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(54) Title: HBV VACCINE AND ANTIBODY COMBINATION THERAPY TO TREAT HBV INFECTIONS

(57) Abstract: The present invention provides a combination product comprising a vector encoding one or more HBV antigens and one or more immune checkpoint modulator(s), a composition and a kit of parts comprising such components as well as methods comprising such combination product, composition or kit of parts. The invention is of very special interest in the field of immunotherapy, and more particular for treating subjects infected with HBV, especially those chronically infected.

HBV vaccine and antibody combination therapy to treat HBV infections

FIELD OF THE INVENTION

The present invention generally relates to the field of immunotherapy and more specifically to compositions and methods to treat, prevent or inhibit HBV infections and associated diseases. Embodiments include a vector encoding one or more HBV antigens in combination with one or more immune checkpoint modulator(s), a composition and a kit of parts comprising such components as well as methods comprising such combination product, composition or kit of parts. The invention is of very special interest in the field of immunotherapy, and more particular for treating subjects infected with HBV, especially those chronically infected.

BACKGROUND OF THE INVENTION

Hepatitis B is a worldwide major public health problem caused by the hepatitis B virus (HBV). It is estimated that approximately 400 million persons are chronically infected by HBV worldwide, 20 to 40% of them being at risk of developing chronic liver disease, cirrhosis and hepatocellular carcinoma. Despite the existence of effective preventive vaccines, HBV infection is still rampant in many countries, even developed ones, with an estimation of 4.5 millions of new cases of infection per year worldwide. National and regional prevalence ranges from over 10% in Asia to under 0.5% in the United States and Northern Europe. Currently hepatitis B is the 10^{th} cause of mortality (around 1 million of deaths/year) and HBV related liver carcinoma, the 5^{th} most frequent cancer.

Conventional treatment of chronic hepatitis B includes pegylated interferon-alpha (IFNα) and nucleoside/nucleotide analogues (NUCs). However, treatment with pegylated IFNα leads to an antiviral responses in only about 30% of patients in specific subgroups and in addition causes serious side effects in a significant percentage of patients. On the other hand, NUCs improve the clinical condition of chronic HBV patients but their efficacy is

limited (3-5% of cure) and in patients not achieving cure, viral rebound occurs immediately after cessation of treatment, requiring patients to stay lifelong under treatment. In addition, long-term efficacy is also hampered by emergence of resistance mutation (e.g. approximately 66% after four years of lamivudine treatment as reported in some studies although newer NUCs showed much fewer occurrences).

Besides antiviral therapies, efforts are currently made to develop supplemental therapies aiming at improved host's immune responses. Several encouraging strategies have focused on immunotherapeutic approaches that simultaneously target multiple HBV antigens (e.g. Depla et al., 2008, J. Virol. 82: 435; WO2005/056051; WO2008/020656). For example, immunization of mice with an adenovirus vaccine encoding HBV polymerase, HBcAg and HBsAg domains was shown to elicit T cell responses against all expressed HBV antigens in preclinical mouse models (see e.g. WO2011/015656; WO2013/007772).

A number of preclinical and clinical studies have emphasized the importance of CD4+ and CD8+ T cell immune responses for effective anti-viral response. It was indeed observed that patients naturally having recovered from hepatitis B mounted multi-specific and sustained responses mediated by T helper (T_H) and cytotoxic T (CTL) lymphocytes which are readily detectable in peripheral blood. Appearance of anti-HBe and anti-HBs antibodies indicates a favorable outcome of infection. HBsAg-specific antibodies are neutralizing, mediate protective immunity and persist for life after clinical recovery.

Chronic HBV infection is only rarely resolved by the immune system that is ineffective to clear viral infection. The reason for this alteration of the effector functions of the cellular immune response in chronic hepatitis B is currently not well-understood even if the involvement of different inhibitory pathways has been described, e.g. presence of regulatory T cells, IL-10, TGF-β as well as various inhibitory receptors that are up-regulated in HBV chronically infected patients (Bertoletti and Gehring, 2013, PLoS Pathogens 9(12): e10003784). Some studies have highlighted that expression of the inhibitory receptor programmed death-1 (PD-1) is upregulated on HBV-specific CD8 T cells in early phase of acute HBV infection (Zhang et al., 2008, Gastroenterology 134: 1938-49). It has also been reported that the interaction of PD-1 with its ligands PDL-1 and PDL-2 plays a critical role in T cell exhaustion (Maier et al., 2007, J. Immunol. 178: 2714-20; Tzeng et al., 2012, PLoS One 7: e39179).

There is increasing interest in the possible benefits of blocking such immune checkpoints as a means of inhibiting immune system tolerance and thus rescue exhausted T cell-mediated immunity. A vast number of antagonistic antibodies have been developed

during the last decade (e.g. anti Tim3, -PD-L1, -CTLA-4, -PD1, etc) including the already marketed anti-CTLA-4 antibody Ipilimumab (Yervoy trade name, Bristol-Myers Squibb) that has been approved for metastatic melanoma.

Preclinical studies with antagonist antibodies are ongoing in infectious disease field (see e.g. Barber et al., 2006, Nature 439: 682-7; Cecchinato et al., 2008, J. Immunol 180: 5439-47) and combinations with different vector platforms (DNA, MVA, lentivirus, vaccinia, etc.) are also envisaged. In particular, the combination of PD-L1 blockade with a vaccinia virus expressing LMCV (Lymphocytic Chronomeningitis Virus) epitope was shown to improve the function of epitope-specific CD8+ T cells during persistent viral infection (Ha et al., 2008, JEM 205: 543-55). Administration of anti-PD-1 antibodies together with a SIV gag adenovirus vector in naive macaques caused increased in Gag-specific T cells (Finnefrock et al., 2009, J. Immunol. 182: 980-7). In chronic HBV infection, a triple combination regimen with additional anti-PD-L1 antibodies improved the function of antigen-specific CD8 T cells compared to NUC treatment in combination with DNA vaccination (Liu et al., 2014, PLoS Pathogens Vol 10: e1003856).

One may expect that HBV will continue to be a serious global health threat for many years due to the chronic and persistent nature of the infection, its high prevalence, the continuing transmission of HBV and the significant morbidity of the associated diseases. Thus, there is an important need to develop effective approaches for improving prevention and especially treatment of HBV infections or HBV-associated diseases or disorders, in particular approaches that potentiate anti-viral responses and/or restore exhausted T cell-mediated immunity generally associated with HBV infection and/or prime de novo novel functional HBV-specific T cells.

SUMMARY OF THE INVENTION

The present invention aids in fulfilling these needs. To achieve such needs, the present invention provides a combination product comprising at least (i) a vector encoding one or more polypeptides from an hepatitis B virus (HBV) and (ii) one or more immune checkpoint modulator(s).

This invention is based on the discovery that administration of an HBV antigen-expressing viral vector in combination with immune checkpoint modulator(s) permit to sustain over time the antiviral responses provided by the HBV antigen-expressing viral

vector. In particular, the present invention provides evidence that multiple administrations of a replication-defective adenovirus encoding a fusion of core, polymerase, and env domains interspersed with administrations of anti-PD-L1 antibody are surprisingly effective to reduce the level of hepatitis B surface antigen (HBsAg) in the sera of HBV-tolerant mouse model. In chronically infected HBV patients, HbsAg quantification can be used as a marker for virus replication and elimination of HBsAg from the sera associated with seroconversion to antiHBs antibodies is considered in the art as the marker indicative of virus clearance and cure of hepatitis B infection. The ability of the combination of the invention to reduce the levels of circulating HBsAg in a mouse model reproducing most of the features of the chronic status of HBV-infected patients is a good indication that the combination of the invention can be useful for treating in the clinic subjects chronically infected with HBV.

Other and further aspects, features and advantages of the present invention will be apparent from the following description of the presently preferred embodiments of the invention. These embodiments are given for the purpose of disclosure.

DEFINITIONS

As used throughout the entire application, the terms "a" and "an" are used in the sense that they mean "at least one", "at least a first", "one or more" or "a plurality" of the referenced components or steps, unless the context clearly dictates otherwise. For example, the term "a cell" includes a plurality of cells, including mixtures thereof.

The term “one or more” refers to either one or a number above one (e.g. 2, 3, 4, 5, etc).

The term "and/or" wherever used herein includes the meaning of "and", "or" and "all or any other combination of the elements connected by said term".

The term "about" or "approximately" as used herein means within 20%, preferably within 10%, and more preferably within 5% of a given value or range.

As used herein, when used to define products, combination of products, compositions and methods, the term "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are open-ended and do not exclude additional, unrecited elements or method steps. Thus, a polypeptide "comprises" an amino acid sequence when the amino acid sequence might be part of the final amino acid sequence of the polypeptide. "Consisting essentially of" means excluding other components or steps of any essential significance. Thus, a composition consisting essentially of the recited components

would not exclude trace contaminants and pharmaceutically acceptable carriers. A polypeptide "consists essentially of" an amino acid sequence when such an amino acid sequence is present with eventually only a few additional amino acid residues. "Consisting of" means excluding more than trace elements of other components or steps. For example, a polypeptide "consists of" an amino acid sequence when the polypeptide does not contain any amino acids but the recited amino acid sequence.

The terms "polypeptide", "peptide" and "protein" refer to polymers of amino acid residues which comprise at least nine or more amino acids bonded via peptide bonds. The polymer can be linear, branched or cyclic and may comprise naturally occurring and/or amino acid analogs and it may be interrupted by non-amino acids. As a general indication, if the amino acid polymer is more than 50 amino acid residues, it is preferably referred to as a polypeptide or a protein whereas if it is 50 amino acids long or less, it is referred to as a "peptide".

Within the context of the present invention, the terms "nucleic acid", "nucleic acid molecule", "polynucleotide" and "nucleotide sequence" are used interchangeably and define a polymer of any length of either polydeoxyribonucleotides (DNA) (e.g. cDNA, genomic DNA, plasmids, vectors, viral genomes, isolated DNA, probes, primers and any mixture thereof) or polyribonucleotides (RNA) (e.g. mRNA, antisense RNA, SiRNA) or mixed polyribo-polydeoxyribonucleotides. They encompass single or double-stranded, linear or circular, natural or synthetic, modified or unmodified polynucleotides. Moreover, a polynucleotide may comprise non-naturally occurring nucleotides and may be interrupted by non-nucleotide components.

The term "analog" as used herein refers to a molecule (polypeptide or nucleic acid) exhibiting one or more modification(s) with respect to the native counterpart. Any modification(s) can be envisaged, including substitution, insertion and/or deletion of one or more nucleotide/amino acid residue(s). When several mutations are contemplated, they can concern consecutive residues and/or non-consecutive residues. Mutation(s) can be generated by a number of ways known to those skilled in the art, such as site-directed mutagenesis (e.g. using the Sculptor(TM) in vitro mutagenesis system of Amersham, Les Ullis, France), PCR mutagenesis, DNA shuffling and by chemical synthetic techniques (e.g. resulting in a synthetic nucleic acid molecule). Preferred are analogs that retain a degree of sequence identity of at least 80%, preferably at least 85%, more preferably at least 90%, and even more preferably at least 98% identity with the sequence of the native counterpart or a portion thereof of at least 30 residues.

In a general manner, the term "identity" refers to an amino acid to amino acid or nucleotide to nucleotide correspondence between two polypeptide or nucleic acid sequences. The percentage of identity between two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps which need to be introduced for optimal alignment and the length of each gap. Various computer programs and mathematical algorithms are available in the art to determine the percentage of identity between amino acid sequences, such as for example the Blast program available at NCBI or ALIGN in Atlas of Protein Sequence and Structure (Dayhoffed, 1981, Suppl., 3: 482-9). Programs for determining identity between nucleotide sequences are also available in specialized data base (e.g. GenBank, the Wisconsin Sequence Analysis Package, BESTFIT, FASTA and GAP programs). For illustrative purposes, "at least 80% identity" means 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%.

The term "combination" as used herein refers to any arrangement possible of various components (e.g. at least the HBV encoding vector and the one or more immune checkpoint modulator(s) described herein). Such an arrangement includes a mixture of the individual components meaning that the individual components of the combination are placed together in a common container before administration to the subject. By contrast, dissociate arrangements refer to the case where the individual components of the combination are in separate containers which can be administered to the subject concomitantly or sequentially. Exemplary combinations include the HBV encoding vector described herein and one or more immune checkpoint modulator(s) which can be in the form of polypeptide(s) (e.g. recombinant polypeptide or mixture of recombinant polypeptides) or nucleic acid molecule(s) (e.g. carried by one or more vector engineered for expressing such one or more immune checkpoint modulator(s) including the vector encoding the HBV antigens) as well as mixture of polypeptide(s) and nucleic acid molecule(s) (e.g. a recombinant immune checkpoint modulator and an expressing vector thereof). The present invention encompasses combinations comprising equal molar concentrations of each immune checkpoint modulator when more than one is used as well as combinations with very different concentrations. It is appreciated that optimal concentration of each component of the combination can be determined by the artisan skilled in the art. Preferably the combination is synergistic providing higher efficacy than each entity alone. In accordance with the present invention, the term combination encompasses one or several administration(s) of each of the various components (for example, one may proceed with one or more administration(s) of the HBV-

encoding vector and one or more administration(s) of the immune checkpoint modulator(s) which can be concomitant, sequential or interspersed).

The term "vector" as used herein refers to a vehicle, preferably a nucleic acid molecule or a viral particle that contains the elements necessary to allow delivery, propagation and/or expression of one or more nucleic acid molecule(s) within a host cell or subject. This term encompasses vectors for expression in various host cells or organisms (expression vectors), extrachromosomal vectors (e.g. multicopy plasmids) or integration vectors (e.g. designed to integrate into the host cell genome and produce additional copies of the nucleic acid molecules when the host cell replicates) as well as shuttle vectors (e.g. functioning in both prokaryotic and/or eukaryotic hosts) and transfer vectors (e.g. for transferring nucleic acid molecule(s) in a viral genome). The vectors may be of naturally occurring genetic sources, synthetic or artificial, or some combination of natural and artificial genetic elements.

As used herein, "HBV" and "hepatitis B virus" are used interchangeably and refer to any member of the *Hepadnaviridae* (see e.g. Ganem and Schneider in Hepadnaviridae (2001) "The viruses and their replication", pp2923-2969, Knipe DM et al, eds. Fields Virology, 4th ed. Philadelphia, Lippincott Williams & Wilkins or subsequent edition). Typically, Hepadnaviruses are small enveloped hepatotropic DNA viruses having a partially double-stranded, circular DNA with a compact gene organization. The prototype member of this family is the human hepatitis B virus (HBV). HBV are classified into eight genotypes (A to H) divided into nine serotypes (ayw1, ayw2, ayw3, ayw4, ayr, adw2, adw4, adrq+ and adqr-) according to HBsAg-associated serology (see review by Mamum-Al Mahtab et al., 2008, Hepatobiliary Pancrease Dis Int 5: 457-64; Schaeffer, 2007, World Gastroenterol. 7: 14). The genotypes show distinct geographic distribution and clinical outcome and differ each other by at least 8% of their sequence. Each genotype and serotype encompasses different HBV strains and isolates. An isolate corresponds to a specific virus isolated from a particular source of HBV (e.g. a patient sample or other biological HBV reservoir) whereas a strain encompasses various isolates which are very close each other in terms of genomic sequences.

The term "immune checkpoint modulator" refers to a molecule capable of modulating the function of an immune checkpoint protein in a positive or negative way (in particular the interaction between an antigen presenting cell (APC) such as an HBV infected cell and an immune T effector cell). "Immune checkpoint" proteins are directly or indirectly involved in an immune pathway that under normal physiological conditions is crucial for preventing uncontrolled immune reactions and thus for the maintenance of self-tolerance and/or tissue

protection. But under pathological conditions (e.g. HBV infection), they play a critical role in T cell exhaustion. The one or more immune checkpoint modulator(s) in use herein may independently act at any step of the T cell-mediated immunity including clonal selection of antigen-specific cells, T cell activation, proliferation, trafficking to sites of antigen and inflammation, execution of direct effector function and signaling through cytokines and membrane ligands. Each of these steps is regulated by counterbalancing stimulatory and inhibitory signals that in fine tune the response. In the context of the present invention, the term encompasses immune checkpoint modulator(s) capable of down-regulating at least partially the function of an inhibitory immune checkpoint (antagonist) and/or immune checkpoint modulator(s) capable of up-regulating at least partially the function of a stimulatory immune checkpoint (agonist).

As used herein, the term “isolated” refers to a component (e.g. a polypeptide, peptide, polynucleotide, vector, etc.), that is removed from its natural environment (i.e. separated from at least one other component(s) with which it is naturally associated or found in nature). An isolated component refers to a component that is maintained in an heterologous context or purified (partially or substantially). For example, a nucleic acid molecule is isolated when it is separated of sequences normally associated with it in nature (e.g. dissociated from a genome) but it can be associated with heterologous sequences (e.g. a recombinant vector).

The term "obtained from", “originating” or “originate” is used to identify the original source of a component (e.g. a polypeptide, peptide, polynucleotide, vector, etc.) but is not meant to limit the method by which the component is made which can be, for example, by chemical synthesis or recombinant means.

As used herein, the term “host cell” should be understood broadly without any limitation concerning particular organization in tissue, organ, or isolated cells. Such cells may be of a unique type of cells or a group of different types of cells such as cultured cell lines, primary cells and dividing cells. In the context of the invention, the term “host cells” include prokaryotic cells, lower eukaryotic cells such as yeast, and other eukaryotic cells such as insect cells, plant and mammalian (e.g. human or non-human) cells as well as cells capable of producing the HBV antigen-encoding adenovirus and/or the immune checkpoint modulator(s) for use in the invention. This term also includes cells which can be or has been the recipient of the combination product described herein as well as progeny of such cells.

The term “subject” generally refers to an organism for whom any product and method of the invention is needed or may be beneficial. Typically, the organism is a mammal, particularly a mammal selected from the group consisting of domestic animals, farm animals,

sport animals, and primates. Preferably, the subject is a human who have been diagnosed as being or at risk of being infected with an HBV and thus are susceptible of having or at risk of having a disease or condition caused by or associated with an HBV infection. In preferred embodiments, the host organism is a human patient chronically infected with an HBV virus or alternatively co-infected with an HBV virus and another virus (e.g. the human immunodeficiency virus HIV). The terms "subject" and "patients" may be used interchangeably when referring to a human organism and encompasses male and female. The subject to be treated may be a newborn, an infant, a young adult or an adult.

