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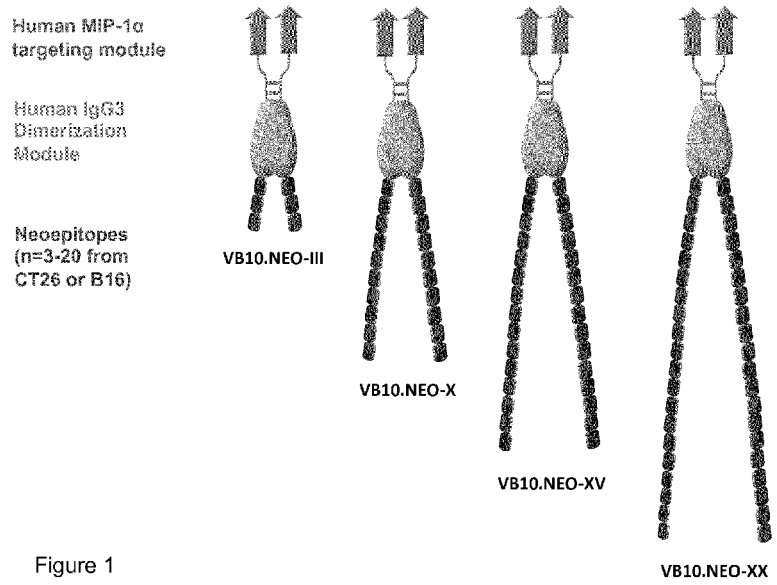

Figure 1

(57) Abstract: The present invention relates to an anticancer vaccine comprising polynucleotides or polypeptides, methods of treatment of cancer wherein such an anticancer vaccine is used as well as methods for producing the vaccine. The vaccine comprises a polynucleotide comprising a nucleotide sequenceencoding a targeting unit, a dimerization unit, a first linker and an antigenic unit, wherein said antigenic unit comprises n-1 antigenic subunits, each subunit comprising at least a part of a cancer neoepitope sequence and a second linker and said antigenic unit further comprising a final cancer neoepitope sequence, wherein n is an integer of from 3 to 50, or the vaccine comprises a polypeptide encoded by the polynucleotide or a dimeric protein consisting of two polypeptides encoded by the polynucleotide.

WO 2017/118695 A1

Therapeutic anticancer neoepitope vaccine

Field of invention

The present invention relates to an anticancer vaccine comprising polynucleotides or polypeptides, 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. Recently also treatment using antibodies directed towards known cancer associated antigens is used.

Within the last few years cancer immune therapies targeting cancer cells with the help of the patient's own immune system, i.e. cancer vaccines, have attracted interest because such therapies may reduce or even eliminate some of the side-effects seen in the traditional cancer treatment.

The foundation of immunology is based on self-nonself 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. One class of tumor antigens are the so-called tumor associated antigens, i.e. antigens expressed at low levels in normal tissues and expressed at a much higher level in tumor tissue. Such tumorassociated antigens have been the target for cancer vaccines for the last decade. However, immunological treatment directed towards tumor associated antigens exhibit several challenges, in that the tumor cells may evade the immune system by downregulating the antigen in question, and the treatment may also lead to toxicities due to normal cell destruction.

Recently, another class of tumor antigens have been identified, the so-called tumor neoantigens or tumor specific-antigens. Tumor neoantigens 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. Since these mutations are not present in normal tissue, the side-effects of the treatment directed towards the tumor associated antigens do not arise with an immunologic treatment towards tumor neoantigens.

The average number of somatic, tumor-specific non-synonymous mutations for malignant melanoma is between 100 and 120. Some of the genetic alterations can be recognized by the immune system, representing ideal antigens. Animal models have confirmed the utility of immunization with tumor neoantigens, and two clinical trials have been initiated, one with a vaccine comprising up to 10 mutated proteins and the other with an RNA vaccine (IVAC MUTANOME). The RNA vaccine comprises 2 RNA molecules each comprising five different mutation-encoding sequences.

However, by administration of either several different proteins or several RNA sequences it is difficult to control the immunological response to the various proteins administered or expressed *in vivo*.

Accordingly, there is a need for a more efficient vaccine ensuring expression of the mutated proteins either *in vivo* or *in vitro* and ensure delivery of the antigen as well as activation of the antigen presenting cells needed to elicit a strong T cell response.

Summary of invention

The present invention relates to a therapeutic anticancer vaccine being directed to a plurality of neoepitopes from tumor neoantigens, wherein the neoepitopes are presented to the immune system as a dimeric protein called a vaccibody. WO 2004/076489 describes dimeric proteins called vaccibodies in detail.

In one embodiment the invention relates to a therapeutic anticancer neoepitope vaccine comprising an immunologically effective amount of

1) a polynucleotide comprising a nucleotide sequence encoding
   - a targeting unit
   - a dimerization unit
   - a first linker
   - an antigenic unit, wherein said antigenic unit comprises n-1 antigenic subunits, each subunit comprising at least a part of a cancer neoepitope sequence and a second linker and said antigenic unit further comprising a final cancer neoepitope sequence, wherein n is an integer of from 3 to 50.

or

2) a polypeptide encoded by the polynucleotide as defined in 1), or
3) a dimeric protein consisting of two polypeptides encoded by the polynucleotide as defined in 1).

In another aspect, the invention relates to the polynucleotide as defined above. Such polynucleotide is e.g. useful in a vaccine according to the invention.

In a third aspect the invention relates to a vector comprising the polynucleotide as defined above, and in a fourth aspect the invention relates to a host cell comprising the polynucleotide or the vector as defined above.

In a fifth aspect the invention relates to a polypeptide encoded by the polynucleotide as defined above. Such polypeptide is e.g. useful in a vaccine according to the invention, and in a sixth aspect the invention relates to a dimeric protein consisting of two polypeptides as defined above.

In a seventh aspect the invention relates to the polypeptide, the dimeric protein, or the polynucleotide as defined above for use as a medicament.

As described above, in some embodiments, the vaccine comprises a polypeptide or a dimeric protein, and accordingly, in an eighth aspect the invention relates to a method for preparing a dimeric protein or an polypeptide as defined above, wherein the method comprises

a) transfecting the polynucleotide as defined above into a cell population;
b) culturing the cell population;
c) collecting and purifying the dimeric protein, or the polypeptide expressed from the cell population.

In other embodiments, the vaccine comprises a polynucleotide, and accordingly, in a ninth aspect the invention relates to a method for preparing a vaccine, such as a DNA or RNA vaccine, comprising an immunologically effective amount of a polynucleotide, wherein said method comprises

a. preparing a polynucleotide as defined above;

b. mixing the polynucleotide obtained under step a) in a pharmaceutically acceptable carrier, diluent, or buffer, thereby obtaining the vaccine.

In a tenth aspect the invention relates to a method of treating cancer in a patient, the method comprising administering to the patient in need thereof, a vaccine as defined above. In an alternative tenth aspect, the invention relates to a vaccine as defined above for use in a method of treating cancer.

Description of Drawings

Figure 1 shows a schematic drawing of a dimeric protein according to the invention having 3, 10 or 20 neoepitopes on each monomer, respectively.

Figure 2 shows that neoantigen-based vaccibody proteins are produced and secreted as functional homodimers after transfection of HEK293 cells with VB10.NEO constructs. Figure 2 upper left panels shows Western blots of VB10.NEO CT26-X (VB4001) and VB10.NEO B16-X (VB4003) comprising 10 neoepitopes and figure 2 lower left panels shows Western blots of VB10.NEO CT26-III (VB4002) and VB10.NEO B16-III (VB4004) comprising 3 neoepitopes. The formation of functional homodimers are shown in the left panels of the western blots for each construct (- reducing agent). The right panels illustrate the monomers (+ reducing agent). Figure 2 right panels shows results from two ELISA experiments detecting vaccibody proteins in the supernatant from HEK293 cells transfected with the VB10.NEO constructs. Upper right panel shows the expression level of the VB10.NEO CT26 constructs, VB4001 and VB4002, and lower right panel shows the expression level of the VB10.NEO B16 constructs, VB4003 and VB4004

Figure 3 illustrates that strong and broad T-cell responses are induced after a single injection with vaccibody DNA vaccines comprising 10 neoepitopes when compared to vaccibody DNA vaccines comprising 3 neoepitopes. The left panel displays IFN-γ responses towards individual neoepitopes in the B16 melanoma model when injecting VB10.NEO B16-III (VB4004) or VB10.NEO B16-X (VB4003) comprising 3 and 10 neoepitopes, respectively. The right panel displays IFN-γ responses towards neoepitopes in the CT26 colon carcinoma model when injecting VB10.NEO CT26-III (VB4002) or VB10.NEO CT26-X (VB4001) comprising 3 and 10 neoepitopes, respectively. The x-axis represents the 10 different neoepitopes, pepM1-M10.

VB10.NEO CT26-X = VB4001 = CT26 pepM1-M10,
VB10.NEO CT26-III = VB4002 = CT26 pepM1-M3,
VB10.NEO B16-X = VB4003 = B16 pepM1-M10,
VB10.NEO B16-III = VB4004 = B16 pepM1-M3.

Figure 4 illustrates that vaccibody DNA vaccines comprising 10 neoepitopes induces a stronger and broader total immune response than vaccibody DNA vaccines comprising only 3 neoepitopes. Upper panel: Comparison of the immune responses towards neoepitopes in the B16 melanoma model when injecting with VB10.NEO B16-X comprising 10 neoepitopes (VB4003) and VB10.NEO B16-III comprising 3 neoepitopes (VB4004), respectively. Lower panel: Comparison of the immune responses towards neoepitopes in the CT26 colon carcinoma model when injecting VB10.NEO CT26-X comprising 10 neoepitopes (VB4001) and VB10.NEO CT26-III comprising 3 neoepitopes (VB4002), respectively.
VB10.NEO CT26-X = VB4001 = CT26 pepM1-M10,
VB10.NEO CT26-III = VB4002 = CT26 pepM1-M3,
VB10.NEO B16-X = VB4003 = B16 pepM1-M10,
VB10.NEO B16-III = VB4004 = B16 pepM1-M3.

Figure 5. Vaccibody DNA vaccines comprising 10 neoepitopes induce a much stronger immune response than a mix of the corresponding 10 peptides plus adjuvant. Upper panel: Comparison of the vaccibody expression level of two variants of VB10.NEO B16-X with varying order of the 10 neoepitopes (VB4003 and VB4014) in the supernatant of HEK293 cells transfected with the corresponding Vaccibody DNA constructs, detected by sandwich ELISA. In VB4003, every other neoepitope is either hydrophobic or hydrophilic, whereas in VB4014, the hydrophobic neoepitopes are placed centrally in the neoepitope antigenic module. A hydrophobic core of neoepitopes in the antigenic module may improve expression and secretion of functional vaccibody proteins in the same constructs. Lower panel: The histogram shows immune responses induced by the DNA vaccines VB10.NEO B16-X VB4003 and VB4014, and a mix of 10 peptides plus adjuvant (the same 10 neoepitopes as encoded in the VB10.NEO B16-X constructs). The order of the neoepitopes within the neoepitope antigenic module does not change the hierarchy of the immunogenicity of the individual neoepitopes.
VB10.NEO B16-X = VB4003 = B16 pepM1-M10,
VB10.NEO B16-X = VB4014 = B16 hydrophobic core (pepM9+pepM5+pepM1+pepM4+pepM6+pepM8+pepM10+pepM3+pepM7+pepM2).

Figure 6. VB10.NEO B16-X DNA vaccine where the 10 neoepitopes are spaced with 10 amino acid (aa) linkers (VB4011), induces a stronger total immune response, compared to VB10.NEO B16-X DNA vaccine where the 10 neoepitopes are spaced with 5 aa linkers (VB4003). Upper panel: Comparison of the vaccibody expression level of VB4003 and VB4011 in the supernatant of HEK293 cells transfected with the corresponding Vaccibody DNA constructs, detected by sandwich ELISA. Similar expression and secretion of functional vaccibody proteins are observed for VB4003 and VB4011. Lower panel: Histogram showing the IFN-γ immune response towards neoepitopes from the B16 melanoma model in mice injected with VB4003 or VB4011. A single injection with vaccibody DNA vaccines comprising 10 neoepitopes spaced

with 10 amino acid linkers resulted in the strongest total immune response. Empty vector was included as a negative control.
VB10.NEO B16-X = VB4003 = B16 pepM1-M10, 5 aa linker
VB10.NEO B16-X = VB4011 = B16 pepM1-M10, 10 aa linker.

Figure 7. Vaccibody DNA vaccine comprising 2x10 neoepitopes (VB4018) induces a broader immune response against individual neoepitopes compared to vaccibody DNA vaccine comprising 1x 10 neoepitopes (VB4003). Upper panel: Comparison of vaccibody expression levels of VB10.NEO B16-X (VB4003) and VB10.NEO B16-XX (VB4018) in the supernatant of HEK293 cells transfected with the corresponding vaccibody DNA constructs, detected by sandwich ELISA. Lower panel: Histogram showing the IFN-γ immune response towards neoepitopes from the B16 melanoma model in mice injected with VB4003 or VB4018. The benefit of including 2 copies of each neoepitope is limited on the total immune response, however, a broader immune response is observed towards individual neoepitopes. Empty vector is included as a negative control.
VB10.NEO B16-X = VB4003 = B16 pepM1-M10, 5 aa linker
VB10.NEO B16-XX = VB4018 = B16 pepM1-M4+M11+M6-M10 x 2, 5 aa linker

Figure 8. Several copies of each neoeptiope in a vaccibody construct gives a more uniform immune response against the 5 selected best neoepitopes. Upper panel: Comparison of vaccibody expression level of VB10.NEO B16-X (VB4003 and VB4011), VB10.NEO B16-XX (VB4018), VB10.NEO B16-Vx2 (VB4019) and VB10.NEO B16-Vx4 in the supernatant of HEK293 cells transfected with the corresponding vaccibody DNA constructs, detected by sandwich ELISA. Lower panel: Histogram showing the IFN-γ immune responses towards 5 neoepitopes from the B16 melanoma model (PepM3, PepM4, PepM7, PepM9 and PepM10) in mice injected with 5 different vaccibody DNA vaccines that all include these 5 neoepitopes, but in different context. Empty vector is included as a negative control. The figure illustrates that several copies of each neoepitope as observed with the vaccibody constructs VB4019 (Vx2) and VB4021 (Vx4) mediate a more evenly immune response towards the 5 shared neoepitopes compared to the decatope VB4003, where the 5 selected neoepitopes are presented once. However, the construct holding 10 different neoepitopes (i.e. just a single copy of the 5 neoepitopes tested in this assay), thus, importantly with an increased length of the linker (10 amino acids, VB4011) induced the strongest total immune response towards the 5 shared neoepitopes.
VB10.NEO B16-X = VB4003 = B16 pepM1-M10, 5 aa linker
VB10.NEO B16-X = VB4011 = B16 pepM1-M10, 10 aa linker
VB10.NEO B16-XX = VB4018 = B16 pepM1-M4+M11+M6-M10 x 2, 5 aa linker
VB10.NEO B16-Vx2 = VB4019 = B16 pepM3+M4+M7+M9+M10 x 2, 5 aa linker
VB10.NEO B16-Vx4 = VB4021 = B16 pepM3+M4+M7+M9+M10 x 4, 5 aa linker

Figure 9 illustrates that vaccibodiy VB4018 comprising 20 neoepitopes are expressed to the same level as vaccibody VB4017 comprising 10 neoepitopes. The vaccibody proteins are detected in the supernatant of HEK293 cells transfected with the different Vaccibody DNA constructs by sandwich ELISA.

VB10.NEO B16-X = VB4017= B16 pepM1-M4+M11+M6-M10, 5 aa linker

VB10.NEO B16-XX = VB4018 = B16 pepM1-M4+M11+M6-M10 x 2, 5 aa linker

Figure 10. Expression levels of different vaccibody constructs comprising 3-neoepitopes are compared. The vaccibody proteins are detected in the supernatant of HEK293 cells transfected with the different Vaccibody DNA constructs by sandwich ELISA Upper panel: Improved expression and secretion of functional vaccibody proteins are observed when the 3 neoepitopes are spaced with an 10 aa linker (VB4012) compared to a 5 aa linker (VB4004). Lower panel:The figure illustrates that changing the order of the neoepitopes may affect expression of the vaccibodies.

VB10.NEO B16-III = VB4004 = B16 pepM1-M3, 5 aa linker

VB10.NEO B16-III = VB4012 = B16 pepM1-M3, 10 aa linker

VB10.NEO B16-III = VB4015 = B16 pepM1+M8+M3, 5 aa linker

VB10.NEO B16-III = VB4016 = B16 pepM1+M3+M2, 5 aa linker

Figure 11 illustrates immune responses in B16 melanoma mice that are induced after a single injection with vaccibody DNA vaccines comprising either 10 neoepitopes (VB4011), 15 neoepitopes (VB4024) or 20 neoepitopes (VB4025). Upper panel: Expression levels of the tested vaccibody constructs comprising 10-, 15- or 20 neoepitopes. The vaccibody proteins are detected in the supernatant of HEK293 cells transfected with the different Vaccibody DNA constructs by sandwich ELISA. Lower panel: Total immune response against neoepitopes in mice injected with the DNA vaccine candidates VB10.NEO B16-XV comprising 15 neoepitopes (VB4024) or VB10.NEO B16-XX comprising 20 neoepitopes (VB4025) compared to the VB10.NEO B16-X comprising 10 neoepitopes (VB4011). The figure shows the total number of IFNγ-spots per 10^6 splenocytes. As a negative control, mice were injected with empty vector not comprising the neoepitopes. The figure illustrates that vaccibody DNA vaccines comprising 20 neoepitopes induces a stronger and broader total immune response than vaccibody DNA vaccines comprising only 10 neoepitopes.

Figure 12 illustrates immune responses in CT26 colon carcinoma mice that are induced after a single injection with vaccibody DNA vaccines comprising either 10 neoepitopes (VB4009), 15 neoepitopes (VB4026) or 20 neoepitopes (VB4027). Upper panel: Expression levels of the tested vaccibody construct VB10.NEO CT26-X comprising 10 neoepitopes (left panel) and vaccibody constructs VB10.NEO CT26-XV and XX comprising 15 and 20 neoepitopes,

respectively (right panel). Lower panel: Total immune response towards neoepitopes in the CT26 colon carcinoma model in mice injected with the DNA vaccine candidates VB10.NEO CT26-XV comprising 15 neoepitopes (VB4026) or VB10.NEO CT26-XX comprising 20 neoepitopes (VB4027) compared to the VB10.NEO CT26-X comprising 10 neoepitopes (VB4009). The figure shows the total number of IFNγ-spots per 10^6 splenocytes. As a negative control, mice were injected with empty vector not comprising the neoepitopes. The figure illustrates that vaccibody DNA vaccines comprising 20 or 15 neoepitopes induces a stronger and broader total immune response than vaccibody DNA vaccines comprising only 10 neoepitopes.

NEO CT26-X = VB4009 = CT26 pepM1-M10, 10 aa linker
NEO CT26-XV = VB4026 = CT26 pepM1-M15, 10 aa linker
NEO CT26-XX = VB4027 = CT26 pepM1-M20, 10 aa linker

Figure 13 illustrates that mice immunized twice with VB10.NEO vaccine candidates comprising 10 neoepitopes are able to significantly delay and reduce tumour growth in the a) B16 melanoma model and b) the CT26 colon carcinoma model compared to negative control mice receiving PBS only. The figure shows the tumour volume development over time. In the CT26 colon carcinoma experiment, mice were divided into responders that were able to stabilize tumour growth and non-responders.

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.

Tumor neoantigen is used for any tumor specific antigen comprising one or more mutations as compared to the host's exome and is used synonymously with the term cancer neoantigen.

Tumor neoepitope is used for any immunogenic mutation in a tumor antigen and is used synonymously with the term cancer neoepitope.

Tumor neoepitope sequence is used to describe the sequence comprising the neoepitope in an antigenic subunit, and is used synonymously with the term cancer neoepitope sequence.

Therapeutic anticancer vaccine is used to describe that the vaccine is used for reducing or destroying tumor cells already present in the patient.

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. This is also the explanation of some of the failures in prior attempts to develop an anticancer vaccine, namely that the vaccine to some extent is also directed to the normal cells in the patient. As discussed above, the approach of attacking a tumor as defined by the present invention is to use the knowledge that any tumor, due to the mutations, expresses mutated proteins, so-called neoantigens that are not identical to any proteins in the normal cells of the patient, and therefore the neoantigens are efficient targets for a therapeutic anticancer vaccine. The mutations found in a tumor are normally highly individual, and accordingly, the vaccine according to the present invention is personalized for use only in the patient having the mutation in question.

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 foreign antigen evokes an immune response specifically towards said foreign antigen by the recognition of specific "non-self" 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 particularly, the vaccine according to the present invention is designed for evoking a cell-mediated immune response through activation of T cells against the neoantigens. T cells recognize neoepitopes when they have been processed and presented complexed to a MHC molecule as discussed below.

Major histocompatibility complex (MHC)

The neoepitopes according to the present invention are designed to be presented in MHC-neoepitope complexes. There are two primary classes of major histocompatibility complex (MHC) molecules, MHC I and MHC II.

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 complex-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 class II peptides are derived from extracellular proteins. Extracellular proteins are endocytosed, digested in lysosomes, and the resulting antigenic 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.

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

Vaccines

The neoantigen 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 called a 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.

The present invention relates to vaccines where the antigenic unit comprises antigenic subunits, wherein each subunit comprises a cancer neoepitope sequence or at least a part of a cancer neoepitope sequence. The neoepitope sequence is obtained by sequencing tumor DNA or RNA and identifying tumor specific mutations representing neoantigens. Thereby, a personalized neoantigen vaccine is obtained that specifically targets the identified tumor antigens.

One aspect of the present invention relates to a therapeutic anticancer neoepitope vaccine comprising an immunologically effective amount of

a polynucleotide comprising a nucleotide sequence encoding

- a targeting unit
- a dimerization unit
- a first linker
- an antigenic unit, wherein said antigenic unit comprises n-1 antigenic subunits, each subunit comprising at least a part of a cancer neoepitope

sequence and a second linker and said antigenic unit further comprising a final cancer neoepitope sequence, wherein n is an integer of from 3 to 50.

or

a polypeptide encoded by the polynucleotide as defined in 1), or

a dimeric protein consisting of two polypeptides encoded by the polynucleotide as defined in 1).

Thus, the vaccine comprises n neoepitopes or neoepitope sequences and n-1 second linkers, wherein n is an integer from 3 to 50.

