQ ID NO: 26 (MVA-SIi-HBV-PreS-Pmut-C-TPA-S(sh)) or a variant thereof. A variant of SEQ ID NO: 26 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 26. The variant of SEQ ID NO: 26 may substantially retain the immunogenicity of SEQ ID NO: 26.

In one example, the viral vector comprises the nucleic acid sequence of SEQ ID NO: 46 (SIi-HBV-CPmutS) or a variant thereof. A variant of SEQ ID NO: 46 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 46.

In one example, the viral vector comprises the nucleic acid sequence of SEQ ID NO: 47 (SIi-HBV-SCPmut) or a variant thereof. A variant of SEQ ID NO: 47 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 47.

In one example, the viral vector comprises the nucleic acid sequence of SEQ ID NO: 48 (SIi-HBV-CPmutPreS-S(sh)) or a variant thereof. A variant of SEQ ID NO: 48 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 48.

In one example, the viral vector comprises the nucleic acid sequence of SEQ ID NO: 49 (SIi-HBV-CPmutPreS-TPA-S(sh)) or a variant thereof. A variant of SEQ ID NO: 49 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 49.

In one example, the viral vector comprises the nucleic acid sequence of SEQ ID NO: 24 (MVA-SIi-HBV-PreS-Pmut-C-S(sh)) or a variant thereof. A variant of SEQ ID NO: 24 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 24.

In one example, the viral vector comprises the nucleic acid sequence of SEQ ID NO: 27 (MVA-SIi-HBV-PreS-Pmut-C-TPA-S(sh)) or a variant thereof. A variant of SEQ ID NO: 27 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 27. In one example, the viral vector comprises the nucleic acid sequence of SEQ ID NO: 58 (MVA-SIi-HBV-PreS-Pmut-C-TPA-S(sh)) or a variant thereof. A variant of SEQ ID NO: 58 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 58.

Promoter

In one example, the promoter is encoded in the immunogen expression cassette, for example the promoter may be encoded at, or adjacent to, the 5' end of the immunogen expression cassette. Alternatively, the promoter may be encoded as part of the viral vector nucleic acid outside of the immunogen expression cassette. For

example the promoter may be encoded upstream (5') of the immunogen expression cassette.

The promoter may promote the expression of all the encoded protein of the immunogen expression cassette. In an alternative example wherein the immunogen expression cassette comprises a secondary promoter, the promoter may be a primary promoter that is arranged to promote expression of at least the HBV core and modified polymerase (Pmut), and optionally not the HBV surface antigen (HbsAg) which may be arranged to be promoted separately by the secondary promoter.

The skilled person will recognise that any suitable promoter may be used as the primary and/or secondary promoter as appropriate for the host for expression. In one example, the promoter comprises a CMV promoter. The CMV promoter may comprise the long or short CMV promoter. In an example wherein the viral vector comprises an Adenoviral vector, such as ChAdOx1 or 2, the promoter may comprise a CMV promoter, SV40 promoter, or EF1a promoter. In an example wherein the viral vector comprises an Adenoviral vector, such as ChAdOx1 or 2, the promoter may comprise a CMV promoter.

The promoter element(s) used in the vector, for example adenoviral vector, (such as ChAdOx1 or 2) may comprise a tetracycline operator (*tetO*) sequence. A tetracycline operator (*tetO*) sequence is helpful for generation of viral vectors that can express foreign proteins that are toxic to cells within which they are generated.

In another example, the promoter comprises a pox viral promoter. In an example wherein the viral vector comprises MVA, the promoter may comprise a pox viral promoter, such as F11. In one example, the promoter comprises early F11 promoter. Alternatively, the pox viral promotor may comprise an early promoter, for example selected from any of B8R, K6L, A44L, C11R, and B2R, a promoter with early and late activity, for example selected from mH5, p7.5, and SSP, or a late promoter, for example FP4b.

Early promoter based transgene expression is can be useful for an immunogen intended primarily for T-cell response (there is a higher magnitude of T-cell induction upon usage of these early promoters). However promoters with both early and late

activity can be used for an immunogen intended for inducing antibody response. However, these early and late activity promoters can also be used for an immunogen intended for T-cell induction.

In one example, the promoter comprises or consists of the nucleic acid sequence of SEQ ID NO: 50 or 52 (CMV long or short promoter) or variants thereof. A variant of SEQ ID NO: 50 or 52 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 50 or 52 respectively. The variant may substantially retain the promoter function of SEQ ID NO: 50 or 52).

In one example, the promoter comprises or consists of a sequence located in the F11 Left flank sequence (SEQ ID NO: 35) or a variant thereof. A variant may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with the promoter sequence located in the F11 Left flank sequence (SEQ ID NO: 35). The variant may substantially retain the promoter function of the promoter located in the F11 Left flank sequence (SEQ ID NO: 35).

Secondary Promoter

In one example, the immunogen expression cassette encodes a secondary promoter. In an example wherein a secondary promoter is provided in addition to the first/primary promoter, the secondary promoter may be encoded by/within the immunogen expression cassette. The secondary promoter may be encoded 3' of the first/primary promoter. The secondary promoter may be encoded 3' of the first protein/antigen to be expressed, for example downstream of the HBV core and HBV modified polymerase and upstream of the HBV surface antigen. The secondary promoter may promote the expression of at least the HBV surface antigen.

The secondary promoter may comprise a pox viral promoter. The secondary promoter may comprise pox viral early promoters, such as any of B8R, K6L, A44L, C11R, and B2R, or pox viral promoters with early and late activity, such as mH5, p7.5 or SSP, or a late promoter such as FP4b.