The term "treatment" (and any form of treatment such as "treating", "treat") as used herein encompasses prophylaxis (e.g. preventive measure in a subject at risk of having the pathological condition to be treated) and/or therapy (e.g. in a subject diagnosed as having the pathological condition), eventually in association with conventional therapeutic modalities. The result of the treatment is to slow down, cure, ameliorate or control the progression of the targeted pathological condition. For example, a subject is successfully treated for an HBV infection or associated diseases if after administration of the combination product as described herein, the subject shows an observable improvement of its clinical status.

The term "administering" (or any form of administration such as "administered") as used herein refers to the delivery to a subject of at least one of the component of the combination product of the invention such as the HBV antigen-encoding vector and/or the immune checkpoint modulator(s) described herein.

DETAILLED DESCRITION OF THE INVENTION

In a first aspect the present invention provides a combination product comprising at least (i) a vector encoding one or more polypeptide(s) of a hepatitis B virus (HBV), preferably one or more HBV immunogenic polypeptide(s) and (ii) one or more immune checkpoint modulator(s).

Vector

In the context of the invention, the term "vector" has to be understood broadly as including plasmid and viral vectors. Typically, such vectors are commercially available (e.g. in Invitrogen, Stratagene, Amersham Biosciences, Promega, etc.) or available from depositary institutions such as the American Type Culture Collection (ATCC, Rockville, Md.) or have been the subject of numerous publications describing their sequence, organization and methods of producing, allowing the artisan to apply them.

A "plasmid vector" as used herein refers to a replicable DNA construct. Usually plasmid vectors contain selectable marker genes that allow host cells carrying the plasmid vector to be selected for or against in the presence of a corresponding selective drug. A variety of positive and negative selectable marker genes are known in the art. By way of illustration, an antibiotic resistance gene can be used as a positive selectable marker gene that allows a host cell to be selected in the presence of the corresponding antibiotic. Representative examples of suitable plasmid vectors include, without limitation, pREP4, pCEP4 (Invitrogen), pCI (Promega), pVAX (Invitrogen) and pgWiz (Gene Therapy System Inc).

The term "viral vector" as used herein refers to a vector that includes at least one element of a virus genome and may be packaged into a viral particle or to a viral particle. The terms “virus”, “virions”, viral particles” and “viral vector particle” are used interchangeably to refer to viral particles that are formed when the vector is transduced into an appropriate cell or cell line according to suitable conditions allowing the generation of infectious viral particles. In the context of the present invention, the term “viral vector” has to be understood broadly as including nucleic acid vector (e.g. DNA viral vector) as well as viral particles generated thereof. The term “infectious” refers to the ability of a viral vector to infect and enter into a host cell or organism. Representative examples of suitable viral vectors are generated from a variety of different viruses (e.g. retrovirus, adenovirus, adenovirus-associated virus (AAV), poxvirus, herpes virus, measles virus, foamy virus, alphavirus, vesicular stomatitis virus, etc).

Viral vectors can be replication-competent or -selective (e.g. engineered to replicate better or selectively in specific host cells), or can be genetically disabled so as to be replication-defective or replication-impaired. The term “replication-defective” or “replication-impaired” refers to a virus which replication is strongly impaired or abolished once the infection has taken place. Replication defectiveness is typically obtained through modification (e.g. mutation or deletion) of a part of the viral genome critical for viral replication. Such a replication-defective virus thus requires a helper virus or a complementation cell line to provide the missing protein(s) for production of new viral particles. The terms "virus", "virions" and viral particles" are used interchangeably to refer to viral particles that are formed when the viral vector genome is delivered into an appropriate cell or cell line according to suitable conditions allowing the generation of infectious viral particles.

Adenoviral vector

In one embodiment, the vector comprised in the combination product of the present invention originates from an adenovirus (i.e. an adenoviral vector). The term “adenovirus” (or Ad) refers to a group of viruses belonging to the *Adenoviridae* family. Typically adenoviruses are non-enveloped and their genome consists of a single molecule of linear, double stranded DNA that codes for more than 30 proteins including the regulatory early proteins participating in the replication and transcription of the viral DNA which are distributed in 4 regions designated E1 to E4 (E denoting "early") dispersed in the adenoviral genome and the late (L) structural proteins (see e.g. Evans and Hearing (2002) in “Adenoviral Vectors for Gene Therapy” pp 39-70, eds. Elsevier Science). Three regions, E1, E2 and E4, respectively, are essential to the viral replication whereas E3 is dispensable and appears to be responsible for inhibition of the host's immune response in the course of adenovirus infection. In the context of the present invention, the term "adenovirus", “adenovirus vector” or “adenoviral vector” has to be understood broadly as including nucleic acid vector as well as viral particles generated thereof.

Any serotype can be employed from any human or animal adenovirus (e.g. canine, ovine, simian, etc). It can also be a chimeric adenovirus (WO2005/001103). One of skill will recognize that elements derived from multiple serotypes can be combined in a single adenovirus.

Desirably, the adenoviral vector originates from a human Ad (e.g. WO03/000283), including those of rare serotypes, or from a primate (e.g. chimpanzee) Ad. Representative examples of human adenoviruses include subgenus C (e.g. Ad2 Ad5 and Ad6), subgenus B (e.g. Ad3, Ad7, Ad11, Ad14, Ad34, Ad35 and Ad50), subgenus D (e.g. Ad19, Ad24, Ad26, Ad48 and Ad49) and subgenus E (Ad4). Representative examples of chimp Ad include without limitation AdCh3 (Peruzzi et al., 2009, Vaccine 27: 1293-300) and AdCh63 (Dudareva et al, 2009, vaccine 27: 3501-4) and any of those described in the art (see for example, WO2010/086189; WO2009/105084; WO2009/073104; WO2009/073103; WO2005/071093; and WO03/046124). A number of adenoviruses are now well characterized genetically and biochemically (Hoffmann et al., 2007, Human Gene Ther. 18: 51-62). An exemplary genome sequence of human adenovirus type 5 (Ad5) is found in GenBank Accession M73260 and in Chroboczek et al., 1992, Virol. 186: 280-5). In a preferred embodiment wherein the adenoviral vector in use in this invention originates from a human adenovirus selected from the group consisting of Ad2, Ad3, Ad4, Ad5, Ad7, Ad11, Ad24,

Ad26, Ad34, Ad35, Ad48, Ad49 and Ad50, with a specific preference for Ad5, or from a chimpanzee adenovirus.

Advantageously, the adenoviral vector comprised in the combination of the invention is replication-defective. Typically, the virus is rendered replication-defective by partial or total deletion or inactivation of regions critical to replication of the virus, such as the E1 region. An appropriate E1 deletion extends from approximately positions 459 to 3510 by reference to the sequence of the Ad5 disclosed in the GenBank under the accession number M 73260. Preferably, the virus retains a functional viral pIX gene. The regions can also be inactivated by mutation or insertion of a heterologous nucleic acid molecule (e.g. the HBV-encoding sequences). The present invention also encompasses adenoviruses having additional deletion(s)/modification(s) within the adenoviral genome (all or part of other essential E2, E4 regions as described in W094/28152; Lusky et al, 1998, J. Virol 72: 2022). In addition, the non-essential E3 region can also be mutated or deleted.

In a preferred embodiment, the adenoviral vector comprised in the combination product of the invention is a replication defective human adenovirus of serotype 5 (Ad5) defective for E1 and E3 functions.

The preparation of recombinant adenoviral vectors is well known in the art. Preparation of recombinant Ad is described, for example in Chartier et al. (1996, J. Virol. 70: 4805-10) and in WO96/17070. The recombinant nucleic acid molecule can be inserted in any location of the adenoviral genome (e.g. in place of the E1 and/or the E3 region of the adenoviral genome), and may be positioned in sense or antisense orientation relative to the natural transcriptional direction of the region in question. Preferably, the HBV-encoding nucleic acid molecule(s) is/are cloned in place of the deleted E1 region and in sense orientation.

Complementing cell lines are used to propagate and produce sufficient amount of recombinant adenoviruses. A complementing cell is a cell that supplies in trans the adenoviral protein(s) encoded by those genes that have been deleted or inactivated in the replication-defective adenovirus, thus allowing the virus to replicate in the cell. Suitable cell lines for complementing E1-deleted adenoviruses include the 293 cells (Graham et al., 1997, J. Gen. Virol. 36: 59-72) as well as the HER-96 and PER-C6 cells (e.g. Fallaux et al., 1998, Human Gene Ther. 9: 1909-1917; WO97/00326) and E1 A549 (Imler et al., 1996, Gene Ther. 3: 75-84) or any derivative of these cell lines. But any other cell line described in the art can also be used in the context of the present invention, especially cell lines approved for producing products for human use.

The infectious viral particles may be recovered from the culture supernatant and/or from the cells after lysis. They can be further purified according to standard techniques (ultracentrifugation in a cesium chloride gradient, chromatography, etc. as described for example in WO96/27677, WO98/00524, WO98/22588, WO98/26048, WO00/40702, EP1016711 and WO00/50573).

Other viral vectors suitable in the context of the invention are poxviral vectors which can be obtained from any member of the poxviridae with a specific preference for a poxviral vector originating from a canarypox, fowlpox or vaccinia virus, the latter being preferred. Suitable vaccinia viruses include without limitation the Copenhagen strain (Goebel et al., 1990, Virol. 179: 247; Johnson et al., 1993, Virol. 196: 381), the Wyeth strain and particularly the modified Ankara (MVA) strain (Antoine et al., 1998, Virol. 244: 365). The general conditions for constructing recombinant poxvirus are well known in the art. The HBV-encoding nucleic acid molecule(s) is preferably inserted within the poxviral genome in a non-essential locus. Thymidine kinase gene is particularly appropriate for insertion in Copenhagen vaccinia vectors and deletion II or III for insertion in MVA vector.

The present invention also encompasses vectors complexed to lipids or polymers to form particulate structures such as liposomes, lipoplexes or nanoparticles as well as polymers (e.g. polyethylene glycol). Ad pegylation is particularly appropriate enable the vector to bypass pre-existing immunity (see e.g. WO01/23001). The present invention also encompasses vectors that have been modified to allow preferential targeting to a specific host cell. A characteristic feature of targeted vectors is the presence at their surface of a ligand capable of recognizing and binding to a cellular and surface-exposed component such as a cell-specific (e.g. an HBV-infected cell), a tissue-specific (e.g. a liver-specific marker), as well as a viral (e.g. HBV antigen) marker. Examples of suitable ligands include antibodies or fragments thereof directed to an HBV antigenic domain. Targeting can be carried out by genetically inserting the ligand into a polypeptide present on the surface of the virus (e.g. in the adenoviral fiber, penton, pIX, vaccinia virus p14 or B5R, etc.).

HBV polypeptide(s)

The HBV genome is a relaxed circular partially double-stranded DNA of approximately 3,200 nucleotides consisting of a full-length negative strand and a shorter positive strand. It contains 4 overlapping open reading frames (ORFs), C, S, P and X. The C ORF encodes the core protein (or HBcAg) constitutive of the nucleocapsid, the S ORF the

envelop proteins, the P ORF the viral polymerase and the X ORF a protein known as the X protein which is thought to be a transcriptional activator.

In accordance with the present invention, each of the one or more HBV polypeptides encoded by the vector described herein may independently originate from any HBV that can be found, isolated, obtained from a source in nature, in particular any HBV readily available to investigators in the field and HBV described in GenBank and PubMed.

Exemplary HBV of genotype A include without limitation isolate HB-JI444AF and strain HB-JI444A (accession number AP007263). Exemplary HBV of genotype B include without limitation clone pJDW233 (accession number D00329), isolate HBV/14611 (accession number AF121243), HBV-B1 (GenBank accession number AF282917.1), HBV strain Whutj-37 (GenBank accession number AY2933309.1), the Chinese HBV strain GDH1 (GenBank accession number AY766463.1) and HBV isolate 57-1 subtype adw (GenBank accession number AY518556.1). Exemplary HBV of genotype C include without limitation isolate AH- 1-ON980424 (accession number AB 113879), strain HCC-3-TT (accession number AB113877), HBV isolate SWT3.3 (GenBank accession number EU916241.1), HBV isolate H85 (GenBank accession number AY306136.1), HBV strain C1248 (GenBank accession number DQ975272.1), HBV isolate CHN-H155 (GenBank accession number DQ478901.1) and HBV isolate GZ28-1 (GenBank accession number EF688062). Exemplary HBV of genotype D include without limitation isolates KAMCHATKA27 (accession number AB 188243), ALTAY136 (accession number AB 188245) and Y07587 (GenBank accession number Y07587 and Stoll-Becker et al, 1997, J. Virol. 71 : 5399) as well as the HBV isolate described under accession number AB267090. Exemplary HBV of genotype E include without limitation isolate HB-JI411F and strain HB-JI411 (accession number AP007262). Exemplary HBV of genotype F include without limitation isolates HBV-BL597 (accession number AB214516) and HBV-BL592 (accession number AB 166850). Exemplary HBV of genotype G include without limitation isolate HB-JI444GF and strain HB-JI444G (accession number AP007264). Exemplary HBV of genotype H include without limitation isolate HBV ST0404 (accession number AB298362) and isolate HB-JI260F and strain HB-JI260 (accession number AP007261).

Amino acid sequences of the various HBV polypeptides and corresponding gene sequences can be found in specialized data banks and in the literature (see e.g. Valenzuela et al., 1980, The nucleotide sequence of the hepatitis B viral genome and the identification of the major viral genes (pp57-70) in "Animal Virus Genetics"; eds B. Fields, et al; Academic Press Inc., New York and Ono et al, 1983, Nucleic Acids Res. 11: 1747-57). It is intended that the

present invention is not limited to these exemplary HBV sequences. Indeed the nucleotide and amino acid sequences of any or all of the HBV polypeptides/peptides encoded in accordance with the present invention can vary between different HBV and this natural genetic variation is included within the scope of the invention. Moreover, such encoded HBV polypeptides/peptides can be representative of a specific genotype, and thus comprise an amino acid sequence corresponding to a consensus or near consensus sequence.

In one embodiment, the one or more HBV polypeptide(s) encoded by the vector comprised in the combination product of the present invention is/are selected from the group consisting of HBV polymerase, HBc (core) and HBs (env) polypeptides. In accordance with the purpose of the present invention, each of such encoded HBV polypeptide(s) can be a native (i.e. naturally-occurring) or modified (e.g. analogs or fragments of native HBV polypeptides).

In addition, if more than one HBV polypeptide is used, they may originate from distinct HBV, especially from HBV of different genotypes. Such a configuration permits to provide protection against a broader range of HBV genotypes or adaptation to a specific geographic region by using HBV genotype(s) that is/are endemic in this region or to a specific population of patients. In this regard, genotypes A and C are the most prevalent in the United States, genotypes A and D in Western European countries and genotype D in the Mediterranean basin whereas genotypes B and C are the most common in China. Limited data from India suggest that genotypes A and D are most prevalent in India. It is within the reach of the skilled person to choose appropriate HBV genotypes, serotypes, strains and/or isolates according to the population and/or geographic region to be treated. Alternatively, the HBV polypeptides encoded by the vector comprised in the combination product of the invention originate from HBV with the same genotype.

In a preferred embodiment, the vector comprised in the combination product of the present invention encodes three HBV polypeptides and more specifically a HBV polymerase polypeptide, HBc (core) polypeptide and HBs (env) domains. Preferably, they all originate from a genotype D HBV virus, with a specific preference for HBV isolate Y07587.

More preferably, the encoded HBV polypeptide(s) are “immunogenic” in the sense that they are able to induce or stimulate an immune response in a subject into which they have been expressed, which can be innate and/or specific (i.e. against at least one antigen/epitope of the infecting HBV), humoral and/or cellular (e.g. production of antibodies and/or cytokines and/or the activation of cytotoxic T cells, B, T lymphocytes, antigen presenting cells, helper T cells, dendritic cells, NK cells, etc). A vast variety of direct or indirect biological assays are

available in the art to evaluate the immunogenic nature of a component either *in vivo* (animal or human being), or *in vitro* (e.g. in a biological sample) as described herein.

Core polypeptide

In the native context, the HBV genome encodes a 183 amino acid-long core protein (or HBcAg), constitutive of the nucleocapsid. A second "core" protein is also found in the serum of patients during virus replication known as HBeAg which contains a precore N-terminal extension and a part of HBcAg. The C-terminus of the core protein is very basic and contains 4 Arg-rich domains which are predicted to bind nucleic acids as well as numerous phosphorylation sites.

As used herein, the term "core polypeptide", "core antigen", "HbcAg" or "HBc" refers to a polypeptide that retains at least 100 amino acid residues comprised in a native HBV core protein (i.e. a naturally-occurring core polypeptide of any HBV that can be found, isolated, obtained from a source of HBV in nature such as those cited above in connection with the term "HBV") as well as an analog or a fragment thereof. Preferably, the encoded core antigen does not include any portion of a precore N-terminal extension.

Any modification(s) of a native HBc can be envisaged, provided that the resulting core polypeptide retains a significant immunogenic activity (preferably in the same range as or higher than the native counterpart). Suitable modifications include truncation of at least 10 amino acid residues and at most 41 amino acid residues normally present at the C-terminus of a native core polypeptide or within the C-terminal part thereof, with a special preference for a C-term truncation extending from the last core residue (position 183) to residue 143, 144, 145, 146, 147, 148 or 149 of the native core.

According to a preferred embodiment, the core polypeptide encoded by the combination product of the present invention comprises (alternatively essentially consists of) an amino acid sequence that is at least 80% (e.g. 85%, 90%, 95%, 98%, 100%) identical to SEQ ID NO: 1 or SEQ ID NO: 2.

Polymerase polypeptide

In the native context, the HBV polymerase is about 832-845 amino acid residues long according to the HBV genotype and it is encoded in a long open reading frame ("P") that overlaps the 3'end of the core gene and all the surface protein genes. The viral polymerase is a multifunctional protein composed of four domains, including three functional domains, respectively the terminal protein, polymerase and RNase H domains that catalyse the major

steps in HBV replication (priming, DNA synthesis and removal of RNA templates) as well as a non-essential spacer domain present between the terminal protein and polymerase domains (see for example Radziwill et al., 1990, J. Virol. 64: 613-20; Bartenschlager et al., 1990, J. Virol. 64: 5324-32).

