Individualized therapeutic anticancer vaccine

Field of invention

The present invention relates to an individualized therapeutic anticancer vaccine, methods of treatment of cancer wherein such an anticancer vaccine is used as well as methods for producing the vaccine.

Background of invention

Although treatment of cancer has been improved over the past few decades in particularly due to early detection and diagnosis, which has significantly increased the survival, only about 60% of patients diagnosed with cancer are alive 5 years after the diagnosis. Most of the cancer treatments in use are surgical procedures, radiation and cytotoxic chemotherapeutics, however they all have serious side effects. Within the last few years cancer immune therapies targeting cancer cells with the help of the patient's own immune system, i.e. anticancer vaccines, have attracted interest because such therapies may reduce or even eliminate some of the side effects seen in traditional cancer treatment.

The foundation of immunology is based on self-non-self-discrimination. Most of the pathogens inducing infectious diseases contain molecular signatures that can be recognized by the host and trigger immune responses. However, tumor cells are derived from normal cells, and do not generally express any molecular signatures, making them more difficult to be distinguished from normal cells. Nevertheless, most tumor cells express different types of tumor antigens. These can be shared tumor antigens, i.e. antigens that are expressed by tumors of the same kind in multiple individuals or expressed by a variety of tumors in multiple individuals or patient-specific antigens that are found in a particular patient.

Shared tumor antigens include overexpressed or aberrantly expressed cellular proteins, mutations in oncogenes or tumor suppressor genes and viral antigens. Patient-specific tumor antigens may arise due to one or more mutations in the tumor genome leading to a change in the amino acid sequence of the protein in question. These include non-synonymous mutations, frameshift mutations, fusion antigens and intron-retention antigens.

Summary of invention

The present inventors have realized that an individualized anticancer vaccine raising immune response against shared tumor antigens that are present in the patient for whom the vaccine is designed and manufactured, and optionally in addition thereto against patient-specific tumor antigens, improves the anticancer immune response necessary to control or inhibit growth of the tumor cells.

Definitions

Tumor is used in the present context for both a solid tumor as well as for tumor cells found in a bodily fluid, such as blood.

Patient-specific tumor antigen, patient-specific cancer antigen and patient-specific antigen are used interchangeably herein for a tumor antigen found in a specific individual/patient, wherein said tumor antigen comprises one or more mutations found in the tumor cell as compared to the patient's normal cells.

Patient-specific tumor epitope, patient-specific cancer epitope, patient-specific epitope and neoepitope are used interchangeably herein for a peptide comprised in a patient-specific tumor antigen, which peptide comprises one or more immunogenic mutations.

Patient-specific tumor epitope sequence, patient-specific cancer epitope sequence, patient-specific epitope sequence and neoepitope sequence are used interchangeably herein to describe a nucleic acid sequence encoding the epitope/neoepitope or an amino acid sequence comprising the epitope/neoepitope.

Shared tumor antigen, shared cancer antigen and shared antigen are used interchangeably herein to describe a tumor antigen expressed by tumors of the same kind in multiple individuals or expressed by a variety of tumors in multiple individuals.

Shared tumor antigen sequence, *shared cancer antigen sequence* and *shared antigen sequence* are used interchangeably herein to describe a nucleic acid sequence encoding or an amino acid sequence comprising a part of or the whole of a shared antigen.

Patient-present shared tumor antigen, patient-present shared cancer antigen and patient-present shared antigen are used interchangeably herein to describe a shared tumor antigen that is or has been identified to be present in said patient.

Patient-present shared tumor antigen sequence, patient-present shared cancer antigen sequence and patient-present shared antigen sequence are used interchangeably herein to describe a nucleic acid sequence encoding or an amino acid sequence comprising a part of or the whole of a patient-present shared antigen.

Individualized therapeutic anticancer vaccine is used to describe a vaccine which is designed and manufactured for a specific individual/patient with the aim to stimulate an immune response that can recognize tumor cells already present in the individual and control or inhibit growth of such tumor cells.

Individual and patient are used interchangeably herein and denote a specific human being having cancer or suspected to have cancer.

Detailed description of the invention

Cancers develop from the patient's normal tissue by one or a few cells starting an abnormal, uncontrolled proliferation of the cells due to mutations. Although the cancer cells are mutated, most of the genome is intact and identical to the remaining cells in the patient. One approach of attacking a tumor is based on the knowledge that any tumor in an individual/patient is unique: patient-specific mutations lead to expression of patient-specific mutated proteins (tumor-specific antigens), that are unique for the particular patient. These tumor specific and patient-specific antigens are not identical to any proteins in the normal cells of the patient. Therefore, such patient-specific antigens should be suitable targets for a therapeutic anticancer vaccine which is manufactured specifically for the patient in question, i.e. an individualized therapeutic anticancer vaccine. The challenge with this type of anticancer vaccine is that, although patient-specific antigens are selected for inclusion into the vaccine according to their predicted therapeutic efficacy, some but not all show the expected therapeutic efficacy in the clinical setting. There is a need for improvement.

Shared tumor antigens have been found to be expressed by many tumors, either across patients with the same cancer type, or across patients and cancer types. An example is the HPV16 antigen, a viral antigen that is expressed in about 50% of all squamous cell carcinoma of the head and neck (SCCHN) patients, but also in patients with different cancers such as cervical cancer and vulvar squamous cell carcinoma (vSCC). Many of these shared antigens have previously been characterized as immunogenic and/or to be presented on specific HLA class I or class II alleles. Shared antigens may be included in an off-the-shelf therapeutic anticancer vaccine to be use in many patients (see for instance WO 2013/092875). However, while this type of anticancer vaccine shows clinical efficacy in some patients, no or little therapeutic efficacy is seen in others.

T cell responses are dependent on processing and presentation of cancer epitopes on each patient's HLA molecules. Due to the diversity of HLA class I and HLA class II molecules, off-the-shelf vaccines used in multiple patients normally include large full-length antigens to optimize the chance that the antigen includes epitopes that are present on a wide range of HLA molecules. However, a patient will only need shorter sequences of the antigen that include those epitopes that match that particular patient's HLA molecules. Hence, it is not possible to design and produce an off-the-shelf anticancer vaccine that covers all tumor antigens in every patient.

Further, off-the-shelf therapeutic anticancer vaccines that only target one tumor antigen do disregard tumor heterogeneity commonly found in a tumor and immune pressure against the one antigen may result tumor clones with different mutations.

The present invention presents an individualized therapeutic anticancer vaccine that targets patient-present shared antigens, optionally in addition thereto patient-specific antigens. This will increase the therapeutic effect compared to an anticancer vaccine comprising only patient-specific antigens or compared to an off-the-self anticancer vaccine including shared antigens.

Patient-present shared antigens to be included in the vaccine of the invention can have known immunogenicity, known expression patterns and known binding to specific HLA class I and/or class II molecules. T cells specific to patient-present shared antigens can travel to the tumor and affect the tumor microenvironment, thus increasing the likelihood that additional tumor-specific T cells are to able attack the tumor cells. Tumors are to various degrees heterogenic and thus may be composed of tumor cells expressing different subsets of the patient-present shared antigens included in the vaccine. Including patient-present shared antigens and optionally patient-specific antigens in the individualized vaccine according to the invention increases the chance of recognition and killing of multiple or all tumor cells due to shift in the immune microenvironment when reaching a threshold of activated T cells trafficking to the tumor.

The vaccines according to the present invention use the normal adaptive immune system to provide immunity against the tumor cells. The adaptive immune system is specific in that every antigen evokes an immune response specifically towards said antigen by the recognition of specific antigens during a process called antigen presentation. The cells of the adaptive immune system are lymphocytes, in particularly B cells and T cells. B cells are involved in the humoral immune response, whereas T cells are involved in cell-mediated immune response.

In particular, the vaccine according to the present invention is designed for evoking a cell-mediated immune response through activation of T cells against the tumor antigens. T cells recognize epitopes when they have been processed and presented complexed to an MHC molecule as discussed below.

The patient-present shared antigens sequences and optionally patient-specific antigens sequences of shared antigens and optionally patient-specific antigens included in the vaccine according to the invention are designed to be presented in the peptide-binding groove of MHC molecules as MHC-peptide complexes. There are two primary classes of major histocompatibility complex (MHC) molecules, MHC I and MHC II. The terms MHC (class) I and

MHC (class) II are interchangeably used herein with HLA (class) I and HLA (class) II. Human leukocyte antigen (HLA) is a major histocompatibility complex in humans.

MHC I is found on the cell surface of all nucleated cells in the body. One function of MHC I is to display peptides of non-self-proteins from within the cell to cytotoxic T cells. The MHC I peptide complex is inserted into the plasma membrane of the cell presenting the peptide to the cytotoxic T cells, whereby an activation of cytotoxic T cells against the particular MHC-peptide complex is triggered. The peptide is positioned in a groove in the MHC I molecule, allowing the peptide to be about 8-10 amino acids long.

MHC II molecules are a family of molecules normally found only on antigen-presenting cells such as dendritic cells, mononuclear phagocytes, some endothelial cells, thymic epithelial cells, and B cells.

As opposed to MHC I, the antigens presented by MHC class II molecules are derived from extracellular proteins. Extracellular proteins are endocytosed, digested in lysosomes, and the resulting peptides are loaded onto MHC class II molecules and then presented at the cell surface. The antigen-binding groove of MHC class II molecules is open at both ends and is able to present longer peptides, generally between 15 and 24 amino acid residues long.

MHC class I molecules are recognized by CD8 and co-receptors on the T cells, normally called CD8+ T cells (or CD8+ cells), whereas MHC class II molecules are recognized by CD4 and co-receptors on the T cells, normally called CD4+ T cells (or CD4+ cells).

The individualized anticancer vaccines of the present invention comprise a polynucleotide encoding a polypeptide comprising three units, i.e. a targeting unit, a dimerization unit and an antigenic unit. Due to the dimerization unit, the polypeptide forms a dimeric protein, a so-called vaccibody.

The genes encoding the three units are genetically engineered to be expressed as one gene. When expressed *in vivo*, the polypeptides/dimeric proteins target antigen presenting cells (APCs), which results in enhanced vaccine potency compared to identical non-targeted antigens.

In a first aspect the invention relates to a polynucleotide comprising a nucleotide sequence encoding a targeting unit, a dimerization unit and an antigenic unit, wherein said antigenic unit comprises at least one patient-present shared antigen sequence or one or more parts thereof, and optionally one or more patient-specific antigen sequences or one or more parts thereof.

In a second aspect the invention relates to a polypeptide encoded by a polynucleotide comprising a nucleotide sequence encoding a targeting unit, a dimerization unit and an antigenic unit, wherein said antigenic unit comprises at least one patient-present shared antigen sequence or one or more parts thereof, and optionally one or more patient-specific antigen sequences or one or more parts thereof.

In a third aspect the invention relates to a dimeric protein consisting of two polypeptides encoded by a polynucleotide comprising a nucleotide sequence encoding a targeting unit, a dimerization unit and an antigenic unit, wherein said antigenic unit comprises at least one patient-present shared antigen sequence or one or more parts thereof, and optionally one or more patient-specific antigen sequences or one or more parts thereof.

In a fourth aspect the invention relates to an individualized therapeutic anticancer vaccine comprising an immunologically effective amount of:

(i) a polynucleotide comprising a nucleotide sequence encoding a targeting unit, a dimerization unit and an antigenic unit, wherein said antigenic unit comprises at least one patient-present shared antigen sequence or one or more parts thereof, and optionally one or more patient-specific antigen sequences or one or more parts thereof; or

(ii) a polypeptide encoded by the polynucleotide as defined in (i), or

(iii) a dimeric protein consisting of two polypeptides encoded by the polynucleotide as defined in (i); and

a pharmaceutically acceptable carrier.

In one embodiment, the invention relates to an individualized therapeutic anticancer vaccine comprising an immunologically effective amount of:

(a) a polynucleotide comprising a nucleotide sequence encoding a targeting unit, a dimerization unit and an antigenic unit, wherein said antigenic unit comprises at least one patient-present shared antigen sequence or one or more parts thereof, and optionally one or more patient-specific antigen sequences or one or more parts thereof; and

(b) a pharmaceutically acceptable carrier.

In another embodiment, the invention relates to an individualized therapeutic anticancer vaccine comprising an immunologically effective amount of

(a) a dimeric protein or polypeptide encoded by a polynucleotide comprising a nucleotide sequence encoding a targeting unit, a dimerization unit and an antigenic unit, wherein said antigenic unit comprises at least one patient-present shared antigen sequence or

one or more parts thereof, and optionally one or more patient-specific antigen sequences or one or more parts thereof; and

(b) a pharmaceutically acceptable carrier.

The antigenic unit comprised in the polynucleotide, polypeptide, dimeric protein and individualized therapeutic anticancer vaccine according to the invention comprises at least one patient-present shared antigen sequence or one or more parts thereof. In one embodiment, the patient-present shared antigen is a shared antigen selected from the group consisting of overexpressed cellular proteins, aberrantly expressed cellular proteins, cancer testis antigens, viral antigens, differentiation antigens, mutated oncogenes, mutated tumor suppressor genes, oncofetal antigens, shared fusion antigens, shared intron retention antigens, dark matter antigens, shared antigens caused by spliceosome mutations and shared antigens caused by frameshift mutations.

In one embodiment, the patient-present shared antigen is an overexpressed or aberrantly expressed human cellular protein, i.e. a cellular protein found at increased levels in tumors compared with normal healthy cells and tissues. Examples of such overexpressed or aberrantly expressed cellular proteins include tumor protein D52, Her-2/neu, hTERT (telomerase) and survivin. In another embodiment, the patient-present shared antigen is a cancer testis antigen whose expression occurs in human malignancies as well as in normal testicular tissue. Examples of cancer testis antigens include MAGE-A, MAGE-B, GAGE, PAGE-1, SSX, HOM-MEL-40 (SSX2), NY-ESO-1, LAGE-1 and SCP-1. In yet another embodiment, the patient-present shared antigen is a differentiation antigen, for example tyrosinase and TRP-2. In yet another embodiment, the patient-present shared antigen is a viral antigen. Examples of viral antigens include human papilloma virus (HPV), hepatitis B virus (HBV), Epstein-Barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), Merkel cell polyomavirus (MCV or MCPyV), human cytomegalovirus (HCMV) and human T-lymphotropic virus (HTLV). In yet another embodiment, the patient-present shared antigen is a mutated oncogene. Examples of mutated oncogenes include RAS mutations, including KRAS, PIK3CA mutations and EGFR mutations. In yet another embodiment, the patient-present shared antigen is a mutated tumor suppressor gene. Examples include mutated p53, mutated pRB, mutated BCL2 and mutated SWI/SNF. In yet another embodiment, the patient-present shared antigen is an oncofetal antigen, for example alpha-fetoprotein or carcinoembryonic antigen. In yet another embodiment, the patient-present shared antigen is a shared intron retention antigen or shared antigen caused by frameshift mutation, for example CDX2 or CALR. In yet another embodiment, the patient-present shared antigen is a shared antigen caused by spliceosome mutations. An example is an antigen caused by mutations like SF3B1 mut.

For any cancer antigen, immune tolerance has likely occurred when a patient presents with cancer. An anticancer vaccine should specifically trigger immune response to the antigens incorporated in the vaccine. The peripheral immune tolerance to the selected antigens may be weak or strong. A patient is more likely to establish central immune tolerance against a shared antigen that is also expressed in normal tissue, i.e. a human cellular protein, such as overexpressed antigens and differentiation antigens. By incorporating such shared antigen sequences or parts thereof in the antigenic unit (either alone or together with other patient-present shared antigen sequences and optionally patient-specific antigen sequences), the vaccine according to the invention comprising the antigenic unit could elicit an immune response which is strong and broad enough to affect the tumor microenvironment and change the patient's immune response against the tumor from a suppressive/tolerated type to a pro-inflammatory type of immune response. This may help to break tolerance to several other antigens, thus representing a considerable clinical benefit for the patient. The afore-described concept may be referred to as tipping the cancer immunity set-point,

Thus, in one embodiment the at least one patient-present shared antigen sequence is a shared antigen that is a human cellular protein, preferably an overexpressed or aberrantly expressed human cellular protein or a differentiation antigen.

The at least one patient-present shared antigen may be detected in the tissue or body fluid of the patient by methods known in the art, including:

- sequencing the patient's genome or exome and optionally searching by tailor-made software in whole genome/exome-seq data to e.g. identify mutated oncogenes or mutated tumor suppressor genes;
- immunohistochemistry of the patient's tumor tissue to detect the presence of mutated proteins;
- RT-PCR to detect the presence of viral antigens or known mutations in oncogenes;
- ELISA using antibodies against mutated tumor proteins in serum samples;
- RNA-seq of tumor tissue and comparison to healthy tissue to detect expression/over-expression of shared antigens;
- searching by tailor-made software in raw RNA sequence data to identify intron retention antigens;
- searching by tailor-made software in whole genome-seq data to identify transposable elements which are elements of dark matter antigens;
- detection of short repeats in raw whole exome/RNA sequence data to identify dark matter antigens;
- RNA-seq data to identify shared viral antigens; and

- comparing RNA-seq of the patient's tumor samples with either patient's own healthy tissue or a cohort/database (e.g. TCGA) versus GTEX/HPA gene expression data.

In a preferred embodiment, the antigenic unit comprises at least one patient-present shared antigen sequence or part(s) of such antigen sequence that is known to be immunogenic, e.g. has previously shown an immunogenic response in other patients, has been described to elicit an immune response in other patients, or is predicted to bind to the particular patient's HLA class I and/or HLA class II alleles. In another preferred embodiment, the antigenic unit comprises one or more parts of at least one patient-present shared antigen sequence, e.g. one or more epitopes that are known to be immunogenic or are predicted to bind to the particular patient's HLA class I and/or HLA class II alleles. In a further preferred embodiment, the antigenic unit comprises one or more parts of at least one patient-present shared antigen sequence, e.g. one or more epitopes that are known to be immunogenic or are predicted to bind to the particular patient's HLA class I alleles.

In one embodiment, the antigenic unit comprises a patient-present shared antigen sequence or one or more parts thereof that have a length suitable for presentation by the particular patient's HLA alleles. Thus, in one embodiment the patient-present shared antigen sequence or the part thereof is from 7 to 30 amino acids long. In another embodiment, the patient-present shared antigen sequence or the part thereof has a length of from 7 to 10 amino acids or a length of from 13 to 30 amino acids, such as 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids e.g. 9 amino acids.

The antigenic unit can comprise the at least one patient-present shared antigen sequence either in full length or can comprise one or more parts thereof. In one embodiment, the antigenic unit comprises one part of a patient-present shared antigen sequence. In another embodiment, the antigenic unit comprises several parts of a patient-present shared antigen sequence. The antigenic unit can comprise more than one patient-present shared antigen sequence, i.e. sequences of several patient-shared antigens, either each in full length or one or more parts of each such antigen. In one embodiment, the antigenic unit comprises one patient-present shared antigen sequence in full length and one or more parts of sequences of one or several other patient-present shared antigens, e.g. one part of a sequence of one other patient-present shared antigen or several parts of a sequence of one other patient-present shared antigen or one part of a sequence of each of the several other patient-present shared antigens or several parts of sequences of some or each of the several other patient-present shared antigens. In a preferred embodiment, the antigenic unit comprises sequences of several patient-present shared antigens, e.g. several parts of sequences of several patient-present shared antigens, more preferably several epitopes of several patient-present shared antigen, which epitopes are

known to be immunogenic or are predicted to bind to the particular patient's HLA class I and/or HLA class II alleles, preferably to the particular patient's HLA class I alleles.

In yet another embodiment, the antigenic unit comprises one or more patient-present shared antigens in full length and one or more parts of one or more patient-present shared cancer antigens. Examples include:

- antigenic units comprising one patient-present shared antigen in full length and one or more epitopes of one patient-present shared cancer antigen; and
- antigenic units comprising several patient-present shared cancer antigens, each of them in full length and one or more epitopes of one patient-present shared cancer antigen; and
- antigenic units comprising one patient-present shared antigen in full length and one or more epitopes of several patient-present shared cancer antigens; and
- antigenic units comprising several patient-present shared cancer antigens, each of them in full length and one or more epitopes of several patient-present shared cancer antigens.

In yet another embodiment, the antigenic unit comprises at least one patient-present shared antigen sequence in full length or more than one patient-present shared antigen sequences in full length. In one embodiment, the antigenic unit comprises 1 to 10 patient-present shared antigen sequences in full length. In another embodiment, the antigenic unit comprises 1 to 30 parts of patient-present shared antigen sequences in the form of long peptide sequences, e.g. from about 28 to 100 amino acids long, or nucleic acid sequences encoding such long peptide sequences, wherein the long peptide sequences include multiple epitopes that are predicted to bind to the patient's HLA class I and/or HLA class II alleles. In yet another embodiment, the antigenic unit comprises 1 to 50 parts of patient-present shared antigen sequences in the form of short peptide sequences/epitopes or nucleic acid sequences encoding such short peptide sequences/epitopes that are predicted to bind to the patient's HLA class I and/or HLA class II alleles.

In one embodiment from 3 to 50 patient-present shared antigen sequences are included in the antigenic unit, such as from 3 to 30 sequences, such as from 3 to 20 sequences, such as from 3 to 15 sequences, or such as from 3 to 10 sequences.

In another embodiment 5 to 50 patient-present shared antigen sequences are included in the antigenic unit, such as from 5 to 30 sequences, such as for example from 5 to 25 sequences, such as from 5 to 20 sequences, such as from 5 to 15 sequences or such as from 5 to 10 sequences.

In a further embodiment 10 to 50 patient-present shared antigen sequences are included in the antigenic unit, such as from 10 to 40 sequences, such as from 10 to 30 sequences, such as from 10 to 25 sequences, such as from 10 to 20 sequences or such as from 10 to 15 sequences.

In order to avoid that tumors escape the immune system by e.g. shutting down expression of an antigen that is the target of the anticancer vaccine, it is preferred to include sequences of a plurality of different patient-present shared antigens and optionally sequences of a plurality of different patient-specific antigens into the antigenic unit. Generally, the more genes the tumor needs to shut down in order to escape the immune system the less likely is it that the tumor is actually capable of shutting down all of them while still be able to proliferate or even survive. Furthermore, the tumor may be heterogeneous in that not each and every patient-present shared antigen or patient-specific antigen is expressed by all of the tumor cells.

Accordingly, in a preferred embodiment, the approach is to include as many patient-present shared antigen sequences and optionally patient-specific antigen sequences as possible into the antigenic unit of the vaccine of the invention in order to attack the tumor efficiently by activating T-cells able to recognize more tumor antigens expressed by the tumor cells. Also, in order to secure that all patient-present shared antigen sequences and optionally patient-specific antigen sequences are taken up efficiently into the same antigen presenting cell they are arranged such that they are one amino acid chain or encode for one amino acid chain (i.e. antigenic unit) instead of discrete peptides. However, as described above, the object of the vaccine of the invention is to activate the patient's T cells against the patient-present shared antigen sequences and optionally patient-specific antigen sequences comprised in the antigenic unit, and including too many of such sequences into the antigenic unit may result in the dilution of T-cells. Therefore, it is important to select the optimal patient-present shared antigen sequences and optionally patient-specific antigen sequences for inclusion into the antigenic unit.

The optimal patient-present shared antigen sequences are those that are known to be immunogenic. In a preferred embodiment, the antigenic unit comprises one or more parts of at least one patient-present shared antigen sequence, e.g. one or more epitopes that are known to be immunogenic or are predicted to bind the particular patient's HLA alleles, preferably to the patient's HLA class I alleles.

It is preferred to make "best use" of the antigenic unit, i.e. the antigenic unit comprises the least possible number of amino acids/sequences which do not contribute to the immunogenicity of the antigenic unit. As an example, it is less preferred to include a full-length patient-present shared antigen sequence into the antigenic unit, if such sequence only contains a few epitopes

that are known to be immunogenic or are predicted to bind the particular patient's HLA alleles and the remainder of the sequence does not contribute to the immunogenicity of the antigenic unit. On the other hand, if such sequence contains several or many such epitopes which are close together, it may make sense to include the full length of the patient-present shared antigen sequence.

The antigenic unit may further comprise one or more patient-specific antigen sequences. Patient-specific antigens may be identified by sequencing the genome or exome of a patient's tumor. Compared to the patient's normal tissue exome, such sequences comprise one or more mutations. The mutation may be any mutation leading to a change in at least one amino acid. Accordingly, the mutation may be one of the following:

- a non-synonymous mutation leading to a change in the amino acid
- a mutation leading to a frame shift and thereby a completely different open reading frame in the direction after the mutation
- a read-through mutation in which a stop codon is modified or deleted leading to a longer protein with a tumor-specific epitope
- splice mutations that lead to a unique tumor-specific protein sequence
- chromosomal rearrangements that give rise to a chimeric protein with a tumor-specific epitope at the junction of the two proteins.

The antigenic unit may comprise one or more patient-specific antigen sequences or one or more parts thereof. In one embodiment, the antigenic unit comprises one or more (several) patient-specific antigen sequences. In another embodiment, the antigenic unit comprises one or more parts of such one or more patient-specific antigen sequences, preferably one or more patient-specific epitopes.

The epitopes preferably have a length suitable for presentation by the MHC molecules discussed above. Thus, in a preferred embodiment the epitope is from 7 to 30 amino acids long. More preferred are epitope sequences having a length of from 7 to 10 amino acids or epitope sequences having a length of from 13 to 30 amino acids, such as 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids.

In a preferred embodiment, the antigenic unit comprises at least one patient-specific epitope or at least 5 patent specific epitopes or at least 10 patient-specific epitopes. In another preferred embodiment, the antigenic unit comprises at least 15 patient-specific epitopes, such as at least 20 patient-specific epitopes.

In one embodiment from 3 to 50 patient-specific antigen sequences are included in the antigenic unit, such as from 3 to 30 sequences, such as from 3 to 20 sequences, such as from 3 to 15 sequences or such as from 3 to 10 sequences. In a preferred embodiment, such sequences are epitopes.

