
HBV antigen formulation for treating Hepatitis B

I. FIELD OF THE INVENTION

[0001] The invention aims at providing a, preferably curative, therapeutic vaccination of a (chronic) Hepatitis B Virus (HBV) infection. In particular, the present invention relates to an Hepatitis B Virus core antigen (HBcAg) particle, comprising Hepatitis B Virus (HBV) core proteins from at least two different HBV genotypes. The present invention relates also to a pharmaceutical composition comprising the HBcAg particle disclosed herein and optionally a pharmaceutically acceptable carrier or excipient. The present invention relates also to a container comprising one or more doses of the pharmaceutical composition comprising the HBcAg particle disclosed herein. The present invention relates also to a kit comprising the pharmaceutical composition comprising the HBcAg particle disclosed herein and a second pharmaceutical composition comprising an HBsAg. The present invention relates also to the HBcAg particle disclosed herein, optionally comprised in the pharmaceutical composition disclosed herein, optionally comprised in the container disclosed herein or in the kit disclosed herein, for use in therapy. The present invention relates also to the HBcAg particle disclosed herein, optionally comprised in the pharmaceutical composition disclosed herein, optionally comprised in the container disclosed herein or in the kit disclosed herein, for use in treating an HBV infection. The present invention relates also to an expression cassette, an mRNA, or a cDNA, encoding an HBV core protein from HBV genotype C and an HBV core protein from HBV genotype D, wherein the expression cassette only comprises coding sequences for two or more HBV core proteins. The present invention relates also to a nucleic acid molecule comprising the expression cassette, mRNA, or cDNA disclosed herein. The present invention relates also to an expression vector comprising the expression cassette or the cDNA disclosed herein or the nucleic acid molecule disclosed herein. The present invention relates also to an expression vector encoding an HBV core protein from HBV genotype C and an HBV core protein from HBV genotype D, wherein the expression vector has at least 90% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 10. The present invention relates also to a vaccine vector wherein the vaccine vector, preferably an MVA viral vector, and wherein the vaccine vector comprises a nucleic acid molecule comprising a nucleotide sequence having at least 90% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 5. The present invention also relates to an mRNA or cDNA comprising a nucleotide sequence having at least 90% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 5. The present invention relates also to the vaccine vector disclosed herein, optionally comprised in the pharmaceutical

composition disclosed herein, optionally comprised in the container disclosed herein, for use in therapy. The present invention relates also to the vaccine vector or mRNA disclosed herein, optionally comprised in the pharmaceutical composition disclosed herein, optionally comprised in the container disclosed herein, for use in treating an HBV infection. The present invention relates also to a pharmaceutical composition comprising the vaccine vector disclosed herein and optionally a pharmaceutically acceptable carrier or excipient. The present invention relates also to a container comprising one or more doses of the pharmaceutical composition comprising the vaccine vector disclosed herein, wherein a dose of the pharmaceutical composition comprises the vaccine vector in an amount from about 1×10^7 ifu to about 1×10^9 ifu. The present invention relates also to a method of vaccination, comprising administering to a human subject (i) a first dose of an HBcAg particle and of an HBsAg, (ii) a second dose of the HBcAg particle and of the HBsAg, (iii) a dose of a vaccine vector wherein the vaccine vector expresses a HBsAg from HBV genotype A, which preferably comprises a sequence having at least 90 % sequence identity to the amino acid sequence set forth in SEQ ID NO: 11; a HBcAg from HBV genotype D, which preferably comprises a sequence having at least 90 % sequence identity to the amino acid sequence set forth in SEQ ID NO: 2 or 12; an HBsAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 7; a HBcAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 8 or 17; and an RT domain of a polymerase from HBV comprising a sequence having at least 90 % sequence identity to the amino acid sequence set forth in SEQ ID NO: 9. The present invention relates also to an HBcAg particle, and optionally an HBsAg, for use in a method of vaccination. The present invention relates also to a vaccine vector expressing a HBsAg from HBV genotype A; a HBcAg from HBV genotype D; a HBsAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 7; a HBcAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 8 or 17; and an RT domain of a polymerase from HBV comprising a sequence having at least 90 % sequence identity to the amino acid sequence set forth in SEQ ID NO: 9. for use in a method of vaccination. The present invention relates also to a use of an HBcAg particle, and optionally an HBsAg, for the manufacture of a medicament. The present invention relates also to a use of a vaccine vector expressing a HBsAg from HBV genotype A; a HBcAg from HBV genotype D; a HBsAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 7; a HBcAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 8 or 17; and an RT domain of a polymerase from HBV having at least 90 % sequence identity to the amino acid sequence set forth in SEQ ID NO: 9, for the manufacture of a medicament. The present invention relates also to a use of the expression cassette, mRNA or cDNA disclosed herein, the nucleic acid sequence disclosed

herein, the expression vector comprising the expression cassette disclosed herein, and/or the expression vector encoding an HBV core protein from HBV genotype C and an HBV core protein from HBV genotype D disclosed herein, for the manufacture of a medicament.

II. BACKGROUND

[0002] Hepatitis B is an inflammatory disease of the liver and is caused by infection with the hepatitis B virus (HBV). HBV is a human pathogenic, hepatotropic DNA virus with transmission by blood-blood contact and vertically from mother to child. HBV carriers usually do not develop an efficient immune response against the virus (immune tolerance) and are therefore not able to fight the infection efficiently. More specifically, they neither produce a detectable amount of neutralizing antibodies directed against HBV surface antigen (HBsAg), nor a significant T cell response directed against HBV antigens. As one of the most common infectious diseases worldwide, HBV is estimated to cause nearly 900,000 deaths annually with numbers predicted to rise in the absence of effective therapies. Despite the availability of a prophylactic vaccine since 40 years, about 1/3 of all human beings still become infected with HBV at some point during their lifetime.

[0003] It is well established that the host adaptive immune system is essential for efficient HBV control. Neutralizing antibodies directed against HBsAg prevent virus spread, e.g., to non-infected hepatocytes and seroconversion from HBsAg to anti-HBs represents the clinical endpoint of HBV-infection (Rehermann and Nascimben, Nat Rev Immunol 2005; 5:215-29). Patients clearing the virus develop strong polyclonal and multispecific CD8+ and CD4+ T-cell responses, whereas chronic infection is associated with depletion and progressive dysfunction of antiviral T-cells (Rehermann et al., J Exp Med 1995; 181:1047-58). So far, therapeutic vaccinations were designed to activate endogenous HBV-specific T-cell responses, but numerous clinical attempts, in particular in chronically infected patients, only had transient effects on anti-HBV immune responses and failed to control HBV (Gehring A and Protzer U., Targeting Innate and Adaptive Immune Responses to Cure Chronic HBV Infection. Gastroenterology, 2019 Jan;156(2):325-337. PMID 30367834). Continued exposure to high levels of circulating viral antigens seems to be a major hurdle for immunotherapeutic approaches because they induce tolerance and effector cell dysfunction. Also, combining therapeutic vaccination with antiviral treatment, that controls viremia but has no effect on circulating antigen levels, did not improve vaccine efficacy and clinical outcome (Michel et al., J Hepatol 2011; 54:1286-96).

[0004] The definition of an HBV cure is not trivial as complete virus elimination can be rarely achieved. For practical reasons, endpoints are used that can be quantitatively evaluated. Thus, a functional HBV cure has been defined by suppression of viremia, normalization of

alanine aminotransferase (ALT) levels and loss of HBsAg – ideally followed by anti-HBs seroconversion. Hence, a sustained therapeutic effect requires both antiviral suppression but in particular restoration of effector immune cell responses. Thus, a cure of an HBV infection should ideally be associated with virus-specific CD4+ and CD8+ T-cell responses. For example, chimpanzees were only able to cure an acute HBV infection, when functional CD4+ and CD8+ T-cell responses were restored after antibody-mediated depletion (cf., e.g., Thimme et al., CD8(+) T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection, J Virol, 2003, 77(1): p. 68-76). The essentials of T-cell responses to achieve an HBV cure is assumed to be based on their ability to eliminate HBV infected cells by their cytotoxic activity but also control HBV persistence, gene expression and replication in a non-cytolytic fashion (Guidotti., The role of cytotoxic T cells and cytokines in the control of hepatitis B virus infection, Vaccine, 2002, 20 Suppl 4: p. A80-2). Thus, a functional immune system and in particular HBV-specific T-cell responses are required to treat and, ideally, cure an HBV infection.

[0005] Currently available treatment strategies can be basically divided into i) concepts focusing on directly acting antivirals affecting different parts of the viral life cycle and ii) immune therapeutic approaches intending to stimulate the host immune response. Approved therapies for an HBV infection comprise nucleoside and/or nucleotide analogues and interferon alpha. Nucleoside and/or nucleotide analogues are costly and have cure rates comparable to cure rates of spontaneous HBV elimination by the immune system, and the use of interferon alpha is declining due to severe side effects when used for treatment. Alternative approaches are in clinical development, which comprise, e.g., attempting to specifically suppress the replication of HBV and/or viral protein expression. Attempts are also being made to stimulate the innate immune system or to induce specific adaptive immune responses against HBV components through the administration of HBV antigens. However, current treatment options are of limited efficacy so far, thus failing to cure an HBV infection.

[0006] Hence, there is still a need to have at hand alternative solutions for being able to treat an HBV infection, in particular a chronic HBV infection, that are preferably capable of providing an efficient immune stimulation.

III. SUMMARY

[0007] The invention aims at providing a, preferably curative, therapeutic vaccination of a Hepatitis B Virus (HBV) infection.

[0008] The present invention relates to a Hepatitis B Virus core antigen (HBcAg) particle, comprising Hepatitis B Virus (HBV) core proteins from at least two different HBV

genotypes, wherein the HBcAg particle is preferably an isolated HBcAg particle. The HBcAg particle is preferably a mosaic HBcAg particle.

[0009] The present invention relates also to a pharmaceutical composition comprising the HBcAg particle disclosed herein and optionally a pharmaceutically acceptable carrier or excipient.

[0010] The present invention relates also to a container comprising one or more doses of the pharmaceutical composition comprising the HBcAg particle disclosed herein.

[0011] The present invention relates also to a kit comprising the pharmaceutical composition comprising the HBcAg particle disclosed herein and a second pharmaceutical composition comprising a HBsAg.

[0012] The present invention relates also to the HBcAg particle disclosed herein, optionally comprised in the pharmaceutical composition disclosed herein, optionally comprised in the container disclosed herein or in the kit disclosed herein, for use in therapy.

[0013] The present invention relates also to the HBcAg particle disclosed herein, optionally comprised in the pharmaceutical composition disclosed herein, optionally comprised in the container disclosed herein or in the kit disclosed herein, for use in treating an HBV infection.

[0014] The present invention relates also to an expression cassette, mRNA, or cDNA encoding an HBV core protein from HBV genotype C and an HBV core protein from HBV genotype D, wherein the expression cassette only comprises coding sequences for two or more HBV core proteins.

[0015] The present invention relates also to a nucleic acid molecule comprising the expression cassette, mRNA, or cDNA disclosed herein.

[0016] The present invention relates also to an expression vector comprising the expression cassette or cDNA disclosed herein or the nucleic acid molecule disclosed herein.

[0017] The present invention relates also to an expression vector or an mRNA encoding full-length or truncated HBV core proteins from two different HBV genotypes.

[0018] The present invention relates also to an expression vector or an mRNA encoding an HBV core protein from HBV genotype C and an HBV core protein from HBV genotype D, wherein the expression vector has at least 90% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 10.

[0019] The present invention relates also to a vaccine vector, wherein the vaccine vector is preferably an MVA viral vector, and wherein the vaccine vector comprises a nucleic acid molecule comprising a nucleotide sequence having at least 90% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 5.

[0020] The present invention relates also to the vaccine vector disclosed herein, optionally comprised in the pharmaceutical composition disclosed herein, optionally comprised in the container disclosed herein, for use in therapy.

[0021] The present invention relates also to the vaccine vector disclosed herein, optionally comprised in the pharmaceutical composition disclosed herein, optionally comprised in the container disclosed herein, for use in treating an HBV infection.

[0022] The present invention relates also to a pharmaceutical composition comprising the vaccine vector or mRNA disclosed herein and optionally a pharmaceutically acceptable carrier or excipient.

[0023] The present invention relates also to a container comprising one or more doses of the pharmaceutical composition comprising the vaccine vector disclosed herein, wherein a dose of the pharmaceutical composition comprises the vaccine vector in an amount from about 1×10^7 ifu to about 1×10^9 ifu.

[0024] The present invention relates also to a method of vaccination, comprising administering to a human subject

- (i) a first dose of an HBcAg particle and of an HBsAg,
- (ii) a second dose of the HBcAg particle and of the HBsAg,
- (iii) a dose of a vaccine vector

wherein the vaccine vector expresses

- a. an HBsAg from HBV genotype A;
- b. an HBcAg from HBV genotype D;
- c. an HBsAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 7;
- d. an HBcAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 8 or 17; and
- e. an RT domain of a polymerase from HBV comprising a sequence having at least 90 % sequence identity to the amino acid sequence set forth in SEQ ID NO: 9.

[0025] The present invention relates also to an HBcAg particle, and optionally an HBsAg, for use in a method of vaccination.

[0026] The present invention relates also to a vaccine vector expressing

- an HBsAg from HBV genotype A;
- an HBcAg from HBV genotype D;
- an HBsAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 7;
- an HBcAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 8 or 17; and
- an RT domain of a polymerase from HBV comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 9.

for use in a method of vaccination.

[0027] The present invention relates also to the use of an HBcAg particle, and optionally an HBsAg, for the manufacture of a medicament.

[0028] The present invention relates also to the use of a vaccine vector expressing

- an HBsAg from HBV genotype A;
- an HBcAg from HBV genotype D;
- an HBsAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 7;
- an HBcAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 8; and
- an RT domain of a polymerase from HBV comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 9,

for the manufacture of a medicament.

[0029] The present invention relates also to the use of the expression cassette, mRNA, or cDNA disclosed herein, the nucleic acid molecule disclosed herein, the expression vector comprising the expression cassette or cDNA disclosed herein, and/or the expression vector encoding an HBV core protein from HBV genotype C and an HBV core protein from HBV genotype D disclosed herein, for the manufacture of a medicament.

IV. DETAILED DESCRIPTION

A. General structure of a Hepatitis B Virus (HBV) particle and HBV types

[0030] HBV particles comprise an outer envelope and an inner core that encloses both viral DNA and a DNA polymerase with reverse transcriptase activity. The outer envelope comprises lipids and embedded proteins, which are involved in viral binding of, and entry into, susceptible cells like human cells. A protein embedded in the outer envelope of an HBV particle is also herein referred to as both an HBV surface protein (HBs). The inner core of an HBV particle is a particulate capsid, more specifically an icosahedral nucleocapsid. A structural

protein of the inner core of an HBV particle, and thus of an HBV nucleocapsid, is herein also referred to as an HBV core protein (HBc).

[0031] Hepatitis B viruses are divided into four major serotypes (adr, adw, ayr, ayw) that can induce differential antibody responses based on antigenic epitopes present on the surface proteins, and into (at least) nine genotypes (A–I) according to overall nucleotide sequence variation of the HBV genome. HBV genotypes have a distinct geographical distribution and are used in tracing the evolution and transmission of the virus. Differences between genotypes affect disease severity, course and likelihood of complications, as well as response to treatment and possibly vaccination. HBV genotypes can be further divided into sub-genotypes, e.g. A1-5. For example, in Central Europe and the United States, the predominant sub-genotype is A2. However, only 1 % of the infected humans carry the A2 sub-genotype, while the majority of patients carry HBV of genotype B, C, or D. Thus, HBV genotypes and/or serotypes may regionally differ in their occurrence.

[0032] It has been shown that conventional HBV vaccines provide a better protection against HBV of the same (sub)genotype as the HBV antigens comprised in a given vaccine than to other (sub)genotypes. Thus, the present invention is directed at an efficient HBV vaccination method using components that ensure protection against more than one HBV (sub-) genotype.

B. The Hepatitis B Virus core antigen (HBcAg) particle of the disclosure

[0033] The present invention addresses the need for a therapy of an HBV infection that is associated with an induction of neutralizing antibodies and of a multi-specific T cell response directed to multiple HBV antigens and/or HBV genotypes by providing the embodiments as recited in the claims.

[0034] In particular, the present invention relates to an Hepatitis B Virus core antigen (HBcAg) particle, comprising Hepatitis B Virus (HBV) core proteins from at least two different HBV genotypes. The HBcAg particle is preferably an isolated HBcAg particle. The HBcAg particle is preferably recombinant. The HBcAg particle is preferably a mosaic HBcAg particle. The present invention relates, inter alia, also to a MVA viral vaccine vector expressing multiple HBV antigens from different HBV genotypes. Furthermore, the present invention relates, inter alia, also to a method of vaccination comprising administering to a human subject a first and a second dose of both the novel HBcAg particle and an HBsAg as two protein priming vaccinations as well as a dose of a vaccine vector expressing multiple HBV antigens from different HBV genotypes as a boost vaccination.

[0035] It may surprisingly be found that a novel HBcAg particle comprising HBV core proteins from multiple HBV genotypes can induce a broad immune response against the HBV

core proteins comprised in said particle. Moreover, the HBcAg particle may represent a powerful protein prime, in particular when combined with an, optionally adjuvanted, HBsAg. The HBsAg is preferably an isolated HBsAg. The HBsAg is preferably recombinant. Thus, balanced CD4⁺ TH1/TH2 T-cell responses for production of neutralizing anti-HBc and anti-HBs antibodies can preferably be induced. Surprisingly, preclinical data suggests that priming with HBcAg also supports anti-HBs immune response. Without wishing to be bound by theory, it is believed that this is caused by a mechanism that is commonly known as "interstructural help". Thus, the inventors of the present application have surprisingly found that simultaneous priming with HBcAg and HBsAg have a synergistic effect. When further combined with a subsequent boost comprising the vaccine vector of the present disclosure, a strong activation of cytotoxic CD8⁺ T-cells for elimination of infected cells can preferably be observed. Thus, the novel HBcAg particle, in combination with an (adjuvanted) HBsAg, and the vaccine vector may preferably constitute key components of a novel therapeutic vaccination regime preferably has the potential to even cure HBV infections.

[0036] More specifically, said novel therapeutic vaccination regime, herein optionally also referred to as VacB (or VacB vaccination regime; cf. e.g. **Figure 1**), was investigated in a first-in-human Phase 1a study conducted in healthy subjects for assessing safety and efficacy. Moreover, a phase 1b/2a trial to assess safety, tolerability and immunogenicity of a heterologous protein prime/MVA boost therapeutic hepatitis B vaccine candidate has been conducted. The present disclosure also provides a therapeutic vaccination comprising a novel (particulate) protein prime and a (recombinant) vaccine vector boost regimen. Priming with both an HBsAg and the novel, particulate HBcAg may induce HBV core- or S-specific CD4⁺ helper T-cell in peripheral blood lymphocytes and/or CD4⁺ T cell cytokines in the blood. These lymphocytes and/or cytokines may support CD8⁺ T-cells and neutralizing anti-HBs antibodies that complex circulating HBV antigens and thus, preferably help to counteract potential skewing of T-cell responses by (high) antigen levels. Furthermore, boosting with a vaccine vector, such as a (recombinant) MVA vector, expressing HBsAg, HBcAg and the RT domain of the HBV polymerase may preferably be found to expand primed cytotoxic CD8⁺ T-cells that may lead to an elimination of infected hepatocytes in the liver. Furthermore, the present invention encompasses the use of a combination of antigens from different HBV genotypes and/or serotypes. This combination may preferably induce a broad immune response against multiple HBV strains and, moreover, also a stronger immune response against each HBV genotype compared to vaccines comprising only single antigens and/or antigens from a single HBV genotype. Thus, the vaccination regime disclosed herein preferably represents an efficient and powerful novel approach to induce immune control of an HBV infection by inducing a strong and polyclonal T-cell response and neutralizing antibodies against different HBV antigens from several HBV genotypes. Thus, the immune system can preferably combat and eliminate HBV.

[0037] Hence, the therapeutic vaccination regime according to the present invention relates to a promising novel approach, preferably with broad applicability and an acceptable risk of side effects. The underlying concept of restoring endogenous immune functions by the therapeutic vaccination regime according to the present invention does preferably not only allow for a cure of an HBV infection, but may preferably also be favorable over costly and cumbersome long-term application of antiviral drugs, which aim at controlling rather than curing an HBV infection. Also, such a long-term application of antiviral drugs requires the patient's compliance, thus bearing the risk of discontinuous uptake, developing resistance and/or undesired side effects. Hence, the novel therapeutic vaccination regime according to the present invention may preferably pave the way to a new treatment strategy that, for the first time, allows to treat, and preferably also to cure, an HBV infection.

[0038] The present invention also relates to an HBV core antigen (HBcAg) particle, comprising HBV core proteins from at least two different HBV genotypes. Thus, said HBcAg particle comprises a combination of B-cell antigens and T-cell epitopes from HBV core proteins of different HBV genotypes. Such a combination may be advantageous to induce a broad immune response. Thus, an immune response against at least two and thus, multiple HBV genotypes, can preferably be induced. Moreover, such a combination is preferably capable of inducing a stronger immune response against each HBV genotype comprised in the combination compared to vaccines comprising only antigens from a single HBV genotype. For example, the HBcAg particle may comprise HBV core proteins from two, three, four or more genotypes. Thus, the HBcAg particle may comprise HBV core proteins from at least two genotypes selected from the group consisting of A, B, C, D, E, F, G, H and I. For example, the HBcAg particle may comprise HBV core proteins from two, three, four or more genotypes selected from the group consisting of A, B, C, D, E, F, G, H and I.

[0039] As used herein, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

[0040] Furthermore, herein the term "antigen" refers to a molecule which contains one or more epitopes that stimulate a host's immune system to make a cellular antigen-specific immune response, or a humoral antibody response. Antigens may include proteins, polypeptides, antigenic protein fragments and the like. Furthermore, the antigen can be derived from any known virus, bacterium, parasite, prion, plants, protozoans, or fungus and can be a whole organism. The term also includes tumor antigens. Synthetic antigens such as polypeptides, flanking epitopes, and other recombinant or synthetically derived antigens are also included in this application. Preferably, the antigen in the present invention is a polypeptide or protein.

[0041] In relation to the term “epitope”, the term “antigen” refers to a (longer) sequence, in particular a (longer) amino acid sequence or protein sequence, whereas the phrase “antigenic epitope” or “an epitope of the antigen” encompasses a stretch of shorter sequence from the longer sequence. The term “antigen” thus encompasses epitopes. The term “antigen” also includes variants of proteins, polypeptides, and antigenic protein fragments as described herein. Also, the term “antigen” encompasses sequences identical to the native sequence as well as modification to the native sequence, such as deletions, additions, insertions and substitutions. Preferably, an antigen variant has at least about 50%, at least about 60% or 65%, at least about 70% or 75%, at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, or 89%, more typically, at least about 90%, 91%, 92%, 93%, or 94% and even more typically at least about 95%, 96%, 97%, 98% or 99%, most typically, at least about 99% amino acid identity with the reference antigen (i.e. the antigen from which it is derived).

[0042] An epitope, also termed herein as “antigenic epitope”, forms part of the antigen that still elicit an immune response in a host. An epitope is, however, not limited to the exact sequence of the antigen from which it is derived. Thus, the term “epitope” encompasses sequences identical to the native sequence as well as modification to the native sequence, such as deletions, additions, insertions and substitutions. Preferably, an epitope variant have at least about 50%, at least about 60% or 65%, at least about 70% or 75%, at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, or 89%, more typically, at least about 90%, 91%, 92%, 93%, or 94% and even more typically at least about 95%, 96%, 97%, 98% or 99%, most typically, at least about 99% amino acid identity with the reference epitope (i.e. the epitope from which it is derived).

[0043] As used herein, a “Hepatitis B Virus core antigen”, “HBV core antigen” or “HBcAg” refers to an HBV core protein or a fragment thereof. A full-length HBV core protein is 183 amino acids in length and consists of an (self-) assembly domain (amino acids 1 to 149) and a nucleic acid-binding domain (amino acids 150 to 183). The 34-residue long nucleic acid-binding domain is extremely basic, with 17 arginines, consistent with its function, thus conferring a positive charge inside the capsid that enables the binding of the viral genome. During the viral life cycle, an RNA transcript of the viral genome (pgRNA) and the viral polymerase are selectively incorporated into the HBcAg capsid (stabilized by the positive charge), where the pgRNA becomes reverse transcribed into the viral genome (rcDNA). The viral genome containing capsids are surrounded by the viral envelop (containing HBsAg) and the so formed mature and infectious virions are secreted from the infected cells. When a (recombinant) HBcAg particle is produced for example in *E. coli* cells and thus, in the absence of pgRNA molecules, cellular RNAs may be bound to the positively charged C-terminal domain and may thus be encapsidated into the HBcAg instead of viral genome. Thus, an HBcAg particle “consisting of”

HBV core protein subunits of at least two different HBV genotypes is preferably understood as referring to an HBcAg particle not comprising any other HBV core protein or fragment thereof except the specifically recited ones while it may nevertheless comprise non-viral nucleic acids enclosed. Such non-viral nucleic acids may be enclosed in an HBcAg particle may be advantageous as it can stabilize the HBcAg particle.

[0044] The term “HBcAg” may thus relate herein to a full-length HBV core protein. Alternatively, the term may relate herein to a truncated HBV core protein and thus, a fragment of an HBV core protein. Preferably, such a fragment is C-terminally truncated, i.e. lacking at least one C-terminal amino acids, compared to a respective full-length HBV core protein, more preferably such a fragment is C-terminally truncated, i.e. lacking at least one C-terminal amino acid, compared to a respective full-length HBV core protein. Thus, such a truncated HBV core protein comprises preferably at least 100, more preferably at least 125, even more preferably at least 149, most preferably at least 163 consecutive amino acids of a respective full-length HBV core protein. Further, such a fragment is usually, and preferably, immunogenic. Preferably, such a truncated, preferably immunogenic, HBV core protein comprises at least amino acids 1 to 149, more preferably said HBV core protein comprises at least amino acids 1 to 163, or is a respective full length HBV core protein. For example, a respective HBV core protein of genotype A may preferably comprise at least amino acids 1 to 163 or be a full-length HBV core protein of genotype A. As a further example, an HBV core protein of genotype B may preferably comprise at least amino acids 1 to 163 or be a full-length HBV core protein of genotype B. As another example, an HBV core protein of genotype C may preferably comprise at least or consist of amino acids 1 to 163 or be a full-length HBV core protein of genotype C. As another example, an HBV core protein of genotype D may preferably comprise at least amino acids 1 to 163 or be an HBV core protein of genotype D.

[0045] As used herein, the term “isolated” HBcAg or HBsAg (particle) refers to an HBcAg or HBsAg (particle) that has been isolated from the environment in which it has been expressed. The isolated HBcAg or HBsAg (particle) comprises HBV core proteins or S-, M-, and/or L- proteins, which may have been expressed by a cell, such as a bacterial or mammalian host cell, or an *in vitro* translation system. Hence, an isolated HBcAg or HBsAg particle may refer to an HBcAg or HBsAg (particle) that has been partially or substantially purified from the environment in which it had been expressed and thus, e.g. from the cell (e.g. a cellular expression system) or the *in vitro* translation system that was used for HBV core or S-, M-, and/or L- protein expression. For example, an isolated HBcAg particle may be substantially free of cellular material (optionally) and/or culture medium or *in vitro* translation medium components, or of chemical precursors or other chemicals when chemically synthesized

(optionally) and may or may not contain host cell RNA. An isolated HBcAg or HBsAg particle is preferably *ex vivo*.

[0046] As used herein, the term “and/or” includes the meaning of “and,” “or,” and “all or any other combination of the elements connected by said term.”

[0047] As used herein, “immunogenic” refers to the ability of a particular substance, such as an antigen or epitope, to provoke an immune response in the body of a human or animal. In other words, immunogenicity is the ability to induce a humoral and/or cell mediated immune response. The ability of an antigen to elicit immune responses is called immunogenicity, which can be humoral and/or cell-mediated immune responses. Without wishing to be bound by theory, it is assumed that any naturally occurring HBV surface antigen, HBV core antigen and/or polymerase is immunogenic. Also, it is assumed that many variants and different genotypes and serotypes of naturally occurring HBV surface antigen, HBV core antigen or polymerase from HBV, in which one or more amino acids are exchanged, deleted or inserted compared to the naturally occurring sequence. As an illustrative example, an immunogenic variant of a naturally occurring HBV surface antigen is an HBV surface antigen, in which the “a” determinant epitope has been replaced with the “a” determinant of an HBs-antigen of another serotype. Typically, an immunogenic variant of a naturally occurring HBV surface antigen, HBV core antigen or polymerase may have 90 % sequence identity with the amino acid sequence of the natural occurring HBV surface antigen, HBV core antigen or polymerase from HBV.

[0048] The HBcAg of the present invention is in the form of a uniform or mosaic capsid particle, which is preferably a uniform or mosaic capsid. Thus, the HBcAg particle comprises HBV core proteins that form a particle such as a capsid. In solution, an HBV nucleocapsid comprises dimers of HBV core proteins. It is to be noted that not only full-length, but also truncated core proteins, comprising, e.g., only amino acids 1 to 149 or 1 to 163 of the respective full-length core protein, are preferably capable of forming capsids. As regards said capsid formation, dimers of HBV core antigens self-assemble into icosahedral (nucleo)capsids in solution comparable to HBV core proteins, forming preferably T3 particulate capsids comprising approximately 90 HBV core protein dimers or T4 particulate capsids comprising approximately 120 HBV core protein dimers. Said icosahedral particulate capsids have usually a size of about 30-34 nm in diameter. Accordingly, also the isolated HBcAg particle has preferably a size of about 20 to about 60 nm, preferably about 25 to about 50 nm, such as about 30-34 nm in diameter. Furthermore, the isolated HbcAg particle preferably has a capsid-like structure comprising HBV core proteins from at least two different HBV genotypes filled with host cell RNA. Such a HbcAg particle may be advantageous to trigger an immune response against multiple HBV genotypes. The isolated HBcAg particle may have a hydrodynamic radius of about

20 nm to about 80 nm, about 30 nm to about 70 nm, about 40 nm to about 60 nm, preferably about 45 nm to about 55 nm, such as about 48 nm to about 52 nm, preferably when measured by dynamic light scattering.

[0049] Herein, a “capsid” in the context of an (isolated) HbcAg particle refers to a capsid comprising, or consisting of, HBV core proteins and optionally nucleic acids like non-viral or viral RNA. For example, the HbcAg particle may be a capsid comprising bacterial or mammalian RNA.

[0050] Preferably, the HbcAg particle is a self-assembling particulate capsid. Preferably, the HbcAg particle is a self-assembling particulate mosaic capsid.

[0051] Preferably, the HBcAg particle comprises about 50 to 200 dimers of HBV core proteins, preferably about 80 to 100 dimers of HBV core proteins or about 110 to 130 dimers of HBV core proteins, even more preferably (about) 90 dimers of HBV core proteins to form a T=3 or (about) 120 dimers of HBV core proteins. This is advantageous as thus, the isolated HbcAg particle can form a T=3 in case of (about) 90 dimers of HBV core proteins, or to form a T=4 icosahedron in case of (about) 120 dimers of HBV core proteins. The HBcAg particle may also consist of about 50 to 200 dimers of HBV core proteins, preferably of about 80 to 100 dimers of HBV core proteins or of about 110 to 130 dimers of HBV core proteins, even more preferably of about 90 dimers of HBV core proteins or of about 120 dimers of HBV core proteins, most preferably of 90 or 120 dimers of HBV core proteins. Thus, the HBcAg particle preferably resembles the capsid of a naturally occurring HBV nucleocapsid that is preferably capable of triggering an immune response while avoiding any (HBV) viral genetic information transfer.

[0052] The HBcAg particle of the present invention comprises HBV core proteins from at least two different HBV genotypes.

[0053] Preferably, the HBV genotypes are selected from the group consisting of A, B, C, and D. Thus, the HBcAg particle may comprise HBV core proteins from at least two different HBV genotypes selected from the group consisting of A, B, C, and D. This preferably has the advantage of inducing an immune response against A, B, C, and/or D HBV genotypes that may be of particular relevance in some regions due to their frequent occurrence. For example, the HBcAg particle may comprise or consist of HBV core proteins from HBV genotypes i) A and B, ii) A and C, iii) A and D, iv) B and C, v) B and D or vi) C and D. As another example, the HbcAg particle may comprise or consist of HBV core proteins from HBV genotypes i) A, B and C, ii) A, B and D, or iii) B, C and D. As a further example, the HbcAg particle may comprise or consist of HBV core proteins from HBV genotypes A, B, C and D. Such an immune response may be determined, e.g. by measuring the level of CD4+ and CD8+ T cells specific for HBV core or S protein(s) and/or by measuring anti-S antibody titers.