The catalytic sites responsible for enzymatic activities have been characterized. In this regard, four residues forming the conserved YMDD motif (residues 538 to 541 numbered with respect to the 832 residue long polymerase) have been shown essential to the DNA- and RNA-dependent DNA polymerase activity. RNase H activity has been mapped within the C-terminal portion (e.g. from position 680 to the C-terminus) and is based on a DEDD motif involving four non-consecutive amino acid residues, respectively Asp (D) in position 689, Glu (E) in position 718, Asp (D) in position 737 and Asp (D) in position 777 as well as few other amino acid residues including Val (V) in position 769 and Thr (T) in position 776. For purpose of illustration, the amino acid residues for HBV polymerase described herein are numbered by reference to a 832 amino acids long polymerase. It is within the reach of the skilled person to adapt the numeration of the amino acid residues to other polymerases (e.g. 843 or 845 amino acid long).

As used herein, the term "polymerase" refers to a polypeptide that retains at least 500 amino acid residues comprised in a native HBV polymerase protein (i.e. a naturally-occurring polymerase of any HBV that can be found, isolated, obtained from a source of HBV in nature such as those cited above in connection with the term "HBV") as well as an analog or a fragment thereof. Desirably, such at least 500 amino acid residues are spread over the three functional domains and preferably over the four domains normally present in a native HBV polymerase.

The polymerase modification(s) contemplated by the present invention preferably encompass deletion(s) and/or substitution(s) of one or more amino acid residue(s) (consecutive or not), in particular those aimed at functionally disrupting at least one of the enzymatic activities carried by a native polymerase (i.e. leading to a weak residual activity less than 20% of the native enzymatic activity). However, it is preferred to avoid modification(s) that can be detrimental to the immunogenic activity, especially in portions rich in B, CTL and/or T_H epitopes so as to retain an immunogenic activity (e.g. in the same range as or higher than the native counterpart).

A preferred embodiment comprises a polymerase polypeptide that is defective for the polymerase enzymatic activity exhibited by the native counterpart. A suitable modification aimed at functionally disrupting the polymerase activity comprises the deletion of at least 4

amino acid residues and at most 30 amino acid residues including the YMDD motif (positions 538-541 of a native polymerase of 832 amino acids), eventually with one or more amino acid residue(s) present at either side or both sides of said YMDD motif. A particularly suitable deletion encompasses positions 538 to 544 including the YMDD motif as well as the neighboring VVL motif that, if present, may contribute to the formation of "junctional" epitopes (e.g. colinearly synthesized new epitopes) which are at risk of reducing or silencing the host's anti-polymerase immune response (deletion encompassing the polymerase amino acids residues normally present between the Ser residue in position 536 and the Gly residue in position 537 of SEQ ID NO: 3). The disruption of the polymerase activity exhibited by the resulting polymerase polypeptide can be evaluated using assays well known in the art (e.g. the endogenous polymerase assays described in Radziwill et al., 1990, J Virol. 64: 613-20).

Alternatively or in combination, another preferred embodiment comprises modification(s) that functionally disrupt the RNaseH activity normally exhibited by a native HBV polymerase and the present invention encompasses the mutation(s) of any residues involved in native RNase H activity such as those cited above. Disruption of the RNase H activity can be evaluated using assays well known in the art (e.g. in vitro RNaseH activity assays or DNA-RNA tandem molecule analysis described in Radziwill et al., 1990, J Virol. 64: 613-20 or in Lee et al., 1997, Biochem. Bioph. Res. Commun. 233(2):401). Suitable modification(s) include(s) without limitation the deletion of at least 8 amino acids and at most 80 amino acids within the RNaseH polymerase domain (e.g positions 680 to 832 of a native 832 long polymerase, advantageously from 690 to 770, desirably from 695 to 765, preferably or 700 to 760, more preferably from 705 to 750 and even more preferably from 710 to 742 and/or the substitution of one or more amino acid residue(s) that participate(s) to RNaseH activity (see above) with an amino acid residue other than the native one. Preferably the substituted residue(s) is/are individually replaced with a His (H) residue or with a Tyr (Y) residue. Particularly preferred modification(s) encompass the deletion within the polymerase RNaseH domain extending from approximately position 710 to approximately 742 (deletion encompassing the polymerase amino acids residues normally present between the Leu residue in position 701 and the Ser residue in position 702 of SEQ ID NO: 3) and the substitution of the Asp residue in position 689 with a His (H) residue (D689H corresponding to the His residue in position 681 of SEQ ID NO: 3), the substitution of the Val residue in position 769 with a Tyr (Y) residue (V769Y corresponding to the Tyr residue in position 728 of SEQ ID NO: 3), the substitution of the Thr residue in position 776 with a Tyr (Y) residue (T776Y corresponding to the Tyr residue in position 735 of SEQ ID NO: 3), and the substitution of the

Asp residue in position 777 with a His (H) residue (D777H corresponding to the His residue in position 736 of SEQ ID NO: 3), and any combination thereof.

More preferably, the polymerase polypeptide encoded by the replication-defective adenovirus comprised in the combination product of the invention comprises (alternatively essentially consists of) an amino acid sequence which exhibits at least 80% (e.g. 85%, 90%, 95%, 98%, 100%) of identity with the amino acid sequence shown in SEQ ID NO: 3.

HBsAg

In the native context, the HBV S ORF encodes three surface proteins all of which have the same C terminus but differ at their N-termini due to the presence of three in-frame ATG start codons that divide the S ORF into three regions, S (226 amino acids), pre-S2 (55 amino acids) and pre-S1 (108 amino acids), respectively. The large-surface antigen protein (L) is produced following translation initiation at the first ATG start codon and comprises 389 amino acid residues (preS1-preS2-S). The middle surface antigen protein (M) results from translation of the S region and the pre-S2 region starting at the second start ATG whereas the small surface antigen protein of 226 amino acids (S, also designated HBsAg) results from translation of the S region initiated at the third start ATG codon. The HBV surface proteins are glycoproteins with carbohydrate side chains (glycans) attached by N-glycosidic linkages.

In a preferred embodiment, the vector comprised in the combination of the present invention encodes HBs antigen in the form of one or more HBsAg peptide(s) (called envelop or HBsAg domain) that originate from a native HBsAg. As used herein, the term "envelop domain" or "HBsAg domain" refers to a polypeptide having from approximately 15 to approximately 100 amino acid residues, and preferably at least 20 and at most 60 consecutive amino acids comprising at least one B and/or T cell epitope specific for T helper (T_H) cells and/or for cytotoxic T (CTL) cells normally present in a native HBsAg protein (i.e. a naturally-occurring HBsAg of any HBV that can be found, isolated, obtained from a source of HBV in nature such as those cited above in connection with the term "HBV"). Moreover such epitope(s) can be restricted to various MHC class I and/or class II antigens (e.g. A2, A24, DR, DP, etc). Preferably, the one or more HBsAg immunogenic domains used in the invention do not include any portions of HBV preS1 and preS2 polypeptides.

A vast choice of envelop domains are available in the art (e.g. WO93/03764; WO94/19011; WO2011/015656; Desombere et al., 2000, Clin. Exp. Immunol 122: 390; Loirat et al., 2000, J. Immunol. 165: 4748; Schirmbeck et al., 2002, J. Immunol 168: 6253; and Depla et al., 2008, J. Virol. 82: 435). Particularly preferred envelop domains include the

env1 and env2 domains described in WO2013/007772. As a general guidance, "Env1" corresponds to the portion of a native HBsAg from approximately position 14 to approximately position 51 and "env2" to the HBsAg portion from approximately position 165 to approximately position 194.

Preferably, the one or more HbsAg envelop domain(s) encoded by the replication-defective adenovirus comprised in the combination product of the invention comprises (alternatively essentially consists of) an amino acid sequence which exhibits at least 80% (e.g. 85%, 90%, 95%, 98% or 100%) of identity, with the amino acid sequence shown in SEQ ID NO: 4 or SEQ ID NO: 5.

Fusion polypeptide

In one embodiment, the vector comprised in the combination product of the invention encodes the HBV polypeptides (e.g. core and polymerase polypeptides and the HBsAg domain(s)) in the form of a fusion protein. The term "fusion" or "fusion protein" as used herein refers to the combination of two or more polypeptides/peptides in a single polypeptide chain. Preferably, the fusion is performed by genetic means, i.e. by fusing in frame the nucleotide sequences encoding each of said polypeptides/peptides. By "fused in frame", it is meant that the expression of the fused coding sequences results in a single protein without any translational terminator between each of the fused polypeptides/peptides. The fusion can be direct (i.e. without any additional amino acid residues in between) or through a linker (e.g. 3 to 30 amino acids long peptide composed of amino acid residues such as glycine, serine, threonine, asparagine, alanine and/or proline). It is within the reach of the skilled person to define accordingly the need and location of the translation-mediating regulatory elements (e.g. the initiator Met and codon STOP).

The various HBV polypeptides encoded by the vector in use in this invention can be included in the fusion in any arrangement (e.g. fusion of the polymerase and core polypeptides; fusion of the polymerase polypeptide and env domains; fusion of the polymerase and core polypeptides and HBs peptides described herein, etc). Desirably, in the encoded fusion, the HBV core polypeptide is C- terminally truncated and especially truncated at residue 148; the polymerase polypeptide comprises internal deletions and/or amino acid substitution(s) aimed at disrupting polymerase and RNaseH activities as described herein and the envelop domains comprises env1 and env2. The various HBV polypeptides/peptides can be individually positioned in the fusion protein (e.g. at N-terminus, C-terminus and/or internally). Fusion proteins of particular interest comprise from the N to the C-terminus (i) the

core polypeptide; (ii) the polymerase polypeptide described herein or fragment thereof, and (iii) one or more HBsAg domain(s). It is preferred to insert the one or more envelop domains internally within the polymerase polypeptide and in particular in place of the deleted polymerase and/or RNaseH portion(s). In a preferred embodiment, the fusion protein encoded by the adenovirus comprised in the combination product of the present invention comprises at its N-terminus, the core polypeptide (e.g. a C-term truncated core having residues 1 to 148 of a native HBc with an initiator Met) fused to the polymerase polypeptide (without initiator Met) comprising env1 domain inserted within the internal deletion aimed at disrupting polymerase activity and env2 within the internal deletion aimed at disrupting RNaseH activity (and if needed a STOP codon).

In a preferred aspect of this embodiment, the fusion protein of the invention comprises, (alternatively essentially consists of) an amino acid sequence which exhibits at least 80% (e.g. 85%, 90%, 95%, 98% or 100%) of identity with any of the amino acid sequence shown in SEQ ID NO: 6.

In the context of the invention, the fusion protein encoded by the vector in use in the invention may further comprise additional structural features.

In one embodiment, it can comprise additional peptide or polypeptide aimed to improve its immunogenic activity in a host organism. Such polypeptides capable of enhancing immunogenicity have been described in the literature and include, without limitation, calreticulin (Cheng et al., 2001, J. Clin. Invest. 108: 669), Mycobacterium tuberculosis heat shock protein 70 (HSP70) (Chen et al., 2000, Cancer Res. 60: 1035), ubiquitin (Rodriguez et al., 1997, J. Virol. 71: 8497), bacterial toxin such as the translocation domain of Pseudomonas aeruginosa exotoxin A (ETA(dIII)) (Hung et al., 2001 Cancer Res. 61: 3698) as well as such as T_H Pan-Dr epitope (Sidney et al., 1994, Immunity 1: 751), pstS1 GCG epitope (Vordermeier et al., 1992, Eur. J. Immunol. 22: 2631), tetanus toxoid P2TT (Panina-Bordignon et al., 1989, Eur. J. Immunol. 19: 2237) and P30TT (Demotz et al., 1993, Eur. J. Immunol. 23: 425) peptides, and influenza epitope (Lamb et al., 1982, Nature 300: 66 ; Rothbard et al., 1989, Int. Immunol. 1: 479).

Other suitable structural features are those which are beneficial to the synthesis, processing, stability and solubility of the encoded fusion protein (e.g. those aimed to modify potential cleavage sites, potential glycosylation sites and/or membrane anchorage so as to improve presentation to the cell membrane).

For example, it could be beneficial for immune response to direct the synthesis of the fusion protein described herein at the cell surface by using appropriate sequences well known in the art such as signal and/or trans-membrane peptides. Briefly, signal peptides usually comprise 15 to 35 essentially hydrophobic amino acids which are then removed by a specific ER (endoplasmic reticulum)-located endopeptidase to give the mature polypeptide. Trans-membrane peptides are also highly hydrophobic in nature and serve to anchor the polypeptides within cell membrane. The choice of the trans-membrane and/or signal peptides which can be used in the context of the present invention is vast. They may be obtained from any membrane-anchored and/or secreted polypeptide (e.g. cellular or viral polypeptides) such as those of immunoglobulins, tissue plasminogen activator, insulin, rabies glycoprotein, the HIV virus envelope glycoprotein or the measles virus F protein or may be synthetic.

Encoding nucleic acid molecule(s)

The nucleic acid molecules encoding the HBV polypeptide(s) and more specifically the fusion protein in use in this invention may be easily generated by a number of ways known to those skilled in the art (e.g. cloning, PCR amplification, DNA shuffling). For example, they can be isolated independently from any source of HBV or biologic materials described in the art (e.g. from HBV-containing cells, cDNA and genomic libraries, viral genomes or any prior art vector known to include it) using sequence data available to the skilled person and the sequence information provided herein, and then suitably linked together by conventional molecular biology or PCR techniques. Alternatively, they can also be generated by chemical synthesis in automatized process (e.g. assembled from overlapping synthetic oligonucleotides or synthetic gene). Modification(s) can be generated by a number of ways known to those skilled in the art, such as chemical synthesis, site-directed mutagenesis, PCR mutagenesis, etc.

In addition, the HBV-encoding nucleic acid molecules can individually be optimized for providing high level expression in a particular host cell or subject. It has been indeed observed that, the codon usage patterns of organisms are highly non-random and the use of codons may be markedly different between different hosts. As the nucleotide sequences encompassed by the invention are mostly of viral origin (HBV), they may have an inappropriate codon usage pattern for efficient expression in higher eukaryotic cells (e.g. human). Typically, codon optimization is performed by replacing one or more "native" (HBV) codon corresponding to a codon infrequently used in the host organism of interest by one or more codon encoding the same amino acid which is more frequently used. It is not

necessary to replace all native codons corresponding to infrequently used codons since increased expression can be achieved even with partial replacement. Moreover, some deviations from strict adherence to optimised codon usage may be made to accommodate suitable restriction site(s) into the resulting nucleic acid molecule.

Alternatively or in combination, the HBV-encoding nucleic acid molecules can be individually degenerated over the full length nucleotide sequence or portion(s) thereof so as to reduce sequence homology between the various HBV nucleotide sequences in use herein. It is indeed advisable to degenerate the portions of nucleic acid sequences that show a high degree of sequence identity (e.g. the overlapping HBV nucleotide sequence) so as to avoid homologous recombination problems during production process and the skilled person is capable of identifying such portions by sequence alignment.

Further, expression in the host cell or organism can be improved through additional modifications of the HBV nucleotide sequences aimed to prevent clustering of rare, non-optimal codons and/or to suppress or modify at least partially negative sequence elements which are expected to negatively influence expression levels (e.g. AT-rich or GC-rich sequence stretches; unstable direct or inverted repeat sequences; RNA secondary structures; and/or internal cryptic regulatory elements such as internal TATA-boxes, chi-sites, ribosome entry sites, and/or splicing donor/acceptor sites).

Expression of the HBV-encoding nucleic acid molecule

In accordance with the present invention, the nucleic acid molecules encoding the various HBV polypeptides in use in the present invention (e.g. the fusion protein described herein) are operably linked to suitable regulatory elements for their expression in a host cell or subject. As used herein, the term "regulatory elements" or "regulatory sequence" refers to any element that allows, contributes or modulates nucleic acid expression in a given host cell or subject, including replication, duplication, transcription, splicing, translation, stability and/or transport of the nucleic acid(s) or its derivative (i.e. m RNA). As used herein, “operably linked” means that the elements being linked are arranged such that they function in concert for their intended purposes.

It will be appreciated by those skilled in the art that the choice of the regulatory sequences can depend on such factors as the gene itself, the virus into which it is inserted, the host cell or subject, the level of expression desired, etc. The promoter is of special importance. The term "promoter" is used herein for its art-recognized meaning to denote a portion of a gene containing DNA sequences that provide for the binding of RNA polymerase

and effects transcription from the transcription initiation to the terminator. Promoter sequences are typically found in the 5' non-coding regions of genes. In the context of the invention, it can be constitutive directing expression of the HBV nucleic acid molecule(s) in many types of host cells or specific to certain host cells (e.g. liver-specific regulatory sequences) or regulated in response to specific events or exogenous factors (e.g. by temperature, nutrient additive, hormone, etc) or according to the phase of a viral cycle (e.g. late or early). One may also use promoters that are repressed during the production step in response to specific events or exogenous factors, in order to optimize virus production and circumvent potential toxicity of the expressed polypeptide(s).

Promoters suitable for constitutive expression in mammalian cells include but are not limited to the cytomegalovirus (CMV) immediate early promoter (US 5,168,062), the RSV promoter, the adenovirus major late promoter, the phosphoglycero kinase (PGK) promoter (Adra et al., 1987, Gene 60: 65-74), the thymidine kinase (TK) promoter of herpes simplex virus (HSV)-1 and the T7 polymerase promoter (WO98/10088). Vaccinia virus promoters are particularly adapted for expression in poxviral vectors. Representative example include without limitation the vaccinia 7.5K, H5R, 11K7.5 (Erbs et al., 2008, Cancer Gene Ther. 15: 18), TK, p28, p11 and K1L promoter, as well as synthetic promoters such as those described in Chakrabarti et al. (1997, Biotechniques 23: 1094), Hammond et al. (1997, J. Virological Methods 66: 135) and Kumar and Boyle (1990, Virology 179: 151) as well as early/late chimeric promoters. Liver-specific promoters include without limitation those of HMG-CoA reductase (Luskey, 1987, Mol. Cell. Biol. 7: 1881); sterol regulatory element 1 (SRE-1; Smith et al., 1990, J. Biol. Chem. 265: 2306); albumin (Pinkert et al., 1987, Genes Dev. 1: 268); phosphoenol pyruvate carboxy kinase (PEPCK) (Eisenberger et al., 1992, Mol. Cell Biol. 12: 1396); alpha-1 antitrypsin (Ciliberto et al., 1985, Cell 41: 531); human transferrin (Mendelzon et al., 1990, Nucleic Acids Res. 18: 5717); and FIX (US 5,814,716) genes.

Those skilled in the art will appreciate that the regulatory elements controlling the expression of the HBV-encoding nucleic acid molecule(s) may further comprise additional elements for proper initiation, regulation and/or termination of transcription (e.g. polyA transcription termination sequences), mRNA transport (e.g. nuclear localization signal sequences), processing (e.g. splicing signals), and stability (e.g. introns and non-coding 5' and 3' sequences), translation (e.g. an initiator Met, tripartite leader sequences, IRES ribosome binding sites, Shine-Dalgarno sequences, etc.) and purification steps (e.g. a tag).