In another embodiment 5 to 50 patient-specific antigen sequences are included in the antigenic unit, such as from 5 to 30 sequences, such as for example from 5 to 25 sequences, such as from 5 to 20 sequences, such as from 5 to 15 sequences or such as from 5 to 10 sequences. In a preferred embodiment, such sequences are epitopes.

In a further embodiment 10 to 50 patient-specific antigen sequences may be included in the antigenic unit, such as from 10 to 40 sequences, such as from 10 to 30 sequences, such as from 10 to 25 sequences, such as from 10 to 20 sequences or such as from 10 to 15 sequences. In a preferred embodiment, such sequences are epitopes.

Particularly when the patient specific antigen sequences to be included in the antigenic unit is a short epitope, e.g. only a few amino acids long, the short epitope is included in the antigenic unit such that it is flanked at both sides by an amino acid sequence. Preferably, the short epitope is positioned essentially in the middle of two flanking sequences, in order to ensure that the epitope is presented by the antigen presenting cells after it has been processed. The flanking sequences are preferably the amino acid sequences flanking the epitope in the antigen.

The following applies to the patient-present shared antigen sequences and parts thereof and, if present, also to the patient-specific antigen sequences and parts thereof. Thus, in the following the term antigen sequence is used and intended to cover both patient-present shared antigen sequences and parts thereof and patient-specific antigen sequences and parts thereof.
In one embodiment, the antigenic unit comprises one copy of each antigen sequence, so that when e.g. 10 different of such sequences are comprised in the antigenic unit, a vaccine comprising said antigenic unit elicits a cell mediated immune response against all 10 different antigen sequences.

However, if only a few antigen sequences are included in the antigenic unit - either because only a few antigens have been identified or because only a few of the identified antigens are sufficiently immunogenic/known to or predicted to bind to the patient's HLA alleles - then the antigenic unit may comprise at least two copies of a particular antigen sequence in order to strengthen the immune response to the antigen.

The length of the antigenic unit is primarily determined by the length of the antigen sequences comprised therein as well as their number. In one embodiment, the antigenic unit comprises

from 7 to 2000 amino acids, e.g. from 21 to 2000 amino acids, preferably from about 30 amino acids to about a 1500 amino acids, more preferably from about 50 to about 1000 amino acids, such as from about 100 to about 500 amino acids or from about 100 to about 400 amino acids or from about 100 to about 300 amino acids.

Although it is possible to obtain a relevant immune response towards the tumor if the antigen sequences are randomly arranged in the antigenic subunit, it is preferred to follow at least one of the following methods for arranging them in the antigenic unit in order to enhance the immune response:

The antigenic unit can be described as a polypeptide having an N-terminal start and a C-terminal end. The antigenic unit is connected to the dimerization unit, preferably via a unit linker. The antigenic unit is either located at the COOH-terminal end or the NH2-terminal end of the polypeptide/dimeric protein. It is preferred that the antigenic unit is in the COOH-terminal end of the polypeptide/dimeric protein.

The antigen sequences are preferably separated by a linker. In one embodiment, all but the terminal antigen sequence, i.e. the antigen sequence located at the end of the antigenic unit that is not connected to the dimerization unit, are arranged in antigenic subunits, each subunit consist of an antigen sequence and a subunit linker. Due to the separation of the antigen sequences by the linkers, each antigen is presented in an optimal way to the immune system.

In one embodiment, the antigen sequences are arranged from most antigenic to least antigenic in the direction from the N-terminal start of the antigenic unit to the C-terminal end of the antigenic unit, preferably in the direction of the dimerization unit towards the C-terminal end of the antigenic unit.

In another embodiment, in particular if the hydrophilicity/hydrophobicity varies greatly among the antigen sequences, it is preferred that the most hydrophobic antigenic sequence(s) is/are positioned substantially in the middle of the antigenic unit and the most hydrophilic antigen sequence(s) is/are positioned at the beginning and/or end of the antigenic unit.

Since a true positioning in the middle of the antigenic unit is only possible if the antigenic unit comprises an odd number of antigen sequences, the term “substantially” in this context refers to antigenic units comprising an even number of antigen sequences, wherein the most hydrophobic antigen sequence is positioned as close to the middle as possible.
By way of example, an antigenic unit comprises 5 antigenic subunits, each comprising a different antigen sequence, which are arranged as follows: 1-2-3*-4-5; with 1, 2, 3*,4 and 5 each

being a different antigen sequence and – being a linker and * indicates the most hydrophobic antigen sequence, which is positioned in the middle of the antigenic unit.

In another example, an antigenic unit comprises 6 antigenic subunits, each comprising a different antigen sequence, which are arranged as follows: 1-2-3*-4-5-6 or, alternatively, as follows: 1-2-4-3*-5-6; with 1, 2, 3*, 4, 5 and 6 each being a different antigen sequence and – being a linker and * indicates the most hydrophobic antigen sequence, which is positioned substantially in the middle of the antigenic unit.

Alternatively, the antigen sequences may be arranged alternating between a hydrophilic and a hydrophobic antigen sequence.

Furthermore, GC rich antigen sequences should not be arranged adjacent to each other to avoid GC clusters. In a preferred embodiment, one GC rich antigen sequence is followed by at least one non-GC rich antigen sequence before a second GC rich antigen sequence follows.

In one embodiment the antigenic unit comprises antigen sequences in the following order: E7 | linker | NY-ESO-1 | linker | E6. In a preferred embodiment the antigenic unit comprises SEQ ID NO: 14.

In another preferred embodiment the antigenic unit comprises SEQ ID NO: 14 and SEQ ID NO: 15. SEQ ID NO: 15 comprises antigen sequences in the following order: T1D320 | linker | T1D814 | linker | T1D182 | linker | T1D689 | linker | E7 | linker | T1D339 | linker | T1D428 | linker |NY-ESO-1 | linker | T1D572 | linker | T1D359 | linker | T1D488 | linker | T1D554 | linker | T1D272 | linker | T1D210 | linker | T1D849 | linker | T1D4 | linker | T1D77 | linker | T1D717 | linker | T1D586 | linker | E6.

In one embodiment the antigenic unit comprises antigen sequences in the following order: E6 | linker | NY-ESO-1 | linker | E7. In a preferred embodiment the antigenic unit comprises SEQ ID NO: 16.

In another preferred embodiment the antigenic unit comprises SEQ ID NO: 16 and SEQ ID NO: 17. SEQ ID NO: 17 comprises antigen sequences in the following order: E6 | linker | T1D323 | linker | T1D506 | linker | T1D12 | linker | T1D315 | linker | T1D302 | linker | T1D700 | linker | NY-ESO-1 | linker | T1D535 | linker | T1D358 | linker | T1D670 | linker | T1D294 | linker | T1D336 | linker | T1D499 | linker | T1D425 | linker T1D491 | linker | T1D314 | linker | T1D430 | linker | E7 | linker | T1D582.

In one embodiment the antigenic unit comprises antigen sequences in the following order: NY-ESO-1 | linker | E7 | linker | E6. In a preferred embodiment the antigenic unit comprises SEQ ID NO: 18.

In another preferred embodiment the antigenic unit comprises SEQ ID NO: 18 and SEQ ID NO: 19. SEQ ID NO: 19 comprises antigen sequences in the following order: T1D223 | linker | T1D164 | linker | T1D56 | linker | T1D36 | linker | T1D129 | linker | T1D274 | linker | T1D62 | linker | T1D5 | linker | T1D144 | linker | T1D441 | linker | T1D368 | linker | NY-ESO-1 | linker | T1D234 | linker | T1D162 | linker | T1D39 | linker | T1D272 | linker | E7 | linker | T1D328 | linker | T1D188 | linker | E6.

The antigenic unit may further comprise one or more linkers, which separate one antigen sequence from the other and a linker which connects the antigenic unit to the dimerization unit (hereinafter also called the unit linker). The one or more linkers ensure that each antigen sequence is presented in an optimal way to the immune system, which, if the antigenic units are included in the vaccine of the invention, increases the vaccine's efficacy.

The one or more linkers are preferably designed to be non-immunogenic and are preferably also flexible, which allows for the antigen sequences to be presented in an optimal manner to the T cells, even if the antigenic unit comprises a high number of antigen sequences.

Preferably, the length of the one or more linkers is from 4 to 20 amino acids to secure flexibility. In another preferred embodiment, the length of the one or more linkers is from 8 to 20 amino acids, such as from 8 to 15 amino acids, for example 8 to 12 amino acids or such as for example from 10 to 15 amino acids. In a particular embodiment, the length of the one or more linkers is 10 amino acids.

In a specific embodiment, the antigenic unit comprises 10 antigen sequences, wherein the linkers between these sequences have a length of from 8 to 20 amino acids, such as from 8 to 15 amino acids, for example 8 to 12 amino acids or such as for example from 10 to 15 amino acids. In a particular embodiment, the antigenic unit comprises 10 antigen sequences and the linkers between these sequences have a length of 10 amino acids.

The one or more linkers have preferably all the same nucleotide or amino acid sequence. If, however, one or more of the antigen sequences comprise an amino acid motif similar to the linker, it may be an advantage to substitute the neighboring linkers of that antigen sequence with linker of a different sequence. Further, if an antigen sequence/ linker junction is predicted to constitute an immunogenic epitope in itself, then a linker of a different sequence might be used.

The one or more linkers are preferably serine (S)-glycine (G) linkers or comprise or consist of nucleotides that encode a serine-glycine amino acid sequence, such as GGGGS, GGGSS, GGGSG, GGGGS or multiple variants thereof such as GGGGSGGGGS or $(GGGGS)_m$, $(GGGSS)_m$, $(GGGSG)_m$, where m is an integer from 1 to 5, from 1 to 4 or from 1 to 3. In a preferred embodiment, m is 2.

In a preferred embodiment, the serine-glycine linker further comprises at least one leucine (L), such as at least 2 or at least 3 leucines. The serine-glycine linker may for example comprise 1, 2, 3 or 4 leucine. Preferably, the serine-glycine linker comprises 1 leucine or 2 leucines.

In one embodiment, the one or more linkers comprise or consist of the sequence LGGGS, GLGGS, GGLGS, GGGLS or GGGGL. In another embodiment, the one or more linkers comprise or consist of the sequence LGGSG, GLGSG, GGLSG, GGGLG or GGGSL. In yet another embodiment, the one or more linkers comprise or consist of the sequence LGGSS, GLGSS, GGLSS, GGGLS or GGGSL.

In yet another embodiment, the one or more linkers comprise or consist of the sequence LGLGS, GLGLS, GLLGS, LGGLS or GLGGL. In yet another embodiment, one or more linkers comprise or consist of the sequence LGLSG, GLLSG, GGLSL, GGLLG or GLGSL. In yet another embodiment, the one or more linkers comprise or consist of the sequence LGLSS, GLGLS, GGLLS, GLGSL or GLGSL.

In another embodiment, the one or more linkers are serine-glycine linkers that have a length of 10 amino acids and comprise 1 leucine or 2 leucines.

In one embodiment, the one or more linkers comprise or consist of the sequence LGGGSGGGGS, GLGGSGGGGS, GGLGSGGGGS, GGGLSGGGGS or GGGGLGGGGS. In another embodiment, the one or more linkers comprise or consist of the sequence LGGSG GGGSG, GLGSGGGGSG, GGLSGGGGSG, GGGLGGGGSG or GGGSLGGGSG. In yet another embodiment, the one or more linkers comprise or consist of the sequence LGGSSGGGSS, GLGSSGGGSS, GGLSSGGGSS, GGGLSGGGSS or GGGSLGGGSS.

In a further embodiment, the one or more linkers comprise or consist of the sequence LGGGSLGGGS, GLGGSGLGGS, GGLGSGGLGS, GGGLSGGGLS or GGGGLGGGGL. In another embodiment, the one or more linkers comprise or consist of the sequence LGGSGLGGSG, GLGSGGLGSG, GGLSGGGLSG, GGGLGGGGLG or GGGSLGGGSL. In yet another embodiment, the one or more linkers comprise or consist of the sequence LGGSSLGGSS, GLGSSGLGSS, GGLSSGGLSS, GGGLSGGGLS or GGGSLGGGSL.

In one embodiment, the antigenic unit comprises 10 antigen sequences that are separated by 9 linkers, i.e. the terminal sequence is an antigen sequence, not a linker. In another embodiment, the antigenic unit comprises 15 antigen sequences that are separated by 14 linkers or 20 antigen sequences that are separated by 19 linkers.

In another embodiment, the antigenic unit comprises from 10 to 20 or from 10 to 25 antigen sequences that are separated by linkers. Preferably, said linkers have a length of 10 amino acids. The linkers may also have any length as defined herein above, such as for example from 5 to 12 amino acids.

Alternatively, the one or more linkers may be selected from the group consisting of GSAT linkers, i.e. a linkers comprising one or more glycine, serine, alanine and threonine residues and SEG linkers, i.e. linkers comprising one or more serine, glutamic acid and glycine residues or multiple variants thereof.

The antigenic unit and the dimerization unit are preferably connected by a unit linker. The unit linker may comprise a restriction site in order to facilitate the construction of the polynucleotide. It is preferred that the unit linker is a GLGGL linker or a GLSGL linker.

The vaccine of the invention comprises a targeting unit that targets antigen-presenting cells. Due to the targeting unit, the polypeptide/dimeric protein/vaccine of the invention leads to attraction of dendritic cells (DCs), neutrophils and other immune cells. Thus, the polypeptide/dimeric protein/vaccine comprising the targeting unit will not only target the antigenic unit to specific cells, but in addition facilitate a response-amplifying effect (adjuvant effect) by recruiting specific immune cells to the administration site of the polynucleotide/polypeptide/dimeric protein/vaccine. This unique mechanism is of great importance in a clinical setting where patients can receive the vaccine of the invention without any additional adjuvants since the vaccine itself provides the adjuvant effect.

The term "targeting unit" as used herein refers to a unit that delivers the polypeptide/dimeric protein/vaccine with its antigenic unit to an antigen presenting cell for MHC class II-restricted presentation to CD4+ T cells or for providing cross presentation to CD8+ T cells by MHC class I restriction.

The targeting unit is connected through the dimerization unit to the antigenic unit, wherein the latter is in either the COOH-terminal or the NH_2-terminal end of the polypeptide/dimeric protein. It is preferred that the antigenic unit is in the COOH-terminal end of the polypeptide/dimeric protein.

The targeting unit is designed to target the polypeptide/dimeric protein/vaccine of the invention to surface molecules expressed on the relevant antigen presenting cells, such as molecules expressed exclusively on subsets of dendritic cells (DC).

Examples of such target surface molecules on APC are HLA, cluster of differentiation 14 (CD14), cluster of differentiation 40 (CD40), chemokine receptors and Toll-like receptors (TLRs). Chemokine receptors include C-C motif chemokine receptor 1 (CCR1), C-C motif chemokine receptor 3 (CCR3) and C-C motif chemokine receptor 5 (CCR5) and XCR1. The Toll-like receptors may for example include TLR-2, TLR-4 and/or TLR-5.

The polypeptide/dimeric protein/vaccine of the invention can be targeted to said surface molecules by means of the targeting unit which comprises or consists, for example, of one or more antibody binding regions with specificity for HLA, CD14, CD40, or Toll- like receptor; ligands, e.g. soluble CD40 ligand; natural ligands like chemokines, e.g. chemokine ligand 5, also called C-C motif ligand 5 (CCL5 or RANTES) or macrophage inflammatory protein alpha (CCL3 or MIP-1a/MIP1-α); chemokine motif ligand 1 or 2 (XCL1 or XCL2) or bacterial antigens like for example flagellin.

In one embodiment, the targeting unit has affinity for an MHC class II protein. Thus, in one embodiment the nucleotide sequence encoding the targeting unit encodes the antibody variable domains (VL and VH) with specificity for MHC class II proteins, selected from the group consisting of anti-HLA-DP, anti-HLA-DR and anti-pan HLA class II.

In another embodiment, the targeting unit has affinity for a surface molecule selected from the group consisting of CD40, TLR-2, TLR-4 and TLR-5. Thus, in one embodiment the nucleotide sequence encoding the targeting unit encodes the antibody variable domains (VL and VH) with specificity for anti-CD40, anti-TLR-2, anti-TLR-4 and anti-TLR-5. In one embodiment, the nucleotide sequence encoding the targeting unit encodes Flagellin. Flagellin has affinity for TLR-5.

Preferably, the targeting unit has affinity for a chemokine receptor selected from CCR1, CCR3 and CCR5. More preferably, the nucleotide sequence encoding the targeting unit encodes the chemokine human macrophage inflammatory protein alpha (hMIP-1alpha, also called LD78beta, and hereinafter also denoted (h)MIP1α and LD78β), which binds to its cognate receptors, CCR1 and CCR5 expressed on the cell surface of APCs.

The binding of the polypeptide/dimeric protein/vaccine of the invention to its cognate receptors leads to internalization in the APC and degradation of the proteins into small peptides that are

loaded onto MHC molecules and presented to CD4+ and CD8+ T cells to induce tumor specific immune responses. Once stimulated and with help from activated CD4+ T cells, CD8+ T cells will target and kill tumor cells expressing the same antigens.

In one embodiment of the present invention, the targeting unit comprises an amino acid sequence having at least 80% sequence identity to the amino acid sequence 24-93 of SEQ ID NO: 1. In a preferred embodiment, the targeting unit comprises an amino acid sequence having at least 85% sequence identity to the amino acid sequence 24-93 of SEQ ID NO:1, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity. In one embodiment, the targeting unit comprises the amino acid sequence 24-93 of SEQ ID NO: 1.

In a more preferred embodiment the targeting unit consists of an amino acid sequence having at least 80% sequence identity to the amino acid sequence 24-93 of SEQ ID NO:1, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as at least 100% sequence identity to the amino acid sequence 24-93 of SEQ ID NO:1.

The term “dimerization unit” as used herein, refers to a sequence of nucleotides or amino acids between the antigenic unit and the targeting unit. Thus, the dimerization unit serves to connect the antigenic unit and the targeting unit and facilitates dimerization of two monomeric polypeptides into a dimeric protein. Furthermore, the dimerization unit also provides the flexibility in the polypeptide/dimeric protein to allow optimal binding of the targeting unit to the surface molecules on the APCs, even if they are located at variable distances. The dimerization unit may be any unit that fulfils these requirements.

Accordingly, in one embodiment the dimerization unit may comprise a hinge region. In another embodiment, the dimerization unit comprises another domain that facilitates dimerization. In yet another embodiment, the dimerization unit comprises a hinge region and another domain that facilitates dimerization. In one embodiment, the hinge region and the other domain may be connected through a linker (dimerization unit linker). In yet another embodiment, the dimerization unit comprises a hinge region, a dimerization unit linker and another domain that facilitates dimerization, wherein the dimerization unit linker is located between the hinge region and the other domain that facilitates dimerization.

The term "hinge region" refers to a peptide sequence of the dimeric protein that facilitates the dimerization. In other words, the term "hinge region" refers to an amino acid sequence comprised in the dimerization unit that contributes to joining two of the polypeptides, i.e. contributes to the formation of a dimeric protein.

Moreover, the hinge region functions as a flexible spacer between the units allowing the two targeting units of the dimeric protein to bind simultaneously to two target molecules on APCs, even if they are expressed with variable distances. The hinge region may be Ig derived, such as derived from IgG3. The hinge region may comprise of one or more parts of an Ig-derived hinge region. The hinge region may contribute to the dimerization through the formation of covalent bond(s), e.g. disulfide bridge(s) between cysteines. Thus, in one embodiment the hinge region has the ability to form one or more covalent bonds. The covalent bond can for example be a disulfide bridge.

In one embodiment, the dimerization unit comprises a hinge exon h1 and hinge exon h4 (human hinge region 1 and human hinge region 4) having an amino acid sequence having at least 80 % sequence identity to the amino acid sequence 94-120 of SEQ ID NO: 2.

In a preferred embodiment, the dimerization unit comprises a hinge exon h1 and hinge exon h4 with an amino acid sequence having at least 85% sequence identity to the amino acid sequence 94-120 of SEQ ID NO: 2, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98% or such as at least 99% sequence identity. In a preferred embodiment, the dimerization unit comprises a hinge exon h1 and hinge exon h4 with the amino acid sequence 94-120 of SEQ ID NO: 2.

In one embodiment, the other domain that facilitates dimerization is an immunoglobulin domain, such as a carboxyterminal C domain (C domain), such as a CH1 domain, a CH2 domain or a carboxyterminal C domain (i.e. a CH3 domain), or a sequence that is substantially identical to the C domain or a variant thereof. Preferably, the other domain that facilitates dimerization is a carboxyterminal C domain derived from IgG. More preferably, the other domain that facilitates dimerization is a carboxyterminal C domain derived from IgG3.

In one embodiment, the dimerization unit comprises a carboxyterminal C domain derived from IgG3 with an amino acid sequence having at least 80 % sequence identity to the amino acid sequence 131-237 of SEQ ID NO: 2.

In a preferred embodiment, the dimerization unit comprises a carboxyterminal C domain derived from IgG3 with an amino acid sequence having at least 85% sequence identity to the amino acid sequence 131-237 of SEQ ID NO: 2, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98% or such as at least 99% sequence identity. In a preferred embodiment, the dimerization unit comprises a carboxyterminal C domain derived from IgG3 with the amino acid sequence 131-237 of SEQ ID NO: 2.

The immunoglobulin domain contributes to dimerization through non-covalent interactions, e.g. hydrophobic interactions. Thus, in one embodiment, the immunoglobulin domain has the ability to form dimers via noncovalent interactions. Preferably, the noncovalent interactions are hydrophobic interactions.

It is preferred that if the dimerization unit comprises a CH3 domain, it does not comprise a CH2 domain. Further, it is preferred that if the dimerization unit comprises a CH2 domain, it does not comprise a CH3 domain.

In a preferred embodiment, the dimerization unit consists of a polypeptide consisting of hinge exon h1 and hinge exon h4, a third linker (or dimerization unit linker) and a CH3 domain of human IgG3.

In one embodiment of the present invention, the dimerization unit comprises an amino acid sequence having at least 80 % sequence identity to the amino acid sequence 94-237 of SEQ ID NO: 2. In a preferred embodiment, the dimerization unit comprises an amino acid sequence having at least 85% sequence identity to the amino acid sequence 94-237 of SEQ ID NO: 2, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, or such as at least 99% sequence identity.

In a more preferred embodiment the dimerization unit consists of an amino acid sequence having at least 80% sequence identity to the amino acid sequence 94-237 of SEQ ID NO: 2, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% sequence identity to the amino acid sequence 94-237 of SEQ ID NO: 2.

In an even more preferred embodiment, the dimerization unit consists of the amino acid sequence 94-237 of SEQ ID NO: 2.

In one embodiment the linker connecting the hinge region to the other domain (the dimerization unit linker) is present in the dimerization unit. In another embodiment, the linker is present and is a G3S2G3SG linker. In an alternative embodiment, the dimerization unit linker is a glycine-serine rich linker, preferably GGGSSGGGSG, i.e. the dimerization unit comprises a glycine-serine rich dimerization unit linker and preferably the dimerization unit linker is GGGSSGGGSG. It is to be understood that the dimerization unit may have any orientation with respect to antigenic unit and targeting unit. In one embodiment, the antigenic unit is in the COOH- terminal end of the dimerization unit (e.g. via a unit linker) with the targeting unit in the N-terminal end of the dimerization unit. In another embodiment, the antigenic unit is in the N-terminal end of the dimerization unit with the targeting unit in the COOH-terminal end of the dimerization unit. It is preferred that the antigenic unit is in the COOH end of the dimerization unit.

In a preferred embodiment, the polynucleotide of the invention further comprises a nucleotide sequence encoding a signal peptide. The signal peptide is either located at the N-terminal end of the targeting unit or the C-terminal end of the targeting unit, depending on the orientation of the targeting unit in the polypeptide. The signal peptide is constructed to allow secretion of the polypeptide encoded by the polynucleotide in the cells transfected with said polynucleotide.

Any suitable signal peptide may be used. Examples of suitable peptides are an Ig VH signal peptide, such as SEQ ID NO: 9 a human TPA signal peptide, such as SEQ ID NO: 10 and a signal peptide comprising an amino acid sequence having at least 80 % sequence identity to the amino acid sequence 1-23 of SEQ ID NO:1. In an alternative embodiment, the signal peptide is a human MIP1-α signal peptide.

In a preferred embodiment, the signal peptide comprises an amino acid sequence having at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% sequence identity to the amino acid sequence 1-23 of SEQ ID NO:1. In an alternative embodiment, the signal peptides comprises the amino acid sequence 1-23 of SEQ ID NO:1.

In a more preferred embodiment, the signal peptide consists of an amino acid sequence having at least 80%, preferably at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%,

such as at least 97%, such as at least 98%, such as at least 99%, such as 100% sequence identity to the amino acid sequence 1-23 of SEQ ID NO:1. In an alternative embodiment, the signal peptides consists of the amino acid sequence 1-23 of SEQ ID NO:1.

Sequence identity may be determined as follows: A high level of sequence identity indicates likelihood that a second sequence is derived from a first sequence. Amino acid sequence identity requires identical amino acid sequences between two aligned sequences. Thus, a candidate sequence sharing 70% amino acid identity with a reference sequence requires that, following alignment, 70% of the amino acids in the candidate sequence are identical to the corresponding amino acids in the reference sequence. Identity may be determined by aid of computer analysis, such as, without limitations, the ClustalW computer alignment program (Higgins D., Thompson J., Gibson T., Thompson J.D., Higgins D.G., Gibson T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673-4680), and the default parameters suggested therein. Using this program with its default settings, the mature (bioactive) part of a query and a reference polypeptide are aligned. The number of fully conserved residues is counted and divided by the length of the reference polypeptide. In doing so, any tags or fusion protein sequences, which form part of the query sequence, are disregarded in the alignment and subsequent determination of sequence identity.

The ClustalW algorithm may similarly be used to align nucleotide sequences. Sequence identities may be calculated in a similar way as indicated for amino acid sequences.

Another preferred mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the FASTA sequence alignment software package (Pearson WR, Methods Mol Biol, 2000, 132:185-219). Align calculates sequence identities based on a global alignment. Align0 does not penalize to gaps in the end of the sequences. When utilizing the ALIGN and Align0 program for comparing amino acid sequences, a BLOSUM50 substitution matrix with gap opening/extension penalties of –12/-2 is preferably used.