[0054] The isolated HBcAg particle according to the present invention relates comprises HBV core proteins from at least two different HBV genotypes. Said at least two genotypes may be comprised in the isolated HbcAg particle with a given ratio. For example, in case of HBV core proteins from two genotypes, such a ratio may be from about 10:90 to about 90:10, about 20:80 to about 80:20, about 30:70 to about 70:30, or about 40:60 to about 60:40. For example, the ratio about 10:90, about 20:80, about 30:70, about 40:60, about 50:50, about 60:40, about 70:30, about 80:20, or about 90:10. Preferably, the HBV core proteins of the at least two different HBV genotypes are substantially balanced and thus, in case of two genotypes, for example, the HBcAg particle may comprise HBV core proteins from said two HBV genotypes in a ratio of, e.g. about 20:80, about 30:70, about 40:60, about 50:50, about 60:40, about 70:30, or about 80:20, preferably of about 40:60, about 50:50, or about 60:40. Ratio specified herein are preferably molar ratios. Preferably, the HBV core protein subunits from the at least two different HBV genotypes are in an approximately equimolar ratio.

[0055] For example, the HBV core proteins from the at least two different HBV genotypes may be in an approximately equimolar ratio. Thus, the isolated HBcAg particle may comprise HBV core proteins from at least two different genotypes, wherein said genotypes are in an approximately equimolar ratio. This may be advantageous to ensure a broad immune response against the HBV genotypes comprised in the HBcAg particle without any (substantial) bias towards a portion of the genotypes comprised in the HbcAg particle compared to the remaining genotypes comprised in said HbcAg particle. For example, in case of an HbcAg particle comprising HBV core proteins from four different HBV genotypes, said four different genotypes would be resembled in said HbcAg in an approximately equimolar ratio and thus, in an approximate ratio of 1:1:1:1. Hence, in the latter example a comparable strong immune response can preferably be observed against the four genotypes, the HBV core proteins of which may be comprised in an HbcAg according to the invention. In case of the genotypes being A, B, C, and D, the HBcAg particle preferably induces a comparable strong immune response against the respective HBV genotypes that may be of particular relevance in some regions due to their (frequent) occurrence. Thus, the HBcAg is well suited for a regionally optimized HBV therapy.

[0056] It is to be noted that herein the terms “about” and/or “approximately” refer to a deviation of 20% or less, preferably of 15% or less, more preferably of 10% or less, even more preferably of 5% or less, most preferably of 1% or less. Thus, said terms relate to a value that is within a deviation of, e.g., maximal 10% or 5% of a given value or range.

[0057] Preferably, the HBcAg particle comprises HBV core proteins of not more than two different HBV genotypes. Thus, said HBcAg particle may comprise HBV core proteins from at least two different HBV genotypes though of not more than two different HBV genotypes.

Hence, said HBcAg particle may comprise, or consist of, HBV core proteins of two different HBV genotypes.

[0058] Preferably, the HBV genotypes are selected from the group consisting of C and D. Thus, the HBcAg particle may comprise HBV core proteins from at least two different HBV genotypes selected from the group consisting of C and D. Thus, the HBcAg particle may comprise, or consist of, HBV core proteins from genotypes C and D. This may be particularly advantageous as thus broad immune response covering >95% of circulating HBV strains can be covered.

[0059] Preferably, the HBcAg particle comprises HBV core proteins from HBV genotypes C and D. The HBV core proteins from said HBV genotypes are preferably in an approximately equimolar ratio. Thus, the HBcAg particle may comprise or consist of HBV core proteins from genotypes C and D, wherein the HBV core proteins from said HBV genotypes are in a ratio from about 10:90 to about 90:10, about 20:80 to about 80:20, about 30:70 to about 70:30, about 40:60 to about 60:40, or in an approximately equimolar ratio. This may be advantageous for inducing a broad immune response against the HBV genotypes C and D without any (substantial) bias towards one of the two genotypes.

[0060] Preferably, the HBV core proteins comprise at least amino acids 1 to 163 or are full-length HBV core proteins. The HBcAg particle may thus comprise HBV core proteins from at least two different HBV genotypes, wherein the HBV core proteins are full-length HBV core proteins. Alternatively, at least a portion of the HBV core proteins may be truncated HBV core proteins. The HBcAg particle may thus comprise HBV core proteins from at least two different HBV genotypes, wherein at least a portion of the HBV core proteins are truncated HBV core proteins. For example, the HBcAg particle may comprise HBV core proteins of in total two genotypes, wherein HBV core proteins of one genotype are full-length and HBV core proteins of the other genotype are truncated. As another example, the HBcAg particle may comprise HBV core proteins of in total two genotypes, wherein both of the HBV core proteins are full length. As another example, the HBcAg particle may comprise HBV core proteins of in total two genotypes, wherein a portion of the HBV core proteins of one of the two genotypes is truncated. As a further example, the HbcAg particle may comprise HBV core proteins of in total two genotypes, wherein a first portion of the HBV core proteins of one of the two genotypes is truncated and a second portion of the HBV core proteins of the other of the two genotypes is truncated. As a further example, the HBcAg particle may comprise HBV core proteins of more than two genotypes, wherein a first portion of the HBV core proteins of one of the genotypes is truncated and the HBV core proteins of the other of the genotypes are truncated. As a further example, the HBcAg particle may comprise HBV core proteins of two or more genotypes, wherein a first portion of the HBV core proteins of one of the genotypes is truncated and the

HBV core proteins of the other of the genotypes are full length. As a further example, the HbcAg particle may comprise HBV core proteins of two or more genotypes, wherein all HBV core proteins are full length. Hence, the HBcAg particle may comprise full-length and truncated HBV core proteins. Alternatively, the HBcAg particle may not comprise full-length HBV core proteins or may consist of truncated HBV core proteins.

[0061] Preferably, the HBcAg particle comprises truncated HBV core proteins from HBV genotype C and full-length HBV core proteins from HBV genotype D. Thus, the HBcAg particle may comprise HBV core proteins, wherein at least a portion of the HBV core proteins of HBV genotype C are truncated HBV core proteins and wherein HBV core proteins of HBV genotype D are full-length HBV core proteins. Preferably, the truncated HBV core proteins from HBV genotype C refer to a deletion of the C-terminal 20aa. Thus, no antigenic epitope is lost by the deletion of the 20aa in genotype C monomers compared to full length genotype D monomers. Hence, a truncation is preferably chosen to not impair the immunogenicity of the HBcAg particle compared to an HBcAg particle comprising no truncated but only full-length HBV core proteins of the same HBV genotypes. Further, it is preferred that the HbcAg particle does not comprise full-length HBV core proteins from HBV genotype C but truncated HBV core proteins from HBV genotype C and full-length HBV core proteins from HBV genotype D. Preferably, the HBcAg particle thus consists of truncated HBV core proteins from HBV genotype C and full-length HBV core proteins from HBV genotype D. This may be advantageous especially from a technical point of view as different protein length allow for an easy and cheap analytical detection of HBV core proteins from different genotypes like genotypes C and D. For example, presence and ratio of the different protein types comprised in an HBcAg particle can be shown, e.g. by a SDS-PAGE after HBcAg particle pull-down.

[0062] The HBcAg particle may comprise a mixture of truncated HBV core proteins and full-length HBV core proteins from different HBV genotypes. Preferably, the HbcAg particle comprises truncated HBV core proteins from one HBV genotype and full-length HBV core proteins from another HBV genotype. The genotypes can be individually from the group consisting of A, B, C, D, E, F, G, H and I, preferably from the group consisting of A, B, C, and D, preferably from the group consisting of C and D. Preferably, the HBcAg particle comprises truncated HBV core proteins from HBV genotype C and full-length HBV core proteins from HBV genotype D (or less preferred *vice versa*). The HBV core proteins from said HBV genotypes are preferably in a ratio from about 10:90 to about 90:10, about 20:80 to about 80:20, about 30:70 to about 70:30, about 40:60 to about 60:40, or in an approximately equimolar ratio. Thus, HBV core proteins comprised in the HBcAg particle may be observed in an approximate ratio of about 30:70 to about 70:30, about 40:60 to about 60:40, or about 50:50 as regards the genotypes C and D. This may be advantageous for inducing a broad immune response against

the HBV genotypes C and D, which may be of particular relevance in some regions due to their (frequent) occurrence, without any (substantial) bias towards one of the two genotypes. Preferably, the HBcAg particle thus consists of truncated HBV core proteins from HBV genotype C and full-length HBV core proteins from HBV genotype D, wherein the HBV core proteins from said HBV genotypes are preferably in a ratio from about 10:90 to about 90:10, about 20:80 to about 80:20, about 30:70 to about 70:30, about 40:60 to about 60:40, or in an approximately equimolar ratio.

[0063] As indicated above, the HbcAg particle is preferably a self-assembling particulate capsid that may comprise about 50 to 200 dimers of HBV core proteins. Thus, the HBcAg particle preferably comprises – or consists of – dimers of HBV core proteins, wherein said dimers are assembled from i) HBV core proteins from HBV genotype C, ii) HBV core proteins from HBV genotype C and HBV core proteins from HBV genotype D, and/or iii) HBV core proteins from HBV genotype D.

[0064] Furthermore, in view of the length of the HBV core proteins comprised in an HBcAg particle, it may be noted that the HBcAg particle preferably comprises dimers of HBV core proteins, wherein said dimers are assembled from i) truncated HBV core proteins from HBV genotype C, ii) truncated HBV core proteins from HBV genotype C and full-length HBV core proteins from HBV genotype D, and/or iii) full-length HBV core proteins from HBV genotype D. Preferably the HBcAg particle even consists of dimers of HBV core proteins, wherein said dimers are assembled from i) truncated HBV core proteins from HBV genotype C, ii) truncated HBV core proteins from HBV genotype C and full-length HBV core proteins from HBV genotype D, and/or iii) full-length HBV core proteins from HBV genotype D. As above, it is preferred that said HBV genotypes are in an approximately equimolar ratio, thus enabling the HBcAg particle to induce an, preferably comparable immune response against HBV core proteins of both genotypes C and D while allowing to distinguish the two HBV core protein types and thus, to assess the HBcAg particle's HBV core protein composition analytically.

[0065] As regards preferred HBV proteins, said proteins are preferably defined based on their sequence identity to a given reference sequence. Techniques for determining sequence identity between two sequences of nucleic acids or amino acids are well known and established in the art. Two or more sequences (polynucleotide or amino acid) can be compared by determining their "percent identity." The percent identity of two sequences, whether nucleic acid or amino acid sequences, is the number of exact matches between two aligned sequences divided by the length of the shorter sequences and multiplied by 100.

[0066] As used herein, the term "sequence identity" or "identity" denotes a property of sequences that measures their similarity or relationship. The term "sequence identity" or

"identity" as used in the present disclosure means the percentage of pair-wise identical residues – following (homologous) alignment of a sequence of a protein or polypeptide of the disclosure with a sequence in question – with respect to the number of residues in the sequence specified as "reference" of these two sequences. Sequence identity is measured by dividing the number of identical amino acid residues by the total number of residues and multiplying the product by 100. A skilled artisan will recognize available computer programs, for example BLAST (Altschul et al., 1997), BLAST2 (Altschul et al., 1990), FASTA (Pearson and Lipman, 1988), GAP (Needleman and Wunsch, 1970), Smith-Waterman (Smith and Waterman, 1981), and Wisconsin GCG Package, for determining sequence identity using standard parameters. The percentage of sequence identity can, for example, be determined herein using the program BLASTP, version 2.2.5, November 16, 2002 (Altschul et al., 1997), calculating the percentage of numbers of "positives" (homologous amino acids) from the total number of amino acids selected for the alignment.

[0067] "Percent (%)sequence identity" with respect to antigens, epitopes and/or proteins described herein is preferably defined on amino acid level and thus, as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the respectively specified reference sequence (i.e. the antigen from which it is derived and/or to which it is compared), after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publically available computer software such as BLAST, ALIGN, or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximum alignment over the full length of the sequences being compared. The same is applicable to nucleotide sequences, mutatis mutandis.

[0068] For example, an appropriate alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman, (1981), *Advances in Applied Mathematics* 2: 482-489. This algorithm can be applied to amino acid sequences by using the scoring matrix developed by Dayhoff, *Atlas of Protein Sequences and Structure*, M. O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA, and normalized by Gribskov (1986), *Nucl. Acids Res.* 14(6): 6745-6763. An exemplary implementation of this algorithm to determine percent identity of a sequence is provided by the Genetics Computer Group (Madison, Wis.) in the "BestFit" utility application. The default parameters for this method are described in the Wisconsin Sequence Analysis Package Program Manual, Version 8 (1995) (available from Genetics Computer Group, Madison, Wis.). A

preferred method of establishing percent identity in the context of the present invention is to use the MPSRCH package of programs copyrighted by the University of Edinburgh, developed by John F. Collins and Shane S. Sturrok, and distributed by IntelliGenetics, Inc. (Mountain View, Calif). From this suite of packages the Smith-Waterman algorithm can be employed where default parameters are used for the scoring table (for example, gap open penalty of 12, gap extension penalty of one, and a gap of six). From the data generated the "Match" value reflects "sequence identity." Other suitable programs for calculating the percent identity or similarity between sequences are generally known in the art, for example, another alignment program is BLAST, used with default parameters. For example, BLASTN and BLASTP can be used using the following default parameters: genetic code=standard; filter=none; strand=both; cutoff=60; expect=10; Matrix=BLOSUM62; Descriptions=50 sequences; sort by=HIGH SCORE; Databases=non-redundant, GenBank+EMBL+DDBJ+PDB+ GenBank CDS translations+Swiss protein+Spupdate+PIR. Details of these programs can be found at the following internet address: <http://www.ncbi.nlm.gov/cgi-bin/BLAST>.

[0069] As regards the HBV core proteins comprised in an HbcAg particle of the invention, the HBV core proteins from HBV genotype C have preferably at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1 or 13. Additionally or alternatively, the HBV core proteins from HBV genotype D have at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence set forth in SEQ ID NO: 2 or 14. Thus, the HBV core proteins from HBV genotype C have preferably at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1 or 13 and/or HBV core proteins from HBV genotype D have at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 2 or 14. Preferably, the HbcAg particle of the invention comprises or consists of i) HBV core proteins from HBV genotype C having at least 95% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1 or 13 and/or ii) HBV core proteins from HBV genotype D having at least 95% sequence identity to the amino acid sequence set forth in SEQ ID NO: 2 or 14. Preferably, the HbcAg particle comprises HBV core proteins, wherein HBV core proteins from HBV genotype C have the amino acid sequence set forth in SEQ ID NO: 1 or 13 and/or HBV core proteins from HBV genotype D have the amino acid sequence set forth in SEQ ID NO: 2 or 14. Preferably, the HbcAg particle consists of HBV core proteins, wherein HBV core proteins from HBV genotype C have the amino acid sequence set forth in SEQ ID NO: 1 or 13 and/or HBV core proteins from HBV genotype D have the amino acid sequence set forth in SEQ ID NO: 2 or 14. Thus, a preferred example of a truncated HBV core protein from HBV genotype C is a truncated HBV core protein that consists of amino acids 1 to 149 or 1 to 163 of the respective full-length HBV core protein set forth in SEQ ID NO: 8, thus having the sequence set forth in SEQ ID NO: 1 or 13.

[0070] Further, as indicated above, the HbcAg particle of the present invention comprises preferably immunogenic HBV core proteins, or fragments thereof, of at least two different HBV genotypes. Thus, the HbcAg particle preferably induces an immune response against the comprised antigenic HBV core proteins of multiple HBV genotypes. Thus, preferably the HbcAg particle is preferably capable of inducing i) an immune response against the HBV core proteins it is composed of and/or ii) an antigen-specific adaptive immune response. Preferably, said immune response is associated with i) anti-HBcAg antibody induction and/or with ii) HBcAg-specific CD4+/CD8+ T-cell induction. Thus, strong polyclonal and multi-specific CD8+ and CD4+ T-cell responses can preferably be induced.

[0071] As regards said immune responses, it is to be noted that an adaptive immunity refers to an antigen-dependent and antigen-specific immune response. An adaptive immune response involves a lag time between exposure to an antigen and maximal response and has the capacity for memory, which enables a rapid and efficient immune response upon subsequent exposure to said antigen. Key functions of an adaptive immune response are the recognition of specific "non-self" antigens, their distinction from "self" antigens, the generation of pathogen-specific immunologic effector pathways that eliminate specific pathogens and/or pathogen-infected cells and the development of an immunologic memory that can quickly eliminate a specific pathogen should subsequent infections occur (cf., e.g., Bonilla and Oettgen, Adaptive immunity. *J Allergy Clin Immunol.* 2010;125(Suppl 2):S33–40). Adaptive immune responses are the basis for effective immunization against infectious diseases like HBV with cells of the adaptive immune system including B cells, which differentiate into plasma cells to produce antibodies, and antigen-specific T cells. Said T cells, can be stimulated to differentiate, e.g., into cytotoxic CD8+ cells or CD4+ T-helper cells. CD8+ cytotoxic T cells are primarily involved in the destruction of cells infected by foreign agents, such as viruses. Upon resolution of the infection, few of these stimulated and differentiated CD8+ cells are retained as memory cells that can quickly differentiate into effector cells upon subsequent encounters with the same antigen. Thus, induction of an immune response, preferably an adaptive immune response, wherein the immune response is ideally associated with HBcAg- and HBsAg-specific CD4+/CD8+ T-cell induction and/or anti-HBsAg antibody production may be highly advantageous for an efficient therapy of an infection like HBV.

[0072] The present invention relates also to a pharmaceutical composition comprising the HBcAg particle as disclosed herein above and optionally a pharmaceutically acceptable carrier or excipient. Such additional factors and/or agents may be included in the pharmaceutical composition comprising the HBcAg particle as disclosed herein to preferably produce a synergistic effect and/or minimize side-effects. Thus, the pharmaceutical composition may comprise one or more excipient and/or one or more pharmaceutically acceptable and/or

approved carrier as additive, optionally also one or more selected from the group consisting of an adjuvant, a preservative, an antibiotic, a diluent, peptides and/or a stabilizing excipient. Such auxiliary substances can be, e.g., water, saline, glycerol, ethanol, wetting or emulsifying agents, a detergent, an amino acid, a sugar, a surfactant, such as a kolliphor, pH buffering substances, or the like.

[0073] The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the HBcAg particle according to the present invention but rather stabilizes it against environmental stress. The characteristics of the carrier will depend on the route of administration and whether the vaccine antigens are lyophilized or used in solution. The pharmaceutical composition may further contain other agents which either enhance the activity or use in treatment. Suitable pharmaceutically acceptable carriers and/or excipients are typically large, slowly metabolized molecules such as modified nucleic acids, proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates, or the like.

[0074] Preferably, the pharmaceutical composition comprising the HBcAg particle as disclosed further comprises an HBV surface antigen (HBsAg), wherein the HBsAg is preferably a particulate HBsAg. This may be advantageous to further strengthen immune response induction. Thus, the pharmaceutical composition can be considered an efficient component of an effective HBV infection therapy. While HBsAg and HBcAg particle are preferably comprised both in the pharmaceutical composition, it may also be envisioned that the HBcAg particle is comprised in a first pharmaceutical composition and the HBsAg in a second pharmaceutical composition, wherein said first and said second pharmaceutical composition can be administered separately, approximately simultaneously or simultaneously. Alternatively, it may be envisioned that said first and said second pharmaceutical compositions are mixed together before, preferably directly before, administration, e.g. via intramuscular injection.

[0075] Herein, a "HBV surface antigen" or "HBsAg" refers to a transmembrane protein of HBV that forms the viral envelop, which comprises a cell-derived lipid bilayer with embedded HBV surface (HBs) proteins, or a fragment thereof. As used herein, "HBsAg" may comprise the S protein only, but may also comprise one or more pre-S regions, such as pre-S1 region and/or pre-S2 region. Thus, as used herein "HBsAg" may contain the small (S) as well as the middle (M) and large (L) envelope proteins. HBV surface proteins confer the binding of the HBV virion to their respective receptors, NTCP, e.g., on hepatocytes. An HBV core or surface protein of either HBV genotype, or a fragment thereof, can act as an HBV antigen. Thus, upon its recognition by the immune system, neutralizing antibodies can be induced that are directed against the HBsAg (anti-HBsAg antibodies). Therefore, HBV surface antigens represent the antigenic component of all prophylactic HBV vaccines to date. More specifically, an HBV

surface protein may relate on any one of the three variants, the small (S), middle (M), and large (L) surface protein, which are translated from distinct mRNAs. Common to all three variants, is S protein containing the “a” determinant that is located at codon positions 124 to 147 within the major hydrophilic region (MHR) of the S gene. This “a” determinant is one of the main targets of anti-HBs antibodies during the course of the initial immune response in acute hepatitis B. On the nucleic acid level in the genome, the pre-S/S gene has three in-frame initiation codons and encodes the small (S) as well as the middle (M) and large (L) envelope proteins, which contain pre-S2 and pre-S (pre-S1 and pre-S2) sequences, respectively. More specifically, the M protein is an extension of the S protein, with an additional 55 amino acids (i.e., pre-S2 region), and the L protein is an extension of the M protein, with an additional 108-119 amino acids depending on the genotype (i.e. pre-S1 region). The amino acids sequence at the C terminus of the L and M protein, respectively, is identical to the S protein and is referred to as the S region. The pre-S (pre-S1 and pre-S2) region of the L protein may be crucial for viral replication.

[0076] An HBsAg of the disclosure is preferably an antigen composed of HBV surface protein monomers or dimers, or a, preferably immunogenic, fragment thereof. The HBsAg is preferably a particulate antigen. A, preferably immunogenic, fragment of a surface protein relates to proteins or peptides derived from any full-length surface protein of any HBV serotype or genotype that is N-terminally and/or C-terminally shortened, i.e. lacking at least one of the N-terminal and/or C-terminal amino acids. Such a fragment comprises preferably at least 70, preferably at least 80, preferably at least 90, preferably at least 100, more preferably at least 125, most preferably at least 150 consecutive amino acids of the primary sequence of a surface protein and is usually immunogenic. Typically, such a, preferably immunogenic, fragment comprises compared to the full-length protein at least amino acids 99 to 168 corresponding to the amino acid positions of the small surface protein.

[0077] Preferably, the pharmaceutical composition comprising the HBcAg particle as disclosed may further comprise a pharmaceutically acceptable carrier and/or excipient, such as a stabilizer and/or a stabilizing agent. For example, such a stabilizing agent may be phosphate buffered saline (PBS). PBS may be advantageous for stabilization of the HBsAg, which may be present in the pharmaceutical composition embedded in a particulate lipid bilayer.

[0078] Accordingly, the pharmaceutical composition comprising the HBcAg particle as disclosed, optionally comprising an HBsAg, preferably comprises further an adjuvant, preferably a nucleosidic adjuvant, more preferably a CpG, most preferably CpG-1018. Further examples of suitable adjuvants comprise adjuvants other than alum, e.g. composite adjuvants containing MPL and QS21, poly-IC, polyIC-LC, SD101 and toll-like, Rig-I-like or other pattern-recognition receptors. The CpG adjuvant CpG-1018 is an unmethylated cytosine phosphoguanosine (CpG) enriched oligodeoxynucleotide (ODN) immunostimulatory adjuvant that mediates its effect by

binding to TLR9. CpG-1018 preferably has the advantage of being comprised for example in the commercially available HEPLISAV-B® and thus, represents a well-studied adjuvant for a HBsAg.

[0079] The present invention relates also to a container comprising one or more doses of the pharmaceutical composition comprising the HBcAg particle as disclosed herein above. Thus, said container may represent a packing unit of the pharmaceutical composition as described herein above.

[0080] A “dose”, and more specifically an “effective dose” or even more specifically, a “therapeutically effective dose”, refers herein to that amount of a given compound, ingredient and/or therapeutic agent that is sufficient to result in amelioration of symptoms, e.g. treatment, healing, prevention or amelioration of a given condition like an HBV infection. Thus, such an amount of a given compound, ingredient and/or therapeutic agent is an amount sufficient to effect beneficial or desired effects of a treatment. Herein, said doses may preferably refer to a therapeutic effect of a given compound or ingredient like an HBcAg particle or an HBsAg, wherein said therapeutic effect is preferably a curative effect. However, also a prophylactic effect may be encompassed. Effective doses affecting the immune response vary depending upon many different factors, including the type of antigen or vaccine, means of administration, addition of adjuvant, target site, whether the subjects human or an animal, and whether treatment is prophylactic or curative. However, the skilled person is aware of suitable techniques to assess therapeutically effective doses for a given combination of component, route of administration etc. Preferred doses of the HBcAg particle or the HBsAg are disclosed herein further below.

[0081] An effective dose can be administered in one or more individual administrations like intramuscular injections. Furthermore, a dose can be administered alone with one agent or in combination with one or more additional agents.

[0082] Accordingly, the present invention relates also to a kit comprising the pharmaceutical composition comprising the HBcAg particle as disclosed herein above and a second pharmaceutical composition comprising an HBsAg. More specifically, in case of said kit, the pharmaceutical composition comprising the HBcAg particle as disclosed herein above does preferably not further comprise an HBsAg. Thus, said kit may refer to a packing unit of a pharmaceutical composition comprising the HBcAg particle and a second pharmaceutical composition comprising an HBsAg, wherein said pharmaceutical compositions can be administered, e.g., separately, approximately simultaneously or simultaneously. Alternatively, it may be envisioned that said pharmaceutical compositions are either lyophilized or formulated

together or mixed together before, preferably directly before, administration, e.g. via intramuscular injection.

[0083] Preferably, the second pharmaceutical composition further comprises an adjuvant. As regards said adjuvant, the same preferably applies as stated herein above in the context of the pharmaceutical composition comprising the HBcAg particle of the invention. Thus, said adjuvant is preferably a nucleosidic adjuvant. Said adjuvant is particularly preferred a CpG, preferably CpG-1018.

[0084] The kit may comprise a first container comprising one or more doses of the pharmaceutical composition comprising the HBcAg particle and a second container comprising one or more doses of the second pharmaceutical composition. Alternatively, the kit may comprise a first container comprising one or more doses of a pharmaceutical composition comprising both, an HBsAg and an HBcAg as disclosed herein. The kit may optionally comprise a third container comprising an adjuvant. Thus, said kit may represent another version of a packing unit, wherein the HBcAg particle, the HBsAg and the adjuvant are comprised in different containers and wherein it is envisioned that said components can be administered, e.g., separately, approximately simultaneously or simultaneously. Alternatively, it may be envisioned that said pharmaceutical compositions are either lyophilized or formulated together or mixed together before, preferably directly before, administration, e.g. via intramuscular injection.

[0085] Preferably, the adjuvant comprised in the second pharmaceutical composition and/or the adjuvant comprised in the third container is a nucleosidic adjuvant, preferably a CpG, even more preferably CpG-1018. Furthermore, as regards said adjuvant preferably the same applies as stated above herein in the context of the pharmaceutical composition comprising the HBcAg particle disclosed herein.

[0086] Preferably of the container or the kit, a dose of the pharmaceutical composition comprises the HBcAg particle in an amount from about 10µg to about 100µg, preferably in an amount from about 10µg to about 75µg, more preferably in an amount from about 20µg to about 100µg, preferably to about 60µg, most preferably in an amount of about 25µg or of about 50µg.

[0087] Preferably, a dose of the pharmaceutical composition in case of the container and/or a dose of the second pharmaceutical composition in case of the kit comprises HBsAg, wherein the HBsAg is preferably a particulate HBsAg.

[0088] Preferably, in case of the container, a dose of the pharmaceutical composition comprises HBsAg in an amount from about 5µg to about 100µg, preferably from about 5µg to about 75µg, preferably in an amount from about 10µg to about 50µg, more preferably in an

amount from 20µg or of about 40µg. As kit and container may be understood as referring to different packaging units of the pharmaceutical composition comprising the HBcAg of the present invention, the same applies mutatis mutandis in case of the kit. Accordingly, in the kit, a dose of the second pharmaceutical composition preferably comprises HBsAg in an amount from about 5µg to about 100µg, preferably from about 5µg to about 75µg, preferably in an amount from about 10µg to about 50µg, more preferably in an amount from 20µg or of about 40µg. This dosing is particularly envisioned and also in line with study reports of recombinant HBsAg produced in yeast that has been used in a number vaccines, like EngerixB, Fendrix, and HEPLISAV-B, at doses up of 20-40 µg for adults and thus, dosing of HBsAg using amounts from about 20µg to about 40µg has a very high and proven safety profile (cf. e.g., Halperin et al., Comparison of the safety and immunogenicity of hepatitis B virus surface antigen co-administered with an immunostimulatory phosphorothioate oligonucleotide and a licensed hepatitis B vaccine in healthy young adults, *Vaccine*. 2006 Jan 9;24(1):20-6. Doi: 10.1016/j.vaccine.2005.08.095. Epub 2005 Sep 12. PMID: 16198027; Halperin et al., Comparison of safety and immunogenicity of two doses of investigational hepatitis B virus surface antigen co-administered with an immunostimulatory phosphorothioate oligodeoxyribonucleotide and three doses of a licensed hepatitis B vaccine in healthy adults 18-55 years of age. *Vaccine*. 2012 Mar 28;30(15):2556-63. Doi: 10.1016/j.vaccine.2012.01.087. Epub 2012 Feb 9. PMID: 22326642; Heyward et al., Immunogenicity and safety of an investigational hepatitis B vaccine with a Toll-like receptor 9 agonist adjuvant (HBsAg-1018) compared to a licensed hepatitis B vaccine in healthy adults 40-70 years of age. *Vaccine*. 2013 Nov 4;31(46):5300-5. Doi: 10.1016/j.vaccine.2013.05.068. Epub 2013 May 30. PMID: 23727002; Jackson et al., HBV-23 Study Group. Immunogenicity of a two-dose investigational hepatitis B vaccine, HbsAg-1018, using a toll-like receptor 9 agonist adjuvant compared with a licensed hepatitis B vaccine in adults. *Vaccine*. 2018 Jan 29;36(5):668-674. Doi: 10.1016/j.vaccine.2017.12.038. Epub 2017 Dec 27. PMID: 29289383; Sablan et al., Demonstration of safety and enhanced seroprotection against hepatitis B with investigational HBsAg-1018 ISS vaccine compared to a licensed hepatitis B vaccine. *Vaccine*. 2012 Mar 30;30(16):2689-96. doi: 10.1016/j.vaccine.2012.02.001. Epub 2012 Feb 14. PMID: 22342916; Janssen et al., Immunogenicity and safety of an investigational hepatitis B vaccine with a toll-like receptor 9 agonist adjuvant (HBsAg-1018) compared with a licensed hepatitis B vaccine in patients with chronic kidney disease. *Vaccine*. 2013 Nov 4;31(46):5306-13. doi: 10.1016/j.vaccine.2013.05.067. Epub 2013 May 30. PMID: 23727422.).

[0089] Furthermore, in the container, a dose of the pharmaceutical composition preferably comprises the HBcAg particle in an amount of about 25µg or of about 50µg and a dose of the pharmaceutical composition comprises, preferably particulate, HBsAg in an amount of about 20µg or of about 40µg, preferably, a dose of the pharmaceutical composition

comprises the HBcAg particle in an amount of about 25µg and a dose of the pharmaceutical composition comprises, preferably particulate, HBsAg in an amount of about 20µg; even more preferably, a dose of the pharmaceutical composition comprises the HBcAg particle in an amount of about 50µg and a dose of the pharmaceutical composition comprises, preferably particulate, HBsAg in an amount of about 40µg. As kit and container may be understood as referring to different packaging units of the pharmaceutical composition comprising the HBcAg of the present invention, the same applies mutatis mutandis in case of the kit. Accordingly, in case of the kit, a dose of the pharmaceutical composition preferably comprises the HBcAg particle in an amount of about 25µg or of about 50µg and a dose of the second pharmaceutical composition comprises HBsAg in an amount of about 20µg or of about 40µg; preferably, a dose of the pharmaceutical composition comprises the HBcAg particle in an amount of about 25µg and a dose of the second pharmaceutical composition comprises HBsAg in an amount of about 20µg; even more preferably, a dose of the pharmaceutical composition comprises the HBcAg particle in an amount of about 50µg and a dose of the second pharmaceutical composition comprises HBsAg in an amount of about 40µg.

[0090] The present invention relates also to the disclosed HBcAg particle or the disclosed pharmaceutical composition comprising the HBcAg particle, or comprised in the disclosed container or in the disclosed kit, for use in therapy. More specifically, said components are preferably used in therapy and/or vaccination, preferably in therapeutic vaccination, preferably against HBV. Furthermore, it is to be noted that herein “therapy” and “therapeutic” may encompass both “cure” and “curative” as well as “prevention” and “preventive”. Thus, while the disclosed HBcAg particle or the disclosed pharmaceutical composition comprising the HBcAg particle, or comprised in the disclosed container or in the disclosed kit may be useful for preventing an HBV infection, preferably said components are preferably useful for curing an HBV infection.