If needed, the HBV-encoding vector comprised in the combination of the present invention can further comprise one or more transgene(s), aimed to improve therapeutic or

protective activity to an HBV infection or any disease or condition caused by or associated with an HBV infection. Suitable transgenes include without limitation immunomodulators such as cytokines (e.g an interleukin or a colony-stimulating factor such as GM-CSF) and any other antigen originating from a potentially co-infecting organism (e.g. human immunodeficiency virus (HIV), hepatitis D virus (HDV), tuberculosis mycobacterium, etc).

A particularly preferred embodiment of the invention is directed to a replication-defective adenovirus (vector or viral particles thereof) comprising inserted in place of the E1 region a nucleic acid molecule placed under the control of a promoter such as the CMV promoter, and encoding a fusion protein comprising core and polymerase polypeptides and two env domains, with a specific preference for a fusion protein comprising an amino acid sequence as shown in SEQ ID NO: 6.

Immune checkpoint modulator(s)

In accordance with the present invention, the one or more immune checkpoint modulator(s) comprised in the combination product of the invention may independently be a polypeptide or a polypeptide-encoding nucleic acid molecule; said polypeptide comprising a domain capable of binding the targeted immune checkpoint and/or inhibiting the binding of a ligand to said targeted immune checkpoint so as to exert an antagonist function (i.e. being capable of antagonizing an immune checkpoint-mediated inhibitory signal) or an agonist function (i.e. being capable of boosting an immune checkpoint-mediated stimulatory signal). Such one or more immune checkpoint modulator(s) can be independently selected from the group consisting of peptides (e.g. peptide ligands), soluble domains of natural receptors, RNAi, antisense molecules, antibodies and protein scaffolds.

Desirably, the one or more immune checkpoint modulator(s) in use in the present invention antagonizes at least partially (e.g. more than 50%) the activity of inhibitory immune checkpoint(s), in particular those mediated by any of the following PD-1, PD-L1, PD-L2, LAG3, Tim3, BTLA, SLAM, 2B4, CD160, KLRG-1 and CTLA4, with a specific preference for a modulator that specifically binds to any of such target proteins. The term "specifically binds to" refers to the capacity to a binding specificity and affinity for a particular target or epitope even in the presence of a heterogeneous population of other proteins and biologics. Thus, under designated assay conditions, the modulator in use in the invention binds preferentially to its target and does not bind in a significant amount to other components present in a test sample or subject. Preferably, such a modulator shows high affinity binding to its target with an equilibrium dissociation constant equal or below $1x10^{-6}$M (e.g. at least

$0.5x10^{-6}$, $1x10^{-7}$, $1x10^{-8}$, $1x10^{-9}$, $1x10^{-10}$, etc). Alternatively, the one or more immune checkpoint modulator(s) in use in the present invention exerts an agonist function in the sense that it is capable of stimulating or reinforcing stimulatory signals, in particular those mediated by CD28 with a specific preference for any of ICOS, CD137 (4-1BB), OX40, CD27, CD40 and GITR immune checkpoints. Standard assays to evaluate the binding ability of such modulators toward immune checkpoints are known in the art, including for example, ELISAs, Western blots, RIAs and flow cytometry. The binding kinetics (e.g., binding affinity) of antibody modulators also can be assessed by standard assays known in the art, such as by Biacore analysis.

In a preferred embodiment, the immune checkpoint modulator is an antibody. In the context of the invention, "antibody" ("Ab") is used in the broadest sense and encompasses naturally occurring and engineered by man as well as full length antibodies or functional fragments or analogs thereof that are capable of binding the target immune checkpoint or epitope (thus retaining the target-binding portion). The antibody in use in the invention can be of any origin, e.g. human, humanized, animal (e.g. rodent or camelid antibody) or chimeric. It may be of any isotype (e.g. IgG1, IgG2, IgG3, IgG4, IgM, etc.). In addition, it may be glycosylated or non- glycosylated. The term antibody also includes bispecific or multispecific antibodies so long as they exhibit the binding specificity described herein.

For illustrative purposes, full length antibodies are glycoproteins comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (VH) and a heavy chain constant region which is made of three CH1, CH2 and CH3 domains (eventually with a hinge between CH1 and CH2). Each light chain is comprised of a light chain variable region (VL) and a light chain constant region which comprises one CL domain. The VH and VL regions comprise hypervariable regions, named complementarity determining regions (CDR), interspersed with more conserved regions named framework regions (FR). Each VH and VL is composed of three CDRs and four FRs in the following order: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. The CDR regions of the heavy and light chains are determinant for the binding specificity.

As used herein, a "humanized antibody" refers to a non-human (e.g. murine, camel, rat, etc) antibody whose protein sequence has been modified to increase its similarity to a human antibody (i.e. produced naturally in humans). The process of humanization is well known in the art (see e.g. Presta et al., 1997, Cancer Res. 57(20): 4593-9; US 5,225,539; US 5,530,101; US 6,180,370; WO2012/110360). For example, a monoclonal antibody developed for human use can be humanized by substituting one or more residue of the FR regions to

look like human immunoglobulin sequence whereas the vast majority of the residues of the variable regions (especially the CDRs) are not modified and correspond to those of a non-human immunoglobulin. For general guidance, the number of these amino acid substitutions in the FR regions is typically no more than 20 in each variable region VH or VL.

As used herein, a "chimeric antibody" refers to an antibody comprising one or more element(s) of one species and one or more element(s) of another species, for example, a non-human antibody comprising at least a portion of a constant region (Fc) of a human immunoglobulin.

Antibody fragments can be engineered for use in the combination of the invention. Representative examples include without limitation Fab, Fab', F(ab')2, dAb, Fd, Fv, scFv, di-scFv and diabody, etc,. More specifically:

(i) a Fab fragment represented by a monovalent fragment consisting of the VL, VH, CL and CH1 domains;

(ii) a F(ab')2 fragment represented by a bivalent fragment comprising two Fab fragments linked by at least one disulfide bridge at the hinge region;

(iii) a Fd fragment consisting of the VH and CH1 domains;

(iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody,

(v) a dAb fragment consisting of a single variable domain fragment (VH or VL domain.;

(vi) a single chain Fv (scFv) comprising the two domains of a Fv fragment, VL and VH, that are fused together, eventually with a linker to make a single protein chain (see e.g. Bird et al., 1988, Science 242: 423-6; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85: 5879-83; US 4,946,778; US 5,258,498); and

(vii) any other artificial antibody.

Methods for preparing antibodies, fragments and analogs thereof are known in the art (see e.g. Harlow and Lane, 1988, Antibodies – A laboratory manual; Cold Spring Harbor Laboratory, Cold Spring Harbor NY). One may cite for example hybridoma technology (as described in Kohler and Milstein, 1975, Nature 256: 495-7; Cote et al., 1983, Proc. Natl. Acad. Sci. USA 80: 2026-30; Cole et al. in Monoclonal antibodies and Cancer Therapy; Alan Liss pp77-96), recombinant techniques (e.g. using phage display methods), peptide synthesis and enzymatic cleavage. Antibody fragments can be produced by recombinant technique as described herein. They may also be produced by proteolytic cleavage with enzymes such as papain to produce Fab fragments or pepsin to produce F(ab')2 fragments as described in the

literature (see e.g. Wahl et al., 1983, J. Nucl. Med. 24: 316-25). Analogs (or fragment thereof) can be generated by conventional molecular biology methods (PCR, mutagenesis techniques). If needed, such fragments and analogs may be screened for functionality in the same manner as with intact antibodies (e.g. by standard ELISA assay).

In a preferred embodiment, at least one of the one or more immune checkpoint modulator(s) for use in the present invention is a monoclonal antibody, with a specific preference for a human (in which both the framework regions are derived from human germline immunoglobin sequences) or a humanized antibody according to well-known humanization process.

In a preferred embodiment, at least one of the one or more checkpoint modulator(s) for use in this invention comprises an antibody capable of antagonizing at least partially the protein Programmed Death 1 (PD-1), and especially an antibody that specifically binds to human PD-1. PD-1 is part of the immunoglobulin (Ig) gene superfamily and a member of the CD28 family. It is a 55 kDa type 1 transmembrane protein expressed on antigen-experienced cells (e.g. activated B cells, T cells, and myeloid cells) (Agata et al., 1996, Int. Immunol. 8: 765-72; Okazaki et al., 2002, Curr. Opin. Immunol. 14: 391779-82; Bennett et al., 2003, J. Immunol 170: 711-8). In normal context, it acts by limiting the activity of T cells at the time of inflammatory response, thereby protecting normal tissues from destruction (Topalian, 2012, Curr. Opin. Immunol. 24: 207-12). Two ligands have been identified for PD-1, respectively PD-L1 (programmed death ligand 1) and PD-L2 (programmed death ligand 2) (Freeman et al., 2000, J. Exp. Med. 192: 1027-34; Carter et al., 2002, Eur. J. Immunol. 32: 634-43). PD-L1 was identified in 20-50% of human cancers (Dong et al., 2002, Nat. Med. 8: 787-9). The interaction between PD-1 and PD-L1 resulted in a decrease in tumor infiltrating lymphocytes, a decrease in T-cell receptor mediated proliferation, and immune evasion by the cancerous cells (Dong et al., 2003, J. MoI. Med. 81: 281-7; Blank et al., 2005, Cancer Immunol. Immunother. 54: 307-314). The complete nucleotide and amino acid PD-1 sequences can be found under GenBank Accession No U64863 and NP_005009.2. A number of anti PD1 antibodies are available in the art (see e.g. those described in WO2004/004771; WO2004/056875; WO2006/121168; WO2008/156712; WO2009/014708; WO2009/114335; WO2013/043569; and WO2014/047350). Preferred anti PD-1 antibodies in the context of this invention are FDA approved or under advanced clinical development and one may use in particular an anti-PD-1 antibody selected from the group consisting of Nivolumab (also termed BMS-936558 under development by Bristol Myer Squibb), Pembrolizumab (also

termed lanbrolizumab or MK-3475; under development by Merck), and Pidilizumab (also termed CT-011 under development by CureTech).

Another preferred example of immune checkpoint modulator is represented by a modulator capable of antagonizing at least partially the PD-1 ligand termed PD-L1, and especially an antibody that specifically binds to human PD-L1. A number of anti PD-L1 antibodies are available in the art (see e.g. those described in EP1907000). Preferred anti PD-L1 antibodies are FDA approved or under advanced clinical development (e.g. MPDL3280A under development by Genentech/Roche and BMS-936559 under development by Bristol Myer Squibb).

Still another preferred example of immune checkpoint modulator is represented by a modulator capable of antagonizing at least partially the CTLA-4 protein, and especially an antibody that specifically binds to human CTLA-4. CTLA4 (for cytotoxic T-lymphocyte-associated antigen 4) also known as CD152 was identified in 1987 (Brunet et al., 1987, Nature 328: 267-70) and is encoded by the CTLA4 gene (Dariavach et al., Eur. J. Immunol. 18: 1901–5). It is expressed on the surface of helper T cells where it primarily regulates the amplitude of the early stages of T cell activation. Recent work has suggested that CTLA-4 may function *in vivo* by capturing and removing B7-1 and B7-2 from the membranes of antigen-presenting cells, thus making these unavailable for triggering of CD28 (Qureshi et al., Science, 2011, 332: 600-3). The complete CTLA-4 nucleic acid sequence can be found under GenBank Accession No Ll 5006. A number of anti CTLA-4 antibodies are available in the art (see e.g. those described in US 8,491,895). Preferred anti CTLA-4 antibodies in the context of this invention are FDA approved or under advanced clinical development. One may cite more particularly Ipilimumab marketed by Bristol Myer Squibb as Yervoy (see e.g. US 6,984,720; US 8,017,114), Tremelimumab under development by Pfizer (see e.g. US 7,109,003 and US 8,143,379) and single chain anti-CTLA4 antibodies (see e.g. WO97/20574 and WO2007/123737).

Immune checkpoint modulator for antagonizing the TIM3 receptor may also be used in the combination of the present invention (see e.g. Ngiow et al., 2011, Cancer res. 71: 3540-51; US2012-0189617).

Another example of immune checkpoint modulator is represented by an OX40 agonist such as agonist ligand of OX40 (OX40L) (see e.g. US 5,457,035, US 7,622,444; WO03/082919) or an antibody directed to the OX40 receptor (see e.g. US 7,291,331 and WO03/106498).

Other examples of immune checkpoint modulators are represented by anti-KIR or anti-CD96 antibody targeting the inhibitory receptors harboured by CD8+ T cells and NK cells.

The present invention encompasses a combination comprising more than one immune checkpoint modulator. A preferred example includes without limitation using an anti-TIM-3 antibody with an anti-PD-1 or an anti-PD-L1 antibody in combination with the HBV-encoding vector described herein.

Production of immune checkpoint modulator

The one or more immune checkpoint modulator(s) for use in this invention can be produced by recombinant means using suitable expression vectors and host cells. Nucleic acid molecules encoding the relevant portion(s) of the desired immune checkpoint modulator can be obtained by standard molecular biology techniques using sequence data accessible in the art and the information provided herein. For example, cDNAs encoding the light and heavy chains of the antibody or their CDRs can be isolated from the producing hybridoma, immunoglobulin gene libraries or any available source.

In one embodiment, the nucleic acid molecule(s) encoding the immune checkpoint modulator(s) can be cloned in a suitable vector and expressed in a host cell to produce said immune checkpoint modulator by recombinant means. Insertion into the expression vector can be performed by routine molecular biology, e.g. as described in Sambrook et al. (2001, Molecular Cloning-A Laboratory Manual, Cold Spring Harbor Laboratory). A variety of host-vector systems may be used or constructed to express the one or more immune checkpoint modulator(s) for use in the present invention, including prokaryotic organisms such as bacteria, yeast, insect cell systems and mammalian cell systems. Typically, such vectors are commercially available (e.g. in Invitrogen, Stratagene, Amersham Biosciences, Promega, etc.) or available from depositary institutions such as the American Type Culture Collection (ATCC, Rockville, Md.) or have been the subject of numerous publications describing their sequence, organization and methods of producing, allowing the artisan to apply them.

If appropriate, the expression vector can be combined with one or more reagents which improve the transfection efficiency and/or stability of the vector. These substances are widely documented in the literature. Representative examples of such reagents include without limitation polycationic polymers (e.g. chitosan, polymethacrylate, PEI, etc), cationic lipids (e.g.DC-Chol/DOPE, transfectam lipofectin now available from Promega) and liposomes.

Suitable vectors for use in prokaryotic systems include without limitation pBR322 (Gibco BRL), pUC (Gibco BRL), pBluescript (Stratagene), p Poly (Lathe et al., 1987, Gene 57: 193-201), pTrc (Amann et al., 1988, Gene 69: 301-15); pET lid (Studier et al., 1990, Gene Expression Technology: Methods in Enzymology 185: 60-89); pIN (Inouye et al., 1985, Nucleic Acids Res. 13: 3101-9; Van Heeke et al., 1989, J. Biol. Chem. 264: 5503-9); and pGEX vectors where the nucleic acid molecule can be expressed in fusion with glutathione S-transferase (GST) (Amersham Biosciences Product). Suitable vectors for expression in yeast (e.g. *S. cerevisiae*) include, but are not limited to pYepSec1 (Baldari et al., 1987, EMBO J. 6: 229-34), pMFa (Kujan et al., 1982, Cell 30: 933-43), pJRY88 (Schultz et al., 1987, Gene 54: 113-23), pYES2 (Invitrogen Corporation) and pTEF-MF (Dualsystems Biotech Product). Suitable plasmid vectors for expression in mammalian host cells include, without limitation, pREP4, pCEP4 (Invitrogene), pCI (Promega), pCDM8 (Seed, 1987, Nature 329: 840) and pMT2PC (Kaufman et al., 1987, EMBO J. 6: 187-95), pVAX and pgWiz (Gene Therapy System Inc; Himoudi et al., 2002, J. Virol. 76: 12735-46). Viral-based expression systems can also be utilized in the context of the invention derived from a variety of different viruses (e.g. baculovirus, retrovirus, adenovirus, AAV, poxvirus, measles virus, and the like).

Moreover, the vector for the expression of immune checkpoint modulator may also comprise one or more additional element(s) enabling maintenance, propagation or expression of the nucleic acid molecule encoding the immune checkpoint modulator in the host cell. Suitable marker genes for expression in prokaryotic host cells include tetracycline and ampicillin-resistance genes. Also, resistance genes can be used for expression in mammalian host cells such as dihydrofolate reductase (dhfr) which confers resistance to methotrexate (Wigler et al., 1980, Proc. Natl. Acad. Sci. USA 77: 3567; O'Hare et al., 1981, Proc. Natl. Acad. Sci. USA 78: 1527); gpt which confers resistance to mycophenolic acid (Mulligan and Berg, 1981, Proc. Natl. Acad. Sci. USA 78: 2072); neo which confers resistance to the aminoglycoside G-418 (Colbeère-Garapin et al., 1981, J. Mol. Biol. 150: 1); zeo which confers resistance to zeomycin, kana which confers resistance to kanamycin and hygro, which confers resistance to hygromycin (Santerre et al., 1984, Gene 30: 147). URA3 and LEU2 genes can be used for expression in yeast systems, which provide for complementation of ura3 or leu2 yeast mutants.

Recombinant DNA technologies can also be used to improve expression of the nucleic acid molecule in the host cell, e.g. by using high-copy number vectors, substituting or modifying one or more transcriptional regulatory sequences (e.g. promoter, enhancer and the

like), optimizing the codon usage and suppressing negative sequences that may destabilize the transcript as described herein in connection with the HBV nucleic acid molecule).

Preferably, the nucleic acid molecule encoding the immune checkpoint modulator is in a form suitable for its expression in a host cell, which means that the nucleic acid molecule is placed under the control of one or more regulatory sequences, appropriate to the vector, the host cell and/or the level of expression desired as described above.