The vaccine of the invention may comprise a polynucleotide as described above. The polynucleotide may comprise a DNA nucleotide sequence or an RNA nucleotide sequence, such as genomic DNA, cDNA, and RNA sequences, either double stranded or single stranded.

It is preferred that the polynucleotide is optimized to the species to express the polypeptide according to the invention, i.e. it is preferred that the polynucleotide sequence is human codon optimized.

The vaccine of the invention may further comprise a polypeptide encoded by the polynucleotide sequence as defined above. The polypeptide may be expressed *in vitro* for production of the vaccine according to the invention, or the polypeptide may be expressed *in vivo* as a result of administration of the polynucleotide as defined above to an individual/patient.

Due to the presence of the dimerization unit, dimeric proteins are formed when the polypeptide is expressed. The dimeric protein may be a homodimer, i.e. wherein the two polypeptide chains are identical and consequently comprise identical antigen sequences, or the dimeric protein may be a heterodimer comprising two different monomeric polypeptides encoded in the antigenic units. The latter may be relevant if the amount of antigen sequences exceeds an upper size limit for the antigenic unit. It is however preferred that the dimeric protein is a homodimeric protein.

In a fifth aspect, the invention relates to a vector comprising a polynucleotide comprising a nucleotide sequence encoding a targeting unit, a dimerization unit and an antigenic unit, wherein said antigenic unit comprises at least one patient-present shared antigen sequence or one or more parts thereof, and optionally one or more patient-specific antigen sequences or one or more parts thereof.

The vector is for transfecting a host cell and expression of a polypeptide/dimeric protein encoded by the polynucleotide described above, i.e. an expression vector, e.g. a DNA plasmid.

It is preferred that the vector allows for easy exchange of the various units described above, particularly the antigenic unit. In one embodiment, the vector may be a pUMVC4a vector or a vector comprising NTC9385R vector backbones. The antigenic unit may be exchanged with an antigenic unit cassette restricted by the SfiI restriction enzyme cassette where the 5' site is incorporated in the GLGGL/GLSGL linker and the 3' site is included after the stop codon in the vector.

In a sixth aspect, the invention relates to a host cell comprising a polynucleotide comprising a nucleotide sequence encoding a targeting unit, a dimerization unit and an antigenic unit, wherein said antigenic unit comprises at least one patient-present shared antigen sequence or one or more parts thereof, and optionally one or more patient-specific antigen sequences or one or more parts thereof.

In a seventh aspect, the invention relates to a host cell comprising a vector comprising a polynucleotide comprising a nucleotide sequence encoding a targeting unit, a dimerization unit

and an antigenic unit, wherein said antigenic unit comprises at least one patient-present shared antigen sequence or one or more parts thereof, and optionally one or more patient-specific antigen sequences or one or more parts thereof.

Suitable host cells include prokaryotes, yeast, insect or higher eukaryotic cells. In a preferred embodiment, the host cell is a human cell, preferably the cell of a cancer patient, more preferably the same cancer patient whose at least one patient-present shared antigen sequence or one or more parts thereof, and optionally whose one or more patient-specific antigen sequences or one or more parts thereof is derived from.

The vaccine according to the invention is an individualized therapeutic anticancer vaccine in the sense that the at least one patient-present shared antigen sequence and optionally one or more patient-specific antigen sequences are identified in the patient who will be vaccinated with said vaccine, in e.g. the patient's tumor tissue or body fluids such as blood.

Accordingly, in an eighth aspect the invention relates to a method for preparing an individualized therapeutic anticancer vaccine comprising an immunologically effective amount of the dimeric protein, or the polypeptide as defined above by producing the polypeptide *in vitro*.

The *in vitro* synthesis of the polypeptides and proteins may be carried out by any suitable method known to the person skilled in the art, such as by peptide synthesis or expression of the polypeptide in any of the suitable expressions systems known in the art, followed by purification.

Accordingly, in one embodiment the invention provides a method for preparing an individualized therapeutic anticancer vaccine comprising an immunologically effective amount of

(i) a dimeric protein consisting of two polypeptides encoded by a polynucleotide comprising a nucleotide sequence encoding a targeting unit, a dimerization unit and an antigenic unit, wherein said antigenic unit comprises at least one patient-present shared antigen sequence or one or more parts thereof, and optionally one or more patient-specific antigen sequences or one or more parts thereof; or

(ii) (ii) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence encoding a targeting unit, a dimerization unit and an antigenic unit, wherein said antigenic unit comprises at least one patient-present shared antigen sequence or one or more parts thereof, and optionally one or more patient-specific antigen sequences or one or more parts thereof by producing the dimeric protein or polypeptide *in vitro*, the method comprises

a) transfecting cells with the polynucleotide;

b) culturing the cells;

c) collecting and purifying the dimeric protein or the polypeptide expressed from the cells, and
d) mixing the dimeric protein or polypeptide obtained from step c) with a pharmaceutically acceptable carrier.

In a preferred embodiment, the dimeric protein or polypeptide from step c) is dissolved in said pharmaceutically acceptable carrier.

The pharmaceutically acceptable carrier is preferably an aqueous pharmaceutically acceptable carrier, such as water or a buffer. In one embodiment, the vaccine comprises further an adjuvant.

Purification may be carried out according to any suitable method, such as chromatography, centrifugation, or differential solubility.

In a ninth aspect the invention relates to a method for preparing an individualized therapeutic anticancer vaccine comprising an immunologically effective amount of polynucleotide as defined above *in vitro*.

Thus, in one embodiment, the invention provides a method for preparing an individualized therapeutic anticancer vaccine comprising an immunologically effective amount of a polynucleotide comprising a nucleotide sequence encoding a targeting unit, a dimerization unit and an antigenic unit, wherein said antigenic unit comprises at least one patient-present shared antigen sequence or one or more parts thereof, and optionally one or more patient-specific antigen sequences or one or more parts thereof, the method comprises

a. preparing the polynucleotide;
b. optionally cloning the polynucleotide into an expression vector and
c. mixing the polynucleotide from step a) or the vector form step b) with a pharmaceutically acceptable carrier.

The polynucleotide may be prepared by any suitable method known to the skilled person. For example, the polynucleotide may be prepared by chemical synthesis using an oligonucleotide synthesizer.

In particularly, smaller nucleotide sequences, such as for example nucleotide sequences encoding the targeting unit, the dimerization unit and/or parts of the antigenic unit may be synthesized individually and then ligated to produce the final polynucleotide for inclusion into the vector backbone.

For the design of the antigenic unit comprised in the individualized therapeutic anticancer vaccine, the method of preparing the vaccine is preceded by a method of identifying the antigen sequences (i.e. patient-present shared antigen sequences and optionally patient-specific antigen sequences) to be included into the antigenic unit.

The patient-present shared antigens may be identified in the (tumor) tissue or body fluid of the patient (obtained by methods known in the art) by methods known in the art, including:

- sequencing the patient's genome or exome and optionally searching by tailor-made software in whole genome/exome-seq data to e.g. identify mutated oncogenes or mutated tumor suppressor genes;
- immunohistochemistry of the patient's tumor tissue to detect the presence of mutated proteins;
- RT-PCR to detect the presence of viral antigens or known mutations in oncogenes;
- ELISA using antibodies against mutated tumor proteins in serum samples;
- RNA-seq of tumor tissue and comparison to healthy tissue to detect expression/over-expression of shared antigens;
- searching by tailor-made software in raw RNA sequence data to identify intron retention antigens;
- searching by tailor-made software in whole genome-seq data to identify transposable elements which are elements of dark matter antigens;
- detection of short repeats in raw whole exome/RNA sequence data to identify dark matter antigens;
- RNA-seq data to identify shared viral antigens; and
- comparing RNA-seq of the patient's tumor samples with either patient's own healthy tissue or a cohort/database (e.g. TCGA) versus GTEX/HPA gene expression data

In a preferred embodiment, the antigenic unit comprises at least one patient-present shared antigen sequence that is known to be immunogenic. In another preferred embodiment, the antigenic unit comprises one or more parts of at least one patient-present shared antigen sequence, e.g. one or multiple epitopes that are known to be immunogenic or are predicted to bind the particular patient's HLA alleles. If patient-specific antigen sequences are included in the antigenic unit, the antigenic unit preferably comprises patient-specific antigen sequences with predicted immunogenicity.

Thus, the identified patient-present shared antigens and patient-specific antigens may be further processed to find those sequences which renders the vaccine of the invention most effective, when such sequences are included into the antigenic unit. The way and sequence such processing is done depends on how said antigens were identified, i.e. the data that form the

basis for such processing. In one embodiment, the processing and selecting of the antigen sequences to be included in the vaccine of the invention is carried out as follows:

1) A search in the literature and/or in one or more databases is carried out to retrieve information about and sequences of shared antigens and preferably information about their expression pattern, immunogenicity, epitopes and HLA presentation. Such search is carried out to determine whether the identified antigen is a patient-present shared antigen or a patient-specific antigen.
2) If it was determined that the identified antigen is a patient-present shared antigen, the sequence thereof is studied to identify epitopes, preferably all epitopes, that are predicted to bind to patient-specific HLA class I and/or class II alleles. The patient's HLA class I and/or II alleles are determined, e.g. by sequencing normal tissue such as blood cells. The prediction may be carried out by prediction tools known in the art, i.e. prediction software known in the art, e.g. NetMHCpan and similar tools.
3) The most promising, i.e. the most immunogenic sequences showing predicted binding to one or more of the patient's HLA class I/II alleles, of the patient-present shared antigen are selected for inclusion into the antigenic unit. In one embodiment, a number of minimal epitopes is selected, e.g. if only a few promising epitopes were identified in step 2 or if longer stretches of non-immunogenic sequences are present between the epitopes. In another embodiment, a longer sequence is selected which comprises several epitopes that bind to patient's specific HLA alleles. In yet another embodiment, the full-length sequence is selected for inclusion into the antigenic unit.
4) Optionally, the most promising patient-specific antigen sequences, e.g. epitopes, are selected for inclusion into the antigenic unit based on predicted immunogenicity and binding to the patient's HLA class I and/or class II alleles of such sequences.

If patient-specific antigen sequences are to be included in the antigenic unit, once such antigens are identified and the patient's HLA class I and/or II alleles are determined, the next step is to select the most promising sequences thereof, e.g. epitopes, based on predicted immunogenicity and binding to the patient's HLA class I and/or class II alleles of such sequences.

Tumor mutations are discovered by sequencing of tumor and normal tissue and make a comparison of the obtained sequences. A variety of methods are available for detecting the presence of a particular mutation or allele in an individual's DNA or RNA. For example, techniques including dynamic allele- specific hybridization (DASH), microplate array diagonal gel electrophoresis (MADGE), pyrosequencing, oligonucleotide- specific ligation, the TaqMan system as well as various DNA "chip" technologies such as the Affymetrix SNP chips may be applied. Alternatively, a method for identifying mutations by direct protein sequencing may be carried out.

Out of the maybe hundreds or thousands of mutations in the tumor exome, the most promising sequences are selected *in silico* on the basis of predictive HLA-binding algorithms. The intention is to identify all relevant epitopes and after a ranking or scoring, determine the sequences to be included in the antigenic unit.

Any suitable algorithm may be used, such as one of the following:
Available free software analysis of peptide-MHC binding (IEDB and NetMHCpan) may be downloaded from the following websites:
http://www.iedb.org/
http://www.cbs.dtu.dk/services/NetMHC/

Commercially available advanced software to predict optimal sequences for vaccine design are found here:
http://www.oncoimmunity.com/
https://omictools.com/t-cell-epitopes-category
https://github.com/griffithlab/pVAC-Seq
http://crdd.osdd.net/raghava/cancertope/help.php
http://www.epivax.com/tag/neoantigen/

Each mutation is scored with respect to its antigenicity, and the most antigenic epitopes are selected and optimally arranged in the antigenic unit.

Thus, in one embodiment, the invention provides a method for preparing an individualized therapeutic anticancer vaccine comprising the steps of:

a) identifying at least one patient-present shared antigen in the tumor tissue or body fluid of a patient
b) determining the patient's HLA class I and/or class II alleles
c) predicting the immunogenicity of the identified at least one antigen or one or more parts thereof by their predicted binding to the patient's HLA class I and/or II alleles
d) selecting at least one antigen or one or more parts thereof based on their immunogenicity predicted in step c); and
e) preparing a polynucleotide sequence comprising an antigenic unit comprising a nucleotide sequence encoding the at least one antigen or one or more parts thereof selected in step d).

In another embodiment, the invention provides a method for preparing an individualized therapeutic anticancer vaccine comprising the steps of:

a) identifying at least one patient-present shared antigens in the tumor tissue or body fluid of a patient and identifying one or more patient-specific antigens in the tumor tissue of said patient;
b) determining the patient's HLA class I and/or class II alleles;
c) predicting the immunogenicity of the identified at least one patient-present shared antigens or parts thereof and the identified one or more patient-specific antigens or one or more parts thereof by their predicted binding to the patient's HLA class I and/or II alleles;
d) selecting at least one patient-specific shared antigens or one or more parts thereof and one or more patient-specific antigens or one or more parts thereof based on their immunogenicity predicted in step c); and
e) preparing a polynucleotide sequence comprising an antigenic unit comprising a nucleotide sequence encoding the at least one patient-specific shared antigens or one or more parts thereof and one or more patient-specific antigens or one or more parts thereof selected in step d).

In one embodiment, the polynucleotide sequence prepared in step e) further comprises nucleotide sequences encoding a targeting unit as described herein and a dimerization unit as described herein.

In a preferred embodiment, the prepared polynucleotide sequence is cloned into an expression vector. In yet another preferred embodiment, the polynucleotide sequence of step e) is cloned into an expression vector comprising nucleotide sequences encoding the dimerization unit and the targeting unit.

In yet another embodiment, the polynucleotide or the vector is mixed with a pharmaceutically acceptable carrier.

The final vaccine is then produced to comprise one of the following:

- the polynucleotide as defined above
- the polypeptide encoded by the polynucleotide as defined above
- the dimeric protein comprising to polypeptide chains encoded by the polynucleotide as defined above

The vaccine further comprises a pharmaceutically acceptable carrier and may further comprise other pharmaceutically acceptable excipients, e.g. stabilizers, adjuvants, buffers and the like.

Pharmaceutically acceptable carriers include, but are not limited to, saline, buffered saline, such as PBS, dextrose, water, glycerol, ethanol, sterile isotonic aqueous buffer(s), and combinations thereof.

In particularly for vaccines comprising polypeptides/proteins, pharmaceutically acceptable excipients include, but are not limited to poly-ICLC, 1018 ISS, aluminum salts, Amplivax, AS 15, BCG, CP-870,893, CpG7909, CyaA, dSLIM, GM-CSF, IC30, IC31, Imiquimod, ImuFact EV1 P321, IS Patch, ISS, ISCOMATRIX, Juvlmmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, OK-432, OM-174, OM-197-MP-EC, ONTAK, PepTel.RTM, vector system, PLGA microparticles, resiquimod, SRL172, virosomes and other virus-like particles, YF-17D, VEGF trap, R848, beta-glucan, Pam3Cys, Aquila's QS21 stimulon, vadimezan, and/or AsA404 (DMXAA).

Particularly for vaccines comprising polynucleotides comprised in an expression vector, the vaccine may comprise molecules that ease the transfection of cells and/or adjuvants in the form of plasmids comprising nucleotide sequences encoding chemokines or cytokines in order to enhance the immune response.

The vaccine is formulated into any suitable formulation for administration to the patient, such as a liquid formulation for intradermal or intramuscular injection.

The vaccine may be administered in any way suitable for either a polypeptide/protein vaccine or a polynucleotide vaccine, such as administered by injection intradermally, intramuscularly, subcutaneously, or by mucosal or epithelial application, such as intranasally, orally, enterally or to the bladder.

In particularly the vaccine is preferably administered intramuscularly or intradermally when the vaccine is a polynucleotide vaccine.

In one embodiment the vaccine is administered by intranodal injection. As used herein, the term “intranodal injection” means that the vaccine is injected into the lymph nodes.

The individualized therapeutic anticancer vaccine of the invention prepared by the methods described above may be obtained within less than 12 weeks, e.g. within less than 9 weeks or less than 8 weeks or less than 6 weeks or within 4 weeks.

The cancer treated may be any cancer, e.g. a cancer wherein the cancer cells comprise alterations resulting in a shared cancer antigen and optionally a patient-specific cancer antigen.

The cancer may be a primary tumor, metastasis or both. The tumor examined for alterations may be a primary tumor or a metastasis. In one embodiment, the cancers to be treated are cancers known to have a high antigen load, such as melanomas, lung cancer, renal, head and neck or bladder cancer. In another embodiment, the cancer treated is a solid cancer or liquid cancer. Examples of solid cancers are cancers forming a solid mass, e.g. a tumor. Examples of liquid cancers are cancers present in body fluid, such as lymphomas or blood cancers. Examples of cancers that can be treated with vaccine of the invention are breast cancer, ovarian cancer, colon cancer, prostate cancer, bone cancer, colorectal cancer, gastric cancer, lymphoma, malignant melanoma, liver cancer, small cell lung cancer, non-small cell lung cancer, pancreatic cancer, thyroid cancers, kidney cancer, cancer of the bile duct, brain cancer, cervical cancer, bladder cancer, esophageal cancer, Hodgkin's disease and adrenocortical cancer.

In a preferred embodiment the treatment is performed with a vaccine comprising the polynucleotide as described above, for example wherein the polynucleotide is DNA or RNA, preferably comprised in a vector.

It is preferred to inject the polynucleotide vaccine of the invention intramuscularly, such as in the big muscles, for example in the shoulder, buttock or thigh. It has been found that the polypeptide/dimeric protein of the invention is produced locally, and relevant immune cells internalize the polypeptide/dimeric protein essentially at the site of their production, i.e. substantially no polypeptide/dimeric protein reaches the blood stream.

Any suitable method for injecting the polynucleotide vaccine may be used, such as by the use of a jet injector or assisted by electroporation.

The vaccine may be administered as a single dosage, or administration may be repeated. When the vaccine administration is repeated, it is preferred that it is administered with at least 3 weeks intervals, to avoid exhaustion of the T cells.

Accordingly, in one embodiment the dosage regimen is vaccination in week 0, 3 and 6 and then every 4 weeks as long as the patient has a clinical benefit. The vaccine may be administered for as long as a year.

The vaccine comprises an immunologically effective amount of the polynucleotide/polypeptide/dimeric protein. By “immunologically effective amount” is meant the amount of the aforementioned compounds required to elicit an immune response in the patient being vaccinated with such compounds. Non-limiting parameters that indicate such an immune response include one or more of the following: stop the growth of a tumor and/or stop its

spreading and/or reduce the size of a tumor, reduction in disease progression or stable disease, i.e. the cancer does progress at a slower rate or does not progress. This includes that a tumor does grow at a slower rate or does not grow and/or does spread slower or does not spread, e.g. to lymph nodes or forming metastases and/or does not become more aggressive. Other non-limiting parameters that indicate such an immune response are tumor shrinkage (in terms of weight and/or volume); a decrease in the number of individual tumor colonies; tumor elimination; and progression-free survival. Ultimately, the physician determines the dosage which may vary and depend on the age, weight, and general condition of the patient being treated, the severity of the cancer being treated, the judgment of the physician and the particular nature and properties the individualized vaccine of the invention. In one embodiment, the dosage typically is in the range of 0.3-6 mg for DNA vaccines, and in the range of 5 µg-5 mg for polypeptide/protein vaccines.

In a tenth aspect, the invention provides a method of treating cancer in a patient, the method comprising administering to the patient an individualized therapeutic anticancer vaccine comprising an immunologically effective amount of:

(i) a polynucleotide comprising a nucleotide sequence encoding a targeting unit, a dimerization unit and an antigenic unit, wherein said antigenic unit comprises at least one shared antigen sequence present in said patient or one or more parts thereof and optionally one or more antigen sequences specific for said patient or one or more parts thereof; or

(ii) a polypeptide encoded by the polynucleotide as defined in (i); or

(iii) a dimeric protein consisting of two polypeptides encoded by the polynucleotide as defined in (i); and

a pharmaceutically acceptable carrier.

Thus, the invention provides a method of treating cancer in a patient, the method comprising administering to the patient an individualized therapeutic anticancer vaccine according to the invention, which has been prepared specifically for the patient.

Alternatively, the invention provides an individualized therapeutic anticancer vaccine comprising an immunologically effective amount of:

(i) a polynucleotide comprising a nucleotide sequence encoding a targeting unit, a dimerization unit and an antigenic unit, wherein said antigenic unit comprises at least one patient-present shared antigen sequence or one or more parts thereof and optionally one or more patient-specific antigen sequences or one or more parts thereof; or

(ii) a polypeptide encoded by the polynucleotide as defined in (i); or

(iii) a dimeric protein consisting of two polypeptides encoded by the polynucleotide as defined in (i); and

a pharmaceutically acceptable carrier for use in a method of treating cancer in a patient, wherein the vaccine has been specifically prepared for the patient.

Further, the invention provides the use of

(i) a polynucleotide comprising a nucleotide sequence encoding a targeting unit, a dimerization unit and an antigenic unit, wherein said antigenic unit comprises at least one patient-present shared antigen sequence or one or more parts thereof and optionally one or more patient-specific antigen sequences or one or more parts thereof; or

(ii) a polypeptide encoded by the polynucleotide as defined in (i); or

(iii) a dimeric protein consisting of two polypeptides encoded by the polynucleotide as defined in (i); for the manufacture of a medicament for the treatment of cancer in a patient, wherein the polynucleotide, polypeptide or dimeric protein has been specifically prepared for the patient.

The vaccine treatment according to the present invention may be combined with any other anticancer treatment, such as radiation therapy, chemotherapy, and surgical treatment.

The vaccine treatment according to the invention may also be combined with checkpoint-blockade inhibitor treatment.

Description of the drawings

Fig. 1 shows the amino acid sequence of HPV16 E6 and all epitopes predicted to bind to the HLA class I alleles of patient 1. The two underlined sequences constitute HLA class I optimized sequences for inclusion into the antigenic unit of a therapeutic anticancer vaccine individualized for patient 1.

Fig. 2 shows the amino acid sequence of HPV16 E6 and all epitopes predicted to bind to the HLA class I and HLA class II alleles of patient 1. The sequence in the box constitutes an HLA class I/HLA class II optimized sequence for inclusion into the antigenic unit of a therapeutic anticancer vaccine individualized for patient 1.

Fig. 3 shows the amino acid sequence of HPV16 E7 and all epitopes predicted to bind to the HLA class I alleles of patient 1. The underlined sequence constitutes an HLA class I optimized sequence for inclusion into the antigenic unit of a therapeutic anticancer vaccine individualized for patient 1.

Fig. 4 shows the amino acid sequence of HPV16 E7 and all epitopes predicted to bind to the HLA class I and HLA class II alleles of patient 1. The sequences in the two boxes constitute

HLA class I/HLA class II optimized sequences for inclusion into the antigenic unit of a therapeutic anticancer vaccine individualized for patient 1.

Fig. 5 shows the amino acid sequence of HPV16 E6 and all epitopes predicted to bind to the HLA class I alleles of patient 2. The underlined sequence constitutes an HLA class I optimized sequence for inclusion into the antigenic unit of a therapeutic anticancer vaccine individualized for patient 2.

Fig. 6 shows the amino acid sequence of HPV16 E6 and all epitopes predicted to bind to the HLA class I and HLA class II alleles of patient 2. The sequence in the box constitutes an HLA class I/HLA class II optimized sequence for inclusion into the antigenic unit of a therapeutic anticancer vaccine individualized for patient 2.

Fig. 7 shows the amino acid sequence of HPV16 E7 and all epitopes predicted to bind to the HLA class I alleles of patient 2. The underlined sequence constitutes an HLA class I optimized sequence for inclusion into the antigenic unit of a therapeutic anticancer vaccine individualized for patient 2.

Fig. 8 shows the amino acid sequence of HPV16 E7 and all epitopes predicted to bind to the HLA class I and HLA class II alleles of patient 2. The sequences in the two boxes constitute HLA class I/HLA class II optimized sequences for inclusion into the antigenic unit of a therapeutic anticancer vaccine individualized for patient 2.

Fig. 9 shows the immunogenicity of DNA vaccines (constructs) VB4097, VB4100 and VB4105 in mice vaccinated with these constructs by way of measuring the IFN-γ immune responses from T cells (total T cell response), compared to the negative control VB1026.

Fig. 10 shows the immunogenicity of DNA vaccines (constructs) VB4100, VB4101 and VB4102 in mice vaccinated with these constructs by way of measuring the IFN-γ immune responses from CD8+ T cells, compared to the negative control VB1026.

Fig. 11 shows the immunogenicity of DNA vaccines (constructs) VB4100 and VB4102 in mice vaccinated with these constructs by way of measuring the IFN-γ immune responses from A) T cells (total T cell response), B) CD8+ T cells and C) CD4+ T cells, compared to the negative control VB1026.

Fig. 12 shows the immunogenicity of DNA vaccines (constructs) VB4118, VB4119, VB4121, VB4127, VB4128 and VB4130 in mice vaccinated with these constructs by way of measuring the IFN-γ immune responses from T cells, compared to the negative control VB1026.

Examples

EXAMPLE 1: Design of the antigenic unit comprised in the polynucleotide/polypeptide/dimeric protein and vaccine of the invention

The antigenic unit may be designed with the following variations in the patient-present shared antigen sequence:

A. Full-length sequence
B. HLA-optimized sequences. The selected sequence for inclusion into the antigenic unit is optimized to cover the most immunogenic epitopes, i.e. those having a high binding affinity to the patient's HLA I and/or HLA II molecules
C. Epitopes with predicted binding to the patient's HLA I and/or HLA II molecules.
D. Combination of A+C
E. Combination of B+C
F. Combination of A+B

The patient-present shared antigen sequence above may for instance be HPV16. In cases D and F, which include a full-length sequence, case D may for instance be the combination of HPV16 E7 as a full-length sequence (A) and epitopes from HPV E6 (C) while case F may for instance be the combination of HPV16 E7 as a full-length sequence (A) and HLA-optimized sequences (B). The aforementioned example includes one single patient-present shared antigen but two different regions thereof. In another embodiment, cases D and F relate to the combination of the full-length sequence (A) of a first patient-present shared antigen, e.g. HPV16 E7 and epitopes (C) or HLA-optimized sequences (B) of a second patient-shared antigen, e.g. KRAS.