[0091] Accordingly, the use is preferably in an immune stimulation method and/or in a vaccination method, preferably in a therapeutic vaccination method. As described herein above, the HBcAg particle is preferably capable of inducing an immune response against HBV core proteins it is composed of such as HBV core proteins of two or more genotypes like C and D. Hence, the disclosed HBcAg particle or the disclosed pharmaceutical composition comprising the HBcAg particle, or comprised in the disclosed container or in the disclosed kit, are preferably well suited for use in an immune stimulation method and/or vaccination. Immune stimulation can be measured by determining anti-HBs antibodies, for example using ELISA-based immunoassays. Immune stimulation can be measured by determining hBcore- and/or HBs-specific CD8 T-cell responses, for example using fluorospot technique. Immune stimulation may be beneficial in cases that require support of the naturally occurring immune response

without being limited to vaccination, especially in cases of an infection with HBV. Thus, anti-HBcAg and— by intrastructural help— anti-HBsAg antibody production can preferably be intensified and HBcAg-specific CD4+/CD8+ T-cell induction ensured. These aspects may also be beneficial in the context of vaccination which may be for example preventive or curative. Thus, the invention relates also to the disclosed HBcAg particle or the disclosed pharmaceutical composition comprising the HBcAg particle, optionally comprised in the disclosed container or in the disclosed kit, for use in therapy, wherein the use is in a therapeutic immune stimulation method, preferably in a therapeutic vaccination method, most preferably in a curative vaccination method.

[0092] The present invention relates also to the disclosed HBcAg particle or the disclosed pharmaceutical composition comprising the HBcAg particle, or comprised in the disclosed container or in the disclosed kit, for use in treating an HBV infection. Thus, the innovative components may be especially advantageous for treating an HBV infection by stimulating an, preferably adaptive, immune response directed against the HBV core proteins and HBV surface proteins comprised therein. Moreover, the innovative components and especially the HBcAg enables the induction of an immune response against multiple HBV genotypes like genotypes C and D that may be of particular relevance in some regions due to their (frequent) occurrence and provides an intrastructural help to induce HBsAg-directed CD4 T- and B-cell responses.

[0093] As used herein, “treat” or “treatment” refers to clinical intervention designed to alter the natural course of the subject being treated during the course of a physiological condition or disorder or clinical pathology. A treatment may be a therapeutic treatment and/or a prophylactic or preventative measure, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the growth, development or spread of a hyperproliferative condition, such as cancer. Desired effects of treatment include, but not limited to, decreasing the rate of disease progression, ameliorating or palliating the disease state, alleviating symptoms, stabilizing or not worsening the disease state, and remission of improved prognosis, whether detectable or undetectable. Desired effects of treatment also include prolonging survival as compared to expected survival if not receiving treatment. A subject in need of a treatment includes a subject already with the condition or disorder or prone to have the condition or disorder or a subject in which the condition or disorder is to be prevented. The subject may have a hepatitis B virus infection. The infection may be acute or chronic. Preferably, the subject has a chronic hepatitis B virus infection.

[0094] Herein, a “subject” is a vertebrate, preferably a mammal, more preferably a human. The term “mammal” is used herein to refer to any animal classified as a mammal, including, without limitation, humans, domestic and farm animals, and zoo, sports, or pet

animals, such as sheep, dogs, horses, cats, cows, rats, pigs, apes such as cynomolgus monkeys, to name only a few illustrative examples. Preferably, the "mammal" used herein is human. Particularly preferred is a "subject" being a human.

[0095] Accordingly, the use preferably comprises inducing anti-HBcAg antibodies and/or inducing HBcAg-specific CD4+/CD8+ T-cells. Further, the use preferably enhances the induction of anti-HBs antibodies and HBsAg-specific CD4+/CD8+ T cells by intrastructural help.

[0096] As regards the generation of an HBcAg particle as disclosed herein, a vector system may be used for expression, e.g., in a cell-based expression system, such as a bacterial expression system, such as *E. coli*, or in a eukaryotic expression system, such as a baculoviral expression system. This preferably has the advantage that production of the HBcAg particle can be easily scaled-up, done cheap and under condition that can ensure conformity with good manufacturing practice (GMP) guidelines. As a first option a vector system can be generated comprising several, e.g. expression, vectors, wherein each of the vectors encodes an HBV core protein of an HBV genotype. Thus, such a vector system may comprise for example two (expression) vectors, wherein one of the two (expression) vectors encodes a full-length HBV core protein from HBV genotype D, and the other one of the two (expression) vectors encodes a truncated HBV core protein of HBV genotype C. When both (expression) vectors are introduced into a bacterial cell like an *E. coli* cell, the two HBV core proteins may be expressed and self-assemble into a (mosaic) HBcAg particle that can be isolated from the cell. Alternatively or additionally, a multicistronic, e.g. expression, vector may be used that encodes for all different HBV core proteins that shall be comprised in the HBcAg particle of the invention. Thus, for example a bicistronic plasmid may be used for generating a HBcAg particle comprising HBV core proteins from genotypes C and D. Hence, a multicistronic vector may be advantageous for obtaining an approximately defined ratio of HBV core proteins like an approximately equimolar ratio of HBV core proteins from genotypes C and D in case of a bicistronic (expression) vector like a plasmid. Alternatively or additionally, an expression cassette may be used, wherein the sequences encoding the different HBV core proteins are under the control of the same promoter. This preferably has the advantage that in case of the same copy number of sequences encoding the individual HBV core protein types, an approximate equimolar ratio of the HBV core proteins can be ensured to be comprised in the HBcAg particle of the invention. Thus, nucleic acid sequences encoding for different HBV core proteins may be comprised in individual expression cassettes, or all together in a single expression cassette.

[0097] The term "expression cassette" as used herein encompasses DNA as well as RNA sequences which are preferably capable of directing expression of a particular nucleotide sequence in an appropriate host cell like *E. coli*. In general, it comprises a promoter operably linked to a polynucleotide of interest, which is optionally operably linked to a termination signal

and/or other regulatory elements. The expression cassette may comprise a transcription regulating nucleotide sequence. An expression cassette may also comprise sequences required for proper translation of the nucleotide sequence. The expression cassette may be one, which is naturally occurring but has preferably been obtained in a recombinant form useful for heterologous expression. The coding region usually codes for a protein of interest. The expression cassette comprising the polynucleotide sequence of interest may also be chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components. Typically, the expression cassette is preferably capable of inducing transcription in respective host cells. The expression of the nucleotide sequence in the expression cassette may be under the control of a constitutive promoter or of an inducible promoter, which initiates transcription only when the host cell is exposed to some particular external stimulus. Nucleic acid sequences disclosed herein and thus, encoding any HBV antigen, are preferably codon optimized. A "codon-optimized" nucleic acid sequence refers to a nucleic acid sequence containing codons that are replaced by codons preferred by the desired host cell, preferably an *E. coli* and/or human host cell depending on the host. A nucleic acid sequence is converted into a codon-optimized nucleic acid sequence having an identical translated polypeptide sequence, but with alternative codon usage, in particular using the most frequently codons of the host organism. The method of creating a codon-optimized nucleic acid sequence of an antigen generally includes identifying codons in the naturally occurring sequence of an antigen that are commonly not associated with high expressing genes in the host and replacing them with codons that are known to be widely used in gene expression of the host. A codon-optimized nucleic acid sequence may show improved expression over the naturally occurring sequence in the desired host cell. Whether a codon optimized sequence will induce an improvement in the protein production over the non-optimized sequence can be examined by a skilled person. Furthermore, also respective techniques for codon optimization are known in the art.

[0098] Accordingly, the present invention relates also to an expression cassette, an mRNA, or a cDNA, encoding an HBV core protein from HBV genotype C and an HBV core protein from HBV genotype D, wherein the expression cassette, mRNA, or cDNA, only comprises coding sequences for two or more HBV core proteins. Such an expression cassette may be highly advantageous for the generation of a HBcAg particle of the present invention, wherein said particle comprises HBV core proteins from HBV genotypes C and D, preferably in a ratio where each core protein is represented by at least about 25%, preferably in a ratio where each core protein is represented by at least about 30%, preferably in an approximately equimolar ratio.

[0099] As regards the encoded HBV core proteins, the expression cassette, mRNA, or cDNA, preferably encodes an HBV core protein from HBV genotype C comprising a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1 or 13. Additionally or alternatively, the expression cassette, mRNA, or cDNA, encodes an HBV core protein from HBV genotype D comprising a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence set forth in SEQ ID NO: 2 or 14. Thus, the expression cassette, mRNA, or cDNA, preferably encodes i) an HBV core protein from HBV genotype C comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1 or 13 and/or ii) an HBV core protein from HBV genotype D comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 2 or 14. Preferably, the expression cassette, mRNA, or cDNA, encodes i) an HBV core protein from HBV genotype C comprising a sequence having at least 95% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1 or 13 and/or ii) an HBV core protein from HBV genotype D comprising a sequence having at least 95% sequence identity to the amino acid sequence set forth in SEQ ID NO: 2 or 14. Preferably, the expression cassette, mRNA, or cDNA, encodes i) an HBV core protein from HBV genotype C comprising a sequence having the amino acid sequence set forth in SEQ ID NO: 1 or 13 and/or ii) an HBV core protein from HBV genotype D comprising a sequence having the amino acid sequence set forth in SEQ ID NO: 2 or 14.

[00100] Accordingly, the expression cassette, mRNA, or cDNA, preferably comprises a first nucleotide sequence encoding an HBV core protein from HBV genotype C, wherein said first nucleotide sequence has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 3 or 15. Additionally or alternatively, the expression cassette, mRNA, or cDNA, comprises a second nucleotide sequence encoding an HBV core protein from HBV genotype D, wherein said second nucleotide sequence has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 4 or 16. Thus, the expression cassette, mRNA, or cDNA, preferably comprises of i) a first nucleotide sequence encoding an HBV core protein from HBV genotype C, wherein said first nucleotide sequence has at least 90% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 3 or 15, and/or ii) a second nucleotide sequence encoding an HBV core protein from HBV genotype D, wherein said second nucleotide sequence has at least 90% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 4 or 16. Preferably, the expression cassette, mRNA, or cDNA, comprises i) a first nucleotide sequence encoding an HBV core protein from HBV genotype C, wherein said first nucleotide sequence has at least 95% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 3 or 15, and/or ii) a second nucleotide sequence

encoding an HBV core protein from HBV genotype D, wherein said second nucleotide sequence has at least 95% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 4 or 16. Preferably, the expression cassette, mRNA, or cDNA, comprises i) a first nucleotide sequence encoding an HBV core protein from HBV genotype C, wherein said first nucleotide sequence has the nucleotide sequence set forth in SEQ ID NO: 3 or 15, and/or ii) a second nucleotide sequence encoding an HBV core protein from HBV genotype D, wherein said second nucleotide sequence has the nucleotide sequence set forth in SEQ ID NO: 4 or 16.

[00101] The present invention relates also to a nucleic acid molecule comprising the disclosed expression cassette, mRNA, or cDNA. Thus, said nucleic acid molecule may be a DNA molecule like a DNA based vector comprising the expression cassette, mRNA, or cDNA disclosed herein above. This may have advantages for introducing genetic information for generating the disclosed HBcAg particle in an expression system disclosed herein, such as *E. coli*. Alternatively, said nucleic acid molecule may be an RNA molecule, preferably an mRNA molecule. Such an RNA molecule may be advantageous for a temporarily restricted generation of the disclosed HBcAg particle. Such an RNA molecule may relate to an mRNA molecule and thus, a transcript of an, e.g., DNA based vector comprising the expression cassette or cDNA disclosed herein. This may be the case, e.g., when splicing sites are encoded in the expression cassette between the HBV core protein encoding sequences comprised therein. Alternatively or additionally, it may refer to a RNA, preferably an mRNA molecule that can be introduced into a cell in a manner comparable to RNA based vaccinations. This has may have advantage that genetic information is not permanently transferred into the target cell. Preferably, the nucleic acid molecule comprising the expression cassette is a DNA molecule, which is preferably transferred into a host cell, such as *E. coli*, for the generation of HBV core proteins that can self-assemble into particulate HBcAg particles that can be isolated as HBcAg particles according to the invention.

[00102] Furthermore, the present invention relates also to an expression vector comprising the disclosed expression cassette, the disclosed cDNA, or the disclosed nucleic acid molecule. Preferably, the expression vector is a recombinant vector. This may be advantageous for using a well-established and well-characterized expression vector like a plasmid for efficient generation of HBcAg particles comprising HBV core proteins from genotypes C and D by recombinantly introducing the respective expression cassette, cDNA, or nucleic acid molecule into the vector backbone. Thus, HBcAg particle generation may be optimized, e.g. also in view of the chosen expression system, and/or host cell. Suitable expression systems and/or host cells are disclosed herein.

[00103] Furthermore, the present invention relates also to an expression vector encoding an HBV core protein from HBV genotype C and an HBV core protein from HBV genotype D,

wherein the expression vector comprises a sequence that has at least 90% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 10. Preferably, expression vector comprises a sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 10. Preferably, the expression vector comprises a sequence that has at least 95% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 10. Preferably, the expression vector comprises a sequence that has the nucleotide sequence set forth in SEQ ID NO: 10. Thus, the expression vector may be a bicistronic plasmid for generating a (mosaic) HBcAg particle comprising HBV core proteins from genotypes C and D. Preferably, the expression vector is a recombinant vector and/or a plasmid.

[00104] Accordingly, the present invention relates also to a host cell comprising the disclosed expression cassette, mRNA, cDNA, optionally comprised in the disclosed nucleic acid molecule and/or the disclosed expression vector, or the disclosed expression vector encoding an HBV core protein from HBV genotype C and an HBV core protein from HBV genotype D. The host cell may be prokaryotic or eukaryotic, such as a bacterial cell, an insect cell, a yeast cell, or a mammalian cell. Preferably, the host cell is an *E. coli* cell or a *Spodoptera frugiperda* cell.

C. The vaccine vector of the disclosure

[00105] The present invention relates also to a vaccine vector, wherein the vaccine vector is preferably a modified vaccinia virus Ankara (MVA) viral vector, and wherein the vaccine vector comprises a nucleic acid molecule comprising a nucleotide sequence having at least 90% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 5.

[00106] A "vaccine vector" as used herein refers to a bacterial or viral vaccine vector, preferably a viral vaccine vector, which can be an attenuated MVA virus. In this context, "vector" refers preferably to a virus and in particular to an MVA virus used as the carrier. Typically, this attenuated virus is used to introduce nucleic acid encoding for antigens to cells of the subject. More specifically, a vaccine vector of the invention may be a viral vector that may be a viral particle having infectivity, which is also a carrier for introducing a gene into a cell. Viral vaccine vectors are familiar to the person skilled in the art. A (recombinant) MVA virus, may be an MVA virus that is produced by standard genetic engineering methods.

[00107] MVA is particularly well-suited as vector system. MVA is related to vaccinia virus, a member of the genera Orthopoxvirus, in the family of Poxviridae. MVA was generated by 516 serial passages on chicken embryo fibroblasts of the Ankara strain of vaccinia virus (CVA) (for review see Mayr, A., et al. Infection 3, 6-14 (1975)). As a consequence of these long-term passages, the genome of the resulting MVA virus had about 31 kilobases of its genomic

sequence deleted and, therefore, was described as highly host cell restricted for replication to avian cells (Meyer, H. et al., J. Gen. Virol. 72, 1031-1038 (1991)). It was shown in a variety of animal models that the resulting MVA was significantly avirulent (Mayr, A. & Danner, K., Dev. Biol. Stand. 41: 225-34 (1978)) but still raised protective immune responses against poxviruses. For example, a preferred MVA strain, MVA-F6 (Sutter and Staib, 2003. Curr. Drug Targets Infect. Disord. 3:263–271), has been shown to grow well in primary Chicken Embryo Fibroblast (CEF) cells while not replicating in human cells. In human cells, genetic information comprised in the MVA are expressed, but no infectious virus is produced. The restricted host range of MVA may explain the non-virulent phenotype observed in vivo in a wide range of mammalian species including humans. Therefore, this MVA strain has been tested in clinical trials as a vaccine to immunize against the human smallpox disease (Mayr et al., Zbl. Bakt. Hyg. I, Abt. Org. B 167, 375-390 (1987); Stickl et al., Dtsch. med. Wschr. 99, 2386-2392 (1974)). These studies involved over 120,000 humans, including high-risk patients, and proved that, compared to vaccinia-based vaccines, MVA had diminished virulence and was well tolerated, while it still induced a good specific immune response. Thus, its safety has been established and its potency in efficiently inducing both humoral and cellular immune responses in a short period of time after vaccination against, for example, small pox virus has been demonstrated. Furthermore, construction, production and use of recombinant MVA have been described in the art, cf. e.g. WO 97/02355, Sutter and Staib, 2003 (supra), and WO 2003/008533. Hence, MVA is a well suited, and herein preferred, vector system that is a safe, well tolerated and immunogenic vaccine platform preferably capable of inducing a multimodal humoral and cell-based immunological antigen response.

[00108] As regards the expression cassette, the same applies as stated herein above in the context of the expression cassette encoding one or more HBV core proteins. Thus, preferably the expression cassette is the expression cassette encoding one or more HBV core proteins disclosed herein. Typically, in case of the expression cassette comprised in the vaccine vector, the expression cassette is not naturally occurring (i.e., heterologous or exogenous or foreign) in the MVA viral vector, though preferably capable of inducing transcription in respective host cells. Hence, said expression cassette is typically generated by means of recombination, resulting in a recombinant MVA viral vector. Furthermore, in case of an expression cassette comprised in the MVA viral vector, the promoter is preferably a poxviral promoter. Such a poxviral promoter may be a natural occurring promoter or a synthetic promoter. As an illustrative example, the poxvirus promoter may be a Pr7.5 promoter, a hybrid early/late promoter, a PrS promoter, a synthetic or natural early or late promoter such as one of the promoters described in WO 2010/102822 or in WO 2005/054484, or cowpox virus ATI promoter. A preferred promoter is, e.g., the promoter PH5 as described in US 2011/0064769.

[00109] More specifically, the expression cassette preferably comprises

- a. a nucleotide sequence encoding an HBsAg from HBV genotype A, which preferably comprises a sequence having at least 90 % sequence identity to the amino acid sequence set forth in SEQ ID NO: 11,
- b. a nucleotide sequence encoding an HBcAg from HBV genotype D, which preferably comprises a sequence having at least 90 % sequence identity to the amino acid sequence set forth in SEQ ID NO: 2 or 12,
- c. a nucleotide sequence encoding a reverse transcriptase (RT) domain of a polymerase from HBV, which preferably comprises a sequence having at least 90 % sequence identity to the amino acid sequence set forth in SEQ ID NO: 9,
- d. a nucleotide sequence encoding HBsAg from HBV genotype C, which preferably comprises a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 7, and
- e. a nucleotide sequence encoding HBcAg from HBV genotype C, which preferably comprises a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 8 or 17.

[00110] Thus, the expression cassette comprised in the vaccine vector preferably encodes for, and is thus preferably capable of expressing, two HBV surface proteins, two HBV core proteins as well as a polymerase from HBV or at least a RT domain of the HBV polymerase. More specifically, surface protein recited in a. may for example be an surface protein of HBV serotype adw, such as of HBV genotype A serotype adw, such as of HBV genotype A2 serotype adw2. The core protein recited in b. may for example be a core protein of HBV serotype ayw, such as of HBV genotype D serotype ayw. Thus, it is preferably envisioned that the vaccine vector comprises an expression cassette encoding for a combination of immunogenic HBV antigens (core, surface and polymerase RT domain) from different HBV genotypes and/or serotypes. Such a combination may be advantageous for inducing a broad immune response against multiple HBV strains as well as at the same time a stronger immune response against each HBV genotype compared to vaccines comprising only antigens from a single HBV genotype.

[00111] However, the presence of multiple antigens bears the risk of homologous recombination that can take place in an MVA vector. The disclosed expression cassette encodes for two HBsAg and two HBcAg with regions of high sequence similarity being naturally present between the two HBsAg encoding sequences as well as the two HBcAg encoding sequences. To ensure a stable expression cassette within MVA vectors, the expression cassette sequence disclosed herein was modified via codon optimization specifically in view of sequence regions of high similarity. Sequence integrity was assessed over several, e.g. 6 to 7,

serial MVA viral passages without any detection of a mutation including any recombination event within the expression cassette. Hence, the disclosed sequence preferably has the advantage of ensuring equimolar expression of several HBV antigens of different HBV genotypes using a stable MVA vector with limited risk of homologous recombination within the expression cassette.

[00112] Preferably, the vaccine vector comprises an expression cassette comprising a nucleotide sequence having at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 5. Preferably, the expression cassette comprises a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 5. Preferably, the expression cassette has a coding sequence having the nucleotide sequence set forth in SEQ ID NO: 5.

[00113] Herein, the term "MHBVac" refers to a vaccine vector with said vector being an MVA viral vector and having a genome set forth in SEQ ID NO: 6. In particular, MHBVac comprises as an insert the nucleotide sequence set forth in SEQ ID NO: 5. Accordingly, the MHBVac is preferably capable of expressing two different HBsAg, two different HBcAg and a RT domain of an HBV polymerase covering in total genotypes A, C, and D. Thus, the MHBVac is particularly well suited for inducing and/or strengthening an immune response against multiple HBV antigens of different HBV genotypes. Hence, the MHBVac refers preferably to a vaccine vector as disclosed herein.

[00114] Preferably, the vaccine vector of the invention comprises a nucleic acid molecule comprising a nucleotide sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 6. Preferably, the vaccine vector comprises a nucleic acid molecule comprising a nucleotide sequence that has at least 90% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 6. Preferably, the vaccine vector comprises a nucleic acid molecule comprising a nucleotide sequence that has at least 95% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 6. Preferably, the vaccine vector comprises a nucleic acid molecule comprising a nucleotide sequence that has the nucleotide sequence set forth in SEQ ID NO: 6.

[00115] Thus, the vaccine vector disclosed herein comprises preferably an expression cassette encoding multiple HBV antigens of different HBV genotypes. Moreover, it is envisioned that all of said HBV antigens are preferably translated as one single polypeptide chain comprising said HBV antigens as schematically depicted in **Figure 2**. On the polypeptide chain, antigen sequences are preferably separated by self-cleavage site sequences such as P2A or T2A. Thus, the polypeptide chain comprising several antigens will be post-translationally cleaved to multiple polypeptide chains, wherein each of the multiple polypeptide chains may

comprise a single HBV antigen. This approach preferably has the advantage that all HBV antigens are expressed in about equimolar amounts and thus, in an approximately equimolar ratio. Shown in **Figure 3** is a preferred nucleotide sequence arrangement encoding a polypeptide chain comprising several antigens from N-terminus to C-terminus: a HBsAg from HBV genotype A/adw, a P2A site, a HBcAg from HBV genotype D/ayw, a P2A site, an immunogenic RT domain of a polymerase from HBV, a T2A site, an immunogenic HBsAg from HBV, a T2A site, and an immunogenic HBcAg from HBV. The two different HBsAg will be located in a cellular membrane and may be secreted as subviral particles. These subviral particles may comprise both HBsAg that are from different HBV genotypes and may be taken up by antigen-presenting cells, which may increase the induced immune response. The HBcAg may form particles and more specifically particulate capsids, wherein the capsids may be empty and may similarly comprise HBcAg from different HBV genotypes. Such a mosaic HBcAg particle will trigger an immune response against multiple HBV genotypes. The polymerase will be degraded in the proteasome and presented by in an HLA context. The disclosed arrangement may further have the advantage that most of the only partially processed proteins, i.e. proteins where a self-cleaving site has not been cleaved for example, will be incorporated into secreted particles, which are preferably capable of further increasing immune stimulation and/or of further enhancing and broadening the induced (adaptive) immune response.

[00116] The present invention relates also to a pharmaceutical composition comprising the vaccine vector disclosed herein above and optionally a pharmaceutically acceptable carrier or excipient. As regards said pharmaceutically acceptable carrier and/or excipients the same applies as stated herein above in the context of the pharmaceutical composition comprising the HBcAg particle disclosed herein above. Thus, said pharmaceutical composition comprising the vaccine vector may comprise one or more excipient and/or one or more pharmaceutically acceptable and/or approved carrier as additive, optionally also one or more selected from the group consisting of an antibiotic, a preservative, an adjuvant, a diluent and/or a stabilizer. Such auxiliary substances can be, e.g., water, saline, glycerol, ethanol, wetting or emulsifying agents, a detergent, an amino acid, a sugar, a surfactant, such as a kolliphor, pH buffering substances, or the like. Furthermore, said pharmaceutically acceptable carrier or excipient preferably refer to a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s), e.g. of the vaccine vector disclosed herein. The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition may further contain other agents which either enhance the activity or use in treatment. Suitable pharmaceutically acceptable carriers and/or excipients are typically large, slowly metabolized molecules such as as modified nucleic acids, proteins, polysaccharides, polylactic acids, 37 dditionallyc acids, polymeric amino acids, amino acid copolymers, lipid aggregates, or the like. Such a

pharmaceutically acceptable carrier or excipient may be preferably advantageous in producing or supporting a synergistic effect and/or to minimize side-effects.

[00117] The present invention relates also to a container comprising one or more doses of the pharmaceutical composition comprising the disclosed vaccine vector, wherein a dose of said pharmaceutical composition comprises the vaccine vector in an amount from about 1×10^7 infectious focus units (ifu) to about 1×10^9 ifu, preferably in an amount from about 5×10^7 ifu to about 1×10^9 ifu, more preferably in an amount of about 6×10^7 ifu or of about 3×10^8 ifu or of about 5×10^8 ifu, most preferably in an amount of about 3×10^8 ifu. Determination of IFUs is well known to the skilled artisan, e.g. by quantifying the number of virus-infected cell spots identified via immunostaining of MVA-vector proteins or the expressed antigen. Alternative quantification methods such as quantification by pfu or TCID₅₀ are also known to the skilled person and are likewise applicable. It is understood that the quantity of the vaccination vector defined herein is not limited by the quantification method used. As regards said one or more doses and thus the term "dose" the same preferably applies as stated herein above in the context of the kit and/or container comprising one or more doses of the disclosed pharmaceutical composition comprising the HBcAg particle. Hence, it refers preferably to that amount of the vaccine vector that is sufficient to result in a beneficial or desired effect of the treatment and thus, preferably to an amount that is sufficient to result in a therapeutic effect considering influencing factors such as means of administration and whether the treatment is intended to be prophylactic or curative.

[00118] The present invention relates also to the disclosed vaccine vector, or the disclosed pharmaceutical composition comprising the same, optionally comprised in the disclosed container, for use in therapy. More specifically, said components are preferably used in therapy and/or vaccination, preferably in therapeutic vaccination, preferably against HBV. As regards said therapy, the same applies as stated herein above in the context of the HBcAg particle and its respective pharmaceutical composition, optionally comprised in a disclosed kit or container. Thus, the disclosed vaccine vector, or the disclosed pharmaceutical composition comprising the same, optionally comprised in the disclosed container, may be advantageous for preventing and/or, even more, curing an HBV infection. Thus, the disclosed vaccine vector, or the disclosed pharmaceutical composition comprising the same, optionally comprised in the disclosed container, is preferably used in therapy and/or vaccination, preferably in therapeutic vaccination, preferably in therapeutic vaccination against HBV.

[00119] Accordingly, the use is preferably in an immune stimulation method and/or in a vaccination method, preferably in a therapeutic vaccination method. As described herein above in the context of the HBcAg particle also the disclosed vaccine vector is preferably capable of inducing an immune response against HBV core proteins, HBV surface proteins and the RT

domain of the HBV polymerase and thus, it is preferably capable of expressing antigens of different type and genotype. As regards the immune stimulation method and/or vaccination method, preferably a therapeutic vaccination method, most preferably a curative vaccination method, the same applies as stated herein above in the context of the HBcAg particle and its respective pharmaceutical composition, optionally comprised in a kit or container. Thus, while the disclosed vaccine vector, or the disclosed pharmaceutical composition comprising the same, optionally comprised in the disclosed container, may be useful for preventing an HBV infection, preferably said disclosed vaccine vector, or disclosed pharmaceutical composition comprising the same, optionally comprised in the disclosed container, is preferably useful for curing an HBV infection.

[00120] Accordingly, the present invention relates also to the disclosed vaccine vector or the disclosed pharmaceutical composition comprising the vaccine vector, optionally comprised in the disclosed container, for use in treating an HBV infection. The vaccine vector may be especially advantageous for treating an HBV infection by stimulating an, preferably adaptive, immune response directed against the HBV core proteins, HBV surface proteins and the RT domain of the HBV polymerase comprised therein, in particular an immune response against multiple HBV genotypes, which may be of particular relevance in some regions due to their (frequent) occurrence. Accordingly, the use preferably comprises inducing anti-HBcAg antibodies and/or inducing HBcAg-specific CD4+/CD8+ T-cells, and also preferably comprises enhancing the induction of anti-HBsAg antibodies and HBsAg-specific CD4+/CD8+ T-cells, which may be caused by in structural help.

D. The vaccination method of the disclosure

[00121] The present invention relates also to a method of vaccination, comprising administering to a human subject

- (i) a first dose of an HBcAg particle and of an HBsAg,
- (ii) a second dose of the HBcAg particle and of the HBsAg,
- (iii) a dose of a vaccine vector

wherein the vaccine vector expresses

- a. an HBsAg from HBV genotype A;
- b. an HBcAg from HBV genotype D;
- c. an HBsAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 7;
- d. an HBcAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 8 or 17; and
- e. an RT comprising a sequence domain of a polymerase from HBV having at least 90 % sequence identity to the amino acid sequence set forth in SEQ ID NO: 9.

[00122] The vaccination method of the present invention, herein optionally also referred to as “VacB” or “VacB vaccination regime”, represents a novel vaccination regime that is based on a pan-genotypic heterologous prime-boost therapeutic vaccine regime. It is particularly envisioned that preferably two priming steps are performed (cf. i and ii), followed by a boost (cf. iii). Priming is crucial for the immune stimulatory, and especially the therapeutic, success. Thus, for priming a, preferably adjuvanted, HBsAg and a particulate novel mosaic HBcAg particle are combined. Priming is followed by a boost using a vaccine vector boosting B and T cell responses.

[00123] As regards the HBcAg particle used in the method of vaccination of the invention the same applies as stated herein above in detail in the context of the HBcAg particle of the invention. Thus, the HBcAg particle used in the method of vaccination of the invention is preferably the HBcAg particle disclosed herein.

[00124] As regards the HBsAg, said HBsAg preferably comprises HBV surface proteins of HBV subtype ayw or adw, genotype A, preferably serotype adw genotype A, more preferably serotype adw (genotype A2). Alternatively, said HBsAg preferably comprises HBV surface proteins only of HBV genotype A, preferably of serotype adw genotype A, more preferably serotype adw (genotype A2). Alternatively or additionally, said isolated HBsAg preferably comprises HBV surface proteins of HBV serotype adw, or only of HBV serotype adw.

[00125] Accordingly, in (i) the first dose of the HBcAg particle and of the HBsAg is preferably a first dose of a pharmaceutical composition disclosed herein, which comprises both, the HBcAg particle disclosed herein and the HBsAg disclosed herein. Alternatively, in (i) the first dose of the HBcAg particle and of the HBsAg may be a first dose of two individual pharmaceutical compositions of the disclosure, one comprising the HBcAg particle disclosed herein and the other comprising the HBsAg disclosed herein. The pharmaceutical composition(s) may be comprised in a container disclosed herein or in a kit disclosed herein.

[00126] Furthermore, in (ii) the second dose of the HBcAg particle and of the HBsAg is preferably a second dose of the pharmaceutical composition disclosed herein which comprises both, the HBcAg particle disclosed herein and the HBsAg disclosed herein. Alternatively, in (ii) the second dose of the HBcAg particle and of the HBsAg may be a second dose of two individual pharmaceutical compositions of the disclosure, one comprising the HBcAg particle disclosed herein and the other comprising the HBsAg disclosed herein. The pharmaceutical composition(s) may be comprised in a container disclosed herein or in a kit disclosed herein.

[00127] Preferably, the same pharmaceutical composition(s) is/are used both in (i) and in (ii). Preferably, the HBsAg is a particulate HBsAg. Preferably, the HBsAg is an HBsAg from

subtype ayw or adw and/or HBV genotype A. Preferably, the HBsAg in (i) and/or (ii) is an HBsAg from HBV subtype adw.