In accordance with the present invention, the immune checkpoint modulator can be modified. Various modifications can be contemplated such as those modifying the amino acid sequence as well as those aimed at increasing its biological half-life, its affinity or its stability. For example, a signal peptide may be included for facilitating secretion of the immune checkpoint modulator in the cell culture. The signal peptide is typically inserted at the N-terminus of the protein immediately after the Met initiator. The choice of signal peptides is wide and is accessible to persons skilled in the art. As an additional example, a tag peptide (typically a short peptide sequence able to be recognized by available antisera or compounds) may be also be added for facilitating purification of the recombinant immune checkpoint modulator. A vast variety of tag peptides can be used in the context of the invention including, without limitation, PK tag, FLAG octapeptide, MYC tag, HIS tag (usually a stretch of 4 to 10 histidine residues) and e-tag (US 6,686,152). The tag peptide(s) may be independently positioned at the N-terminus of the protein or alternatively at its C-terminus or alternatively internally or at any of these positions when several tags are employed. Tag peptides can be detected by immunodetection assays using anti-tag antibodies.

Another approach that may be pursued in the context of the present invention is coupling of the immune checkpoint modulator to an external agent such as a radiosensitizer agent, a cytotoxic agent and/or a labelling agent. The coupling can be covalent or not. As used herein, the term "radiosensitizer" refers to a molecule that makes cells more sensitive to radiation therapy. Radiosensitizer includes, but are not limited to, metronidazole, misonidazole, desmethylmisonidazole, pimonidazole, etanidazole, nimorazole, mitomycin C, RSU 1069, SR 4233, E09, RB 6145, nicotinamide, 5-bromodeoxyuridine (BUdR), 5-iododeoxyuhdine (IUdR), bromodeoxycytidine, fluorodeoxyuridine (FUdR), hydroxyurea and cisplatin. As used herein, the term “cytotoxic agent” refers to a compound that is directly toxic to cells, preventing their reproduction or growth such as toxins (e. g. an enzymatically active toxin of bacterial, fungal, plant or animal origin, or fragments thereof). As used herein, "a labeling agent" refers to a detectable compound. The labeling agent may be detectable by

itself (e. g., radioactive isotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical modification of a substrate compound which is detectable.

The methods for recombinantly producing the immune checkpoint modulator are conventional in the art. Typically such methods comprise (a) introducing the vector expressing the desired immune checkpoint modulator into a suitable producer cell to produce a transfected or infected producer cell, (b) culturing in-vitro said transfected or infected producer cell under conditions suitable for its growth, (c) recovering the immune checkpoint modulator from the cell culture, and (d) optionally, purifying the recovered immune checkpoint modulator. In the context of the invention, producer cells include prokaryotic cells, lower eukaryotic cells such as yeast, and other eukaryotic cells such as insect cells and mammalian (e.g. human or non-human) cells. Preferred prokaryotic organism include *E. coli* (e.g. *E. coli* BL21; Amersham Biosciences) and *B. subtilis*. Preferred yeast producer cells include without limitation *Saccharomyces cerevisiae*, *Saccharomyces pombe* and *Pichia pastoris*. Preferred mammalian producer cells include without limitation BHK-21 (baby hamster kidney), CV-1 (African monkey kidney cell line), COS (e.g. COS-7) cells, Chinese hamster ovary (CHO) cells, mouse NIH/3T3 cells, mouse NSO myeloma cells, HeLa cells, Vero cells, HEK293 cells and PERC.6 cells as well as the corresponding hybridoma cells.

The producer cells can be cultured in conventional fermentation bioreactors, flasks, and petri plates. Culturing can be carried out at a temperature, pH and oxygen content appropriate for a given host cell. No attempts to describe in detail the various methods known for the production of proteins in prokaryote and eukaryote cells will be made here. Production of the immune checkpoint modulator can be periplasmic, intracellular or preferably secreted outside the producer cell (e.g. in the culture medium).

The immune checkpoint modulator can then be purified by well-known purification methods including ammonium sulfate precipitation, acid extraction, gel electrophoresis, filtration and chromatographic methods (e.g. reverse phase, size exclusion, ion exchange, affinity, phosphocellulose, hydrophobic-interaction or hydroxylapatite chromatography,etc). The conditions and technology used to purify a particular protein will depend on factors such as net charge, molecular weight, hydrophobicity, hydrophilicity and will be apparent to those having skill in the art. Moreover, the level of purification will depend on the intended use. It is also understood that depending upon the producer cell, the immune checkpoint modulator proteins can have various glycosylation patterns, or may be non-glycosylated (e.g. when produced in bacteria). Desirably, the immune checkpoint modulator in use in the present invention is at least partially purified in the sense that it is substantially free of other

antibodies having different antigenic specificities and/or other cellular material. Further, the immune checkpoint modulator may be formulated according to the conditions conventionally used in the art (e.g. WO2009/073569).

Combination therapy

"Combination therapy" refers to the action of administering in the same subject at least the two entities (or active agent) being an object of the invention. In the context of the invention, the HBV-encoding vector and/or the immune check point modulator(s) may be administered together or separately to the subject and in a single dose or multiple doses. In particular, the HBV-encoding vector and/or the immune check point modulator(s) may be mixed in the same composition or in different compositions administered concomitantly (at approximately the same time), sequentially (virus followed by immune checkpoint modulator or *vice versa*) or interspersed (intermixed administrations at various intervals). The administrations of the HBV-encoding vector and/or the immune check point modulator(s) may be performed by the same or different routes and may take place at the same site or at alternative sites.

Therefore, such a combination can be in the form of (a) a single composition (e.g. a mixture of the HBV-encoding vector with one or more immune checkpoint modulator(s); a mixture of the HBV-encoding vector with vector(s) encoding the one or more immune checkpoint modulator(s)), (b) separate compositions that may be administered concurrently or sequentially each once or several times and via the same or different routes or (c) through specific vector design, e.g. where the vector encodes both the HBV polypeptides (e.g. HBV fusion) described herein and the one or more immune checkpoint modulator(s). In this context, the HBV polypeptides and the immune checkpoint modulator(s) are preferably expressed independently in the same vector using distinct regulatory elements (e.g. distinct promoter and termination sequences).

In one embodiment, the composition(s) comprising the HBV-encoding vector and/or the one or more immune checkpoint modulator comprises a pharmaceutically acceptable vehicle in addition to a therapeutically effective amount of such entities.

As used herein a "therapeutically effective amount" is a dose sufficient for the alleviation of one or more symptoms normally associated with an HBV infection or any disease or condition caused by or associated with an HBV infection. When prophylactic use is concerned, this term means a dose sufficient to prevent or to delay the establishment of an

HBV infection. "Therapeutic" compositions are designed and administered to a subject already infected by an HBV with the goal of reducing or ameliorating at least one disease or condition caused by or associated with said HBV infection, eventually in combination with one or more conventional therapeutic modalities as described herein (e.g. treatment with nucleoside, nucleotide analogs and/or IFN-based therapy). For example, a therapeutically effective amount for inducing an immune response could be that amount necessary to cause activation of the immune system (e.g. resulting in the development of an anti-HBV response).

The term "pharmaceutically acceptable vehicle" is intended to include any and all carriers, solvents, diluents, excipients, adjuvants, dispersion media, coatings, antibacterial and antifungal agents, absorption agents and the like compatible with administration in mammals and in particular human subjects.

In other embodiments, each of the HBV-encoding vector and the immune check point modulator composition(s) is suitably buffered for human use. Suitable buffers include without limitation phosphate buffer (e.g. PBS), bicarbonate buffer and/or Tris buffer capable of maintaining a physiological or slightly basic pH (e.g. from approximately pH 7 to approximately pH 9).

Each of the HBV-encoding vector and the one or more immune check point modulator(s) composition can independently be placed in a solvent or diluent appropriate for human or animal use. The solvent or diluent is preferably isotonic, hypotonic or weakly hypertonic and has a relatively low ionic strength. In particular, the formulation of each entity comprised in the combination of the present invention is designed with the goal of improving its stability in particular under the conditions of manufacture and long-term storage (i.e. for at least 6 months, with a preference for at least two years) at freezing (e.g. -70°C, -20°C), refrigerated (e.g. 4°C) or ambient temperatures. Various formulations are available in the art either in frozen, liquid form or lyophilized form.

Liquid and frozen formulations generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, (e.g. sodium chloride), Ringer's solution, Hank's solution, saccharide solution (e.g. glucose, trehalose, saccharose, dextrose, etc) and other aqueous physiologically balanced salt solutions as well as glycols (such as ethylene glycol, propylene glycol or polyethylene glycol) may be included (see for example the most current edition of Remington: The Science and Practice of Pharmacy, A. Gennaro, Lippincott, Williams&Wilkins and WO98/02522, WO01/66137. Solid (e.g. dry powdered or lyophilized) compositions can be obtained by a process involving

vacuum drying and freeze-drying (see e.g. WO03/053463; WO2006/0850082; WO2007/056847; WO2008/114021 and WO2014/053571). For illustrative purposes, sterile histidine, acetate citrate or phosphate saline buffers containing surfactant such as polysorbate 80 and stabilizers such as sucrose or mannitol are adapted to the preservation of recombinant antibodies and buffered formulations including NaCl and/or sugar are particularly adapted to virus preservation (e.g. Tris 10 mM pH 8 with saccharose 5 % (W/V), Sodium glutamate 10 mM, and NaCl 50 mM or phosphate-buffered saline with glycerol (10%) and NaCl).

In certain embodiments, the HBV-encoding vector and/or immune checkpoint modulator(s) can be formulated to ensure proper distribution or a delayed release *in vivo*. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, polylactic acid and polyethylene glycol. Many methods for the preparation of such formulations are described in the art (e.g. J. R. Robinson in "Sustained and Controlled Release Drug Delivery Systems", ed., Marcel Dekker, Inc., New York, 1978; WO01/23001; WO2006/93924; WO2009/53937). Gastro-resistant capsules and granules are particularly appropriate for oral administration, suppositories for rectal or vaginal administration, eventually in combination with absorption enhancers useful to increase the pore size of the mucosal membranes. Such absorption enhancers are typically substances having structural similarities to the phospholipid domains of the mucosal membranes such as sodium deoxycholate, sodium glycocholate, dimethyl-beta-cyclodextrin, lauryl-1-lysophosphatidylcholine).

Each of the HBV-encoding vector and/or the immune check point modulator composition(s) may also contain other pharmaceutically acceptable excipients for providing desirable pharmaceutical or pharmacodynamic properties, including for example osmolarity, viscosity, clarity, colour, sterility, stability, rate of dissolution of the formulation, modifying or maintaining release or absorption into an the human or animal subject, promoting transport across the blood barrier or penetration in a particular organ (e.g. liver).

The appropriate dosage of the HBV-encoding vector and one or more immune checkpoint modulator(s) can be adapted as a function of various parameters such as the mode of administration; the age, health, and weight of the subject; the nature and extent of symptoms; kind of concurrent treatment; the frequency of treatment; etc. The optimal ratios of each component may be determined by techniques well known in the art. Further refinement of the calculations necessary to determine the appropriate dosage for treatment is routinely made by a practitioner, in the light of the relevant circumstances.

For general guidance, suitable dosage of the immune checkpoint modulator(s) varies from about 0.01mg/kg to about 50mg/kg, advantageously from about 0.1mg/kg to about 30mg/kg, desirably from about 0.5mg/kg to about 25mg/kg, preferably from about 1mg/kg to about 20mg/kg, more preferably from about 2mg/kg to about 15mg/kg, with a specific preference for doses from about 3mg/kg to about 10mg/kg. In some embodiments, two or more monoclonal antibodies with different binding specificities are administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated.

Suitable dosage for the HBV-encoding vector varies from approximately 10^5 to approximately 10^{13} vp (viral particles), iu (infectious unit) or pfu (plaque-forming units) depending on the quantitative technique used. As a general guidance, viral (e.g. adenovirus) doses from approximately 10^6 to approximately $5x10^{12}$ vp are suitable, preferably from approximately 10^7 vp to approximately 10^{12} vp, more preferably from approximately 10^8 vp to approximately $5x10^{11}$ vp; doses from approximately $5x10^8$vp to approximately 10^{11} vp being particularly preferred especially for human use. The quantity of virus present in a sample can be determined by routine titration techniques, e.g. by counting the number of plaques following infection of permissive cells, immunostaining (e.g. using anti-virus antibodies), by measuring the A260 absorbance (vp titers), or still by quantitative immunofluorescence (iu titers).

Administration

Any of the conventional administration routes are applicable in the context of the invention including parenteral, topical or mucosal routes, for each of the active agents comprised in the combination product of the invention. Parenteral routes are intended for administration as an injection or infusion and encompass systemic as well as local routes. Common parenteral injection types are intravenous (into a vein, such as the portal vein feeding liver), intravascular (into a blood vessel), intra-arterial (into an artery such as hepatic artery), intradermal (into the dermis), subcutaneous (under the skin), intramuscular (into muscle) and intraperitoneal (into the peritoneum) or still by scarification. Infusions typically are given by intravenous route. Mucosal administrations include without limitation oral/alimentary, intranasal, intratracheal, intrapulmonary, intravaginal or intra-rectal route. Topical administration can also be performed using transdermal means (e.g. patch and the like). Preferred routes of administration for the immune checkpoint modulator(s) include intravenous (e.g. intravenous injection or infusion), intramuscular, subcutaneous and

intraperitoneal. Preferred routes of administration for the HBV-encoding vector include intramuscular and subcutaneous but oral administration can also be envisaged in particular for a vector originating from Ad4 or Ad7 adenovirus.

Administrations may use conventional syringes and needles (e.g. Quadrafuse injection needles) or any compound or device available in the art capable of facilitating or improving delivery of the active agent(s) in the subject (e.g. electroporation for facilitating intramuscular administration). An alternative is the use of a needleless injection device to administer at least one of the active agent comprised in the combination product of the invention (e.g. Biojector TM device). Transdermal patches may also be envisaged.

In one embodiment, the HBV-encoding vector and the one or more immune checkpoint modulator(s) are administered sequentially, such as the vector being administered first and the immune checkpoint modulator(s) second, or vise-versa (immune checkpoint modulator(s) being administered first and the vector second). If more than one immune checkpoint modulator(s) is used (e.g. anti-PD-L1 and anti-TIM-3 antibodies), they may be administered simultaneously or sequentially, in which case the dosage of each antibody administered falls within the ranges indicated herein. Furthermore, if the combination therapy is administered sequentially, the order of the sequential administration can be reversed or kept in the same order at each time point of administration. Moreover, sequential administrations may be combined with concurrent administrations. It is also possible to proceed via sequential cycles of administrations that are repeated after a rest period. Intervals between each administration can be from one hour to one year (e.g. 24h, 48h, 72h, weekly, every two weeks, monthly or yearly). Intervals can also be irregular (e.g. following the measurement of monoclonal antibodies in the patient blood levels). The doses can vary for each administration within the range described above. Preferably, the time interval between each vector administration can vary from approximately 1 day to approximately 8 weeks, advantageously from approximately 2 days to approximately 6 weeks, preferably from approximately 3 days to approximately 4 weeks and even more preferably from approximately 1 week to approximately 3 weeks. In combination, the time interval between each administration of immune check point modulator(s) can vary from approximately 2 days to approximately 8 weeks, advantageously from approximately 1 week to approximately 6 weeks, preferably every 3 weeks.

In a preferred embodiment, the administrations of the HBV-encoding vector and the immune checkpoint modulator(s) are interspersed. A preferred therapeutic scheme involves

from 2 to 8 (e.g. 3) intramuscular or subcutaneous administrations of 10^8 or 10^9 vp of vector at approximately 1 week interval followed by or interspersed with 2 to 6 (e.g. 3 or 4) intravenous administrations of 3 to 10 mg/kg of anti-immune checkpoint antibody(ies)(s) every 2 or 3 weeks. The period of time between the first administration of the vector and the first administration of the immune check point modulator(s) may vary from approximately several minutes to several week(s).

The present invention also provides a composition comprising effective amounts of the vector encoding one or more HBV polypeptide(s) and of the one or more immune checkpoint modulator described herein.

The present invention also relates to a kit of parts comprising at least (i) said vector encoding one or more HBV polypeptide(s) and said one or more immune checkpoint modulator(s). In one embodiment, the kit includes at least the HBV-encoding vector described herein in one container (e.g., in a sterile glass or plastic vial), and one or more immune checkpoint modulator(s) described herein in another container (e.g., in a sterile glass or plastic vial). A preferred kit comprises a replication-defective adenovirus encoding one or more HBV polypeptide(s) (e.g. an adenoviral vector encoding the HBV fusion protein of SEQ ID NO: 6) and an immune checkpoint modulator(s) which specifically binds PD-L1 (e.g. an anti-PD-L1 antibody). Optionally, the kit can include a device for performing the administration of the active agents. The kit can also include a package insert including information concerning the compositions or individual component and dosage forms in the kit.

The combination product, composition and kit of parts of the invention may be employed in methods for treating a variety of diseases and pathologic conditions, especially those caused by or associated with an HBV infection. Accordingly, the combination product or composition of the invention is for use for treating or preventing HBV infections, HBV-associated diseases and pathologic conditions, according to the modalities described herein, and particularly chronic HBV infection.

The present invention also relates to a method of treatment comprising administering an HBV-encoding vector and one or more immune checkpoint modulator(s) in an amount sufficient to treat or prevent HBV infections (e.g. especially to treat a chronic HBV infection) in a subject in need thereof or alleviate one or more symptoms related to HBV-associated diseases and pathologic conditions, according to the modalities described herein. The present invention also relates to a method for decreasing the levels of HBsAg in the serum of a

subject diagnosed as having an HBV infection comprising administering an HBV-encoding vector and one or more immune checkpoint modulator(s) according to the modalities described herein. The present invention also relates to a method for decreasing HBV viral load in the serum of a subject diagnosed as having an HBV infection comprising administering an HBV-encoding vector and one or more immune checkpoint modulator(s) according to the modalities described herein. In a particular embodiment, the combination product, composition and method of the invention may be employed according to the modalities described herein to break HBV-specific immune tolerance usually encountered in HBV chronic subjects.

Typically, upon administration according to the modalities described herein, the combination product, composition or kit of parts of the invention provides a therapeutic benefit to the treated subject over the baseline status or over the expected status if not treated. The therapeutic benefit can be evidenced by any relevant clinical measurement typically used by physicians or other skilled healthcare staff, including, for instance, a decrease of the HBV viral load quantified in blood, plasma, or sera of a treated subject, and/or a decrease of the level of liver enzyme activity (e.g. alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST)), and/or a stabilized (not worsening) state of disease (e.g. stabilization or decrease of conditions typically associated with HBV infection such as liver inflammation/steatosis/fibrosis), and/or the reduction of the level of sero markers such as HBeAg or HBsAg (e.g. HBe or HBs seroconversion) and/or the appearance or the modification of the level of antibodies to any HBV antigen and/or an improved response of the treated subject to conventional therapies and/or a survival extension as compared to expected survival if not receiving the combination treatment.