In one example, the secondary promoter comprises early/late promoter mH5. In one example, the secondary promoter comprises or consists of the nucleic acid sequence of SEQ ID NO: 28 (mH5 promoter) or a variant thereof. A variant of SEQ ID NO: 28 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 28. The variant may substantially retain the secondary promoter function of SEQ ID NO: 28).

In another example, the secondary promoter may comprise a SV40 promoter or EF1a promoter.

Cleavage domain

In one example, the immunogen expression cassette encodes a cleavage domain. In one example, the cleavage domain is a post-translation proteolytic cleavage domain, which allows for the cleavage of a translated protein, for example by a proteolytic cleavage enzyme. The cleavage enzyme may be provided by the host, for example the host being vaccinated, such as a human. In another example, the cleavage enzyme may be encoded in the immunogen expression cassette or viral vector. In one example, the cleavage domain comprises a non-HBV sequence, for example, a mammalian sequence. In one example, the cleavage domain comprises a human derived sequence.

In one example, the cleavage domain comprises a ribosome skipping cleavage domain. In one example, the cleavage domain comprises a Furin recognition site comprising or consisting of the sequence RXRR, where X could be any amino acid. In one example, the cleavage domain comprises a Furin recognition site and a 2A peptide sequence. The 2A peptide sequence may comprise FMDV (Foot and Mouth Disease Virus) 2A peptide sequence (APVKQTLNFDLLKLAGDVESNPGP – SEQ ID NO: 43). Alternatively, 2A peptides may be selected from P2A (porcine teschovirus-1 2A), T2A (Thoseaasigna virus 2A), and E2A (equine rhinitis A virus [ERAV] 2A). Any picorna virus 2A peptide sequence may be provided for, and function as, a peptide cleavage site. Therefore, the cleavage domain may comprise a sequence encoding picorna virus 2A peptide sequence.

In one example, the cleavage domain comprises or consists of Furin-2A (F2A) peptide sequence or a functional variant thereof. The cleavage domain may comprise or consists of the sequence of SEQ ID NO: 9 or a variant thereof. A variant of SEQ ID NO: 9 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 9. The variant of SEQ ID NO: 9 may substantially retain the cleavage function of SEQ ID NO: 9.

F2A is advantageously short (only 28 amino acids), which helps to provide appropriate inserts and express more than one protein from vectors that can have a size limit, such as Adenovirus based vectors, including ChAdOx1/2.

In an example where the viral vector is MVA, or otherwise a viral vector is used as a boost vaccination following a prime vaccination, a different cleavage domain may be provided to avoid any potential boosting of T-cell responses to the cleavage domain of the prime vaccination. Alternatively a secondary promoter may be used instead of a cleavage domain in order to avoid any potential boosting of T-cell responses to a cleavage domain of a prime vaccination.

IRES (Internal Ribosomal Entry Site)

In one example, the immunogen expression cassette encodes an Internal Ribosome Entry Site (IRES). The skilled person will recognise that an IRES is an RNA structures that allows cap independent initiation of translation, and is able to initiate translation in the middle of a messenger RNA.

Splicing Signal

In one example, the immunogen expression cassette encodes an mRNA splicing signal. The skilled person will be familiar with commonly used splicing signals that are appropriate for this use. One such example of an mRNA splicing signal that may be used is a chimeric intron composed of the 5' donor site from the first intron of the human β-globin gene and the branch and 3' acceptor site from the intron of an immunoglobulin gene heavy chain variable region in the expression vector pCI-neo Mammalian Expression Vector (Promega).

HBV Pre-Core (PreC)

LLWFHISCLTFGRETVLEYLVSFGVWIRTPPAYRPPNAPILSTLPETTVV (SEQ ID NO: 17).

The HBV e-Antigen (HBeAg) may comprise or consist of the sequence of SEQ ID NO: 17 or a variant thereof. A variant of SEQ ID NO: 17 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 17. The variant of SEQ ID NO: 17 may substantially retain the immunogenicity of SEQ ID NO: 17. The variant of SEQ ID NO: 17 may substantially retain the tertiary structure of SEQ ID NO: 17.

Modified HBV polymerase

The modified HBV polymerase (P_{mut}) may comprise or consist of a truncated HBV polymerase. In particular, the mutation to wild-type HBV polymerase to substantially remove polymerase function may comprise a sequence encoding a truncated HBV polymerase. Alternatively or additionally, the mutation comprises one or more point mutations to the encoded HBV polymerase sequence. The modification may comprise one or more amino acid substitutions, deletions or additions in the encoded HBV polymerase sequence. In one example, the modified HBV polymerase (Pmut) is not a truncated form of HBV polymerase (i.e. it is full length relative to wildtype HBV polymerase).

The modified HBV polymerase (P_{mut}) may comprise or consist of the sequence of SEQ ID NO: 8 or a variant thereof. A variant of SEQ ID NO: 8 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 8. The variant of SEQ ID NO: 8 may substantially retain the immunogenicity of SEQ ID NO: 8. The variant of SEQ ID NO: 8 may substantially retain the tertiary structure of SEQ ID NO: 8.