[00128] Furthermore, it is particularly preferred that both in (i) and in (ii) the HBcAg particle comprises HBV core proteins from HBV genotypes C and D and/or the HBsAg is an HBsAg from HBV subtype adw. This may be particularly advantageous as priming is done in view of HBV core and surface antigens from HBV genotypes A, B, C, and D. Thus, a pan-genotypic priming may be ensured that is preferably well-suited especially for broad immune stimulation.

[00129] Preferably, in a. the HBsAg is a small or large surface protein from HBV subtype adw, preferably a small or large surface protein from HBV subtype adw, even more preferably a small surface protein from HBV genotype A subtype adw, most preferably a small surface protein from HBV genotype A2 serotype adw. Preferably, in a. the HBsAg comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence set forth in SEQ ID NO: 11. Thus, preferably in a. the HBsAg comprises an amino acid sequence having at least 90% or 95% sequence identity to the amino acid sequence set forth in SEQ ID NO: 11. Preferably, in a. the HBsAg has the amino acid sequence set forth in SEQ ID NO: 11.

[00130] Preferably, in b. the HBcAg is from HBV genotype D, preferably from HBV genotype D serotype ayw. Preferably, in b. the HBcAg comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence set forth in SEQ ID NO: 12. Thus, in b. the HBcAg has preferably an amino acid sequence having at least 90% or 95% sequence identity to the amino acid sequence set forth in SEQ ID NO: 12. Preferably, in b. the HBcAg has the amino acid sequence set forth in SEQ ID NO: 12.

[00131] Preferably, in c. the HBsAg comprises an amino acid sequence having at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 7. The HBsAg preferably comprises the amino acid sequence set forth in SEQ ID NO: 7. Of note, said sequence represents a 400 amino acid long consensus sequence of a large surface protein of HBV genotype C. More specifically, SEQ ID NO: 7 is a consensus sequence of large surface proteins of genotype C strains, which was generated based on an alignment of 500 HBV sequences representing the worldwide distribution of HBV strains.

[00132] Preferably, in d. the HBcAg comprises an amino acid sequence having at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 8 or 17. The HBcAg preferably comprises the amino acid sequence set forth in SEQ ID NO: 8 or 17. Of note SEQ ID NO: 8 represents a 183 amino acid

long consensus sequence of an HBV core protein of HBV genotype C, while SEQ ID NO: 17 is a truncated version of SEQ ID NO:8 comprising amino acids 1-149. More specifically, SEQ ID NO: 8 is a consensus sequence of core proteins of genotype C strains that was generated based on alignment of 500 HBV-sequences representing the worldwide distribution of HBV strains.

[00133] Preferably, in e. the RT domain comprises an amino acid sequence having at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 9. The RT domain preferably comprises the amino acid sequence set forth in SEQ ID NO: 9. Of note, said sequence represents a 343 amino acid long consensus sequence across genotypes A, B, C and D of the reverse transcriptase (RT) domain of the HBV polymerase. More specifically, SEQ ID NO: 9 is a consensus sequence of RT domains of genotype A, B, C, and D strains that was generated based on alignment of 500 HBV-sequences representing the worldwide distribution of HBV strains.

[00134] Preferably, the vaccine vector is the vaccine vector disclosed herein above, optionally comprised in the respective disclosed pharmaceutical composition comprising the same and/or in the respective disclosed container. Thus, the vaccine vector may be the vaccine vector disclosed herein above, comprised in the respective disclosed pharmaceutical composition or in the respective disclosed container.

[00135] Preferably, the first dose is administered to the human subject in a first injection, the second dose in a second injection, and/or the dose of the vaccine vector in a third injection wherein the first, second and/or third injection are preferably intramuscular injections. Thus, (i), the first dose, (ii) the second dose, and (iii) the dose of the vaccine vector are preferably administered intramuscularly. As indicated herein above, (i) the first dose and/or (ii) the second dose of the HBcAg particle and of the HBsAg is preferably a dose of a pharmaceutical composition disclosed herein, which comprises both, the HBcAg particle disclosed herein and the HBsAg disclosed herein. Alternatively, (i) the first dose and/or (ii) the second dose of the HBcAg particle and of the HBsAg may be a dose of two individual pharmaceutical compositions of the disclosure, one comprising the HBcAg particle disclosed herein and the other comprising the HBsAg disclosed herein. The pharmaceutical composition(s) may be comprised in a container disclosed herein or in a kit disclosed herein. Thus, the skilled artisan is aware that administration of said first dose may encompass both a) administration of an amount of a single solution comprising both a first dose of the HBcAg particle and the HBsAg and b) administration of a first dose of the HBcAg particle together with the administration of the particulate and optionally adjuvanted HBsAg. Hence, considering said just mentioned options a) and b), step (i) can encompass a single injection in case of a) and two or more injections in case of b). In case of two or more injections, it is preferred that said two or more injections of (i) are done

preferably in the same arm of the human subject. Also two or more injections of (i) are preferably done within less than 3 days, preferably within less than 3 days, 2 days or 1 day, even more preferably within less than 1 hour, most preferably within less than 30, 20 or 10 minutes. Particularly preferably, said two or more injections of (i) are done approximately simultaneously or simultaneously. Furthermore, the skilled artisan is aware that the same as stated herein as regards (i) applies mutatis mutandis as disclosed herein as regards (ii) and thus, the second dose of the HBcAg particle and of the HBsAg. Thus, the second dose preferably comprises the pharmaceutical composition comprising the HBcAg particle disclosed herein, optionally comprised in the respective disclosed container, or of the pharmaceutical composition of the HBcAg particle disclosed herein not comprising a particulate HBsAg but comprised in the respective disclosed kit. The injections of this paragraph are preferably intramuscular injections.

[00136] Preferably, the first dose and the second dose each comprise the HBcAg particle in an amount from about 10µg to about 100µg and the HBsAg in an amount from about 5µg to about 100µg. Preferably, the first dose and the second dose each comprise the HBcAg particle in an amount from about 10µg to about 75µg and the HBsAg in an amount from about 5µg to about 75µg. Preferably, the first dose and the second dose each comprise the HBcAg particle in an amount from about 20µg to about 100µg, preferably to about 60µg, and the HBsAg in an amount from about 5µg to about 75µg. Preferably, the first dose and the second dose each comprise the HBcAg particle in an amount of about 25µg or of about 50µ and the HBsAg in an amount from about 5µg to about 75µg. Preferably, the first dose and the second dose each comprise the HBcAg particle in an amount from about 10µg to about 100µg and the HBsAg in an amount from about 10µg to about 50µg. Preferably, the first dose and the second dose each comprise the HBcAg particle in an amount from about 10µg to about 75µg and the HBsAg in an amount from about 10µg to about 50µg. Preferably, the first dose and the second dose each comprise the HBcAg particle in an amount from about 20µg to about 100µg, preferably to 60µg, and the HBsAg in an amount from about 10µg to about 50µg. Preferably, the first dose and the second dose each comprise the HBcAg particle in an amount of about 25µg or of about 50µ and the HBsAg in an amount from about 10µg to about 50µg. Preferably, the first dose and the second dose each comprise the HBcAg particle in an amount from about 10µg to about 100µg and the HBsAg in an amount of about 20µg or of about 40µg. Preferably, the first dose and the second dose each comprise the HBcAg particle in an amount from about 10µg to about 75µg and the HBsAg in an amount of about 20µg or of about 40µg. Preferably, the first dose and the second dose each comprise the HBcAg particle in an amount from about 20µg to about 100µg, preferably to about 60µg, and the HBsAg in an amount of about 20µg or of about 40µg. Preferably, the first dose and the second dose each comprise the HBcAg particle in an amount of about 25µg or of about 50µg and the HBsAg in an amount of about 20µg or of about 40µg.

Preferably, the first dose and the second dose each comprise the HBcAg particle in an amount from about 25µg and the HBsAg in an amount of about 20µg. Preferably, the first dose and the second dose each comprise the HBcAg particle in an amount from about 50µg and the HBsAg in an amount of about 40µg. Amounts of HBcAg and/or HBsAg are preferably determined by UV spectrophotometry.

[00137] Preferably, the dose of the vaccine vector comprises the vaccine vector in an amount from about 1×10^7 ifu to about 1×10^9 ifu, preferably in an amount from about 5×10^7 ifu to about 5×10^8 ifu, more preferably in an amount of about 6×10^7 ifu or of about 3×10^8 ifu or of about 5×10^8 ifu, most preferably in an amount of about 3×10^8 ifu or of about 5×10^8 ifu.

[00138] Preferably, the method of vaccination comprises administering the second dose about 1 week to about 8 weeks after the first dose, preferably about 2 weeks to about 6 weeks after the first dose, more preferably about 4 weeks after the first dose. Thus, preferably the second dose is administered 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 days after the first dose, preferably 25, 26, 27, 28, 29, 30, 31, or 32 days after the first dose, more preferably 27, 28 or 29 days after the first dose. This may be a particularly advantageous administration scheme as, e.g., also supported by other vaccination regimes as in the case of the commercially available HEPLISAV-B®. The latter is a CpG-adjuvanted recombinant yeast-derived HBsAg vaccine that has been shown to induce neutralizing antibody responses against different HBV genotypes after two intramuscular injections four weeks apart (Splawn et al., Hepelisav-B vaccination for the prevention of hepatitis B virus infection in adults in the United States. *Drugs Today (Barc)*. 2018 Jul;54(7):399-405. doi: 10.1358/dot.2018.54.7.2833984. PMID: 30090877).

[00139] Preferably, the method of vaccination comprises administering the dose of the vaccine vector about 2 weeks to about 24 weeks after the first dose, preferably about 4 weeks to about 12 weeks after the first dose, more preferably about 8 weeks after the first dose.

[00140] In **Table 1** dosing schemes are given, of which every one may be a preferred dosing scheme. Thus, the vaccination method preferably comprises administering to a human subject a first dose of the HBcAg particle and of the HBsAg, a second dose of the HBcAg particle and of the HBsAg, and a dose of a vaccine vector as disclosed herein above, wherein

- a. the first and the second dose each comprise the HBcAg particle in an amount (1) from about 20µg to about 100µg, preferably to about 60µg, more preferably in an amount (2) of about 25µg or (3) of about 50µg,

- b. the first and the second dose each comprise the HBsAg in an amount (4) from about 10µg to about 50µg, more preferably in an amount (5) of about 20µg or (6) of about 40µg,
- c. the dose of the vaccination vector comprises the vaccine vector in an amount (7) of about 6×10^7 ifu or (8) of about 3×10^8 ifu,
- d. wherein the second dose is preferably administered (9) about 2 weeks to about 6, preferably (10) about 4 weeks after the first dose, and
- e. wherein the dose of the vaccine vector is preferably administered (11) about 4 weeks to about 12 weeks after the first dose, preferably (12) about 8 weeks after the first dose.

Table 1: Overview of preferred dosing schemes.

a	b	c	d	e
1	4	7	9	11
2 or 3	4	7	9	11
1	5 or 6	7	9	11
2 or 3	5 or 6	7	9	11
1	4	8	9	11
2 or 3	4	8	9	11
1	5 or 6	8	9	11
2 or 3	5 or 6	8	9	11
1	4	7	10	11
2 or 3	4	7	10	11
1	5 or 6	7	10	11
2 or 3	5 or 6	7	10	11
1	4	8	10	11
2 or 3	4	8	10	11
1	5 or 6	8	10	11
2 or 3	5 or 6	8	10	11
1	4	7	9	12
2 or 3	4	7	9	12
1	5 or 6	7	9	12
2 or 3	5 or 6	7	9	12
1	4	8	9	12
2 or 3	4	8	9	12
1	5 or 6	8	9	12
2 or 3	5 or 6	8	9	12
1	4	7	10	12
2 or 3	4	7	10	12
1	5 or 6	7	10	12
2 or 3	5 or 6	7	10	12
1	4	8	10	12
2 or 3	4	8	10	12
1	5 or 6	8	10	12

2 or 3 5 or 6 8 10 12

[00141] Preferably, the first dose and the second dose each comprise the HBcAg particle in an amount of about 25µg and the HBsAg in an amount of about 20µg, and the dose of the vaccine vector comprises the vaccine vector in an amount of about 6×10^7 ifu, wherein the second dose is administered about 4 weeks after the first dose, and wherein the dose of the vaccine vector is administered about 8 weeks after the first dose. Preferably, the first dose and the second dose each comprise the HBcAg particle in an amount of about 50µg and the HBsAg in an amount of about 40µg, and the dose of the vaccine vector comprises the vaccine vector in an amount of about 3×10^8 ifu, wherein the second dose is administered about 4 weeks after the first dose, and wherein the dose of the vaccine vector is administered about 8 weeks after the first dose. Preferably, the first dose and the second dose each comprise the HBcAg particle in an amount of about 25µg and the HBsAg in an amount of about 20µg, and the dose of the vaccine vector comprises the vaccine vector in an amount of about 3×10^8 ifu, wherein the second dose is administered about 4 weeks after the first dose, and wherein the dose of the vaccine vector is administered about 8 weeks after the first dose.

[00142] The greatest challenge for an HBV vaccine is that it is able to induce both a humoral as well as a cellular immune response and that this immune response is preferably directed to multiple genotypes and/or serotypes of HBV. The choice of the priming protein(s), the adjuvant, the vaccine vector, and/or the vaccination regime may all contribute to the effectiveness of the vaccination.

[00143] As it may be shown based on the (expected) results of the first-in-human phase 1a study presented herein, the disclosed vaccination method is preferably highly efficient in immune stimulation and/or in inducing a strong and broad immune response against multiple HBV antigens of different HBV geno- and/or serotypes that may be of particular relevance in some regions due to their (frequent) occurrence. Thus, the method of vaccination is preferably inducing an immune response against the HBV antigens used for priming and boosting. Furthermore, the results obtained from the clinical study may show that the method of vaccination disclosed herein preferably induces a strong polyclonal and multispecific CD8+ and CD4+ T-cell responses. Thus, the disclosed vaccination method preferably relates to a therapeutic HBV vaccination with the potential to cure HBV infections.

[00144] Accordingly, the method of vaccination results preferably in an induction of an immune response against the HBV antigens it is composed of, preferably an antigen-specific adaptive immune response. In particular, the vaccination method preferably induces a, preferably antigen-specific adaptive, immune response against HBcAg of genotypes C and D,

HBsAg of genotypes A and C as well as the RT domain of the HBV polymerase. Hence, a pan-genotypic immune response is preferably induced.

[00145] Preferably, the method of vaccination results in an induction of an antigen-specific adaptive immune response. Accordingly, the method of vaccination preferably results in an induction of anti-HBs antibodies and/or in an induction of HBcAg- and HBsAg-specific CD4+/CD8+ T-cells. Additionally or alternatively, the method of vaccination may result in an induction of anti-HBcAg antibodies. Additionally or alternatively, the method of vaccination may result in an induction of anti-RT antibodies and/or in an induction of RT-specific CD4+/CD8+ T-cells. Thus, the method of vaccination results preferably in an induction of anti-HBsAg antibodies and/or in an induction of CD4+/CD8+ T-cells against HBsAg, HBcAg and/or a polymerase from HBV like a RT from HBV.

[00146] Herein, an induction of an immune response may encompass the induction of anti-HBs antibodies. In the context of an adaptive immune response, an induction thereof may encompass induction of antibodies that neutralize HBsAg and HBV virions and prevent further spread of the virus. Furthermore, herein, induction in the context of an antigen-specific adaptive immune response may encompass induction of HBsAg- and HBcAg-specific and preferably also RT-specific CD4+/CD8+ T-cells.

[00147] Preferably, the method of vaccination is associated with a blood level increase of neutralizing and/or immune activating anti-HBs antibodies. Preferably, the method of vaccination is associated with a blood level increase of neutralizing antibodies that neutralize HBsAg. The blood level increase may preferably be detectable by anti-HBs antibodies becoming detectable in peripheral blood at a titer ≥ 10 IU/ml or preferably at a titer ≥ 100 IU/ml. Alternatively or additionally, the method of vaccination is associated with a blood level increase of antibodies by a factor of at least about 2.

[00148] Preferably, the method of vaccination is associated with induction of anti-HBs antibodies, which may be detectable by a decrease in HBsAg (in peripheral blood) of at least a factor of 2, preferably by $\geq 1\log 10$ or preferably by HBsAg becoming undetectable in peripheral blood. Preferably, the method of vaccination is associated with a decrease in HBsAg (in peripheral blood) of at least a factor of 2, preferably by $\geq 1\log 10$ or preferably by HBsAg becoming undetectable in peripheral blood.

[00149] A blood level decrease of HBsAg and / or the induction or alternatively an increase of anti-HBs antibodies can preferably be detected in serum or plasma of a given individual. Such an increase or decrease can be measured by various techniques known in the art such as enzyme-linked immunoassays (EIA). Preferably, said increase is measured using

an EIA accredited to quantify anti-HBs antibodies. Preferred is a detection of HBsAg decrease or an increase of anti-HBs antibodies in blood.

[00150] Preferably, the method of vaccination is associated with a blood level increase of anti-HBsAg antibodies. Preferably, the method of vaccination is associated with a blood level increase of anti-HBsAg antibodies in peripheral blood at a titer ≥ 10 IU/ml or preferably at a titer ≥ 100 IU/ml.

[00151] Preferably, the method of vaccination is associated with a blood level increase of anti-HBcAg antibodies. Preferably, the method of vaccination is associated with a blood level increase of anti-HBcAg antibodies by a factor of at least about 2.

[00152] Preferably, the method of vaccination is associated with a blood level increase of anti-RT antibodies. Preferably, the method of vaccination is associated with a blood level increase of anti-RT antibodies to amounts that are detectable.

[00153] Preferably, the method of vaccination results in a blood level increase of CD4+/CD8+ T-cells against HBsAg, HBcAg and/or a polymerase from HBV.

[00154] Such an increase of CD4+/CD8+ T-cells against a given HBV antigen can be measured by various techniques known in the art such as EliSpot or FlouroSpot techniques or by flow cytometry following intracellular cytokine staining after antigen specific T-cell stimulation using peptide libraries or by staining of antigen-specific T-cell using HLA-multimers. Preferably, said increase is measured using EliSpot or FlouroSpot techniques detecting cytokine-secreting T cells after antigen-specific immune stimulation using peptide pools.

[00155] Preferably, the method of vaccination is associated with a blood level increase of HBsAg-specific CD4+ and/or CD8+ T-cells. Preferably, the method of vaccination is associated with a blood level increase of HBsAg-specific CD4+ and/or CD8+ T-cells to at least about 10, preferably at least about 100 spots/500.000 peripheral blood mononuclear cells, as preferably measured by EliSpot technique.

[00156] Preferably, the method of vaccination is associated with a blood level increase of HBcAg-specific CD4+ and/or CD8+ T-cells. Preferably, the method of vaccination is associated with a blood level increase of HBcAg-specific CD4+ and/or CD8+ T-cells to at least about 10, preferably at least about 100 spots/500.000 peripheral blood mononuclear cells, as preferably measured by EliSpot technique.

[00157] Preferably, the method of vaccination is associated with a blood level increase of RT-specific CD4+ and/or CD8+ T-cells. Preferably, the method of vaccination is associated with a blood level increase of RT-specific CD4+ and/or CD8+ T-cells to at least about 10, preferably

at least about 100 spots/500.000 peripheral blood mononuclear cells, as preferably measured by EliSpot technique.

[00158] Preferably, the method of vaccination is associated with a blood level increase of HBcAg- and HBsAg-specific CD4+ and/or CD8+ T-cells. Preferably, the method of vaccination is associated with a blood level increase of HBcAg- and HBsAg-specific CD4+ and/or CD8+ T-cells at least about 10, preferably at least about 100 spots/500.000 peripheral blood mononuclear cells, as preferably measured by EliSpot technique.

[00159] Preferably, the method of vaccination is associated with a blood level increase of HBsAg-specific CD4+ and/or CD8+ T-cells. Preferably, the method of vaccination is associated with a blood level increase of HBsAg-specific CD4+ and/or CD8+ T-cells by a factor of at least about 2 as detected by flow cytometry analysis after intracellular cytokine staining.

[00160] Preferably, the method of vaccination is associated with a blood level increase of HBcAg-specific CD4+ and/or CD8+ T-cells. Preferably, the method of vaccination is associated with a blood level increase of HBsAg-specific CD4+ and/or CD8+ T-cells by a factor of at least about 2 as detected by flow cytometry analysis after intracellular cytokine staining.

[00161] Preferably, the method of vaccination is associated with a blood level increase of HBsAg- and HBcAg-specific CD4+ and/or CD8+ T-cells. Preferably, the method of vaccination is associated with a blood level increase of HBsAg- and HBcAg-specific CD4+ and/or CD8+ T-cells by a factor of at least about 2 as detected by flow cytometry analysis after intracellular cytokine staining.

[00162] Preferably, the method of vaccination is not associated with dose limiting toxicity. More preferably, the method of vaccination is not associated with dose limiting toxicity with the first dose and the second dose each comprising the HBcAg particle in an amount from about 10µg to about 100µg and the HBsAg in an amount from about 5µg to about 100µg, and the dose of the vaccine vector comprising the vaccine vector in an amount from about 1×10^7 ifu to about 1×10^9 ifu. Even more preferably, the method of vaccination is not associated with dose limiting toxicity with the first dose and the second dose each comprising the HBcAg particle in an amount of about 25µg or of about 50µg and the HBsAg in an amount of about 20µg or of about 40µg, and the dose of the vaccine vector comprising the vaccine vector in an amount of about 6×10^7 ifu or of about 3×10^8 ifu. For example, the method of vaccination may not be associated with dose limiting toxicity with the first dose and the second dose each comprising the HBcAg particle in an amount of about 25µg and the HBsAg in an amount of about 20µg, and the dose of the vaccine vector comprising the vaccine vector in an amount of about 6×10^7 ifu. In another example, the method of vaccination may not be associated with dose limiting toxicity with the first dose and the second dose each comprising the HBcAg particle in an amount of

about 50µg and the HBsAg in an amount of about 40µg, and the dose of the vaccine vector comprising the vaccine vector in an amount of about 3×10^8 ifu.

[00163] Preferably, the method of vaccination is associated with a reduction of HBsAg titers in peripheral blood by at least about 1 log₁₀ from start of the treatment. Preferably, the method of vaccination is associated with a reduction of HBsAg titers in peripheral blood below the limit of quantification. Preferably, the method of vaccination is associated with a reduction of HBsAg titers in peripheral blood below the limit of detection. HBsAg titers are preferably measured by chemiluminescent immunoassay (CLIA).

[00164] Preferably, the method of vaccination is associated with an increased frequency of total HBV-specific, cytokine secreting T cells compared to pretreatment values, preferably as measured in a FluoroSpot assay. Preferably, the increase is at least about two-fold. Preferably, the increase is at least about five-fold.

[00165] Preferably, the method of vaccination is associated with increased number of cytokine secreting, HBV S-, core- and/or pol-specific T cells compared to pretreatment values, preferably as measured in an FluoroSpot assay or an intracellular cytokine staining (ICS) assay. Preferably, the increase is at least about two-fold. Preferably, the increase is at least about five-fold.

[00166] Preferably, the method of vaccination is associated with increased number of cytokine secreting, HBV S-specific T cells compared to pretreatment values, preferably as measured in an FluoroSpot assay or an intracellular cytokine staining (ICS) assay.

[00167] Preferably, the method of vaccination is associated with increased number of cytokine secreting, core-specific T cells compared to pretreatment values, preferably as measured in an FluoroSpot assay or an intracellular cytokine staining (ICS) assay.

[00168] Preferably, the method of vaccination is associated with increased number of cytokine secreting, pol-specific T cells compared to pretreatment values, preferably as measured in an FluoroSpot assay or an intracellular cytokine staining (ICS) assay.

[00169] Preferably, the method of vaccination is associated with an at least about 20% reduction of HBcore-related antigen (HBcr-Ag) compared to pretreatment values or stable suppression (over >6 weeks), preferably as measured by chemiluminescent enzyme immunoassay (CLEIA). The reduction of HBcr-Ag can even be higher, such as about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, or about 99%. The reduction of HBcr-Ag can be to below the limit of detection.

[00170] Preferably, the method of vaccination is associated with an at least about a ≥ 0.5 log₁₀ reduction of HBV-RNA in serum or stable suppression (over >6 weeks), preferably as measured by RT-qPCR. The of reduction of HBV-RNA in serum or stable suppression can even be higher, such as at least about ≥ 0.5 log₁₀, at least about ≥ 0.6 log₁₀, at least about ≥ 0.7 log₁₀, at least about ≥ 0.8 log₁₀, at least about ≥ 0.9 log₁₀, at least about ≥ 1.0 log₁₀, at least about ≥ 1.5 log₁₀, at least about ≥ 2.0 log₁₀, at least about ≥ 2.5 log₁₀, at least about ≥ 3.0 log₁₀, at least about ≥ 3.5 log₁₀, or at least about ≥ 4.0 log₁₀. The reduction of HBV-RNA in serum or stable suppression can be to below the limit of detection.

[00171] Preferably, the method of vaccination is a therapeutic vaccination method. The method of vaccination disclosed herein offers thus an alternative vaccination method that preferably has the advantage of providing a strong stimulation of the immune system, including preferably CD4+/CD8+ T-cell induction and/or anti-HBcAg antibody production. Thus, the disclosed method of vaccination is particularly well suited for applications in view of HBV and in particular in view of cases associated with an infection of an HBV genotype that may be of particular relevance in some regions due to their (frequent) occurrence.

[00172] Preferably, the method of vaccination is a vaccination method for treating an HBV infection. The HBV infection can be acute or chronic. Preferably, the method of vaccination is a vaccination method for treating a chronic HBV infection. Thus, by administering to a human subject the method of vaccination as disclosed herein, CD4+ TH1/TH2 T-cell responses can be induced for neutralizing anti-HBc antibody and anti-HBs antibody production by the two protein prime vaccinations and thus, a first and a second dose of both the HBcAg particle and an HBsAg. Additionally, cytotoxic CD8+ T-cells can be activated for an efficient elimination of infected cells by the subsequent vaccine vector administration that serves as a further boost. Hence, the method of vaccination disclosed herein represents a novel therapeutic, preferably curative, vaccination regime for treating an HBV infection.

[00173] The present invention relates also to an HBcAg particle and/or an HBsAg, for use in a method of vaccination. Said method preferably comprises administering to a human subject

- (i) a first dose of the HBcAg particle and of the HBsAg,
- (ii) a second dose of the HBcAg particle and of the HBsAg,
- (iii) a dose of a vaccine vector;

wherein the vaccine vector expresses

- a. an HBsAg from HBV genotype A;
- b. an HBcAg from HBV genotype D;
- c. an HBsAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 7;

- d. an HBcAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 8 or 17; and
- e. an RT domain of a polymerase from HBV comprising a sequence having at least 90 % sequence identity to the amino acid sequence set forth in SEQ ID NO: 9.

[00174] As regards the HBcAg particle, the HBsAg, the vaccine vector and the method of vaccination, the same applies as stated herein above in the context of the respective products and methods, respectively. For example, the vaccine vector is preferably the vaccine vector disclosed herein. Alternatively or additionally, the HBcAg particle is preferably the HBcAg particle disclosed herein. Alternatively or additionally, the HBsAg is preferably the, preferably particulate, HBsAg disclosed herein, more preferably an HBsAg from HBV genotype A and/or an, optionally nucleosidic, adjuvanted HBsAg, preferably a CpG, more preferably a CpG-1018, adjuvanted HBsAg. Alternatively or additionally, the method of vaccination is preferably the method of vaccination disclosed herein. Furthermore, the HBcAg particle, and optionally the HBsAg, may be comprised in the respective disclosed pharmaceutical composition, optionally comprised in the respective disclosed container or in the respective disclosed kit. Additionally or alternatively, the vaccine vector may be comprised in the respective disclosed pharmaceutical composition, optionally comprised in the respective disclosed container. Particularly preferred, the HBcAg particle is the HBcAg particle disclosed herein, the HBsAg is the HBsAg disclosed herein, the vaccine vector is the vaccine vector disclosed herein and the method of vaccination is the method of vaccination disclosed herein.

[00175] The present invention relates also to a vaccine vector expressing

- a. an HBsAg from HBV genotype A;
- b. an HBcAg from HBV genotype D;
- c. an HBsAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 7;
- d. an HBcAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 8 or 17; and
- e. an RT domain of a polymerase from HBV comprising a sequence having at least 90 % sequence identity to the amino acid sequence set forth in SEQ ID NO: 9.

for use in a method of vaccination, said method preferably comprising administering to a human subject

- (i) a first dose of an HBcAg particle and of an HBsAg,
- (ii) a second dose of the HBcAg particle and of the HBsAg, and
- (iii) a dose of the vaccine vector.

[00176] As regards the HBcAg particle, the HBsAg, the vaccine vector and the method of vaccination, the same applies as stated herein above in the context of the respective products

and methods, respectively. For example, the vaccine vector is preferably the vaccine vector disclosed herein. Alternatively or additionally, the HBcAg particle is preferably the HBcAg particle disclosed herein. Alternatively or additionally, the HBsAg is preferably the, preferably particulate, HBsAg disclosed herein, more preferably an HBsAg from HBV genotype A and/or an, optionally nucleosidic, adjuvanted HBsAg, preferably a CpG, more preferably a CpG-1018, adjuvanted HBsAg. Alternatively or additionally, the method of vaccination is preferably the method of vaccination disclosed herein. Furthermore, the HBcAg particle, and optionally the HBsAg, may be comprised in the respective disclosed pharmaceutical composition, optionally comprised in the respective disclosed container or in the respective disclosed kit. Additionally or alternatively, the vaccine vector may be comprised in the respective disclosed pharmaceutical composition, optionally comprised in the respective disclosed container. Particularly preferred, the HBcAg particle is the HBcAg particle disclosed herein, the HBsAg is the HBsAg disclosed herein, the vaccine vector is the vaccine vector disclosed herein and the method of vaccination is the method of vaccination disclosed herein.

[00177] The present invention relates also to a use of an HBcAg particle and/or an HBsAg, for the manufacture of a medicament. Preferably, said medicament is a medicament for a method of vaccination, wherein said method preferably comprises administering to a human subject

- (i) a first dose of the HBcAg particle and of the HBsAg,
- (ii) a second dose of the HBcAg particle and of the HBsAg, and
- (iii) a dose of a vaccine vector,

wherein the vaccine vector expresses

- a. an HBsAg from HBV genotype A;
- b. an HBcAg from HBV genotype D;
- c. an HBsAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 7;
- d. an HBcAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 8 or 17; and
- e. an RT domain of a polymerase from HBV comprising a sequence having at least 90 % sequence identity to the amino acid sequence set forth in SEQ ID NO: 9.

[00178] As regards the HBcAg particle, the HBsAg, the vaccine vector and the method of vaccination, the same applies as stated herein above in the context of the respective products and methods, respectively. For example, the vaccine vector is preferably the vaccine vector disclosed herein. Alternatively or additionally, the HBcAg particle is preferably the HBcAg particle disclosed herein. Alternatively or additionally, the HBsAg is preferably the, preferably particulate, HBsAg disclosed herein, more preferably an HBsAg from HBV genotype A and/or

an, optionally nucleosidic, adjuvanted HBsAg, preferably a CpG, more preferably a CpG-1018, adjuvanted HBsAg. Alternatively or additionally, the method of vaccination is preferably the method of vaccination disclosed herein. Furthermore, the HBcAg particle, and optionally the HBsAg, may be comprised in the respective disclosed pharmaceutical composition, optionally comprised in the respective disclosed container or in the respective disclosed kit. Additionally or alternatively, the vaccine vector may be comprised in the respective disclosed pharmaceutical composition, optionally comprised in the respective disclosed container. Particularly preferred, the HBcAg particle is the HBcAg particle disclosed herein, the HBsAg is the HBsAg disclosed herein, the vaccine vector is the vaccine vector disclosed herein and the method of vaccination is the method of vaccination disclosed herein.

[00179] The present invention relates also to a use of a vaccine vector expressing

- a. an HBsAg from HBV genotype A;
- b. an HBcAg from HBV genotype D;
- c. an HBsAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 7;
- d. an HBcAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 8 or 17; and
- e. an RT domain of a polymerase from HBV comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 9,

for the manufacture of a medicament. Said medicament is preferably a medicament for a method of vaccination, wherein said method preferably comprises administering to a human subject

- (i) a first dose of an HBcAg particle and of an HBsAg,
- (ii) a second dose of the HBcAg particle and of the HBsAg, and
- (iii) a dose of the vaccine vector.