Antigenic unit

The antigenic unit according to the invention comprises a plurality of tumor neoepitopes, wherein each neoepitope corresponds to a mutation identified in a tumor neoantigen. 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 neoepitope
- splice mutations that lead to a unique tumor-specific protein sequence
- chromosomal rearrangements that give rise to a chimeric protein with a tumor-specific neoepitope at the junction of the two proteins

In the antigenic unit, all but the last of the tumor neoepitopes are arranged in antigenic subunits, wherein each subunit consists of a tumor neoepitope sequence and a second linker, whereas the last subunit comprises a neoepitope only, i.e. no such second linker. Due to the separation of the tumor neoepitope sequences by said second linker, each neoepitope is presented in an optimal way to the immune system, whereby the efficiency of the vaccine is ensured as discussed below.

The cancer neoepitope sequence preferably has a length suitable for presentation by the MHC molecules discussed above. Thus, in a preferred embodiment the cancer neoepitope is from 7 to 30 amino acids long. More preferred are cancer neoepitope sequences having a length of from 7 to 10 amino acids or cancer neoepitope sequences having a length of from 13 to 30 amino acids.

In order to avoid that tumors escape the immune system by shutting down expression of a mutated gene if the vaccine is directed towards the expression product of said gene, it is preferred to include a plurality of different neoepitopes into the antigenic unit. In general the more genes the tumor has to shut down the less likely is it that the tumor is capable of shutting down all of them and still be able to proliferate or even survive. Furthermore, the tumor may be heterogeneous in that not each and every neoantigen is expressed by all the tumor cells. Accordingly, in accordance with the present invention, the approach is to include as many neoepitopes as possible into the vaccine in order to attack the tumor efficiently. Also, in order to secure that all neoepitopes are loaded efficiently to the same antigen presenting cell they are arranged as one amino acid chain instead of as discrete peptides. However, as described above, the object of the vaccine is to activate the T cells against the neoepitopes, and the T cells may be diluted in case too many neoepitopes are included into the vaccine, and therefore it is a balance to provide the vaccine with an optimal number of neoepitopes in the antigenic unit.

As discussed below in more details, the tumor exome is analysed to identify neoantigens and subsequently the most antigenic neoepitopes are selected. The present inventor has found that at least 3 neoepitopes should be selected to be incorporated into the vaccine, such as at least 5 neoepitopes, such as at least 7 neoepitopes, such as at least 10 neoepitopes, in order to efficiently be able to “hit” substantially all tumor cells.

In addition, the inventors of the present invention have found that increasing the numbers of neoepitopes in the vaccine constructs from 3 neoepitopes to 10 neoepitopes leads to a surprising increase in the immune response (see Figure 4). In addition, it has been found that increasing the number of neoepitopes in the vaccine constructs from 10 neoepitopes to 15 or 20 neoepitopes leads to a further increase in the immune response (see Figures 11 and 12).

Thus, in a preferred embodiment the vaccine according to the present invention comprises at least 10 neoepitopes. In another preferred embodiment the vaccine according to the present invention comprises at least 15 neoepitopes, such as at least 20 neoepitopes.

In one embodiment from 3 to 50 neoepitopes are included in the vaccine in order to obtain the most efficient immune response without diluting the T cells, such as from 3 to 30 neoepitopes, such as from 3 to 20 neoepitopes, such as from 3 to 15 neoepitopes, such as from 3 to 10 neoepitopes, and consequently n is preferably an integer of from 3 to 50, such as from 3 to 30, such as from 5 to 25, such as from 3 to 20, such as from 3 to 15, such as from 3 to 10.

In another embodiment 5 to 50 neoepitopes may be included in the vaccine in order to obtain the most efficient immune response without diluting the T cells, such as from 5 to 30

neoepitopes, such as for example from 5 to 25 neoepitopes, such as from 5 to 20 neoepitopes, such as from 5 to 15 neoepitopes, such as from 5 to 10 neoepitopes, and consequently n is preferably an integer of from 5 to 50, such as from 5 to 30, such as from 5 to 25, such as from 5 to 20, such as from 5 to 15, such as from 5 to 10.

In a further embodiment 10 to 50 neoepitopes may be included in the vaccine in order to obtain the most efficient immune response without diluting the T cells, such as from 10 to 40 neoepitopes, such as from 10 to 30 neoepitopes, such as from 10 to 25 neoepitopes, such as from 10 to 20 neoepitopes, such as from 10 to 15 neoepitopes, and consequently n is preferably an integer of from 10 to 50, such as from 10 to 30, such as from 10 to 20, such as from 10 to 15 neoepitopes.

The inventors of the present invention have shown that vaccibody DNA vaccines comprising 10 neoepitopes induces a stronger and broader total immune response than vaccibody DNA vaccines comprising only 3 neoepitopes (see Figure 4 and Example 2). Further, increasing the number of neoepitopes to more than 20 may result in a less efficient vaccine due to a dilution of the T cells. Further, it can be associated with technical difficulties to include more than 20 neoepitopes.

Accordingly, in a preferred embodiment of the present invention the vaccine comprises from 10 to 20 neoepitopes.

In yet another embodiment 15 to 50 neoepitopes are included in the vaccine in order to obtain the most efficient immune response without diluting the T cells, such as from 15 to 30 neoepitopes or such as from 15 to 20 neoepitopes and consequently n is preferably an integer of from 15 to 50, such as from 15 to 30 or such as from 15 to 20 neoepitopes.

In one embodiment, the antigenic unit comprises one copy of each cancer neoepitope, so that when 10 neoepitopes are included in the vaccine a cell-mediated immune response against 10 different neoepitopes can be evoked.

If however only a few relevant antigenic mutations are identified, then the antigenic unit may comprise at least two copies of at least one neoepitope in order to strengthen the immune response to these neoepitopes. Also for manufacturing and regulatory reasons it may be an advantage to keep the length of plasmid and i.e. the antigenic unit constant, and therefore it may be advantageously to include more than one copy of the same neoepitope in the antigenic unit.

As discussed above, it may be an advantage to keep the length of the antigenic unit constant, and therefore it is preferred in one embodiment that all the cancer neoepitope sequences have identical length. However, if one or more of the neoepitopes result from a mutation leading to a frame shift or stop codon mutation, the neoepitope may have a substantial length, such as consisting of at least the mutated part of the protein, the most antigenic portion of the mutated protein or maybe of the whole mutated protein, whereby the length of at least one of the neoepitopes is substantially longer than the neoepitopes arising from a non-synonymous point mutation.

The length of the antigenic unit is primarily determined by the length of the neoepitopes and the number of neoepitopes arranged in the antigenic unit and is from about 21 to 1500, preferably from about 30 amino acids to about a 1000 amino acids, more preferably from about 50 to about 500 amino acids, such as from about 100 to about 400 amino acids, from about 100 to about 300 amino acids.

In particularly when the neoepitope is short, such as a few amino acids long, the cancer neoepitope sequence comprises the neoepitope flanked at both sides by an amino acid sequence. Preferably, the neoepitope is positioned essentially in the middle of a cancer neoepitope sequence, in order to ensure that the neoepitope is presented by the antigen presenting cells after processing. The amino acid sequences flanking the neoepitope are preferably the amino acid sequences flanking the neoepitope in the neoantigen, whereby the cancer neoepitope sequence is a true subsequence of the cancer neoantigen amino acid sequence.

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

In one embodiment, depending on the selected neoepitopes, the antigenic subunits are arranged in the order of more antigenic to less antigenic in the direction from the first linker towards the final neoepitope.

In another embodiment, in particularly if the hydrophilicity/hydrophobicity varies greatly among the neoepitopes, it is preferred that the most hydrophobic antigenic subunit(s) is/are substantially positioned in the middle of the antigenic unit and the most hydrophilic antigenic subunit(s) is/are positioned at the beginning and/or end of the antigenic unit. Alternatively, the neoepitopes may be arranged alternating between a hydrophilic and a hydrophobic neoepitope.

Furthermore, GC rich neoepitopes should be spaced so that GC clusters are avoided, preferably GC rich neoepitopes are spaced by at least one subunit.

The second linker is designed to be non-immunogenic and is preferably also a flexible linker, whereby the tumor neoepitopes, in spite of the high numbers of antigenic subunits present in the antigenic unit, are presented in an optimal manner to the T cells. Preferably, the length of the second linker is from 4 to 20 amino acids to secure the flexibility. In another preferred embodiment, the length of the second linker 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 second linker is 10 amino acids.

In a specific embodiment, the vaccine of the present invention comprises 10 neoepitopes, wherein the second linkers 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 vaccine of the present invention comprises 10 neoepitopes and wherein the second linkers have a length of 10 amino acids.

The second linker is preferably identical in all antigenic subunits. If, however, one or more of the neoepitopes comprise an amino acid motif similar to the linker, it may be an advantage to substitute the neighbouring second linkers with a second linker of a different sequence. Also, if a neoepitope-second linker junction is predicted to constitute an epitope in itself, then a second linker of a different sequence might be used.

The second linker is preferably a serine-glycine linker, such as a flexible GGGGS linker, such as 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 second linker comprises or consists of the sequence LGGGS, GLGGS, GGLGS, GGGLS or GGGGL. In another embodiment the second linker comprises or consists of the sequence LGGSG, GLGSG, GGLSG, GGGLG or GGGSL. In yet another embodiment the second linker comprises or consists of the sequence LGGSS, GLGSS, GGLSS, GGGLS or GGGSL.

In yet another embodiment the second linker comprises or consists of the sequence LGLGS, GLGLS, GLLGS, LGGLS or GLGGL. In another embodiment the second linker comprises or consists of the sequence LGLSG, GLLSG, GGLSL, GGLLG or GLGSL. In yet another embodiment the second linker comprises or consists of the sequence LGLSS, GLGLS, GGLLS, GLGSL or GLGSL.

In another embodiment of the present invention the second serine-glycine linker has a length of 10 amino acids and comprises 1 leucine or 2 leucines.

In one embodiment the second linker comprises or consists of the sequence LGGGSGGGGS, GLGGSGGGGS, GGLGSGGGGS, GGGLSGGGGS or GGGGLGGGGS. In another embodiment the second linker comprises or consists of the sequence LGGSG GGGSG, GLGSGGGGSG, GGLSGGGGSG, GGGLGGGGSG or GGGSLGGGSG. In yet another embodiment the second linker comprises or consists of the sequence LGGSSGGGSS, GLGSSGGGSS, GGLSSGGGSS, GGGLSGGGSS or GGGSLGGGSS.

In a further embodiment the second linker comprises or consists of the sequence LGGGSLGGGS, GLGGSGLGGS, GGLGSGGLGS, GGGLSGGGLS or GGGGLGGGGL. In another embodiment the second linker comprises or consists of the sequence LGGSGLGGSG, GLGSGGLGSG, GGLSGGGLSG, GGGLGGGGLG or GGGSLGGGSL. In yet another embodiment the second linker comprises or consists of the sequence LGGSSLGGSS, GLGSSGLGSS, GGLSSGGLSS, GGGLSGGGLS or GGGSLGGGSL.

In a preferred embodiment the vaccine according to the present invention comprises at least 10 neoepitopes that are separated by 10 amino acid linkers. In another preferred embodiment the vaccine according to the present invention comprises at least 15 neoepitopes that are separated by 10 amino acid linkers, such as at least 20 neoepitopes that are separated by 10 amino acid linkers.

In another preferred embodiment the vaccine comprises from 10 to 20 or from 10 to 25 neoepitopes that are separated by second linkers. Preferably, said second linkers are 10 amino acids. The second linker may also have any length as defined herein above, such as for example from 8 to 12 amino acids.

Alternative linkers may be selected from the group consisting of GSAT linkers and SEG linkers, or multiple variants thereof.

Targeting unit

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

The term "targeting unit" as used herein refers to a unit that delivers the polypeptide/protein with its antigen 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 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 targeting unit is designed to target the polypeptide/dimeric protein 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 human leukocyte antigen (HLA), cluster of differentiation 14 (CD14), cluster of differentiation 40 (CD40), chemokine receptors and Toll-like receptors (TLRs). HLA is a major histocompatibility complex (MHC) in humans. The Toll-like receptors may for example include TLR-2, TLR-4 and/or TLR-5.

The polypeptide/dimeric protein of the invention can be targeted to said surface molecules by means of targeting units comprising for example antibody binding regions with specificity for CD14, CD40, or Toll- like receptor; ligands, e.g. soluble CD40 ligand; natural ligands like chemokines, e.g. RANTES or MIP-1a ; 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 an 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-HLA-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 hMIP-1alpha (LD78beta), which binds to its cognate receptors, CCR1, CCR3 and CCR5 expressed on the cell surface of APCs.

The binding of the polypeptide/dimeric protein 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 neoantigens.

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 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.

Dimerization unit

The term “dimerization unit” as used herein, refers to a sequence of 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 polpeptide/dimeric protein to allow optimal binding of the targeting unit to the surface molecules on the antigen presenting cells (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 and optionally another domain that facilitates dimerization, and the hinge region and the other domain may be connected through a third linker.

The term "hinge region" refers to a peptide sequence of the dimeric protein that facilitates the dimerization. The hinge region functions as a flexible spacer between the units allowing the two targeting units 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 contribute to the dimerization through the formation of covalent bond(s), e.g. disulfide bridge(s). 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 other domain that facilitates dimerization is an immunoglobulin domain, such as a carboxyterminal C 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.

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

It is preferred that the dimerization unit does not comprise a CH2 domain.

In a preferred embodiment, the dimerization unit consists of hinge exons h1 and h4 connected through a third linker to 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 :3. In a preferred embodiment, the dimerization unit comprises an amino acid sequence having at least 85% sequence identity to the amino acid sequence94-237 of SEQ ID NO :3, 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 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 :3, 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 94-237 of SEQ ID NO :3.
In one embodiment the third linker is a G3S2G3SG linker.

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 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.

First linker

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

Signal peptide

In a preferred embodiment, the polynucleotide further comprises a nucleotide sequence encoding a signal peptide. The signal peptide is constructed to allow secretion of the polypeptide encoded by the polynucleotide of the inventionin 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: 31, a human TPA signal peptide, such as SEQ ID NO: 32, 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 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 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.

Sequence identity

Sequence identity may be determined as follows: A high level of sequence identity indicates likelihood that the first sequence is derived from the second 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, non-limiting example of a 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 penalise to gaps in the end of the sequences. When utilizing the ALIGN og Align0 program for comparing amino

acid sequences, a BLOSUM50 substitution matrix with gap opening/extension penalties of –12/-2 is preferably used.

Polynucleotides

The invention also relates to a polynucleotide as described above. The polynucleotide may comprise a DNA nucleotide sequence or a 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.

Polypeptides and dimeric proteins

The invention further relates to 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.

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 neoepitopes, 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 neoepitopes exceeds an upper size limit for the antigenic unit. It is however preferred that the dimeric protein is a homodimeric protein.

Vector

Furthermore, the invention relates to a vector comprising a nucleotide sequence as defined above. It is preferred that the vector allows for easy exchange of the various units described above, in particularly the antigenic unit. In particularly, the expression vector may be pUMVC4a vector or 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.

Host cell

The invention also relates to a host cell comprising a nucleotide sequence as defined above or comprising a vector as defined above for expression of the polypeptide according to the invention.

Suitable host cells include prokaryotes, yeast, insect or higher eukaryotic cells.

Methods for preparing the vaccine

The vaccine according to the invention is preferably a personalized vaccine in the sense that the neoantigens are identified in the patient's tumor and accordingly, the vaccine is directed exactly against the specific mutated proteins in the patient's tumor.

Accordingly, in one aspect the invention relates to a method for preparing a vaccine comprising an immunologically effective amount of the dimeric protein, or the polypeptide as defined above by producing the polypeptides *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 a through peptide synthesis or expression of the polypeptide in any of a variety of expressions systems followed by purification. Accordingly, in one embodiment the method comprises

a) transfecting the polynucleotide as defined above into a cell population;

b) culturing the cell population;

c) collecting and purifying the dimeric protein, or the polypeptide expressed from the cell population, and

d) mixing the dimeric protein or polypeptide obtained under step c) with a pharmaceutically acceptable carrier, thereby obtaining the vaccine.

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

Furthermore, an adjuvant or buffer may be added to the vaccine.

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

In another aspect the invention relates to a method for preparing a vaccine comprising an immunologically effective amount of the polynucleotide as defined above. In one embodiment the method comprises

a. preparing the polynucleotide as defined above;

b. mixing the polynucleotide obtained under step a) with a pharmaceutically acceptable carrier thereby obtaining the vaccine.

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 the subunits of the antigenic unit may be synthesized individually and then ligated to produce the final polynucleotide into the vector backbone.

For the design of a personalized vaccine the methods above are preceded by a method of identifying the neoepitopes to be included into the polynucleotide.

This method preferably includes the steps of

- sequencing the genome, or exome of a tumor
- identifying tumor neoantigens comprising neoepitopes from said tumor,
- selecting neoepitopes based on predicted antigenicity.

The tumor or tumor part may be by through any suitable method, such as by obtaining a biopsy of the tumor or by excision of the tumor, or from any suitable body fluid, such as a blood sample or a urine sample.

Sequencing of tumor genome or exome

The genome or the exome, i.e. the coding part of the genome, may be sequenced using any suitable method, such as whole exome sequencing. In particularly the sequencer may be an Illumina HiSeq2500), using Paired-end 2x100-125 or PE100-125 (read length), multiplex.

Identifying tumor antigens

Once the tumor specific mutations are identified the next step is to select predicted antigenic peptides comprising the neoepitopes.

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 neoepitopes are selected *in silico* on the basis of predictive HLA-binding algorithms. The intention is to identify all relevant neoepitopes and after a ranking or scoring determine the neoepitopes to be included in the vaccine for the specific patient in question.

Any suitable algorithms may be used, such as one of the following:

Available free software analysis of peptide-MHC binding (IEDB and NetMHC) may be downloadedfrom the following websites:

http://www.iedb.org/

http://www.cbs.dtu.dk/services/NetMHC/

Commercially available advanced software to predict optimal peptides 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 neoepitopes are selected and optimally designed in the polynucleotide. As discussed above from 3 to 50 neoepitopes are preferred according to the present invention.

Vaccine

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

The vaccine may further comprise a pharmaceutically acceptable carrier, diluent, adjuvant or buffer.

Pharmaceutically acceptable carriers, diluents, and buffers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, sterile isotonic aqueous buffer, and combinations thereof.

In particularly for vaccines comprising polypeptides/proteins, pharmaceutically acceptable adjuvants 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 EV1P321, 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).

In particularly for vaccines comprising polynucleotides the carriers may include molecules that ease transfection of cells and adjuvants may include plasmids comprising nucleotide sequences encoding chemokines or cytokines in order to enhance the immune response.

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

Administration

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

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

In a specific 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.

Treatment

The polynucleotides, polypeptides and dimeric proteins are preferably for use in the treatment of cancer, and formulated in a vaccine as discussed above. By the methods described herein it is possible to treat a patient suffering from cancer by examining any mutations present in the tumor in the patient, producing the vaccine and then immunizing the patient with the vaccine directed exactly to neoantigens present in his or her tumor. Due to the fast and reliable methods for sequencing, epitope-determining and producing nucleotide sequences today, it has become likely that a patient may receive the vaccine within 12 weeks from having the tumor resected

The cancer may be any cancer wherein the cancer cells comprise mutations. The cancer may be a primary tumor, metastasis or both. The tumor examined for mutations may be a primary tumor or a metastasis. The cancers to be treated are in particularly the cancers known to have a high mutational load, such as melanomas, lung cancer, breast cancer, prostate cancer or colonic cancer.

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

It is preferred to inject a polynucleotide vaccine intramuscular, such as in the big muscles, for example in the shoulder, buttock or thigh. It has been found that the polypeptides are produced locally and relevant immune cells internalize the polypeptides/proteins essentially at the site of production, and substantially no polypeptides or proteins reach the blood stream.

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

Dosage regimen

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

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

The vaccine is administered in an immunologically effective amount. By “immunologically effective amount” is meant the amount of the vaccine required to establish a tumor reducing

effect. Ultimately, the physician determines the dosage that 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.

Combination treatments

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.

Specific embodiments

1. A therapeutic anticancer neoepitope vaccine comprising an immunologically effective amount of

   a polynucleotide comprising a nucleotide sequence encoding

   - a targeting unit
   - a dimerization unit
   - a first linker
   - an antigenic unit, wherein said antigenic unit comprises n-1 antigenic subunits, each subunit comprising at least a part of a cancer neoepitope sequence and a second linker and said antigenic unit further comprising a final cancer neoepitope sequence, wherein n is an integer of from 3 to 50.

   or

   a polypeptide encoded by the polynucleotide as defined in 1), or

   a dimeric protein consisting of two polypeptides encoded by the polynucleotide as defined in 1).

2. The vaccine according to embodiment 1, wherein the antigenic unit comprises one copy of each cancer neoepitope.

3. The vaccine according to embodiment 1, wherein the antigenic unit comprises at least two copies of at least one neoepitope.

4. The vaccine according to any of the preceding embodiments, wherein the cancer neoepitope sequence has a length of from 7 to 30 amino acids.

5. The vaccine according to embodiment 4, wherein the cancer neoepitope sequence has a length of from 7 to 10 amino acids.

6. The vaccine according to embodiment 4, wherein the cancer neoepitope sequence has a length of from 13 to 30 amino acids.

7. The vaccine according to any of the preceding embodiments, wherein each cancer neoepitope sequence has identical length.

8. The vaccine according to any of the preceding embodiments, wherein the cancer neoepitope is positioned essentially in the middle of the cancer neoepitope sequence.

9. The vaccine according to any of the preceding embodiments, wherein the cancer neoepitope sequence is a subsequence of a cancer neoantigen.

10. The vaccine according to any of the preceding embodiments, wherein the antigenic subunits are in the order of more antigenic to less antigenic from the first linker.

11. The vaccine according to any of the preceding embodiments, wherein the most hydrophobic antigenic subunit(s) is(are)substantially the middle of the antigenic unit and the most hydrophilic antigenic subunit(s) is/are at the ends of the antigenic unit.

12. The vaccine according to any of the preceding embodiments, wherein the second linker is a flexible linker.

13. The vaccine according to any of the preceding embodiments, wherein the second linker is non-immunogenic.

14. The vaccine according to any of the preceding embodiments, wherein the second linker is identical in all antigenic subunits.

15. The vaccine according to any of the preceding embodiments, wherein the second linker is a Serine-Glycine linker.

16. The vaccine according to any of the preceding embodiments, wherein the length of the second linker is from 4 to 20 amino acids.

17. The vaccine according to any of the preceding embodiments, wherein the length of the second linker is 10 amino acids.

18. The vaccine according to any of the preceding embodiments, wherein the length of the antigenic unit is from about 100 amino acids to about a 1000 amino acids.