The modification may be a mutation that prevents protein priming. Additionally or alternatively mutations may be provided in the reverse transcriptase and/or RNAase domains to prevent their function. Further additionally or alternatively the mutation may be structural such that is disrupts the correct protein folding of the polymerase. The modifications may comprise one or more, or all of Y63, C323, C334, C338, C352, R714, D788, R792, or equivalent residues thereof, by reference to wild-type HBV

polymerase consensus sequence herein (i.e. SEQ ID NO: 19). In one example the modifications may comprise one or more, or all of Y63A, C323A, C334A, C338A, C352A, R714A, D788A, R792A, or equivalent residues thereof, by reference to wild-type HBV polymerase consensus sequence herein (i.e. SEQ ID NO: 19). In another example, the modifications may comprise one or more, or all of R714, D788, and R792, or equivalent residues thereof, by reference to wild-type HBV polymerase consensus sequence herein (i.e. SEQ ID NO: 19). In one example the modifications may comprise one or more, or all of R714A, D788A, and R792A, or equivalent residues thereof, by reference to wild-type HBV polymerase consensus sequence herein (i.e. SEQ ID NO: 19). Reference to equivalent residues is understood to mean that the HBV polymerase may be based on an alternative sequence than the HBV polymerase sequence provided herein, and that numbering or identity of amino acid residues may differ between the sequences. Such differences and equivalency may be readily determined by an alignment of the HBV polymerase sequence with the wild-type HBV polymerase consensus sequence herein (i.e. SEQ ID NO: 19).

Mutations R714A, D788A, R792A advantageously stop polymerase function. However, one or more, or all of, additional mutations of Y63 and other cysteine mutations of C323, C334, C338, and C352 may be added as an extra measure in the event of a reversion within those functional mutations. The additional mutations may comprise Y63A, C323A, C334A, C338A, and/or C352A.

The mutations advantageously provide that the HBV polymerase function is disrupted by (a) RNAase H functional mutations, to stop its enzyme activity, and/or (b) Y63A mutation to stop the first step of replication (Priming of DNA synthesis), and/or (c) cysteine mutations to disrupt its native conformation and stop the HBV polymerase from participating in the initial steps of viral replication (protein priming, RNA binding and RNA packaging).

The skilled person will be familiar with various HBV polymerase modifications that can be provided which significantly reduce or ablate function, and can be provided in the modified polymerase according to the disclosure. Such modifications are for example described in WO2016020538, WO2013007772, and WO2011015656, which are incorporated herein by reference.

In one example, the modified polymerase is modified so as to exhibit a reduced reverse-transcriptase (RTase) enzymatic activity with respect to a wild-type HBV polymerase. The reduction of RTase activity may be provided by one or more mutation(s) in the domain responsible for RTase enzymatic activity. Four residues have been found to be involved in RTase activity, forming a motif "YMDD" (for Tyr, Met, Asp and Asp residues) at approximately position 538 to approximately position 541 of a wild-type HBV polymerase. The present disclosure encompasses any mutation(s) in this motif, or elsewhere in the RTase domain, that provides a significant reduction (e.g. at least a 10 fold reduction) or ablation of the RTase activity. Representative examples of suitable RTase-deficient polymerase mutants are described in Radziwill et al. (1990, J. Virol. 64:613), in Bartenschlager et al. (1990, J. Virol. 64:5324) and in Jeong et al. (1996, Biochem Bioph Res Commun. 223(2):264), which are incorporated herein by reference. In one example, the modified polymerase may comprise the substitution of the first Asp residue of the YMDD motif or of the amino acid residue located in an equivalent position in a native HBV polymerase to any amino acid residue other than Asp, with an optional substitution to a His residue (D540H mutation).

Additionally or alternatively, the modified polymerase may be modified to provide a reduced RNase H enzymatic activity with respect to wild-type HBV polymerase. The reduction of RNase H activity may be provided by one or more mutation(s) in the domain responsible for RNase H enzymatic activity. The functional domain involved in RNase H activity is within the C-terminal portion of HBV polymerase, approximately from position 680 to the C-terminal position 832 of wild-type polymerase, and the modified polymerase of the present disclosure may encompasses any mutation(s) in this domain that provides a significant reduction (e.g. at least a 10 fold reduction) or ablation of the RNase H activity. Representative examples of suitable RNase H-deftcient polymerase mutants are described in Radziwill et ai. (1990, J. Virol. 64:613), in Bartenschlager at al. (1990, J. Virol. 64:5324).

HBV surface antigen (HbsAg)

The skilled person will understand that PreS1 and PreS2 are components of the Large (L) form of HBV surface protein (e.g. L form = PreS1+PreS2+S). The medium (M)

form of HBV surface protein has PreS2+S. Having such sequences together means that T-Cell epitopes are included in those sequential order.

The HbsAg may comprise or consist of a full length wild-type HbsAg sequence, or a variant thereof. The HbsAg variant may comprise or consist of a truncated HbsAg sequence.

The HbsAg including the PreS1 and PreS2 sequences may comprise or consist of the sequence of SEQ ID NO: 10 or a variant thereof. A variant of SEQ ID NO: 10 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 10. The variant of SEQ ID NO: 10 may substantially retain the immunogenicity of SEQ ID NO: 10. The variant of SEQ ID NO: 10 may substantially retain the tertiary structure of SEQ ID NO: 10.

In another example, the HbsAg may comprise the surface antigen without PreS1 and/or PreS2. The HbsAg may comprise or consist of the sequence of SEQ ID NO: 18 or a variant thereof. A variant of SEQ ID NO: 18 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 18. The variant of SEQ ID NO: 18 may substantially retain the immunogenicity of SEQ ID NO: 18. The variant of SEQ ID NO: 18 may substantially retain the tertiary structure of SEQ ID NO: 18.