Thus, the antigenic unit may comprise A-F from one patient-present shared antigen or comprise A-F from several patient-present shared antigens.

Sequences A-F are arranged in the antigenic unit according to the methods provided herein. Different antigenic unit designs may be evaluated in animal models, e.g. as described in Example 3 to determine the optimal antigenic unit design. Breadth, strength and kinetics for the antigen-specific immunogenicity can be determined by IFN-gamma ELISPOT analysis. Anti-tumor efficacy can be tested in a tumor challenge experiment.

EXAMPLE 2: **Construction of a polynucleotide according to the invention**

A polynucleotide according to the invention is designed and comprises the following units and components:

1: Native leader sequence for human LD78b	Signal peptide
2: Full-length LD78b sequence	Targeting unit
3: Human hinge-region 1 from IgG3 4: Human hinge region 4 from IgG3 5: Glycine-Serine linker 6: Human CH3 domain from IgG3	Dimerization unit
7: Glycine-Leucine linker	Unit linker
8: Patient-present shared antigen sequence(s) 9: Optionally: patient-specific antigen sequence(s)	Antigenic unit

Table 1

EXAMPLE 3: Selecting sequences of patient-present shared antigens for inclusion into the antigenic unit comprised in the polynucleotides/polypeptides/dimeric peptides and vaccines according to the invention

Blood samples and tumor tissue samples of two patients – patient 1 and 2 - presenting with squamous cell carcinoma of the head and neck were obtained. The blood sample was analyzed for exome sequencing to characterize the exons in the healthy cells. The tumor tissue sample was analyzed for exome sequencing to characterize the exons in the tumor tissue and RNA-seq to evaluate RNA expression level of each gene. The presence of HPV16 shared antigen was identified using anti-HPV16 antibodies in an ELISA.

To find the most immunogenic sequences, each patients HLA class I and II alleles were determined by sequencing normal tissue (blood cells). The following HLA class I and II alleles were found:

Patient 1 - HLA class I alleles	**Patient 1 - HLA class II alleles**
HLA-A01:01	DRB1_0701
HLA-A24:02	DRB1_1301
HLA-B08:01	DRB3_0202
HLA-B40:01	DRB4_0101
HLA-C03:04	HLA-DQA10103-DQB10202
HLA-C07:01	HLA-DQA10103-DQB10603
	HLA-DQA10201-DQB10202
	HLA-DQA10201-DQB10603

Patient 2 -HLA class I alleles	Patient 2 - HLA class II alleles
HLA-A01:01	DRB1_0101
HLA-A24:02	DRB1_1301
HLA-B40:01	DRB3_0202
HLA-C03:04	HLA-DPA10103-DPB10401
	HLA-DQA10101-DQB10501
	HLA-DQA10101-DQB10603
	HLA-DQA10103-DQB10501
	HLA-DQA10103-DQB10603

Table 2

HPVs have circular, double-stranded DNA genomes that are approximately 8 kb in size and encode eight genes, of which E6 and E7 have transforming properties. The viral E6 and E7 proteins are known to be involved in conversion of healthy cells into malignant cells. The abilities of HPV16 E6 and E7 proteins to associate with the tumor suppressors p53 and pRB, respectively, have been suggested as a mechanism by which these viral proteins induce tumors. Thus, the E6 and E7 sequences of HPV16 are known shared tumor antigens and were selected for finding sequences therein for inclusion into the antigenic unit. Prediction of binding to the patients' HLA class I and HLA class II alleles was carried out using the NetMHCpan 4.0 software.

Patient 1:

HPV 16 E6:

A total of 16 epitopes, each 9 amino acids long, were predicted to bind to HLA class I alleles of patient 1 and a total of 16 epitopes, each 9 amino acids long, were predicted to bind to HLA class II alleles of patient 1 (Fig. 2 and table 3)

Thus, an antigenic unit for inclusion into an individualized therapeutic anticancer vaccine for patient 1 is designed, which may comprise:

A. The full-length HPV16 E6 sequence (151 amino acids)
B. An HLA class I optimized sequence comprising some of the 16 epitopes, e.g. the 12 underlined epitopes shown on the left side of Fig. 1. This sequence contains 65 amino acids, i.e. 43% of the full-length sequence.
C. Two HLA class I optimized sequences, wherein the first sequence comprises 12 of the 16 epitopes and the second sequence comprises 3 of the epitopes (Fig. 1). Thus, the two sequences combined include 15 of the 16 epitopes and contain 92 amino acids, i.e. 61% of the full-length sequence.

D. An HLA class I/HLA class II optimized sequence, comprising 12 of the 16 HLA class I epitopes and 9 of the 16 HLA class II epitopes, thus including 21 of the 32 HLA class I/class II epitopes (Fig. 2, box). This sequence contains 65 amino acids, i.e. 43% of the full-length sequence.

Inclusion of C or D into the antigenic unit is preferred.

HPV E6: MFQDPQERPRKLPQLCTELQTTIHDIILECVYCKQQLLRREVYDFARRDLCIVYRDGNPYAV RDKCLKFYSKISEYRHYCYSLYGTTLEQQYNKPLCDLLIRCINRQKPLCPEEKQRHLDKKQR FHNIRGRWTGRCMSCCRSSRTRRETQL	
Epitope corresponding to amino acids in full length sequence	Sequence of epitope
6-15	QERPRKLPQ
8-17	RPRKLPQLC
23-32	IHDIILECV
24-33	HDIILECVY
26-35	IILECVYCK
35-44	QQLLRREVY
42-51	VYDFARRDL
43-52	YDFARRDLC
44-53	DFARRDLCI
45-54	FARRDLCIV
60-69	YAVRDKCLK
62-71	VRDKCLKFY
81-90	YSLYGTTLE
125-134	FHNIRGRWT
131-140	RWTGRCMSC
143-152	SRTRRETQL
16-31	CTELQTTIHDIILEC
17-32	TELQTTIHDIILECV
18-33	ELQTTIHDIILECVY
19-34	LQTTIHDIILECVYC
20-35	QTTIHDIILECVYCK
34-49	KQQLLRREVYDFARR
49-64	DLCIVYRDGNPYAVR
50-65	LCIVYRDGNPYAVRD
51-66	CIVYRDGNPYAVRDK

78-93	HYCYSLYGTTLEQQY
95-110	PLCDLLIRCINRQKP
96-111	LCDLLIRCINRQKPL
97-112	CDLLIRCINRQKPLC
98-113	DLLIRCINRQKPLCP
99-114	LLIRCINRQKPLCPE
121-136	KKQRFHNIRGRWTGR

Table 3

HPV16 E7:

A total of 9 epitopes, each 9 amino acids long, were predicted to bind to HLA class I alleles of patient 1 and a total of 12 epitopes, each 9 amino acids long, were predicted to bind to HLA class II alleles of patient 1 (Fig. 4 and table 4)

Thus, an antigenic unit for inclusion into an individualized therapeutic anticancer vaccine for patient 1 is designed, which may comprise:

E. The full length HPV16 E7 sequence (98 amino acids)
F. An HLA class I optimized sequence comprising some of the 9 epitopes, e.g. the underlined epitopes shown in Fig. 3. This sequence contains 56 amino acids, i.e. 57% of the full-length sequence
G. Two HLA class I/HLA class II optimized sequences, wherein the first sequence comprises 2 of the 9 HLA class I epitopes and 7 of the 16 HLA class II epitopes and the second sequence comprises 4 of the 9 HLA class I epitopes and 5 of the 16 HLA class II epitopes (Fig. 4, boxes). Thus, the two sequences combined include 6 of the 9 HLA class I epitopes and all of the HLA class II epitopes. The two sequences combined contain 54 amino acids, i.e. 55% of the full-length sequence.

Inclusion of F or G into the antigenic unit is preferred.

Based on the above, an antigenic unit for inclusion into an individualized therapeutic anticancer vaccine for patient 1 is designed, which comprises at least one of C, D, F and G or all of C, D, F and G or any combination thereof between these two described extrema.

HPV E7: MHGDTPTLHEYMLDLQPETTDLYGYGQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFCCKC DSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP	
Epitope corresponding to amino acids in full length sequence	Sequence of epitope
7-16	TLHEYMLDL
22-31	LYGYGQLND

38-47	IDGPAGQAE
48-57	DRAHYNIVT
55-64	VTFCCKCDS
66-75	RLCVQSTHV
72-81	THVDIRTLE
73-82	HVDIRTLED
85-94	GTLGIVCPI
4-19	DTPTLHEYMLDLQPE
5-20	TPTLHEYMLDLQPET
6-21	PTLHEYMLDLQPETT
7-22	TLHEYMLDLQPETTD
8-23	LHEYMLDLQPETTDL
9-24	HEYMLDLQPETTDLY
10-25	EYMLDLQPETTDLYG
70-85	QSTHVDIRTLEDLLM
71-86	STHVDIRTLEDLLMG
72-87	THVDIRTLEDLLMGT
73-88	HVDIRTLEDLLMGTL
74-89	VDIRTLEDLLMGTLG

Table 4

Patient 2:

HPV16 E6

A total of 14 epitopes, each 9 amino acids long, were predicted to bind to HLA class I alleles of patient 2 and a total of 14 epitopes, each 9 amino acids long, were predicted to bind to HLA class II alleles of patient 2 (Fig. 6 and table 5)

Thus, an antigenic unit for inclusion into an individualized therapeutic anticancer vaccine for patient 2 is designed, which may comprise;

H. The full-length HPV16 E6 sequence (151 amino acids)

I. An HLA class I optimized sequence comprising some of the 14 epitopes, e.g. the 11 underlined epitopes shown in Fig. 5. This sequence contains 57 amino acids, i.e. 38% of the full-length sequence.

J. An HLA class I/HLA class II optimized sequence, comprising 11 of the 14 HLA class I epitopes and 8 of the 14 HLA class II epitopes, thus including 19 of the 28 HLA class I/class II epitopes (Fig. 6, box). This sequence contains 59 amino acids, i.e. 39% of the full-length sequence.

Inclusion of J into the antigenic unit is preferred.

HPV E6: MFQDPQERPRKLPQLCTELQTTIHDIILECVYCKQQLLRREVYDFARRDLCIVYRDGNPYAV RDKCLKFYSKISEYRHYCYSLYGTTLEQQYNKPLCDLLIRCINRQKPLCPEEKQRHLDKKQR FHNIRGRWTGRCMSCCRSSRTRRETQL	
Epitope corresponding to amino acids in full length sequence	Sequence of epitope
16-25	CTELQTTIH
42-51	VYDFARRDL
45-54	FARRDLCIV
52-61	IVYRDGNPY
54-63	YRDGNPYAV
68-77	KFYSKISEY
73-82	ISEYRHYCY
75-84	EYRHYCYSL
80-89	CYSLYGTTL
84-93	YGTTLEQQY
88-97	LEQQYNKPL
91-100	QYNKPLCDL
11-20	RHLDKKQRF
12-21	RFHNIRGRW
18-33	ELQTTIHDIILECVY
49-64	DLCIVYRDGNPYAVR
50-65	LCIVYRDGNPYAVRD
51-66	CIVYRDGNPYAVRDK
74-89	SEYRHYCYSLYGTTL
75-90	EYRHYCYSLYGTTLE
76-91	YRHYCYSLYGTTLEQ
77-92	RHYCYSLYGTTLEQQ
78-93	HYCYSLYGTTLEQQY
96-111	LCDLLIRCINRQKPL
97-112	CDLLIRCINRQKPLC
98-113	DLLIRCINRQKPLCP
99-114	LLIRCINRQKPLCPE
121-136	KKQRFHNIRGRWTGR

Table 5

HPV16 E7

A total of 10 epitopes, each 9 amino acids long, were predicted to bind to HLA class I alleles of patient 2 and a total of 11 epitopes, each 9 amino acids long, were predicted to bind to HLA class II alleles of patient 2 (Fig. 8 and table 6)

Thus, an antigenic unit for inclusion into an individualized therapeutic anticancer vaccine for patient 2 may be designed which comprises:

A. The full length HPV16 E7 sequence (98 amino acids)
B. An HLA class I optimized sequence comprising some of the 10 epitopes, e.g. the 6 underlined epitopes shown in Fig. 7. This sequence contains 26 amino acids, i.e. 27% of the full-length sequence.
C. Two HLA class I/HLA class II optimized sequences, wherein the first sequence comprises 6 of the 10 HLA class I epitopes and 6 of the 11 HLA class II epitopes and the second sequence comprises 2 of the 10 HLA class I epitopes and 5 of the 11 HLA class II epitopes (Fig. 8, boxes). Thus, the two sequences combined include 8 of the 11 HLA class I epitopes and all of the HLA class II epitopes. The two sequences combined contain 45 amino acids, i.e. 45% of the full-length sequence.

Inclusion of L or M into the antigenic unit is preferred.

Based on the above, an antigenic unit for inclusion into an individualized therapeutic anticancer vaccine for patient 2 is designed, which comprises at least one of J, L and M or all of J, L and M or any combination thereof between these two described extrema.

HPV E7: MHGDTPTLHEYMLDLQPETTDLYGYGQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFCCKC DSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP	
Epitope corresponding to amino acids in full length sequence	Sequence of epitope
3-12	GDTPTLHEY
7-16	TLHEYMLDL
9-18	HEYMLDLQP
15-24	LQPETTDLY
19-28	TTDLYGYGQ
20-29	TDLYGYGQL
44-53	QAEPDRAHY
49-58	RAHYNIVTF
71-80	STHVDIRTL
79-88	LEDLLMGTL
3-18	GDTPTLHEYMLDLQP

4-19	DTPTLHEYMLDLQPE
5-20	TPTLHEYMLDLQPET
6-21	PTLHEYMLDLQPETT
7-22	TLHEYMLDLQPETTD
8-23	LHEYMLDLQPETTDL
70-85	QSTHVDIRTLEDLLM
71-86	STHVDIRTLEDLLMG
72-87	THVDIRTLEDLLMGT
73-88	HVDIRTLEDLLMGTL
74-89	VDIRTLEDLLMGTLG

Table 6

Comparing patient 1 and 2, i.e. Fig. 2 and 6 and Fig. 4 and 8, it is apparent that the optimal sequences for inclusion into a vaccine according to the invention differ considerably between the two patients.

EXAMPLE 4: **Comparing vaccines comprising patient-specific antigens and patient-present shared antigens**

To compare the efficacy of vaccines comprising only patient-specific antigen sequences with vaccines according to the invention comprising patient-present shared antigen sequences and optionally patient-specific antigen sequences, a mouse TC-2 tumor model is used.

Shared antigens and specific antigens present in the TC-2 tumor cell line are identified, processed and selected as described herein, i.e. shared antigen sequences for inclusion into the antigenic unit are selected based on their binding to MHC molecules while specific antigen sequences are selected based on additional parameters in an *in silico* predicted immunogenicity method. The shared antigen which is selected for inclusion into the antigenic unit is the viral antigen HPV16 and sequences encoding parts of the E6 and E7 proteins thereof were selected.

All selected antigen sequences are ordered from a commercial supplier, e.g. from Genscript (New Jersey, US) and cloned into the expression vector pUMVC4a, which comprises sequences encoding the LD78beta targeting unit and the hIgG3 dimerization unit.

The antigenic unit of vector 1 only comprises shared antigen sequences while the antigenic unit of vector 2 comprises both shared antigen sequences and specific antigen sequences.

To verify correct vaccibody formation, HEK293 cells are transfected with the vectors and vaccibody proteins in the supernatant are identified by Western blot and/or sandwich ELISA. The empty pUMVC4a vector is included as a negative control. Intact homodimeric protein formation is confirmed as follows: the proteins in the supernatant from transfected cells are detected in a Western blot by an anti-hMIP-1alpha antibody, in either the presence of reducing agents, which result in dimeric proteins being reduced to monomers, or absence of reducing agents.

A vaccine is prepared by mixing 20 µg of the vector 1 and 2, respectively, with an aqueous buffer. The vaccine is injected intramuscularly in the tibial anterior muscle of the mouse followed by electroporation using TriGrid, Ichor, (US). At day 13, the mice are euthanized, and spleens are harvested.

The T cell responses are evaluated by IFN-gamma ELISpot. We observe that vaccines according to the present invention induce broader T cell responses that are higher compared to vaccines comprising only specific antigen sequences.

EXAMPLE 5: Individual therapeutic anticancer DNA vaccine according to the invention

An individual therapeutic anticancer DNA vaccine according to the invention may be prepared by GMP manufacturing of the vector comprising the polynucleotide according to the invention according to regulatory authorities' guidelines, and Fill & Finish of the DNA vaccine. The vector may be formulated by dissolving it in a sterile saline solution, such as PBS, at a concentration of 2-6 mg/ml. The vaccine may be administered either intradermally or intramuscularly with or without following electroporation or may alternatively be administered with a jet injector.

EXAMPLE 6: Selecting sequences of patient-present shared antigens (and patient-specific antigens) for inclusion into the antigenic unit comprised in the polynucleotides/polypeptides/dimeric peptides and vaccines according to the invention

Blood samples and tumor tissue samples of three patients (patient 1, 2 and 3) presenting with squamous cell carcinoma of the head and neck were obtained. The blood sample was analyzed for exome sequencing to characterize the exons in the healthy cells. The tumor tissue sample was analyzed for exome sequencing to characterize the exons in the tumor tissue and RNA-seq to evaluate RNA expression level of each gene.

The presence of HPV16 shared antigen was identified using PCR. The ectopic expression of NY-ESO-1 was determined by anti-NY-ESO-1 antibodies in an ELISA. Patient-specific antigen

sequences for each patient were identified as previously described in this application and in WO 2017/118695, which is included herein by reference.

NY-ESO-1 (also known as cancer/testis antigen 1B) is a protein belonging to the family of cancer-testis antigens (CTAs) that have been found to be re-expressed in a variety of malignant tumors at the mRNA and protein levels, while its normal expression in adult tissue is restricted to germ cells and placental cells. NY-ESO-1 expression has been reported in a wide range of tumor types.

For HPV, the E6 and E7 sequences of HPV16 are known shared tumor antigens and were selected for finding sequences therein for inclusion into the antigenic unit.

To find the most immunogenic sequences, each patients HLA class I and II alleles were determined by sequencing normal tissue (blood cells). Prediction of binding of HPV16 E6/E7 sequences and NY-ESO-1 sequences to the patients' HLA class I and HLA class II alleles was carried out using the NetMHCpan 4.0 software. Included in the analysis were sequences from the IEDB database which are known from the literature to have elicited a positive T cell response (not matched to patients' HLA alleles).

The HLA class I and II alleles which were found for patients 1, 2 and 3 are listed in the table below:

Patient 1 - HLA class I alleles	**Patient 1 - HLA class II alleles**
HLA-A02:01	DRB1_0701
HLA-A31:01	DRB1_1301
HLA-B37:01	DRB3_0202
HLA-B51:01	DRB4_0101
HLA-C06:02	HLA-DQA10103-DQB10202
HLA-C15:02	HLA-DQA10103-DQB10603
	HLA-DQA10201-DQB10202
	HLA-DQA10201-DQB10603
Patient 2 - HLA class I alleles	**Patient 2 - HLA class II alleles**
HLA-A01:01	DRB1_0101
HLA-A24:02	DRB1_1301
HLA-B08:01	DRB3_0202
HLA-B40:01	HLA-DPA10103-DPB10401
HLA-C03:04	HLA-DQA10101-DQB10501
HLA-C07:01	HLA-DQA10101-DQB10603

	HLA-DQA10103-DQB10501 HLA-DQA10103-DQB10603
Patient 3 - HLA class I alleles	**Patient 3 - HLA class II alleles**
HLA-A02:01 HLA-B44:02 HLA-B57:01 HLA-C05:01 HLA-C06:02	DRB1_0112 DRB1_0480 DRB5_0202 HLA-DPA10201-DPB10402 HLA-DQA10102-DQB10301 HLA-DQA10101-DQB10603 HLA-DQA10201-DQB10501 HLA-DQA10103-DQB10603

Table 7.

Patient 1:

Protein/Alleles/ IEDB epitopes	**Total number of epitopes**	**Epitopes selected for inclusion into antigenic unit**	**Remarks**
HPV16 E6			Full length E6 sequence selected, i.e. SEQ ID NO: 11
HLA I	25*	25	
HLA II	16*	16	
IEDB HLA I	26	26	
IEDB HLA II	18	18	
HPV 16 E7			Amino acid 69-98 of SEQ ID NO: 12 selected
HLA I	16*	9	
HLA II	12*	5	
IEDB HLA I	20	7	
IEDB HLA II	22	7	
NY-ESO-1			Amino acid 80-101 of SEQ ID NO: 13 selected
HLA I	26*		
HLA II	43*		
IEDB HLA I	7		
IEDB HLA II	6		

Table 8. * total number of epitopes, each 9 amino acids long, predicted to bind to HLA class I and II alleles of patient 1, respectively.

An antigenic unit for inclusion into an individualized therapeutic anticancer vaccine for patient 1 was designed, which comprises SEQ ID NO: 14, comprising the sequences set forth in the right hand column of the table above in the following order: E7 | linker | NY-ESO-1 | linker | E6.

A second antigenic unit for inclusion into an individualized therapeutic anticancer vaccine for patient 1 was designed, which comprises SEQ ID NO: 15, comprising the sequences set forth in the right hand column of the table above and in addition 17 patient-specific antigen sequences. The most hydrophobic sequences were positioned substantially in the middle of the antigenic unit and the most hydrophilic sequence were positioned at the beginning and at end of the antigenic unit. Glycine-serine linkers were inserted between the sequences. The antigenic unit comprises the sequences in the following order, with T1D denoting the patient-specific antigen sequences:
T1D320 | linker | T1D814 | linker | T1D182 | linker | T1D689 | linker | E7 | linker | T1D339 | linker | T1D428 | linker |NY-ESO-1 | linker | T1D572 | linker | T1D359 | linker | T1D488 | linker | T1D554 | linker | T1D272 | linker | T1D210 | linker | T1D849 | linker | T1D4 | linker | T1D77 | linker | T1D717 | linker | T1D586 | linker | E6.

T1D320	HNEGDDQQGSRYSLIPQIQKVCEVVDG
T1D814	SEQERMKSSLLKEHMLRKQAELESAQC
T1D182	ELRENLLTYLPDSVTQLRRLEELDLGN
T1D689	QEVFSSYKFNHLVRRLVLQREKHFHYL
T1D339	IEKNADLCYLSTVHWSLILDAVSNNYI
T1D428	LATAGEPYHDIRFKLMAVVPDRRIKYE
T1D572	MLRLPTVFRQIRPVSRVLAPHLTR
T1D359	IQIMENPFVQSMLWNPDLMRQLIMANP
T1D488	LLRFLFLGLSALALPSRAQLQLHLPAN
T1D554	MGKNPVRPPRAFPPVPSIDDIPLSR
T1D272	GHVDFTIEVERALTVLDGAVLVLCAVG
T1D210	EYKLMYGMLFSIRLFVSKMSPLDMKDG
T1D849	SQGAGVESLDFRLYLRYEFLMLGIQPV
T1D4	AAKTLVLCVSDIILLSANISETSSNKT
T1D77	CKDLQLYLSNLANHIDRETGIGDVPLV
T1D717	QSKHTEARELMYSAALLFFSHGQQNSA
T1D586	MTLAESYAQYVYNLCNSLSIKVEES

Table 9.

Patient 2:

Protein/Alleles/ IEDB epitopes	Total number of epitopes	Epitopes selected for inclusion into antigenic unit	Remarks
HPV16 E6			Amino acid 62-151 of SEQ ID NO: 11 selected
HLA I	25*	15	
HLA II	14*	10	
IEDB HLA I	26	15	
IEDB HLA II	18	13	
HPV 16 E7			Amino acid 42-87 of SEQ ID NO: 12 selected
HLA I	13*	5	
HLA II	11*	5	
IEDB HLA I	20	6	
IEDB HLA II	22	11	
NY-ESO-1			Amino acid 79-149 of SEQ ID NO: 13 selected
HLA I	19*	19	
HLA II	11*	11	
IEDB HLA I	7	4	
IEDB HLA II	6	3	

Table 10. * total number of epitopes, each 9 amino acids long, predicted to bind to HLA class I and II alleles of patient 2, respectively.

An antigenic unit for inclusion into an individualized therapeutic anticancer vaccine for patient 2 was designed, which comprises SEQ ID NO: 16, comprising the sequences set forth in the right hand column of the table above in the following order: E6 | linker | NY-ESO-1 | linker | E7.

A second antigenic unit for inclusion into an individualized therapeutic anticancer vaccine for patient 2 was designed, which comprises SEQ ID NO: 17, comprising the sequences set forth in the right hand column of the table above and in addition 17 patient-specific antigen sequences. The most hydrophobic sequences were positioned substantially in the middle of the antigenic unit and the most hydrophilic sequence were positioned at the beginning and at end of the antigenic unit. Glycine-serine linkers were inserted between the sequences. The antigenic unit comprises the sequences in the following order, with T1D denoting the patient-specific antigen sequences:

E6 | linker | T1D323 | linker | T1D506 | linker | T1D12 | linker | T1D315 | linker | T1D302 | linker | T1D700 | linker | NY-ESO-1 | linker | T1D535 | linker | T1D358 | linker | T1D670 | linker | T1D294 | linker | T1D336 | linker | T1D499 | linker | T1D425 | linker T1D491 | linker | T1D314 | linker | T1D430 | linker | E7 | linker | T1D582.