19. The vaccine according to any of the preceding embodiments, wherein n is an integer between 3 and 30.

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

21. The vaccine according to embodiment 20, wherein the hinge region is Ig derived.

22. The vaccine according to any one of embodiments 20-21, wherein the hinge region has the ability to form one or more covalent bonds.

23. The vaccine according to embodiment 22, wherein the covalent bond is a disulfide bridge.

24. The vaccine according to any one of embodiments 20-23, 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.

25. The vaccine according to embodiment 24, wherein the carboxyterminal C domain is derived from IgG.

26. The vaccine according to any one of embodiments 24-25, wherein the immunoglobulin domain of the dimerization unit has the ability to homodimerize.

27. The vaccine according to any one of embodiments 24-26, wherein said immunoglobulin domain has the ability to homodimerize via noncovalent interactions.

28. The vaccine according to embodiment 27, wherein said noncovalent interactions are hydrophobic interactions.

29. The vaccine according to any one of embodiments 20-28, wherein said dimerization unit does not comprise a CH2 domain.

30. The vaccine according to any one of embodiments 20-29, 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 20-30, 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 one of embodiments 30-31, wherein said third linker is a $G_3S_2G_3SG$ linker.

33. The vaccine according to any of the preceding embodiments, wherein said antigenic unit and the dimerization unit is connected through said first linker.

34. The vaccine according to embodiment 33, wherein the first linker comprises a restriction site.

35. The vaccine according to embodiment 33 or 34, wherein the first linker is a GLGGL linker or a GLSGL linker.

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

37. The vaccine according to any of the preceding embodiments, wherein said 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.

38. The vaccine according to any of the preceding embodiments, wherein said targeting unit consists of an amino acid sequence having at least 85% sequence identity to the amino acid sequence 24-93 of SEQ ID NO:1.

39. The vaccine according to any of the preceding embodiments, wherein said nucleotide sequence further encodes a signal peptide.

40. The vaccine according to embodiment 39, wherein said signal peptide comprises an amino acid sequence having at least 80 % sequence identity to the amino acid sequence 1-23 of SEQ ID NO:1.

41. The vaccine according to embodiment 39 or 40, wherein said 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.

42. The vaccine according to any of the preceding embodiments, 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.

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

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

45. The vaccine according to any of the preceding embodiments, further comprising a pharmaceutically acceptable carrier and/or adjuvant.

46. A polynucleotide as defined in any of the embodiments 1-45.

47. A vector comprising the nucleotide sequence as defined in any of the embodiments 1-45.

48. A host cell comprising the nucleotide sequence as defined in any of the embodiments 1-45 or comprising the vector as defined in embodiment 47.

49. The polynucleotide according to embodiment 46 formulated for administration to a patient to induce production of the dimeric protein in said patient.

50. A polypeptide encoded by the nucleotide sequence as defined in any of the embodiments 1-45.

51. A dimeric protein consisting of two polypeptides as defined by embodiment 50.

52. The dimeric protein according to embodiment 51, being a homodimeric protein.

53. The polypeptide as defined in embodiment 50, the dimeric protein as defined in embodiment 51-52, or the polynucleotide as defined in embodiment 46 for use as a medicament.

54. A method for preparing a vaccine comprising an immunologically effective amount of the dimeric protein as defined in embodiment 50, or the polypeptide as defined in embodiment 50, the method comprising
    e) transfecting the polynucleotide as defined in embodiment 46 into a cell population;
    f) culturing the cell population;
    g) collecting and purifying the dimeric protein, or the polypeptide expressed from the cell population
    h) mixing the dimeric protein or polypeptide obtained under step c) with a pharmaceutically acceptable carrier thereby obtaining the vaccine.

55. A method for preparing a vaccine comprising an immunologically effective amount of the polynucleotide according to embodiment 46, said method comprising
    a. preparing the polynucleotide according to embodiment 46;
    b. mixing the polynucleotide obtained under step a)with a pharmaceutically acceptable carrier, thereby obtaining the vaccine.

56. The method according to embodiment 55, including the steps of:
    - sequencing the exome of a tumor
    - identifying tumor neoantigens comprising neoepitopes from said tumor,
    - selecting neoepitopes based on antigenicity,

prior to the step of preparing the polynucleotide.

57. A method of treating cancer in a patient, the method comprising administering to the patient in need thereof, the vaccine as defined in any of the embodiments 1-45.

58. The method according to embodiments 57, wherein the vaccine comprises a polynucleotide and is administered intradermally or intramuscular.

59. The method according to embodiment 58 wherein the polynucleotide is a DNA.

60. The method according to embodiment 59 wherein the polynucleotide is a RNA.

61. The method according to embodiments 57 to 60, wherein administration is carried out with a jet injector.

62. The method according to embodiments 57 to 60, wherein administration is assisted by electroporation.

Examples

EXAMPLE 1: Construction and expression of the vaccines.

Gene sequences were designed according to the following structure:

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.	First linker
8: Neoepitope sequence (see below)	Antigenic unit

Previously described exome sequencing and RNA sequencing of the mouse melanoma cancer cell line B16-F10 and the mouse colon cancer cell line CT26 revealed hundreds to thousands of tumor-specific non-synonymous mutations (Castle et al 2012, Castle et al 2014 and Kreiter et al 2015*). In silico*-based methods were used to identify potential immunogenic neo-epitopes. Mice were immunized with peptides encoding the mutated epitopes, and their immunogenicity was observed as specific T cell immune responses (ELISpot assay). Furthermore, vaccination of mice with the most immunogenic epitopes selected from the ELISpot conferred strong anti-tumor activity (Castle et al 2012 and Kreiter et al 2015).

Each of the neoepitopes are peptides of 27 amino acids separated by a flexible GGGGS linker. Short peptides (<20 amino acids) are processed and novel epitopes may be presented on MHC class I molecules and activate CD8+ T cells. However, it is preferred that the vaccine activates CD8+ and CD4+ T cells and therefore neoepitopes encoding for long peptides (>20 amino acids) are chosen. That may allow for efficient peptide processing and presentation on both MHC class I and II (Kreiter et al 2015). In the first two VB10.NEO-X constructs the selected hydrophobic and hydrophilic neoepitopes are evenly distributed. A neutral, flexible GGGGS linker between the 27mer neoepitopes is important to avoid generation of new immunogenic epitopes in the junctions of the combined neoepitopes.

The sequences of the neoepitopes found in the B16-F10 and CT26 cell lines are shown in Table 1 and 2.

Table 1 - CT26 cell line

Mutation number polypeptide (Vaccibody)	**Gene**	**Mutated sequence used for vaccination**	**Sub.WT, AA#, Mut)**	**Reactive T cell subtype**	**MHC I score (best prediction)**
CT26-PepM1	E2f8	VILPQAPSGPSYATYLQPAQA QMLTPP (SEQ ID NO: 14)	I522T	CD8+	0,1
CT26-PepM2	Aldh18a1	LHSGQNHLKEMAISVLEARA CAAAGQS (SEQ ID NO: 15)	P154S		
CT26-PepM3	Slc4a3	PLLPFYPPDEALEIGLELNSS ALPPTE (SEQ ID NO: 16)	T373I	CD4+	0,9
CT26-PepM4	Nphp3	AGTQCEYWASRALDSEHSIG SMIQLPQ (SEQ ID NO: 17)	G234D	CD4+	0,1
CT26-PepM5	Tdg	AAYKGHHYPGPGNYFWKCL FMSGLSEV (SEQ ID NO: 18)	H169Y	CD4+	0,3
CT26-PepM6	Ubqln1	DTLSAMSNPRAMQVLLQIQQ GLQTLAT (SEQ ID NO: 19)	A62V		
CT26-PepM7	Slc20a1	DKPLRRNNSYTSYIMAICGMP LDSFRA (SEQ ID NO: 20)	T425I	CD4+	0,3
CT26-PepM8	Dhx35	EVIQTSKYYMRDVIAIESAWLL ELAPH (SEQ ID NO: 21)	T646I	CD4+	0,1
CT26-PepM9	Als2	GYISRVTAGKDSYIALVDKNI MGYIAS (SEQ ID NO: 22)	L675I	CD8+	0,2
CT26-PepM10	Agxt2l2	EHIHRAGGLFVADAIQVGFGR IGKHFW (SEQ ID NO: 23)	E247A	CD4+	0,2
CT26-PepM11	Tmem87a	QAIVRGCSMPGPWRSGRLLV SRRWSVE (SEQ ID NO: 50)	G63R	CD8+	0.7
CT26-PepM12	Ppp6r1	DGQLELLAQGALDNALSSMG ALHALRP (SEQ ID NO: 51)	D309N	CD4+	
CT26-PepM13	Deptor	SHDSRKSTSFMSVNPSKEIKI VSAVRR (SEQ ID NO: 52)	S253N	CD4+	0.3
CT26-	Nap1l4	HTPSSYIETLPKAIKRRINALK	V63I	CD4+	0.7

PepM14		QLQVR (SEQ ID NO: 53)			
CT26-PepM15	Cxcr7	MKAFIFKYSAKTGFTKLIDASR VSETE (SEQ ID NO: 54)	L340F	CD4+	1.8
CT26-PepM16	Dkk2	EGDPCLRSSDCIDEFCCARH FWTKICK (SEQ ID NO: 55)	G192E	CD4+	9.7
CT26-PepM17	Trip12	WKGGPVKIDPLALMQAIERYL VVRGYG (SEQ ID NO: 56)	V1328M	CD8+	
CT26-PepM18	Steap2	VTSIPSVSNALNWKEFSFIQS TLGYVA (SEQ ID NO: 57)	R388K	CD4+	6.8
Ct26-PepM19	Gpc1	YRGANLHLEETLAGFWARLL ERLFKQL(SEQ ID NO: 58)	E165G	CD8+	1.9
CT26-PepM20	Usp26	KTTLSHTQDSSQSLQSSSDS SKSSRCS (SEQ ID NO: 59)	S715L	n.d.	5.8

Table 2 - B16-F10 cell line

Mutation number polypep-tide (Vacci-body)	**Gene**	**Mutated sequence used for vaccination**	**Substi.WT, AA#, Mut)**	**Reactive T cell subtype**	**MHC I score (best predi ction)**
B16-PepM1	Kif18b	PSKPSFQEFVDWENVSPELNSTD QPFL (SEQ ID NO: 4)	K739N	CD4+	1,2
B16-PepM2	Obsl1	REGVELCPGNKYEMRRHGTTHSL VIHD (SEQ ID NO: 5)	T176M	CD8+	2,3
B16-PepM3	Def8	SHCHWNDLAVIPAGVVHNWDFEP RKVS (SEQ ID NO: 6)	R255G	CD4+	3,8
B16-PepM4	Rpl13a	GRGHLLGRLAAIVGKQVLLGRKVV VVR (SEQ ID NO: 7)	A24G	CD4+	0,5
B16-PepM5	Tubb3	FRRKAFLHWYTGEAMDEMEFTEA ESNM (SEQ ID NO: 8)	G402A	CD4+	1,9
B16-PepM6	Tnpo3	VVDRNPQFLDPVLAYLMKGLCEK PLAS (SEQ ID NO: 9)	G504A	CD4+	1
B16-PepM7	Atp11a	SSPDEVALVEGVQSLGFTYLRLKD NYM (SEQ ID NO: 10)	R552S	CD4+	0,1
B16-PepM8	Cpsf3l	EFKHIKAFDRTFANNPGPMVVFAT PGM (SEQ ID NO: 11)	D314N	CD4+	0,5

B16-PepM9	Plod1	STANYNTSHLNNDVWQIFENPVD WKEK (SEQ ID NO: 12)	F530V	CD4+	0,1
B16-PepM10	Pbk	DSGSPFPAAVILRDALHMARGLKY LHQ (SEQ ID NO: 13)	V145D	CD8+	0,1
B16-PepM11	Ddx23	ANFESGKHKYRQTAMFTATMPPA VERL (SEQ ID NO: 36)	V602A	CD4+	1,3
B16-PepM12	Actn4	NHSGLVTFQAFIDVMSRETTDTDT ADQ (SEQ ID NO: 60)	F835V	CD4+	0.2
B16-PepM13	Tm9sf3	CGTAFFINFIAIYHHASRAIPFGTM VA (SEQ ID NO: 61)	Y382H	CD4+	0.2
B16-PepM14	Eef2	FVVKAYLPVNESFAFTADLRSNTG GQA (SEQ ID NO: 62)	G795A	CD4+	1.1
B16-PepM15	Gnas	TPPPEEAMPFEFNGPAQGDHSQP PLQV (SEQ ID NO: 63)	S111G	CD4+	1.2
B16-PepM16	Asf1b	PKPDFSQLQRNILPSNPRVTRFHI NWD (SEQ ID NO: 64)	A141P	CD4+	1.7
B16-PepM17	Mthfd1l	IPSGTTILNCFHDVLSGKLSGGSP GVP (SEQ ID NO: 65)	F294V	CD4+	1.7
B16-PepM18	Sema3b	GFSQPLRRLVLHVVSAAQAERLA RAEE (SEQ ID NO: 66)	L663V	CD4+	2.9
B16-PepM19	Mkm1	ECRITSNFVIPSEYWVEEKEEKQK LIQ (SEQ ID NO: 67)	N346Y	CD4+	1.4
B16-PepM20	Ppp1r7	NIEGIDKLTQLKKPFLVNNKINKIEN I(SEQ ID NO: 68)	L170P	CD4+	3.2

EXAMPLE 2: Comparing Vaccibodies comprising 3 or 10 neoepitopes

Vaccibody vaccines containing either 3 or10 neoepitopes were compared. In the 10 neoepitope Vaccibody DNA construct the place and order for the 3 first (N-terminal) peptides are similar as in the 3 neoepitope Vaccibody DNA construct. This is done to be able to compare the immunogenicity of these 3 neoepitopes in the context with 3 and in the context containing 7 more epitopes.

VB4001 (VB10.NEO CT26-X), VB4002 (VB10.NEO CT26-III), VB4003 (VB10.NEO B16-X) and VB4004 (VB10.NEO B16-III) were selected as vaccine candidates. A schematic drawing of the vaccibodies are shown in Figure 1.

The neoepitopes used for the vaccines VB4001-VB4021 are shown below.For example, VB4015 comprises three neoepitopes, B16 pepM1+pepM8+pepM3 that are separated by 5 amino acid linkers. VB4018 comprises 2 copies of the 10 neoepitopes, B16 pepM1+pepM2+pepM3+pepM4+pepM11+pepM6+pepM7+pepM8+pepM9+pepM10 that are separated by 5 amino acid linkers. The neoepitope sequences are shown in Tables 1 and 2.

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VB4001 = VB10.NEO CT26-X = CT26 pepM1-M10, 5 aa linker
VB4002 = VB10.NEO CT26-III = CT26 pepM1-M3, 5 aa linker
VB4003 = VB10.NEO B16-X = B16 pepM1-M10, 5 aa linker
VB4004 = VB10.NEO B16-III = B16 pepM1-M3, 5 aa linker
VB4011 = VB10.NEO B16-X = B16 pepM1-M10, 10 aa linker
VB4012 = VB10.NEO B16-III = B16 pepM1-M3, 10 aa linker
VB4014 = VB10.NEO B16-X = B16 hydrophobic core, (pepM9+pepM5+pepM1+pepM4+pepM6+pepM8+pepM10+pepM3+pepM7+pepM2), 5 aa linker
VB4015 = VB10.NEO B16-III = B16 pepM1+M8+M3, 5 aa linker
VB4016 = VB10.NEO B16-III = B16 pepM1+M3+M2, 5 aa linker
VB4017 = VB10.NEO B16-X = B16 pepM1-M4+M11+M6-M10, 5 aa linker
VB4018 = VB10.NEO B16-XX = B16 pepM1-M4+M11+M6-M10 x 2, 5 aa linker
VB4019 = VB10.NEO B16-Vx2 = B16 pepM3+M4+M7+M9+M10 x 2, 5 aa linker
VB4021 = VB10.NEO B16-Vx4 = B16 pepM3+M4+M7+M9-M10 x 4, 5 aa linker

All neoepitope gene sequences were ordered from Genescript (New Jersey, US) and cloned into the expression vector pUMVC4a holding the LD78beta targeting unit and the hIgG3 dimerization unit.

All constructs were transfected into HEK293 cells and Vaccibody proteins in the supernatant were verified by Western blot and/or sandwich ELISA. Empty pUMVC4a vector was included as a negative control. Figure 2, left panels: To illustrate the formation of intact homodimeric proteins, the proteins in the supernatant from transfected cells were detected in a Western blot by an anti-hMIP-1alpha antibody, in either the presence or absence of reducing agents. The formation of homodimers are shown in the left lane (-reducing agent) whereas the monomers are illustrated in the right lane (+ reducing agent). Figure 2, right panel shows the expression level of the Vaccibody proteins in the supernatant of HEK293 cells transfected with the different VB10.NEO constructs detected by a sandwich ELISA using antibodies against both hMIP-1alpha and hIgG3. Right, upper panel shows the expression level of the VB10.NEO CT26-X (VB4001) and VB10.NEO CT26-III (VB4002) constructs, comprising 10 or 3 neoepitopes, respectively. Right, lower panel shows the expression level of the VB10.NEO B16-X (VB4003)

and VB10.NEO B16-III (VB4004) constructs, comprising 10 or 3 neoepitopes, respectively. To compare the immunogenicity of vaccibodies comprising 3 or 10 neoepitopes, 20 µg plasmid DNA of each vaccibody candidate were injected intramuscularly in the tibial anterior muscle of C57Bl/6-mice (for B16 constructs) or BALB/c- mice (for CT26 constructs), followed by electroporation using TriGrid, Ichor, (US). At day 13, the mice were euthanized and spleens were harvested.

The T cell responses were evaluated by IFN-gamma ELISpot. The results are shown in Figure 3 where the T cell responses are indicated as the number of IFN-γ spots/10^6 splenocytes. We observe that vaccibodies comprising 10 neoepitopes induces significant T cell responses towards 4-6 of 10 included neoepitopes in the same mice. The peptides stimulating the strongest IFN-γ response generally have the best MHC I binding score.

The total neoantigen-specific immune responses induced by vaccibody constructs comprising 3 or 10 neoepitopes are depicted in Figure 4. Vaccibodies comprising 10 neoepitopes (VB10.NEO B16-X and VB10.NEO CT26-X) resulted in an increased total neoantigen-specific immune response when compared with vaccibodies comprising 3 neoepitopes (VB10.NEO B16-III and VB10.NEO CT26-III).

EXAMPLE 3: Comparing immunogenicity of vaccibody DNA vaccines and corresponding peptide plus adjuvant vaccines.

Before the VB10.NEO constructs are used in mice vaccination studies, Vaccibody protein expression and secretion in HEK293 cells are verified using a sandwich ELISA assay, as previously described in detail in the text for Figure 2. The order of the neoepitopes could have an impact on the expression and secretion of functional Vaccibodies. In Figure 5, upper panel we observe that the VB10.NEO B16-X construct VB4014 has a slightly improved expression and secretion of functional vaccibody proteins compared to the VB10.NEO B16-X construct VB4003. The 10 neopitopes in VB4014 is similar as for VB4003, however the order of the neoepitopes are changed and the most hydrophobic neoepitopes are located in the core in the neoepitope antigenic module. To test immunogenicity of Vaccibody DNA vaccines VB4003 and VB4014 compared with peptides comprising only neoepitopes delivered in combination with the poly (I:C) adjuvant, C57/Bl6 mice were injected with 20 µg of the VB10.NEO B16-X constructs VB4003 and VB4014 (The induced immune responses were compared with immune responses of mice s.c. injected with 20 µg or 200 µg peptide mix + 50 µg poly I:C comprising the 10 neoepitopes encoded by VB4003 and VB4014. The T cell responses were evaluated by IFN-gamma ELISpot. The results, shown in Figure 5 lower panel, illustrate that the vaccibodies clearly induces a much stronger response than peptide+adjuvant. Moreover, some of the animals immunized with the VB10.NEO B16-X VB4014 construct responded to all 10 neoepitopes included in the vaccine.

EXAMPLE 4: Comparing vaccibodies comprising second linkers with a length of 5 or 10 amino acids.

Each of the neoepitopes is separated by a second linker. In the present example the second linker is a flexible GGGGS linker. To test if the length of the second linker has any effect on the expression level, HEK293 cells were transfected with VB10.NEO B16-X constructs comprising second linkers with a length of either 5 or 10 amino acids. Figure 6 illustrates that changing the linker length from 5 (VB4003) to 10 (VB4011) amino acids does not affect expression of vaccibodies comprising 10 neoepitopes (Figure 6, upper panel). To test if the length of the second linker has any effect on the immune response, C57Bl/6 mice were injected with VB10.NEO B16-X constructs comprising 10 neoepitopes with either 5 (VB4003) or 10 (VB4011) amino acid linkers. At day 13, the mice were sacrificed and splenocytes harvested, stimulated with the individual corresponding neoepitope peptides for 24 hours and T cell responses were quantified in an IFN-gamma ELISpot assay. The results are shown in Figure 6, lower panel, and demonstrate that vaccibody constructs comprising 10 amino acid linkers (VB4011) lead to an increased total immune response when compared to vaccibodies comprising 5 amino acid linkers (VB4003). Empty vector was included as a negative control.

EXAMPLE 5: Comparing vaccibodies comprising different number of copies of identical neoepitopes.

The following constructs were tested:
VB4003 = VB10.NEO B16-X = B16 pepM1-M10, 5 aa linker
VB4018 = VB10.NEO B16-XX = B16 pepM1-M4+M11+M6-M10 x 2, 5 aa linker

The expression level of VB10.NEO B16-X (VB4003) construct comprising 10 neoepitopes was compared to the expression level of VB10.NEO B16-XX (VB4018) comprising 2x10 neoepitopes. The results demonstrate that VB10.NEO B16-XX (VB4018) comprising 20 neoepitopes are slightly less expressed compared to VB10.NEO B16-X (VB4003) comprising 10 neoepitopes (Figure 7, upper panel).

The immunogenicity of Vaccibodies comprising either 10 or 20 neoepitopes was tested by intramuscular injection of C57Bl/6 mice with the Vaccibody DNA vaccine VB10.NEO B16-X (VB4003) and VB10.NEO B16-XX (VB4018) At day 13, the mice were sacrificed and splenocytes harvested, stimulated with the individual corresponding neoepitope peptides for 24 hours and T cell responses were quantified in an IFN-gamma ELISpot assay. The results

shown in Figure 7, lower panel illustrate that the benefit of including 2 copies per neoepitope (2x10 neoepitopes) is limited on the total immune response, however, a broader immune response is observed towards individual neoepitopes.