[00180] As regards the HBcAg particle, the HBsAg, the vaccine vector and the method of vaccination, the same applies as stated herein above in the context of the respective products and methods, respectively. For example, the vaccine vector is preferably the vaccine vector disclosed herein. Alternatively or additionally, the HBcAg particle is preferably the HBcAg particle disclosed herein. Alternatively or additionally, the HBsAg is preferably the, preferably particulate, HBsAg disclosed herein, more preferably an HBsAg from HBV genotype A and/or an, optionally nucleosidic, adjuvanted HBsAg, preferably a CpG, more preferably a CpG-1018, adjuvanted HBsAg. Alternatively or additionally, the method of vaccination is preferably the method of vaccination disclosed herein. Furthermore, the HBcAg particle, and optionally the HBsAg, may be comprised in the respective disclosed pharmaceutical composition, optionally comprised in the respective disclosed container or in the respective disclosed kit. Additionally or

alternatively, the vaccine vector may be comprised in the respective disclosed pharmaceutical composition, optionally comprised in the respectively disclosed container. Particularly preferred, the HBcAg particle is the HBcAg particle disclosed herein, the HBsAg is the HBsAg disclosed herein, the vaccine vector is the vaccine vector disclosed herein and the method of vaccination is the method of vaccination disclosed herein.

[00181] The present invention relates also to a use of the expression cassette encoding an HBV core protein from HBV genotype C and an HBV core protein from HBV genotype D as disclosed herein, the nucleic acid sequence comprising the expression cassette as disclosed herein, the expression vector comprising the expression cassette as disclosed herein, and/or the expression vector encoding an HBV core protein from HBV genotype C and an HBV core protein from HBV genotype D as disclosed herein, for the manufacture of a medicament. Said medicament is preferably a medicament for a method of vaccination, wherein said method preferably comprises administering to a human subject

- (i) a first dose of the HBcAg particle or the expression cassette and of the HBsAg,
 - (ii) a second dose of the HBcAg particle or the expression cassette and of the HBsAg, and
 - (iii) a dose of a vaccine vector,
- wherein the vaccine vector expresses
- a. an HBsAg from HBV genotype A;
 - b. an HBcAg from HBV genotype D;
 - c. an HBsAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 7;
 - d. an HBcAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 8 or 17; and
 - e. an RT domain of a polymerase from HBV comprising a sequence having at least 90 % sequence identity to the amino acid sequence set forth in SEQ ID NO: 9.

[00182] As regards the HBcAg particle, the HBsAg, the vaccine vector and the method of vaccination, the same applies as stated herein above in the context of the respective products and methods, respectively. For example, the vaccine vector is preferably the vaccine vector disclosed herein. Alternatively or additionally, the HBcAg particle is preferably the HBcAg particle disclosed herein. Alternatively or additionally, the HBsAg is preferably the, preferably particulate, HBsAg disclosed herein, more preferably an HBsAg from HBV genotype A and/or an, optionally nucleosidic, adjuvanted HBsAg, preferably a CpG, more preferably a CpG-1018, adjuvanted HBsAg. Alternatively or additionally, the method of vaccination is preferably the method of vaccination disclosed herein. Furthermore, the HBcAg particle, and optionally the HBsAg, may be comprised in the respective disclosed pharmaceutical composition, optionally

comprised in the respective disclosed container or in the respective disclosed kit. Additionally or alternatively, the vaccine vector may be comprised in the respective disclosed pharmaceutical composition, optionally comprised in the respectively disclosed container. Particularly preferred, the HBcAg particle is the HBcAg particle disclosed herein, the HBsAg is the HBsAg disclosed herein, the vaccine vector is the vaccine vector disclosed herein and the method of vaccination is the method of vaccination disclosed herein. The invention is further characterized by the following items:

1. A Hepatitis B Virus core antigen (HBcAg) particle, comprising Hepatitis B Virus (HBV) core proteins from at least two different HBV genotypes, wherein the HBcAg particle is preferably an isolated HBcAg particle.
2. The HBcAg particle of item 1, wherein the HBV genotypes are selected from the group consisting of A, B, C, and D.
3. The HBcAg particle of item 1 or 2, wherein the HBV core proteins from the at least two different HBV genotypes are in an approximately equimolar ratio.
4. The HBcAg particle of any one of the preceding items, comprising HBV core proteins of not more than two different HBV genotypes.
5. The HBcAg particle of any one of the preceding items, wherein the HBV genotypes are selected from the group consisting of C and D.
6. The HBcAg particle of any one of the preceding items, wherein the HBcAg particle comprises HBV core proteins from HBV genotypes C and D and wherein the HBV core proteins from said HBV genotypes are in a ratio of about 10:90 to about 90:10.
7. The HBcAg particle of any one of the preceding items, wherein at least a portion of the HBV core proteins are full-length HBV core proteins.
8. The HBcAg particle of items 1 to 6, wherein at least a portion of the HBV core proteins are truncated HBV core proteins.
9. The HBcAg particle of any one of items 1 to 6 and 8, wherein the HBcAg particle comprises truncated HBV core proteins from HBV genotype C and full-length HBV core proteins from HBV genotype D.
10. The HBcAg particle of any one of items 1 to 6 and 8 to 9, wherein the HBcAg particle comprises truncated HBV core proteins from HBV genotype C and full-length HBV core

proteins from HBV genotype D and wherein the HBV core proteins from said HBV genotypes are in a ratio of about 10:90 to about 90:10.

11. The HBcAg particle of any one of items 1 to 6 and 8 to 10, wherein the HBcAg particle consists of truncated HBV core proteins from HBV genotype C and full-length HBV core proteins from HBV genotype D.
12. The HBcAg particle of any one of items 1 to 6 and 8 to 11, wherein the HBcAg particle consists of truncated HBV core proteins from HBV genotype C and full-length HBV core proteins from HBV genotype D and wherein the HBV core proteins from said HBV genotypes are in a ratio of about 10:90 to about 90:10.
13. The HBcAg particle of any one of the preceding items, wherein the HBcAg particle is a self-assembling particulate capsid.
14. The HBcAg particle of any one of the preceding items, wherein the HBcAg particle comprises about 50 to 200 dimers of HBV core proteins and/or wherein the HBcAg particle has a size of about 20 to about 60 nm in diameter.
15. The HBcAg particle of any one of the preceding items, wherein the HBcAg particle comprises about 80 to 100 dimers of HBV core proteins or about 110 to 130 dimers of HBV core proteins and/or wherein the HBcAg particle has a size of about 25 to about 50 nm, preferably a size of about 30 to about 34 nm in diameter.
16. The HBcAg particle of any one of the preceding items, wherein the HBcAg particle comprises about 90 dimers of HBV core proteins or about 120 dimers of HBV core proteins and/or wherein the HBcAg particle has a size of about 30 or about 34 nm in diameter.
17. The HBcAg particle of any one of the preceding items, wherein the hydrodynamic radius of the particle is about 40 nm to about 60 nm, preferably about 45 nm to about 55 nm, preferably about 48 nm to about 52 nm, preferably as measured by dynamic light scattering.
18. The HBcAg particle of any one of the preceding items, wherein the HBcAg particle comprises dimers of HBV core proteins, wherein said dimers are assembled from i) HBV core proteins from HBV genotype C, ii) HBV core proteins from HBV genotype C and HBV core proteins from HBV genotype D, and/or iii) HBV core proteins from HBV genotype D.
19. The HBcAg particle of any one of items 1 to 6 and 8 to 18, wherein the HBcAg particle comprises dimers of HBV core proteins, wherein said dimers are assembled from i) truncated HBV core proteins from HBV genotype C, ii) truncated HBV core proteins from

- HBV genotype C and full-length HBV core proteins from HBV genotype D, and/or iii) full-length HBV core proteins from HBV genotype D.
20. The HBcAg particle of any one of items 1 to 6 and 8 to 19, wherein the HBcAg particle consists of dimers of HBV core proteins, wherein said dimers are assembled from i) truncated HBV core proteins from HBV genotype C, ii) truncated HBV core proteins from HBV genotype C and full-length HBV core proteins from HBV genotype D, and/or iii) full-length HBV core proteins from HBV genotype D.
21. The HBcAg particle of any one of the preceding items, wherein HBV core proteins from HBV genotype C comprise a sequence that has at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1 or 13 and/or HBV core proteins from HBV genotype D comprise a sequence that has at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 2 or 14.
22. The HBcAg particle of any one of the preceding items, wherein HBV core proteins from HBV genotype C comprise a sequence that has at least 95% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1 or 13 and/or HBV core proteins from HBV genotype D comprise a sequence that has at least 95% sequence identity to the amino acid sequence set forth in SEQ ID NO: 2 or 14.
23. The HBcAg particle of any one of the preceding items, wherein HBV core proteins from HBV genotype C comprise the amino acid sequence set forth in SEQ ID NO: 1 or 13 and/or HBV core proteins from HBV genotype D comprise the amino acid sequence set forth in SEQ ID NO: 2 or 14.
24. The HBcAg particle of any one of the preceding items, wherein the HBcAg particle is capable of inducing an immune response against the HBV core proteins it is composed of.
25. The HBcAg particle of any one of the preceding items, wherein the HBcAg particle is capable of inducing an antigen-specific adaptive immune response.
26. The HBcAg particle of item 24 or 25, wherein the immune response is associated with anti-HBcAg antibody induction.
27. The HBcAg particle of any one of items 24 to 26, wherein the immune response is associated with HBcAg-specific CD4⁺/CD8⁺ T-cell induction.
28. A pharmaceutical composition comprising the HBcAg particle of any one of the preceding items and optionally a pharmaceutically acceptable carrier or excipient.

29. The pharmaceutical composition of item 28 further comprising an HBV surface antigen (HBsAg), wherein the HBsAg is preferably a particulate HBsAg.
30. The pharmaceutical composition of item 28 or 29 further comprising an adjuvant.
31. The pharmaceutical composition of item 30, wherein the adjuvant is a nucleosidic adjuvant.
32. The pharmaceutical composition of item 31, wherein the adjuvant is a CpG.
33. The pharmaceutical composition of item 32, wherein the adjuvant is CpG-1018.
34. A container comprising one or more doses of the pharmaceutical composition of any one of items 28 to 33.
35. A kit comprising the pharmaceutical composition of item 28 and a second pharmaceutical composition comprising an HBsAg, wherein the HBsAg is preferably a particulate HBsAg.
36. The kit of item 35, wherein the second pharmaceutical composition further comprises an adjuvant.
37. The kit of items 35 or 36, wherein the kit comprises a first container comprising one or more doses of the pharmaceutical composition comprising the HBsAg particle and a second container comprising one or more doses of the second pharmaceutical composition, and optionally a third container comprising an adjuvant.
38. The kit of any one of items 35 to 37, wherein the adjuvant comprised in the second pharmaceutical composition and/or the adjuvant comprised in the third container is a nucleosidic adjuvant.
39. The kit of any one of items 35 to 38, wherein the adjuvant comprised in the second pharmaceutical composition and/or the adjuvant comprised in the third container is a CpG.
40. The kit of any one of items 35 to 39, wherein the adjuvant comprised in the second pharmaceutical composition and/or the adjuvant comprised in the third container is CpG-1018.
41. The container of item 34 or the kit of any one of items 35 to 40, wherein a dose of the pharmaceutical composition comprises the HBsAg particle in an amount from about 10 μ g to about 100 μ g.

42. The container of item 34 or the kit of any one of items 35 to 51, wherein a dose of the pharmaceutical composition comprises the HBcAg particle in an amount from about 10µg to about 75µg.
43. The container of item 34 or the kit of any one of items 35 to 42, wherein a dose of the pharmaceutical composition comprises the HBcAg particle in an amount from about 20µg to about 100µg, preferably to about 60µg.
44. The container of item 34 or the kit of any one of items 35 to 43, wherein a dose of the pharmaceutical composition comprises the HBcAg particle in an amount of about 25µg or of about 50µg.
45. The container of item 34 or the kit of any one of items 35 to 44, wherein in case of the container a dose of the pharmaceutical composition or in case of the kit a dose of the second pharmaceutical composition comprises an HBsAg in an amount from about 5µg to about 100µg, preferably in an amount from about 5µg to about 75µg, wherein the HBsAg is preferably an particulate HBsAg.
46. The container of item 34 or the kit of any one of items 35 to 45, wherein in case of the container a dose of the pharmaceutical composition or in case of the kit a dose of the second pharmaceutical composition comprises HBsAg in an amount from about 10µg to about 50µg.
47. The container of item 34 or the kit of any one of items 35 to 46, wherein in case of the container a dose of the pharmaceutical composition or in case of the kit a dose of the second pharmaceutical composition comprises HBsAg in an amount of about 20µg or of about 40µg.
48. The container of item 34 or the kit of any one of items 35 to 47, wherein a dose of the pharmaceutical composition comprises the HBcAg particle in an amount of about 25µg or of about 50µg and wherein in case of the container a dose of the pharmaceutical composition or in case of the kit a dose of the second pharmaceutical composition comprises HBsAg in an amount of about 20µg or of about 40µg.
49. The container of item 34 or the kit of any one of items 35 to 48, wherein a dose of the pharmaceutical composition comprises the HBcAg particle in an amount of about 25µg and wherein in case of the container a dose of the pharmaceutical composition or in case of the kit a dose of the second pharmaceutical composition comprises HBsAg in an amount of about 20µg.

50. The container of item 34 or the kit of any one of items 35 to 49, wherein a dose of the pharmaceutical composition comprises the HBcAg particle in an amount of about 50µg and wherein in case of the container a dose of the pharmaceutical composition or in case of the kit a dose of the second pharmaceutical composition comprises HBsAg in an amount of about 40µg.
51. The HBcAg particle of any one of items 1 to 27, or the pharmaceutical composition of any one of items 28 to 33, or comprised in the container of any one of items 34 or 41 to 50 or in the kit of any one of items 35 to 50, for use in therapy.
52. The HBcAg particle for the use of item 51, or the pharmaceutical composition for the use of item 51, or comprised in the container for the use of item 51 or in the kit for the use of item 51, wherein the use is in an immune stimulation method.
53. The HBcAg particle for the use of item 51 or 52, or the pharmaceutical composition for the use of item 51 or 52, or comprised in the container for the use of item 51 or 52 or in the kit for the use of item 51 or 52, wherein the use is in a vaccination method.
54. The HBcAg particle for the use of any one of items 51 to 53, or the pharmaceutical composition for the use of any one of items 51 to 53, or comprised in the container for the use of any one of items 51 to 53 or in the kit for the use of any one of items 51 to 53, wherein the vaccination method is a therapeutic vaccination method.
55. The HBcAg particle for the use of any one of items 51 to 54, or the pharmaceutical composition for the use of any one of items 51 to 54, or comprised in the container for the use of any one of items 51 to 54 or in the kit for the use of any one of items 51 to 54, for use in treating an HBV infection.
56. The HBcAg particle for the use of item 55, or the pharmaceutical composition for the use of item 55, or comprised in the container for the use of item 55 or in the kit for the use of item 55, wherein the use comprises inducing anti-HBcAg antibodies.
57. The HBcAg particle for the use of item 55 or 56, or the pharmaceutical composition of item 55 or 56, or comprised in the container for the use of item 55 or 56 or in the kit for the use of item 55 or 56, wherein the use comprises inducing HBcAg-specific CD4+/CD8+ T-cells.
58. An expression cassette, an mRNA, or a cDNA, wherein the expression cassette, mRNA, or cDNA, encodes an HBV core protein from HBV genotype C and an HBV core protein from HBV genotype D, wherein the expression cassette, mRNA, or cDNA, only comprises coding sequences for two or more HBV core proteins.

59. The expression cassette, mRNA, or cDNA, of item 58, wherein the expression cassette, mRNA, or cDNA, encodes an HBV core protein from HBV genotype C comprising a sequence that has at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1 or 13 and/or an HBV core protein from HBV genotype D comprising a sequence that has at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 2 or 14.
60. The expression cassette, mRNA, or cDNA, of item 58 or 59, wherein the expression cassette, mRNA, or cDNA, encodes an HBV core protein from HBV genotype C comprising a sequence that has at least 95% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1 or 13 and/or an HBV core protein from HBV genotype D comprising a sequence that has at least 95% sequence identity to the amino acid sequence set forth in SEQ ID NO: 2 or 14.
61. The expression cassette, mRNA, or cDNA, of any one of items 58 to 60, wherein the expression cassette, mRNA, or cDNA, encodes an HBV core protein from HBV genotype C comprising a sequence that has the amino acid sequence set forth in SEQ ID NO: 1 or 13 and/or an HBV core protein from HBV genotype D comprising a sequence that has the amino acid sequence set forth in SEQ ID NO: 2 or 14.
62. itemThe expression cassette, mRNA, or cDNA, of any one of items 58 to 61, wherein the expression cassette, mRNA, or cDNA, comprises a first nucleotide sequence encoding an HBV core protein from HBV genotype C, wherein said first nucleotide sequence comprises a sequence that has at least 90% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 3 or 15, and/or wherein the expression cassette, mRNA, or cDNA, comprises a second nucleotide sequence encoding an HBV core protein from HBV genotype D, wherein said second nucleotide sequence comprises a sequence that has at least 90% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 4 or 16.
63. The expression cassette, mRNA, or cDNA, of any one of items 58 to 62, wherein the expression cassette, mRNA, or cDNA, comprises a first nucleotide sequence encoding a HBV core protein from HBV genotype C, wherein said first nucleotide sequence comprises a sequence that has at least 95% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 3 or 15, and/or wherein the expression cassette, mRNA, or cDNA, comprises a second nucleotide sequence encoding a HBV core protein from HBV genotype D, wherein said second nucleotide sequence comprises a sequence that has at least 95% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 4 or 16.

64. The expression cassette, mRNA, or cDNA, of any one of items 58 to 63, wherein the expression cassette, mRNA, or cDNA, comprises a first nucleotide sequence encoding a HBV core protein from HBV genotype C, wherein said first nucleotide comprises the nucleotide sequence set forth in SEQ ID NO: 3 or 15, and/or wherein the expression cassette, mRNA, or cDNA, comprises a second nucleotide sequence encoding a HBV core protein from HBV genotype D, wherein said second nucleotide sequence comprises the nucleotide sequence set forth in SEQ ID NO: 4 or 16.
65. itemA nucleic acid molecule comprising the expression cassette, the mRNA, or the cDNA, of any one of items 58 to 64.
66. The nucleic acid molecule of item 65, wherein the nucleic acid molecule is a DNA molecule.
67. The nucleic acid molecule of item 66, wherein the nucleic acid molecule is an mRNA molecule.
68. An expression vector comprising the expression cassette or the cDNA of any one of items 58 to 64 or the nucleic acid molecule of item 65 or 66.
69. The expression vector of item 68, wherein the expression vector is a recombinant vector.
70. An expression vector encoding an HBV core protein from HBV genotype C and an HBV core protein from HBV genotype D, wherein the expression vector comprises a nucleotide sequence that has at least 90% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 10.
71. The expression vector of item 70, wherein the expression vector comprises a nucleotide sequence that has at least 95% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 10.
72. The expression vector of item 70 or 71, wherein the expression vector comprises the nucleotide sequence set forth in SEQ ID NO: 10.
73. The expression vector of any one of items 70 to 72, wherein the expression vector is a recombinant vector.
74. A vaccine vector, wherein the vaccine vector is preferably an MVA viral vector, and wherein the vaccine vector comprises a nucleic acid molecule comprising a nucleotide sequence having at least 90% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 5.

75. The vaccine vector of item 74, wherein the nucleic acid molecule comprises
- a nucleotide sequence encoding a HBsAg from HBV genotype A,
 - a nucleotide sequence encoding a HBcAg from HBV genotype D,
 - a nucleotide sequence encoding a reverse transcriptase (RT) domain of a polymerase from HBV,
 - a nucleotide sequence encoding HBsAg from HBV genotype C, and
 - a nucleotide sequence encoding HBcAg from HBV genotype C.
76. The vaccine vector of item 74 or 75, wherein the nucleic acid molecule comprises a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 5.
77. The vaccine vector of any one of items 74 to 76, wherein the nucleic acid molecule comprises the nucleotide sequence set forth in SEQ ID NO: 5.
78. The vaccine vector of any one of items 74 to 77, wherein the vaccine vector comprises a nucleic acid molecule comprising a nucleotide sequence that has at least 90% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 6.
79. The vaccine vector of any one of items 74 to 78, wherein the vaccine vector comprises a nucleic acid molecule comprising a nucleotide sequence that has at least 95% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 6.
80. The vaccine vector of any one of items 74 to 79, wherein the vaccine vector comprises a nucleic acid molecule comprising a nucleotide sequence that has the nucleotide sequence set forth in SEQ ID NO: 6.
81. A pharmaceutical composition comprising the vaccine vector of any one of items 74 to 80 and optionally a pharmaceutically acceptable carrier or excipient.
82. A container comprising one or more doses of the pharmaceutical composition of item 81, wherein a dose of the pharmaceutical composition comprises the vaccine vector in an amount from about 1×10^7 ifu to about 1×10^9 ifu.
83. The container of item 82, wherein a dose of the pharmaceutical composition comprises the vaccine vector in an amount from about 5×10^7 ifu to about 1×10^9 ifu.
84. The container of item 82 or 83, wherein a dose of the pharmaceutical composition comprises the vaccine vector in an amount of about 6×10^7 ifu or of about 3×10^8 ifu or of about 5×10^8 ifu.

85. The container of any one of items 82 to 84, wherein a dose of the pharmaceutical composition comprises the vaccine vector in an amount of about 3×10^8 ifu.
86. The vaccine vector of any one of items 74 to 80, or the pharmaceutical composition of item 81, or comprised in the container of any one of items 82 to 85, for use in therapy.
87. The vaccine vector for the use of item 86, or the pharmaceutical composition for the use of item 86, or comprised in the container for the use of item 86, wherein the use is in an immune stimulation method.
88. The vaccine vector for the use of item 86 or 87, or the pharmaceutical composition for the use of item 86 or 87, or comprised in the container for the use of item 85 or 86, wherein the use is in a vaccination method.
89. The vaccine vector for the use of any one of items 86 to 88, or the pharmaceutical composition the use of any one of items 86 to 89, or comprised in the container of any one of items 86 to 89, wherein the vaccination method is a therapeutic vaccination method.
90. The vaccine vector for the use of any one of items 86 to 89, or pharmaceutical composition for the use of any one of items 86 to 89, or comprised in the container for the use of any one of items 86 to 89, for use in treating an HBV infection.
91. The vaccine vector for the use of item 90, or the pharmaceutical composition for the use of item 90, or comprised in the container for the use of item 90, wherein the use comprises inducing anti-HBcAg antibodies.
92. The vaccine vector for the use of item 90 or 91, or the pharmaceutical composition for the use of item 90 or 91, or comprised in the container for the use of item 90 or 91, wherein the use comprises inducing HBcAg-specific CD4⁺/CD8⁺ T-cells.
93. A method of vaccination, comprising administering to a human subject
- (i) a first dose of an HBcAg particle and of an HBsAg,
 - (ii) a second dose of the HBcAg particle and of the HBsAg,
 - (iii) a dose of a vaccine vector
- wherein the vaccine vector expresses
- a. an HBsAg from HBV genotype A;
 - b. an HBcAg from HBV genotype D;
 - c. an HBsAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 7;

- d. an HBcAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 8 or 17; and
 - e. an RT domain of a polymerase from HBV comprising a sequence having at least 90 % sequence identity to the amino acid sequence set forth in SEQ ID NO: 9.
94. The method of vaccination of item 93, wherein in (i) the first dose of the HBcAg particle and of the HBsAg is a first dose of the pharmaceutical composition of any one of items 29 to 33, optionally comprised in the container of any one of items 34 or 41 to 50, or of the pharmaceutical composition of item 28 comprised in the kit of any one of items 35 to 50, wherein the HBsAg is preferably an particulate HBsAg.
95. The method of vaccination of item 93 or 94, wherein in (ii) the second dose of the HBcAg and of the HBsAg is a second dose of the pharmaceutical composition of any one of items 29 to 33, optionally comprised in the container of any one of items 34 or 41 to 50, or of the pharmaceutical composition of item 28 comprised in the kit of any one of items 35 to 50, wherein the HBsAg is preferably an particulate HBsAg.
96. The method of vaccination of any one of items 93 to 95, wherein in a. the HBsAg is a surface protein from HBV genotype A, preferably a small or large surface protein from HBV genotype A.
97. The method of vaccination of any one of items 93 to 96, wherein in a. the HBsAg is a small or large surface protein from HBV genotype A serotype adw.
98. The method of vaccination of any one of items 93 to 97, wherein in a. the HBsAg is a small surface protein from HBV genotype A serotype adw.
99. The method of vaccination of any one of items 93 to 98, wherein in b. the HBcAg is from HBV genotype D.
100. The method of vaccination of any one of items 93 to 99, wherein in b. the HBcAg is from HBV genotype D serotype ayw.
101. The method of vaccination of any one of items 93 to 100, wherein in c. the HBsAg comprises the amino acid sequence set forth in SEQ ID NO: 7.
102. The method of vaccination of any one of items 93 to 101, wherein in d. the HBcAg comprises the amino acid sequence set forth in SEQ ID NO: 8 or 17.
103. The method of vaccination of any one of items 93 to 102, wherein in e. the RT domain comprises the amino acid sequence set forth in SEQ ID NO: 9.

104. The method of vaccination of any one of items 93 to 103, wherein the vaccine vector is the vaccine vector of any one of items 74 to 80, or comprised in the pharmaceutical composition of item 80 or in the container of any one of items 82 to 85.
105. The method of vaccination of any one of items 93 to 104, wherein the first dose is administered to the human subject in a first injection, the second dose in a second injection, and/or the dose of the vaccine vector in a third injection, wherein the first, second and third injection are preferably intramuscular injections.
106. The method of vaccination of any one of items 93 to 105, wherein the first dose and the second dose each comprise the HBcAg particle in an amount from about 10 μ g to about 100 μ g and the HBsAg in an amount from about 5 μ g to about 100 μ g.
107. The method of vaccination of item 93 or 106, wherein the first dose and the second dose each comprise the HBcAg particle in an amount from about 10 μ g to about 75 μ g and the HBsAg in an amount from about 5 μ g to about 75 μ g.
108. The method of vaccination of any one of items 93 to 107, wherein the first dose and the second dose each comprise the HBcAg particle in an amount from about 20 μ g to about 100 μ g, preferably to about 60 μ g, and the HBsAg in an amount from about 5 μ g to about 75 μ g.
109. The method of vaccination of any one of items 93 to 108, wherein the first dose and the second dose each comprise the HBcAg particle in an amount of about 25 μ g or of about 50 μ and the HBsAg in an amount from about 5 μ g to about 75 μ g.
110. The method of vaccination of items 93 to 109, wherein the first dose and the second dose each comprise the HBcAg particle in an amount from about 10 μ g to about 100 μ g and the HBsAg in an amount from about 10 μ g to about 50 μ g.
111. The method of vaccination of items 93 to 110, wherein the first dose and the second dose each comprise the HBcAg particle in an amount from about 10 μ g to about 75 μ g and the HBsAg in an amount from about 10 μ g to about 50 μ g.
112. The method of vaccination of items 93 to 111, wherein the first dose and the second dose each comprise the HBcAg particle in an amount from about 20 μ g to about 100 μ g, preferably to about 60 μ g, and the HBsAg in an amount from about 10 μ g to about 50 μ g.
113. The method of vaccination of items 93 to 112, wherein the first dose and the second dose each comprise the HBcAg particle in an amount of about 25 μ g or of about 50 μ and the HBsAg in an amount from about 10 μ g to about 50 μ g.

114. The method of vaccination of items 93 to 113, wherein the first dose and the second dose each comprise the HBcAg particle in an amount from about 10µg to about 100µg and the HBsAg in an amount of about 20µg or of about 40µg.
115. The method of vaccination of items 93 to 114, wherein the first dose and the second dose each comprise the HBcAg particle in an amount from about 10µg to about 75µg and the HBsAg in an amount of about 20µg or of about 40µg.
116. The method of vaccination of items 93 to 115, wherein the first dose and the second dose each comprise the HBcAg particle in an amount from about 20µg to about 100µg, preferably to about 60µg, and the HBsAg in an amount of about 20µg or of about 40µg.
117. The method of vaccination of items 93 to 116, wherein the first dose and the second dose each comprise the HBcAg particle in an amount of about 25µg or of about 50µg and the HBsAg in an amount of about 20µg or of about 40µg.
118. The method of vaccination of items 93 to 117, wherein the first dose and the second dose each comprise the HBcAg particle in an amount from about 25µg and the HBsAg in an amount of about 20µg.
119. The method of vaccination of items 93 to 118, wherein the first dose and the second dose each comprise the HBcAg particle in an amount from about 50µ and the HBsAg in an amount of about 40µg.
120. The method of vaccination of any one of items 93 to 119, wherein the dose of the vaccine vector comprises the vaccine vector in an amount from about 1×10^7 ifu to about 1×10^9 ifu.
121. The method of vaccination of any one of items 93 to 120, wherein the dose of the vaccine vector comprises the vaccine vector in an amount from about 5×10^7 ifu to about 5×10^8 ifu.
122. The method of vaccination of any one of items 93 to 121, wherein the dose of the vaccine vector comprises the vaccine vector in an amount of about 6×10^7 ifu or of about 3×10^8 ifu or of about 5×10^8 ifu.
123. The method of vaccination of any one of items 93 to 122, wherein the dose of the vaccine vector comprises the vaccine vector in an amount of about 3×10^8 ifu or of about 5×10^8 ifu.

124. The method of vaccination of any one of items 93 to 123, wherein the method of vaccination comprises administering the second dose about 1 week to about 8 weeks after the first dose.
125. The method of vaccination of any one of items 93 to 124, wherein the method of vaccination comprises administering the second dose about 2 weeks to about 6 weeks after the first dose.
126. The method of vaccination of any one of items 93 to 125, wherein the method of vaccination comprises administering the second dose about 4 weeks after the first dose.
127. The method of vaccination of any one of items 93 to 126, wherein the method of vaccination comprises administering the dose of the vaccine vector about 2 weeks to about 24 weeks after the first dose.
128. The method of vaccination of any one of items 93 to 127, wherein the method of vaccination comprises administering the dose of the vaccine vector about 4 weeks to about 12 weeks after the first dose.
129. The method of vaccination of any one of items 93 to 128, wherein the method of vaccination comprises administering the dose of the vaccine vector about 8 weeks after the first dose.
130. The method of vaccination of any one of items 93 to 129, wherein the first dose and the second dose each comprise the HBcAg particle in an amount of about 25 μ g and the HBsAg in an amount of about 20 μ g, wherein the dose of the vaccine vector comprises the vaccine vector in an amount of about 6×10^7 ifu, wherein the second dose is administered about 4 weeks after the first dose, and wherein the dose of the vaccine vector is administered about 8 weeks after the first dose.
131. The method of vaccination of any one of items 93 to 130, wherein the first dose and the second dose each comprise the HBcAg particle in an amount of about 50 μ g and the HBsAg in an amount of about 40 μ g, wherein the dose of the vaccine vector comprises the vaccine vector in an amount of about 3×10^8 ifu, wherein the second dose is administered about 4 weeks after the first dose, and wherein the dose of the vaccine vector is administered about 8 weeks after the first dose.
132. The method of vaccination of any one of items 93 to 131, wherein the first dose and the second dose each comprise the HBcAg particle in an amount of about 25 μ g and the HBsAg in an amount of about 20 μ g, wherein the dose of the vaccine vector comprises the

vaccine vector in an amount of about 3×10^8 ifu, wherein the second dose is administered about 4 weeks after the first dose, and wherein the dose of the vaccine vector is administered about 8 weeks after the first dose.

133. The method of vaccination of any one of items 93 to 132, wherein the method of vaccination results in an induction of an immune response against the HBV antigens it is composed of.
134. The method of vaccination of any one of items 93 to 133, wherein the method of vaccination results in an induction of an antigen-specific adaptive immune response.
135. The method of vaccination of any one of items 93 to 134, wherein the method of vaccination results in an induction of anti-HBcAg antibodies, anti-HBsAg antibodies and/or anti-RT antibodies.
136. The method of vaccination of any one of items 93 to 135, wherein the method of vaccination results in an induction of CD4+/CD8+ T-cells against HBsAg, HBcAg and/or a polymerase from HBV.
137. The method of vaccination of any one of items 93 to 136, wherein the method of vaccination is associated with a blood level increase of neutralizing and/or immune activating antibodies, wherein the antibodies are detectable in peripheral blood at a titer ≥ 10 IU/ml or wherein the blood level increase of neutralizing and/or immune activating antibodies results in a drop in HBsAg of at least about $1\log_{10}$.
138. The method of vaccination of any one of items 93 to 137, wherein the method of vaccination is associated with a blood level increase of anti-HBsAg antibodies, wherein the antibodies are detectable in peripheral blood at a titer of at least about 10 IU/ml or wherein the blood level increase of neutralizing and/or immune activating antibodies results in a drop in HBsAg of at least about $1\log_{10}$.
139. The method of vaccination of any one of items 93 to 138, wherein the method of vaccination results in a blood level increase of CD4+/CD8+ T-cells against HBsAg, HBcAg and/or a polymerase from HBV to at least about 10 spots/500.000 peripheral blood mononuclear cells measurable in an EliSpot or FlouroSpot assay.
140. The method of vaccination of any one of items 93 to 139, wherein the method of vaccination is not associated with dose limiting toxicity.
141. The method of vaccination of any one of items 93 to 140 wherein the method of vaccination is a therapeutic vaccination method.