In one example, the HbsAg may comprise or consist of any one or all of the four known transmembrane regions in HbsAg (amino acids 1-226), which are amino acids (8-32) FLGPLLVLQAGFFLLTRILTIPQSL (SEQ ID NO: 54), amino acids (80-98) FIIFLFILLLCLIFLLVLL (SEQ ID NO: 55), amino acids (160-184) RFLWEWASVRFSWLSLLVPFVQWFV (SEQ ID NO: 56), and amino acids (189-210) TVWLSVIWMMWYWGPSLYNILS (SEQ ID NO: 57) respectively. In one example, the HbsAg may at least comprise or consist of the HbsAg transmembrane region of amino acids (8-32) FLGPLLVLQAGFFLLTRILTIPQSL (SEQ ID NO: 54).

HBV PreS1

The HBV PreS1 may comprise or consist of a full length wild-type HBV PreS1 sequence, or a variant thereof. The HBV PreS1 may comprise or consist of the

sequence of SEQ ID NO: 52 or a variant thereof. A variant of SEQ ID NO: 52 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 52. The variant of SEQ ID NO: 52 may substantially retain the immunogenicity of SEQ ID NO: 52. The variant of SEQ ID NO: 52 may substantially retain the tertiary structure of SEQ ID NO: 52.

The HBV PreS1 variant may comprise or consist of a truncated HBV PreS1 sequence, for example CΔPreS1 or NΔPreS1 described herein (CΔPreS1 refers to C-terminal truncated PreS1 and NΔPreS1 refers to N-terminal truncated PreS1). In one example, the immunogen expression cassette may encode both NΔPreS1 and CΔPreS1. In an example wherein the immunogen expression cassette encodes both NΔPreS1 and CΔPreS1, the NΔPreS1 may be encoded upstream (5') of intergenic sequence and the CΔPreS1 may be encoded downstream (3') of intergenic sequence. In an example wherein the immunogen expression cassette encodes NΔPreS1 upstream (5') of intergenic sequence, it may be fused with PreS2 and/or the modified polymerase (Pmut). In an example wherein the immunogen expression cassette encodes NΔPreS1 upstream (5') of intergenic sequence, it may be fused with PreS2 and/or the modified polymerase (Pmut) and the CΔPreS1 encoded downstream (3') of intergenic sequence may be fused with the surface antigen (HbsAg).

CΔPreS1

In one example, the immunogen expression cassette encodes a truncated form of HBV PreS1. The truncation may comprise a C-terminal truncation. In one example, the truncated HBV PreS1 comprises CΔPreS1 (SEQ ID NO: 21) described herein. In one example, the truncated PreS1, such as CΔPreS1 described herein, is arranged to be expressed a fusion protein with the HBV surface antigen (S / HbsAg). A linker sequence, such as a linker described herein, may be provided between the truncated PreS1 and surface antigen (S / HbsAg). For example, CΔPreS1 + linker + S (described herein as S(sh)). The nucleotide sequence encoding S(sh) may comprise or consist of SEQ ID NO: 61 or SEQ ID NO: 38.

The CΔPreS1 may comprise or consist of the sequence of SEQ ID NO: 21 or a variant thereof. A variant of SEQ ID NO: 21 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 21. The

variant of SEQ ID NO: 21 may substantially retain the immunogenicity of SEQ ID NO: 21. The variant of SEQ ID NO: 21 may substantially retain the tertiary structure of SEQ ID NO: 21.

In one example, the truncated PreS1, such as CΔPreS1 described herein, optionally with a fused surface antigen, is encoded downstream (3') of the intergenic sequence.

The PreS1 truncation advantageously favours antibody generation for both T-cell and antibody response for the HBV antigens provided in the immunogen expression cassette. Without being bound by theory, antibody generation is possibly due to proper folding of PreS1 and Surface antigens.

NΔPreS1

In one example, the truncated PreS1 comprises NΔPreS1 as described herein. The NΔPreS1 and PreS2 fusion may comprise or consist of SEQ ID NO: 15/38, or a variant thereof. A variant of SEQ ID NO: 15/38 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 15/38. The variant of SEQ ID NO: 15 may substantially retain the immunogenicity of SEQ ID NO: 15/38.

The NΔPreS1 and PreS2 fusion sequence advantageously provides a good T-cell response. NΔPreS1 advantageously includes amino acids in a sequence that would still preserve T-cell epitopes (8-11 amino acids for CD8 T-cell epitopes and slightly longer (12-16) for CD4 T-cell epitopes.

In an example wherein the immunogen expression cassette encodes NΔPreS1 fused with PreS2 upstream (5') of intergenic sequence, it may be further fused with the modified polymerase (Pmut). A linker sequence, such as a linker described herein, may be provided between the PreS2 and modified polymerase (Pmut). For example, NΔPreS1 + PreS2 + linker + Pmut.

HBV PreS2

The HBV PreS2 may comprise or consist of a full length wild-type HBV PreS2 sequence, or a variant thereof. The HBV PreS2 variant may comprise or consist of a truncated HBV PreS2 sequence.

The HBV PreS2 may comprise or consist of the sequence of SEQ ID NO: 53 or a variant thereof. A variant of SEQ ID NO: 53 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 53. The variant of SEQ ID NO: 53 may substantially retain the immunogenicity of SEQ ID NO: 53. The variant of SEQ ID NO: 53 may substantially retain the tertiary structure of SEQ ID NO: 53.

Peptide Adjuvant

The immunogen expression cassette may further encode a peptide adjuvant. The peptide adjuvant may comprise TPA (tissue plasminogen activator). In one example, the peptide adjuvant may comprise a human or non-human invariant chain (Ii), or a fragment thereof. A fragment of the long isoform (isoform (b)) of the human CD74 molecule, is also known as the invariant chain (Nucleic Acids Res. 1985 December 20; 13(24): 8827-8841). It is known that N-terminal fragments of the invariant chain (Ii) which comprise at least the transmembrane domain thereof, provide a surprisingly effective adjuvant function when expressed as a fusion protein with an antigen of interest. Fragments encompassing the transmembrane domain and the cytoplasmic domain, and preferably including the N-terminal 16 amino acids of the long isoform of the protein are particularly efficacious.