Next, the expression levels of Vaccibody constructs comprising one or more copies of the 5 selected neoepitopes, PepM3, PepM4, PepM7, PepM9 and PepM10, were tested (Figure 8, upper panel).

C57Bl/6 mice were injected with the following Vaccibody constructs:

VB4003 = VB10.NEO B16-X = B16 pepM1-M10, 5 aa linker

VB4011 = VB10.NEO B16-X = B16 pepM1-M10, 10 aa linker

VB4018 = VB10.NEO B16-XX = B16 pepM1-M4+M11+M6-M10 x 2, 5 aa linker

VB4019 = VB10.NEO B16-Vx2 = B16 pepM3+M4+M7+M9+M10 x 2, 5 aa linker

VB4021 = VB10.NEO B16-Vx4 = B16 pepM3+M4+M7+M9+M10 x 4, 5 aa linker

The immune responses of the Vaccibody candidates for each of the five selected neoepitopes are shown in Figure 8, lower panel. Multiple copies of the five neoepitopes had limited effect on the total immune response. However, several copies of each neoepitope (VB4018, VB4019 and VB4021) gives a more evenly immune response towards the 5 shared neoepitopes compared to the decatope VB4003, where the 5 neoepitopes are presented once. Interestingly, Vaccibodies comprising a 10 amino acid second linker and the neoepitopes only once (VB4011) displayed a better total immune response than Vaccibodies comprising multiple copies of the five neoepitopes.

EXAMPLE 6: Comparing vaccibodies comprising different number of neoepitopes

The immune response of vaccibody constructs comprising different numbers of neoepitopes were compared to test the immunological effect of adding further neoepitopes.

The total immune response was tested in the B16 melanoma mouse model using the following constructs:

NEO B16-X = VB4011 = B16 pepM1-M10, 10 aa linker

NEO B16-XV = VB4024 = B16 pepM1-M15, 10 aa linker

NEO B16-XX = VB4025 = B16 pepM1-M20, 10 aa linker

The neoepitope sequences are shown in Table 2.

The expression levels of the three tested vaccibody constructs are shown in Figure 11, upper panel.

C57Bl/6 mice were injected with the DNA vaccine candidates VB10.NEO B16-XV comprising 15 neoepitopes (VB4024) or VB10.NEO B16-XX comprising 20 neoepitopes (VB4025) compared to the VB10.NEO B16-X comprising 10 neoepitopes (VB4011). Figure 11, lower panel, shows the total number of IFNγ-spots per 10^6 splenocytes. Constructs with 15 and 20 neoepitopes resulted in a broader immune response against more individual neoepitopes and a higher total T cell response when compared to constructs with only 10 neoepitopes. As a negative control, mice were injected with empty vector not comprising the neoepitopes. As seen from Figure 11, lower panel, injections with empty vector did not lead to any significant immune response against the individual neoepitopes.

Further, the total immune response was tested in the CT26 melanoma mouse model using the following constructs

NEO CT26-X = VB4009 = CT26 pepM1-M10, 10 aa linker
NEO CT26-XV = VB4026 = CT26 pepM1-M15, 10 aa linker
NEO CT26-XX = VB4027 = CT26 pepM1-M20, 10 aa linker

The neoepitope sequences are shown in Table 1.

BALB/c mice were injected with the DNA vaccine candidates VB10.NEO CT26-XV comprising 15 neoepitopes (VB4026) or VB10.NEO CT26-XX comprising 20 neoepitopes (VB4027) compared to the VB10.NEO CT26-X comprising 10 neoepitopes (VB4009). Figure 12, lower panel, shows the total number of IFNγ-spots per 10^6 splenocytes. Constructs with 15 and 20 neoepitopes resulted in a broader immune response against more individual neoepitopes and a higher total T cell response when compared to constructs with only 10 neoepitopes. As a negative control, mice were injected with empty vector not comprising the neoepitopes. As seen from Figure 12, lower panel, injections with empty vector did not lead to any significant immune response against the individual neoepitopes.

EXAMPLE 7: Expression levels of different vaccibody constructs - are compared.

The following constructs were tested:
VB4004 = VB10.NEO B16-III = B16 pepM1-M3, 5 aa linker
VB4012 = VB10.NEO B16-III = B16 pepM1-M3, 10 aa linker
VB4015 = VB10.NEO B16-III = B16 pepM1+M8+M3, 5 aa linker

VB4016 = VB10.NEO B16-III = B16 pepM1+M3+M2, 5 aa linker
VB4017 = VB10.NEO B16-X = B16 pepM1-M4+M11+M6-M10, 5 aa linker
VB4018 = VB10.NEO B16-XX = B16 pepM1-M4+M11+M6-M10 x 2, 5 aa linker

Similar expression and secretion of functional vaccibody proteins are observed for VB10.NEO B16-X (VB4017) and VB10.NEO B16-XX (VB4018) (Figure 9).

Improved expression and secretion of functional vaccibody proteins are observed when the 3 neoepitopes are spaced with a 10 aa linker as in the VB10.NEO B16-III (VB4012) construct compared to a 5 aa linker in the VB10.NEO B16-III (VB4004) construct (Figure 10, upper panel). Moreover, by changing the order of the three neoepitopes as shown by comparing VB4004, VB4015 and VB4016 (Figure 10, lower panel), may affect the expression levels of the vaccibodies.

EXAMPLE 8: Therapeutic effect

VB10.NEO were used as vaccine candidates for therapeutic vaccine studies.

$7.5x10^4$ B16.F10 cells or $1x10^5$ CT26 cells (ATCC) was injected in the thigh region of C57Bl/6 mice or BALB/c mice. After 1 and 8 days, the mice were vaccinated with 20 µg plasmid DNA followed by electroporation, TriGrid, Ichor, US. Tumor sizes were measured two to three times a week. Figure 13 shows that VB10.NEO DNA vaccine candidates comprising 10 neoepitopes are able to significantly delay and reduce tumour growth.

EXAMPLE 9: Therapeutic DNA vaccine

A therapeutic DNA vaccine to be used may be prepared by GMP manufacturing of the plasmid vaccine according to regulatory authorities' guidelines, and Fill & Finish of the DNA vaccine. The DNA vaccine may be formulated by dissolving in a saline solution, such as PBS at a concentration of 2-6 mg/ml. The vaccine may be administered either intradermal or intramuscular with or without following electroporation or alternatively with a jet injector.

SEQUENCES

SEQ ID NO : 1

C-C motif chemokine 3-like 1 precursor including signal peptide and mature peptide (LD78-beta), aa 24-93:

MQVSTAALAVLLCTMALCNQVLSAPLAADTPTACCFSYTSRQIPQNFIADYFETSSQCSKPSVIF LTKRGRQVCADPSEEWVQKYVSDLELSA

SEQ ID NO : 2

DNA sequence of constant coding part of all VB10.NEO constructs

For the purpose of illustration only, the different domains of the constructs are separated by an "I"with the domains in the following order: Signal peptide | human MIP-Iα | Hinge hi | Hinge h4 I Gly-Ser Linker or Gly-Leu linker | hCH3 IgG3 | Gly-Ser Linker or Gly-Leu linker |
The construct is a standard construct that can be used to insert neoepitopes. Neoepitope sequences can be added after the linker GGCCTCGGTGGCCTG.

ATGCAGGTCTCCACTGCTGCCCTTGCCGTCCTCCTCTGCACCATGGCTCTCTGCAACCAG
GTCCTCTCT | GCACCACTT
GCTGCTGACACGCCGACCGCCTGCTGCTTCAGCTACACCTCCCGACAGATTCCACAGAAT
TTCATAGCTGACTACTTTG
AGACGAGCAGCCAGTGCTCCAAGCCCAGTGTCATCTTCCTAACCAAGAGAGGCCGGCAGG
TCTGTGCTGACCCCAGTGA
GGAGTGGGTCCAGAAATACGTCAGTGACCTGGAGCTGAGTGCC |
GAGCTCAAAACCCCACTTGGTGACACAACTCACAC A I
GAGCCCAAATCTTGTGACACACCTCCCCCGTGCCCAAGGTGCCCA |
GGCGGTGGAAGCAGCGGAGGTGGAAGTGGA |
GGACAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAA
GAACCAGGTCAGCCTGACCT
GCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAGCGGGCAG
CCGGAGAACAACTACAACAC
CACGCCTCCCATGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGA
CAAGAGCAGGTGGCAGCAG
GGGAACATCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCGCTTCACGCAGAAG
AGCCTCTCCCTGTCTCCGG GTAAA I GGCCTCGGTGGCCTG |

SEQ ID NO: 3

Amino acid sequence of constant coding part of all VB10.NEO proteins:B4001

MQVSTAALAVLLCTMALCNQVLS | APLAADTPTACCFSYTSRQIPQNFIAD
YFETSSQCSKPSVIFLTKRGRQVCADPSEEWVQKYVSDLELSA | ELKTPLG
DTTHT I EPKSCDTPPPCPRCP | GGGSSGGGSG | GQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK | GLGGL |

SEQ ID NO: 4

B16-F10 mutated epitope, B16-PepM1, amino acid sequence
PSKPSFQEFVDWENVSPELNSTDQPFL

SEQ ID NO: 5

B16-F10 mutated epitope, B16-PepM2, amino acid sequence
REGVELCPGNKYEMRRHGTTHSLVIHD

SEQ ID NO: 6

B16-F10 mutated epitope, B16-PepM3, amino acid sequence
SHCHWNDLAVIPAGVVHNWDFEPRKVS

SEQ ID NO: 7

B16-F10 mutated epitope, B16-PepM4, amino acid sequence
GRGHLLGRLAAIVGKQVLLGRKVVVVR

SEQ ID NO: 8

B16-F10 mutated epitope, B16-PepM5, amino acid sequence
FRRKAFLHWYTGEAMDEMEFTEAESNM

SEQ ID NO: 9

B16-F10 mutated epitope, B16-PepM6, amino acid sequence
VVDRNPQFLDPVLAYLMKGLCEKPLAS

SEQ ID NO: 10

B16-F10 mutated epitope, B16-PepM7, amino acid sequence
SSPDEVALVEGVQSLGFTYLRLKDNYM

SEQ ID NO: 11

B16-F10 mutated epitope, B16-PepM8, amino acid sequence

EFKHIKAFDRTFANNPGPMVVFATPGM

SEQ ID NO: 12

B16-F10 mutated epitope, B16-PepM9, amino acid sequence

STANYNTSHLNNDVWQIFENPVDWKEK

SEQ ID NO: 13

B16-F10 mutated epitope, B16-PepM10, amino acid sequence

DSGSPFPAAVILRDALHMARGLKYLHQ

SEQ ID NO: 14

CT26 mutated epitope, CT26-PepM1, amino acid sequence

VILPQAPSGPSYATYLQPAQAQMLTPP

SEQ ID NO: 15

CT26 mutated epitope, CT26-PepM2, amino acid sequence

LHSGQNHLKEMAISVLEARACAAAGQS

SEQ ID NO: 16

CT26 mutated epitope, CT26-PepM3, amino acid sequence

PLLPFYPPDEALEIGLELNSSALPPTE

SEQ ID NO: 17

CT26 mutated epitope, CT26-PepM4, amino acid sequence

AGTQCEYWASRALDSEHSIGSMIQLPQ

SEQ ID NO: 18

CT26 mutated epitope, CT26-PepM5, amino acid sequence

AAYKGHHYPGPGNYFWKCLFMSGLSEV

SEQ ID NO: 19

CT26 mutated epitope, CT26-PepM6, amino acid sequence

DTLSAMSNPRAMQVLLQIQQGLQTLAT

SEQ ID NO: 20

CT26 mutated epitope, CT26-PepM7, amino acid sequence

DKPLRRNNSYTSYIMAICGMPLDSFRA

SEQ ID NO: 21

CT26 mutated epitope, CT26-PepM8, amino acid sequence

EVIQTSKYYMRDVIAIESAWLLELAPH

SEQ ID NO: 22

CT26 mutated epitope, CT26-PepM9, amino acid sequence

GYISRVTAGKDSYIALVDKNIMGYIAS

SEQ ID NO: 23

CT26 mutated epitope, CT26-PepM10, amino acid sequence

EHIHRAGGLFVADAIQVGFGRIGKHFW

SEQ ID NO: 24

First linker, amino acid sequence: GLSGL

SEQ ID NO: 25

First linker, amino acid sequence: GLGGL

SEQ ID NO: 26

Hinge regions (IgG3 UH hinge), 12 amino acids: ELKTPLGDTTHT

SEQ ID NO: 27

Hinge region (IgG3, MH hinge, 15 amino acids): EPKSCDTPPPCPRCP

SEQ ID NO: 28

Gly-Ser Linker: GGGSSGGGSG

SEQ ID NO: 29

hCH3 IgG3, amino acid sequence:

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDG SFFLYSKL TVDKSRWQQG NIFSCSVM H EALH N RFTQKSLSLSPGK

SEQ ID NO: 30

Amino acid sequence of VB4001 = VB10.NEO CT26-X = CT26 pepM1-M10, 5 aa linker

The neoepitope sequences are inserted after GGGSSGGGSG.

MQVSTAALAVLLCTMALCNQVLS | APLAADTPTACCFSYTSRQIPQNFIAD YFETSSQCSKPSVIFLTKRGRQVCADPSEEWVQKYVSDLELSA | ELKTPLG DTTHT I EPKSCDTPPPCPRCP | GGGSSGGGSG | GQPREPQVYTLPPSREEMTK

NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK | GLGGL |
MHGDTPTLHEYMLDLQPETTDLYGYGQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFC
CKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP | GGGSSGGGSG |
VILPQAPSGPSYATYLQPAQAQMLTPP**GGGGS**LHSGQNHLKEMAISVLEARACAAAGQS**GGG**
GSPLLPFYPPDEALEIGLELNSSALPPTE**GGGGS**AGTQCEYWASRALDSEHSIGSMIQLPQ**GG**
GGSAAYKGHHYPGPGNYFWKCLFMSGLSEV**GGGGS**DTLSAMSNPRAMQVLLQIQQGLQTLA
T**GGGGS**DKPLRRNNSYTSYIMAICGMPLDSFRA**GGGGS**EVIQTSKYYMRDVIAIESAWLLELAP
H**GGGGS**GYISRVTAGKDSYIALVDKNIMGYIAS**GGGGS**EHIHRAGGLFVADAIQVGFGRIGKHF
W

SEQ ID NO: 31

Amino acid sequence of VB4002 VB10.NEO CT26-III = CT26 pepM1-M3, 5 aa linker

MQVSTAALAVLLCTMALCNQVLS | APLAADTPTACCFSYTSRQIPQNFIAD
YFETSSQCSKPSVIFLTKRGRQVCADPSEEWVQKYVSDLELSA | ELKTPLG
DTTHT I EPKSCDTPPPCPRCP | GGGSSGGGSG | GQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK | GLGGL | MHGDTPTL
HEYMLDLQPETTDLYGYGQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFC
CKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP | GGGSSGGGSG |
VILPQAPSGPSYATYLQPAQAQMLTPP**GGGGS**LHSGQNHLKEMAISVLEARACAAAGQS**GGG**
GSPLLPFYPPDEALEIGLELNSSALPPTE

SEQ ID NO: 32

Amino acid sequence of VB4003 = VB10.NEO B16-X = B16 pepM1-M10, 5 aa linker (VB10.Neo-10B)

MQVSTAALAVLLCTMALCNQVLS | APLAADTPTACCFSYTSRQIPQNFIAD
YFETSSQCSKPSVIFLTKRGRQVCADPSEEWVQKYVSDLELSA | ELKTPLG
DTTHT I EPKSCDTPPPCPRCP | GGGSSGGGSG | GQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK | GLGGL | MHGDTPTL
HEYMLDLQPETTDLYGYGQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFC
CKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP | GGGSSGGGSG |
PSKPSFQEFVDWENVSPELNSTDQPFL**GGGGS**REGVELCPGNKYEMRRHGTTHSLVIHD**GG**
GGSSHCHWNDLAVIPAGVVHNWDFEPRKVS**GGGGS**GRGHLLGRLAAIVGKQVLLGRKVVVV
R**GGGGS**FRRKAFLHWYTGEAMDEMEFTEAESNM**GGGGS**VVDRNPQFLDPVLAYLMKGLCE
KPLAS**GGGGS**SSPDEVALVEGVQSLGFTYLRLKDNYM**GGGGS**EFKHIKAFDRTFANNPGPMV

VFATPGM**GGGGS**STANYNTSHLNNDVWQIFENPVDWKEK**GGGGS**DSGSPFPAAVILRDALH
MARGLKYLHQ

SEQ ID NO: 33

Amino acid sequence of **VB4004 = VB10.NEO B16-III = B16 pepM1-M3, 5 aa linker**

MQVSTAALAVLLCTMALCNQVLS | APLAADTPTACCFSYTSRQIPQNFIAD
YFETSSQCSKPSVIFLTKRGRQVCADPSEEWVQKYVSDLELSA | ELKTPLG
DTTHT I EPKSCDTPPPCPRCP | GGGSSGGGSG | GQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK | GLGGL | MHGDTPTL
HEYMLDLQPETTDLYGYGQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFC
CKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP | GGGSSGGGSG |
PSKPSFQEFVDWENVSPELNSTDQPFL**GGGGS**REGVELCPGNKYEMRRHGTTHSLVIHD**GG**
GGSSHCHWNDLAVIPAGVVHNWDFEPRKVS

SEQ ID NO: 34

Signal peptide

MNFGLRLIFLVLTLKGVQC

SEQ ID NO: 35

Signal peptide

MDAMKRGLCCVLLLCGAVFVSP

SEQ ID NO: 36

B16-F10 mutated epitope, B16-pepM11, amino acid sequence

ANFESGKHKYRQTAMFTATMPPAVERL

SEQ ID NO: 37

Amino acid sequence of **VB4011 = VB10.NEO B16-X = B16 pepM1-M10, 10 aa linker**

MQVSTAALAVLLCTMALCNQVLS | APLAADTPTACCFSYTSRQIPQNFIAD
YFETSSQCSKPSVIFLTKRGRQVCADPSEEWVQKYVSDLELSA | ELKTPLG
DTTHT I EPKSCDTPPPCPRCP | GGGSSGGGSG | GQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK | GLGGL | MHGDTPTL
HEYMLDLQPETTDLYGYGQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFC
CKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP | GGGSSGGGSG |

PSKPSFQEFVDWENVSPELNSTDQPFL**GGGGSGGGGS**REGVELCPGNKYEMRRHGTTHSLV
IHD**GGGGSGGGGS**SHCHWNDLAVIPAGVVHNWDFEPRKVS**GGGGSGGGGS**GRGHLLGRLA
AIVGKQVLLGRKVVVVR**GGGGSGGGGS**FRRKAFLHWYTGEAMDEMEFTEAESNM**GGGGSG**
GGGSVVDRNPQFLDPVLAYLMKGLCEKPLAS**GGGGSGGGGS**SSPDEVALVEGVQSLGFTYL
RLKDNYM**GGGGSGGGGS**EFKHIKAFDRTFANNPGPMVVFATPGM**GGGGSGGGGS**STANYN
TSHLNNDVWQIFENPVDWKEK**GGGGSGGGGS**DSGSPFPAAVILRDALHMARGLKYLHQ

SEQ ID NO: 38

Amino acid sequence of VB4012 = VB10.NEO B16-III = B16 pepM1-M3, 10 aa linker

MQVSTAALAVLLCTMALCNQVLS | APLAADTPTACCFSYTSRQIPQNFIAD
YFETSSQCSKPSVIFLTKRGRQVCADPSEEWVQKYVSDLELSA | ELKTPLG
DTTHT I EPKSCDTPPPCPRCP | GGGSSGGGSG | GQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK | GLGGL | MHGDTPTL
HEYMLDLQPETTDLYGYGQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFC
CKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP | GGGSSGGGSG |
PSKPSFQEFVDWENVSPELNSTDQPFL**GGGGSGGGGS**REGVELCPGNKYEMRRHGTTHSLV
IHD**GGGGSGGGGS**SHCHWNDLAVIPAGVVHNWDFEPRKVS

SEQ ID NO: 39

Amino acid sequence of VB4014 = VB10.NEO B16-X = B16 hydrophobic core,
(pepM9+M5+M1+M4+M6+M8+M10+M3+M7+M2), 5 aa linker

MQVSTAALAVLLCTMALCNQVLS | APLAADTPTACCFSYTSRQIPQNFIAD
YFETSSQCSKPSVIFLTKRGRQVCADPSEEWVQKYVSDLELSA | ELKTPLG
DTTHT I EPKSCDTPPPCPRCP | GGGSSGGGSG | GQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK | GLGGL | MHGDTPTL
HEYMLDLQPETTDLYGYGQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFC
CKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP | GGGSSGGGSG |
STANYNTSHLNNDVWQIFENPVDWKEK**GGGGS**FRRKAFLHWYTGEAMDEMEFTEAESNM**G**
GGGSPSKPSFQEFVDWENVSPELNSTDQPFL**GGGGS**GRGHLLGRLAAIVGKQVLLGRKVVVV
R**GGGGS**VVDRNPQFLDPVLAYLMKGLCEKPLAS**GGGGS**EFKHIKAFDRTFANNPGPMVVFAT
PGM**GGGGS**DSGSPFPAAVILRDALHMARGLKYLHQ**GGGGS**SHCHWNDLAVIPAGVVHNWDF
EPRKVS**GGGGS**SSPDEVALVEGVQSLGFTYLRLKDNYM**GGGGS**REGVELCPGNKYEMRRHG
TTHSLVIHD

SEQ ID NO: 40

Amino acid sequence of VB4015 = VB10.NEO B16-III = B16 pepM1-M8-M3, 5 aa linker

MQVSTAALAVLLCTMALCNQVLS | APLAADTPTACCFSYTSRQIPQNFIAD
YFETSSQCSKPSVIFLTKRGRQVCADPSEEWVQKYVSDLELSA | ELKTPLG
DTTHT I EPKSCDTPPPCPRCP | GGGSSGGGSG | GQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK | GLGGL | MHGDTPTL
HEYMLDLQPETTDLYGYGQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFC
CKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP | GGGSSGGGSG |
PSKPSFQEFVDWENVSPELNSTDQPFL**GGGGS**EFKHIKAFDRTFANNPGPMVVFATPGM**GGG**
GSSHCHWNDLAVIPAGVVHNWDFEPRKVS