142. The method of vaccination of any one of items 93 to 141, wherein the method of vaccination is a vaccination method for treating a HBV infection.
143. The method of any one of items 93 to 142, wherein the subject has a chronic hepatitis B virus infection.
144. The method of any one of items 93 to 143, wherein the method is associated with a reduction of HBsAg titers in peripheral blood by at least about 1 log₁₀ from start of the treatment, preferably as measured by chemiluminescent immunoassay (CLIA).
145. The method of any one of items 93 to 144, wherein the method is associated with a reduction of HBsAg titers in peripheral blood below the limit of quantification or below the limit of detection.
146. The method of any one of items 93 to 145, wherein the method is associated with an increased frequency of total HBV-specific, cytokine secreting T cells compared to pretreatment values, preferably as measured in a FluoroSpot assay, wherein the increase is preferably at least about two-fold, preferably at least about five-fold.
147. The method of any one of items 93 to 146, wherein the method is associated with increased number of cytokine secreting, HBV S-, core- and/or pol-specific T cells compared to pretreatment values, preferably as measured in an FluoroSpot assay or an intracellular cytokine staining (ICS) assay, wherein the increase is preferably at least about two-fold, preferably at least about five-fold.
148. The method of any one of items 93 to 147, wherein the method is associated with an at least about 20% reduction of HBcore-related antigen (HBcr-Ag) compared to pretreatment values or stable suppression (over >6 weeks), preferably as measured by chemiluminescent enzyme immunoassay (CLEIA).
149. The method of any one of items 93 to 148, wherein the method is associated with an at least about a ≥ 0.5 log₁₀ reduction of HBV-RNA in serum or stable suppression (over >6 weeks), preferably as measured by RT-qPCR.
150. An HBcAg particle, and optionally an HBsAg, for use in a method of vaccination, said method preferably comprising administering to a human subject
- (i) a first dose of the HBcAg particle and of the HBsAg,
 - (ii) a second dose of the HBcAg particle and of the HBsAg,
 - (iii) a dose of a vaccine vector;
- wherein the vaccine vector expresses

- a. an HBsAg from HBV genotype A;
- b. an HBcAg from HBV genotype D;
- c. an HBsAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 7;
- d. an HBcAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 8 or 17; and
- e. an RT domain of a polymerase from HBV comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 9.

151. A vaccine vector expressing

- a. an HBsAg from HBV genotype A;
- b. an HBcAg from HBV genotype D;
- c. an HBsAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 7;
- d. an HBcAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 8 or 17; and
- e. an RT domain of a polymerase from HBV comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 9.

for use in a method of vaccination, said method preferably comprising administering to a human subject

- (i) a first dose of an HBcAg particle and of an HBsAg,
- (ii) a second dose of the HBcAg particle and of the HBsAg, and
- (iii) a dose of the vaccine vector.

152. The HBcAg particle, and optionally the HBsAg, for use in a method of vaccination of item 150, or the vaccine vector for use in a method of vaccination of item 151, wherein the vaccine vector is defined as in any one of items 74 to 80.

153. The HBcAg particle, and optionally the HBsAg, for use in a method of vaccination of item 150 or 152, or the vaccine vector for use in a method of vaccination of item 151 or 152, wherein the HBcAg particle is defined as in any one of items 1 to 26.

154. The HBcAg particle, and optionally the HBsAg, for use in a method of vaccination of any one of items 150 or 152 to 153, or the vaccine vector for use in a method of vaccination of any one of items 151 to 153, wherein the HBsAg is an particulate HBsAg and/or an HBsAg from HBV genotype A.

155. The HBcAg particle, and optionally the HBsAg, for use in a method of vaccination of any one of items 150 or 152 to 154, or the vaccine vector for use in a method of vaccination of

any one of items 151 to 154, wherein the HBsAg is an, optionally nucleosidic, adjuvanted HBsAg, optionally a CpG adjuvanted HBsAg.

156. The HBcAg particle, and optionally the HBsAg, for use in a method of vaccination of any one of items 150 or 152 to 155, or the vaccine vector for use in a method of vaccination of any one of items 151 to 155, wherein the method of vaccination is the method of any one of items 93 to 149.

157. The HBcAg particle, and optionally the HBsAg, for use in a method of vaccination of any one of items 150 or 152 to 156, or the vaccine vector for use in a method of vaccination of any one of items 151 to 156, wherein the HBcAg particle, and optionally the HBsAg is comprised in the pharmaceutical composition of any one of items 28 to 33, or comprised in a container of any one of items 34 or 41 to 50 or in a kit of any one of items 35 to 50.

158. The HBcAg particle, and optionally the HBsAg, for use in a method of vaccination of any one of items 150 or 152 to 157, or the vaccine vector for use in a method of vaccination of any one of items 151 to 157, wherein the vaccine vector is comprised in the pharmaceutical composition of item 81, or comprised in a container of any one of items 82 to 85.

159. Use of an HBcAg particle, and optionally an HBsAg, for the manufacture of a medicament, wherein said medicament is preferably a medicament for a method of vaccination, wherein said method is preferably a method comprising administering to a human subject

- (i) a first dose of the HBcAg particle and of the HBsAg,
- (ii) a second dose of the HBcAg particle and of the HBsAg, and
- (iii) a dose of a vaccine vector,

wherein the vaccine vector expresses

- a. an HBsAg from HBV genotype A;
- b. an HBcAg from HBV genotype D;
- c. an HBsAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 7;
- d. an HBcAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 8 or 17; and
- e. an RT domain of a polymerase from HBV comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 9.

160. Use of a vaccine vector expressing

- a. an HBsAg from HBV genotype A;

- b. an HBcAg from HBV genotype D;
- c. an HBsAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 7;
- d. an HBcAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 8 or 17; and
- e. an RT domain of a polymerase from HBV comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 9,

for the manufacture of a medicament, wherein said medicament is preferably a medicament for a method of vaccination, wherein said method is preferably a method comprising administering to a human subject

- (i) a first dose of an HBcAg particle and of an HBsAg,
- (ii) a second dose of the HBcAg particle and of the HBsAg, and
- (iii) a dose of a vaccine vector.

161. Use of the expression cassette, mRNA, or cDNA, of any one of items 58 to 64, the nucleic acid molecule of any one of items 65 to 67, the expression vector of item 68 or 69, or the expression vector of any one of items 70 to 73, for the manufacture of a medicament, wherein said medicament is preferably a medicament for a method of vaccination, wherein said method is preferably a method comprising administering to a human subject

- (i) a first dose of the HBcAg particle and of the HBsAg,
- (ii) a second dose of the HBcAg particle and of the HBsAg, and
- (iii) a dose of a vaccine vector,

wherein the vaccine vector expresses

- a. a HBsAg from HBV genotype A;
- b. a HBcAg from HBV genotype D;
- c. a HBsAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 7;
- d. a HBcAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 8 or 17; and
- e. an RT domain of a polymerase from HBV comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 9.

162. The use of any one of items 159 to 161, wherein the vaccine vector is defined as in any one of items 74 to 80.

163. The use of any one of items 159 to 162, wherein the HBcAg particle is defined as in any one of items 1 to 27.

164. The use of any one of items 159 to 163, wherein the HBsAg is an particulate HBsAg and/or an HBsAg from HBV genotype A.
165. The use of any one of items 159 to 164, wherein the HBsAg is an, optionally nucleosidic, adjuvanted HBsAg, optionally a CpG adjuvanted HBsAg.
166. The use of any one of items 159 to 165, wherein the method of vaccination is the method of any one of items 93 to 149.
167. The use of any one of items 159 to 166, wherein the HBcAg particle, and optionally the HBsAg is comprised in the pharmaceutical composition of any one of items 28 to 33, or comprised in a container of any one of items 34 or 41 to 50 or in a kit of any one of items 35 to 50, and/or wherein the vaccine vector is comprised in the pharmaceutical composition of item 81, or comprised in a container of any one of items 82 to 85.
168. The use of any one of items 159 to 167, wherein the medicament is a medicament for treating an HBV infection.
169. The use of any one of items 159 to 168, wherein the medicament is used in the method of any one of items 93 to 149.
170. An mRNA or cDNA comprising a nucleotide sequence having at least 90% sequence identity, preferably at least 95% sequence identity, or is preferably identical to the sequence set forth in SEQ ID NO: 5.

[00183] It is to be noted that in case of any definition given herein, the respective definition of a term, phrase, and/or abbreviation applies vice versa throughout the specification. Furthermore, all definition given herein are intended to encompass all grammatical forms.

[00184] Additional objects, advantages, and features of this disclosure will become apparent to those skilled in the art upon examination of the following Examples and the attached Figures thereof, which are not intended to be limiting. Thus, it should be understood that although the present disclosure is specifically disclosed by exemplary embodiments and optional features, modification and variation of the disclosures embodied therein herein disclosed may be resorted to by those skilled in the art and that such modifications and variations are considered to be within the scope of this disclosure.

V. BRIEF DESCRIPTION OF THE FIGURES

[00185] **Figure 1:** VacB as a novel therapeutic, preferably curative, vaccination regime for treating an HBV infection. (A) Schematic representation of the VacB vaccination regime

comprising 2x protein prime vaccinations (day 0 and approximately day 28) comprising a novel HBcAg particle and a HBsAg, followed by a vaccine vector boost vaccination (approximately day 56). (B) Schematic representation of the immunological principle underlying the novel vaccination regime that is associated with an induction of a balanced CD4+ TH1/TH2 T-cell responses for a production of neutralizing anti-HBc and anti-HBs antibodies by the two protein prime vaccinations comprising the HBcAg particle and optionally (adjuvanted) HBsAg, and activation of cytotoxic CD8+ T-cells for an elimination of infected cells by the vaccine vector. Mosaic core protein: HBcAg particle disclosed herein comprising HBV core proteins from at least two different HBV genotypes; MVA-vector as well as MHBVac: vaccine vector disclosed herein expressing multiple HBV antigens from different HBV genotypes and being preferably an MVA vector like MHBVac.

[00186] **Figure 2:** Multi-antigenic open reading frame. Depicted is the structure of the multi-antigenic polypeptide chain preferably comprised in the vaccine vector disclosed herein. Schematically depicted are the formation of subviral particles comprising HBV surface A/adw and C/ayw antigens, and the formation of empty capsids comprising HBV core D/ayw and C/ayw antigens. Partially unprocessed antigens are assumed to increase immune responses (especially enhance and broaden adaptive immune responses) due to incorporation into secreted virus-like particles.

[00187] **Figure 3:** Preferred nucleotide sequence that may be comprised in the vaccine vector disclosed herein encoding a multi-antigenic polypeptide chain as depicted in **Figure 2**. Coding regions are indicated as follows: bold: nucleotide sequence encoding an HBV surface antigen from A2/adw, italic: nucleotide sequence encoding a P2A site, italic and bold: nucleotide sequence encoding an HBV core antigen from D/ayw, regular: consensus nucleotide sequence of a RT domain of the HBV polymerase, italic and underlined: nucleotide sequence encoding a T2A site, bold and underlined: nucleotide sequence encoding an HBV surface antigen from C/ayw, and bold and italic and underlined: nucleotide sequence encoding an HBV core antigen from genotype C.

[00188] **Figure 4:** Plasmid map of the vector used for production in E.coli of the disclosed HBcAg particle. The vector was generated for expression of both HBV core proteins from genotype C (truncated 1-163aa / 18.54 kDa) and genotype D (full length 1-183aa / 21.12 kDa) that self-assemble into the disclosed HBcAg particle comprising HBV core proteins from two or more HBV genotypes.

[00189] **Figure 5:** Nucleotide sequence of the plasmid shown in **Figure 4**. Coding regions are indicated as follows: bold: nucleotide sequence encoding a truncated HBV core protein from HBV genotype C with the truncated HBV core protein consisting of 1-163aa, bold

and underlined: nucleotide sequence encoding a full-length HBV core protein from HBV genotype D with the full-length HBV core protein consisting of 1-183aa.

[00190] **Figure 6:** Schematic overview of a pull-down experiment to demonstrate the presence of different HBV core proteins in HBcAg particles obtained from E.coli.

[00191] **Figure 7:** Schematic representation of theoretically possible HBcAg particle assemblies. (a) Alignment of amino acid sequences of HBV core proteins from HBV genotypes C and D with polymorphisms summarized in the table. The C-terminal truncated residues of HBV core proteins from genotype C are indicated in grey. Shown are in the alignment SEQ ID NO: 18 (upper rows) and SEQ ID NO: 2 (lower rows). (b) Side view of the assembly domain of the HBV core protein dimer from genotype D (two full-length 1-183 aa monomers). The eight residues unique to each genotype are numbered according to primary sequence. (c-f) Schematic representation of theoretically expectable HBV core protein capsids when HBV core proteins from both genotypes are co-expressed.

[00192] **Figure 8:** NMR analysis of the HBcAg particle. (a) Schematic representation of the arrangement of HBV core proteins into a T=4 HBV capsid (left) and zoom on a quasi-six-fold vertex, with subunits "A", "B", "C" and "D", respectively [generated from PBD entry 1qgt]. Of note, said subunits do not relate to genotypes but in this Figure to asymmetric units which is a kind of "structural" unit within in an icosahedron (an icosahedron is usually composed of 60 so-called asymmetric units, e.g. in the T4 capsid an asymmetric unit consists of 4 monomers (or 2 dimers), while in the subunits A, B, C, D each denote a monomer in the asymmetric unit shown in color). The bases of the HBV core protein dimers form *interdimer* contacts, while the spikes of the HBV core protein dimers represent *intradimer* contacts. Residues with spectral perturbations attributed to mosaic assemblies are highlighted as black spheres. (b) Side view of the "AB" dimer. Residues highlighted as dark spheres report on the existence of heterodimers (and mosaic assemblies, respectively). (c) Zoom on selected regions from the 2D DARR and NCA spectra of Cp163C, Cp183D and mixed Cp163C183D, showing representative peaks of residues sensitive to *interdimer*, *intradimer* and both inter- and *intradimer* contacts, as well as residues unique for each genotype.

[00193] **Figure 9:** Comparison of homologous vs. heterologous prime / boost vaccination. C57BL/6 mice (n=3) were vaccinated with (A) 15µg CpG-adjuvanted HBsAg or HBcAg particle, (B) 1x10⁸ IFU MVA-S, MVA-core or wildtype MVA (one shot: filled bars, two shots on day 0 and 21: striped bars), (C) 15µg CpG adjuvanted HBsAg or HBcAg particle (day 0) and 1x10⁸ IFU MVA-S or MVA-core (day 21). Analysis was done one week after (last) vaccination by flow cytometry after intracellular cytokine staining (ICS). IFNγ+ CD8+ T-cell responses against HBV peptides S190, S208 and C93 or MVA peptide B8R are shown. (D) Sera of mice from (C) were

analyzed for anti-HBs by EIA or for anti-HBc using a competitive enzyme-linked immuno sorbent assay (ELISA).

[00194] **Figure 10:** Combination of HBsAg and the HBcAg particle increased anti-HBs immune response. C57BL/6 mice (n=4) were vaccinated twice with 10µg CpG-adjuvanted HBsAg or and the HBcAg particle or only CpG-adjuvanted HBsAg. On day 21, i.e. one week after the second immunization, sera of mice from were analyzed for anti-HBc (A) or for anti-HBs (B).

[00195] **Figure 11:** Comparison of DNA, mRNA and Protein for VacB priming. Groups of C57BL/6 mice (n>2) were primed twice either with mixture of HBsAg and the HBcAg particle adjuvanted with CpG (Protein), mRNA or DNA vaccines at day 0 and 14, at indicated doses. At day 28 mice were boosted with MVA expressing HBV antigens. Groups of mice receiving PBS injections served as controls. Vaccine-induced immune responses were evaluated at day 35. (A-B) Levels of serum anti-HBc (A) and anti-HBs (B). (C) S-specific and core-specific CD8+ T-cell response in spleen detected by ICS.

[00196] **Figure 12:** Immunogenicity of VacB with Alum or CpG adjuvants for protein priming. Groups of C57BL/6 mice (n>3) were transduced with AAV-HBV to establish persistent HBV replication. After 6 weeks mice were primed twice either with mixture of HBsAg and the HBcAg particle adjuvanted with Alum or CpG. At week 4 mice were boosted with MVA expressing HBV antigens. Groups of mice without vaccination or primed without adjuvant served as controls. Vaccine-induced immune response was evaluated 6 weeks after MVA boost. (A) Levels of serum anti-HBs. (B-C) S-specific CD4+ (B) and CD8+ (C) T-cell response in liver.

[00197] **Figure 13:** VacB results in long-term HBV control in AAV-HBV mice. Groups of male and female C57BL/6 mice (n=5) were transduced with AAV-HBV to establish persistent HBV replication. After 6 weeks mice were primed twice with mixture of HBsAg and the HBcAg particle adjuvanted with CpG-1018. At week 4 mice were boosted with MHBVac and afterwards monitored for 14 weeks. AAV-HBV-transduced mice without vaccination served as controls. (upper panel) Time kinetics of serum HBsAg throughout the monitoring period in mice immunized with CpG formulation. (lower panel) Serum HBeAg levels detected at the beginning of VacB (week 0) and at the end of the experiment – week 18.

[00198] **Figure 14** schematically depicts the overall Phase 1a clinical trial design.

[00199] **Figure 15** illustrates the immunization scheme of the Phase 1a clinical trial.

[00200] **Figure 16** schematically depicts the Phase 1b/2a clinical trial design.

[00201] Figure 17 Mosaic HBcoreAg particles are stable antigens and foster HBV-specific adaptive immune responses. (A) Determination of antigenicity and stability of Δ CD mosaic HBcoreAg particles by ELISA. 2.5 μ g of Δ CD HBcoreAg particles were stored at RT for an extended period of time and were assessed for their ability to bind monoclonal 8C9 antibodies at indicated time points. **(B)** Activation of TCR-grafted human CD4⁺ T cells (2F2 TCR) that recognize a peptide from HBV genotype A-D. TCR-grafted T cells were activated *ex vivo* by co-culture with primary human dendritic cells supplemented with either 10 μ g of RIGA HBcAg, full-length HBV core protein genotype D (D) or Δ CD HBcoreAg particles. T-cell activation was determined via TNF α secretion measured by flow cytometry after intracellular cytokine staining. **(C)** Naïve C57/BL6 mice (n=4) were immunized were immunized at intervals of 2 weeks with the Δ CD mosaic HBcoreAg in combination with adjuvanted HBsAg twice, followed by a booster vaccination with an MVA-HBVac expressing HBV S and core from different genotypes. The final analysis was performed at week 5 after the first immunization. **(D)** HBV-specific CD4⁺ T-cell responses (upper panel) and CD8⁺ T-cell responses directed against immunodominant peptides from HBV S (peptide pool) protein (lower panel) were determined. Active T cells were determined as IFN γ ⁺ HBV-specific T cells by intracellular cytokine staining and flow cytometry. d: Days; TNF α : Tumor necrosis factor α ; D: full-length HBcoreAg, genotype D; Δ CD: Mosaic HBcoreAg particles; IFN γ : Interferon γ ; No Vac: non-vaccinated mice; ns: not significant. Asterisks mark statistical significances: * P <0.05; ** P <0.01; *** P <0.001; **** P <0.0001.

[00202] Figure 18 TherVacB containing Δ CD mosaic HBcoreAg induces HBV-specific T-cell response against HBV genotype B. (A) C57/BL6 mice (n=5) were infected with AAV-HBV genotype B to establish persistent HBV replication. After 6 weeks, mice were immunized at intervals of 2 weeks, twice with the Δ CD mosaic HBcoreAg in combination with adjuvanted HBsAg, followed by a booster vaccination with an MVA-HBVac expressing HBV S and core from different genotypes. The final analysis was performed at week 6 after the first immunization. **(B)** HBsAg- and HBcAg-specific CD4⁺ and CD8⁺ T cell responses from the spleen (left panel) and liver (right panel). Isolated lymphocytes were stimulated overnight with overlapping peptide pools covering HBV S protein or the Core protein (either genotype B/C or D) to determine HBV-specific effector T cells. Effector T cells were determined as IFN γ ⁺ HBV-specific T cells by intracellular cytokine staining and flow cytometry. The mean of all mice is shown, and error bars indicate SEM. Statistical differences were calculated using unpaired t-tests. No Vac – non-vaccinated mice; D: genotype D HBcoreAg (full-length core protein); Δ CD: mosaic HBcoreAg; IFN γ : Interferon γ ; AAV-HBVgtB: AAV-HBV genotype B; pool: Overlapping peptide pools; ns: not significant. Asterisks mark statistical significances: * P <0.05; ** P <0.01; *** P <0.001.

VI. EXAMPLES

Example 1: Generation of an (recombinant) HBcAg particle of the disclosure

[00203] For generating a recombinant HBcAg particle, a bicistronic plasmid was generated for simultaneous expression of HBV core proteins from genotype C (truncated 1-163aa / 18.54 kDa, "HBc gtC 1-163aa", **SEQ ID NO: 1**) and genotype D (full length 1-183aa / 21.12 kDa, "HBc gtD 1-183aa", **SEQ ID NO: 2**). Sequence of HBc gtD 1-183aa was derived from GenBank Acc. No. V01460 and codon-optimized for expression in humans (GeneART, Regensburg/Germany). As regards the sequence of HBc gtC 1-163aa, a truncated monomer sequence from genotype C was chosen to have analytical means to proof the presence of monomers from both genotypes in a given HBcAg capsid. The truncation and thus, the measurable difference in molecular mass, provided hence options to clearly differentiate both monomer variants (genotype D vs. genotype C) by e.g. SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) after pull-down of capsids, cf. **Example 2**. Furthermore, the truncation was chosen to not impair the immunogenicity of the recombinant HBcAg particle, as the C-terminal deleted 20 amino acids do not contain any differences in the amino acid sequence between both genotypes C and D. Therefore, no antigenic epitope was lost by the deletion of the C-terminal 20 amino acids in genotype C monomers compared to full length genotype D monomers. The open reading frame (ORF) sequence of HBc gtC 1-163aa was also synthesized by GeneART.

[00204] Both HBcAg insert sequences, HBc gtC 1-163aa and HBc gtD 1-183aa, were initially cloned into pET28a2 (Novagen Inc.) for testing of functionality and protein expression. After verification of protein expression, both insert sequences were cloned into an intermediate donor plasmid, which contained a Tetracycline (Tet) repressor for conditional expression in mammalian cell culture. To enable large scale expression in bacteria like E.coli, the insert containing both ORFs of the HBV core antigens was cloned into a pRSF-Duet-1 plasmid via *AgeI*/*PfoI*. The obtained final plasmid "pRSF_T7-HBc163UP_T7-HBc183opt_noTetRep" was proven to be free of the Tet repressor. Presence of the correct insert sequences was verified by Sanger sequencing and measurement of protein expression in bacteria of both HBV core antigen variants was demonstrated by Western Blot.

[00205] The final plasmid (**Figure 4**, **Figure 5**, **SEQ ID NO: 10**) had a size of 4825 bp and was generated for simultaneous expression of HBV core proteins from genotype C (truncated 1-163aa / 18.54 kDa) and genotype D (full length 1-183aa / 21.12 kDa). The expression of both genotype sequences was induced by the identical T7 promotor to obtain equimolar expression and finally led to the assembly of "mosaic" HBcAg particle consisting of HBV core proteins from both genotypes.

Example 2: Obtaining a recombinant HBcAg particle and its pharmaceutical composition

[00206] The recombinant HBcAg particle was recombinantly produced in E.coli transduced with a plasmid described in **Example 1** for IPTG (Isopropyl- β -D-thiogalactopyranosid) inducible expression of the HBV core proteins from genotype C (1-163aa) and genotype D (1-183). The simultaneous expression of HBV core proteins from both genotypes led to self-assembly of HBcAg particle in the E.coli cells. The HBcAg particles were collected by cell lysis and afterwards processed by a cascaded downstream process (including ammonium sulfate (AMS) precipitation, two chromatography steps, diafiltration) Thus obtained recombinant HBcAg particles were sterile filtered and aseptically filled in 2R glass vials (1.2 ml) to produce the investigational medicinal product (IMP) for clinical application.

[00207] For quality control, starting material as well as intermediate products were extensively tested prior and throughout the manufacturing process. Based on material and process step, testing was performed with regard to microbial purity and sterility, physico-chemical properties, identity, concentration as well as product- and process-related impurities.

Example 3: Characterization of the obtained recombinant HBcAg particleTransmission Electron Microscopy (TEM)

[00208] The recombinant HBcAg particle obtained from **Example 2** was characterized with regard to particulate structure, size, composition of both genotypes, and encapsidated nucleic acids.

[00209] Transmission Electron Microscopy (TEM) was used for visualization of the recombinant HBcAg particle to analyze appearance and integrity of this particulate structure as well as homogeneity of the obtained recombinant HBcAg particle. Samples comprising recombinant HBcAg particles were diluted to 0.1 mg/ml, loaded onto copper grid and incubated. Grids were washed with HEPES buffer and stained with uranyl acetate. Images were taken using a TEM with 60.000x magnification (0.275 nm/pix). Scale bar was set to 200 nm. TEM analysis showed a uniform pattern of all recombinant HBcAg particles concerning size, shape and integrity of the particles.

Pull-down assays

[00210] To proof whether the recombinant HBcAg particle indeed consists of HBV core proteins from both genotypes C and D, a pull-down assay specific for genotype D HBV core proteins was performed as illustrated in **Figure 6**. Therefore, the expression plasmid was modified by adding a 15 aa AviTag™-sequence to the N-terminal part of the sequence for the full-length genotype D HBV core protein. During expression in E. coli, biotin ligases bound biotin to the

AviTag peptide, thus leading to biotinylation of the HBV core proteins from genotype D, while HBV core proteins from genotype C were not labelled. Theoretically, 3 capsid species could be obtained: (A) capsids assembled of truncated genotype C HBV core proteins exclusively (18.54 kDa) – which would not be biotinylated, (B) capsids assembled of full-length genotype D HBV core proteins exclusively (24 kDa; the larger molecular mass of 24 kDa compared to the typical 21.16 kDa of genotype D HBV core proteins was due to the used biotin tag) – which would be biotinylated, and (C) capsids assembled of both genotype C and genotype D HBV core proteins (18.54 + 24 kDa) – which would be biotinylated. Thus, a pull-down assay with Avidin-magnetic beads was performed after expression in *E.coli* to collect recombinant HBcAg particles comprising biotinylated genotype D HBV core proteins (i.e. species (B) and (C)) in the eluate, while recombinant HBcAg particles not comprising any biotinylated HBV core proteins (i.e. species (A)) were removed and collected in the flow-through. The eluate as well as the flow-through were analyzed by Western Blot (WB) with anti-HBc antibody recognizing the HBV core proteins from the two genotypes. When no biotinylation was applied during expression (control experiment using the non-modified expression plasmid also used during GMP production), the flow-through contained HBV core proteins of 18 and 21 kDa, i.e. truncated HBV core proteins from genotype C and full-length HBV core proteins from genotype D. However, this did not reveal which kind of species (A) to (C) was present in the preparation. When the modified expression plasmid was used (i.e. expression with biotinylation of genotype D HBV core proteins), the WB of the eluate revealed a strong 24 kDa as well as a 18 kDa band. Thus, the recombinant HBcAg particles that were pulled-down were not only composed of the 24 kDa biotinylated HBV core proteins from genotype D but also consisted of HBV core proteins from genotype C. Thus, it was proven that the expression of the used bicistronic plasmid indeed led to an assembly of “mosaic” capsids that comprised HBV core proteins from both genotypes. In contrast, the Western Blot of the flow through only revealed a very weak 18 kDa band, which indicated either that the capsids were solely composed of genotype C HBV core proteins (however, given the weak band, the overall amount amongst all capsids would be very minor and thus negligible) or the presence of non-assembled HBV core proteins from genotype C in the preparation.

NMR analyses

[00211] While the pull-down assay was able to prove the presence of recombinant “mosaic” HBcAg particles comprising HBV core proteins from both genotypes C and D, the assembly of HBV core proteins within said particles could not be defined by pull-down assays. Thus, to corroborate the presence of recombinant HBcAg particles further, and to reveal the geometrical arrangement of HBV core proteins (180 or 240 HBV core proteins per capsid, i.e. 90 or 120 HBV core protein dimers, respectively), solid-state nuclear magnetic resonance spectroscopy (NMR) was performed. This technique can probe the local environment around

individual amino acid residues, such as the structural features of capsids assembled in the presence of a mixture of HBV core proteins from genotypes C and D. Theoretically, three types of capsids could arise comprising HBV core proteins from both genotypes (truncated 1-163 aa genotype C and full-length 1-183 aa genotype D monomers; cf. **Figure 7**): (1) HBcAg particles consisting of heterodimers only, i.e. in which two HBV core proteins from genotype C and D form mixed dimers (cf. **Figure 7 d**), (2) HBcAg particles consisting of homodimers only, i.e. in which HBV core protein dimers are formed from two HBV core proteins from the same genotype C or D, but with homodimers from both genotypes being comprised in the icosahedral assembly (cf. **Figure 7 e**), and (3) HBcAg particles consisting of both, homo- and heterodimers (1 and 2; cf. **Figure 7 f**).

[00212] NMR experiments were carried out as published previously (Taji et al., Transient RNA Interactions Leave a Covalent Imprint on a Viral Capsid Protein. *J Am Chem Soc* 2022. <https://doi.org/10.1021/jacs.1c12439>). Briefly, uniformly ^{13}C , ^{15}N labelled recombinant HBcAg particles were sediment into 1.9 mm rotors and measured at magnetic field of 17.6 T (750 MHz ^1H Larmor frequency) at a Bruker AVANCE III NMR spectrometer equipped with triple-resonance (^1H , ^{13}C , ^{15}N) probe at a magic angle spinning frequency of 16,650 Hz and a sample temperature of 14°C. Two-dimensional (2D) dipolar-assisted rotational resonance (DARR) and NCA experiments were recorded as they provide simple access to ^{13}C and ^{15}N chemical shifts. All spectra were referenced to sodium trimethylsilylpropanesulfonate and the peaks in 2D spectra were assigned based on the reported chemical shifts (BMRB entry 2731; Lecoq et al., *Biomol. NMR Assign.* 12 (2018) 205-214). ^{13}C - ^{13}C DARR (; Lecoq et al., *Biomol. NMR Assign.* 12 (2018) 205-214) and ^{13}C - ^{15}N NCA (Lecoq et al. Localizing conformational hinges by NMR: where do HBV core proteins adapt for capsid assembly? *ChemPhysChem* (2018) doi:10.1002/cphc.201800211). Both approaches can probe the local environment around individual amino acid residues, such as the structural features of HBV capsids assembled in the presence of a mixture of HBV core proteins from both genotypes C and D.

[00213] The rationale for distinguishing the theoretically possible types of HBV capsids assembled from HBV core proteins of mixed genotypes is as follows: Mosaic capsids consisting of homodimers only (**Figure 7 e**) underlie structural perturbations reflected in the NMR spectra in a form of a visible peak splitting or CSPs (chemical shift perturbations) for the residues located to the assembly regions of the dimer base and are thus sensitive to interdimer contacts (residues 6-40, 105-149). Along the same lines, spectral perturbations were expected for residues located in the spike of the capsid and thus sensitive to intradimer contacts (residues 4-5, 43-48, 60-100) for mosaic capsids consisting of heterodimers only (**Figure 7 d**). The existence of mosaic capsids consisting of both homo- and heterodimers (**Figure 7 f**) would entail simultaneous peak splitting for residues in the spike and at the base of the dimers. Finally, the spectral pattern of a

hypothetical sample in which each capsid consists only of pure genotypes HBV core proteins, i.e. no mosaic assembly (**Figure 7 c**) would resemble a superposition of spectra of Cp163C and Cp183D. Between these limiting cases, it is conceivable that assembly does not exclusively but preferably result in mixed or pure dimers or assemblies.

[00214] Overall, the solid-state NMR spectra of capsids assembled in the presence of mixed genotype HBV core proteins did not resemble a combination of spectra from samples with single genotypes. The residues affected by structural perturbations, evident in peak splitting and CSPs, were located at both the base and the spike regions of the dimer, suggesting the existence of capsids containing both heterodimers and mosaic assemblies (**Figure 8**). The assembly of HBV core proteins into dimers and of dimers into the icosahedral capsid lattice apparently did not underlie strict selectivity for either pure or mixed genotypes.