The invariant chain may comprise or consist of a shark invariant chain (SIi), or fragment or a functional variant thereof. The variant shark invariant chain (SIi) may comprise a truncated invariant shark invariant chain. Other non-human animal sources of an invariant chain, or fragment thereof, include chicken, quail, trout, zebrafish, carp, frog, grouper, shark, mandarin fish or mallard.The skilled person will be familiar with appropriate invariant chains, or fragments thereof, for use as peptide adjuvants encoded in an expression cassette/vector, for example the invariant chain may be any invariant chain as provided in WO2015082922, which is incorporated herein by reference.

The HBV may be HBV genotype C. In another example, the HBV may be of any one of the 10 genotypes (A-J). All of the encoded HBV proteins/antigens may be derived from one genotype, for example HBV genotype C.

Linkers

In one example, linker residues may be encoded between one or more, or all, of the protein/antigen sequences that are provided in a fusion protein (e.g. providing junctions between the sequences in the protein). In an example comprising a peptide adjuvant, a linker may be encoded between the peptide adjuvant and the downstream encoded protein/antigen. In one example, a linker is encoded between the sequences of the HBV core and modified HBV polymerase.

The linker residues may comprise random amino acid sequences, or amino-acids that have been selected to be non-immunogenic based on epitope prediction computer programs or experiments in animal models. For example, a linker may not be considered if it is predicted or known to be an epitope (i.e. in order to avoid an immune response to epitopes, e.g. artificial epitopes, not found in HBV. The linker may be flexible. The linker may comprise or consist of K, G, P or S amino acid residues, or combinations thereof. In one example, the linker may comprise or consist of G and/or P amino acid residues. The linker residues may be between 1 and 10 amino acids in length. In another example, the linker residues may be between 2 and 8 residues in length. In another example, the linker residues may be between 1 and 6 residues in length.

A linker may comprise or consist of any of the sequences KGGGPGGG (SEQ ID NO: 5), GGGSGGG (SEQ ID NO: 7), KGGS (SEQ ID NO: 14), KSP, GSKGK (SEQ ID NO: 20), LEGGSGG (SEQ ID NO: 22), SKSGPPSGKS (SEQ ID NO: 31), GSKSGSK (SEQ ID NO: 32), SKSPGSGPP (SEQ ID NO: 33), or ASKGGKSG (SEQ ID NO: 34).

In one example, a linker may comprise the sequence KGGGPGGG (SEQ ID NO: 5). In another example, a linker may comprise the sequence GGGSGGG (SEQ ID NO: 7). In another example, a linker may comprise the sequence KGGS (SEQ ID NO: 14). In another example, a linker may comprise the sequence KSP. In another example, a linker may comprise the sequence GSKGK (SEQ ID NO: 20). In

another example, a linker may comprise the sequence LEGGSGG (SEQ ID NO: 22). In another example, a linker may comprise the sequence SKSGPPSGKS (SEQ ID NO: 31). In another example, a linker may comprise the sequence GSKSGSK (SEQ ID NO: 32). In another example, a linker may comprise the sequence SKSPGSGPP (SEQ ID NO: 33). In another example, a linker may comprise the sequence ASKGGKSG (SEQ ID NO: 34).

Advantageously, the use of linkers can avoid generation of peptides with homology to human proteome (which could potentially generate immune response to self-antigen) and they avoid immune-dominant artificial epitopes. Additionally, linkers can provide a flexible hinge between segments of protein, so that they can fold into their native conformation. For example, a linker between CΔPreS1 and S allows independent folding of CΔPreS1 and S, so that they could generate antibodies for conformational epitopes for the respective proteins.

Linkers may be varied between different immunogen expression cassettes. Using different linkers avoids boosting of any T-cell response created to any potential artificial epitopes (i.e. by changing linkers or changing junctions). For example, when vaccines are used in prime boost vaccination strategies, changing the linkers or the order of proteins within the immunogen layout, helps to overcome boosting of artificial epitope response.

Other elements of the immunogen expression cassette(s)

In an example wherein the immunogen expression cassette is for use in an MVA vector, the immunogen expression cassette may further comprise F11 left and right flanking sequences to allow insertion into the MVA F11 locus by homologous recombination.

The F11-left flank sequence may comprise or consist of the sequence of SEQ ID NO: 35, or a variant thereof. The F11-right flank sequence may comprise or consist of the sequence of SEQ ID NO: 36, or a variant thereof. A variant of SEQ ID NO: 35 or 36 may comprise a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% identity with SEQ ID NO: 35 or 36 respectively. The variant of SEQ ID NO: 35 or 36 may substantially retain the homologous recombination function of SEQ ID

NO: 35 or 36 respectively. The skilled person will appreciate that 1, 2, 3, 4, 5 or more amino acid residues may be substituted, added or removed without affecting function. For example, conservative substitutions may be considered.

The immunogen expression cassette may further comprise a transcription terminator sequence. The transcription terminator sequence may be provided in examples of the immunogen expression cassette comprising a secondary promoter. For example, the transcription terminator sequence may be provided downstream of a protein expressed by the primary promoter, but before (upstream of) the secondary promoter. The transcription terminator sequence may comprise or consist of the sequence TTTTTGT, or variants thereof.