In the context of the invention, the therapeutic benefit can be transient (for one or a couple of months after cessation of administration) or sustained (for several months or years). As the natural course of clinical status which may vary considerably from a subject to another, it is not required that the therapeutic benefit be observed in each subject treated but in a significant number of subjects (e.g. statistically significant differences between two groups can be determined by any statistical test known in the art, such as a Tukey parametric test, the Kruskal-Wallis test the U test according to Mann and Whitney, the Student's t-test, the Wilcoxon test, etc).

Such measurements can be performed before the administration of the described herein (baseline) and at various time points during treatment and after cessation of the

treatment. For general guidance, the viral load can be determined using a quantitative PCR assay or any other methodology accepted in the art (e.g. Roche Ampli Prep/Cobas taqman assay v2.0, Abbott real-time hepatitis B virus performance assay). In preferred embodiments, the administration of the combination product, composition or kit of parts of the invention results in a reduction of the viral load whether transient or sustained of at least one $\log_{10}$, preferably at least 1.5 $\log_{10}$ and more preferably at least 2 $\log_{10}$ as compared to the viral load measured at baseline or as compared to the control group (non-treated subjects). Such administration can result in at least transient return to normal ALT and/or AST values as compared to baseline or to the control group. The levels of liver enzyme activity can be evaluated routinely in medical laboratories and hospitals. Alternatively, the administration of the combination product, composition or kit of parts as described herein results in at least transient reduction of seromarker HBe and/or HBs of at least 30%, preferably at least 40% and more preferably at least 50% as compared to the seromarker level measured at baseline or as compared to the control group. The levels of HBV seromarker can be evaluated routinely in medical laboratories and hospitals and a large number of kits are available commercially (e.g. immunoassays developed by Abbott Laboratories, Organon Technika).

The infecting HBV can be from the same genotype, strain or isolate as any HBV from which originates the HBV polypeptides/peptides in use in the present invention (e.g. genotype D) or it can be from a different genotype (e.g. genotype B, C, A or E).

Preferably, the combination product, composition or kit of parts of the invention is used or administered for eliciting or stimulating and/or re-orienting an immune response in the treated subject. Accordingly, the present invention also encompasses a method for eliciting or stimulating and/or re-orienting an immune response against HBV upon administration of an HBV-encoding vector and one or more immune checkpoint modulator(s) in an amount sufficient according to the modalities described herein.

The elicited or stimulated immune response can be specific (i.e. directed to HBV epitopes/antigens) and/or non-specific (innate), humoral and/or cellular. In the context of the invention, the immune response is preferably a T cell response CD4+ or CD8+-mediated or both, directed to an HBV polypeptide/epitope.

In a preferred embodiment, the combination product, composition or kit of parts of the invention is used for increasing the number of intrahepatic lymphocytes in a patient treated with said combination product, composition or kit of parts, and especially with a combination product, composition or kit of parts comprising a recombinant adenovirus encoding one or more HBV antigens (e.g. core, pol polypeptides and/or HBs domains) and an anti-PD-L1

antibody. Preferably, the increase is of at least 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, or 2.5 fold or even more as compared to the administration of the recombinant adenovirus in the absence of immune checkpoint modulator.

In another preferred embodiment, the combination product, composition or kit of parts of the invention is used for increasing the number of intrahepatic HBV-specific CD8+ T cells in a patient treated with said combination product, composition or kit of parts, and especially with a combination product, composition or kit of parts comprising a recombinant adenovirus encoding one or more HBV antigens (e.g. core, pol polypeptides and/or HBs domains) and an anti-PD-L1 antibody. Preferably, the increase is of at least 1.5, 2.0, 2.5, 3, 3.5 or 4 fold or even more as compared to the administration of the recombinant adenovirus in the absence of immune checkpoint modulator and the induced CD8+ T cells are preferably specific of HBV polymerase epitope(s).

In still another preferred embodiment, the combination product, composition or kit of parts of the invention is used for increasing the number of intrahepatic HBV-specific CD4+ T cells, in particular functional CD4+ T cells producing IFNγ, in a patient treated with said combination product, composition or kit of parts, and especially with a combination product, composition or kit of parts comprising a recombinant adenovirus encoding one or more HBV antigens (e.g. core, pol polypeptides and/or HBs domains) and an anti-PD-L1 antibody. Preferably, the increase is of at least 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4 or 2.5 fold or even more as compared to the administration of the recombinant adenovirus in the absence of immune checkpoint modulator and the induced CD4+ T cells are preferably specific of HBV core epitope(s).

In still another preferred embodiment, the combination product, composition or kit of parts of the invention is used for reducing the proportion of intrahepatic classical CD4+ T cells which are CD25+ among the population of FOXP3+ CD4+ T cells in a patient treated with said combination product, composition or kit of parts, and especially with a combination product, composition or kit of parts comprising a recombinant adenovirus encoding one or more HBV antigens (e.g. core, pol polypeptides and/or HBs domains) and an anti-PD-L1 antibody. Preferably, the reduction of CD25+ cells among intrahepatic FOXP3+ CD4+T cells is of at least 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9 or 3 fold or even more as compared to the administration of the recombinant adenovirus in the absence of immune checkpoint modulator.

The ability of the combination product, composition or kit of parts of the invention to elicit an immune response can be evaluated either *in vitro* or *in vivo* using a variety of direct

or indirect assays which are standard in the art. For a general description of techniques available to evaluate the onset and activation of an immune response, see for example Coligan et al. (1992 and 1994, Current Protocols in Immunology ; ed J Wiley & Sons Inc, National Institute of Health or subsequent editions). The ability to stimulate a humoral response may be determined by antibody binding and/or competition in binding (see for example Harlow, 1989, Antibodies, Cold Spring Harbor Press). Evaluation of non-specific immunity can be performed by for example measurement of the NK/NKT-cells (e.g. representativity and level of activation), as well as, IFN-related cytokine and/or chemokine producing cascades, activation of TLRs and other markers of innate immunity (Scott-Algara et al., 2010 PLOS One 5(1), e8761; Zhou et al., 2006, Blood 107, 2461-2469; Chan, 2008, Eur. J. Immunol. 38, 2964-2968). Evaluation of cellular immunity can be performed for example by quantification of cytokine(s) produced by activated T cells including those derived from CD4+ and CD8+ T-cells using routine bioassays (e.g. characterization and/or quantification of T cells by ELISpot, by multiparameters flow cytometry or ICS, by cytokine profile analysis using multiplex technologies or ELISA), by determination of the proliferative capacity of T cells (e.g. T cell proliferation assays by [^{3}H] thymidine incorporation assay), by assaying cytotoxic capacity for antigen-specific T lymphocytes in a sensitized subject or by immunization of appropriate animal models.

If desired, the combination product, composition, kit of parts or method of the invention can be carried out in combination with one or more conventional therapeutic modalities (e.g. anti-viral therapy, radiation, and/or surgery).

It may be advisable to associate the combination product, composition, kit of parts or method of the invention with the one or more drugs which are available for treating or preventing HBV infections, HBV-associated diseases and pathologic conditions. Their administration may precede, be concomitant, or subsequent to the administration of the combination product of the invention. Representative examples of suitable drugs include without limitation polymerase inhibitors, RNase H inhibitors, nucleoside analogs, nucleotide analogs, IL-10 inhibitors, TLR agonists, IFN, N-glycosylation inhibitors, siRNA, antisense oligonucleotides, anti-HBV antibodies (e.g. anti-capsid antibodies), immune modulators, therapeutic vaccines and antitumor agents usually used in the treatment of HBV-associated liver cancers (e.g. adriamycin, adriamycin with lipiodol or sorasenib). Moreover, the combination product may also be used in association with other therapeutic vaccines such as synthetic peptides, recombinant antigens, VLPs, vectors encoding HBV proteins (Core,

preS1, PreS2, S and/or polymerase) which are particularly suited to trigger an anti-HBV humoral response.

A particularly suitable association in the context of the invention is with standard of care treatment routinely used to treat HBV infections and especially chronic ones. Representative examples of such standard of care treatment includes without limitation cytokines (e.g. IFNalpha, pegylated IFNa2a or 2b such as Pegasys (Roche), Pegintron (Schering Plough) or IntronA (Schering Plough)) and nucleotide or nucleoside analogs (NUCs) such as lamivudine, entecavir, telbivudine, adefovir, adefovir dipivoxil or tenofovir. The treatment with NUCs is only partially effective (infection resolution is observed in only 3-5% of subjects after 1 year of treatment) and needs long term therapy (may be life-long). It is expected that association with the combination product of the invention brings an immune dimension that would permit to complement NUC's action on viral replication, thus resulting in an improvement of such treatment (e.g. by decreasing doses of NUCs or length of NUC treatment required to achieve a therapeutic benefit) or an increase of the percentage of infection resolution (greater than 5%).

The combination product of the invention may also be used in association with adjuvant(s) to enhance immunity (especially a T cell-mediated immunity), e.g. through toll-like receptors (TLR) such as TLR-7, TLR-8 and TLR-9. For illustrative purposes, such adjuvants include, without limitation, alum, mineral oil emulsion such as, Freunds complete and incomplete (IFA), lipopolysaccharides (Ribi et al., 1986, Immunology and Immunopharmacology of Bacterial Endotoxins, Plenum Publ. Corp., NY, p407-419), saponins such as ISCOMATRIX, AbISCO, QS21 (Sumino et al., 1998, J.Virol. 72: 4931; WO98/56415), imidazo-quinoline compounds such as Imiquimod (Suader, 2000, J. Am Acad Dermatol. 43:S6), S-27609 (Smorlesi, 2005, Gene Ther. 12: 1324) and related compounds such as those described in WO2007/147529, cytosine phosphate guanosine oligodeoxynucleotides such as CpG and CpG ODN (Chu et al., 1997, J. Exp. Med. 186: 1623; Tritel et al., 2003, J. Immunol. 171: 2358); cationic peptides such as IC-31 (Kritsch et al., 2005, J. Chromatogr Anal. Technol. Biomed. Life Sci. 822: 263-70), polysaccharides such as Adjuvax and squalenes such as MF59.

All of the above cited disclosures of patents, publications and database entries are specifically incorporated herein by reference in their entirety to the same extent as if each such individual patent, publication or entry were specifically and individually indicated to be incorporated by reference.

Brief Description of the Drawings

Figure 1 illustrates the HbsAg expression in sera of HBV-tolerant mice immunized with AdTG18201 alone, anti-PD-L1 antibodies alone or both AdTG18201 and anti-PD-L1 antibodies or with a negative control antibody (isotype) at different time points, respectively (A) day 40, (B) day 47, (C) day 61 and (D) day 82. In each case, HbsAg levels are given as a percentage of pre-treatment level (D28).

Figure 2 illustrates the number of intrahepatic lymphocytes observed per liver (Figure 2A), the number of polymerase-specific CD8+ T cells detected in the liver with a tetramer specific of a class I epitope located in the HBV polymerase protein (VSA epitope) (Figure 2B) and the percentage of HBV or adenovirus specific CD4+ T cells producing IFNgamma in the liver (Figure 2C). All these parameters were monitored 12 days after the cell transfer, in naïve HBV transgenic mice (group 1), HBV transgenic mice receiving an adoptive transfer of cells from C57BL/6J mice injected with AdT1G8201 (group 2), HBV transgenic mice receiving the same cell transfer and being treated with anti-PD-L1 antibody (group 3). Each symbol represents an individual mouse, means of each groups are represented with a thick black bar. When needed the threshold of positivity (or cut-off) is represented by a horizontal dotted line. Statistical analyses were performed using non parametric tests, a Kruskal-Wallis test followed by a Mann-Whitney test if p value of Kruskal-Wallis test was below 0.05. Result is considered as significant when the p value is below 0.05. Only significant p values are indicated.

Figure 3 illustrates the percentage of HBV or adenovirus specific CD4+ T cells producing IFNg in the liver (Figure 3A), the percentage of FOXP3+ cells among CD4+ T cells in the liver (Figure 3B) and the percentage of CD25+ cells among FOXP3+ CD4+ T cells in the liver (Figure 3C). All these parameters were monitored 12 days after the cell transfer, in C57BL/6J mice receiving an adoptive transfer of cells from C57BL/6J mice injected with AdTG18201 (group 1), HBV transgenic mice receiving an adoptive transfer of cells from C57BL/6J mice injected with AdTG18201 (group 2), HBV transgenic mice receiving the same cell transfer and being treated with anti-PD-L1 antibody (group 3), HBV transgenic mice receiving an adoptive transfer of cells from C57BL/6J mice injected with AdTG15149 (empty adenovirus) and being treated with anti-PD-L1 antibody (group 4), HBV transgenic mice receiving an adoptive transfer of cells from naïve C57BL/6J mice and being treated with anti-PD-L1 antibody (group 5). Each symbol represents an individual mouse, means of each groups are represented with a thick black bar. When needed the threshold of

positivity (or cut-off) is represented by a horizontal dotted line. Statistical analyses were performed using non parametric tests, a Kruskal-Wallis test followed by a Mann-Whitney test if p value of Kruskal-Wallis test was below 0.05. Result is considered as significant when the p value is below 0.05. Only significant p values are indicated.

EXAMPLES

Among immune mechanisms impaired in chronically infected patients, immunologists have described that patients' T cells can over-express inhibitor immune checkpoint receptors. We set out to combine immune checkpoint blocking approaches with a HBV-encoding adenovirus with the goal of improving the host immune response and thus the antiviral capacity of the HBV-encoding adenovirus. The beneficial effect of the combined use of such adenovirus and antibodies was assessed in an HBV-tolerant mouse model based on a recombinant adenovirus-associated virus (AAV) encoding an over length genome of HBV that can induce in mice the chronic production of HBV DNA, HBsAg, HBeAg and infectious HBV particles (Dion et al., 2013, J Virol. 87(10): 5554-63).

The adenovirus chosen for these studies (AdTG18201) is based on a non-replicative Adenovirus serotype 5 and encoding a unique large fusion protein composed of a truncated HBV Core, a enzymatically inactive HBV Polymerase and 2 envelop domains (amino acid sequence set out in SEQ ID NO: 6). Early preclinical data in naïve mice have shown that this vaccine is capable to induce robust, multi-specific and long-lasting HBV-specific T cells. These cells target all three encoded immunogens and display capacity to produce both IFNγ and TNFα as well as cytolytic functions. This adenovirus by itself has in addition been shown in the AAV-HBV to exert antiviral activities such as impact on both viral DNA and HBsAg levels (Martin et al., 2013, J. Hepatol. 58: S55-S56).

It was first chosen to target the ligand PD-L1 of the immune checkpoint blocker PD-1 with an appropriate antibody. The rat antibody 10F.9G2 (BioXcell) directed to mouse PD-L1 was chosen for this study.

Combination of AdTG18201 with anti-PDL-1 Mab: AAV-HBV mouse model

The combination of mPDL-1 inhibitor (BioXCell; commercial clone 10F.9G2) with AdTG18201 was tested *in vivo* in the HBV AAV mouse model. HLA-A2/HLA-DR1 transgenic mice (Pajot et al., 2004, Eur. J. Immunol. 34: 3060-9) were intravenously injected

with AAV-HBV (5.10^{10} viral genome (vg) / mouse) at day 0 of the experiment to establish a chronic carrier stage (HBsAg, HBeAg, viral DNA can be detected in the sera of AAV-HBV injected mice). The mice are then subcutaneously (sc) immunized with either $1.4\text{-}1.6x10^{9}$ vp of AdTG18201 (corresponding to 10^{8} iu) (6 weekly sc administrations of at days 33, 40, 47, 54, 61, and 68) in combination with 4 intraperitoneal (ip) administrations every 3.5 days of 200μg of either anti-PDL-1 antibody (BioXCell commercial clone 10F.9G2) or the isotype rat IgG2 control antibody (BioXCell clone LTF-2) at days 33, 36, 40 and 43.

In other terms 4 groups of 6 mice were tested; a first group treated with AdTG18201 receiving 6 sc injections of AdTG18201 (group 1); a second group treated with both AdTG18201 and anti-PDL-1 antibody receiving 6 sc injections of AdTG18201 and 4 ip injections of anti-PDL-1 antibody (group 2); a third group treated with the anti-PDL-1 antibody receiving 4 ip injections of anti-PDL-1 antibody (group 3) and a control group receiving 4 ip injections of isotype antibody (group 4). Blood samples were taken before treatment (at days 0 before AAV HBV administrations, at day 28 before Ad and/or anti-PDL-1 treatment) and post treatment for about 3 months (at days 40, 47, 61, 82, 103 and 124).

HBV infection was monitored by the detection in sera of treated mice of hepatitis B surface antigen (HBsAg) which is one of the first detectable antigen appearing upon infection with HBV.

Figure 1 illustrates HBsAg percentage at different time points of the experiment, respectively at day 40 (concomitant with the third antibody injection and the second virus injection), at day 47 (after the antibody treatment and at the 3th virus injection), at day 61 (after the end of antibody treatment and at the 5th virus injection) and at day 82 (after completion of virus and antibody treatment). At day 40, HBsAg levels are approximately the same in all groups. At day 47, HBsAg peaked in the control group and the anti-PDL-1-treated group, respectively at approximately 200 and 130% of the level before any treatment (day 28) whereas HBsAg levels remain stable in animal groups treated with AdTG18201 alone or in combination with anti-PDL-1 antibodies. At day 61, HBsAg levels return to the pretreatment levels in control and anti-PDL-1-treated groups whereas HBsAg is below the pretreatment level (respectively 80 and 55%) in the AdTG18201 and AdTG18201+anti-PDL-1-treated groups. At day 82 after completion of the treatment, HBsAg levels continue to decrease in all groups but the decrease is more pronounced in the doubly treated group 2 reaching approximately 40% of the pretreatment concentrations.

To summary, HBsAg percentage increased very rapidly in control group 4 to peak at day 47 to reach a double level as that before treatment and then return at the pretreatment

level at day 61 and then slightly decreased over time. Although less dramatic, HbsAg levels increased and peaked at day 47 in the sera of mice only treated with the anti-PDL-1 antibody (group 3) after what the levels decreased regularly overtime. In marked contrast, no HBsAg peak was observed in the animal groups treated with AdTG18201 alone (group 1) or in combination with anti-PDL-1 antibodies (group 2). In these two groups, HbsAg decreased regularly over time and the decreased is more pronounced in the groups having received both AdTG18201 and anti-PDL-1 antibodies especially at day 61.