Analysis of encapsidated nucleic acids (NAs)

[00215] The biological function of HBV core proteins is to encapsidate the HBV genome. Therefore, the C-terminal region of the HBV core proteins possesses an arginine-rich domain that confers a positive charge in the capsid to enable/facilitate the binding and encapsidation of the RNA transcript of the viral genome (pgRNA) into the capsid. During maturation of the virions, the pgRNA is reverse transcribed into the viral genome (rcDNA).

[00216] Hence, HBV core proteins are prone to nucleic acid binding and when recombinant HBcAg particles are produced in E.coli cells (i.e. in the absence of pgRNA molecules), the biochemical properties and the inherent biological function provoking the binding of cellular RNA to the positively charged C-terminal domain and, thus, the encapsidation of host cell nucleic acids into the HBcAg (instead of the viral genome). This encapsidation of E.coli RNAs is inherent to the generated recombinant HBcAg particle and cannot be prevented during assembly of capsids in expression cultures. Furthermore, the binding of NAs into the capsid also stabilizes this particulate structure and is therefore necessary to obtain stable recombinant HBcAg particle preparations.

[00217] Recombinant HBcAg particles produced with the production process described herein in **Example 1** was used for qualitative and quantitative analysis of the encapsidated NAs. Therefore, total RNA and total DNA was recombinant from batch 101764. Recombinant NAs were characterized by i) quantification using Qubit fluorometric quantification, ii) determination of fragment length using microchip electrophoresis, and iii) sequencing using cDNA-library (made from RNA) and shotgun library (made from DNA) using the Illumina technology.

[00218] The encapsidated nucleic acid was composed of 99.59% RNA, while the amount of DNA made only 0.41% of the total nucleic acid content. In relation to the overall protein amount, RNA made 9.44% w/w of the recombinant HBcAg particle sample (i.e. 94.4 ng RNA/ μ g protein).

The size of the recombinant NAs ranged for RNA between 100 and 3,000-4,000 nt and for DNA between 160 and 1,200 nt. The RNA present in the HBcAg was constituted by mRNA (45.46%), rRNA (53.17%), tRNA (0.39%), tmRNA (0.73%) and ncRNA (0.24%).

Example 4: Heterologous prime-boost vaccination outperforms homologous approaches

[00219] In a first set of the experiments, it was investigated whether a heterologous protein-prime / MVA-boost approach would be more efficient in inducing HBV-specific T-cell responses than homologous prime-boost regimens.

[00220] Homologous prime-boost immunization of HBV-naïve mice with either recombinant HBsAg or the recombinant HBcAg particle resulted in poor HBV surface- or core protein-specific CD8+ T-cell response in spleen, even though CpG-1018 as a potent adjuvant was included in the formulation (**Figure 9 A**). Two sequential immunizations with recombinant MVA, expressing HBV core protein (MVA-core) or HBV surface protein (MVA-S) resulted in strong CD8+ T-cell response against the vector. However, said two sequential immunizations did not induce the desired immune response against HBV antigens (**Figure 9 B**). Only heterologous regimens based on priming with CpG-adjuvanted HBsAg or the recombinant HBcAg particle and boosting with MVA-S or MVA-core, respectively, simultaneously elicited strong HBV-specific CD8+ T-cell responses (**Figure 9 C**) and high HBV-specific antibody titers (**Figure 9 D**).

[00221] The results demonstrated that the heterologous prime-boost regimen of VacB was superior compared to a single-component immunization and thus homologous approaches.

Example 5: Optimizing the prime vaccination by combining protein antigens

[00222] In a further set of experiments the question was addressed whether combining HBsAg and the recombinant HBcAg particle in the protein prime may improve VacB-mediated immune responses. To this purpose, the groups of HBV-naïve mice were immunized twice with CpG-adjuvanted either HBsAg alone or with combination of both antigens and measured anti-HBs and anti-HBc titers in mouse sera one week after second immunization.

[00223] Anti-HBc antibodies could only be detected in the group of mice receiving the vaccine formulation containing the recombinant HBcAg particle (**Figure 10 A**). The addition of the recombinant HBcAg particle to the vaccine formulation, however, significantly improved anti-HBs responses (**Figure 10 B**).

[00224] These data demonstrated that immunization with both HBV antigens not only enhanced the breadth of induced immune responses, but also resulted in a stronger anti-HBs antibody response, a prerequisite of a functional HBV cure.

Example 6: Protein superior over DNA or RNA for prime vaccination

[00225] Recombinant proteins are known to elicit strong antibody responses, but rather low CD8+ T-cell response, unless a potent Th1/Th2 adjuvant is used. Thereby, it was investigated whether employing alternative vaccines, such as DNA and mRNA-based vaccines that do not require additional adjuvants, could improve the immunogenicity of VacB. In addition, mRNA- or DNA-based vaccines would allow the expression of several antigens at a time and avoid the complicated and expensive purification of recombinant proteins for clinical use. Thus, protein-, DNA- and RNA-based vaccines were compared for VacB priming followed by a boost with a recombinant vaccine vector like a recombinant MVA vaccine vector like MHBVac.

[00226] Mice were immunized twice with CpG-adjuvanted HBsAg and the recombinant HBcAg particle and for comparison with various doses of an mRNA vaccine, formulated in lipid nanoparticles, or a plasmid DNA vaccine. All demonstrated high levels of anti-HBc antibodies (**Figure 11 A**), but priming with the different vaccines resulted in various levels of anti-HBs (**Figure 11 B**). Adjuvanted protein HBsAg induced the strongest anti-HBs response, followed by a dose-dependent response to mRNA. Priming with DNA vaccine resulted in very low anti-HBs responses, independently of the dose used. Priming with recombinant proteins, followed by the recombinant vaccine vector boost, elicited strong S- and core-specific CD8+ T-cell response, which was comparable in magnitude to the one induced by a high dose of DNA vaccine (**Figure 11 C**). Priming with mRNA, however, resulted in overall poor HBV-specific CD8+ T-cell response.

[00227] These results demonstrated the VacB regimen, comprising novel protein prime and boost vaccinations, was superior to priming with DNA or mRNA since it induced both neutralizing antibodies and vigorous CD8+ T-cell response directed against both HBV antigens.

Example 7: Defining the optimal adjuvant class for protein prime

[00228] To choose the optimal adjuvant for efficient protein priming, VacB immunogenicity and antiviral efficacy were compared in AAV-HBV mice after formulating HBsAg and the recombinant HBcAg particle with traditional Th2-activating adjuvant aluminum hydroxide (alum) or with CpG that also allows for inducing Th1 response.

[00229] Immunization with alum and CpG formulations generated significantly higher levels of anti-HBs compared to immunization with the antigens without adjuvant (**Figure 12 A**). Accordingly, VacB immunization using alum and CpG formulations stimulated comparable intrahepatic S-specific CD4+ T-cell responses (**Figure 12 B**). Strong S-specific CD8+ T-cell responses were detected in the livers of mice primed with the CpG formulation (**Figure 12 C**). However, no S-specific CD8+ T-cells were detectable when using the alum formulation despite the boost with a recombinant MVA vaccine vector like MHBVac (**Figure 12 C**).

[00230] These results demonstrated that an immunization using Th2-biased adjuvant alum was not sufficient to prime HBV-specific CD8⁺ T-cell responses. By contrast, adjuvants capable of inducing balanced Th1/Th2 immune responses, such as CpG, were crucial to elicit effective CD8⁺ T-cell responses by VacB immunization. Thereby, CpG adjuvant became an important clinical candidate for VacB.

Example 8: Long-term efficacy of VacB

[00231] Following the proof-of-concept in two relevant preclinical models of HBV infection, the question was addressed whether the antiviral effect of VacB could be longitudinally sustained in AAV-HBV infected mice. To this purpose, persistent HBV replication in male and female C57BL/6 mice was established by and immunized them according to VacB regimen using CpG adjuvanted HBV antigen formulations for priming with the mice being monitored for 14 weeks after boost.

[00232] In contrast to not-vaccinated AAV-HBV mice, mice receiving VacB demonstrated a long-term, sustained decrease in serum HBsAg levels over the monitoring period (**Figure 13 A-B**). Moreover, in all immunized mice serum HBeAg levels were significantly reduced until end of follow-up (**Figure 13 C**). VacB performed equally well, independently of gender and type of clinical candidate Th1/Th2 adjuvant that were used for immunizations.

[00233] These results demonstrated that VacB resulted in long-term immune control of persistent HBV infection.

Example 9: Toxicology

[00234] A GLP standard repeat-dose toxicity study was performed in Wistar rats to assess toxicity of the VacB vaccine components. The study was conducted according to "WHO guideline on nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines".

[00235] A shortened but n+1 repeated dose vaccination regime with 3-fold protein prime on day 1, 8 and 15 followed by a 2-fold vector boost on day 29 and 36 was used. According to the clinical application, all vaccine components were administered by i.m. injection. Blood samples were collected on day 2, 16 and at time point of final analysis, that was done on day 37 for the main group and day 50 for the recovery group (i.e. after a 14-day recovery period for assessment of possible findings).

[00236] **Table 2** provides an overview of the study groups and **Table 3** indicates the quality/batches of the vaccine components used for the GLP study.

[00237] The maximum dose that was applied from each vaccine component corresponded to the highest planned full human dose (FHD) in the clinics, except for the CpG-1018 adjuvant,

which is part of the HEPLISAV-B® formulation and provokes its adjuvant effect by binding on TLR9 receptors. A single human dose of HEPLISAV-B® contains 20 µg HBsAg and 3,000 µg CpG-1018. In the herein disclosed phase 1a trial, a two-fold dose of HEPLISAV-B® (corresponding to 40 µg HBsAg and 6,000 µg CpG-1018) is foreseen for study group B0.2.

[00238] A FHD of 3,000 µg / 6,000 µg CpG-1018 is not applicable in rats due to differences in the expression pattern of TLR9 between humans and rodents with much broader expression of TLR9 in rodent tissues. Therefore, and in agreement with PEI, the maximum immunogenic dose of CpG-1018 (30 µg) was applied in case of the rats. However, applying an allometric scaling from human to rodents, the 30 µg dose corresponds to the foreseen two-fold dose of HEPLISAV-B® in humans. In addition, given that HEPLISAV-B® is already a marketed product (prophylactic vaccine for Hepatitis B), extensive safety data in humans exists.

Table 2: Overview of the GLP-toxicology study*.

Group	Vaccine components	Prime (day 1, 8, 15)			Boost (day 29, 36)	No. of animals	
		HBsAg	HBcoreAg	Adjuvant	MVA-HBVac	main group (I day 37)	recovery group (I day 50)
G1	Vehicle control	vehicle			vehicle	10♂ + 10♀	5♂ + 5♀
G2	HEPLISAV-B	20µg		30µg CpG1018	3x10 ⁸ ifu	10♂ + 10♀	5♂ + 5♀
G3	2x HEPLISAV-B + HBcoreAg	40µg	75µg*	30µg CpG1018	3x10 ⁸ ifu	10♂ + 10♀	5♂ + 5♀

[00239] In preparation of the GLP study, the single vaccine components were provided by the respective GMP manufacturers. Ready-to-use formulations of the test items were prepared by mixing the vaccine components needed for the protein prime of the different groups in the respective concentration and volume (administration of 200 µl per animal). Test item formulations were labelled and shipped to the CRO of the GLP study (ATRC Aurigon Toxicological Research Center Ltd.) 24 - 72 hours prior to application.

[00240] A comprehensive in-use-stability study was performed that has proven a sufficient stability of the single vaccine components in the ready-to-use test item formulations for the relevant time between preparation and application in the GLP study.

Proof of vaccination

[00241] Successful conduction of the vaccination was proven by detection of anti-HBs antibodies as well as anti-HBc antibodies during final analysis to assess proper exposure of the animals with the vaccine components. All animals in groups receiving HBsAg vaccination (i.e. HEPLISAV-B® in Group 2 and the recombinant HBcAg particle in Group 3) were highly positive for anti-HBs antibodies and anti-HBc antibodies, respectively. In contrast, no antibodies were detectable in the vehicle control (Group 1).

Mortality and clinical observation

[00242] There was no mortality during the dosing or recovery periods and all animals survived until the scheduled euthanasia. Also, no noticeable clinical observations were made for both sexes except for some animals in group 3 that lifted their hind limb upon i.m. injection of the test items into the legs. These observations were made during approximately the first 3 minutes after dosing and disappeared thereafter. No test item related clinical signs were observed during the recovery period.

Body temperature, body weight and food consumption

[00243] There were no test items related body temperature changes during the dosing period determined 2 and 6h after dosing for both sexes. Also, there were no test items related significant differences in the mean values of body weight in all groups and all animals of both sexes gained similar weight throughout the study (including the recovery period). Also, there were no test item related effects noted on food consumption during dosing and recovery period for all groups and both sexes.

Haematology and clinical chemistry

[00244] At interim measurement on day 16 (i.e. 24h after the third protein prime), changes in white blood cell parameters (higher neutrophils, lower lymphocytes) in Group 2 and 3 in both sexes and higher eosinophils in Group 2 and 3 females were detected and considered to be a test item related non-adverse finding as there were no other changes in these animals to suggest a toxicological effect on these parameters. These test item related non-adverse findings were likely due to vaccination effect, i.e. the activation of local inflammation with increase of granulocytes in the injection side (increase of neutrophils and eosinophils) and concomitant recruitment of lymphocytes and monocytes into lymph nodes for activation of B- and T-cells (decrease of lymphocytes and monocytes in periphery). Findings seen in white blood cell parameters on Day 16 were also seen at final analysis on Day 37 (i.e. 24h after the second recombinant vaccine vector boost using MHBVac), but completely disappeared at the end of the recovery period on Day 50.

[00245] No test item formulation related findings were seen in clinical chemistry and coagulation parameters.

Macroscopic findings

[00246] There were no treatment related macroscopic findings at the end of the dosing (Day 37) and recovery periods (Day 50) for both sexes.

Organ weight

[00247] No test item formulation related organ weight differences compared with controls were noted for both sexes in main group animals at the end of the dosing period (Day 37) and in the recovery groups (Day 50).

Histopathology

[00248] Findings at the injection sites at the end of the dosing period were regarded as primarily low grade acute/subacute tissue responses to the injection of foreign material. The low incidence and severity of toxicity in the form of myofiber necrosis indicated a good local tolerability of the formulations. A lymphoid inflammatory infiltrate in the sciatic nerve of minimal to moderate degree in most animals of Groups 2 and 3 and of minimal degree in four animals of the control group (Group 1) were regarded as an extension of the injection site reactions, involved only the epineurium (nerve sheath), with no indication of neuronal damage. After the 14-day recovery period, the acute responses were reduced, indicating an ongoing resolution of the inflammation. In addition, there was no indication of systemic effects of the test item formulations in the extensive list of organs and tissues examined during the recovery period.

Conclusion

[00249] In conclusion, no adverse, test-item related effects were observed in any of the groups of Wistar rats receiving the VacB vaccination regime (Group 2-3) following intramuscular (i.m.) administration. Successful vaccination was proven by detection of high levels of anti-HBs and anti-HBc antibodies in all animals receiving HBsAg and the recombinant HBcAg particle during protein prime. The most noticeable findings were reversible histopathological alterations at the injection site (myofiber necrosis, lymphoid inflammatory infiltrate). However, they were only graded mild-to-moderate and spontaneously recovered during the recovery period.

[00250] Taken together, VacB vaccination regimens showed a good tolerability of all vaccine components and the formulations.

Example 10: Clinical Study

A. Study Objectives and Overview

[00251] A phase 1a clinical trial will be conducted to test safety and immunogenicity in healthy human subjects. Vaccination will follow a heterologous protein-prime / viral vector boost regime. The clinical trial preferably evidences that the VacB vaccination regimen represents a safe and well-tolerated therapeutic vaccination and to support its efficacy in treating and curing HBV infections.

[00252] For protein priming, in total two injections (day 0, day 28) of (a) the novel HBV core antigen (HBcAg) according to the present invention to prime HBV core-specific CD4+ and CD8+ T-cells covering different HBV genotypes as well as (b) a recombinant HBV surface antigen (HBsAg) will be used. Both HBcAg and HBsAg form spontaneously particulate antigens. Priming will be administered in the form of two intramuscular (i.m.) injections. To allow induction of neutralizing, anti-HBs antibodies and prime a T-cell response, a higher protein amount of HBcAg and HBsAg than typically used in a prophylactic HBV vaccine will be applied.

[00253] As regards HBsAg, a HBsAg adjuvanted with CpG-1018 will be used, herein in the form of the recombinant yeast-derived, prophylactic vaccine HEPLISAV-B®. HEPLISAV-B® will be used as an FDA and EMA approved HBsAg vaccine. It was shown to induce neutralizing antibody responses against different HBV genotypes after two i.m. injections four weeks apart (Splawn, et al., Heplisav-B vaccination for the prevention of hepatitis B virus infection in adults in the United States, *Drugs Today (Barc)*, 2018. 54(7): p. 399-405.). HEPLISAV-B® has an excellent safety profile (Prescribing Information HEPLISAV-B®), a clinical track record of safe use of the recombinant HBcAg particle (cf., e.g., Al Mahtab, et al., Treatment of chronic hepatitis B naïve patients with a therapeutic vaccine containing HBV surface and core antigens (a randomized, open and treatment-controlled phase III clinical trial), *PLoS One*, 2018. 13(8): p. e0201236; and Betancourt, et al., Phase I clinical trial in healthy adults of a nasal vaccine candidate containing recombinant hepatitis B surface and core antigens, *Int J Infect Dis*, 2007. 11(5): p. 394-401) and a plethora of MVA-derived vaccines in clinical development (cf., e.g., ClinicalTrials.gov Search Results for "MVA vaccine") and beyond.

[00254] For viral vector boosting after the two prime injections, the vaccine vector disclosed herein in the form of a modified vaccinia virus Ankara vector (MHBVAc) will be used that expresses HBV antigens covering HBV surface, core and polymerase proteins of all major HBV genotypes. Boosting will be done by administering MHBVAc in the form of one intramuscular (i.m.) injection (day 56) at an amount known to be safe in comparable constructs and able to expand HBV-specific, cytotoxic T-cells.

B. Study Overview

[00255] All VacB vaccine components were produced under GMP conditions, and preclinical GLP-conform safety / tolerability studies were completed in 2021. The VacB vaccination approach was preclinically developed in C57BL/6 mice to select the most suitable vaccine components, vaccination schedule, dosage and application route.

[00256] The complete VacB vaccination regime will be applied to humans for the first time in the phase 1a clinical trial disclosed herein. More specifically, a single-center, open-label, first-in-human phase 1a clinical trial will be performed to evaluate an investigational medicinal product in

healthy volunteers. The test product will be a vaccination regime consisting of a two-fold protein-prime using a combination of recombinant HBsAg and the recombinant HBcAg particle, followed by a single recombinant vaccination-vector boost. Hereby, the protein prime will be varied to establish the most immunogenic but safe protein antigen dose.

[00257] The study design is summarized in **Table 4**.

Table 4: Overview of study outline of phase 1a trial with N indicating number of healthy subjects to be enrolled per study arm. In total, 11 healthy subjects will be participating. HBsAg will be used in the form of the commercially available and EMA and FDA approved HEPLISAV-B®.

Study arm		2x Prime vaccination (day 0, 28)	Boost vaccination (day 56)
A0	N = 3	HBsAg 20µg	Vaccine vector (MHBVAc) 3×10^8 IFU
B0.1	N = 5	HBcAg 25µg + HBsAg 20µg	Vaccine vector (MHBVAc) 6×10^7 IFU
B0.2	N = 5	HBcAg 50µg + HBsAg 40µg	Vaccine vector (MHBVAc) 3×10^8 IFU

[00258] Study arm A0 healthy subjects will receive 20 µg HEPLISAV-B® (0.5 mL) at two time points (day 0 and day 28) and subsequently 3×10^8 ifu MHBVAc (in 0.5 mL; day 56). Study arm B0.1 healthy subjects will receive 20 µg HEPLISAV-B® (0.5 mL) and 25 µg recombinant HBcAg particle (in 0.25 mL) into the same arm at two time points (day 0 and day 28) and subsequently 6×10^7 ifu MHBVAc (in 0.5 mL; day 56). Study arm B0.2 healthy subjects will receive 2 doses of 20 µg HEPLISAV-B® (0.5 mL) and 50 µg recombinant HBcAg particle (in 0.5 mL) into the same arm at two time points (day 0 and day 28) and subsequently 3×10^8 ifu MHBVAc (in 0.5 mL; day 56). As indicated in **Figure 14**, all healthy subjects will receive in total three intramuscular injections, namely two injections of protein ± adjuvant on day 0 and day 28, and an MVA injection on day 56. All healthy subjects will be followed up until day 224 ± 7 .

[00259] The study will be conducted in a staggered approach in ascending order of the different study arms. Each study arm differs by a parameter like dose escalation or addition of a new vaccine component as indicated in **Table 4** and **Table 5**, and will only be initiated after safety evaluation of the previous study arm. Safety assessments (cf., e.g., **Figure 14**) will be performed by review of safety data (adverse events (AEs), serious adverse events (SAEs), safety laboratory and vital signs) that will be collected at all study visits. Laboratory safety tests (biochemistry, hematology, and dipstick urinalysis) will be performed at screening, pre-dose and at study follow-up visits.

[00260] **Table 5** summarizes the rationale of each study arm.

Table 5: Rationale for study outline

Arm	Approach	Rationale
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A0	Combining hepatitis B vaccine HEPLISAV-B® with the novel MHBVac vector (high dose)	- induction of neutralizing (anti-HBs) antibodies and priming of HBV-S-specific T-cell responses - analysis of immunogenicity (boost of CD8+ T-cell response) and safety of MHBVac
B0.1	Addition of the HBcAg particle as protein prime component, combined with a low-dose boost with MHBVac	- broadening of the immune response by additional priming of HBV-core-specific T-cell response - boost with low-dose MHBVac to assess if a lower dose maybe sufficient
B0.2	Doubling the amount of both protein antigens combined with high dose of MHBVac	- increasing the overall humoral and cellular immune stimulation

[00261] Subjects will be vaccinated in all three study arms in a staggered manner as indicated in **Figure 15**. There will be a minimum of 1 day for arm A0 and 2 days for arms B0.1 and B0.2, respectively, between the vaccinations of the 1st subject and the 2nd and 3rd subject within a study arm. The 2nd and 3rd subject within a study arm may be vaccinated on the same day. In case of study arm B0.2, the same applies for the 4th and 5th subject. Thus, the 4th and 5th subject may be vaccinated on the same day, but min. 1 day after the 2nd and 3rd subject.

[00262] Monitoring of healthy subjects will be done for study arms A0 and B0.1 1h and for study arm B0.2 2h after prime vaccination on day 0 and day 28, respectively. Monitoring after boost vaccination will be done 4h after said boost vaccination on day 56.

C. Study Populations

[00263] Twenty-four healthy male and female subjects aged between 18 and 65 years will be enrolled in this clinical trial. The subjects will be recruited from the clinical trial center's subject pools and public advertisement. Allocation to a certain treatment number (or subject number) will be done in successive order following screening and based on the subjects' availability.

[00264] Key inclusion criteria will be the following:

1. Ability to understand the subject information and to personally name, sign and date the informed consent to participate in the clinical trial.
2. Provided written informed consent.
3. Healthy male and female subjects aged 18-65 years at time of informed consent.
4. No clinically significant health problems as determined during medical history and physical examination and clinical laboratory results at screening visit. The following laboratory parameters should be within normal limits: White blood cells (WBC), absolute neutrophil count (ANC), platelets. Aspartate transaminase (AST) and alanine transferase (ALT) should be ≤ upper limit of normal (ULN), Creatinine clearance (CrCL) >60mL/min and total

bilirubin should not exceed 1.5 x ULN. Non-clinically significant, minor deviations of laboratory measurements can be tolerated as they will not increase the risk of the individual having an adverse outcome from participating in this clinical trial as judged by the investigator.

5. Participant may be on chronic or as needed medications if, in the opinion of the investigator, they pose no additional risk to participant safety or assessment of reactogenicity and immunogenicity and do not indicate worsening of a pre-existing medical condition.
6. Body mass index 18.5-32.0 kg/m² and weight >50 kg at screening.
7. Women of child-bearing potential (WOCBP) only: non-pregnant, non-lactating women with negative pregnancy test.
8. WOCBP who agree to comply with the applicable contraceptive requirements of the protocol from at least 14 days prior to vaccination until end of clinical trial or females who are permanently sterilized (at least 6 weeks post-sterilization).
9. The participant is co-operative and available for the entire clinical trial.

[00265] Key exclusion criteria will be the following:

1. Receipt of any vaccine in the 2 weeks prior to first trial vaccination (4 weeks for live vaccines), during trial or planned receipt of any vaccine in the 3 weeks following last trial vaccination. Exception: Required recommended pandemic vaccines are allowed.
2. Previous hepatitis B vaccination or an anti-HBs positive serum status.
3. Smallpox vaccination or immunization with a poxvirus-based viral vector or a suspected or confirmed monkeypox infection within the last 10 years.
4. Known allergy to components of the vaccine products (incl. hypersensitivity to yeast) or history of life-threatening reactions to vaccines containing one of the substances.
5. Known history of anaphylaxis to vaccination or any allergy likely to be exacerbated by any component of the trial vaccines.
6. History of previous HBV infection (serostatus: anti-HBc negative).
7. Clinically relevant findings in ECG or significant thromboembolic events in medical history.
8. Evidence for a condition in the subject's medical history or during medical examination that might influence either the safety of the subject or the absorption, distribution, metabolism or excretion of vaccine products.
9. Any confirmed or suspected immunosuppressive or immune-deficient condition, cytotoxic therapy in the previous 5 years.
10. Any chronic or active neurologic disorder, including seizures, and epilepsy, excluding a febrile seizure as a child and occasional migraine headaches.

11. Subjects with inflammatory, infectious and neuroinflammatory underlying disease which could cause an expected impairment of the blood brain barrier such as meningitis, multiple sclerosis, epilepsy, or Alzheimer's disease.
12. Participation in another clinical trial at the same time or use of an investigational product within 30 days or five times the half-life of the investigational product -whichever is longer- prior to receiving the first dose within this clinical trial.
13. Any positive result for HIV1/2, HCV antibody or HBs antigen testing.
14. Known history of Guillain-Barré Syndrome.
15. Active malignancy or history of metastatic or hematologic malignancy.
16. Suspected or known alcohol or illicit drug abuse within the past 5 years.
17. Moderate or severe illness and fever $\geq 38.0^{\circ}\text{C}$ within 1 week prior to vaccination.
18. Administration of immunoglobulins or any blood products within 120 days preceding clinical trial entry or planned administration during the clinical trial period.
19. Blood donation within 60 days before, during or up to 30 days after participation in the clinical trial.
20. Receipt of chronic (defined as more than 14 days) immune suppressants or other immune-modifying drugs within 6 months before enrollment or during the clinical trial.
 - For corticosteroids, this will mean prednisone, or equivalent, greater than or equal to 0.5 mg/kg/day.
 - Intranasal and inhaled steroids are allowed. Topical steroids are permitted provided they are not required to be applied to injection site.
21. Subjects with skin lesions close to the injection site.
22. Thrombocytopenia, contraindicating intramuscular vaccination based on investigator's judgment.
23. Subjects with a significant infection or known inflammation.
24. Subjects who are known or suspected not to comply with the clinical trial directives.
25. Any other significant finding or underlying medical condition, that in the opinion of the investigator, would increase the risk of the individual having an adverse outcome from participating in this clinical trial.
26. Investigator or employee of the clinical trial site or sponsor with direct involvement in the proposed clinical trial, or identified as an immediate family member (i.e., parent, natural or adopted child) of the investigator or employee with direct involvement in the proposed clinical trial.
27. Subjects who have been placed in an institution by court order or by order of the authorities.
28. Subjects with diminished mental capacity.

D. Pharmaceutical Properties and Formulation

[00266] **Table 6** provides an overview of the properties of the different VacB vaccine components applied in the phase 1a trial. Of note, HEPLISAV-B® was manufactured as a solution for intramuscular injection, labelled and packaged by Dynavax GmbH, Germany; recombinant HBcAg particles were manufactured as a solution for intramuscular injection by Eurofins Amatsigroup, Ghent (Belgium) / Idron (France), labelled by the Pharmacy of the University Medical Center Hamburg-Eppendorf, Germany, and packed by Eurofins Amatsigroup, Idron (France); and MHBVac was manufactured as a solution for intramuscular injection, labelled and packaged by ReiThera, Italy. HEPLISAV-B® comprises a HBsAg that is produced in yeast cells (*Hansenula polymorpha*) by recombinant DNA technology as well as the adjuvant CpG-1018 that is chemically synthesized. HEPLISAV-B® is provided as solution for intramuscular injection (0.5 mL) in pre-filled syringes without needle, wherein one syringe represents one dose.

Table 6: Vaccine components of VacB.

	Component	Properties and Formulation	Study group	Remarks
Protein-Prime	Formulated HBsAg (here: HEPLISAV-B)	Prefilled syringe with a sterile solution for i.m. injection containing 20µg protein (HBsAg) and 3,000µg adjuvant (CpG-1018). Stored at 2-8°C.	A0, B0.1, B0.2	Prophylactic vaccine for hepatitis B (FDA- and EMA-approved, marketed product).
	HBcAg particle	Sterile solution for i.m. injection containing 0.1mg/ml recombinant protein. Stored at -20°C but stable at room temperature for at least 6h.	B0.1, B0.2	HBcAg particle, not mixed with formulated HBsAg but injected into a separate injection site in the same arm.
Vector-Boost	MHBVac	Sterile suspension for i.m. injection containing 5.83 x 10e8 ifu/mL of the viral vector MHBVac. Stored at -20°C but stable at room temperature for at least 4h.	all	

[00267] Chemical properties and details on formulation used are given for the recombinant HBcAg particles in **Table 7**, for HEPLISAV-B® in **Table 8** and for the recombinant vaccine vector MHBVac in **Table 9**.

Table 7: Overview of general properties of the HBcAg particles.

Active Ingredient	HBcAg particle
Chemical nature	Protein
Buffer formulation	L-Methionine, α,α -Trehalose, L-Arginine-HCl, Kolliphor HS15, L-Histidine, L-Histidine-HCl, EDTA in water at pH 6.8.
Color	Colourless
Clarity	Transparent
pH	6.3 – 7.3 (at 20-25°C)
Protein concentration	0.09 – 0.11 mg/ml
Molecular mass	Genotype C monomers (1-163aa): 18.5 kDa Genotype D monomers (1-183aa): 21.16 kDa
Extinction coefficient	1.501 (considering 50:50 ratio of HBV core proteins from genotype C and genotype D)
Biological activity, MoA	Upon immunization with the HBcAg particle, the immune system recognizes this particulate capsid as foreign protein and induces an adaptive immune response against this antigen. Uptake, processing and MHC-presentation by antigen-presenting cells lead to activation of humoral and cellular immune responses with production of anti-HBc antibodies and HBc-specific CD4+/CD8+ T-cells.

Table 8: Overview of general properties of HEPLISAV-B®.

Active Ingredient	20 µg HBsAg /& 3,000 µg CpG-1018
Chemical nature	Protein antigen, subtype adw /& 22-mer oligonucleotide adjuvant
Buffer formulation	Sodium chloride, disodium phosphate dodecahydrate, sodium dihydrogen phosphate dihydrate, polysorbate 80, water for injections
Color	Colourless to slightly yellow solution
Clarity	Clear to slightly opalescent
Biological	Upon immunization with HEPLISAV-B®, the immune system recognizes the

activity, MoA	HBsAg as foreign protein and induces an adaptive immune response against the antigen. Uptake, processing and MHC-presentation by antigen-presenting cells lead to activation of humoral and cellular immune responses with production of anti-HBs antibodies and HBs-specific CD4+/CD8+ T-cells. The CpG-1018 adjuvant contained in HEPLISAV-B® acts as TLR9 agonist for additional stimulation of the immune system (e.g. activation of dendritic cells, production of IFN-α and IL-12) that lead to an increased antibody titer and promotes the development of cytotoxic CD8+ T-cells (that would be reduced without the CpG-1018 adjuvant).
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Table 9: Overview of general properties of the recombinant vaccine vector MHBVac.

Active Ingredient	Vaccine vector MHBVac
Chemical nature	Viral vector
Buffer formulation	Liquid frozen formulation of the active ingredient in 10 mM TRIS, 140 mM NaCl and water for injections
Appearance	Slightly to moderately cloudy solution. May contain a fine precipitate that settles.
Infectious titer	5.83 x 10e8 ifu/mL
pH	7.7 ±1.0
Biological activity, MoA	Upon i.m. injection of MHBVac as a booster dose skeletal muscle cells and fibrocytes are infected, followed by transgene expression and antigen presentation for a period of 1-2 days. The immune system recognizes infected cells expressing HBV antigens and directs an adaptive immune response against the respective antigens. In this way, humoral and cellular immune responses against HBV HBsAg, HBcAg and polymerase are boosted.