In one example, the immunogen expression cassette described herein may be isolated or provided in a non-viral vector.

Therefore, according to another example of the present disclosure there is provided a nucleic acid comprising the immunogen expression cassette described herein, optionally wherein the nucleic acid is isolated nucleic acid.

In another aspect, the present disclosure refers to a nucleic acid comprising or consisting of an HBV immunogen expression cassette, wherein the immunogen expression cassette encodes:

a) HBV Core;

b) a modified HBV polymerase (P_{mut}), wherein the modification is a mutation to wild-type HBV polymerase to substantially remove polymerase function;

c) HBV surface antigen (HbsAg); and

d) an intergenic sequence that is arranged to cause expression of at least the HBV surface antigen (HbsAg) as a separate protein from the HBV core and the modified HBV polymerase (P_{mut}),

wherein the intergenic sequence is downstream (3') of the sequences encoding the HBV core and the modified HBV polymerase (P_{mut}) and upstream (5') of the sequence encoding the HBV surface antigen (HbsAg).

According to another example of the disclosure there is provided a composition comprising the viral vector according to the disclosure, optionally wherein the composition is a pharmaceutically acceptable composition.

In another aspect, the present disclosure refers to a composition comprising the viral vector as disclosed herein or the nucleic acid as disclosed herein, optionally wherein the composition is a pharmaceutically acceptable composition.

The composition may be immunogenic, for example in a mammal, such as a human. The composition may comprise a pharmaceutically acceptable carrier. The composition may be a pharmaceutical composition comprising a pharmaceutically acceptable carrier. The composition may be for use in the prophylaxis or treatment of HBV infection.

According to another example of the disclosure there is provided a method of treatment or prophylaxis of HBV infection comprising the administration of the viral vector, nucleic acid or composition according to the disclosure.

The method of treatment or prophylaxis of HBV infection may be a method of vaccination.

According to another example of the disclosure there is provided a viral vector, nucleic acid or composition according to the disclosure for use in the treatment or prevention of HBV infection, optionally wherein the use is in a vaccine.

In another aspect, the present disclosure refers to the composition as disclosed herein, the viral vector as disclosed herein or the nucleic acid as disclosed herein, for use in the prophylaxis or treatment of HBV infection in a subject; optionally wherein the use is as a vaccine.

In another aspect, the present disclosure refers to use of the composition as disclosed herein, the viral vector as disclosed herein or the nucleic acid as disclosed herein, in the manufacture of a medicament for the treatment or prophylaxis of HBV infection, wherein the medicament is to be administered to a subject.

According to another example of the disclosure there is provided a vaccine comprising the viral vector, nucleic acid or composition according to the disclosure.

The vaccine may be a prime vaccine. The vaccine may be a boost vaccine. Where a boost vaccine is provided following a prime vaccine, the viral vector may be a different viral vector according to the disclosure.

Advantageously, the provision of a different viral vector between prime and boost vaccines can avoid the provision of “false” epitopes formed across junctions of one protein/antigen sequence with another. i.e. the same junction may not occur in a re-ordered protein.

According to another example of the disclosure, there is provided a prime boost vaccination kit comprising

-a prime vaccination according to the disclosure;

-a boost vaccination according to the disclosure.

In another aspect, the present disclosure refers to a prime boost vaccination kit comprising

-a prime vaccination comprising the composition as disclosed herein, the viral vector as disclosed herein or the nucleic acid as disclosed herein; and

-a boost vaccination comprising the composition as disclosed herein, the viral vector as disclosed herein or the nucleic acid as disclosed herein.

The prime and boost vaccination may comprise different viral vectors.

The viral vector may be used in a vaccine in combination with another therapeutically or prophylactically active ingredient. The viral vector may be used in a vaccine in combination with an adjuvant.

The viral vector, nucleic acid encoding the viral vector may be provided in a pharmaceutically acceptable carrier.

The viral vector or composition according to the disclosure may not comprise wild-type HBV, or the nucleic acid according to the disclosure may not encode wild-type HBV.

Reference to sequence “identity” used herein may refer to the percentage identity between two aligned sequences using standard NCBI BLASTp parameters (http://blast.ncbi.nlm.nih.gov).

The term "immunogenic", when applied to the viral vector, nucleic acid or composition of the present disclosure means capable of eliciting an immune response in a human or animal body. The immune response may be protective of HBV. The term "protective" means prevention of Hepatitis B disease, a reduced risk of Hepatitis B disease, reduced risk of HBV infection, transmission and/or progression, reduced severity of Hepatitis B disease, a cure of Hepatitis B, an alleviation of symptoms of Hepatitis B, or a reduction in severity of Hepatitis B disease symptoms.

The term “prophylaxis” means prevention of or protective treatment for Hepatitis B. The prophylaxis may include a reduced risk of HBV infection, transmission and/or Hepatitis B disease progression, or reduced severity of Hepatitis B disease.

The term “treatment”, means a cure of Hepatitis B, an alleviation of symptoms, or a reduction in severity of Hepatitis B disease or Hepatitis B disease symptoms.

With reference to “variant” nucleic acid sequences, the skilled person will appreciate that 1, 2, 3, 4, 5 or more codons may be substituted, added or removed without affecting function. For example, conservative substitutions may be considered.

With reference to “variant” amino acid sequences, the skilled person will appreciate that 1, 2, 3, 4, 5 or more amino acids may be substituted, added or removed without affecting function. For example, conservative substitutions may be considered.

The skilled person will understand that optional features of one example or aspect of the disclosure may be applicable, where appropriate, to other examples or aspects of the disclosure.