SEQ ID NO: 41

Amino acid sequence of VB4016 = VB10.NEO B16-III = B16 pepM1-M3-M2, 5 aa linker

MQVSTAALAVLLCTMALCNQVLS | APLAADTPTACCFSYTSRQIPQNFIAD
YFETSSQCSKPSVIFLTKRGRQVCADPSEEWVQKYVSDLELSA | ELKTPLG
DTTHT I EPKSCDTPPPCPRCP | GGGSSGGGSG | GQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK | GLGGL | MHGDTPTL
HEYMLDLQPETTDLYGYGQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFC
CKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP | GGGSSGGGSG |
PSKPSFQEFVDWENVSPELNSTDQPFL**GGGGS**SHCHWNDLAVIPAGVVHNWDFEPRKVS**GG**
GGSREGVELCPGNKYEMRRHGTTHSLVIHD

SEQ ID NO: 42

Amino acid sequence of VB4017 = VB10.NEO B16-X = B16 pepM1-M4+M11+M6-M10, 5 aa linker

MQVSTAALAVLLCTMALCNQVLS | APLAADTPTACCFSYTSRQIPQNFIAD
YFETSSQCSKPSVIFLTKRGRQVCADPSEEWVQKYVSDLELSA | ELKTPLG
DTTHT I EPKSCDTPPPCPRCP | GGGSSGGGSG | GQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK | GLGGL | MHGDTPTL
HEYMLDLQPETTDLYGYGQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFC
CKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP | GGGSSGGGSG |
PSKPSFQEFVDWENVSPELNSTDQPFL**GGGGS**REGVELCPGNKYEMRRHGTTHSLVIHD**GG**
GGSSHCHWNDLAVIPAGVVHNWDFEPRKVS**GGGGS**GRGHLLGRLAAIVGKQVLLGRKVVVV
R**GGGGS**ANFESGKHKYRQTAMFTATMPPAVERL**GGGGS**VVDRNPQFLDPVLAYLMKGLCEK

PLAS**GGGGS**SSPDEVALVEGVQSLGFTYLRLKDNYM**GGGGS**EFKHIKAFDRTFANNPGPMVV FATPGM**GGGGS**STANYNTSHLNNDVWQIFENPVDWKEK**GGGGS**DSGSPFPAAVILRDALHM ARGLKYLHQ

SEQ ID NO: 43

Amino acid sequence of VB4018 = VB10.NEO B16-XX = B16 pepM1-M4+M11+M6-M10 x 2, 5 aa linker

MQVSTAALAVLLCTMALCNQVLS | APLAADTPTACCFSYTSRQIPQNFIAD YFETSSQCSKPSVIFLTKRGRQVCADPSEEWVQKYVSDLELSA | ELKTPLG DTTHT I EPKSCDTPPPCPRCP | GGGSSGGGSG | GQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKL TVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK | GLGGL | MHGDTPTL HEYMLDLQPETTDLYGYGQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFC CKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP | GGGSSGGGSG | PSKPSFQEFVDWENVSPELNSTDQPFL**GGGGS**REGVELCPGNKYEMRRHGTTHSLVIHD**GG GGS**SHCHWNDLAVIPAGVVHNWDFEPRKVS**GGGGS**GRGHLLGRLAAIVGKQVLLGRKVVVV R**GGGGS**ANFESGKHKYRQTAMFTATMPPAVERL**GGGGS**VVDRNPQFLDPVLAYLMKGLCEK PLAS**GGGGS**SSPDEVALVEGVQSLGFTYLRLKDNYM**GGGGS**EFKHIKAFDRTFANNPGPMVV FATPGM**GGGGS**STANYNTSHLNNDVWQIFENPVDWKEK**GGGGS**DSGSPFPAAVILRDALHM ARGLKYLHQ**GGGGS**PSKPSFQEFVDWENVSPELNSTDQPFL**GGGGS**REGVELCPGNKYEMR RHGTTHSLVIHD**GGGGS**SHCHWNDLAVIPAGVVHNWDFEPRKVS**GGGGS**GRGHLLGRLAAIV GKQVLLGRKVVVVR**GGGGS**ANFESGKHKYRQTAMFTATMPPAVERL**GGGGS**VVDRNPQFLD PVLAYLMKGLCEKPLAS**GGGGS**SSPDEVALVEGVQSLGFTYLRLKDNYM**GGGGS**EFKHIKAF DRTFANNPGPMVVFATPGM**GGGGS**STANYNTSHLNNDVWQIFENPVDWKEK**GGGGS**DSGS PFPAAVILRDALHMARGLKYLHQ

SEQ ID NO: 44

Amino acid sequence of VB4019 = VB10.NEO B16-Vx2 = B16 pepM3-M4-M7-M9-M10 x 2, 5 aa linker

MQVSTAALAVLLCTMALCNQVLS | APLAADTPTACCFSYTSRQIPQNFIAD YFETSSQCSKPSVIFLTKRGRQVCADPSEEWVQKYVSDLELSA | ELKTPLG DTTHT I EPKSCDTPPPCPRCP | GGGSSGGGSG | GQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKL TVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK | GLGGL | MHGDTPTL HEYMLDLQPETTDLYGYGQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFC CKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP | GGGSSGGGSG | SHCHWNDLAVIPAGVVHNWDFEPRKVS**GGGGS**GRGHLLGRLAAIVGKQVLLGRKVVVVR**GG GGS**SSPDEVALVEGVQSLGFTYLRLKDNYM**GGGGS**STANYNTSHLNNDVWQIFENPVDWKE K**GGGGS**DSGSPFPAAVILRDALHMARGLKYLHQ**GGGGS**SHCHWNDLAVIPAGVVHNWDFEP

RKVS**GGGGS**GRGHLLGRLAAIVGKQVLLGRKVVVVR**GGGGS**SSPDEVALVEGVQSLGFTYLR
LKDNYM**GGGGS**STANYNTSHLNNDVWQIFENPVDWKEK**GGGGS**DSGSPFPAAVILRDALHM
ARGLKYLHQ

SEQ ID NO: 45

Amino acid sequence of VB4021 = VB10.NEO B16-Vx4 = B16 pepM3-M4-M7-M9-M10 x 4, 5 aa linker

MQVSTAALAVLLCTMALCNQVLS | APLAADTPTACCFSYTSRQIPQNFIAD
YFETSSQCSKPSVIFLTKRGRQVCADPSEEWVQKYVSDLELSA | ELKTPLG
DTTHT I EPKSCDTPPPCPRCP | GGGSSGGGSG | GQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK | GLGGL | MHGDTPTL
HEYMLDLQPETTDLYGYGQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFC
CKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP | GGGSSGGGSG |
SHCHWNDLAVIPAGVVHNWDFEPRKVS**GGGGS**GRGHLLGRLAAIVGKQVLLGRKVVVVR**GG**
GGSSSPDEVALVEGVQSLGFTYLRLKDNYM**GGGGS**STANYNTSHLNNDVWQIFENPVDWKE
K**GGGGS**DSGSPFPAAVILRDALHMARGLKYLHQ**GGGGS**SHCHWNDLAVIPAGVVHNWDFEP
RKVS**GGGGS**GRGHLLGRLAAIVGKQVLLGRKVVVVR**GGGGS**SSPDEVALVEGVQSLGFTYLR
LKDNYM**GGGGS**STANYNTSHLNNDVWQIFENPVDWKEK**GGGGS**DSGSPFPAAVILRDALHM
ARGLKYLHQ**GGGGS**SHCHWNDLAVIPAGVVHNWDFEPRKVS**GGGGS**GRGHLLGRLAAIVGK
QVLLGRKVVVVR**GGGGS**SSPDEVALVEGVQSLGFTYLRLKDNYM**GGGGS**STANYNTSHLNN
DVWQIFENPVDWKEK**GGGGS**DSGSPFPAAVILRDALHMARGLKYLHQ

SEQ ID NO: 46

Amino acid sequence of **VB4024 = VB10.NEO B16-XV = B16 pepM1-M15, 10 aa linker**

MQVSTAALAVLLCTMALCNQVLS | APLAADTPTACCFSYTSRQIPQNFIAD
YFETSSQCSKPSVIFLTKRGRQVCADPSEEWVQKYVSDLELSA | ELKTPLG
DTTHT I EPKSCDTPPPCPRCP | GGGSSGGGSG | GQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK | GLGGL | MHGDTPTL
HEYMLDLQPETTDLYGYGQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFC
CKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP | GGGSSGGGSG |
PSKPSFQEFVDWENVSPELNSTDQPFL**GGGGSGGGGS**REGVELCPGNKYEMRRHGTTHSLV
IHD**GGGGSGGGGS**SHCHWNDLAVIPAGVVHNWDFEPRKVS**GGGGSGGGGS**GRGHLLGRLA
AIVGKQVLLGRKVVVVR**GGGGSGGGGS**FRRKAFLHWYTGEAMDEMEFTEAESNM**GGGGSG**
GGGSVVDRNPQFLDPVLAYLMKGLCEKPLAS**GGGGSGGGGS**SSPDEVALVEGVQSLGFTYL
RLKDNYM**GGGGSGGGGS**EFKHIKAFDRTFANNPGPMVVFATPGM**GGGGSGGGGS**STANYN
TSHLNNDVWQIFENPVDWKEK**GGGGSGGGGS**DSGSPFPAAVILRDALHMARGLKYLHQ**GGG**

GSGGGGSANFESGKHKYRQTAMFTATMPPAVERL**GGGGSGGGGS**NHSGLVTFQAFIDVMSR
ETTDTDTADQ**GGGGSGGGGS**CGTAFFINFIAIYHHASRAIPFGTMVA**GGGGSGGGGS**FVVKA
YLPVNESFAFTADLRSNTGGQA**GGGGSGGGGS**TPPPEEAMPFEFNGPAQGDHSQPPLQV

SEQ ID NO: 47

Amino acid sequence of **VB4025 = VB10.NEO B16-XX = B16 pepM1-M20, 10 aa linker**

MQVSTAALAVLLCTMALCNQVLS | APLAADTPTACCFSYTSRQIPQNFIAD
YFETSSQCSKPSVIFLTKRGRQVCADPSEEWVQKYVSDLELSA | ELKTPLG
DTTHT I EPKSCDTPPPCPRCP | GGGSSGGGSG | GQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK | GLGGL | MHGDTPTL
HEYMLDLQPETTDLYGYGQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFC
CKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP | GGGSSGGGSG |
PSKPSFQEFVDWENVSPELNSTDQPFL**GGGGSGGGGS**REGVELCPGNKYEMRRHGTTHSLV
IHD**GGGGSGGGGS**SHCHWNDLAVIPAGVVHNWDFEPRKVS**GGGGSGGGGS**GRGHLLGRLA
AIVGKQVLLGRKVVVVR**GGGGSGGGGS**FRRKAFLHWYTGEAMDEMEFTEAESNM**GGGGSG**
GGGSVVDRNPQFLDPVLAYLMKGLCEKPLAS**GGGGSGGGGS**SSPDEVALVEGVQSLGFTYL
RLKDNYM**GGGGSGGGGS**EFKHIKAFDRTFANNPGPMVVFATPGM**GGGGSGGGGS**STANYN
TSHLNNDVWQIFENPVDWKEK**GGGGSGGGGS**DSGSPFPAAVILRDALHMARGLKYLHQ**GGG**
GSGGGGSANFESGKHKYRQTAMFTATMPPAVERL**GGGGSGGGGS**NHSGLVTFQAFIDVMSR
ETTDTDTADQ**GGGGSGGGGS**CGTAFFINFIAIYHHASRAIPFGTMVA**GGGGSGGGGS**FVVKA
YLPVNESFAFTADLRSNTGGQA**GGGGSGGGGS**TPPPEEAMPFEFNGPAQGDHSQPPLQV**GG**
GGSGGGGSPKPDFSQLQRNILPSNPRVTRFHINWD**GGGGSGGGGS**IPSGTTILNCFHDVLSG
KLSGGSPGVP**GGGGSGGGGS**GFSQPLRRLVLHVVSAAQAERLARAEE**GGGGSGGGGS**ECRI
TSNFVIPSEYWVEEKEEKQKLIQ**GGGGSGGGGS**NIEGIDKLTQLKKPFLVNNKINKIENI

SEQ ID NO: 48

Amino acid sequence of **VB4026 = VB10.NEO CT26-XV = CT26 pepM1-M15, 10 aa linker**

MQVSTAALAVLLCTMALCNQVLS | APLAADTPTACCFSYTSRQIPQNFIAD
YFETSSQCSKPSVIFLTKRGRQVCADPSEEWVQKYVSDLELSA | ELKTPLG
DTTHT I EPKSCDTPPPCPRCP | GGGSSGGGSG | GQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK | GLGGL | MHGDTPTL
HEYMLDLQPETTDLYGYGQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFC
CKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP | GGGSSGGGSG |
VILPQAPSGPSYATYLQPAQAQMLTPP**GGGGSGGGGS**LHSGQNHLKEMAISVLEARACAAAG
QS**GGGGSGGGGS**PLLPFYPPDEALEIGLELNSSALPPTE**GGGGSGGGGS**AGTQCEYWASRA

LDSEHSIGSMIQLPQ**GGGGSGGGGS**AAYKGHHYPGPGNYFWKCLFMSGLSEV**GGGGSGGG**
GSDTLSAMSNPRAMQVLLQIQQGLQTLAT**GGGGSGGGGS**DKPLRRNNSYTSYIMAICGMPLD
SFRA**GGGGSGGGGS**EVIQTSKYYMRDVIAIESAWLLELAPH**GGGGSGGGGS**GYISRVTAGKD
SYIALVDKNIMGYIAS**GGGGSGGGGS**EHIHRAGGLFVADAIQVGFGRIGKHFW**GGGGSGGGG**
SQAIVRGCSMPGPWRSGRLLVSRRWSVE**GGGGSGGGGS**DGQLELLAQGALDNALSSMGAL
HALRP**GGGGSGGGGS**SHDSRKSTSFMSVNPSKEIKIVSAVRR**GGGGSGGGGS**HTPSSYIETL
PKAIKRRINALKQLQVR**GGGGSGGGGS**MKAFIFKYSAKTGFTKLIDASRVSETE

SEQ ID NO: 49

Amino acid sequence of **VB4027 = VB10.NEO CT26-XX = CT26 pepM1-M20, 10 aa linker**

MQVSTAALAVLLCTMALCNQVLS | APLAADTPTACCFSYTSRQIPQNFIAD
YFETSSQCSKPSVIFLTKRGRQVCADPSEEWVQKYVSDLELSA | ELKTPLG
DTTHT I EPKSCDTPPPCPRCP | GGGSSGGGSG | GQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK | GLGGL | MHGDTPTL
HEYMLDLQPETTDLYGYGQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFC
CKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP | GGGSSGGGSG |
VILPQAPSGPSYATYLQPAQAQMLTPP**GGGGSGGGGS**LHSGQNHLKEMAISVLEARACAAAG
QS**GGGGSGGGGS**PLLPFYPPDEALEIGLELNSSALPPTE**GGGGSGGGGS**AGTQCEYWASRA
LDSEHSIGSMIQLPQ**GGGGSGGGGS**AAYKGHHYPGPGNYFWKCLFMSGLSEV**GGGGSGGG**
GSDTLSAMSNPRAMQVLLQIQQGLQTLAT**GGGGSGGGGS**DKPLRRNNSYTSYIMAICGMPLD
SFRA**GGGGSGGGGS**EVIQTSKYYMRDVIAIESAWLLELAPH**GGGGSGGGGS**GYISRVTAGKD
SYIALVDKNIMGYIAS**GGGGSGGGGS**EHIHRAGGLFVADAIQVGFGRIGKHFW**GGGGSGGGG**
SQAIVRGCSMPGPWRSGRLLVSRRWSVE**GGGGSGGGGS**DGQLELLAQGALDNALSSMGAL
HALRP**GGGGSGGGGS**SHDSRKSTSFMSVNPSKEIKIVSAVRR**GGGGSGGGGS**HTPSSYIETL
PKAIKRRINALKQLQVR**GGGGSGGGGS**MKAFIFKYSAKTGFTKLIDASRVSETE**GGGGSGGGG**
SEGDPCLRSSDCIDEFCCARHFWTKICK**GGGGSGGGGS**WKGGPVKIDPLALMQAIERYLVVR
GY**GGGGSGGGGS**VTSIPSVSNALNWKEFSFIQSTLGYVA**GGGGSGGGGS**YRGANLHLEET
LAGFWARLLERLFKQL**GGGGSGGGGS**KTTLSHTQDSSQSLQSSSDSSKSSRCS

SEQ ID NO: 50

CT26 mutated epitope, CT26-PepM11, amino acid sequence
QAIVRGCSMPGPWRSGRLLVSRRWSVE

SEQ ID NO: 51

CT26 mutated epitope, CT26-PepM12, amino acid sequence
DGQLELLAQGALDNALSSMGALHALRP

SEQ ID NO: 52

CT26 mutated epitope, CT26-PepM13, amino acid sequence

SHDSRKSTSFMSVNPSKEIKIVSAVRR

SEQ ID NO: 53

CT26 mutated epitope, CT26-PepM14, amino acid sequence

HTPSSYIETLPKAIKRRINALKQLQVR

SEQ ID NO: 54

CT26 mutated epitope, CT26-PepM15, amino acid sequence

MKAFIFKYSAKTGFTKLIDASRVSETE

SEQ ID NO: 55

CT26 mutated epitope, CT26-PepM16, amino acid sequence

EGDPCLRSSDCIDEFCCARHFWTKICK

SEQ ID NO: 56

CT26 mutated epitope, CT26-PepM17, amino acid sequence

WKGGPVKIDPLALMQAIERYLVVRGYG

SEQ ID NO: 57

CT26 mutated epitope, CT26-PepM18, amino acid sequence

VTSIPSVSNALNWKEFSFIQSTLGYVA

SEQ ID NO: 58

CT26 mutated epitope, CT26-PepM19, amino acid sequence

YRGANLHLEETLAGFWARLLERLFKQL

SEQ ID NO: 59

CT26 mutated epitope, CT26-PepM20, amino acid sequence

KTTLSHTQDSSQSLQSSSDSSKSSRCS

SEQ ID NO: 60

B16-F10 mutated epitope, B16-PepM12, amino acid sequence

NHSGLVTFQAFIDVMSRETTDTDTADQ

SEQ ID NO: 61

B16-F10 mutated epitope, B16-PepM13, amino acid sequence

CGTAFFINFIAIYHHASRAIPFGTMVA

SEQ ID NO: 62

B16-F10 mutated epitope, B16-PepM14, amino acid sequence

FVVKAYLPVNESFAFTADLRSNTGGQA

SEQ ID NO: 63

B16-F10 mutated epitope, B16-PepM15, amino acid sequence

TPPPEEAMPFEFNGPAQGDHSQPPLQV

SEQ ID NO: 64

B16-F10 mutated epitope, B16-PepM16, amino acid sequence

PKPDFSQLQRNILPSNPRVTRFHINWD

SEQ ID NO: 65

B16-F10 mutated epitope, B16-PepM17, amino acid sequence

IPSGTTILNCFHDVLSGKLSGGSPGVP

SEQ ID NO: 66

B16-F10 mutated epitope, B16-PepM18, amino acid sequence

GFSQPLRRLVLHVVSAAQAERLARAEE

SEQ ID NO: 67

B16-F10 mutated epitope, B16-PepM19, amino acid sequence

ECRITSNFVIPSEYWVEEKEEKQKLIQ

SEQ ID NO: 68

B16-F10 mutated epitope, B16-PepM20, amino acid sequence

NIEGIDKLTQLKKPFLVNNKINKIENI

SEQ ID NO: 69. Linker: GGGSS

SEQ ID NO: 70. Linker: GGGSG

SEQ ID NO: 71. Linker: GGGGS

SEQ ID NO: 72. Linker: LGGGS

SEQ ID NO: 73. Linker: GLGGS

SEQ ID NO: 74. Linker: GGLGS

SEQ ID NO: 75. Linker: GGGLS

SEQ ID NO: 76. Linker: GGGGL

SEQ ID NO: 77. Linker: LGGSG

SEQ ID NO: 78. Linker: GLGSG
SEQ ID NO: 79. Linker: GGLSG
SEQ ID NO: 80. Linker: GGGLG
SEQ ID NO: 81. Linker: GGGSL
SEQ ID NO: 82. Linker: LGGSS
SEQ ID NO: 83. Linker: GLGSS
SEQ ID NO: 84. Linker: GGLSS
SEQ ID NO: 85. Linker: GGGLS
SEQ ID NO: 86. Linker: GGGSL
SEQ ID NO: 87. Linker: LGLGS
SEQ ID NO: 88. Linker: GLGLS
SEQ ID NO: 89. Linker: GLLGS
SEQ ID NO: 90. Linker: LGGLS
SEQ ID NO: 91. Linker: GLGGL
SEQ ID NO: 92. Linker: LGLSG
SEQ ID NO: 93. Linker: GLLSG
SEQ ID NO: 94. Linker: GGLSL
SEQ ID NO: 95. Linker: GGLLG
SEQ ID NO: 96. Linker: GLGSL
SEQ ID NO: 97. Linker: LGLSS
SEQ ID NO: 98. Linker: GLGLS
SEQ ID NO: 99. Linker: GGLLS
SEQ ID NO: 100. Linker: GLGSL
SEQ ID NO: 101. Linker: GLGSL
SEQ ID NO: 102. Linker: LGGGSGGGGS
SEQ ID NO: 103. Linker: GLGGSGGGGS
SEQ ID NO: 104. Linker: GGLGSGGGGS
SEQ ID NO: 105. Linker: GGGLSGGGGS
SEQ ID NO: 106. Linker: GGGGLGGGGS
SEQ ID NO: 107. Linker: LGGSGGGGSG
SEQ ID NO: 108. Linker: GLGSGGGGSG
SEQ ID NO: 109. Linker: GGLSGGGGSG
SEQ ID NO: 110. Linker: GGGLGGGGSG
SEQ ID NO: 111. Linker: GGGSLGGGSG
SEQ ID NO: 112. Linker: GGGSLGGGSG
SEQ ID NO: 113. Linker: GLGSSGGGSS
SEQ ID NO: 114. Linker: GGLSSGGGSS
SEQ ID NO: 115. Linker: GGGLSGGGSS
SEQ ID NO: 116. Linker: GGGSLGGGSS

SEQ ID NO: 117. Linker: LGGGSLGGGS
SEQ ID NO: 118. Linker: GLGGSGLGGS
SEQ ID NO: 119. Linker: GGLGSGGLGS
SEQ ID NO: 120. Linker: GGGLSGGGLS
SEQ ID NO: 121. Linker: GGGGLGGGGL
SEQ ID NO: 122. Linker: LGGSGLGGSG
SEQ ID NO: 123. Linker: GLGSGGLGSG
SEQ ID NO: 124. Linker: GGLSGGGLSG
SEQ ID NO: 125. Linker: GGGLGGGGLG
SEQ ID NO: 126. Linker: GGGSLGGGSL
SEQ ID NO: 127. Linker: LGGSSLGGSS
SEQ ID NO: 128. Linker: GLGSSGLGSS
SEQ ID NO: 129. Linker: GGLSSGGLSS
SEQ ID NO: 130. Linker: GGGLSGGGLS
SEQ ID NO: 131. Linker: GGGSLGGGSL

The term “comprise” and variants of the term such as “comprises” or “comprising” are used herein to denote the inclusion of a stated integer or stated integers but not to exclude any other integer or any other integers, unless in the context or usage an exclusive interpretation of the term is required.