[00268] The vaccination schedule was established using particulate, recombinant HBsAg and the HBcAg particle injected either separately or in parallel and with or without a viral-vector boost. The optimal vaccination regimen was shown to consist of two protein-prime immunizations followed by a booster vaccination with MHBVac applied i.m. A comparison of the established adjuvant alum with nucleosidic and combination adjuvants showed that either nucleosidic adjuvants like CpG, but not alum, were suited as formulation for the protein prime.

[00269] In initial proof-of-concept studies, a heterologous vaccination regime was essential to induce T-cell responses in preclinical models of chronic HBV infection, and an MVA-based

vector proved most efficient. Design of the vaccine components was optimized to broadly cover all HBV genotypes circulating worldwide and the optimized vaccine components showed maximum efficacy in the preclinical models without detectable toxicity.

[00270] Therapeutic efficacy was associated with induction of anti-HBs antibodies able to neutralize HBV as well as HBV-specific helper and cytotoxic T cell responses, and detailed studies proved that this could clear HBV from circulation and from the livers of mice replicating HBeAg-positive and -negative HBV. These data supported the hypothesis that the optimized VacB vaccination regime is able to cure HBV in chronic hepatitis B patients.

[00271] A GLP standard repeat-dose toxicity study in Wistar rats was performed as disclosed herein above. Successful conduction of the vaccination was proven by detection of anti-HBs antibodies during final analysis with values of >1000 IU/L for the groups receiving vaccination whereas no antibodies were detected in vehicle control and adjuvant only groups. No major adverse effects related to the test items were observed and there were no indications of systemic effects in the extensive list of organs and tissues examined. Some minor findings in the histopathology of the injection site (myofiber necrosis) had a low incidence and disappeared within the 14-day recovery period. Taken together, VacB vaccination regimens were considered non-toxic and well tolerated, causing mild to moderate injection site reactions.

[00272] However, in the clinical trial disclosed herein, subjects may be withdrawn from vaccination, i.e. will not receive second and/or third immunizations if:

1. A clinically significant acute illness occurs before vaccination. Minor illnesses (e.g., diarrhea or mild upper respiratory tract infection) do not affect immunizations.
2. Fever (body temperature $\geq 38.0^{\circ}\text{C}$) within 1 week prior to the planned time of vaccination.
3. Allergic/anaphylactic reaction after vaccination.
4. A contraindication at the discretion of the investigator exists.

In case one or two of the events occur before the first immunization, immunization may be postponed if screening window will not be exceeded. In case subject does not receive day 28 vaccination, no ambulatory visit on day 35 will take place. In case subject does not receive day 56 vaccination, no ambulatory visit on day 63 will take place. Furthermore, in case a healthy subject discontinues the trial early (before 2nd and/or 3rd immunization) the subject will be replaced.

E. Dosing

[00273] The amount of the recombinant vaccine vector used for the boost selected in this clinical trial will represent amounts with optimal immunogenicity and safety profile as observed in prior clinical trials using MVA-vector-based vaccines (Koch et al., Safety and immunogenicity of a modified vaccinia virus Ankara vector vaccine candidate for Middle East respiratory syndrome: an open-label, phase 1 trial. *Lancet Infect Dis.* 2020 Jul;20(7):827-838. doi: 10.1016/S1473-

3099(20)30248-6. Epub 2020 Apr 21. PMID: 32325037; PMCID: PMC7172913) and available MVA-based vaccines (e.g., Imvanex). The amount of the heterologous protein prime selected in this clinical trial represent immunogenic amounts as observed in prior clinical trials. 20 µg and 40 µg of HBsAg are contained in commercially available vaccines (EngerixB Erwachsene: 20 µg, HepVaxPRO: 40 µg; HEPLISAV B®: 20 µg; Fendrix: 20 µg; div. adjuvants). The most common side effects (seen in more than 1 patient in 10) are headache, pain, redness, swelling at the injection site and fatigue (tiredness). In a study by the Cuban CIGB, 100 µg HBcAg combined with 100 µg HBsAg were applied in several hundred individuals in several smaller studies i.m. and intranasally and have been licensed in Cuba as Hepemasvac™. In none of the published studies any toxicity has been reported. To allow induction of neutralizing, anti-HBs antibodies and prime a T-cell response, a higher protein amount than typically used in a prophylactic hepatitis B vaccine will be applied.

F. Endpoints and Assessments

[00274] The clinical trial is designed to investigate the safety and immunogenicity of the heterologous protein prime / MVA boost therapeutic HBV vaccination method according to the present invention with two ascending amount levels of the HBcAg particle and adjuvanted HBsAg in healthy subjects, using for the latter HEPLISAV-B® including its adjuvant CpG-1018, boosted with the vaccine vector.

[00275] Primary endpoints will be assessed in view of the nature, frequency and severity of adverse events associated with the vaccine with said primary endpoints being collected and measured as followed:

- Occurrence of solicited local reactogenicity signs and symptoms (AEs) for 7 days after each vaccination
- Occurrence of solicited systemic reactogenicity signs and symptoms (AEs) for 7 days after each vaccination
- Occurrence of unsolicited AEs for 28 days after each vaccination
- Change from baseline of safety laboratory measures
- Occurrence of SAEs throughout the period of the clinical trial

[00276] Secondary endpoints will be assessed in view of an evaluation of an HBV-specific immunity with said secondary endpoints being collected and measured as followed:

- Humoral immunity: Magnitude of anti-HBs antibody responses determined by an accredited serological immuno-assay
- Percentage of participants who seroconvert to anti-HBs (>10 IU/l), anti-HBc or anti-HBs and anti-HBc
- Magnitude of HBV-specific T-cell responses determined by cytokine release

- MVA-specific neutralizing antibodies

[00277] Exploratory endpoints will be:

- Functional and geno-/phenotypic characterization of HBV-specific CD4+ and CD8+ T-cell responses
- Magnitude of antibody responses against HBV core protein (anti-HBc)
- Quality of HBV-specific antibodies including neutralizing and non-neutralizing antibody functions
- Magnitude and quality of HBV-specific B-cell responses
- Characterization of the B- and T-cell receptor repertoire
- MVA-specific B- and T-cell immunity including immunity to other orthopoxviruses
- Activation of innate immunity signatures

G. Data Analysis / Methods

[00278] As regards the clinical chemistry, blood samples (4.9 mL) for serum biochemistry will be collected at different time points and the following parameters will be assessed: Sodium, aspartate transaminase (AST), potassium, alanine transaminase (ALT), calcium, alkaline phosphatase (ALP), urea, gamma glutamyl transferase (GGT), creatine kinase (total), total bilirubin, creatin kinase-MB (only in case creatine kinase is elevated), glucose, albumin, pancreas specific amylase, total protein, lactate dehydrogenase (LDH), creatinine, C-reactive protein (CRP), and creatinine clearance (only at screening).

[00279] As regards the hematology, blood samples (2.7 mL) for hematology will be collected at different time points described and the following parameters will be assessed: hemoglobin, mean corpuscular hemoglobin concentration (MCHC), hematocrit, white blood cell (WBC) count (total and differential); red blood cells (RBC), neutrophils, mean corpuscular volume (MCV), lymphocytes, platelet count, monocytes, mean corpuscular hemoglobin (MCH), eosinophils, and basophils.

[00280] As regards a safety urinalysis, the following parameters will be analyzed in fresh midstream urine at different time points: pH, ketones, specific gravity, bilirubin, protein, blood, and glucose. Microscopic examination will be conducted if blood is detected during urinalysis. The microscopic examination will comprise of RBC, WBC, casts, and bacteria.

[00281] As regards serology, blood samples (1 tube of approximately 7.5 mL) to test for the presence of HIV, HBsAg, and HCV antibody will be collected at different time points and the following parameter will be assessed: HIV testing (HIV I and HIV II), HCV antibody screen, and HBV testing (HBsAg, anti-HBc, anti-HBs).

[00282] As regards immunogenicity assays, the information on the blood volume drawn

for immunogenicity assays given below in **Table 10** refers to maximum amounts. Humoral and cellular immunogenicity assays may include, but are not limited to, those shown in **Table 10**.

Table 10: Humoral and cellular immunogenicity assays

Test parameter	Method	Assay specification	Blood volume	Expected result
Anti-HBs antibodies	CLIA	Architect Anti-HBs quant (IgG)	2 mL	>100 IU/l
Anti-HBc antibodies	CLIA	Architect Anti-HBc II (total Ig)	2 mL	S/CO $\geq$ 1.1
HBV specific T-cell responses	FluoroSpot	ImmunoSpot multiplex FluoroSpot (IFN- γ , TNF, GzB, IL-2)	30 mL	>LLOD/LLOQ (>10 spot forming cells/106 PBMC

[00283] Data will be analyzed using SAS 9.4 or later, Stata 17.0 or later, or R 3.6.3 or later by a statistician of the Institute of Medical Biometry and Epidemiology, University Medical Center Hamburg-Eppendorf.

[00284] The primary objective of the clinical trial will be the assessment of safety and reactogenicity following injection. Exposure to study medication will be summarized by number of injections and amounts injected using descriptive statistics. Demographics and baseline characteristics, as well as all primary end secondary endpoints will be presented by means of descriptive statistics. Continuous data will be summarized with number, mean or geometric mean, standard deviation as appropriate, as well as minimum, Q25, median, Q75 and maximum. Categorical data will be summarized by absolute and relative frequencies (number and percent). Related adverse events (classified as defined in the primary endpoints) and subjects suffering from those adverse events will be presented for each study arm and summarized according to system organ class and preferred term using MedDRA coding as well as severity. All safety information will be assessed by participant and by study arm. The baseline for calculation of change from baseline of safety laboratory measures will be defined as the time point closest but prior to the respective vaccination.

Example 11: Clinical Study II

[00285] This clinical trial is designed to investigate the safety and immunogenicity of a

heterologous protein prime/ MVA boost therapeutic hepatitis B vaccine candidate with ascending dose levels of the candidate vaccine adjuvanted HBsAg ± HBcoreAg in chronic hepatitis infected subjects in two parts of the trial.

[00286] In Part A of the study the adjuvant CpG1018 (an ingredient of HEPLISAV B®) will be added to protein antigen vaccinations in two ascending dose levels. All vaccinees will be boosted with an MVA-based vectored vaccine. The safety and tolerability will be assessed by collecting safety data (local and systemic reactogenicity, AEs and vital signs) at all study visits throughout the study. Efficacy of the vaccination will be assumed if there is a drop in HBsAg levels $\geq 1 \log_{10}$ or loss of HBsAg two weeks, two and six months after the last vaccination. Details concerning analysis will be defined in the statistical analysis plan (SAP). The HBcAg, HBsAg, and modified vaccinia virus Ankara vector are the same as described in Example 10.

A. Clinical Trial Objectives

[00287] The aim of this clinical trial is to assess the safety, tolerability and immunogenicity of a heterologous protein prime/MVA boost therapeutic hepatitis B vaccine candidate in chronic hepatitis patients.

Primary objective

[00288] To assess safety and tolerability of a heterologous protein prime/MVA boost therapeutic hepatitis B vaccine candidate.

- Safety and tolerability of the vaccine components of the vaccination scheme
- Safety and tolerability of the vaccination scheme in terms of liver toxicity

Secondary objective

[00289] Evaluation of the efficacy and immunogenicity of the vaccine components and the vaccination scheme.

- Evaluation of the antiviral effect of the vaccination scheme with the goal of 30% of patients achieving a $\geq 1 \log_{10}$ HBsAg drop
- Evaluation of the immunogenicity of the vaccine components and the vaccination scheme with the goal of $\geq 30\%$ of patients developing increasing antibody titers or T-cell responses

B. Endpoints

Primary Endpoints

[00290] The primary endpoint concerns safety. Safety endpoints will be stratified by study

arms, vaccine regimens and doses, following prime vaccination and for the entire prime/boost regimen. Primary safety endpoints include:

- Frequencies and magnitudes of unsolicited adverse events
- Frequencies and magnitudes of serious adverse events (SAEs)
- Frequencies and magnitudes of adverse event of special interest (AESI) and Suspected Unexpected Serious Adverse Reaction (SUSAR)
- Frequencies and magnitudes of solicited local reactogenicity signs and symptoms within 7 days after each vaccination
- Frequencies and magnitudes of solicited systemic reactogenicity signs and symptoms within 7 days after each vaccination
- Frequencies and magnitudes of liver toxicity (ALT flare-ups) stratified by severity
- Change from baseline of safety laboratory measures

Secondary Endpoints

[00291] The secondary endpoints concern efficacy. Antiviral efficacy and immunogenicity endpoints will be assessed continuously throughout the trial, following prime vaccination and for the entire prime/boost regimen stratified by study arms, vaccine regimens and doses. The final efficacy and immunogenicity endpoints will apply two weeks, two and six months after completion of the vaccination regimen (last study visit).

[00292] Efficacy endpoints:

- Frequency of subjects with HBsAg drop below the lower limit of quantification
- Frequency of subjects with a ≥ 1 log₁₀ drop in HBsAg titers from day 0 (start of study medication)

[00293] Immunogenicity endpoints:

- Frequency of subjects with HBsAg drop below the lower limit of quantification
- Frequency of subjects with a ≥ 1 log₁₀ drop in HBsAg titers from day 0 (start of study medication)
- Frequency of subjects with an induction of anti-HBs titers ≥ 10 IU/L
- Frequency of subjects developing any anti-HBs antibody response
- Frequency of subjects with an increased signal in the HBV-specific cytokine-secretion assay compared to pretreatment values
- Frequency of subjects with an increased frequency of total HBV-specific, cytokine-secreting T cells compared to pretreatment values

[00294] Exploratory endpoints:

- Frequency of subjects with a ≥ 2 -fold increase in anti-HBe and / or anti-HBc antibody titers compared to pretreatment values
- Frequency of subjects with an induction of MVA- or poxvirus neutralizing antibodies after MVA boost vaccination compared to day 56 (pre-boost)
- Frequency of subjects with increased cytokine secreting, HBV S-, core- or pol-specific T cells compared to pretreatment values
- Frequency of subjects with cytokine secreting, MVA-specific T cells after MVA boost vaccination
- Frequency of subjects with a $\geq 20\%$ drop of HBcore-related antigen (HBcr-Ag) compared to pretreatment values or stable suppression (over >6 weeks)
- Frequency of subjects with a ≥ 0.5 log₁₀ drop of HBV-RNA in serum or stable suppression (over >6 weeks)

C. Trial Design

[00295] This trial will be a multi-center, open-label, ascending dose phase 1b/2a trial in 89 chronic hepatitis B infected subjects aged 18-70 years. The intervention is a heterologous prime-boost vaccine consisting of two protein-based primes (day 0 and day 28) and an MHBV vector boost (day 56), all given as i.m. injections.

[00296] The clinical trial is separated into two Parts (A & B) each with different study arms.

- Part A (including study arms A1, A2, A3 and A4) are considered first-in-CHB (Phase 1b), followed by dose consolidating arm A5 and A6 (Phase 2a).

Table 11: Part A (n=57)

Study arm		Prime vaccinations: 2x (day 0 and 28)	Boost vaccination: 1x (day 56)
A1	n=5	HEPLISAV B®	6x 10 ⁷ ifu Vaccine vector (MHBVac)
A2	n=5	HEPLISAV B® & HBcoreAg 25µg	6x 10 ⁷ ifu Vaccine vector (MHBVac)
A3	n=5	HEPLISAV B® & HBcoreAg 25µg	3x 10 ⁸ ifu Vaccine vector (MHBVac)
A4	n=5	2x HEPLISAV B® & HBcoreAg 50µg	3x 10 ⁸ ifu Vaccine vector (MHBVac)

A5*	n=17	HEPLISAV B® & HBcoreAg 25µg	3x 10 ⁸ ifu Vaccine vector (MHBVac)
		or	
		2x HEPLISAV B® & HBcoreAg 50µg	3x 10 ⁸ ifu Vaccine vector (MHBVac)
A6*	n=20	HEPLISAV B® & HBcoreAg 25µg	3x 10 ⁸ ifu Vaccine vector (MHBVac)
		or	
		2x HEPLISAV B® & HBcoreAg 50µg	3x 10 ⁸ ifu Vaccine vector (MHBVac)

* based on below safety/immunogenicity considerations for dosing and dose-escalation

[00297] A schematic overview of the vaccination design is represented in Figure 16.

D. Study population

[00298] Key inclusion criteria:

1. Ability to understand the subject information and to personally name, sign and date the informed consent to participate in the clinical trial.
2. Provided written informed consent.
3. Confirmed chronic hepatitis B virus (HBV) infection (CHB) that fulfills the following criteria:
 - HBsAg positive for ≥ 6 months
 - Anti-HBs negative
 - HBsAg levels 100-2000 IU/mL
 - HBV nucleos(t)ide analog (NUC) treatment for ≥ 6 months
 - HBV load <100 IU/ml at least twice within the last 6 months
4. Males and non-pregnant, non-lactating female with negative pregnancy test aged 18-70 years at time of informed consent.
5. Apart from CHB no other clinically significant health problems as determined during medical history and physical examination and clinical laboratory results at the screening visit. The following abnormal laboratory parameters will be permitted:
 - leukocyte count ≥2.500/µl
 - platelet count ≥150.000/µl
 - ALT elevation ≤ 60 U/L

- AST should be ≤ 40 U/L
- bilirubin should be $\leq$ ULN
- INR should be $\leq$ ULN
- CrCL >60 mL/min/1.73 m²

Non-clinically significant, minor deviations of laboratory measurements can be tolerated as they will not increase the risk of the individual having an adverse outcome from participating in this clinical trial as judged by the investigator.

6. Subject may be on chronic or as needed medications if, in the opinion of the investigator, they pose no additional risk to subject safety or assessment of reactogenicity and immunogenicity and do not indicate worsening of a pre-existing medical condition.
7. Body mass index 18.5-32.0 kg/m² and weight >50 kg at screening.

[00299] Key exclusion criteria:

1. Advanced liver fibrosis or cirrhosis (demonstrated by ultrasound or transient elastography ≥ 8 kP in fasting condition)
2. WOCBP who don't agree to comply with the applicable contraceptive requirements of the protocol
3. History of hepatocellular carcinoma
4. Coinfection with Hepatitis C Virus (HCV) (RNA positive), or Human Immunodeficiency Virus (HIV) or with a history of hepatitis Delta (anti-Delta positive)
5. Other known liver disease
6. Regular alcohol intake >30 g/d (male), >20 g/d (female) or any other known drug addiction.
7. Donation of blood or blood products (e.g., 450 mL or more of plasma or platelets) within 60 days prior to receiving the first dose of the investigational medicinal product (IMP).
8. Receipt of any vaccine in the 2 weeks prior to first trial vaccination (4 weeks for live vaccines), during trial or planned receipt of any vaccine in the 3 weeks following last trial vaccination. Exception: Required recommended pandemic vaccines or emergency vaccines (e.g., tetanus) are allowed.
9. Known allergy to components of the vaccine products (incl. hypersensitivity to yeast) or history of life-threatening reactions to vaccines containing one of the substances.
10. Known history of anaphylaxis to vaccination or any allergy likely to be exacerbated by any component of the trial vaccines.
11. Clinically relevant findings in ECG or significant thromboembolic events in medical history.

12. Evidence for a condition in the subject's medical history or during medical examination that might influence either the safety of the subject or the absorption, distribution, metabolism or excretion of vaccine products.
13. Administration of immunoglobulins and/or any blood products within the 3 months preceding the administration of the first dose of the trial vaccine.
14. Any confirmed or suspected immunosuppressive or immunodeficient condition, cytotoxic therapy in the previous 3 years.
15. Any treatment with immunosuppressants or other immune-modifying drugs (including, but not limited to systemic corticosteroids, biologicals and Methotrexate) within the last 3 years. Exception: topical corticosteroids, e.g. occasional asthma sprays or systemic corticosteroids for medical emergencies.
16. Any chronic or active neurologic disorder, including diagnosis of migraine, seizures and epilepsy. Exception: a febrile seizure as a child and occasional headaches.
17. Participation in a clinical investigation within the past 4 weeks or five times the half-life of the previously taken IMP.
18. Investigator or employee of the study site with direct involvement in the proposed study, or identified as an immediate family member (i.e., parent, natural or adopted child) of the investigator or employee with direct involvement in the proposed study.
19. Subjects who are known or suspected
 - not to comply with the clinical trial directives.
 - not to be reliable or trustworthy.
 - not to be capable of understanding and evaluating the information given to them
 as part of the formal information policy (informed consent), in particular regarding the risks and discomfort to which they would agree to be exposed.

E. Humoral and cellular immunogenicity assays

Table 10

Test parameter	Method	Assay specification	Expected result
HBsAg	CLIA	HBsAg quantitative	>LLOQ, <ULOQ, numeric
HBeAg	CMIA	HBeAg qualitative	
Anti-HBs antibodies	CLIA	Anti-HBs quantitative	>10 IU/l, numeric
Anti-HBc antibodies	CLIA	Architect Anti-HBc II (total Ig)	S/CO $\geq$ 1.1

Anti-HBe antibodies	CLIA	Architect Anti-HBe (total Ig)	
HBV specific T-cell responses	FluoroSpot	Multiplex FluoroSpot (IFN- γ , TNF, GzB, IL-2)	>LLOD/LLOQ (>10 spot forming cells/ 10^6 PBMC, numeric)
MVA/Poxvirus specific T-cell responses	FluoroSpot	Multiplex FluoroSpot (IFN- γ , TNF, GzB, IL-2)	>LLOD/LLOQ (>10 spot forming cells/ 10^6 PBMC, numeric)
MVA-NAb	Infection neutralization	Neutralization of infection with MVA-GFP	Inhibitory concentration 50 (IC50) of a serum dilution
HBV-RNA	RT-qPCR	Quantification of pregenomic viral RNA and viral mRNAs	
HBcrAg	CLEIA	HBcrAg quantitative	

CLIA=Chemiluminescent immunoassay; HB=Hepatitis B; HBV=Hepatitis B Virus; IFN=Interferon; IgG=immunoglobulin G; IL=Interleukin; LLOD=lower limit of detection; LLOQ=lower limit of quantification; PBMC=peripheral blood mononuclear cell; S/CO=signal cut off ratio; TNF=Tumor Necrosis Factor

CLEIA = chemiluminescent enzyme immunoassay; CMLA = chemiluminescent microparticle immunoassay

Example 11: Comparing mosaic HBcoreAg with HBcoreAg of genotype D

[00300] Antigenicity and stability of Δ CD mosaic HBcoreAg particles were analyzed by ELISA. For this, 2.5 μ g of Δ CD HBcoreAg particles were stored at RT for an extended period of time and were assessed for their ability to bind monoclonal 8C9 antibodies at indicated time points (**Figure 17A**). Further Activation of TCR-grafted human CD4⁺ T cells (2F2 TCR) that recognize a peptide from HBV genotype A-D was tested. TCR-grafted T cells were activated *ex vivo* by co-culture with primary human dendritic cells supplemented with 10 μ g of RIGA HBcAg (genotype D; D) or Δ CD HBcoreAg particles. T-cell activation was determined via TNF α secretion measured by flow cytometry after intracellular cytokine staining (**Figure 17B**). Naïve C57/BL6 mice (n=4) were immunized at intervals of 2 weeks with the Δ CD mosaic HBcoreAg in combination with adjuvanted HBsAg twice, followed by a booster vaccination with an MVA-HBVac expressing HBV S and core from different genotypes. The final analysis was performed at week 5 after the first immunization (**Figure 17C**). HBV-specific CD4⁺ T-cell responses (upper panel) and CD8⁺ T-cell responses directed against an immunodominant peptide from HBV S protein (lower panel) were determined. Active T cells were determined as IFN γ ⁺ HBV-specific T

cells by intracellular cytokine staining and flow cytometry. The Δ CD mosaic HBcoreAg induced stronger CD8⁺ T-cell responses against HBV S than HBcoreAg of genotype D (**Figure 17D**).

[00301] In a further study, C57/BL6 mice (n=5) were infected with AAV-HBV genotype B to establish persistent HBV replication. After 6 weeks, mice were immunized at intervals of 2 weeks, twice with the Δ CD mosaic HBcoreAg in combination with adjuvanted HBsAg, followed by a booster vaccination with an MVA-HBVac expressing HBV S and core from different genotypes. The final analysis was performed at week 6 after the first immunization (**Figure 18A**). HBsAg- and HBcAg-specific CD4⁺ and CD8⁺ T cell responses from the spleen (left panel) and liver (right panel). Isolated lymphocytes were stimulated overnight with overlapping peptide pools covering HBV S protein or the Core protein (either genotype C or D) to determine HBV-specific effector T cells. Effector T cells were determined as IFN γ ⁺ HBV-specific T cells by intracellular cytokine staining and flow cytometry. The mean of all mice is shown, and error bars indicate SEM. Statistical differences were calculated using unpaired t-tests. Overall, it can be derived that Δ CD mosaic HBcoreAg administered within TherVacB is superior in inducing HBV-S-specific T-cell responses (**Figure 18B**).

[00302] It will be readily apparent to a person skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

[00303] All patents, patent applications and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

[00304] The inventions illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including", "containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention. The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention

with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group. Further embodiments of the invention will become apparent from the following claims.

[00305] Equivalents: Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

Claims

1. A method of vaccination, comprising administering to a human subject
 - (i) a first dose of an HBcAg particle and of an HBsAg,
 - (ii) a second dose of the HBcAg particle and of the HBsAg,
 - (iii) a dose of a vaccine vectorwherein the vaccine vector expresses
 - a. an HBsAg from HBV genotype A;
 - b. an HBcAg from HBV genotype D;
 - c. an HBsAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 7;
 - d. an HBcAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 8 or 17; and
 - e. an RT domain of a polymerase from HBV comprising a sequence having at least 90 % sequence identity to the amino acid sequence set forth in SEQ ID NO: 9.
2. The method of vaccination of claim 1, wherein the first dose and the second dose each comprise the HBcAg particle in an amount from about 10µg to about 100µg and the HBsAg in an amount from about 5µg to about 100µg.
3. The method of vaccination of claim 1 or 2, wherein the dose of the vaccine vector comprises the vaccine vector in an amount from about 1×10^7 ifu to about 1×10^9 ifu.
4. The method of vaccination of any one of claims 1 to 3, wherein the method of vaccination comprises administering the second dose about 1 week to about 8 weeks after the first dose.
5. The method of vaccination of any one of claims 1 to 4, wherein the method of vaccination comprises administering the second dose about 4 weeks after the first dose.
6. The method of vaccination of any one of claims 1 to 5, wherein the method of vaccination comprises administering the dose of the vaccine vector about 2 weeks to about 24 weeks after the first dose.
7. The method of vaccination of any one of claims 1 to 6, wherein the method of vaccination comprises administering the dose of the vaccine vector about 8 weeks after the first dose.
8. The method of vaccination of any one of claims 1 to 7, wherein the first dose and the second dose each comprise the HBcAg particle in an amount of about 25µg and the

HBsAg in an amount of about 20 μ g, wherein the dose of the vaccine vector comprises the vaccine vector in an amount of about 6×10^7 ifu, wherein the second dose is administered about 4 weeks after the first dose, and wherein the dose of the vaccine vector is administered about 8 weeks after the first dose.

9. The method of vaccination of any one of claims 1 to 8, wherein the first dose and the second dose each comprise the HBcAg particle in an amount of about 50 μ g and the HBsAg in an amount of about 40 μ g, wherein the dose of the vaccine vector comprises the vaccine vector in an amount of about 3×10^8 ifu, wherein the second dose is administered about 4 weeks after the first dose, and wherein the dose of the vaccine vector is administered about 8 weeks after the first dose.
10. The method of vaccination of any one of claims 1 to 9, wherein the method of vaccination is associated with a blood level increase of neutralizing and/or immune activating antibodies, wherein the antibodies are detectable in peripheral blood at a titer ≥ 10 IU/ml or wherein the blood level increase of neutralizing and/or immune activating antibodies results in a drop in HBsAg of at least about $1 \log_{10}$.
11. The method of vaccination of any one of claims 1 to 10, wherein the method of vaccination is associated with a blood level increase of anti-HBsAg antibodies, wherein the antibodies are detectable in peripheral blood at a titer of at least about 10 IU/ml or wherein the blood level increase of neutralizing and/or immune activating antibodies results in a drop in HBsAg of at least about $1 \log_{10}$.
12. The method of vaccination of any one of claims 1 to 11, wherein the method of vaccination results in a blood level increase of CD4+/CD8+ T-cells against HBsAg, HBcAg and/or a polymerase from HBV to at least about 10 spots/500.000 peripheral blood mononuclear cells measurable in an EliSpot or FlouroSpot assay.
13. The method of vaccination of any one of claims 1 to 12, wherein the method of vaccination is not associated with dose limiting toxicity.
14. The method of vaccination of any one of claims 1 to 13, wherein the HBcAg particle comprises HBV core proteins from HBV genotypes C and D.
15. A Hepatitis B Virus core antigen (HBcAg) particle, comprising Hepatitis B Virus (HBV) core proteins from at least two different HBV genotypes, wherein the HBcAg particle is preferably an isolated HBcAg particle.

16. A pharmaceutical composition comprising the HBcAg particle of any one of the preceding claims and optionally a pharmaceutically acceptable carrier or excipient.
17. The pharmaceutical composition of claim 15 further comprising an HBV surface antigen (HBsAg), wherein the HBsAg is preferably a particulate HBsAg.
18. A container comprising one or more doses of the pharmaceutical composition of claim 15 or 16.
19. A kit comprising the pharmaceutical composition of claim 15 and a second pharmaceutical composition comprising an HBsAg, wherein the HBsAg is preferably a particulate HBsAg.
20. An expression cassette, an mRNA, or a cDNA, wherein the expression cassette, mRNA, or cDNA, encodes an HBV core protein from HBV genotype C and an HBV core protein from HBV genotype D, wherein the expression cassette, mRNA, or cDNA, only comprises coding sequences for two or more HBV core proteins.
21. A nucleic acid molecule comprising the expression cassette, the mRNA, or the cDNA, of claim 19.
22. An expression vector comprising the expression cassette or the cDNA of claim 19 or the nucleic acid molecule of claim 20.
23. A vaccine vector, wherein the vaccine vector is preferably an MVA viral vector, and wherein the vaccine vector comprises a nucleic acid molecule comprising a nucleotide sequence having at least 90% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 5.
24. A pharmaceutical composition comprising the vaccine vector of claim 22 and optionally a pharmaceutically acceptable carrier or excipient.
25. A container comprising one or more doses of the pharmaceutical composition of claim 23, wherein a dose of the pharmaceutical composition comprises the vaccine vector in an amount from about 1×10^7 ifu to about 1×10^9 ifu.
26. The HBcAg particle of claim 15, or the pharmaceutical composition of claim 16 or 17, or comprised in the container of claim 18 or in the kit of claim 19, the vaccine vector as defined in any one of claims 1 to 14 and 23, the pharmaceutical composition of claim 24, or comprised in the container of claim 25, for use in treating an HBV infection.

27. An HBcAg particle, an HBsAg, and/or a vaccine vector for use in a method of vaccination, said method preferably comprising administering to a human subject

- (i) a first dose of the HBcAg particle and of the HBsAg,
- (ii) a second dose of the HBcAg particle and of the HBsAg,
- (iii) a dose of a vaccine vector;

wherein the vaccine vector expresses

- a. an HBsAg from HBV genotype A;
- b. an HBcAg from HBV genotype D;
- c. an HBsAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 7;
- d. an HBcAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 8 or 17; and
- e. an RT domain of a polymerase from HBV comprising a sequence having at least 90 % sequence identity to the amino acid sequence set forth in SEQ ID NO: 9.

28. Use of an HBcAg particle, an HBsAg, and/or a vaccine vector for the manufacture of a medicament, wherein said medicament is preferably a medicament for a method of vaccination, wherein said method is preferably a method comprising administering to a human subject

- (i) a first dose of the HBcAg particle and of the HBsAg,
- (ii) a second dose of the HBcAg particle and of the HBsAg, and
- (iii) a dose of a vaccine vector,

wherein the vaccine vector expresses

- a. an HBsAg from HBV genotype A;
- b. an HBcAg from HBV genotype D;
- c. an HBsAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 7;
- d. an HBcAg comprising a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 8 or 17; and
- e. an RT domain of a polymerase from HBV comprising a sequence having at least 90 % sequence identity to the amino acid sequence set forth in SEQ ID NO: 9.