Embodiments of the invention will now be described in more detail, by way of example only, with reference to the accompanying drawings.

CLAIMS

1. A multi-HBV immunogen viral vector vaccine comprising:

a viral vector comprising an immunogen expression cassette, wherein the expression of a protein encoded by the expression cassette is arranged to be driven by a promoter, wherein the immunogen expression cassette encodes:

a) HBV Core;

b) a modified HBV polymerase (P_{mut}), wherein the modification is a mutation to wild-type HBV polymerase to substantially remove polymerase function;

c) HBV surface antigen (HbsAg); and

d) an intergenic sequence that is arranged to cause expression of at least the HBV surface antigen (HbsAg) as a separate protein from the HBV core and the modified HBV polymerase (P_{mut}),

wherein the intergenic sequence is downstream (3') of the sequences encoding the HBV core and the modified HBV polymerase (P_{mut}) and upstream (5') of the sequence encoding the HBV surface antigen (HbsAg).

2. The multi-HBV immunogen viral vector vaccine according to claim 1, wherein the intergenic sequence comprises a cleavage domain, an IRES (Internal Ribosomal Entry Site), a splicing signal, or a secondary promoter.

3. The multi-HBV immunogen viral vector vaccine according to claim 1, wherein the intergenic sequence comprises a cleavage domain;

optionally wherein the cleavage domain comprises a ribosome skipping cleavage domain;

further optionally, wherein the cleavage domain comprises or consists of Furin-2A (F2A) peptide sequence or a functional variant thereof.

4. The multi-HBV immunogen viral vector vaccine according to claim 1, wherein the intergenic sequence comprises a secondary promoter to promote expression of at least the surface antigen (HbsAg).

5. The multi-HBV immunogen viral vector vaccine according to any preceding claim, wherein the immunogen expression cassette further encodes HBV Pre-Core (PreC).

6. The multi-HBV immunogen viral vector vaccine according to any preceding claim, wherein the immunogen expression cassette further encodes HBV PreS1, and/or a truncated form thereof.

7. The multi-HBV immunogen viral vector vaccine according to any preceding claim, wherein the immunogen expression cassette further encodes HBV PreS2.

8. The multi-HBV immunogen viral vector vaccine according to any preceding claim, wherein the immunogen expression cassette encodes HBV Pre-Core (PreC) and HBV PreS1, and a truncated form of PreS1.

9. The multi-HBV immunogen viral vector vaccine according to any preceding claim, wherein the immunogen expression cassette is capable of expressing HBV e-Antigen.

10. The multi-HBV immunogen viral vector vaccine according to any preceding claim, wherein the HBV Core and modified polymerase (Pmut) are arranged to be expressed as a fusion protein.

11. The multi-HBV immunogen viral vector vaccine according to claims 5 to 10, wherein the HBV Pre-core, HBV Core and modified polymerase (Pmut) are arranged to be expressed as a fusion protein.

12. The multi-HBV immunogen viral vector vaccine according to any preceding claim, wherein the immunogen expression cassette does not encode HBV X protein.

13. The multi-HBV immunogen viral vector vaccine according to any preceding claim, wherein the immunogen expression cassette comprises nucleic acid comprising the sequence of:

SEQ ID NO: 46 (SIi-HBV-CPmutS) or a variant thereof;

SEQ ID NO: 47 (SIi-HBV-SCPmut) or a variant thereof;

SEQ ID NO: 48 (SIi-HBV-CPmutPreS-S(sh)) or a variant thereof;

SEQ ID NO: 49 (SIi-HBV-CPmutPreS-TPA-S(sh)) or a variant thereof; SEQ ID NO: 24 (MVA-SIi-HBV-PreS-Pmut-C-S(sh)) or a variant thereof; or

SEQ ID NO: 27 or SEQ ID NO: 58 (MVA-SIi-HBV-PreS-Pmut-C-TPA-S(sh)) or a variant thereof.

14. The multi-HBV immunogen viral vector vaccine according to any preceding claim, wherein the viral vector encodes the amino acid sequence of:

SEQ ID NO: 3 (SIi-HBV-CPmutS) or a variant thereof.

SEQ ID NO: 11 (SIi-HBV-SCPmut) or a variant thereof.

SEQ ID NO: 13 (SIi-HBV-CPmutPreS-S(sh)) or a variant thereof.

SEQ ID NO: 25 (SIi-HBV-CPmutPreS-TPA-S(sh)) or a variant thereof.

SEQ ID NO: 23 (MVA-SIi-HBV-PreS-Pmut-C-S(sh)) or a variant thereof.

SEQ ID NO: 26 (MVA-SIi-HBV-PreS-Pmut-C-TPA-S(sh)) or a variant thereof.

15. The multi-HBV immunogen viral vector vaccine according to any preceding claim, wherein the viral vector comprises an adenovirus vector or Modified Vaccinia Ankara (MVA) vector.

16. The multi-HBV immunogen viral vector vaccine according to any preceding claim, wherein the viral vector may comprise a group E simian adenovirus vector.

17. The multi-HBV immunogen viral vector vaccine according to any preceding claim, wherein the promoter is encoded in the immunogen expression cassette, for example the promoter may be encoded at, or adjacent to, the 5' end of the immunogen expression cassette; or

wherein the promoter may be encoded as part of the viral vector nucleic acid outside of the immunogen expression cassette.

18. The multi-HBV immunogen viral vector vaccine according to any preceding claim, wherein the promoter promotes the expression of all the encoded protein of the immunogen expression cassette.