During the natural course of infection, the hepatitis B surface antigen (HBsAg) is the first detectable viral antigen to appear during HBV infection and HbsAg clearance is indicative of evolution towards HBV infection resolution and . This experiment highlights the benefit of treating subjects with a combination of a therapeutic Ad-based vaccine and immune checkpoint inhibitors. Anti-PD-L1 antibody can sustain over time the antiviral effect of AdTG18201 measured here for HBsAg.

Combination of AdTG18201 with anti-PDL-1 Mab: adoptive transfer of cells in Tg1.4 HBV –S-Mut-1 transgenic mouse model

Combination of the AdTG18201 with an anti-PD-L1 antibody was further investigated in a context of adoptive transfer of cells, primed by AdTG18201 in C57BL/6J mice, in HBV transgenic mice treated with an anti-PD-L1 antibody._Mice used in these studies were C57BL6/J female mice purchased from Charles River Laboratories (L'Arbresle, France) and Tg1.4 HBV-S-Mut-1 transgenic mice, also referred to as HBV transgenic mice, produced as described by Halverscheid et al. (2008, J. Med. Virol. 80(4): 583-90) and bred at Charles River Laboratories.

Antibodies used to treat HBV transgenic mice were the followings: the Rat IgG2b anti-mPDL1 clone 10F.9G2 purchased from BioXCell (batch#5039/1113B; 4.12mg/mL) and an isotype control antibody being the rat IgG2b clone LTF-2 purchased at bioXCell (batch#4689-2/1013; 4.68mg/mL).

C57BL6/J mice were either injected by AdTG18201 or by AdTG15149 being the counterpart of AdTG18201 without any foreign antigen encoded and used as negative control.

Two separate experiments were performed and are described below.

The first experiment was composed of 3 groups of HBV transgenic mice either not treated (group 1, 3 mice), either receiving the adoptive transfer of cells from AdTG18201 injected C57BL/6J mice (group 2, 7 mice) or treated with anti-PD-L1 antibody and transferred with cells from AdTG18201 injected C57BL/6J mice (group 3, 7 mice). In order

to obtain cells for adoptive transfer in HBV transgenic mice, C57BL/6 mice received one subcutaneous injection of 2 x 10^9 vp of AdTG18201 at the base of the tail. Fourteen days after immunization, mice were sacrificed and splenocytes were isolated to be transferred into HBV transgenic mice. HBV transgenic mice of group 3 were treated once with anti-PD-L1 antibody the day before the adoptive transfer and then treated with the same antibody every 3 days (total of 4 injections of anti-PD-L1 antibody). This antibody was administered through intraperitoneal route (200µg/injection). The adoptive transfer of cells was performed by intravenous injection of spleen cells of AdTG18201 vaccinated C57BL/6J mice in HBV transgenic mice either treated or not by the anti-PD-L1 antibody (80.10^6 spleen cells transferred in each HBV transgenic mouse). Then 12 days after transfer, mice were sacrificed and intrahepatic lymphocytes were analysed by flow cytometry, in particular to assess the number of total intrahepatic lymphocytes, the number of HBV polymerase specific cells (cells specific of the HBV epitope VSAAFYHLPL detected using a VSA-H2Kb dextramer stained with PE, Immudex, JD3639PE), the frequency of CD4+ T cells specific of HBV Core, Polymerase or adenovirus and being functional ie able to produce IFN-gamma.

As shown in Figure 2A, a mean number of 4.78 x 10^6 of intrahepatic lymphocytes (IHL) per liver was found in group 1 mice (not treated HBV transgenic mice) whereas an increase by a factor of approximately 2 (mean number of 8.58 x 10^6 IHL per liver) was observed in the liver of HBV transgenic mice transferred with cells of AdTG18201-injected C57BL/6J mice (group 2). Treatment of the same mice with an anti-pDL-L1 antibody (group 3 corresponding to HBV transgenic mice transferred with cells of C57BL/6J mice injected with AdT1G8201 and treated with anti-PD-L1 antibody) allowed an additional 2-foldincrease in the mean number of IHL as compared to group 2 mice (mean number of 19.97 x 10^6 IHL per liver), the latest difference between groups 2 and 3 being statistically significant.

In addition, as shown in Figure 2B, a clearly detectable number of polymerase-specific CD8+ T cells was measured in HBV transgenic mice transferred with cells of C57BL/6J mice injected with AdTG18201 (groups 2 and 3) as compared to non-treated mice (group 1). Although the same number of cells of C57BL/6J mice injected with AdTG18201 was transferred in mice of groups 2 and 3, a significantly higher number of polymerase-specific CD8+ T cells was detected in the liver of mice of group 3, which were, in addition to transfer, treated with the anti-PD-L1 antibody. This result suggests that the combination of anti-PD-L1 antibody with AdTG18201 is favourable to obtain a higher number of HBV-polymerase specific CD8+ T cells in the liver in such a HBV model.

Finally, as illustrated in Figure 2C, an increase in the percentage of IFNgamma producing CD4+ T cells specific of HBV Core was found in HBV transgenic mice transferred with cells from C57BL/6 mice injected with AdTG18201 (groups 2 and 3) as compared to non-transferred mice (group 1) with the highest percentage obtained in anti-PD-L1 treated mice (group 3). Of note the observation is limited by the fact that only 2 mice could be analysed in group 2.

A second experiment was conducted in HBV transgenic mice. Animals were divided in five groups, group 1 being a control group composed of C57BL/6J mice transferred with spleen cells of AdTG18201 injected C57BL/6 J mice (7 mice) and four groups of HBV transgenic mice, respectively a second group being treated with the isotype control antibody and transferred with spleen cells of AdTG18201 injected C57BL/6J mice (group 2, 7 mice), the third group treated with anti-PD-L1 antibody and transferred with spleen cells of AdTG18201-injected C57BL/6J mice (group 3, 5 mice), the fourth group treated with anti-PD-L1 antibody and transferred with spleen cells of AdTG15149 (empty Ad)-injected C57BL/6J mice (group 4, 5 mice), and the fifth group treated with anti-PD-L1 antibody and transferred with spleen cells from naïve C57BL/6J mice (group 5, 5 mice).

In order to obtain cells for the adoptive transfer, C57BL/6J mice were injected either with 2 x 10^9 vp of AdTG18201 or 2 x 10^9 vp of AdTG15149 at the base of the tail or left naïve and 14 days after immunization, mice were sacrificed and splenocytes were isolated. Mice treated by an antibody (isotype control or anti-PD-L1) were injected intraperitonealy 4 times, at 3 days interval (200µg/injection), the first time being the day before adoptive transfer. Mice receiving an adoptive transfer were injected intravenously with 80.10^6 spleen cells collected from AdTG18201-injected C57BL/6 mice (groups 1 to 3) or with 80.10^6 spleen cells collected from AdTG15149-injected C57BL/6J mice (group 4) or 80.10^6 spleen cells of naïve C57BL/6J mice (group 5).

Then 12 days after transfer, mice were sacrificed and intrahepatic lymphocytes were analysed by flow cytometry, in particular the frequency of CD4+ T cells specific of HBV Core, Polymerase or adenovirus and being functional ie able to produce IFN-gamma. The population of regulatory CD4+ T cells (characterized by markers CD25 and/or FoxP3) was also characterized in the liver of mice of the various groups.

As shown in Figure 3A, IFNg producing CD4+ T cells specific of HBV Core were detected in the liver of HBV transgenic mice transferred with cells from C57BL/6 mice injected with AdT1G8201 (groups 2 and 3). However the percentage of functional CD4+ T

cells was much higher in animals treated with the anti-PD-L1 antibody (group 3) than in animals treated with the isotype control antibody (group 2). This result confirms the role of anti-PD-L1 in the increase of functional specific CD4+ T cells induced by the AdTG18201. In addition the detection of IFNg producing CD4+ T cells is specific of the HBV transgenic mice as CD4+ T cells were not detected in the liver when cells are transferred in HBV-free C57BL/6 mice (group 1). The response is also specific of AdTG18201 as no IFNg-producing CD4+ T cells could be observed in HBV transgenic mice transferred with cells of naïve C57BL6/J mice (group 5) or cells from AdTG15149-injected C57BL/6J mice (group 4).

The observed effect is then specific of the combination of anti-PD-L1 antibody and immune cells induced by AdTG18201 in a liver environment expressing HBV antigens.

The population of CD4+ regulatory T cells was also monitored in the various groups. As shown in Figure 3B, the transfer of cells from C57BL/6J mice injected with AdTG18201 in HBV transgenic mice specifically induce FoxP3+ CD4+ T cells detected in the liver, the HBV transgenic mice being treated or not by anti-PD-L1 antibody (Figure 3B). But surprisingly, as illustrated in Figure 3C, the proportion of FOXP3+ CD4+ T cells displaying the CD25 marker is significantly reduced in mice transferred with cells from C57BL/6J mice injected with AdTG18201 and treated with the anti-PD-L1 antibody (group 3) compared with HBV transgenic mice receiving the same transfer and treated by the isotype control antibody (group 2,) or with HBV transgenic mice being treated with the anti-PD-L1 antibody and being transferred by either cells from C57BL/6 mice injected with AdTG15149 (group 4) or with cells from naïve C57BL/6J mice (group 5).

These results suggest that the combination of AdTG18201 with an anti-PD-L1 antibody could reduce the proportion of classical CD25+FOXP3+ CD4+ T cell population among FOXP3+ CD4+ T cell population (probably by shifting a part of CD25+ FOXP3+ CD4+ T cells to CD25- FOXP3+ CD4+ T cells). With this latest observation, it could be hypothesized that the combination of anti-PD-L1 antibody with AdTG18201 by acting on regulatory T cell population could be favourable for induction of an active and functional immune response.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific method and reagents described herein, including alternatives, variants, additions, deletions, modifications and substitutions. Such equivalents are considered to be within the scope of this invention and are covered by the following claims.

Claims

1. A combination product comprising at least (i) a vector encoding one or more polypeptide(s) of a hepatitis B virus (HBV) and (ii) one or more immune checkpoint modulator(s).

2. The combination product of claim 1, wherein said vector is a plasmid or a viral vector.

3. The combination product of claim 2, wherein said vector is an adenoviral vector, and preferably, said adenoviral vector originates from a human adenovirus selected from the group consisting of Ad2, Ad3, Ad4, Ad5, Ad7, Ad11, Ad24, Ad26, Ad34, Ad35, Ad48, Ad49 and Ad50 or from a chimpanzee adenovirus.

4. The combination product of claim 3, wherein said adenoviral vector is a replication defective human adenovirus of serotype 5 (Ad5) defective for E1 and E3 functions.

5. The combination product of anyone of claims 1 to 4, wherein said one or more HBV polypeptide(s) is selected from the group consisting of HBV polymerase polypeptide, HBc (core) polypeptide and one or more HBs (env) domains.

6. The combination product of claim 5, wherein said vector encodes HBV polymerase, HBc (core) and HBs (env) polypeptides and preferably originating from a genotype D HBV virus.

7. The combination product of claim 5 or 6, wherein said core antigen comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 1 or SEQ ID NO: 2.

8. The combination product of anyone of claims 5 to 7, wherein said polymerase polypeptide is defective for the polymerase enzymatic activity and/or for the RNaseH activity exhibited by the native counterpart, preferably said polymerase polypeptide comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 3.

9. The combination product of anyone of claims 5 to 8, wherein said one or more envelop domain(s) comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 4 or SEQ ID NO: 5.

10. The combination product of anyone of claims 1 to 9, wherein said HBV polypeptides are in the form of a fusion polypeptide, and preferably a fusion protein comprising from the N to the C-terminus (i) the core polypeptide; (ii) the polymerase polypeptide or fragment thereof, and (iii) one or more HBsAg domain(s).

11. The combination product of claim 10, wherein said fusion protein comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 6.

12. The combination product of claim 11, wherein said vector is a replication-defective adenovirus comprising inserted in place of the E1 region a nucleic acid molecule placed under the control of a promoter such as the CMV promoter, and encoding a fusion protein comprising an amino acid sequence as shown in SEQ ID NO: 6.

13. The combination product of anyone of claims 1 to 12, wherein said one or more immune checkpoint modulator(s) antagonizes the activity of inhibitory immune checkpoint(s), in particular PD-1, PD-L1, PD-L2, LAG3, Tim3, BTLA, SLAM, 2B4, CD160, KLRG-1 and CTLA4.

14. The combination product of claim 13, wherein said one or more immune checkpoint modulator(s) comprises an antibody, preferably a monoclonal antibody, in particular a human or a humanized antibody.

15. The combination product of claim 14, wherein said one or more immune checkpoint modulator(s) comprises an antibody that specifically binds to human PD-1 or an antibody that specifically binds to human PD-L1.

16. The combination product of anyone of claims 1 to 15, wherein said vector encoding one or more HBV polypeptide(s) and said one or more immune check point modulator(s) are mixed in the same composition or are comprised in different compositions that can be administered concomitantly, sequentially or interspersed.

17. The combination product of anyone of claims 13 to 16, comprising:
    - from about 1mg/kg to about 20mg/kg, preferably from about 2mg/kg to about 15mg/kg, and more preferably from about 3mg/kg to about 10mg/kg of said one or more immune checkpoint modulator(s), and/or
    - from approximately 10^7 vp to approximately 10^{12} vp, preferably from approximately 10^8 vp to approximately $5x10^{11}$ vp; and more preferably from approximately $5x10^8$vp to approximately 10^{11} vp of said vector encoding one or more HBV polypeptide(s).

18. The combination product of anyone of claims 16 to 17, which is formulated for parenteral injection in a subject.

19. A composition comprising effective amounts of said vector encoding one or more HBV polypeptide(s) and of said one or more immune checkpoint modulator of anyone of claims 1 to 18.

20. A kit of parts comprising at least (i) said vector encoding one or more HBV polypeptide(s) as defined in anyone of claims 2 to 12 and 17 to 18 in a container and one or more immune checkpoint modulator(s) as defined in anyone of claims 13 to 15 and 17 to 18 in another container.

21. The combination product of anyone of claims 1 to 18, the composition of claim 19, or the kit of parts of claim 20 for use for:
    - treating or preventing an HBV infection, an HBV-associated disease or pathologic condition, and particularly chronic HBV infection;
    - eliciting or stimulating and/or re-orienting an immune response in a subject in need thereof, especially a chronically HBV infected subject;
    - controlling sera HBs levels in a subject in need thereof, especially a chronically HBV infected subject;
    - controlling viral clearance in a subject in need thereof, especially a chronically HBV infected subject;
    - increasing the number of intrahepatic lymphocytes in a subject in need thereof, especially a chronically HBV infected subject;

- increasing the number of intrahepatic HBV-specific CD8+ T cells in a subject in need thereof, especially a chronically HBV infected subject;
- increasing the number of intrahepatic HBV-specific CD4+ T cells producing IFNγ in a subject in need thereof, especially a chronically HBV infected subject; or
- reducing the proportion of intrahepatic classical CD4+ T cells which are CD25+ among the population of FOXP3+ CD4+ T cells in a subject in need thereof, especially a chronically HBV infected subject.

22. The combination product of anyone of claims 1 to 18, the composition of claim 19, or the kit of parts of claim 20, for use according to claim 21, wherein said immune checkpoint modulator(s) is administered by intravenous, intramuscular, subcutaneous or intraperitoneal route and wherein said vector encoding one or more HBV polypeptide(s) is administered by intramuscular or subcutaneous route.

23. The combination product of anyone of claims 1 to 18, the composition of claim 19, or the kit of parts of claim 20, for use according to claim 21, wherein the administration(s) of said vector encoding one or more HBV polypeptide(s) and the administration(s) of said one or more immune checkpoint modulator(s) are interspersed.

24. The combination product of anyone of claims 1 to 18, the composition of claim 19, or the kit of parts of claim 20, for use according to claim 21, wherein said use comprises from 2 to 8 intramuscular or subcutaneous administrations of 10^8 or 10^9 vp of vector encoding one or more HBV polypeptide(s) at approximately 1 week interval interspersed with 2 to 6 intravenous administrations of 3 to 10 mg/kg of one or more anti-immune checkpoint antibody(ies) every 2 or 3 weeks.