The reference to any prior art in this specification is not, and should not be taken as an acknowledgement or any form of suggestion that the referenced prior art forms part of the common general knowledge in Australia.

2017205270 06 Dec 2023

The claims defining the invention are as follows:

1. A therapeutic anticancer neoepitope vaccine comprising an immunologically effective amount of

   1) a polynucleotide comprising a nucleotide sequence encoding
      - a targeting unit
      - a dimerization unit
      - a first linker and
      - an antigenic unit, wherein said antigenic unit comprises n cancer neoepitope sequences and n-1 antigenic subunits, each subunit comprising a cancer neoepitope sequence and a second linker and said antigenic unit further comprising a final cancer neoepitope sequence, wherein n is an integer of from 3 to 50, wherein the antigenic unit and the dimerization unit are connected through said first linker

   or

   2) a polypeptide encoded by the polynucleotide as defined in 1), or
   3) a dimeric protein consisting of two polypeptides encoded by the polynucleotide as defined in 1).

2. The vaccine according to claim 1, wherein the antigenic unit comprises one copy of each cancer neoepitope sequence or wherein the antigenic unit comprises at least two copies of at least one cancer neoepitope sequence.

3. The vaccine according to any of the preceding claims, wherein the cancer neoepitope sequence has a length of from 7 to 30 amino acids.

4. The vaccine according to any of the preceding claims, wherein each cancer neoepitope sequence has identical length and/or wherein the cancer neoepitope is positioned essentially in the middle of the cancer neoepitope sequence.

5. The vaccine according to any of the preceding claims, wherein the cancer neoepitope sequence is a subsequence of a cancer neoantigen.

6. The vaccine according to any of the preceding claims, wherein the antigenic subunits are in the order of more antigenic to less antigenic from the first linker.

7. The vaccine according to any of the preceding claims, wherein the antigenic subunit(s) comprising the most hydrophobic cancer neoepitope sequences is/are substantially the

middle of the antigenic unit and the antigenic subunit(s) comprising the most hydrophilic cancer neoepitope sequences is/are at the ends of the antigenic unit.

8. The vaccine according to any of the preceding claims, wherein the second linker is a flexible linker.

9. The vaccine according to any of the preceding claims, wherein the second linker is identical in all antigenic subunits.

10. The vaccine according to any of the preceding claims, wherein the second linker is a Serine-Glycine linker.

11. The vaccine according to any of the preceding claims, wherein the dimerization unit comprises an Ig-derived hinge region and a C_H3 domain.

12. The vaccine according to any of the preceding claims, wherein the dimerization unit consists of hinge exons h1 and h4 connected through a third linker to a C_H3 domain of human IgG3.

13. The vaccine according to any of the preceding claims, wherein the dimerization unit comprises an amino acid sequence having at least 80% sequence identity to the amino acid sequences of SEQ ID NOs: 26-29, preferably having 100% sequence identity to the amino acid sequences of SEQ ID NOs: 26-29.

14. The vaccine according to any of the preceding claims, wherein the dimerization unit consists of the amino acid sequences of SEQ ID NOs: 26-29.

15. The vaccine according to any of the preceding claims, wherein the targeting unit targets surface molecules on antigen presenting cells.

16. The vaccine according to claim 15, wherein the targeting unit has affinity for a chemokine receptor selected from CCR1, CCR3 and CCR5.

17. The vaccine according to claim 16, wherein the targeting unit encodes hMIP-1alpha.

18. The vaccine according to claim 17, wherein the targeting unit comprises an amino acid sequence having at least 80% sequence identity to the amino acid sequence 24-93 SEQ ID NO:1, preferably having 100% sequence identity to the amino acid sequence 24-93 of SEQ ID NO:1.

19. The vaccine according to any of claims 17 or 18, wherein the targeting unit consists of the amino acid sequence 24-93 of SEQ ID NO: 1.

20. The vaccine according to any of the preceding claims, wherein said nucleotide sequence further encodes a signal peptide.

21. The vaccine according to claim 20, wherein said signal peptide comprises an amino acid sequence having at least 80% sequence identity to the amino acid sequence 1-23 of SEQ ID NO:1, preferably having 100% sequence identity to the amino acid sequence 1-23 of SEQ ID NO:1.

22. The vaccine according to any of claims 20 or 21, wherein the signal peptide consist of the amino acid sequence 1-23 of SEQ ID NO:1.

23. The vaccine according to any of the preceding claims, 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.

24. The vaccine according to any of the preceding claims, wherein said polynucleotide sequence is a DNA nucleotide sequence or an RNA nucleotide sequence, preferably a DNA nucleotide sequence.

25. The vaccine according to any of the preceding claims, wherein the vaccine comprises a DNA polynucleotide comprising the nucleotide sequence of SEQ ID NO: 2.

26. The vaccine according to any of the preceding claims, wherein the vaccine comprises a polypeptide comprising the amino acid sequence 24-93 of SEQ ID NO: 1, SEQ ID NOs: 26-29 and SEQ ID NO: 25.

27. The vaccine according to any of the preceding claims, wherein n is an integer of from 10 to 20.

28. The vaccine according to any of claims 1 to 25 or 27, wherein the vaccine comprises a DNA polynucleotide comprised in a vector.

29. The vaccine according to any of the preceding claims, further comprising a pharmaceutically acceptable carrier and/or adjuvant.

30. A vector comprising the nucleotide sequence as defined in any of claims 1 to 28.

31. Use of a polynucleotide comprising a nucleotide sequence as defined in any of claims 1 to 28 or use of the vector as defined in claim 30 in the manufacture of a vaccine for treatment of cancer.

32. A method for treating cancer in a patient, the method comprising administering to the patient in need thereof the vaccine as defined in any of claims 1 to 29 or the vector as defined in claim 30.

33. The use according to claim 31 or the method according to claim 32, wherein said cancer results in a tumor.

34. The use or the method according to any one of claims 31 to 33, wherein said cancer has a high mutational load.

35. The use or the method according to any of claims 31 to 34, wherein the cancer is melanoma, lung cancer, breast cancer, prostate cancer or colon cancer.

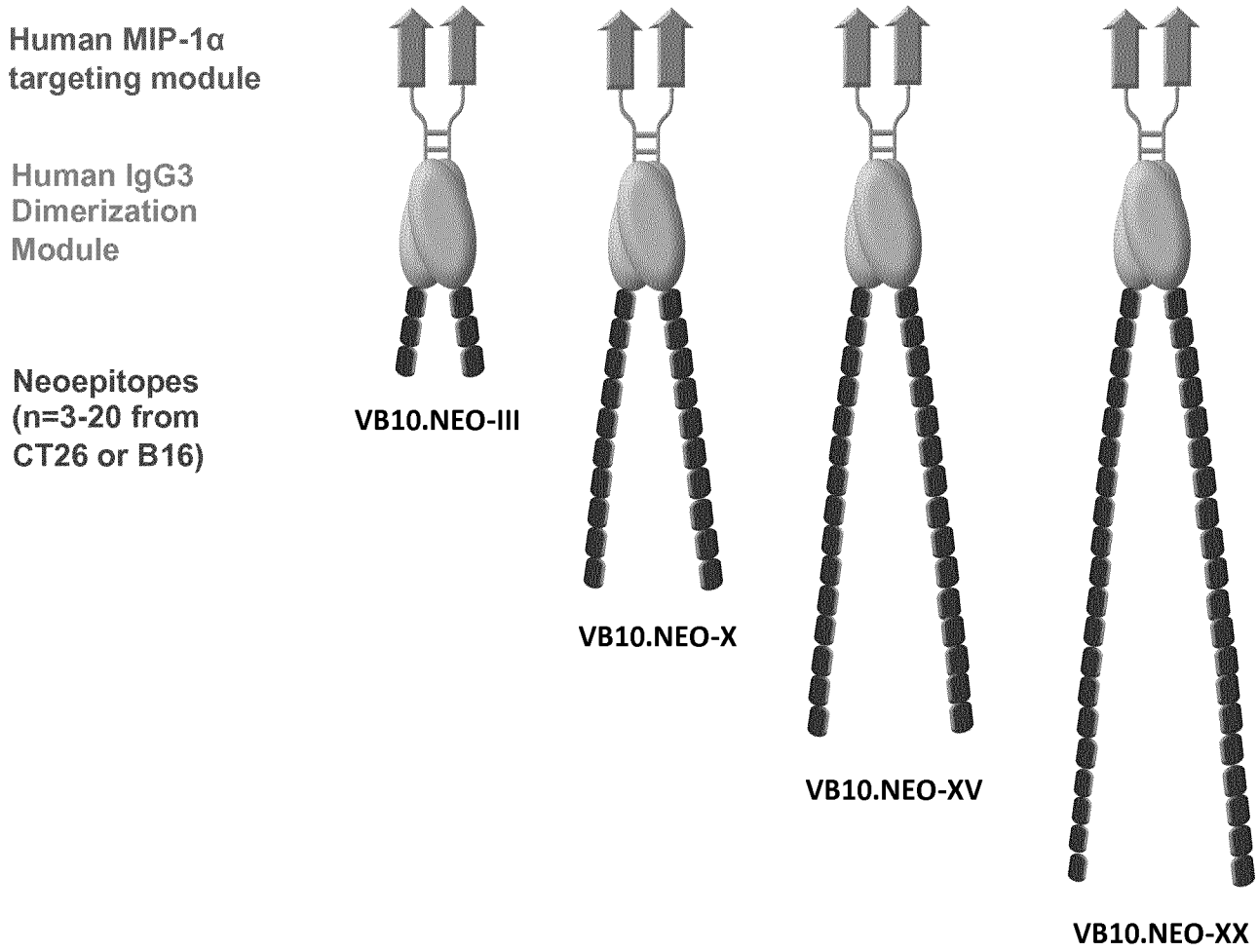
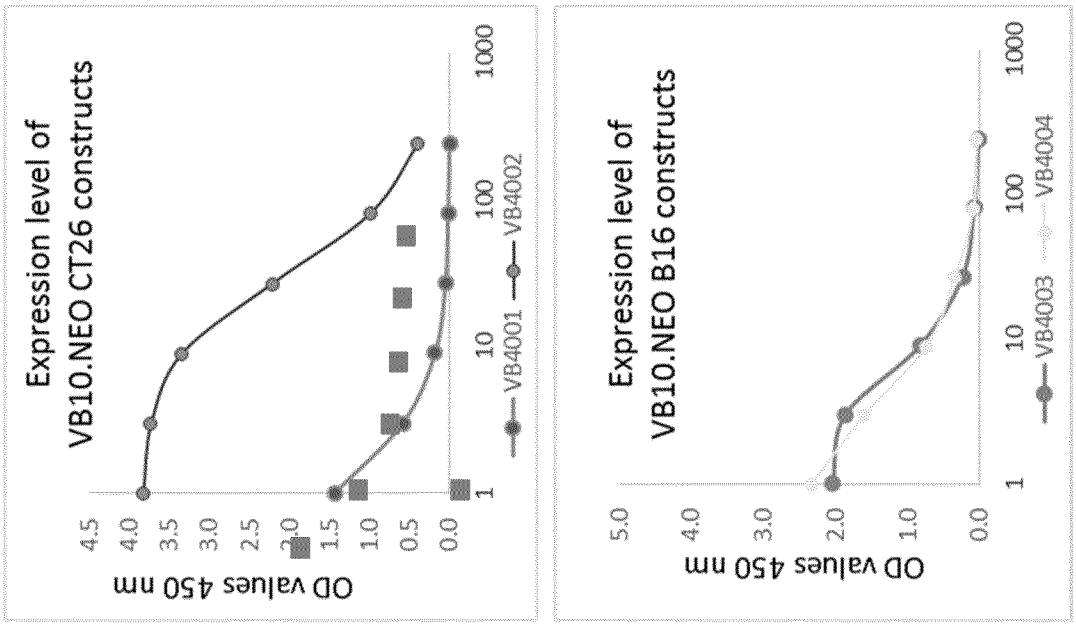

Human MIP-1α targeting module
Human IgG3 Dimerization Module
Neoepitopes (n=3-20 from CT26 or B16)
VB10.NEO-III
VB10.NEO-X
VB10.NEO-XV
VB10.NEO-XX

Figure 1


Expression level of
VB10.NEO CT26 constructs
OD values 450 nm
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1
10
100
1000
VB4001
VB4002
Expression level of
VB10.NEO B16 constructs
OD values 450 nm
5.0
4.0
3.0
2.0
1.0
0.0
1
10
100
1000
VB4003
VB4004

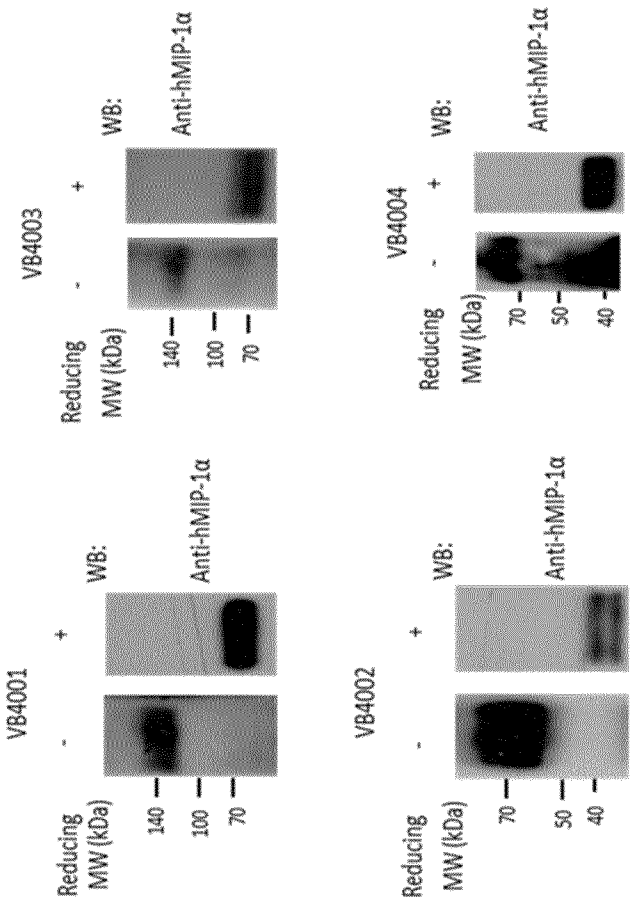

VB4001
VB4002
VB4003
VB4004
WB:
Anti-hMIP-1α
Reducing
MW (kDa)
140
100
70
50
40

Figure 2

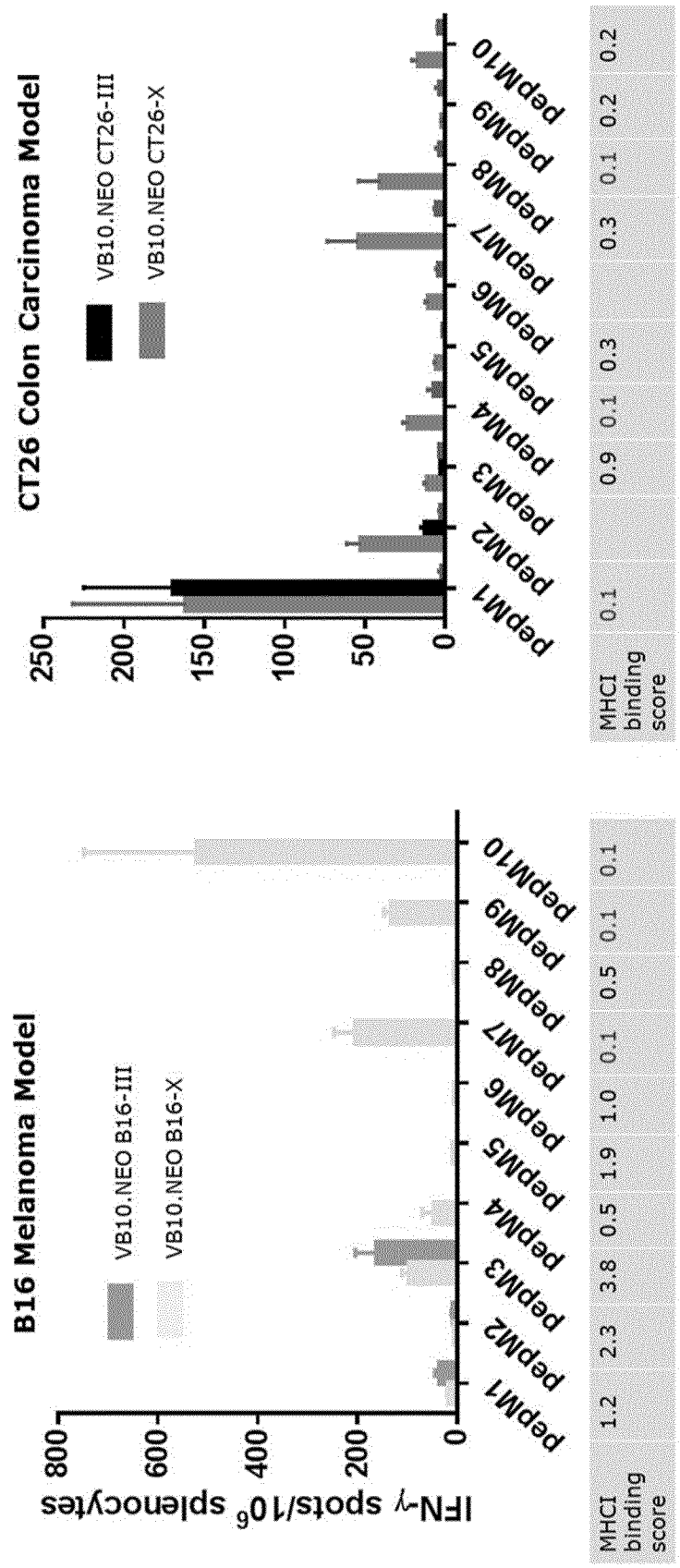

B16 Melanoma Model
IFN-γ spots/10^6 splenocytes
VB10.NEO B16-III
VB10.NEO B16-X
0
200
400
600
800
pepM1
pepM2
pepM3
pepM4
pepM5
pepM6
pepM7
pepM8
pepM9
pepM10
MHCI binding score
1.2
2.3
3.8
0.5
1.9
1.0
0.1
0.5
0.1
0.1
CT26 Colon Carcinoma Model
VB10.NEO CT26-III
VB10.NEO CT26-X
0
50
100
150
200
250
pepM1
pepM2
pepM3
pepM4
pepM5
pepM6
pepM7
pepM8
pepM9
pepM10
MHCI binding score
0.1
0.9
0.1
0.3
0.3
0.1
0.2
0.2

Figure 3

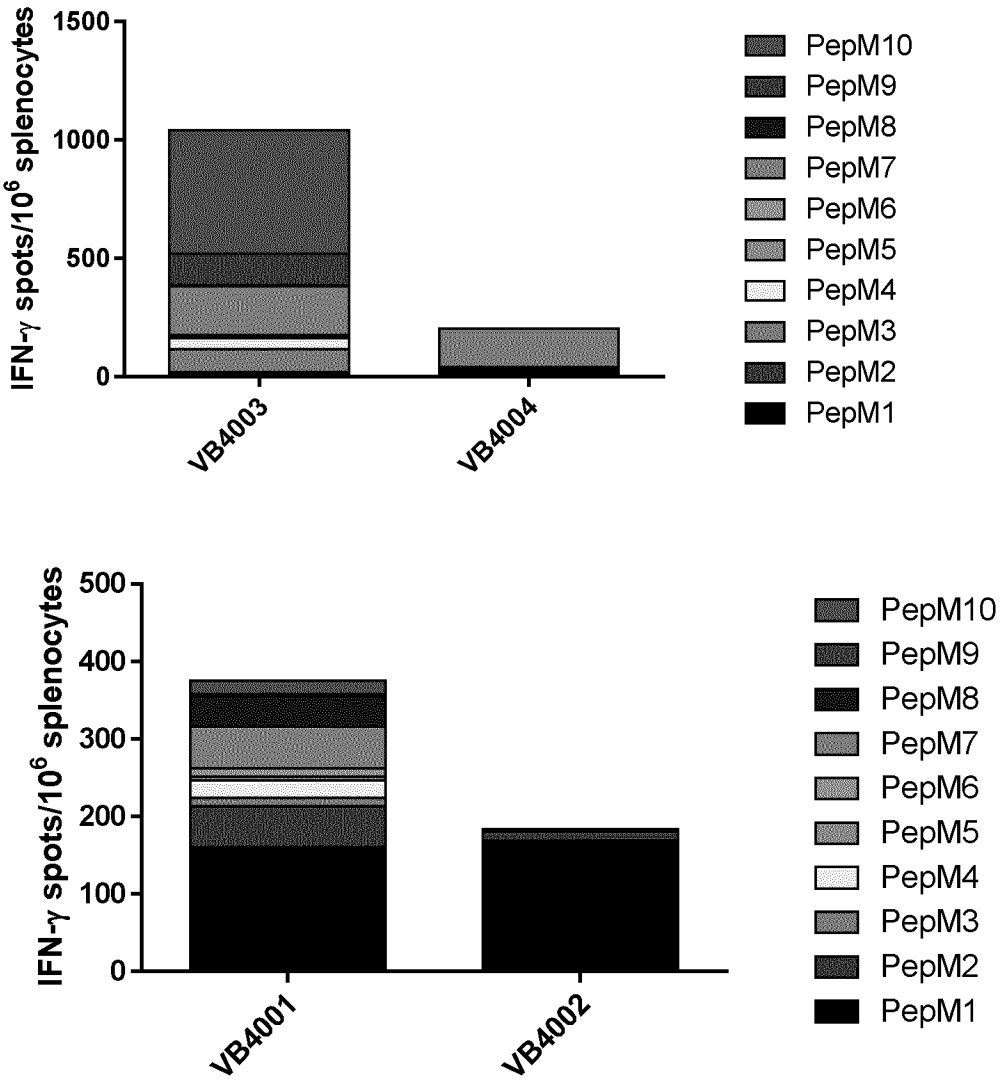
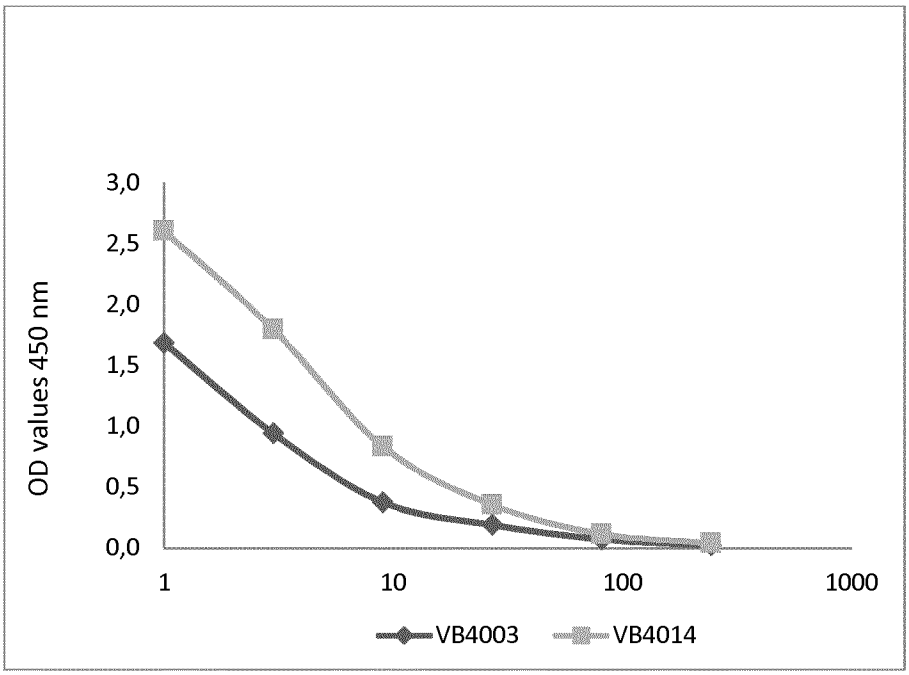