19. The multi-HBV immunogen viral vector vaccine according to claims 1 to 17, wherein the immunogen expression cassette comprises a secondary promoter, wherein the promoter is a primary promoter that is arranged to promote expression of at least the HBV core and modified polymerase (Pmut), and not the HBV surface antigen (HbsAg) which is arranged to be promoted separately by the secondary promoter.

20. The multi-HBV immunogen viral vector vaccine according to any preceding claim, wherein the promoter comprises a CMV promoter or a pox viral promoter.

21. The multi-HBV immunogen viral vector vaccine according to any preceding claim, wherein the HBV Core comprises or consists of a full length wild-type HBV Core sequence.

22. The multi-HBV immunogen viral vector vaccine according to any preceding claim, wherein the modified HBV polymerase (P_{mut}) is not a truncated form of HBV polymerase (i.e. it is full length relative to wildtype HBV polymerase).

23. The multi-HBV immunogen viral vector vaccine according to any preceding claim, wherein the modified HBV polymerase (P_{mut}) comprises or consists of the sequence of SEQ ID NO: 8 or a variant thereof.

24. The multi-HBV immunogen viral vector vaccine according to any preceding claim, wherein the HbsAg comprises or consists of a full length wild-type HbsAg sequence, or a variant thereof.

25. The multi-HBV immunogen viral vector vaccine according to any preceding claim, wherein the immunogen expression cassette encodes a truncated form of HBV PreS1 and the truncated PreS1 is arranged to be expressed a fusion protein with the HBV surface antigen (S / HbsAg).

26. The multi-HBV immunogen viral vector vaccine according to claim 25, further comprising a linker sequence provided between the truncated PreS1 and surface antigen (S / HbsAg).

27. The multi-HBV immunogen viral vector vaccine according to claim 25 or 26, wherein the truncated PreS1 with a fused surface antigen is encoded downstream (3') of the intergenic sequence.

28. The multi-HBV immunogen viral vector vaccine according to any preceding claim, wherein the expression cassette encodes a NΔPreS1 and PreS2 fusion sequence.

29. The multi-HBV immunogen viral vector vaccine according to claim 28, wherein the encoded NΔPreS1 and PreS2 fusion sequence is encoded upstream (5') of the intergenic sequence.

30. The multi-HBV immunogen viral vector vaccine according to claim 29, wherein the encoded NΔPreS1 and PreS2 fusion sequence is further fused with the modified polymerase (Pmut).

31. The multi-HBV immunogen viral vector vaccine according to claim 30, wherein a linker sequence is provided between the PreS2 and modified polymerase (Pmut).

32. The multi-HBV immunogen viral vector vaccine according to any preceding claim, wherein the immunogen expression cassette further encodes a peptide adjuvant.

33. The multi-HBV immunogen viral vector vaccine according to claim 32, wherein the peptide adjuvant comprises TPA (tissue plasminogen activator) or a human or non-human invariant chain (Ii), or a fragment thereof.

34. The multi-HBV immunogen viral vector vaccine according to any preceding claim, wherein linker residues are encoded between one or more, or all, of the protein/antigen sequences that are provided in a fusion protein.

35. A nucleic acid comprising or consisting of an HBV immunogen expression cassette, wherein the immunogen expression cassette encodes:

a) HBV Core;

b) a modified HBV polymerase (P_{mut}), wherein the modification is a mutation to wild-type HBV polymerase to substantially remove polymerase function;

c) HBV surface antigen (HbsAg); and

d) an intergenic sequence that is arranged to cause expression of at least the HBV surface antigen (HbsAg) as a separate protein from the HBV core and the modified HBV polymerase (P_{mut}),

wherein the intergenic sequence is downstream (3') of the sequences encoding the HBV core and the modified HBV polymerase (P_{mut}) and upstream (5') of the sequence encoding the HBV surface antigen (HbsAg).

36. The nucleic acid according to claim 35, wherein the immunogen expression cassette further encodes a promoter.

37. The nucleic acid according to claim 35 or 36, wherein the immunogen expression cassette is isolated or provided in a non-viral vector.

38. A composition comprising the viral vector according to any of claims 1 to 34 or the nucleic acid according to any of claims 35 to 37, optionally wherein the composition is a pharmaceutically acceptable composition.

39. The composition according to any of claim 38, further comprising another therapeutically or prophylactically active ingredient and/or an adjuvant.

40. The composition according to claim 38 or 39, wherein the composition comprises a pharmaceutically acceptable carrier and/or wherein the composition is a vaccine composition.

41. The composition according to any of claims 38 to 40, the viral vector according to any of claims 1 to 34 or the nucleic acid according to any of claims 35 to 37, for use in the prophylaxis or treatment of HBV infection in a subject; optionally wherein the use is as a vaccine.

42. Use of the composition according to any of claims 38 to 40, the viral vector according to any of claims 1 to 34 or the nucleic acid according to any of claims 35 to 37, in the manufacture of a medicament for the treatment or prophylaxis of HBV infection, wherein the medicament is to be administered to a subject.

43. The composition, the viral vector or the nucleic acid for use according to claim 41, or the use according to claim 42, wherein the use or administration is in combination with the use or administration of another therapeutically or prophylactically active ingredient.

44. A prime boost vaccination kit comprising

-a prime vaccination comprising the composition according to any of claims 38 to 40, the viral vector according to any of claims 1 to 34 or the nucleic acid according to any of claims 35 to 37; and

-a boost vaccination comprising the composition according to any of claims 38 to 40, the viral vector according to any of claims 1 to 34 or the nucleic acid according to any of claims 35 to 37.

45. The prime and boost vaccination kit according to claim 44, wherein the prime and boost vaccinations comprise different viral vectors.