T11D323	KKVSKTRHTRETVFRRAKRRWAPIPCS
T11D506	NLKHENILQFLTAQERKTELGKQYWLI
T11D12	VQVFGLYFGEEFHETFDCPIK
T11D315	GQAAASQAGGARGYARGAQLWPPGSDP
T11D302	SIMQTWFTLFTPTDATSIVATTVMSNS
T11D700	QENVRFVLVRSEALLPNAGPRSAEARV
T11D535	DAFPNLKDFISRFQVMPPSSFLFDAPC
T11D358	PSSLQVKPETPASEAVAVAAAAAPTTT
T11D670	RFFHLADLFLSSSQLPAYLVAAFAKRL
T11D294	LVFLWLHSLRRLFGCLYVSVFSNVMIH
T11D336	WENAIAALFRRHIAVSWLIRATLSESE
T11D499	RYIFVKSAGSRIEEGVLQFLVLLVAGR
T11D425	QEIEWLPFRCIKCLKLSFSTAELLCMH
T11D491	LVARCPPCLRLLRQARDFQAARYDRHD
T11D314	ALGTPEDLDSYIDLSLESLNQMILELD
T11D430	SQDMLSIMEKLEFLDFSYDLNLCGLTE
T11D582	RREQYIPNEEFLHFDLLEDSKYRKIYS

Table 11.

Patient 3:

Protein/Alleles/ IEDB epitopes	**Total number of epitopes**	**Epitopes selected for inclusion into antigenic unit**	**Remarks**
HPV16 E6			Amino acid 68-138 of SEQ ID NO: 11 selected
HLA I	18*	6	
HLA II	17*	8	
IEDB HLA I	26	11	
IEDB HLA II	18	6	
HPV 16 E7			Amino acid 42-79 of SEQ ID NO: 12 selected

HLA I	11*	3	
HLA II	0*	0	
IEDB HLA I	20	5	
IEDB HLA II	22	8	
NY-ESO-1			Amino acid 79-170 of SEQ ID NO: 13 selected
HLA I	20*	20	
HLA II	58*	42	
IEDB HLA I	7	6	
IEDB HLA II	6	6	

Table 12. * total number of epitopes, each 9 amino acids long, predicted to bind to HLA class I and II alleles of patient 3, respectively.

An antigenic unit for inclusion into an individualized therapeutic anticancer vaccine for patient 3 was designed, which comprises SEQ ID NO: 18, comprising the sequences set forth in the right hand column of the table above in the following order: NY-ESO-1 | linker | E7 | linker | E6.

A second antigenic unit for inclusion into an individualized therapeutic anticancer vaccine for patient 3 was designed, which comprises SEQ ID NO: 19, comprising the sequences set forth in the right hand column of the table above and in addition 17 patient-specific antigen sequences. The most hydrophobic sequences were positioned substantially in the middle of the antigenic unit and the most hydrophilic sequence were positioned at the beginning and at end of the antigenic unit. Glycine-serine linkers were inserted between the sequences. The antigenic unit comprises the sequences in the following order, with T1D denoting the patient-specific antigen sequences:
T1D223 | linker | T1D164 | linker | T1D56 | linker | T1D36 | linker | T1D129 | linker | T1D274 | linker | T1D62 | linker | T1D5 | linker | T1D144 | linker | T1D441 | linker | T1D368 | linker | NY-ESO-1 | linker | T1D234 | linker | T1D162 | linker | T1D39 | linker | T1D272 | linker | E7 | linker | T1D328 | linker | T1D188 | linker | E6.

T1D223	GPSYRSNSVSSLDLEGE
T1D164	LKEEKENLQGLVTHQTYIIQELEKQLN
T1D56	QRQKTAGKIFRAEVSTGQDAPRQAQAR
T1D36	ILRPSTVNELESGEISYDEVGERIKDF
T1D129	DTCINEDVESLRKMVQDLLAKLQEAKR
T1D274	LREEGTKYKSFVQRARLVFREEGYLAF
T1D62	HEGARPMRAIFLANGNVFTTGFSRMSE
T1D5	IPDAAFIQAARRKCELARAQDDYISLD

T1D144	MEIAKMRAGRRLWVHLIEKMFQPKNSK
T1D441	MELVSFRDVAIEFSPEEW
T1D368	TCVHEIPFHFDLMELLPQCQQLQMFFL
T1D234	VRRVFITDDFHDMIPKYLNFVKGVVDS
T1D162	NFHAHREKAPSLFCSRILNKAVYLFYG
T1D39	DANKEGMFLFRAAHKLRQFLKMNSTGD
T1D272	LLSLGWSVDVGRHSGWTGHVSTSWSIN
T1D328	NTIFSLRKDLRQGKARRLRCMEEKEMF
T1D188	NHFNDFEGDPAMTQFLEEFEKNLEDTK

Table 13.

EXAMPLE 7: Immunogenicity of DNA vaccines according to the invention

Design of DNA vaccines comprising shared antigen sequences and/or neoepitopes:

Five DNA vaccines (constructs) were designed comprising nucleotide sequences encoding the units/parts shown in table 14:

Unit/Part	**Function**
1: Native leader sequence for human MIP1α (LD78β)	Signal peptide
2: Full-length human MIP1α (LD78β) sequence	Targeting unit
3: Human hinge-region 1 from IgG3 4: Human hinge region 4 from IgG3 5: Glycine-Serine linker 6: Human CH3 domain from IgG3	Dimerization unit
7: Glycine-Leucine linker	Unit linker
8: Patient-present shared antigen sequences: **VB4100, VB4101, VB4102** 9: Patient-specific antigen sequences (neoepitopes) **VB4097** 10: Patient-present shared antigen sequences and patient-specific antigen sequences (neoepitopes): **VB4105**	Antigenic unit

Table 14

DNA vaccine VB4097 comprising 10 CT26 neoepitopes:

This construct was chosen as a model of an individualized DNA vaccine comprising patient-specific antigen sequences, i.e., neoepitopes.

Previously described exome sequencing and RNA sequencing of the mouse colon cancer cell line CT26 revealed hundreds to thousands of tumor-specific non-synonymous mutations. *In silico* methods were used to identify potential immunogenic sequences, i.e., neoepitopes, and 10 of them (table 15) were chosen for inclusion into the antigenic unit of VB4097. Each of the identified 10 neoepitopes is a peptide consisting of 27 amino acids. All but the terminal neoepitope were arranged in subunits, each subunit consisting of one neoepitope and one flexible glycine-serine linker (GGGGSGGGGS).

VB4097 consists of a DNA sequence encoding the polypeptide with amino acid sequence of SEQ ID NO: 20.

Neoantigen	**Gene**	**Sequence**	**Reactive T cell subtype**
C-pepM1	E2f8	VILPQAPSGPSYATYLQPAQAQMLTPP	CD8+/CD4+
C-pepM6	Ubqln1	DTLSAMSNPRAMQVLLQIQQGLQTLAT	CD4+
C-pepM8	Dhx35	EVIQTSKYYMRDVIAIESAWLLELAPH	CD4+
C-pepM29	Anapc1	GSLFGSSRVQYVVNPAVKIVFLNIDPS	ND
C-pepM31	Ptpn13	AEYGDYQPEVHGVPYFRLEHYLPARVM	CD4+
C-pepM43	Mtch1	KSWIHCWKYLSVQSQLFRGSSLLFRRV	CD8+/CD4+
C-pepM89	Mmachc	TLAFLVLSTPAMFNRALKPFLKSCHFQ	CD4+
C-pep149	3110057O12Rik	FVSPMAHYVPGIMAIESVVARFQFIVP	CD8+
C-pepM171	Gdf11	LWVYLRPVPRPATIYLQILRLKPLTGE	CD8+
C-pepM173	Top3a	KIYEFDYHLYGQNITMIMTSVSGHLLA	CD4+

Table 15

DNA vaccines VB4100, VB4101 and VB4102 comprising NY-ESO-1 sequences or parts thereof.

These constructs were chosen as models of individualized DNA vaccines comprising patient-present shared antigen sequences.

Human New York esophageal squamous cell carcinoma 1 (NY-ESO-1) has been shown to be a highly immunogenic cancer testis antigen aberrantly expressed in several cancer types. NY-ESO-1 is not endogenously expressed in the CT26 cancer cell line, but using *in silico* methods, several

immunogenic sequences were predicted to bind mouse MHC class I and II in BALB/c mice. The above-mentioned constructs were chosen as a model of an individualized DNA vaccine comprising patient-present shared antigen sequences.

Three NY-ESO-1 constructs were thus designed:

- VB4100 having an antigenic unit that comprises NY-ESO-1 in full length. VB4100 consists of a DNA sequence encoding the polypeptide with amino acid sequence of SEQ ID NO: 21.
- VB4101 having an antigenic unit that comprises amino acids 81-88 of the NY-ESO-1 sequence which is predicted to be an MHC class I antigen. VB4101 consists of a DNA sequence encoding the polypeptide with amino acid sequence of SEQ ID NO: 22.
- VB4102 having an antigenic unit that comprises amino acids 81-126 of the NY-ESO-1 sequence which contains several antigens that are predicted to be MHC class and class II antigens. VB4102 consists of a DNA sequence encoding the polypeptide with amino acid sequence of SEQ ID NO: 23.

The various NY-ESO-1 sequences comprised in the constructs are shown in table 16.

Construct	**NY-ESO-1**	**Sequence**	**Length**	**Reactive T cell subtype**
VB4100	Full length	MQAEGRGTGGSTGDADGPGGPG IPDGPGGNAGGPGEAGATGGRG PRGAGAARASGPGGGAPRGPHG GAASGLNGCCRCGARGPESRLLE FYLAMPFATPMEAELARRSLAQD APPLPVPGVLLKEFTVSGNILTIRL TAADHRQLQLSISSCLQQLSLLMW ITQCFLPVFLAQPPSGQRR (SEQ ID NO: 13)	180 amino acids	Both CD4+ and CD8+
VB4101	Amino acids 81-88	RGPESRLL	8 amino acids	CD8+
VB4102	Amino Acids 81-126	RGPESRLLEFYLAMPFATPMEAEL ARRSLAQDAPPLPVPGVLLKEF	46 amino acids	Both CD4+ and CD8+

Table 16

DNA vaccine VB4105 comprising 10 CT26 neoepitopes and the full-length sequence of NY-ESO-1 sequence

This construct was chosen as a model of an individualized DNA vaccine comprising patient-present shared antigen sequences and patient-specific antigen sequences (neoepitopes). It comprises an antigenic unit comprising the full-length sequence of NY-ESO-1 and the 10 CT26 neoepitopes shown in table 1, each of them separated from the following neoepitope or the NY-ESO-1 sequence by a flexible glycine-serine linker (GGGGSGGGGS). VB4105 consists of a DNA sequence encoding the polypeptide with amino acid sequence of SEQ ID NO: 24.

Negative control VB1026:

This construct is identical to the aforementioned constructs, but comprises neither a unit linker, nor an antigenic unit. It serves as a negative control. VB1026 consists of a DNA sequence encoding the polypeptide with amino acid sequence of SEQ ID NO: 25.

Construction of expression vectors comprising the constructs and confirmation of expression and secretion of intact dimeric proteins encoded by the constructs:

The sequences of the antigenic units of all aforementioned constructs were ordered from Genscript (New Jersey, USA) and cloned into the expression vector pUMVC4a; a master plasmid comprising a nucleotide sequence encoding the signal peptide, targeting unit and dimerization unit described in table 14 above.

HEK293 cells (ATCC) were transiently transfected with the above-mentioned constructs. Briefly, $2x10^5$cells/well were plated in 24-well tissue culture plates with 10% FBS growth medium and transfected with 1 µg of respective DNA plasmid using Lipofectamine® 2000 reagent under the conditions suggested by the manufacturer (Invitrogen, Thermo Fischer Scientific). The transfected cells were then maintained for up to 5 days at 37°C with 5% CO_2. Later the cell supernatants were harvested for characterization of the expression of the proteins encoded by the constructs by sandwich ELISA of the supernatant using antibodies specific for anti hIgG (CH3 domain), hMIP-1α and the respective antigenic units.

Assessment of immunogenicity of the constructs:

Immunogenicity of the constructs was determined by way of measuring the T cell immune response elicited *in vivo* in mice vaccinated with the constructs.

Female, 6-week-old BALB/c mice were obtained from Janvier Labs (France). All animals were housed in the animal facility at the Radium Hospital (Oslo, Norway). All animal protocols were approved by the Norwegian Food Safety Authority (Oslo, Norway). 5 mice/group were used for the testing of the constructs comprising an antigenic unit, whereas 3 mice/group were used for the negative control.

20 µg of the construct was administered intramuscularly twice, i.e., on day 0 and on day 21, followed by electroporation. Spleens were collected on day 28.

Spleens were mashed in cell strainer to obtain a single cell suspension. For each construct tested, a portion of the single cell suspension was used to purify CD4+ and CD8+ T cells using Dynabeads™ depletion. Total splenocytes, CD4 depleted splenocytes and CD8 depleted splenocytes were then tested for production of INF-γ in ELISpot assays. The 10 neoepitopes

shown in table 15 were used to re-stimulate the splenocytes harvested from mice vaccinated with constructs VB4097 and VB4105, while the NY-ESO-1 peptides shown in table 17 below were used to re-stimulate the splenocytes harvested from mice vaccinated with the construct indicated in said table.

NY-ESO-1 peptide ID	Sequence	Length (amino acids)	Used for constructs
NY-ESO_Pep-1	MQAEGRGTGGSTGDA	15	VB4100, VB4105
NY-ESO_Pep-2	TGGSTGDADGPGGPG	15	VB4100, VB4105
NY-ESO_Pep-3	ADGPGGPGIPDGPGG	15	VB4100, VB4105
NY-ESO_Pep-4	GIPDGPGGNAGGPGE	15	VB4100, VB4105
NY-ESO_Pep-5	GNAGGPGEAGATGGR	15	VB4100, VB4105
NY-ESO_Pep-6	EAGATGGRGPRGAGA	15	VB4100, VB4105
NY-ESO_Pep-7	RGPRGAGAARASGPG	15	VB4100, VB4105
NY-ESO_Pep-8	AARASGPGGGAPRGP	15	VB4100, VB4105
NY-ESO_Pep-9	GGGAPRGPHGGAASG	15	VB4100, VB4105
NY-ESO_Pep-10	PHGGAASGLNGCCRC	15	VB4100, VB4105
NY-ESO_Pep-11	GLNGCCRCGARGPES	15	VB4100, VB4105
NY-ESO_Pep-12	CGARGPESRLLEFYL	15	VB4100, VB4102, VB4105
NY-ESO_Pep-13	RGPESRLLEFYLAMP	15	VB4100, VB4102, VB4105
NY-ESO_Pep-14	SRLLEFYLAMPFATP	15	VB4100, VB4102, VB4105
NY-ESO_Pep-15	LAMPFATPMEAELAR	15	VB4100, VB4102, VB4105
NY-ESO_Pep-16	PMEAELARRSLAQDA	15	VB4100, VB4102, VB4105
NY-ESO_Pep-17	RRSLAQDAPPLPVPG	15	VB4100, VB4102, VB4105
NY-ESO_Pep-18	DAPPLPVPGVLLKEF	15	VB4100, VB4102, VB4105
NY-ESO_Pep-19	APPLPVPGVLLKEFT	15	VB4100, VB4102, VB4105
NY-ESO_Pep-20	GVLLKEFTVSGNILT	15	VB4100, VB4105
NY-ESO_Pep-21	TVSGNILTIRLTAAD	15	VB4100, VB4105
NY-ESO_Pep-22	TIRLTAADHRQLQLS	15	VB4100, VB4105
NY-ESO_Pep-23	DHRQLQLSISSCLQQ	15	VB4100, VB4105
NY-ESO_Pep-24	SISSCLQQLSLLMWI	15	VB4100, VB4105
NY-ESO_Pep-25	QLSLLMWITQCFLPV	15	VB4100, VB4105
NY-ESO_Pep-26	ITQCFLPVFLAQPPS	15	VB4100, VB4105
NY-ESO_Pep-27	VFLAQPPSGQRR	12	VB4100, VB4105
NY-ESO_Pep-28	RGPESRLL	8	VB4100, VB4101, VB4102, VB4105

Table 17: NY-ESO-1 peptides used for stimulation of splenocytes.

Comparison of immunogenicity of constructs VB4097, VB4100 and VB4105

Constructs VB4097 (10 neoepitopes), VB4100 (NY-ESO-1 full length), and VB4105 (10 neoepitopes and NY-ESO-1 full length) were compared for their ability to elicit T cell immune response against the peptides in tables 15 (VB4097 and VB4105) and 17 (VB4100 and VB4105).

As shown in Fig. 9, mice vaccinated with the negative control VB1026 showed low basal immunogenicity against both the neoepitopes and the NY-ESO-peptide sequences.

VB4097 and VB4105, both comprising the same 10 CT26 neoepitopes, induce a similar a total T cell response (INF-γ response) against the 10 neoepitopes (Fig. 9, grey bars), independent of whether the antigenic unit only comprises the 10 neoepitopes (VB4097) or comprises in addition the full-length sequence of NY-ESO-1 (VB4105).

Also, VB4100 and VB4105, both comprising the full-length sequence of NY-ESO-1 induce a similar a total T cell response (INF-γ response) against the peptides used for re-stimulation as shown in table 17 (Fig. 9, black bars), independent of whether the antigenic unit only comprises the full-length sequence of NY-ESO-1 (VB4100) or comprises in addition the 10 neoepitopes (VB4105).

For VB4105, the addition of the NY-ESO-1 full length sequence to the antigenic unit of VB4097 comprising the 10 neoepitopes did elicit a higher total T cell response compared to that elicited by vaccination with VB4097, due to the added immunogenicity against NY-ESO-1.

These results indicate that a vaccine according to the invention comprising a patient-present shared antigen is able to elicit an immune response which is similar to that of a vaccine comprising patient-specific antigens (neoepitopes). Moreover, these results indicate that there is a benefit in including both a patient-present shared antigen and patients-specific antigens (neoepitopes) into the antigenic unit.

Comparison of immunogenicity of constructs VB4100, VB4101 and VB4102

Using *in silico* methods, the epitope consisting of amino acids 81-88 of NY-ESO-1 was predicted to strongly bind to MHC class I to active CD8+ T cells, whereas the peptide consisting of amino acids 81 to 126 of NY-ESO-1 was predicted to contain several MHC class I and class II antigens. Therefore, constructs VB4100 (NY-ESO-1 full length), VB4101 (NY-ESO-1 amino acids 81-88) and VB4102 (NY-ESO-1 amino acids 81-126) were constructed and compared for their ability to elicit T cells immune response.

First, the three constructs were compared for their ability to elicit CD8+ T cell immune response against the predicted amino acid 81-88 region. The CD8+ T cells isolated from splenocytes of mice vaccinated with the constructs were re-stimulated with the amino acid 81-88 minimal epitope shown in table 16. As shown in Fig. 10, the experiments confirmed that the epitope consisting of amino acids 81-88 of NY-ESO-1 is indeed a strong CD8+ T cell epitope. Moreover, the immunogenicity for this region is independent of whether the epitope is the only sequence in the

antigenic unit (VB4101) or the antigenic unit comprising a longer NY-ESO-1 sequence or the full NY-ESO-1 sequence.

Next, VB4102 was compared to VB4100 to assess whether the several predicted MHC class I and class II antigens in the amino acid sequence 81-126 of NY-ESO-1 elicit a similar response as the full-length NY-ESO-1 sequence. The CD4+ T cells and CD8+ T cells isolated from splenocytes of mice vaccinated with the constructs were re-stimulated with the peptides shown in table 17. As shown in Fig. 11, the amino acid sequence 81-126 of NY-ESO-1 elicits a stronger response than the NY-ESO-1 full length sequence, both from isolated CD8+ (Fig. 11B) and CD4+ T cells (Fig. 11C) and in terms of total T cell response (Fig. 11 A).

These results suggest that by using *in silico* methods, short(er) sequences/epitopes of patient-present shared antigens can be identified that are predicted to elicit strong immune responses. By including such sequences/epitopes into the antigenic unit of the vaccine of the invention instead of longer sequences or the full-length sequence of a patient-present shared antigen, there is space left in the antigenic unit for including sequences of other patient-present shared antigens and/or patient-specific antigens/neoepitopes. This will enhance the chance that the patient receiving such an individual anticancer vaccine will show a strong immune response to the vaccine.

EXAMPLE 8: Immunogenicity of DNA vaccines according to the invention
Design of DNA vaccines comprising shared antigen sequences and/or neoepitopes:

Six DNA vaccines (constructs) were designed comprising nucleotide sequences encoding the units/parts shown in table 18:

Unit/Part	Function
1: Native leader sequence for human MIP1α (LD78β)	Signal peptide
2: Full-length human MIP1α (LD78β) sequence	Targeting unit
3: Human hinge-region 1 from IgG3 4: Human hinge region 4 from IgG3 5: Glycine-Serine linker 6: Human CH3 domain from IgG3	Dimerization unit
7: Glycine-Leucine linker	Unit linker
8: Patient-present shared antigen sequences: **VB4119, VB4127** 9: Patient-specific antigen sequences (neoepitopes)	Antigenic unit

VB4118 10: Patient-present shared antigen sequences and patient-specific antigen sequences (neoepitopes): **VB4121, VB4128, VB4130**	

Table 18.

DNA vaccine VB4118 comprising 10 B16 neoepitopes:

This construct was chosen as a model of an individualized DNA vaccine comprising patient-specific antigen sequences, i.e. neoepitopes.

Previously described exome sequencing and RNA sequencing of the mouse melanoma cell line B16.F10 revealed hundreds to thousands of tumor-specific non-synonymous mutations. *In silico* methods were used to identify potential immunogenic sequences, i.e. neoepitopes, and 10 of them (table 19) were chosen for inclusion into the antigenic unit of VB4118. Each of the identified 10 neoepitopes is a peptide consisting of 27 amino acids. All but the terminal neoepitope were arranged in subunits, each subunit consisting of one neoepitope and one flexible glycine-serine linker (GGGGSGGGGS).

VB4118 consists of a DNA sequence encoding the polypeptide with amino acid sequence of SEQ ID NO: 26.

Neoantigen	Gene	Sequence	Reactive T cell subtype
B-pepM2	Obsl1	REGVELCPGNKYEMRRHGTTHSLVIHD	CD8+
B-pepM7	Atp11a	SSPDEVALVEGVQSLGFTYLRLKDNYM	CD8+
B-pepM36	Pcmtd1	KNILAVSFAPLVQLSKNDNGTPDSVGL	CD4+
B-pepM78	Klhl26	PAADRWEPRAPMRAPRVLHAMLGAAGR	CD4+
B-pepM79	B3galt6	VLSADLVHYLRLSLEYLRAWHSEDVSL	CD4+
B-pepM82	Mta1	LEAVLRYLETHPRLPKPDPVKSSSSVL	CD4+
B-pepM83	Ddit4l	LKDFFFSRGRFSSALKRTLILSSGFRL	CD4+
B-pepM84	Wdr3	AGKDHKIKQWDADTFEHIQTLEGHHQE	CD8+
B-pepM85	Rfx3	PATIEMAIETLQKFDGLSTHRSSLLNS	ND
B-pepM86	Map1s	YLPGGGAGHLDQNVFLRVRALCYVISG	CD8+

Table 19.

DNA vaccines VB4119 comprising a TRP-2 sequence and VB4127 comprising frameshift antigens

These constructs were chosen as models of individualized DNA vaccine comprising patient-present shared antigen sequences.

Tyrosinase related protein 2 (TRP-2) is a melanocyte lineage normal differentiation protein. This shared antigen (differentiation antigen) is known to induce tumor rejection of B16 melanoma cells in C57BL/6 mice *in vivo*. In the literature, the 9 amino acid long MHC class I epitope shown in table 20 (amino acids 180-188 of TRP-2) has been identified as the immunogenic sequence responsible for the anti-tumor effect of TRP-2.

VB4119 consists of a DNA sequence encoding the polypeptide with amino acid sequence of SEQ ID NO: 27

Construct	**TRP-2 epitope**	**Sequence**	**Length**	**Reactive T cell subtype**
VB4119	Amino acids 180-188	SVYDFFVWL	9 amino acids	CD8+

Table 20

Frameshift mutations are DNA mutations that arise when indels (insertions or deletions) of nucleotides lead to a shift in the DNA reading frame. Consequently, the entire DNA sequence following the indel will be read incorrectly and the resulting protein will be altered. Frameshift mutations arising in tumor cells generate novel peptide sequences that could be highly immunogenic, and, moreover, identical frameshift antigens could arise across different patients, and thus represent promising targets for shared antigen cancer vaccines (see for instance Ballhausen et al., Nat. Commun. 11, 2020, 1-13). The three frameshift antigens shown in table 21 were identified as immunogenic by *in silico* methods and are encoded in the VB4127 construct. In the antigenic unit, they are separated from each other by a flexible glycine-serine linker (GGGGSGGGGS).

VB4127 consists of a DNA sequence encoding the polypeptide with amino acid sequence of SEQ ID NO: 28.

Frameshift antigen	**Gene**	**Sequence**	**Reactive T cell subtype**
B-pepM108	Maz	YISDHMKVHSPSPCL	CD4+
B-pepM115-M122	Dync1h1	EGWQTCWGRSRKHWGSTWNGSARLSPGSTLWVMRICLRSLGIARTWLSCRSTSRKCSPAFPASS	CD4+/CD8+
B-pepM141-M142	Prtg	LFRLLPSGPKVNDGHRSRRWHSLDLYPHLCSHLDLPKQSQEVIRL	CD8+

Table 21

DNA vaccines comprising 10 B16 neoepitopes and the TRP-2 epitope (VB4121) or 10 B16 neoepitopes and the 3 frameshift antigens (VB4128) or 10 B16 neoepitopes, the TRP-2 epitope and the 3 frameshift antigens (VB4130)

These constructs were chosen as models of individualized DNA vaccines comprising patient-present shared antigen sequences and patient-specific antigen sequences (neoepitopes). Each construct comprises an antigenic unit comprising the 10 B16 neoepitopes shown in table 19, each of them separated from the following neoepitope or the shared antigen sequence by a flexible glycine-serine linker (GGGGSGGGGS).The same linker was used to separate the shared antigens sequences in constructs comprising several of such sequences. VB4121 consists of a DNA sequence encoding the polypeptide with amino acid sequence of SEQ ID NO: 29; VB4128 consists of a DNA sequence encoding the polypeptide with amino acid sequence of SEQ ID NO: 30 and VB4130 consists of a DNA sequence encoding the polypeptide with amino acid sequence of SEQ ID NO: 31.