IFN-γ spots/10^6 splenocytes
1500
1000
500
0
VB4003
VB4004
PepM10
PepM9
PepM8
PepM7
PepM6
PepM5
PepM4
PepM3
PepM2
PepM1
IFN-γ spots/10^6 splenocytes
500
400
300
200
100
0
VB4001
VB4002
PepM10
PepM9
PepM8
PepM7
PepM6
PepM5
PepM4
PepM3
PepM2
PepM1

Figure 4


OD values 450 nm
3,0
2,5
2,0
1,5
1,0
0,5
0,0
1
10
100
1000
VB4003
VB4014

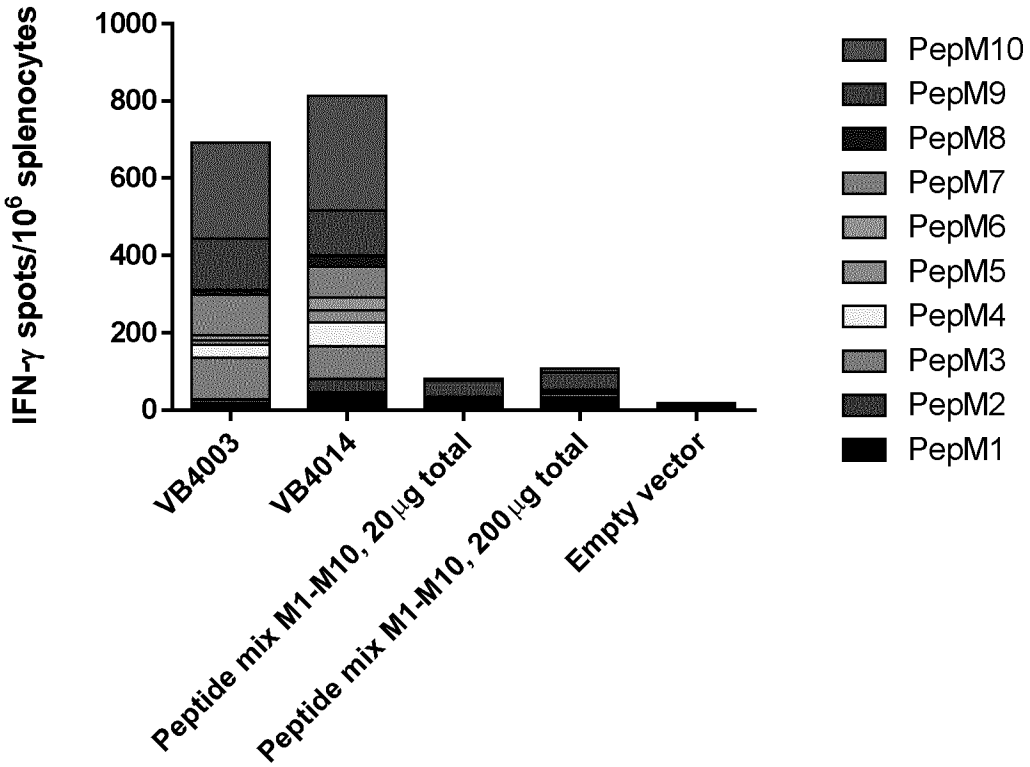
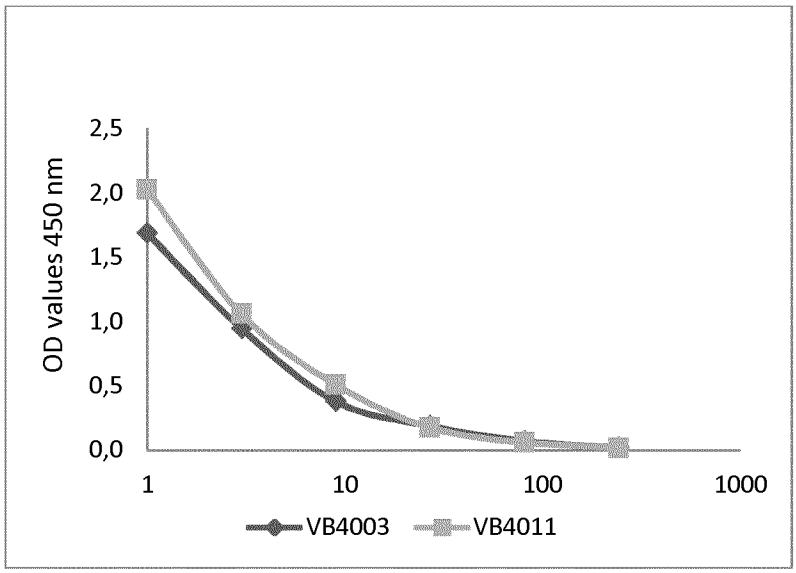

IFN-γ spots/10^6 splenocytes
1000
800
600
400
200
0
VB4003
VB4014
Peptide mix M1-M10, 20 µg total
Peptide mix M1-M10, 200 µg total
Empty vector
PepM10
PepM9
PepM8
PepM7
PepM6
PepM5
PepM4
PepM3
PepM2
PepM1

Figure 5


OD values 450 nm
2,5
2,0
1,5
1,0
0,5
0,0
1
10
100
1000
VB4003
VB4011

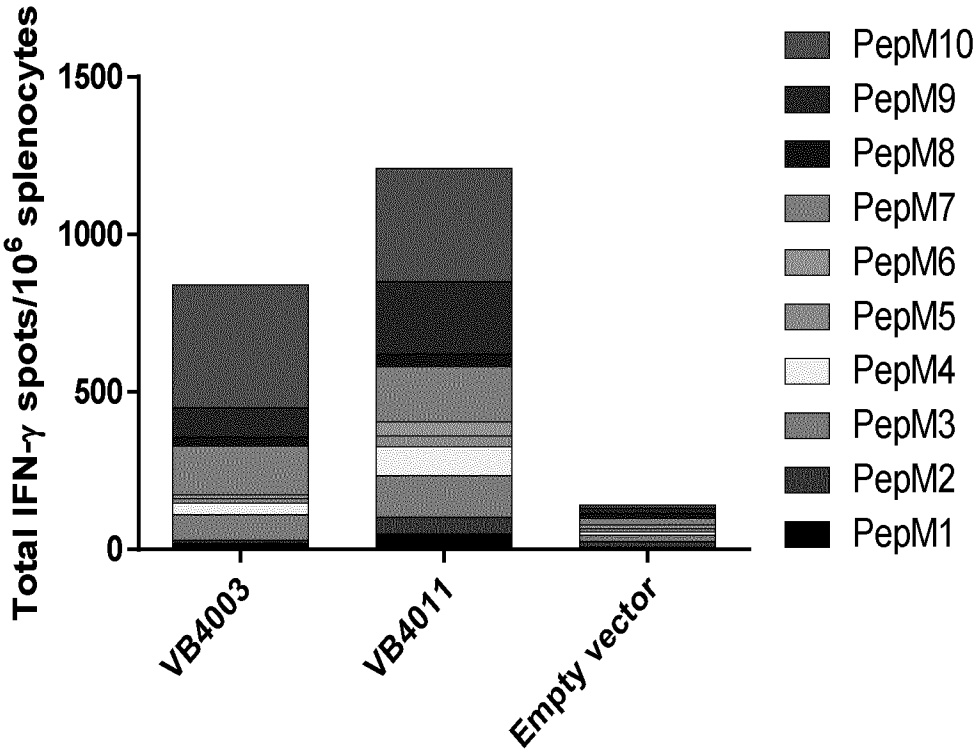
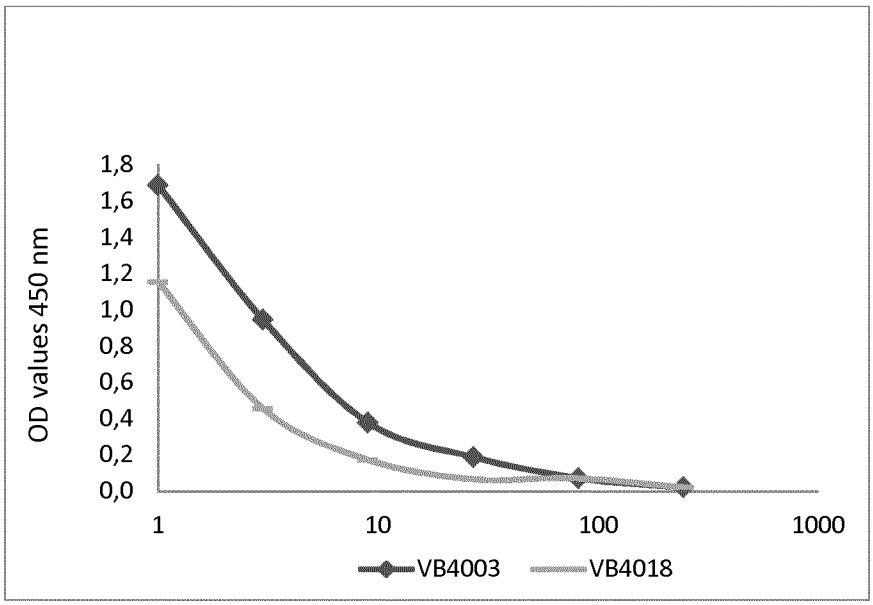

Total IFN-γ spots/10^6 splenocytes
1500
1000
500
0
VB4003
VB4011
Empty vector
PepM10
PepM9
PepM8
PepM7
PepM6
PepM5
PepM4
PepM3
PepM2
PepM1

Figure 6


OD values 450 nm
1,8
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
1
10
100
1000
VB4003
VB4018

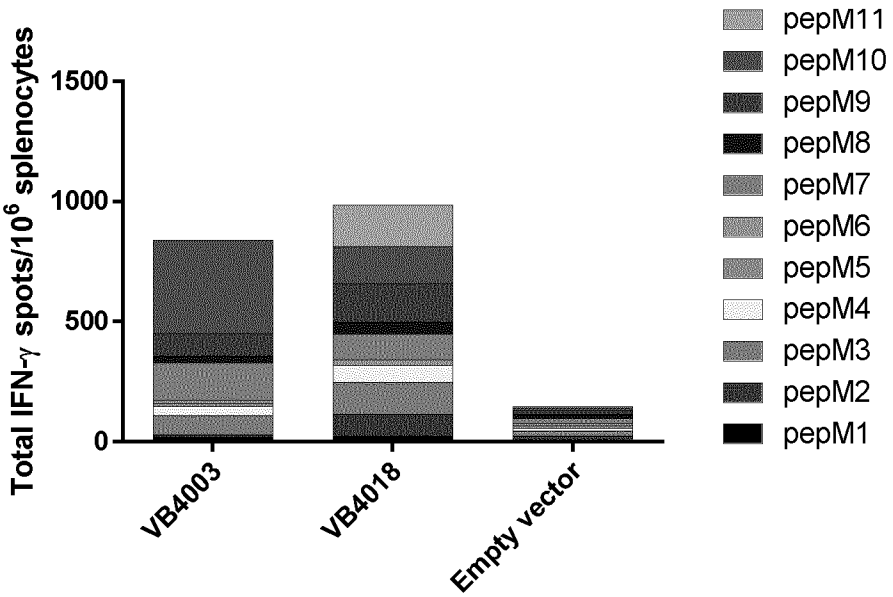
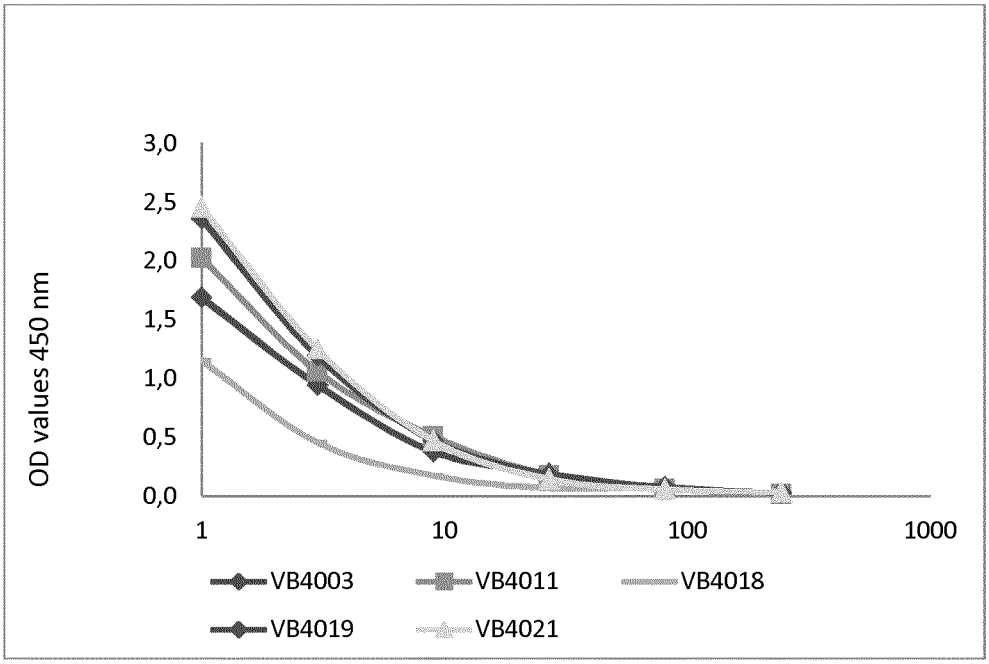

Total IFN-γ spots/10^6 splenocytes
1500
1000
500
0
VB4003
VB4018
Empty vector
pepM11
pepM10
pepM9
pepM8
pepM7
pepM6
pepM5
pepM4
pepM3
pepM2
pepM1

Figure 7


OD values 450 nm
3,0
2,5
2,0
1,5
1,0
0,5
0,0
1
10
100
1000
VB4003
VB4011
VB4018
VB4019
VB4021

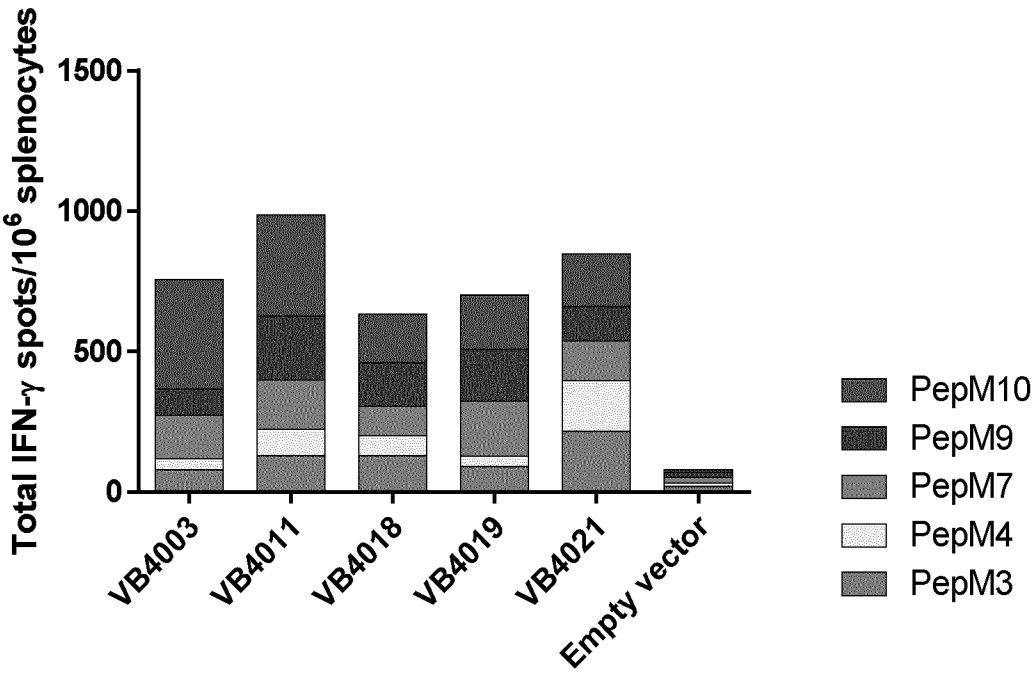

Total IFN-γ spots/10^6 splenocytes
1500
1000
500
0
VB4003
VB4011
VB4018
VB4019
VB4021
Empty vector
PepM10
PepM9
PepM7
PepM4
PepM3

Figure 8

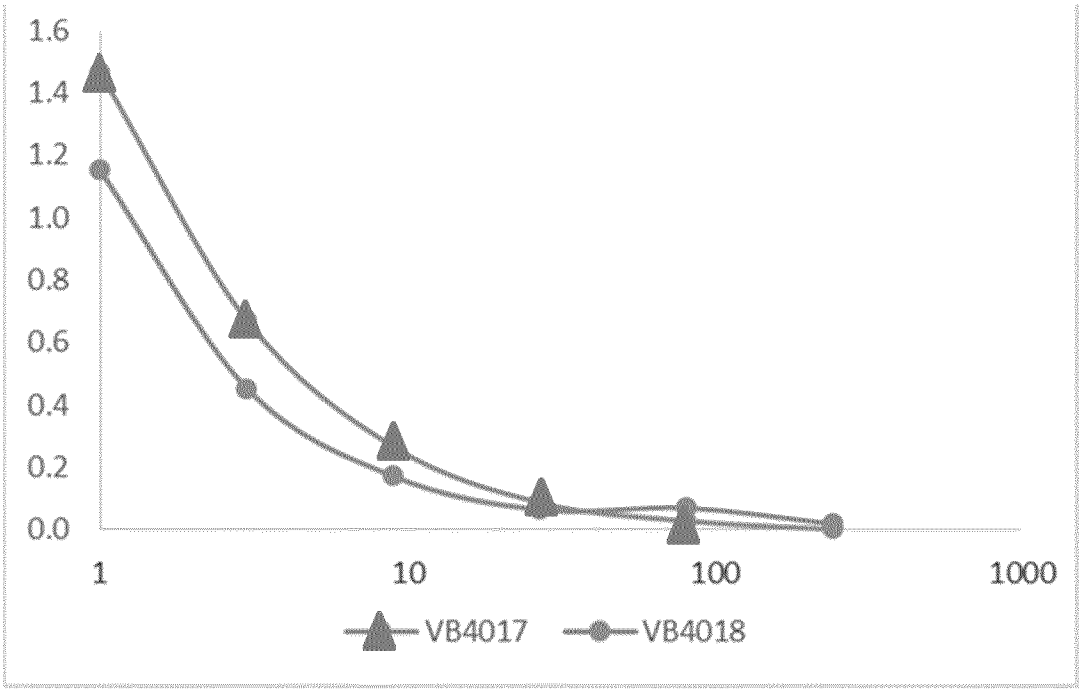
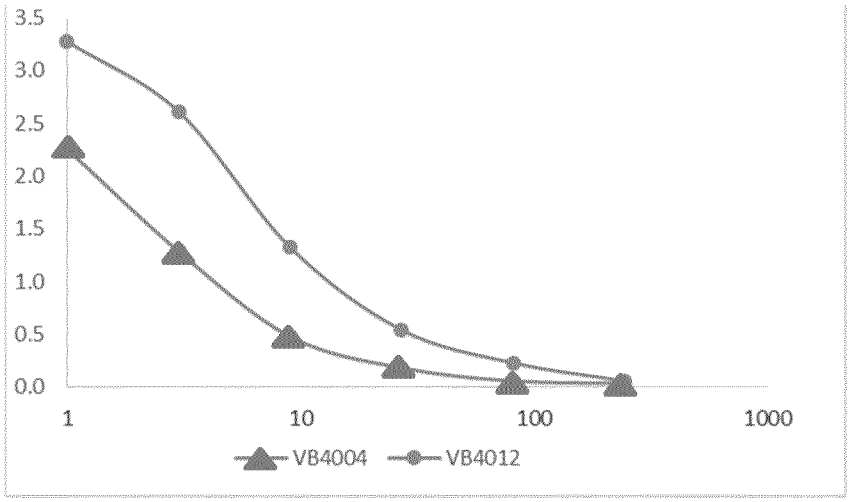


Figure 9


3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1
10
100
1000
VB4004
VB4012