Figure 1

Figure 1A

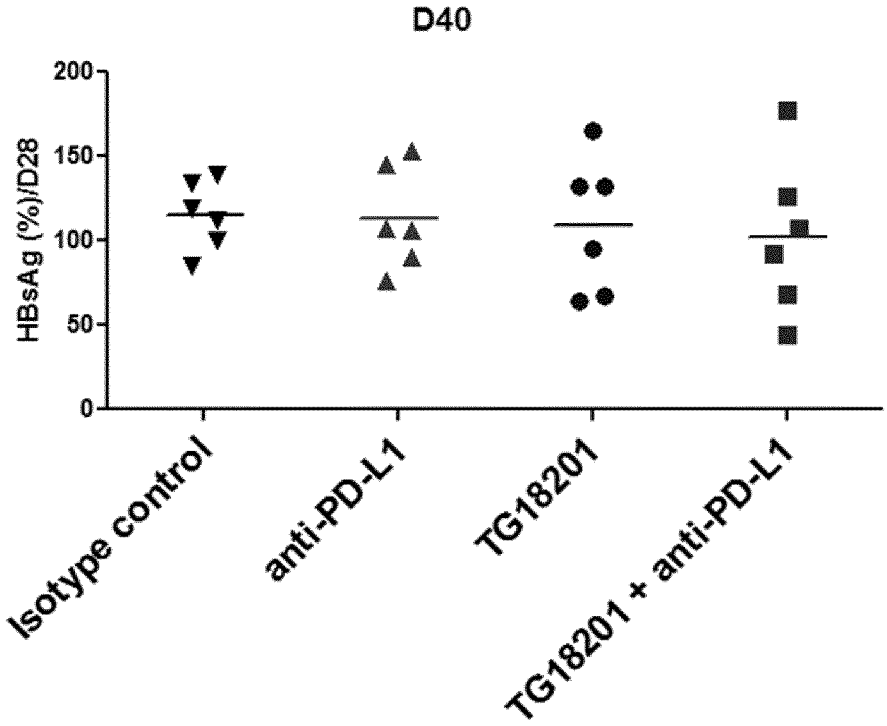


Figure 1B

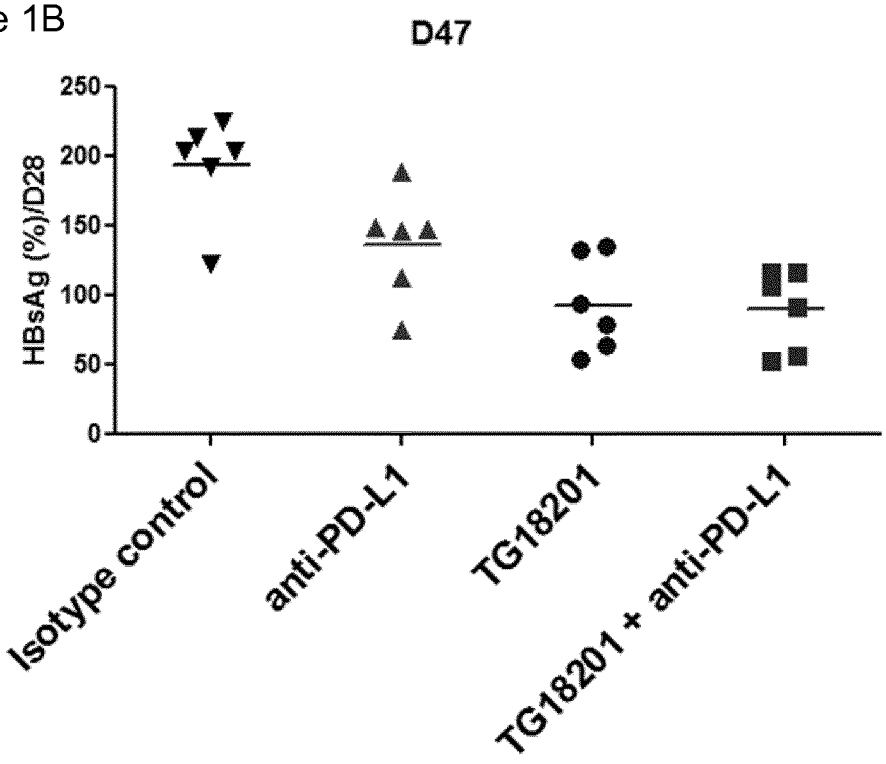


Figure 1

Figure 1C

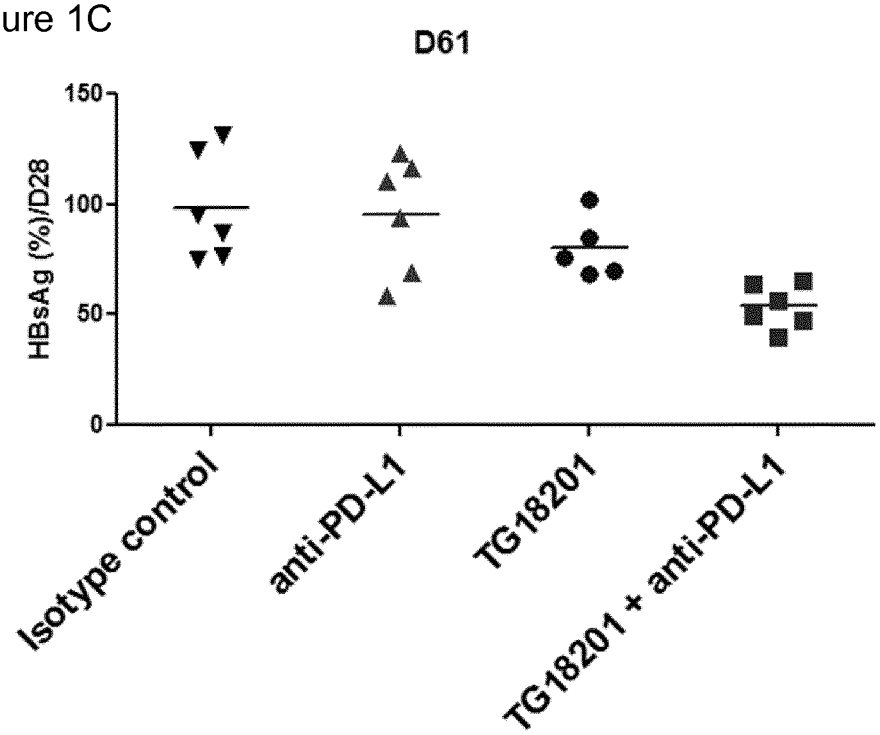


Figure 1D

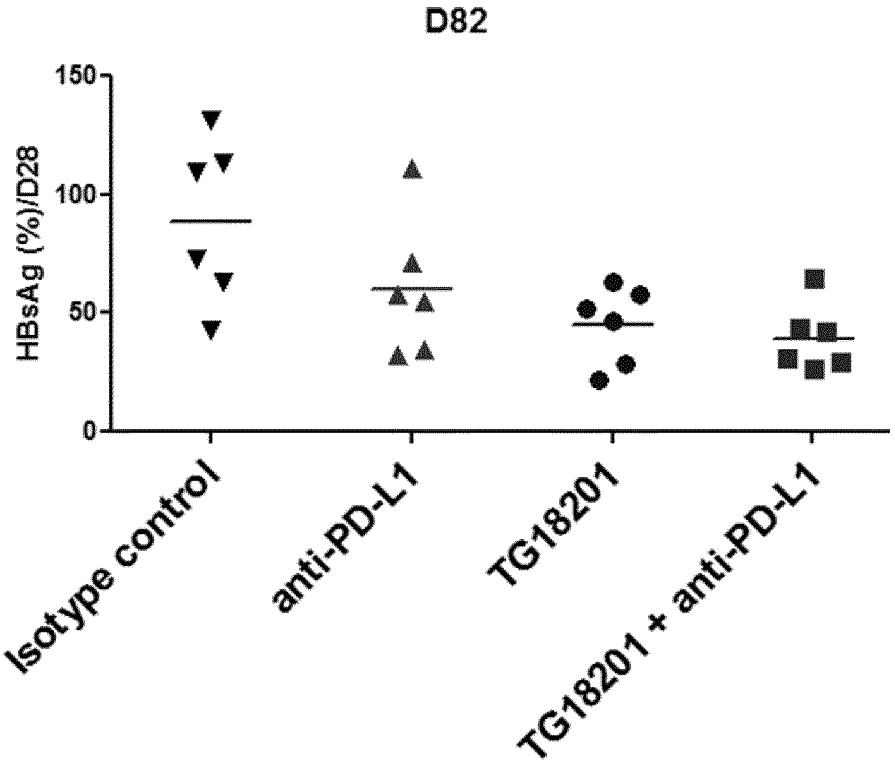


Figure 2

Figure 2A

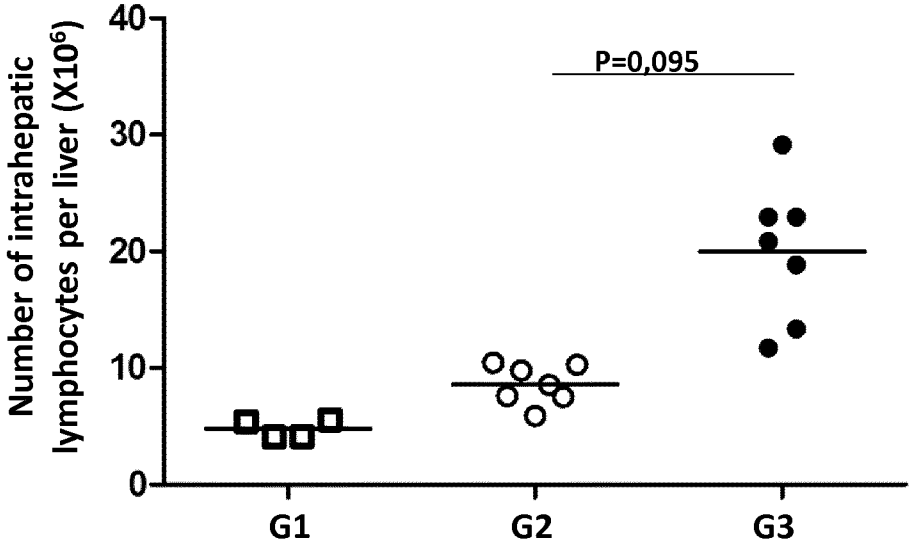
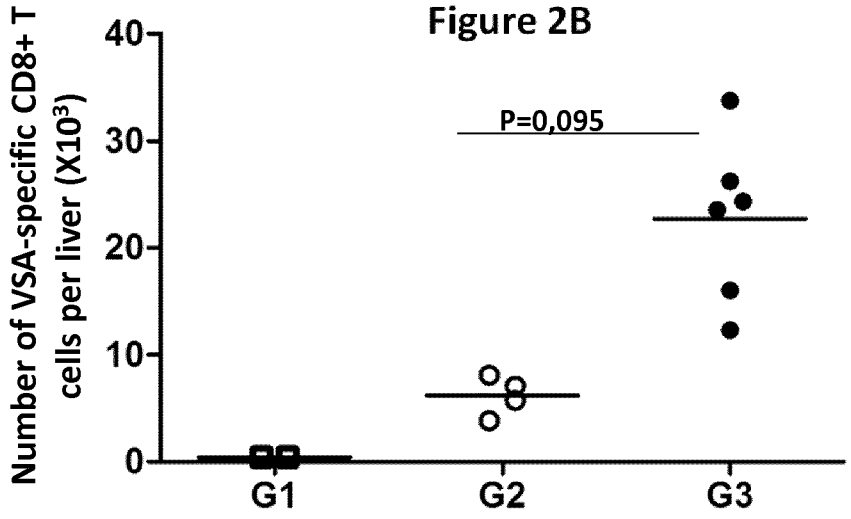
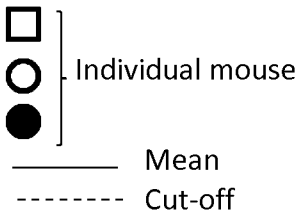


Figure 2B

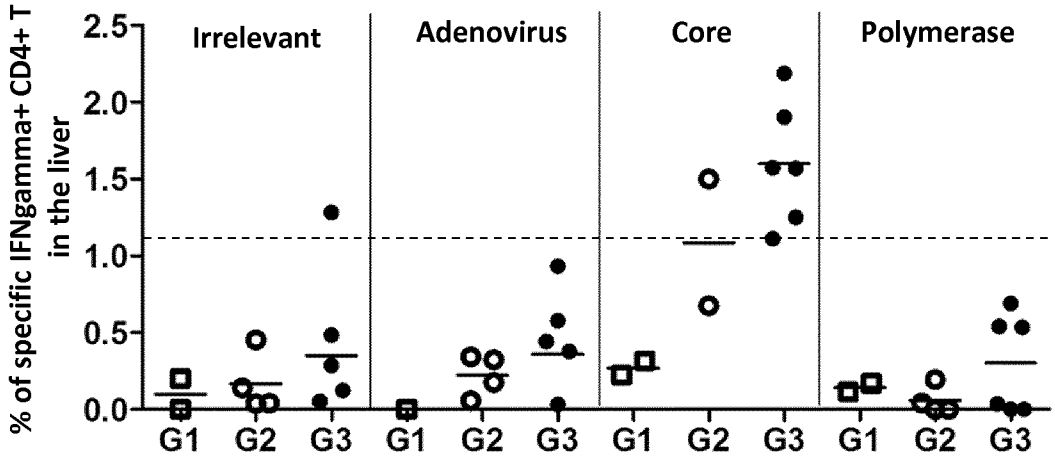


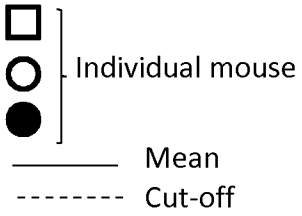


G1 : HBV tg mice not treated
G2 : HBV tg mice transferred with cells of C57BL/6J mice injected with AdTG18201
G3 : HBV tg mice transferred with cells of C57BL/6J mice injected with AdTG18201 and treated by anti-PD-L1 antibody

Figure 2

Figure 2C

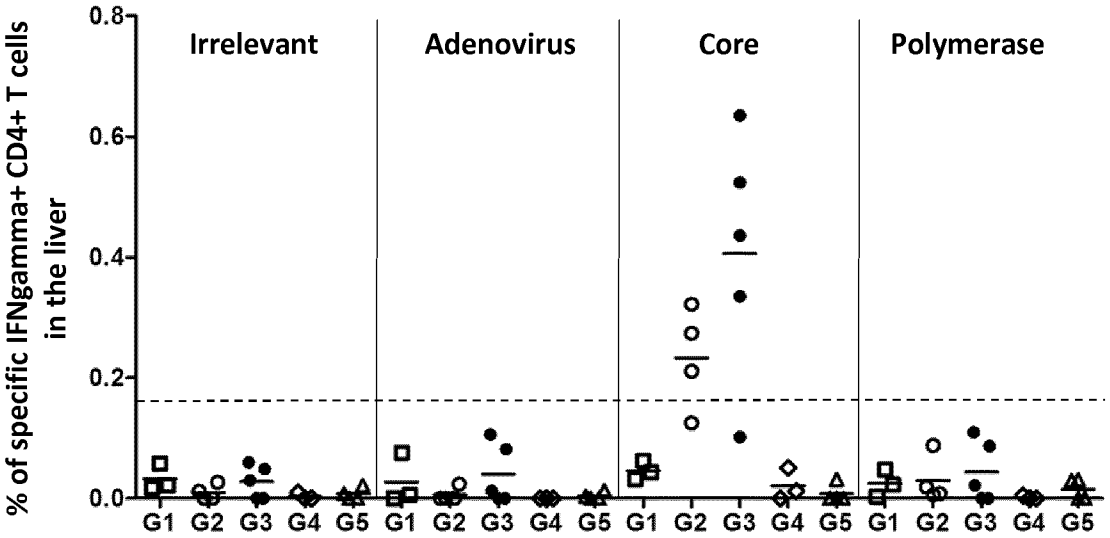




G1 : HBV tg mice not treated
G2 : HBV tg mice transferred with cells of C57BL/6J mice injected with AdTG18201
G3 : HBV tg mice transferred with cells of C57BL/6J mice injected with AdTG18201 and treated by anti-PD-L1 antibody

Figure 3

Figure 3A



Individual mouse

Mean

Cut-off

G1 : C57BL/6J mice transferred with cells of C57BL/6J mice injected with AdTG18201

G2 : HBV tg mice transferred with cells of C57BL/6J mice injected with AdTG18201 and treated with an isotype control antibody

G3 : HBV tg mice transferred with cells of C57BL/6J mice injected with AdTG18201 and treated by anti-PD-L1 antibody

G4 : HBV transgenic mice transferred with cells of C57BL/6J mice injected with AdTG1549 (control) and treated with anti-PD-L1 antibody

G5 : HBV transgenic mice transferred with cells of naive C57BL/6J mice and treated with anti-PD-L1 antibody

Figure 3

Figure 3B

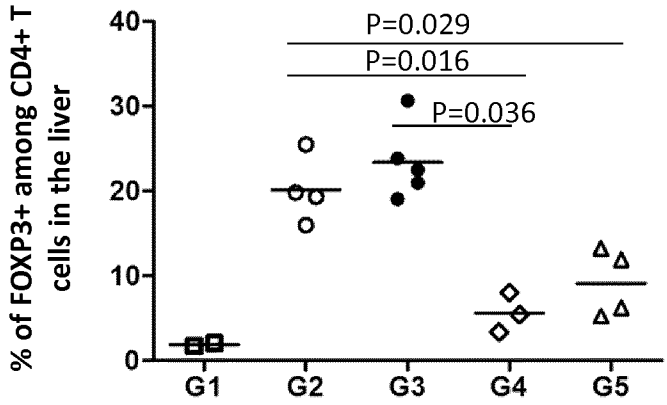
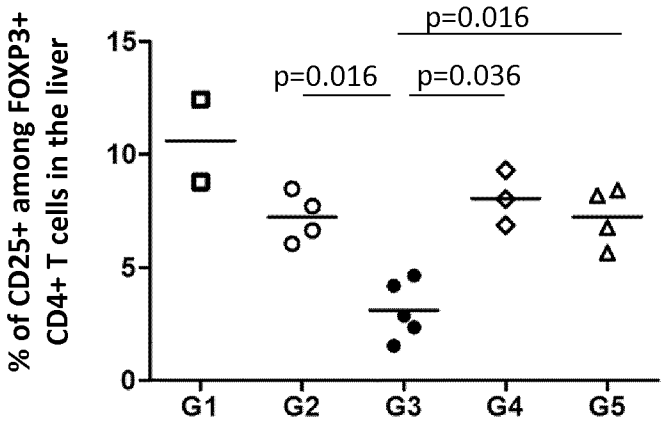


Figure 3C



Individual mouse

Mean

Cut-off

G1 : C57BL/6J mice transferred with cells of mice injected with AdTG18201

G2 : HBV tg mice transferred with cells of mice injected with AdTG18201 and treated with an isotype control antibody

G3 : HBV tg mice transferred with cells of mice injected with AdTG18201 and treated by anti-PD-L1 antibody

G4 : HBV transgenic mice transferred with cells of mice injected with AdTG1549 (control) and treated with anti-PD-L1 antibody

G5 : HBV transgenic mice transferred with cells of naive C57BL/6J mice and treated with anti-PD-L1 antibody

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2015/068282

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/068282

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/29 A61K39/39 C07K16/28 C12N15/86 A61K39/00
A61K39/12
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 2008/011344 A2 (CHILDRENS HOSPITAL INC [US]; WALKER CHRISTOPHER [US] NATIONWIDE CHILDR) 24 January 2008 (2008-01-24) page 27, line 22 - line 25; claims 1-6; example 3 page 12, line 20 - line 27 page 24, line 12 - line 22 -----	1,2, 13-16, 18-23 3-12,17, 24
Y	WO 2011/015656 A2 (TRANSGENE SA [FR]; MARTIN PERRINE [FR]; INCHAUSPE GENEVIEVE [FR]; SILV) 10 February 2011 (2011-02-10) cited in the application page 17 - page 31; claims 1-40; sequences 2,10,12,13 ----- -/--	3-12,17, 24

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 October 2015	09/10/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Deleu, Laurent

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	FISICARO P ET AL: "Antiviral Intrahepatic T-Cell Responses Can Be Restored by Blocking Programmed Death-1 Pathway in Chronic Hepatitis B", GASTROENTEROLOGY, ELSEVIER, PHILADELPHIA, PA, vol. 138, no. 2, February 2010 (2010-02), pages 682-693.e4, XP026876444, ISSN: 0016-5085 [retrieved on 2009-09-30] page 688 - page 692	1-24

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/068282

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	SARAH KUTSCHER ET AL: "Design of therapeutic vaccines: hepatitis B as an example", MICROBIAL BIOTECHNOLOGY, vol. 5, no. 2, 29 September 2011 (2011-09-29), pages 270-282, XP055217521, ISSN: 1751-7915, DOI: 10.1111/j.1751-7915.2011.00303.x page 273 - page 274	1-24

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2015/068282

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