The construct VB1026, described in Example 7, was used as a negative control.

Construction of expression vectors comprising the constructs and confirmation of expression and secretion of intact dimeric proteins encoded by the constructs

The expression vectors comprising the aforementioned constructs were constructed as described in Example 7. Expression and secretion of intact dimeric proteins encoded by the constructs was confirmed as described in Example 7.

Assessment of immunogenicity of the constructs

Immunogenicity of the constructs was determined by way of measuring the T cell immune response elicited *in vivo* in mice vaccinated with the constructs.

Female, 6-week-old C57BL/6 mice were obtained from Janvier Labs (France). All animals were housed in the animal facility at the Radium Hospital (Oslo, Norway). All animal protocols were approved by the Norwegian Food Safety Authority (Oslo, Norway). 5 mice/group were used for the testing of the constructs comprising an antigenic unit, whereas 3 mice/group were used for the negative control.

20 µg of the construct was administered intramuscularly on day 0 followed by electroporation. The spleens from the vaccinated mice were collected on day 14. The collected spleens were processed and ELISpot assays were carried out as described in Example 7.

The 10 neoepitopes shown in table 19 were used to re-stimulate the splenocytes harvested from mice vaccinated with the constructs VB4118, VB4121, VB4128, and VB4130. The TRP-2 peptide in table 20 was used to re-stimulate the splenocytes harvested from mice vaccinated with the constructs VB4119, VB4121, and VB4130. The frameshift peptides shown in table 22 below were used to re-stimulate the splenocytes harvested from mice vaccinated with the constructs VB4127, VB4128, and VB4130.

Gene	Frameshift peptide ID	Sequence	Length
Maz	B-pepM108	YISDHMKVHSPSPCL	
Dync1h1	B-pepM115	EGWQTCWGRSRKHWG	15 amino acids
Dync1h1	B-pepM116	GRSRKHWGSTWNGSA	15 amino acids
Dync1h1	B-pepM117	GSTWNGSARLSPGST	15 amino acids
Dync1h1	B-pepM118	ARLSPGSTLWVMRIC	15 amino acids
Dync1h1	B-pepM119	TLWVMRICLRSLGIA	15 amino acids
Dync1h1	B-pepM120	CLRSLGIARTWLSCR	15 amino acids
Dync1h1	B-pepM121	ARTWLSCRSTSRKCS	15 amino acids
Dync1h1	B-pepM122	RSTSRKCSPAFPASS	15 amino acids
Prtg	B-pepM141	LFRLLPSGPKVNDGHRSRRWHSLDLYP	27 amino acids
Prtg	B-pepM142	SRRWHSLDLYPHLCSHLDLPKQSQEVIRL	29 amino acids

Table 22

Comparison of immunogenicity of constructs VB4118, VB4119, VB4121, VB4127, VB4128, and VB4130

Constructs VB4118 (10 neoepitopes), VB4119 (TRP-2), VB4121 (10 neoepitopes and TRP-2), VB4127 (3 frameshift antigens), VB4128 (10 neoantigens and 3 frameshift antigens), and VB4130 (10 neoepitopes, 3 frameshift antigens and TRP-2) were compared for their ability to elicit T cell immune response against the peptides in tables 19, 20, and 22, as applicable.

As shown in Fig. 12, mice vaccinated with the negative control VB1026 showed low basal immunogenicity against all the peptides tested.

Both the model construct of a vaccine comprising only patient-specific antigens (VB4118) and the model constructs of vaccines comprising only patient-present shared antigen(s) (VB4119 and VB4127) elicit an immune response in the vaccinated mice.

VB4118, VB4121, VB4128, and VB4130, all comprising the same 10 B16 neoepitopes, induce a similar total T cell response (INF-γ response) against the 10 neoepitopes (Fig. 12, black bars), independent of whether the antigenic unit only comprises the 10 neoepitopes (VB4118) or

comprises in addition the TRP-2 epitope (VB4121), the 3 frameshift antigens (VB4128) or the TRP-2 epitope plus the 3 frameshift antigens (VB4130).

Further, Fig. 12 shows that adding the shared antigens to a neoepitope construct leads to a broader and increased total T cell response with the highest total T cell response observed for the construct encoding 10 neoepitopes, 3 frameshift antigens and the TRP-2 epitope (VB4130).

Also the results of this study indicate that a vaccine according to the invention comprising a patient-present shared antigen can elicit an immune response which is similar to that of a vaccine comprising patient-specific antigens (neoepitopes). Moreover, these results indicate that there is the benefit of an increased broader and total T cell response by including different types of shared antigens and patients-specific antigens (neoepitopes) into the antigenic unit.

SEQUENCES

SEQ ID NO: 1
C-C motif chemokine 3-like 1 precursor including signal peptide (aa 1-23) and mature peptide (hMIP1α/LD78-beta, aa 24-93):

MQVSTAALAVLLCTMALCNQVLSAPLAADTPTACCFSYTSRQIPQNFIADYFETSSQCSKPSVIF LTKRGRQVCADPSEEWVQKYVSDLELSA

SEQ ID NO: 2
MQVSTAALAVLLCTMALCNQVLS | APLAADTPTACCFSYTSRQIPQNFIAD YFETSSQCSKPSVIFLTKRGRQVCADPSEEWVQKYVSDLELSA | ELKTPLG DTTHT I EPKSCDTPPPCPRCP | GGGSSGGGSG | GQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKL TVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK | GLGGL |

SEQ ID NO: 3
Linker, amino acid sequence: GLSGL

SEQ ID NO: 4
Linker, amino acid sequence: GLGGL

SEQ ID NO: 5
Hinge region 1 (human IgG3 UH hinge), 12 amino acids: ELKTPLGDTTHT

SEQ ID NO: 6
Hinge region 4 (human IgG3, MH hinge, 15 amino acids): EPKSCDTPPPCPRCP

SEQ ID NO: 7
Gly-Ser Linker: GGGSSGGGSG

SEQ ID NO: 8
hCH3 IgG3, amino acid sequence:
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDG SFFLYSKL TVDKSRWQQG NIFSCSVM H EALH N RFTQKSLSLSPGK

SEQ ID NO: 9
Signal peptide
MNFGLRLIFLVLTLKGVQC

SEQ ID NO: 10
Signal peptide
MDAMKRGLCCVLLLCGAVFVSP

SEQ ID NO: 11: HPV16 E6
MFQDPQERPRKLPQLCTELQTTIHDIILECVYCKQQLLRREVYDFARRDLCIVYRDGNPYAVRD
KCLKFYSKISEYRHYCYSLYGTTLEQQYNKPLCDLLIRCINRQKPLCPEEKQRHLDKKQRFHNIR
GRWTGRCMSCCRSSRTRRETQL

SEQ ID NO: 12: HPV16 E7
MHGDTPTLHEYMLDLQPETTDLYGYGQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFCCKCDS
TLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP

SEQ ID NO: 13: NY-ESO-1
MQAEGRGTGGSTGDADGPGGPGIPDGPGGNAGGPGEAGATGGRGPRGAGAARASGPGGG
APRGPHGGAASGLNGCCRCGARGPESRLLEFYLAMPFATPMEAELARRSLAQDAPPLPVPGV
LLKEFTVSGNILTIRLTAADHRQLQLSISSCLQQLSLLMWITQCFLPVFLAQPPSGQRR

SEQ ID NO: 14 (302 amino acids)
Antigenic unit comprising antigen sequences in the following order: E7 | linker | NY-ESO-1 | linker | E6.
VQSTHVDIRTLEDLLMGTLGIVCPICSQKPGGGGSGGGGSARGPESRLLEFYLAMPFATPMEA
ELARRSLAQDAPPLPVPGVLLKEFTVSGNILTIRLTAADHRQLQLSISSCLQQLSLLMWITQCFLP
VFLAQPPSGQRRSGGGGSGGGGMFQDPQERPRKLPQLCTELQTTIHDIILECVYCKQQLLRRE
VYDFARRDLCIVYRDGNPYAVRDKCLKFYSKISEYRHYCYSLYGTTLEQQYNKPLCDLLIRCINR
QKPLCPEEKQRHLDKKQRFHNIRGRWTGRCMSCCRSSRTRRETQL

SEQ ID NO: 15 (924 amino acids)
Antigenic unit comprising antigen sequences in the following order: T1D320 | linker | T1D814 | linker | T1D182 | linker | T1D689 | linker | E7 | linker | T1D339 | linker | T1D428 | linker |NY-ESO-1 | linker | T1D572 | linker | T1D359 | linker | T1D488 | linker | T1D554 | linker | T1D272 | linker | T1D210 | linker | T1D849 | linker | T1D4 | linker | T1D77 | linker | T1D717 | linker | T1D586 | linker | E6.
HNEGDDQQGSRYSLIPQIQKVCEVVDGGSSGGGSSGGSEQERMKSSLLKEHMLRKQAELES
AQCSGGGGSGGGGELRENLLTYLPDSVTQLRRLEELDLGNGGGGSGGGGSQEVFSSYKFNH

LVRRLVLQREKHFHYLSGGGGSGGGGVQSTHVDIRTLEDLLMGTLGIVCPICSQKPGGGGSG
GGGSIEKNADLCYLSTVHWSLILDAVSNNYIGGGGSGGGGSLATAGEPYHDIRFKLMAVVPDR
RIKYEGGGGSGGGGSARGPESRLLEFYLAMPFATPMEAELARRSLAQDAPPLPVPGVLLKEFT
VSGNILTIRLTAADHRQLQLSISSCLQQLSLLMWITQCFLPVFLAQPPSGQRRSGGGGSGGGG
MLRLPTVFRQIRPVSRVLAPHLTRSGGGGSGGGGIQIMENPFVQSMLWNPDLMRQLIMANPG
GGGSGGGGSLLRFLFLGLSALALPSRAQLQLHLPANGGGGSGGGGSMGKNPVRPPRAFPPV
PSIDDIPLSRGSGGGGSGGGGHVDFTIEVERALTVLDGAVLVLCAVGSSGGGSSGGGEYKLMY
GMLFSIRLFVSKMSPLDMKDGGSSGGGSSGGSQGAGVESLDFRLYLRYEFLMLGIQPVGGGG
SGGGGSAAKTLVLCVSDIILLSANISETSSNKTSGGGGSGGGGCKDLQLYLSNLANHIDRETGI
GDVPLVGGGGSGGGGSQSKHTEARELMYSAALLFFSHGQQNSAGGGGSGGGGSMTLAESY
AQYVYNLCNSLSIKVEESSGGGGSGGGGMFQDPQERPRKLPQLCTELQTTIHDIILECVYCKQ
QLLRREVYDFARRDLCIVYRDGNPYAVRDKCLKFYSKISEYRHYCYSLYGTTLEQQYNKPLCDL
LIRCINRQKPLCPEEKQRHLDKKQRFHNIRGRWTGRCMSCCRSSRTRRETQL

SEQ ID NO: 16 (227 amino acids)
Antigenic unit comprising antigen sequences in the following order: E6 | linker | NY-ESO-1 | linker | E7
VRDKCLKFYSKISEYRHYCYSLYGTTLEQQYNKPLCDLLIRCINRQKPLCPEEKQRHLDKKQRF
HNIRGRWTGRCMSCCRSSRTRRETQLSGGGGSGGGGGARGPESRLLEFYLAMPFATPMEAE
LARRSLAQDAPPLPVPGVLLKEFTVSGNILTIRLTAADHRQLQLSIGGGGSGGGGSAGQAEPDR
AHYNIVTFCCKCDSTLRLCVQSTHVDIRTLEDLLMGTL

SEQ ID NO: 17 (850 amino acids)
Antigenic unit comprising antigen sequences in the following order: E6 | linker | T1D323 | linker | T1D506 | linker | T1D12 | linker | T1D315 | linker | T1D302 | linker | T1D700 | linker | NY-ESO-1 | linker | T1D535 | linker | T1D358 | linker | T1D670 | linker | T1D294 | linker | T1D336 | linker | T1D499 | linker | T1D425 | linker T1D491 | linker | T1D314 | linker | T1D430 | linker | E7 | linker | T1D582.
VRDKCLKFYSKISEYRHYCYSLYGTTLEQQYNKPLCDLLIRCINRQKPLCPEEKQRHLDKKQRF
HNIRGRWTGRCMSCCRSSRTRRETQLSGGGGSGGGGKKVSKTRHTRETVFRRAKRRWAPIP
CSSGGGGSGGGGNLKHENILQFLTAQERKTELGKQYWLIGGGGSGGGGSVQVFGLYFGEEF
HETFDCPIKGSGGGGSGGGGQAAASQAGGARGYARGAQLWPPGSDPGGGGSGGGGSSIM
QTWFTLFTPTDATSIVATTVMSNSSGGGGSGGGGQENVRFVLVRSEALLPNAGPRSAEARVG
GGGSGGGGSGARGPESRLLEFYLAMPFATPMEAELARRSLAQDAPPLPVPGVLLKEFTVSGNI
LTIRLTAADHRQLQLSIGGGGSGGGGSDAFPNLKDFISRFQVMPPSSFLFDAPCSGGGGSGGG
GPSSLQVKPETPASEAVAVAAAAAPTTTSGGGGSGGGGRFFHLADLFLSSSQLPAYLVAAFAK
RLSGGGGSGGGGLVFLWLHSLRRLFGCLYVSVFSNVMIHSGGGGSGGGGWENAIAALFRRHI
AVSWLIRATLSESEGGGGSGGGGSRYIFVKSAGSRIEEGVLQFLVLLVAGRSGGGGSGGGGQ
EIEWLPFRCIKCLKLSFSTAELLCMHGGGGSGGGGSLVARCPPCLRLLRQARDFQAARYDRHD

GGGGSGGGGSALGTPEDLDSYIDLSLESLNQMILELDGGGGSGGGGSSQDMLSIMEKLEFLD
FSYDLNLCGLTEGGGGSGGGGSAGQAEPDRAHYNIVTFCCKCDSTLRLCVQSTHVDIRTLEDL
LMGTLGGGSGGGGSGRREQYIPNEEFLHFDLLEDSKYRKIYS

SEQ ID NO: 18 (221 amino acids):
Antigenic unit comprising antigen sequences in the following order: NY-ESO-1 | linker | E7 | linker | E6.
GARGPESRLLEFYLAMPFATPMEAELARRSLAQDAPPLPVPGVLLKEFTVSGNILTIRLTAADHR
QLQLSISSCLQQLSLLMWITQCFLPVFGGGGSGGGGSAGQAEPDRAHYNIVTFCCKCDSTLRL
CVQSTHVDIRTLSGGGGSGGGGKFYSKISEYRHYCYSLYGTTLEQQYNKPLCDLLIRCINRQKP
LCPEEKQRHLDKKQRFHNIRGRWTGRCMS

SEQ ID NO: 19 (831 amino acids)
Antigenic unit comprising antigen sequences in the following order: T1D223 | linker | T1D164 | linker | T1D56 | linker | T1D36 | linker | T1D129 | linker | T1D274 | linker | T1D62 | linker | T1D5 | linker | T1D144 | linker | T1D441 | linker | T1D368 | linker | NY-ESO-1 | linker | T1D234 | linker | T1D162 | linker | T1D39 | linker | T1D272 | linker | E7 | linker | T1D328 | linker | T1D188 | linker | E6.
GPSYRSNSVSSLDLEGEGGGGSGGGGSLKEEKENLQGLVTHQTYIIQELEKQLNGGGGSGGG
GSQRQKTAGKIFRAEVSTGQDAPRQAQARGGGSSGGGSSILRPSTVNELESGEISYDEVGERI
KDFGGGGSGGGGSDTCINEDVESLRKMVQDLLAKLQEAKRSGGGGSGGGGLREEGTKYKSF
VQRARLVFREEGYLAFGGGGSGGGGSHEGARPMRAIFLANGNVFTTGFSRMSEGGGGSGG
GGSIPDAAFIQAARRKCELARAQDDYISLDGGGGSGGGGSMEIAKMRAGRRLWVHLIEKMFQP
KNSKGGGGSGGGGSMELVSFRDVAIEFSPEEWGGGGSGGGGSTCVHEIPFHFDLMELLPQC
QQLQMFFLGGGSGGGGSGGARGPESRLLEFYLAMPFATPMEAELARRSLAQDAPPLPVPGVL
LKEFTVSGNILTIRLTAADHRQLQLSISSCLQQLSLLMWITQCFLPVFGGGGSGGGGSVRRVFIT
DDFHDMIPKYLNFVKGVVDSSGGGGSGGGGNFHAHREKAPSLFCSRILNKAVYLFYGSSGGG
SSGGGDANKEGMFLFRAAHKLRQFLKMNSTGDGGGGSGGGGSLLSLGWSVDVGRHSGWTG
HVSTSWSINGGGGSGGGGSAGQAEPDRAHYNIVTFCCKCDSTLRLCVQSTHVDIRTLSGGGG
SGGGGNTIFSLRKDLRQGKARRLRCMEEKEMFGGGGSGGGGSNHFNDFEGDPAMTQFLEEF
EKNLEDTKGGGGSGGGGSKFYSKISEYRHYCYSLYGTTLEQQYNKPLCDLLIRCINRQKPLCP
EEKQRHLDKKQRFHNIRGRWTGRCMS

SEQ ID NO: 20
Amino acid sequence of VB4097
MQVSTAALAVLLCTMALCNQVLSAPLAADTPTACCFSYTSRQIPQNFIADYFETSSQCSKPSVIF
LTKRGRQVCADPSEEWVQKYVSDLELSAELKTPLGDTTHTEPKSCDTPPPCPRCPGGGSSGG
GSGGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLD
SDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGKGLGGLKIYEFDYHLY

GQNITMIMTSVSGHLLAGGGGSGGGGSAEYGDYQPEVHGVPYFRLEHYLPARVMGGGGSGG
GGSGSLFGSSRVQYVVNPAVKIVFLNIDPSGGGGSGGGGSLWVYLRPVPRPATIYLQILRLKPL
TGEGGGGSGGGGSTLAFLVLSTPAMFNRALKPFLKSCHFQGGGGSGGGGSFVSPMAHYVPG
IMAIESVVARFQFIVPGGGGSGGGGSVILPQAPSGPSYATYLQPAQAQMLTPPGGGGSGGGG
SEVIQTSKYYMRDVIAIESAWLLELAPHGGGGSGGGGSDTLSAMSNPRAMQVLLQIQQGLQTL
ATGGGGSGGGGSKSWIHCWKYLSVQSQLFRGSSLLFRRV

SEQ ID NO: 21
Amino acid sequence of VB4100
MQVSTAALAVLLCTMALCNQVLSAPLAADTPTACCFSYTSRQIPQNFIADYFETSSQCSKPSVIF
LTKRGRQVCADPSEEWVQKYVSDLELSAELKTPLGDTTHTEPKSCDTPPPCPRCPGGGSSGG
GSGGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLD
SDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGKGLGGLMQAEGRGTG
GSTGDADGPGGPGIPDGPGGNAGGPGEAGATGGRGPRGAGAARASGPGGGAPRGPHGGA
ASGLNGCCRCGARGPESRLLEFYLAMPFATPMEAELARRSLAQDAPPLPVPGVLLKEFTVSGN
ILTIRLTAADHRQLQLSISSCLQQLSLLMWITQCFLPVFLAQPPSGQRR

SEQ ID NO: 22
Amino acid sequence of VB4101
MQVSTAALAVLLCTMALCNQVLSAPLAADTPTACCFSYTSRQIPQNFIADYFETSSQCSKPSVIF
LTKRGRQVCADPSEEWVQKYVSDLELSAELKTPLGDTTHTEPKSCDTPPPCPRCPGGGSSGG
GSGGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLD
SDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGKGLGGLRGPESRLL

SEQ ID NO: 23
Amino acid sequence of VB4102
MQVSTAALAVLLCTMALCNQVLSAPLAADTPTACCFSYTSRQIPQNFIADYFETSSQCSKPSVIF
LTKRGRQVCADPSEEWVQKYVSDLELSAELKTPLGDTTHTEPKSCDTPPPCPRCPGGGSSGG
GSGGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLD
SDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGKGLGGLRGPESRLLEF
YLAMPFATPMEAELARRSLAQDAPPLPVPGVLLKEF

SEQ ID NO: 24
Amino acid sequence of VB4105
MQVSTAALAVLLCTMALCNQVLSAPLAADTPTACCFSYTSRQIPQNFIADYFETSSQCSKPSVIF
LTKRGRQVCADPSEEWVQKYVSDLELSAELKTPLGDTTHTEPKSCDTPPPCPRCPGGGSSGG
GSGGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLD
SDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGKGLGGLKIYEFDYHLY
GQNITMIMTSVSGHLLAGGGGSGGGGSAEYGDYQPEVHGVPYFRLEHYLPARVMGGGGSGG

GGSGSLFGSSRVQYVVNPAVKIVFLNIDPSGGGGSGGGGSLWVYLRPVPRPATIYLQILRLKPL
TGEGGGGSGGGGSTLAFLVLSTPAMFNRALKPFLKSCHFQGGGGSGGGGSFVSPMAHYVPG
IMAIESVVARFQFIVPGGGGSGGGGSVILPQAPSGPSYATYLQPAQAQMLTPPGGGGSGGGG
SEVIQTSKYYMRDVIAIESAWLLELAPHGGGGSGGGGSDTLSAMSNPRAMQVLLQIQQGLQTL
ATGGGGSGGGGSKSWIHCWKYLSVQSQLFRGSSLLFRRVGGGGSGGGGSMQAEGRGTGG
STGDADGPGGPGIPDGPGGNAGGPGEAGATGGRGPRGAGAARASGPGGGAPRGPHGGAA
SGLNGCCRCGARGPESRLLEFYLAMPFATPMEAELARRSLAQDAPPLPVPGVLLKEFTVSGNI
LTIRLTAADHRQLQLSISSCLQQLSLLMWITQCFLPVFLAQPPSGQRR

SEQ ID NO: 25
Amino acid sequence of VB1026
MQVSTAALAVLLCTMALCNQVLSAPLAADTPTACCFSYTSRQIPQNFIADYFETSSQCSKPSVIF
LTKRGRQVCADPSEEWVQKYVSDLELSAELKTPLGDTTHTEPKSCDTPPPCPRCPGGGSSGG
GSGGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLD
SDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK

SEQ ID NO: 26
Amino acid sequence of VB4118
MQVSTAALAVLLCTMALCNQVLSAPLAADTPTACCFSYTSRQIPQNFIADYFETSSQCSKPSVIF
LTKRGRQVCADPSEEWVQKYVSDLELSAELKTPLGDTTHTEPKSCDTPPPCPRCPGGGSSGG
GSGGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLD
SDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGKGLGGLREGVELCPG
NKYEMRRHGTTHSLVIHDGGGGSGGGGSSSPDEVALVEGVQSLGFTYLRLKDNYMGGGGSG
GGGSLKDFFFSRGRFSSALKRTLILSSGFRLGGGGSGGGGSKNILAVSFAPLVQLSKNDNGTP
DSVGLGGGGSGGGGSLEAVLRYLETHPRLPKPDPVKSSSSVLGGGGSGGGGSPAADRWEP
RAPMRAPRVLHAMLGAAGRGGGGSGGGGSVLSADLVHYLRLSLEYLRAWHSEDVSLGGGGS
GGGGSYLPGGGAGHLDQNVFLRVRALCYVISGGGGGSGGGGSPATIEMAIETLQKFDGLSTH
RSSLLNSGGGGSGGGGSAGKDHKIKQWDADTFEHIQTLEGHHQE

SEQ ID NO: 27
Amino acid sequence of VB4119
MQVSTAALAVLLCTMALCNQVLSAPLAADTPTACCFSYTSRQIPQNFIADYFETSSQCSKPSVIF
LTKRGRQVCADPSEEWVQKYVSDLELSAELKTPLGDTTHTEPKSCDTPPPCPRCPGGGSSGG
GSGGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLD
SDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGKGLGGLSVYDFFVWL

SEQ ID NO: 28
Amino acid sequence of VB4127

MQVSTAALAVLLCTMALCNQVLSAPLAADTPTACCFSYTSRQIPQNFIADYFETSSQCSKPSVIF
LTKRGRQVCADPSEEWVQKYVSDLELSAELKTPLGDTTHTEPKSCDTPPPCPRCPGGGSSGG
GSGGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLD
SDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGKGLGGLYISDHMKVHS
PSPCLGGGGSGGGGSEGWQTCWGRSRKHWGSTWNGSARLSPGSTLWVMRICLRSLGIART
WLSCRSTSRKCSPAFPASSGGGGGSGGGGSLFRLLPSGPKVNDGHRSRRWHSLDLYPHLCS
HLDLPKQSQEVIRL

SEQ ID NO: 29
Amino acid sequence of VB4121
MQVSTAALAVLLCTMALCNQVLSAPLAADTPTACCFSYTSRQIPQNFIADYFETSSQCSKPSVIF
LTKRGRQVCADPSEEWVQKYVSDLELSAELKTPLGDTTHTEPKSCDTPPPCPRCPGGGSSGG
GSGGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLD
SDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGKGLGGLREGVELCPG
NKYEMRRHGTTHSLVIHDGGGGSGGGGSSSPDEVALVEGVQSLGFTYLRLKDNYMGGGGSG
GGGSLKDFFFSRGRFSSALKRTLILSSGFRLGGGGSGGGGSKNILAVSFAPLVQLSKNDNGTP
DSVGLGGGGSGGGGSLEAVLRYLETHPRLPKPDPVKSSSSVLGGGGSGGGGSPAADRWEP
RAPMRAPRVLHAMLGAAGRGGGGSGGGGSVLSADLVHYLRLSLEYLRAWHSEDVSLGGGGS
GGGGSYLPGGGAGHLDQNVFLRVRALCYVISGGGGGSGGGGSPATIEMAIETLQKFDGLSTH
RSSLLNSGGGGSGGGGSAGKDHKIKQWDADTFEHIQTLEGHHQEGGGGSGGGGSSVYDFFV
WL

SEQ ID NO: 30
Amino acid sequence of VB4128
MQVSTAALAVLLCTMALCNQVLSAPLAADTPTACCFSYTSRQIPQNFIADYFETSSQCSKPSVIF
LTKRGRQVCADPSEEWVQKYVSDLELSAELKTPLGDTTHTEPKSCDTPPPCPRCPGGGSSGG
GSGGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLD
SDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGKGLGGLYISDHMKVHS
PSPCLGGGGSGGGGSEGWQTCWGRSRKHWGSTWNGSARLSPGSTLWVMRICLRSLGIART
WLSCRSTSRKCSPAFPASSGGGGGSGGGGSLFRLLPSGPKVNDGHRSRRWHSLDLYPHLCS
HLDLPKQSQEVIRLGGGGSGGGGSREGVELCPGNKYEMRRHGTTHSLVIHDGGGGSGGGGS
SSPDEVALVEGVQSLGFTYLRLKDNYMGGGGSGGGGSLKDFFFSRGRFSSALKRTLILSSGFR
LGGGGSGGGGSKNILAVSFAPLVQLSKNDNGTPDSVGLGGGGSGGGGSLEAVLRYLETHPRL
PKPDPVKSSSSVLGGGGSGGGGSPAADRWEPRAPMRAPRVLHAMLGAAGRGGGGSGGGG
SVLSADLVHYLRLSLEYLRAWHSEDVSLGGGGSGGGGSYLPGGGAGHLDQNVFLRVRALCYV
ISGGGGGSGGGGSPATIEMAIETLQKFDGLSTHRSSLLNSGGGGSGGGGSAGKDHKIKQWDA
DTFEHIQTLEGHHQE

SEQ ID NO: 31
Amino acid sequence of VB4130
MQVSTAALAVLLCTMALCNQVLSAPLAADTPTACCFSYTSRQIPQNFIADYFETSSQCSKPSVIF
LTKRGRQVCADPSEEWVQKYVSDLELSAELKTPLGDTTHTEPKSCDTPPPCPRCPGGGSSGG
GSGGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLD
SDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGKGLGGLYISDHMKVHS
PSPCLGGGGSGGGGSEGWQTCWGRSRKHWGSTWNGSARLSPGSTLWVMRICLRSLGIART
WLSCRSTSRKCSPAFPASSGGGGGSGGGGSLFRLLPSGPKVNDGHRSRRWHSLDLYPHLCS
HLDLPKQSQEVIRLGGGGSGGGGSREGVELCPGNKYEMRRHGTTHSLVIHDGGGGSGGGGS
SSPDEVALVEGVQSLGFTYLRLKDNYMGGGGSGGGGSLKDFFFSRGRFSSALKRTLILSSGFR
LGGGGSGGGGSKNILAVSFAPLVQLSKNDNGTPDSVGLGGGGSGGGGSLEAVLRYLETHPRL
PKPDPVKSSSSVLGGGGSGGGGSPAADRWEPRAPMRAPRVLHAMLGAAGRGGGGSGGGG
SVLSADLVHYLRLSLEYLRAWHSEDVSLGGGGSGGGGSYLPGGGAGHLDQNVFLRVRALCYV
ISGGGGGSGGGGSPATIEMAIETLQKFDGLSTHRSSLLNSGGGGSGGGGSAGKDHKIKQWDA
DTFEHIQTLEGHHQEGGGGSGGGGSSVYDFFVWLGGGGSGGGGS

SEQ ID NO: 32
Amino acids 6-15 of HPV E6
QERPRKLPQ

SEQ ID NO: 33
Amino acids 8-17 of HPV E6
RPRKLPQLC

SEQ ID NO: 34
Amino acids 23-32 of HPV E6
IHDIILECV

SEQ ID NO: 35
Amino acids 24-33 of HPV E6
HDIILECVY

SEQ ID NO: 36
Amino acids 26-35 of HPV E6
IILECVYCK

SEQ ID NO: 37
Amino acids 35-44 of HPV E6
QQLLRREVY

SEQ ID NO: 38
Amino acids 42-51 of HPV E6
VYDFARRDL

SEQ ID NO: 39
Amino acids 43-52 of HPV E6
YDFARRDLC

SEQ ID NO: 40
Amino acids 44-53 of HPV E6
DFARRDLCI

SEQ ID NO: 41
Amino acids 45-54 of HPV E6
FARRDLCIV

SEQ ID NO: 42
Amino acids 60-69 of HPV E6
YAVRDKCLK

SEQ ID NO: 43
Amino acids 62-71 of HPV E6
VRDKCLKFY

SEQ ID NO: 44
Amino acids 81-90 of HPV E6
YSLYGTTLE

SEQ ID NO: 45
Amino acids 125-134 of HPV E6
FHNIRGRWT

SEQ ID NO: 46
Amino acids 131-140 of HPV E6
RWTGRCMSC

SEQ ID NO: 47
Amino acids 143-152 of HPV E6
SRTRRETQL

SEQ ID NO: 48
Amino acids 16-31 of HPV E6
CTELQTTIHDIILEC

SEQ ID NO: 49
Amino acids 17-32 of HPV E6
TELQTTIHDIILECV

SEQ ID NO: 50
Amino acids 18-33 of HPV E6
ELQTTIHDIILECVY

SEQ ID NO: 51
Amino acids 19-34 of HPV E6
LQTTIHDIILECVYC

SEQ ID NO: 52
Amino acids 20-35 of HPV E6
QTTIHDIILECVYCK

SEQ ID NO: 53
Amino acids 34-49 of HPV E6
KQQLLRREVYDFARR

SEQ ID NO: 54
Amino acids 49-64 of HPV E6
DLCIVYRDGNPYAVR

SEQ ID NO: 55
Amino acids 50-65 of HPV E6
LCIVYRDGNPYAVRD

SEQ ID NO: 56
Amino acids 51-66 of HPV E6
CIVYRDGNPYAVRDK

SEQ ID NO: 57
Amino acids 78-93 of HPV E6
HYCYSLYGTTLEQQY

SEQ ID NO: 58
Amino acids 95-110 of HPV E6
PLCDLLIRCINRQKP

SEQ ID NO: 59
Amino acids 96-111 of HPV E6
LCDLLIRCINRQKPL

SEQ ID NO: 60
Amino acids 97-112 of HPV E6
CDLLIRCINRQKPLC

SEQ ID NO: 61
Amino acids 98-113 of HPV E6
DLLIRCINRQKPLCP

SEQ ID NO: 62
Amino acids 99-114 of HPV E6
LLIRCINRQKPLCPE

SEQ ID NO: 63
Amino acids 121-136 of HPV E6
KKQRFHNIRGRWTGR

SEQ ID NO: 64
Amino acids 7-16 of HPV E7
TLHEYMLDL

SEQ ID NO: 65
Amino acids 22-31 of HPV E7
LYGYGQLND

SEQ ID NO: 66
Amino acids 38-47 of HPV E7
IDGPAGQAE

SEQ ID NO: 67
Amino acids 48-57 of HPV E7
DRAHYNIVT

SEQ ID NO: 68
Amino acids 55-64 of HPV E7
VTFCCKCDS

SEQ ID NO: 69
Amino acids 66-75 of HPV E7
RLCVQSTHV

SEQ ID NO: 70
Amino acids 72-81 of HPV E7
THVDIRTLE

SEQ ID NO: 71
Amino acids 73-82 of HPV E7
HVDIRTLED

SEQ ID NO: 72
Amino acids 85-94 of HPV E7
GTLGIVCPI

SEQ ID NO: 73
Amino acids 4-19 of HPV E7
DTPTLHEYMLDLQPE

SEQ ID NO: 74
Amino acids 5-20 of HPV E7
TPTLHEYMLDLQPET

SEQ ID NO: 75
Amino acids 6-21 of HPV E7
PTLHEYMLDLQPETT

SEQ ID NO: 76
Amino acids 7-22 of HPV E7
TLHEYMLDLQPETTD

SEQ ID NO: 77
Amino acids 8-23 of HPV E7
LHEYMLDLQPETTDL

SEQ ID NO: 78
Amino acids 9-24 of HPV E7
HEYMLDLQPETTDLY

SEQ ID NO: 79
Amino acids 10-25 of HPV E7
EYMLDLQPETTDLYG

SEQ ID NO: 80
Amino acids 70-85 of HPV E7
QSTHVDIRTLEDLLM

SEQ ID NO: 81
Amino acids 71-86 of HPV E7
STHVDIRTLEDLLMG

SEQ ID NO: 82
Amino acids 72-87 of HPV E7
THVDIRTLEDLLMGT

SEQ ID NO: 83
Amino acids 73-88 of HPV E7
HVDIRTLEDLLMGTL

SEQ ID NO: 84
Amino acids 74-89 of HPV E7
VDIRTLEDLLMGTLG

SEQ ID NO: 85
Amino acids 16-25 of HPV E6
CTELQTTIH

SEQ ID NO: 86
Amino acids 42-51 of HPV E6
VYDFARRDL

SEQ ID NO: 87
Amino acids 45-54 of HPV E6
FARRDLCIV

SEQ ID NO: 88
Amino acids 52-61 of HPV E6
IVYRDGNPY

SEQ ID NO: 89
Amino acids 54-63 of HPV E6
YRDGNPYAV

SEQ ID NO: 90
Amino acids 68-77 of HPV E6
KFYSKISEY

SEQ ID NO: 91
Amino acids 73-82 of HPV E6
ISEYRHYCY

SEQ ID NO: 92
Amino acids 75-84 of HPV E6
EYRHYCYSL

SEQ ID NO: 93
Amino acids 80-89 of HPV E6
CYSLYGTTL

SEQ ID NO: 94
Amino acids 84-93 of HPV E6
YGTTLEQQY

SEQ ID NO: 95
Amino acids 88-97 of HPV E6
LEQQYNKPL

SEQ ID NO: 96
Amino acids 91-100 of HPV E6
QYNKPLCDL

SEQ ID NO: 97
Amino acids 11-20 of HPV E6
RHLDKKQRF

SEQ ID NO: 98
Amino acids 12-21 of HPV E6
RFHNIRGRW

SEQ ID NO: 99
Amino acids 18-33 of HPV E6
ELQTTIHDIILECVY

SEQ ID NO: 100
Amino acids 49-64 of HPV E6
DLCIVYRDGNPYAVR

SEQ ID NO: 101
Amino acids 50-65 of HPV E6
LCIVYRDGNPYAVRD

SEQ ID NO: 102
Amino acids 51-66 of HPV E6
CIVYRDGNPYAVRDK

SEQ ID NO: 103
Amino acids 74-89 of HPV E6
SEYRHYCYSLYGTTL

SEQ ID NO: 104
Amino acids 75-90 of HPV E6
EYRHYCYSLYGTTLE

SEQ ID NO: 105
Amino acids 76-91 of HPV E6
YRHYCYSLYGTTLEQ

SEQ ID NO: 106
Amino acids 77-92 of HPV E6
RHYCYSLYGTTLEQQ

SEQ ID NO: 107
Amino acids 78-93 of HPV E6
HYCYSLYGTTLEQQY

SEQ ID NO: 108
Amino acids 96-111 of HPV E6
LCDLLIRCINRQKPL

SEQ ID NO: 109
Amino acids 97-112 of HPV E6
CDLLIRCINRQKPLC

SEQ ID NO: 110
Amino acids 98-113 of HPV E6
DLLIRCINRQKPLCP

SEQ ID NO: 111
Amino acids 99-114 of HPV E6
LLIRCINRQKPLCPE

SEQ ID NO: 112
Amino acids 121-136 of HPV E6
KKQRFHNIRGRWTGR

SEQ ID NO: 113
Amino acids 3-12 of HPV E7
GDTPTLHEY

SEQ ID NO: 114
Amino acids 7-16 of HPV E7
TLHEYMLDL

SEQ ID NO: 115
Amino acids 9-18 of HPV E6
HEYMLDLQP

SEQ ID NO: 116
Amino acids 15-24 of HPV E6
LQPETTDLY

SEQ ID NO: 117
Amino acids 19-28 of HPV E6
TTDLYGYGQ

SEQ ID NO: 118
Amino acids 20-29 of HPV E6
TDLYGYGQL

SEQ ID NO: 119
Amino acids 44-53 of HPV E6
QAEPDRAHY

SEQ ID NO: 120
Amino acids 49-58 of HPV E6
RAHYNIVTF

SEQ ID NO: 121
Amino acids 71-80 of HPV E6
STHVDIRTL

SEQ ID NO: 122
Amino acids 79-88 of HPV E6
LEDLLMGTL

SEQ ID NO: 123
Amino acids ´03-18 of HPV E6
GDTPTLHEYMLDLQP

SEQ ID NO: 124
Amino acids 4-19 of HPV E6
DTPTLHEYMLDLQPE

SEQ ID NO: 125
Amino acids 5-20 of HPV E6
TPTLHEYMLDLQPET

SEQ ID NO: 126
Amino acids 6-21 of HPV E6
PTLHEYMLDLQPETT

SEQ ID NO: 127
Amino acids 7-22 of HPV E6
TLHEYMLDLQPETTD

SEQ ID NO: 128
Amino acids 8-23 of HPV E6
LHEYMLDLQPETTDL

SEQ ID NO: 129
Amino acids 70-85 of HPV E6
QSTHVDIRTLEDLLM

SEQ ID NO: 130
Amino acids 71-86 of HPV E6
STHVDIRTLEDLLMG

SEQ ID NO: 131
Amino acids 72-87 of HPV E6
THVDIRTLEDLLMGT

SEQ ID NO: 132
Amino acids 73-88 of HPV E6
HVDIRTLEDLLMGTL

SEQ ID NO: 133
Amino acids 74-89 of HPV E6
VDIRTLEDLLMGTLG

SEQ ID NO: 134
T1D320
HNEGDDQQGSRYSLIPQIQKVCEVVDG

SEQ ID NO: 135
T1D814
SEQERMKSSLLKEHMLRKQAELESAQC

SEQ ID NO: 136
T1D182
ELRENLLTYLPDSVTQLRRLEELDLGN

SEQ ID NO: 137
T1D689
QEVFSSYKFNHLVRRLVLQREKHFHYL

SEQ ID NO: 138
T1D339
IEKNADLCYLSTVHWSLILDAVSNNYI

SEQ ID NO: 139
T1D428
LATAGEPYHDIRFKLMAVVPDRRIKYE

SEQ ID NO: 140
T1D572
MLRLPTVFRQIRPVSRVLAPHLTR

SEQ ID NO: 141
T1D359
IQIMENPFVQSMLWNPDLMRQLIMANP

SEQ ID NO: 142
T1D488
LLRFLFLGLSALALPSRAQLQLHLPAN

SEQ ID NO: 143
T1D554
MGKNPVRPPRAFPPVPSIDDIPLSR

SEQ ID NO: 144
T1D272
GHVDFTIEVERALTVLDGAVLVLCAVG

SEQ ID NO: 145
T1D210
EYKLMYGMLFSIRLFVSKMSPLDMKDG

SEQ ID NO: 146
T1D849
SQGAGVESLDFRLYLRYEFLMLGIQPV

SEQ ID NO: 147
T1D4
AAKTLVLCVSDIILLSANISETSSNKT

SEQ ID NO: 148
T1D77
CKDLQLYLSNLANHIDRETGIGDVPLV

SEQ ID NO: 149
T1D717
QSKHTEARELMYSAALLFFSHGQQNSA

SEQ ID NO: 150
T1D586
MTLAESYAQYVYNLCNSLSIKVEES

SEQ ID NO: 151
T11D323

KKVSKTRHTRETVFRRAKRRWAPIPCS

SEQ ID NO: 152
T11D506
NLKHENILQFLTAQERKTELGKQYWLI

SEQ ID NO: 153
T11D12
VQVFGLYFGEEFHETFDCPIK

SEQ ID NO: 154
T11D315
GQAAASQAGGARGYARGAQLWPPGSDP

SEQ ID NO: 155
T11D302
SIMQTWFTLFTPTDATSIVATTVMSNS

SEQ ID NO: 156
T11D700
QENVRFVLVRSEALLPNAGPRSAEARV

SEQ ID NO: 157
T11D535
DAFPNLKDFISRFQVMPPSSFLFDAPC

SEQ ID NO: 158
T11D358
PSSLQVKPETPASEAVAVAAAAAPTTT

SEQ ID NO: 159
T11D670
RFFHLADLFLSSSQLPAYLVAAFAKRL

SEQ ID NO: 160
T11D294
LVFLWLHSLRRLFGCLYVSVFSNVMIH

SEQ ID NO: 161

T11D336
WENAIAALFRRHIAVSWLIRATLSESE

SEQ ID NO: 162
T11D499
RYIFVKSAGSRIEEGVLQFLVLLVAGR

SEQ ID NO: 163
T11D425
QEIEWLPFRCIKCLKLSFSTAELLCMH

SEQ ID NO: 164
T11D491
LVARCPPCLRLLRQARDFQAARYDRHD

SEQ ID NO: 165
T11D314
ALGTPEDLDSYIDLSLESLNQMILELD

SEQ ID NO: 166
T11D430
SQDMLSIMEKLEFLDFSYDLNLCGLTE

SEQ ID NO: 167
T11D582
RREQYIPNEEFLHFDLLEDSKYRKIYS

SEQ ID NO: 168
T1D223
GPSYRSNSVSSLDLEGE

SEQ ID NO: 169
T1D164
LKEEKENLQGLVTHQTYIIQELEKQLN

SEQ ID NO: 170
T1D56
QRQKTAGKIFRAEVSTGQDAPRQAQAR

SEQ ID NO: 171
T1D36
ILRPSTVNELESGEISYDEVGERIKDF

SEQ ID NO: 172
T1D129
DTCINEDVESLRKMVQDLLAKLQEAKR

SEQ ID NO: 173
T1D274
LREEGTKYKSFVQRARLVFREEGYLAF

SEQ ID NO: 174
T1D62
HEGARPMRAIFLANGNVFTTGFSRMSE

SEQ ID NO: 175
T1D5
IPDAAFIQAARRKCELARAQDDYISLD

SEQ ID NO: 176
T1D144
MEIAKMRAGRRLWVHLIEKMFQPKNSK

SEQ ID NO: 177
T1D441
MELVSFRDVAIEFSPEEW

SEQ ID NO: 178
T1D368
TCVHEIPFHFDLMELLPQCQQLQMFFL

SEQ ID NO: 179
T1D234
VRRVFITDDFHDMIPKYLNFVKGVVDS

SEQ ID NO: 180
T1D162
NFHAHREKAPSLFCSRILNKAVYLFYG

SEQ ID NO: 181
T1D39
DANKEGMFLFRAAHKLRQFLKMNSTGD

SEQ ID NO: 182
T1D272
LLSLGWSVDVGRHSGWTGHVSTSWSIN

SEQ ID NO: 183
T1D328
NTIFSLRKDLRQGKARRLRCMEEKEMF

SEQ ID NO: 184
T1D188
NHFNDFEGDPAMTQFLEEFEKNLEDTK

SEQ ID NO: 185
C-pepM1
VILPQAPSGPSYATYLQPAQAQMLTPP

SEQ ID NO: 186
C-pepM6
DTLSAMSNPRAMQVLLQIQQGLQTLAT

SEQ ID NO: 187
C-pepM8
EVIQTSKYYMRDVIAIESAWLLELAPH

SEQ ID NO: 188
C-pepM29
GSLFGSSRVQYVVNPAVKIVFLNIDPS

SEQ ID NO: 189
C-pepM31
AEYGDYQPEVHGVPYFRLEHYLPARVM

SEQ ID NO: 190
C-pepM43
KSWIHCWKYLSVQSQLFRGSSLLFRRV

SEQ ID NO: 191
C-pepM89
TLAFLVLSTPAMFNRALKPFLKSCHFQ

SEQ ID NO: 192
C-pep149
FVSPMAHYVPGIMAIESVVARFQFIVP

SEQ ID NO: 193
C-pepM171
LWVYLRPVPRPATIYLQILRLKPLTGE

SEQ ID NO: 194
C-pepM173
KIYEFDYHLYGQNITMIMTSVSGHLLA

SEQ ID NO: 195
VB 4101 Amino acids 81-88
RGPESRLL

SEQ ID NO: 196
VB4102 Amino Acids 81-126
RGPESRLLEFYLAMPFATPMEAELARRSLAQDAPPLPVPGVLLKEF

SEQ ID NO: 197
NY-ESO_Pep-1
MQAEGRGTGGSTGDA

SEQ ID NO: 198
NY-ESO_Pep-2
TGGSTGDADGPGGPG

SEQ ID NO: 199
NY-ESO_Pep-3
ADGPGGPGIPDGPGG

SEQ ID NO: 200
NY-ESO_Pep-4
GIPDGPGGNAGGPGE

SEQ ID NO: 201
NY-ESO_Pep-5
GNAGGPGEAGATGGR

SEQ ID NO: 202
NY-ESO_Pep-6
EAGATGGRGPRGAGA

SEQ ID NO: 203
NY-ESO_Pep-7
RGPRGAGAARASGPG

SEQ ID NO: 204
NY-ESO_Pep-8
AARASGPGGGAPRGP

SEQ ID NO: 205
NY-ESO_Pep-9
GGGAPRGPHGGAASG

SEQ ID NO: 206
NY-ESO_Pep-10
PHGGAASGLNGCCRC

SEQ ID NO: 207
NY-ESO_Pep-11
GLNGCCRCGARGPES

SEQ ID NO: 208
NY-ESO_Pep-12
CGARGPESRLLEFYL

SEQ ID NO: 209
NY-ESO_Pep-13

RGPESRLLEFYLAMP

SEQ ID NO: 210
NY-ESO_Pep-14
SRLLEFYLAMPFATP

SEQ ID NO: 211
NY-ESO_Pep-15
LAMPFATPMEAELAR

SEQ ID NO: 212
NY-ESO_Pep-16
PMEAELARRSLAQDA

SEQ ID NO: 213
NY-ESO_Pep-17
RRSLAQDAPPLPVPG

SEQ ID NO: 214
NY-ESO_Pep-18
DAPPLPVPGVLLKEF

SEQ ID NO: 215
NY-ESO_Pep-19
APPLPVPGVLLKEFT

SEQ ID NO: 216
NY-ESO_Pep-20
GVLLKEFTVSGNILT

SEQ ID NO: 217
NY-ESO_Pep-21
TVSGNILTIRLTAAD

SEQ ID NO: 218
NY-ESO_Pep-22
TIRLTAADHRQLQLS

SEQ ID NO: 219

NY-ESO_Pep-23
DHRQLQLSISSCLQQ

SEQ ID NO: 220
NY-ESO_Pep-24
SISSCLQQLSLLMWI

SEQ ID NO: 221
NY-ESO_Pep-25
QLSLLMWITQCFLPV

SEQ ID NO: 222
NY-ESO_Pep-26
ITQCFLPVFLAQPPS

SEQ ID NO: 223
NY-ESO_Pep-27
VFLAQPPSGQRR

SEQ ID NO: 224
NY-ESO_Pep-28
RGPESRLL

SEQ ID NO: 225
B-pepM2
REGVELCPGNKYEMRRHGTTHSLVIHD

SEQ ID NO: 226
B-pepM7
SSPDEVALVEGVQSLGFTYLRLKDNYM

SEQ ID NO: 227
B-pepM36
KNILAVSFAPLVQLSKNDNGTPDSVGL

SEQ ID NO: 228
B-pepM78
PAADRWEPRAPMRAPRVLHAMLGAAGR

SEQ ID NO: 229
B-pepM79
VLSADLVHYLRLSLEYLRAWHSEDVSL

SEQ ID NO: 230
B-pepM82
LEAVLRYLETHPRLPKPDPVKSSSSVL

SEQ ID NO: 231
B-pepM83
LKDFFFSRGRFSSALKRTLILSSGFRL

SEQ ID NO: 232
B-pepM84
AGKDHKIKQWDADTFEHIQTLEGHHQE

SEQ ID NO: 233
B-pepM85
PATIEMAIETLQKFDGLSTHRSSLLNS

SEQ ID NO: 234
B-pepM86
YLPGGGAGHLDQNVFLRVRALCYVISG

SEQ ID NO: 235
VB4119 Amino acids 180-188
SVYDFFVWL

SEQ ID NO: 236
B-pepM108
YISDHMKVHSPSPCL

SEQ ID NO: 237
B-pepM115-M122
EGWQTCWGRSRKHWGSTWNGSARLSPGSTLWVMRICLRSLGIARTWLSCRSTSRKCSPAFP
ASS

SEQ ID NO: 238
B-pepM141-M142

LFRLLPSGPKVNDGHRSRRWHSLDLYPHLCSHLDLPKQSQEVIRL

SEQ ID NO: 239
B-pepM108
YISDHMKVHSPSPCL

SEQ ID NO: 240
B-pepM115
EGWQTCWGRSRKHWG

SEQ ID NO: 241
B-pepM116
GRSRKHWGSTWNGSA

SEQ ID NO: 242
B-pepM117
GSTWNGSARLSPGST

SEQ ID NO: 243
B-pepM118
ARLSPGSTLWVMRIC

SEQ ID NO: 244
B-pepM119
TLWVMRICLRSLGIA

SEQ ID NO: 245
B-pepM120
CLRSLGIARTWLSCR

SEQ ID NO: 246
B-pepM121
ARTWLSCRSTSRKCS

SEQ ID NO: 247
B-pepM122
RSTSRKCSPAFPASS

SEQ ID NO: 248
B-pepM141
LFRLLPSGPKVNDGHRSRRWHSLDLYP

SEQ ID NO: 249
B-pepM142
SRRWHSLDLYPHLCSHLDLPKQSQEVIRL

Embodiments A:

1. An individualized therapeutic anticancer vaccine comprising an immunologically effective amount of:
   (i) a polynucleotide comprising a nucleotide sequence encoding a targeting unit, a dimerization unit and an antigenic unit, wherein said antigenic unit comprises at least one patient-present shared antigen sequence or one or more parts thereof, and optionally one or more patient-specific antigen sequences or one or more parts thereof; or
   (ii) a polypeptide encoded by the polynucleotide as defined in (i), or
   (iii) a dimeric protein consisting of two polypeptides encoded by the polynucleotide as defined in (i); and
   a pharmaceutically acceptable carrier.

2. The vaccine according to embodiment A1, wherein said at least one patient-present shared antigen sequence is a shared antigen selected from the group consisting of overexpressed cellular proteins, aberrantly expressed cellular proteins, cancer testis antigens, viral antigens, differentiation antigens, mutated oncogenes and mutated tumor suppressor genes, oncofetal antigens, shared fusion antigens, shared intron retention antigens, dark matter antigens and shared antigens caused by spliceosome mutations or frameshift mutations

3. The vaccine according to any of embodiments A1 or A2, wherein said at least one patient-present shared antigen sequence is a shared antigen that is a human cellular protein, preferably an overexpressed or aberrantly expressed human cellular protein or a differentiation antigen.

4. The vaccine according to any of embodiments A1 to A3, wherein said at least one patient-present shared antigen sequence is known to be immunogenic or wherein one or more parts thereof are known to be immunogenic or are predicted to bind to the patient's HLA class I or HLA class II alleles, preferably to the patient's HLA class I alleles.

5. The vaccine according to any of embodiments A1 to A4, wherein said at least one patient-present shared antigen sequence has a length suitable for presentation by the patient's HLA alleles, preferably a length of from 7 to 30 amino acids.

6. The vaccine according to any of embodiments A1 to A5, comprising more than one patient-present shared antigen sequence or one or more parts thereof.

7. The vaccine according to embodiment A6, comprising sequences of several patient-present shared antigens or one or more parts thereof, preferably several parts of sequences of several patient-present shared antigens, more preferably several epitopes of several patient-present shared antigen, which epitopes are known to be immunogenic or are predicted to bind to the particular patient's HLA class I and HLA class II alleles.

8. The vaccine according to any of embodiments A1 to A7, wherein the antigenic unit comprises one or more patient-present shared antigen sequences in full length, preferably 1 to 10 patient-present shared antigen sequences in full length.

9. The vaccine according to any of embodiments A1 to A7, wherein the antigenic unit comprises 1 to 30 parts of patient-present shared antigen sequences in the form of long peptide sequences, preferably peptide sequences of from about 28 to 100 amino acids.

10. The vaccine according to embodiment A9, wherein the long peptide sequences include multiple epitopes that are predicted to bind to the patient's HLA class I or HLA class II alleles.

11. The vaccine according to any of embodiments A1 to A7, wherein the antigenic unit comprises 1 to 50 parts of patient-present shared antigen sequences in the form of short peptide sequences/epitopes.

12. The vaccine according to embodiment A11, wherein the short peptide sequences/epitopes that are predicted to bind to the patient's HLA class I or HLA class II alleles.

13. The vaccine according to any of embodiments A11 to A12, wherein the short peptide sequences/epitopes have a length of from 7 to 30 amino acids, e.g. 7 to 10 or 13 to 30.

14. The vaccine according to any of the preceding embodiments A1 to A13, comprising one or more patient-specific antigen sequences or one or more parts thereof.

15. The vaccine according to embodiment A14, comprising several patient-specific antigen sequences or one or more parts thereof.

16. The vaccine according to embodiment A15, comprising one or more parts of said patient-specific antigen sequences, preferably one or more patient-specific epitope.

17. The vaccine according to embodiment A16, comprising one or more patient-specific epitopes having a length of from 7 to 30 amino acids, e.g. 7 to 10 or 13 to 30.

18. The vaccine according to any of embodiments A14 to A17, wherein antigenic unit comprises at least 10 patient-specific epitopes, preferably at least 15 patient-specific epitopes, such as at least 20 patient-specific epitopes.

19. The vaccine according to any of the preceding embodiments A1 to A18, wherein the antigenic unit comprises from 21 to 2000 amino acids, preferably from about 30 amino acids to about a 1500 amino acids, more preferably from about 50 to about 1000 amino acids, such as from about 100 to about 500 amino acids or from about 100 to about 400 amino acids or from about 100 to about 300 amino acids.

20. The vaccine according to any of the preceding embodiments A1 to A19, wherein the antigenic unit comprises one or more linkers, preferably one or more non-immunogenic and/or flexible linkers.

21. The vaccine according to embodiment A20, wherein the length of the one or more linkers is from 4 to 20 amino acids.

22. The vaccine according to any of embodiments A20 to A21, wherein the one or more linkers separate the antigen sequences from each other.

23. The vaccine according to any of the preceding embodiments A1 to A22, wherein the dimerization unit comprises a hinge region and optionally another domain that facilitates dimerization, optionally connected through a linker.

24. The vaccine according to embodiment A23, wherein the hinge region is Ig-derived.

25. The vaccine according to any one of embodiments A23 and A24, wherein the hinge region has the ability to form one or more covalent bonds, preferably a covalent bond in the form of a disulfide bridge.

26. The vaccine according to any one of embodiments A23 to A25, wherein the another domain that facilitates dimerization is an immunoglobulin domain, preferably a carboxyterminal C domain, or a sequence that is substantially identical to said C domain or a variant thereof.

27. The vaccine according to embodiment A26, wherein the carboxyterminal C domain is derived from IgG.

28. The vaccine according to any one of embodiments A26 and A37, wherein the immunoglobulin domain of the dimerization unit has the ability to homodimerize, preferably via noncovalent interactions and more preferably via noncovalent interactions that are hydrophobic interactions.

29. The vaccine according to any one of embodiments A23 to A28, wherein said dimerization unit does not comprise a CH2 domain.

30. The vaccine according to any one of embodiments A23 to A29, wherein the dimerization unit consist of hinge exons h1 and h4 connected through said third linker to a C_H3 domain of human IgG3.

31. The vaccine according to any one of embodiments A23 to A30, wherein the dimerization unit comprises an amino acid sequence having at least 80 % sequence identity to the amino acid sequence 94-237 of SEQ ID NO.: 3.

32. The vaccine according to any of the preceding embodiments A1 to A31, wherein the antigenic unit and the dimerization unit are connected through a linker, preferably a linker that comprises a restriction site.

33. The vaccine according to any of the preceding embodiments A1 to A32, wherein the targeting unit has affinity for a chemokine receptor selected from CCR1, CCR3 and CCR5.

34. The vaccine according to any of the preceding embodiments A1 to A33, wherein said targeting unit comprises antibody binding regions with specificity for CD14, CD40, or Toll- like receptor or ligands, such as soluble CD40 ligand, or chemokines, such as RANTES or MIP-1a or bacterial antigens, such as flagellin.

35. The vaccine according to any of embodiments A1 to A33, wherein said targeting unit has an affinity for MHC class II proteins, preferably MHC class II proteins, selected from the group consisting of anti-HLA-DP, anti-HLA-DR and anti-pan HLA class II

36. The vaccine according to any of embodiments A1 to A33, wherein said targeting unit has an amino acid sequence having at least 80 % sequence identity to the amino acid sequence 24-93 of SEQ ID NO.: 1.

37. The vaccine according to any of the preceding embodiments A1 to A36, wherein said polynucleotide further encodes a signal peptide.

38. The vaccine according to any of the preceding embodiments A1 to A37, wherein said targeting unit, dimerization unit and antigenic unit in said peptide are in the N-terminal to C-terminal order of targeting unit, dimerization unit and antigenic unit.

39. The vaccine according to any of the preceding embodiments A1 to A38, wherein said polynucleotide sequence is human codon optimized.

40. The vaccine according to any of the preceding embodiments A1 to A39, wherein said polynucleotide sequence is a DNA nucleotide sequence or RNA nucleotide sequence.

41. A polynucleotide as defined in any of the embodiments A1 to A40.

42. A vector comprising the polynucleotide according to embodiment A41.

43. A host cell comprising the polynucleotide as defined in any of the embodiments A1 to A40 or comprising the vector according to embodiment A42.

44. The polynucleotide according to embodiment A41 formulated for administration to a patient to induce production of a dimeric protein in said patient.

45. A polypeptide encoded by the polynucleotide sequence as defined in any of the embodiments A1 to A40.

46. A dimeric protein consisting of two polypeptides according to embodiment A45.

47. The dimeric protein according to embodiment A46, being a homodimeric protein.

48. The polynucleotide according to embodiment A41 or the polypeptide according to embodiment A45 or the dimeric protein according to any of embodiments A46 to A47 for use as a medicament.

49. A method for preparing an individualized therapeutic anticancer vaccine comprising an immunologically effective amount of

(i) a dimeric protein consisting of two polypeptides encoded by a polynucleotide comprising a nucleotide sequence encoding a targeting unit, a dimerization unit

and an antigenic unit, wherein said antigenic unit comprises at least one patient-present shared antigen sequence or one or more parts thereof, and optionally one or more patient-specific antigen sequences or one or more parts thereof; or

(ii) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence encoding a targeting unit, a dimerization unit and an antigenic unit, wherein said antigenic unit comprises at least one patient-present shared antigen sequence or one or more parts thereof, and optionally one or more patient-specific antigen sequences or one or more parts thereof, the method comprises:

a) transfecting cells with the polynucleotide;
b) culturing the cells;
c) collecting and purifying the dimeric protein or the polypeptide expressed from the cells, and
d) mixing the dimeric protein or polypeptide obtained from step c) with a pharmaceutically acceptable carrier.

50. A method for preparing an individualized therapeutic anticancer vaccine comprising an immunologically effective amount of a polynucleotide comprising a nucleotide sequence encoding a targeting unit, a dimerization unit and an antigenic unit, wherein said antigenic unit comprises at least one patient-present shared antigen sequence or one or more parts thereof, and optionally one or more patient-specific antigen sequences or one or more parts thereof, the method comprises:
    a. preparing the polynucleotide;
    b. optionally cloning the polynucleotide into an expression vector and
    c. mixing the polynucleotide from step a) or the vector form step b) with a pharmaceutically acceptable carrier.

51. The method according to embodiments A49 or A50, including the steps of identifying patient-present shared antigens and patient-specific antigens, identifying the patient's HLA class I and HLA class II alleles, select patient-present shared antigen sequences and optionally patient-specific antigen sequences based on immunogenicity prior to preparing the polynucleotide.

52. A method of treating cancer in a patient, the method comprising administering to the said patient the vaccine according to any of embodiments A1 to A40.

53. The method according to embodiment A52, wherein the vaccine comprises a polynucleotide and is administered intradermally or intramuscularly.

54. The method according to embodiment A53 wherein the polynucleotide is a DNA.

55. The method according to embodiment A53 wherein the polynucleotide is an RNA.

56. The method according to any of claims A52 to A55, wherein administration is carried out with a jet injector.

57. The method according to any of claims A52 to A56, wherein administration is assisted by electroporation.

Embodiments B

1. An individualized therapeutic anticancer vaccine comprising an immunologically effective amount of:

(i) a polynucleotide comprising a nucleotide sequence encoding a targeting unit, a dimerization unit and an antigenic unit, wherein said antigenic unit comprises at least one patient-present shared antigen sequence or one or more parts thereof; or

(ii) a polypeptide encoded by the polynucleotide as defined in (i); or

(iii) a dimeric protein consisting of two polypeptides encoded by the polynucleotide as defined in (i); and

a pharmaceutically acceptable carrier.

2. The vaccine according to embodiment B1, wherein said antigenic unit further comprises one or more patient-specific antigen sequences or one or more parts thereof.

3. The vaccine according to any of embodiments B1 or B2, wherein said at least one patient-present shared antigen sequence is a sequence of a shared antigen selected from the group consisting of overexpressed cellular proteins, aberrantly expressed cellular proteins, cancer testis antigens, viral antigens, differentiation antigens, mutated oncogenes, mutated tumor suppressor genes, oncofetal antigens, shared fusion antigens, shared intron retention antigens, dark matter antigens, shared antigens caused by spliceosome mutations and shared antigens caused by frameshift mutations.

4. The vaccine according to any of embodiments B1 to B3, wherein said at least one patient-present shared antigen sequence is a sequence of a shared antigen that is a human cellular protein.

5. The vaccine according to embodiment B4, wherein said human cellular protein is an overexpressed or aberrantly expressed human cellular protein or a differentiation antigen.

6. The vaccine according to any of embodiments B1 to B5, wherein said at least one patient-present shared antigen sequence or one or more parts thereof are known to be immunogenic or are predicted to bind to the patient's HLA class I and/or HLA class II alleles.

7. The vaccine according to embodiment B6, wherein said at least one patient-present shared antigen sequence or one or more parts thereof are predicted to bind to the patient's HLA class I alleles.

8. The vaccine according to any of embodiments B1 to B7, wherein said at least one patient-present shared antigen sequence or the one or more parts thereof have a length suitable for presentation by the patient's HLA alleles.

9. The vaccine according to embodiment B8, wherein said at least one patient-present shared antigen sequence or the one or more parts thereof have a length of from 7 to 30 amino acids.

10. The vaccine according to any of embodiments B1 to B9, wherein the antigenic unit comprises more than one patient-present shared antigen sequence or one or more parts thereof.

11. The vaccine according to embodiment B10, wherein the antigenic unit comprises sequences of several patient-present shared antigens or one or more parts thereof.

12. The vaccine according to embodiment B11, wherein the antigenic unit comprises several parts of sequences of several patient-present shared antigens.

13. The vaccine according to embodiment B12, wherein the antigenic unit comprises several epitopes of several patient-present shared antigens, which epitopes are known to be immunogenic or are predicted to bind to the patient's HLA class I and/or HLA class II alleles.

14. The vaccine according to any of embodiments B1 to B13, wherein the antigenic unit comprises one or more patient-present shared antigen sequences in full length.

15. The vaccine according to embodiment B14, wherein the antigenic unit comprises 1 to 10 patient-present shared antigen sequences in full length.

16. The vaccine according to any of embodiments B1 to B15, wherein the antigenic unit comprises 1 to 30 parts of at least one patient-present shared antigen sequence.

17. The vaccine according to embodiment B16, wherein said 1 to 30 parts have a length of from 28 to 100 amino acids.

18. The vaccine according to embodiment B17, wherein the parts include multiple epitopes that are predicted to bind to the patient's HLA class I and/or HLA class II alleles.

19. The vaccine according to any of embodiments B1 to B18, wherein the antigenic unit comprises 1 to 50 patient-present shared antigen sequences in the form of epitopes.

20. The vaccine according to embodiment B19, wherein the epitopes are predicted to bind to the patient's HLA class I and/or HLA class II alleles.

21. The vaccine according to any of embodiments B19 to B20, wherein the epitopes have a length of from 7 to 30 amino acids.

22. The vaccine according to any of embodiments B2 to B21, wherein the antigenic unit comprises several patient-specific antigen sequences or one or more parts thereof.

23. The vaccine according to embodiment B22, wherein the antigenic unit comprises one or more parts of said several patient-specific antigen sequences.

24. The vaccine according to any of embodiments B2 to B23, wherein the antigenic unit comprises one or more patient-specific epitopes.

25. The vaccine according to embodiment B24, wherein the one or more patient-specific epitopes have a length of from 7 to 30 amino acids.

26. The vaccine according to any of embodiments B24 to B25, wherein antigenic unit comprises at least 5 patient-specific epitopes.

27. The vaccine according to any of embodiments B24 to B25, wherein the antigenic unit comprises at least 10 patient-specific epitopes.

28. The vaccine according to any of embodiments B24 to B25, wherein the antigenic unit comprises at least 15 patient-specific epitopes.

29. The vaccine according to any of the preceding embodiments B1 to B28, wherein the antigenic unit comprises from 7 to 2000 amino acids.

30. The vaccine according to embodiment B29, wherein the antigenic unit comprises from 30 to 1500 amino acids.

31. The vaccine according to embodiment B29, wherein the antigenic unit comprises from 50 to 1000 amino acids.

32. The vaccine according to any of the preceding embodiments B1 to B31, wherein the antigenic unit comprises one or more linkers.

33. The vaccine according to embodiment B32, wherein the one or more linkers are non-immunogenic and/or flexible linkers.

34. The vaccine according to any of embodiments B32 or B33, wherein the length of the one or more linkers is from 4 to 20 amino acids.

35. The vaccine according to any of embodiments B32 to B34, wherein the one or more linkers separate the antigen sequences from each other.

36. The vaccine according to any of the preceding embodiments B1 to B35, wherein the dimerization unit comprises a hinge region.

37. The vaccine according to embodiment B36, wherein the hinge region has the ability to form one or more covalent bonds, preferably in the form of a disulfide bridge.

38. The vaccine according to any of embodiments B36 or 3B7, wherein the hinge region is Ig derived.

39. The vaccine according to any of embodiments B36 to B38, wherein the dimerization unit further comprises another domain that facilitates dimerization.

40. The vaccine according to embodiment B39, wherein the other domain is an immunoglobulin domain, preferably an immunoglobulin constant domain.

41. The vaccine according to any of embodiments B39 or B40, wherein the other domain is a carboxyterminal C domain derived from IgG, preferably derived from IgG3.

42. The vaccine according to any of embodiments B36 to B41, wherein the dimerization unit further comprises a linker, preferably a linker that connects the hinge region and the other domain that facilitates dimerization.

43. The vaccine according to any of embodiments B36 to B42, wherein the dimerization unit comprises hinge exons h1 and h4 connected through a linker to a CH3 domain of human IgG3.

44. The vaccine according to any of embodiments B36 to B43, wherein the dimerization unit comprises an amino acid sequence having at least 80 % sequence identity to the amino acid sequence 94-237 of SEQ ID NO: 3.

45. The vaccine according to any of embodiments B36 to B44, wherein the dimerization unit consists of the amino acid sequence 94-237 of SEQ ID NO: 3.

46. The vaccine according to any of the preceding embodiments B1 to B45, wherein the antigenic unit and the dimerization unit are connected through a linker, preferably a linker that comprises a restriction site.

47. The vaccine according to any of the preceding embodiments B1 to B46, wherein the targeting unit targets antigen presenting cells.

48. The vaccine according to embodiment B47, wherein the targeting unit is or comprises a moiety that interacts with surface molecules on the antigen presenting cells.

49. The vaccine according to embodiment B48, wherein the surface molecule is selected from the group consisting of HLA, CD14, CD40, chemokine receptors and Toll-like receptors.

50. The vaccine according to any of embodiments B47 to B49, wherein the targeting unit comprises or consists of soluble CD40 ligand, RANTES, MIP-1α, XCL1, XCL2, flagellin, anti-HLA-DP, anti-HLA-DR, anti-pan HLA class II or an antibody variable domain with specificity for anti-CD40, anti-TLR-2, anti-TLR-4 or anti-TLR-5.

51. The vaccine according to embodiment B50, wherein the targeting unit comprises or consists of MIP-1α.

52. The vaccine according to embodiment B51, wherein the targeting unit comprises an amino acid sequence having at least 80 % sequence identity to the amino acid sequence 24-93 of SEQ ID NO: 1.

53. The vaccine according to embodiment B52, wherein the targeting unit consists of an amino acid sequence having at least 80% sequence identity to the amino acid sequence 24-93 of SEQ ID NO: 1.

54. The vaccine according to embodiment B53, wherein the targeting unit consist of the amino acid sequence 24-93 of SEQ ID NO: 1.

55. The vaccine according to any of the preceding embodiments B1 to B54, wherein said vaccine comprises a polynucleotide, preferably an RNA or DNA.

56. The vaccine according to embodiment B55, wherein said polynucleotide is human codon optimized.

57. The vaccine according to any of embodiments B55 or B56, wherein the polynucleotide further comprises a nucleotide sequence encoding a signal peptide.

58. The vaccine according to embodiment B57, wherein the signal peptide is selected from the list consisting of Ig VH signal peptide, human TPA signal peptide and human MIP1-α signal peptide.

59. The vaccine according to any of embodiments B57 to B58, wherein the signal peptide comprises an amino acid sequence having at least 85% sequence identity to the amino acid sequence 1-23 of SEQ ID NO: 1.

60. The vaccine according to embodiment B59, wherein the signal peptide consists of an amino acid sequence having at least 85% sequence identity to the amino acid sequence 1-23 of SEQ ID NO: 1.

61. The vaccine according to embodiment B60, wherein the signal peptide consists of the amino acid sequence 1-23 of SEQ ID NO: 1.

62. The vaccine according to any of the preceding embodiments B1 to B61, wherein said targeting unit, dimerization unit and antigenic unit in said polypeptide are in the N-terminal to C-terminal order of targeting unit, dimerization unit and antigenic unit or wherein said targeting unit, dimerization unit and antigenic unit in said polynucleotide are in the 5' to 3' order of targeting unit, dimerization unit and antigenic unit.

63. The vaccine according to any of the preceding embodiments B1 to B62, wherein the pharmaceutically acceptable carrier is selected from the group consisting of saline, buffered saline, PBS, dextrose, water, glycerol, ethanol, sterile isotonic aqueous buffers, and combinations thereof.

64. A method for preparing the individualized therapeutic anticancer vaccine of embodiment B1, said method comprises the steps of:

a) identifying at least one patient-present shared antigen in the tumor tissue or body fluid of a patient
b) determining the patient's HLA class I and/or class II alleles

c) predicting the immunogenicity of the identified at least one antigen or one or more parts thereof by their predicted binding to the patient's HLA class I and/or II alleles
d) selecting at least one antigen or one or more parts thereof based on their immunogenicity predicted in step c);
e) preparing a polynucleotide sequence comprising an antigenic unit comprising a nucleotide sequence encoding the at least one antigen or one or more parts thereof selected in step d);
f) cloning the polynucleotide sequence into an expression vector comprising nucleotide sequences encoding a targeting unit and a dimerization unit; and
g) mixing the expression vector obtained in step f with a pharmaceutically acceptable carrier.

65. The method according to embodiment B64 for preparing the individualized anticancer vaccine of embodiment B2, wherein said method further comprises:
in step a) identifying one or more patient-specific antigens in the tumor tissue of the patient
in step c) predicting the immunogenicity of the identified one or more patient-specific antigens or one or more parts thereof by their predicted binding to the patient's HLA class I and/or II alleles
in step d) selecting one or more patient-specific antigens or one or more parts thereof based on their immunogenicity predicted in step c);
and wherein the polynucleotide sequence of step e) further comprises nucleotide sequences encoding the one or more patient-specific antigens or one or more parts thereof selected in step d).

66. A polynucleotide as defined in any of the embodiments B1 to B62.

67. A vector comprising the polynucleotide according to embodiment B66.

68. A host cell comprising the polynucleotide as defined in any of the embodiments B1 to B62 or comprising the vector according to embodiment B67.

69. The polynucleotide according to embodiment B66 formulated for administration to a patient to induce production of a dimeric protein in said patient.

70. A polypeptide encoded by the polynucleotide sequence as defined in any of the embodiments B1 to B62.

71. A dimeric protein consisting of two polypeptides as defined in embodiment B70.

72. The dimeric protein according to embodiment B71, being a homodimeric protein.

73. The polynucleotide according to embodiment B66 or the polypeptide according to embodiment B70 or the dimeric protein according to any of embodiments B71 or B72 for use as a medicament.

74. A method for preparing an individualized therapeutic anticancer vaccine comprising an immunologically effective amount of a dimeric protein as defined in any of embodiments B1 to B54 or a polypeptide as defined in any of embodiment B1 to B54, the method comprises:

a) transfecting cells with the polynucleotide as defined in any of embodiments B1 to B62;
b) culturing the cells;
c) collecting and purifying the dimeric protein or the polypeptide expressed from the cells; and
d) mixing the dimeric protein or polypeptide obtained from step c) with a pharmaceutically acceptable carrier.

75. A method for preparing an individualized therapeutic anticancer vaccine comprising an immunologically effective amount of the polynucleotide as defined in any of embodiments B1 to B62, the method comprises

a) preparing the polynucleotide;
b) optionally cloning the polynucleotide into an expression vector and
c) mixing the polynucleotide from step a) or the vector form step b) with a pharmaceutically acceptable carrier.

76. A method of treating a patient having cancer, the method comprising administering to the patient the vaccine according to any of embodiments B1 to B63, which has been prepared specifically for the patient.

77. The method according to embodiment B76, wherein the vaccine comprises a polynucleotide and is administered intradermally or intramuscularly.

78. The method according to embodiment B77 wherein the polynucleotide is a DNA.

79. The method according to embodiment B78 wherein the polynucleotide is an RNA.

80. The method according to any of embodiments B76 to B79, wherein administration is carried out with a jet injector.

81. The method according to any of embodiments B76 to B80, wherein administration is assisted by electroporation.

82. The vaccine according to any of embodiments B1 to B63 for use in a method of treating cancer in a patient, wherein the vaccine has been specifically prepared for the patient.

83. Use of the polynucleotide according to embodiment B66 or the polypeptide according to embodiment 7B0 or the dimeric protein according to any of embodiments B71 or B72 for the manufacture of a medicament for the treatment of cancer in a patient, wherein the polynucleotide, polypeptide or dimeric protein has been specifically prepared for the patient.

84. A method for preparing the polynucleotide of embodiment B66, said method comprises the steps of:

a) identifying at least one patient-present shared antigen in the tumor tissue or body fluid of a patient
b) determining the patient's HLA class I and/or class II alleles
c) predicting the immunogenicity of the identified at least one antigen or one or more parts thereof by their predicted binding to the patient's HLA class I and/or II alleles
d) selecting at least one antigen or one or more parts thereof based on their immunogenicity predicted in step c);
e) preparing a polynucleotide sequence comprising an antigenic unit comprising a nucleotide sequence encoding the at least one antigen or one or more parts thereof selected in step d); and
f) cloning the polynucleotide sequence into an expression vector comprising nucleotide sequences encoding a targeting unit and a dimerization unit.

85. The method according to embodiment B84, wherein said method further comprises:
in step a) identifying one or more patient-specific antigens in the tumor tissue of the patient
in step c) predicting the immunogenicity of the identified one or more patient-specific antigens or one or more parts thereof by their predicted binding to the patient's HLA class I and/or II alleles
in step d) selecting one or more patient-specific antigens or one or more parts thereof based on their immunogenicity predicted in step c);
and wherein the polynucleotide sequence of step e) further comprises nucleotide sequences encoding the one or more patient-specific antigens or one or more parts thereof selected in step d).