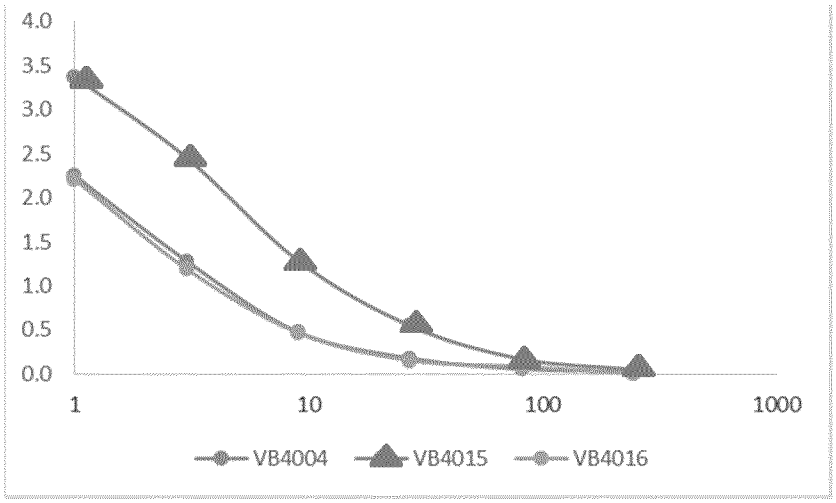
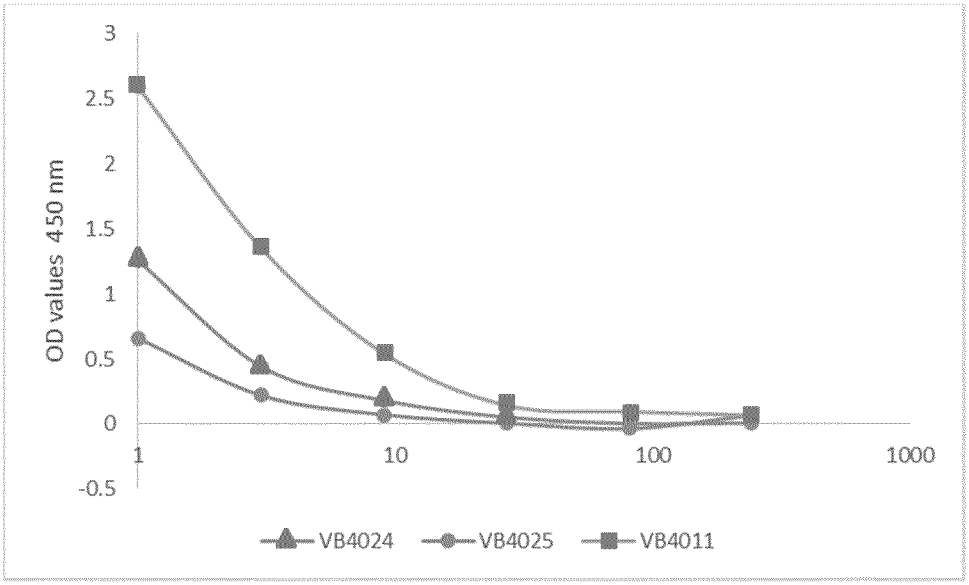

4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1
10
100
1000
VB4004
VB4015
VB4016

Figure 10


OD values 450 nm
3
2.5
2
1.5
1
0.5
0
-0.5
1
10
100
1000
VB4024
VB4025
VB4011

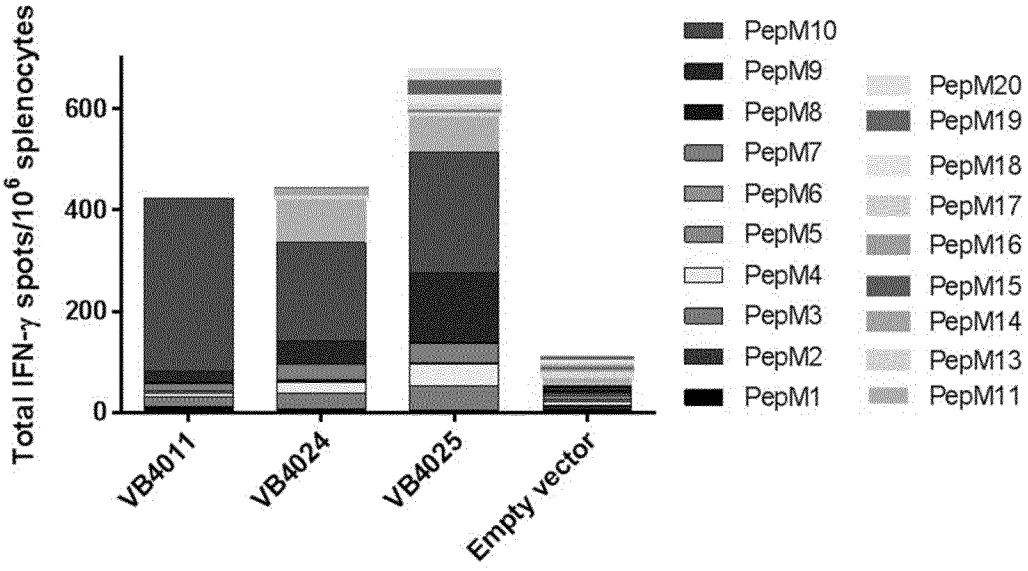
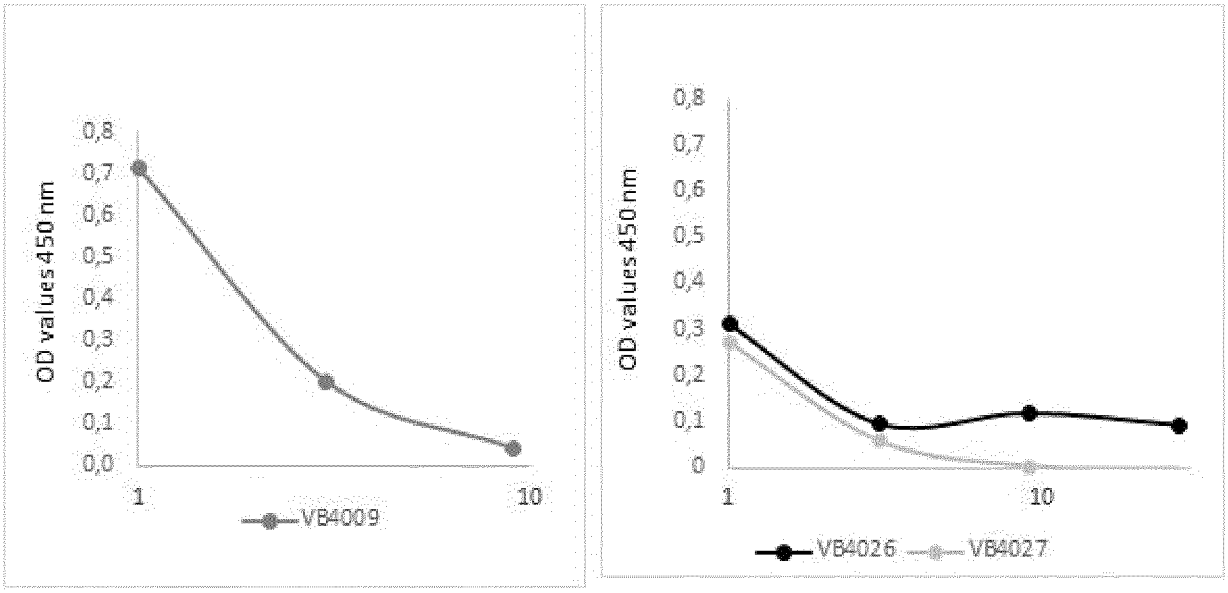

Total IFN-γ spots/10^6 splenocytes
600
400
200
0
VB4011
VB4024
VB4025
Empty vector
PepM10
PepM9
PepM8
PepM7
PepM6
PepM5
PepM4
PepM3
PepM2
PepM1
PepM20
PepM19
PepM18
PepM17
PepM16
PepM15
PepM14
PepM13
PepM11

Figure 11


OD values 450 nm
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0,0
1
10
VB4009
OD values 450 nm
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
1
10
VB4026
VB4027

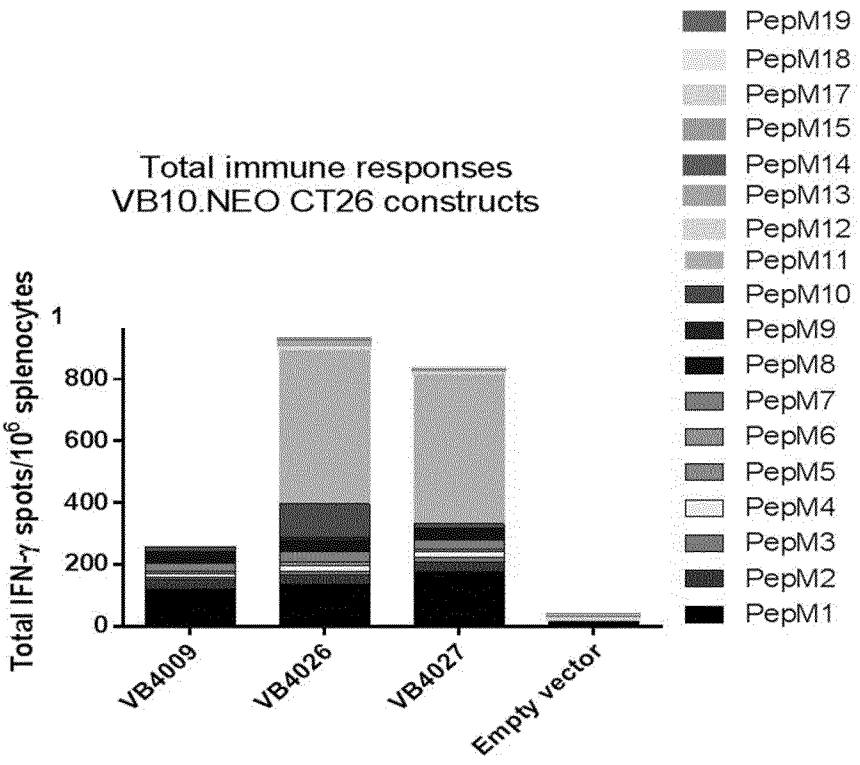
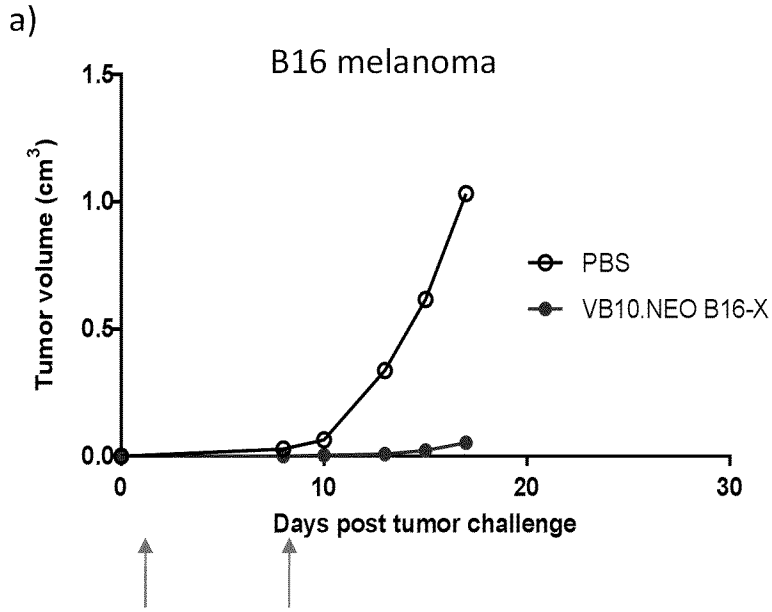

Total immune responses
VB10.NEO CT26 constructs
Total IFN-γ spots/10^6 splenocytes
1
800
600
400
200
0
VB4009
VB4026
VB4027
Empty vector
PepM19
PepM18
PepM17
PepM15
PepM14
PepM13
PepM12
PepM11
PepM10
PepM9
PepM8
PepM7
PepM6
PepM5
PepM4
PepM3
PepM2
PepM1

Figure 12


a)
B16 melanoma
Tumor volume (cm3)
1.5
1.0
0.5
0.0
0
10
20
30
Days post tumor challenge
PBS
VB10.NEO B16-X
Vaccination
VB10.NEO or
PBS

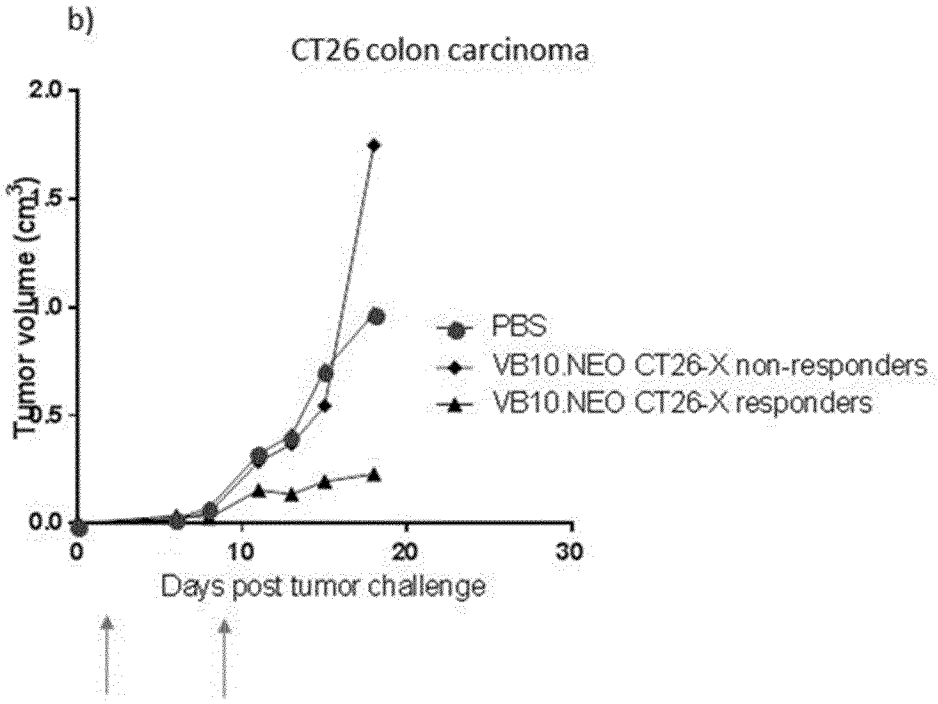

b)
CT26 colon carcinoma
Tumor volume (cm3)
2.0
1.5
1.0
0.5
0.0
0
10
20
30
Days post tumor challenge
PBS
VB10.NEO CT26-X non-responders
VB10.NEO CT26-X responders

Figure 13

eolf-seql
SEQUENCE LISTING

<110> Vaccibody AS

<120> Therapeutic anticancer neoepitope vaccine

<130> P4068PC00

<160> 131

<170> PatentIn version 3.5

<210> 1
<211> 93
<212> PRT
<213> Homo sapiens

<400> 1

Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala
1 5 10 15

Leu Cys Asn Gln Val Leu Ser Ala Pro Leu Ala Ala Asp Thr Pro Thr
20 25 30

Ala Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile
35 40 45

Ala Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Ser Val Ile
50 55 60

Phe Leu Thr Lys Arg Gly Arg Gln Val Cys Ala Asp Pro Ser Glu Glu
65 70 75 80

Trp Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala
85 90

<210> 2
<211> 726
<212> DNA
<213> Artificial Sequence

<220>
<223> Vector

<400> 2
atgcaggtct ccactgctgc ccttgccgtc ctcctctgca ccatggctct ctgcaaccag 60

gtcctctctg caccacttgc tgctgacacg ccgaccgcct gctgcttcag ctacacctcc 120

cgacagattc cacagaattt catagctgac tactttgaga cgagcagcca gtgctccaag 180

cccagtgtca tcttcctaac caagagaggc cggcaggtct gtgctgaccc cagtgaggag 240

tgggtccaga aatacgtcag tgacctggag ctgagtgccg agctcaaaac cccacttggt 300

gacacaactc acacagagcc caaatcttgt gacacacctc ccccgtgccc aaggtgccca 360

ggcggtggaa gcagcggagg tggaagtgga ggacagcccc gagaaccaca ggtgtacacc 420

ctgcccccat cccgggagga gatgaccaag aaccaggtca gcctgacctg cctggtcaaa 480

ggcttctacc ccagcgacat cgccgtggag tgggagagca gcgggcagcc ggagaacaac 540

tacaacacca cgcctcccat gctggactcc gacggctcct tcttcctcta cagcaagctc 600

accgtggaca agagcaggtg gcagcagggg aacatcttct catgctccgt gatgcatgag 660

gctctgcaca accgcttcac gcagaagagc ctctccctgt ctccgggtaa aggcctcggt 720

ggcctg 726

<210> 3
<211> 243
<212> PRT
<213> Artificial Sequence

<220>
<223> Homodimeric construct

<400> 3

Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala
1 5 10 15

Leu Cys Asn Gln Val Leu Ser Ala Pro Leu Ala Ala Asp Thr Pro Thr
20 25 30

Ala Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile
35 40 45

Ala Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Ser Val Ile
50 55 60

Phe Leu Thr Lys Arg Gly Arg Gln Val Cys Ala Asp Pro Ser Glu Glu
65 70 75 80

Trp Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala Glu Leu Lys
85 90 95

Thr Pro Leu Gly Asp Thr Thr His Thr Ile Glu Pro Lys Ser Cys Asp
100 105 110

Thr Pro Pro Pro Cys Pro Arg Cys Pro Gly Gly Gly Ser Ser Gly Gly
115 120 125

Gly Ser Gly Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
130 135 140

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
145 150 155 160

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly
165 170 175

Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp
180 185 190

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
195 200 205

Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His
210 215 220

Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Gly Leu
225 230 235 240

Gly Gly Leu

<210> 4
<211> 27
<212> PRT
<213> Mus musculus

<400> 4

Pro Ser Lys Pro Ser Phe Gln Glu Phe Val Asp Trp Glu Asn Val Ser
1 5 10 15

Pro Glu Leu Asn Ser Thr Asp Gln Pro Phe Leu
20 25

<210> 5
<211> 27
<212> PRT
<213> Mus musculus

<400> 5

Arg Glu Gly Val Glu Leu Cys Pro Gly Asn Lys Tyr Glu Met Arg Arg
1 5 10 15

His Gly Thr Thr His Ser Leu Val Ile His Asp
20 25

<210> 6
<211> 27
<212> PRT
<213> Mus musculus

<400> 6

Ser His Cys His Trp Asn Asp Leu Ala Val Ile Pro Ala Gly Val Val
1 5 10 15

His Asn Trp Asp Phe Glu Pro Arg Lys Val Ser
20 25

<210> 7
<211> 27
<212> PRT
<213> Mus musculus

<400> 7

Gly Arg Gly His Leu Leu Gly Arg Leu Ala Ala Ile Val Gly Lys Gln
1 5 10 15

Val Leu Leu Gly Arg Lys Val Val Val Val Arg
20 25

<210> 8
<211> 27
<212> PRT
<213> Mus musculus

<400> 8

Phe Arg Arg Lys Ala Phe Leu His Trp Tyr Thr Gly Glu Ala Met Asp
1 5 10 15

Glu Met Glu Phe Thr Glu Ala Glu Ser Asn Met
20 25

<210> 9
<211> 27
<212> PRT
<213> Mus musculus

<400> 9

Val Val Asp Arg Asn Pro Gln Phe Leu Asp Pro Val Leu Ala Tyr Leu
1 5 10 15

Met Lys Gly Leu Cys Glu Lys Pro Leu Ala Ser
20 25

<210> 10
<211> 27
<212> PRT
<213> Mus musculus

<400> 10

Ser Ser Pro Asp Glu Val Ala Leu Val Glu Gly Val Gln Ser Leu Gly
1 5 10 15

Phe Thr Tyr Leu Arg Leu Lys Asp Asn Tyr Met
20 25

<210> 11
<211> 27
<212> PRT
<213> Mus musculus

<400> 11

Glu Phe Lys His Ile Lys Ala Phe Asp Arg Thr Phe Ala Asn Asn Pro
1 5 10 15

Gly Pro Met Val Val Phe Ala Thr Pro Gly Met
20 25

<210> 12
<211> 27
<212> PRT
<213> Mus musculus

<400> 12

Ser Thr Ala Asn Tyr Asn Thr Ser His Leu Asn Asn Asp Val Trp Gln
1 5 10 15

Ile Phe Glu Asn Pro Val Asp Trp Lys Glu Lys
20 25

<210> 13
<211> 27
<212> PRT
<213> Mus musculus

<400> 13

Asp Ser Gly Ser Pro Phe Pro Ala Ala Val Ile Leu Arg Asp Ala Leu
1 5 10 15

His Met Ala Arg Gly Leu Lys Tyr Leu His Gln
20 25

<210> 14
<211> 27
<212> PRT
<213> Mus musculus

<400> 14

Val Ile Leu Pro Gln Ala Pro Ser Gly Pro Ser Tyr Ala Thr Tyr Leu
1 5 10 15

Gln Pro Ala Gln Ala Gln Met Leu Thr Pro Pro
20 25

<210> 15
<211> 27
<212> PRT
<213> Mus musculus

<400> 15

Leu His Ser Gly Gln Asn His Leu Lys Glu Met Ala Ile Ser Val Leu
1 5 10 15

Glu Ala Arg Ala Cys Ala Ala Ala Gly Gln Ser
20 25

<210> 16
<211> 27
<212> PRT
<213> Mus musculus

<400> 16

Pro Leu Leu Pro Phe Tyr Pro Pro Asp Glu Ala Leu Glu Ile Gly Leu
1 5 10 15

Glu Leu Asn Ser Ser Ala Leu Pro Pro Thr Glu
20 25

<210> 17
<211> 27
<212> PRT
<213> Mus musculus

<400> 17

Ala Gly Thr Gln Cys Glu Tyr Trp Ala Ser Arg Ala Leu Asp Ser Glu
1 5 10 15

His Ser Ile Gly Ser Met Ile Gln Leu Pro Gln
20 25

<210> 18
<211> 27
<212> PRT
<213> Mus musculus

<400> 18

Ala Ala Tyr Lys Gly His His Tyr Pro Gly Pro Gly Asn Tyr Phe Trp
1 5 10 15

Lys Cys Leu Phe Met Ser Gly Leu Ser Glu Val
20 25

<210> 19
<211> 27
<212> PRT
<213> Mus musculus

<400> 19

Asp Thr Leu Ser Ala Met Ser Asn Pro Arg Ala Met Gln Val Leu Leu
1 5 10 15

Gln Ile Gln Gln Gly Leu Gln Thr Leu Ala Thr
20 25

<210> 20
<211> 27
<212> PRT
<213> Mus musculus

<400> 20

Asp Lys Pro Leu Arg Arg Asn Asn Ser Tyr Thr Ser Tyr Ile Met Ala
1 5 10 15

Ile Cys Gly Met Pro Leu Asp Ser Phe Arg Ala
20 25

<210> 21
<211> 27
<212> PRT
<213> Mus musculus

<400> 21

Glu Val Ile Gln Thr Ser Lys Tyr Tyr Met Arg Asp Val Ile Ala Ile
1 5 10 15

Glu Ser Ala Trp Leu Leu Glu Leu Ala Pro His
20 25

<210> 22
<211> 27
<212> PRT
<213> Mus musculus

<400> 22

Gly Tyr Ile Ser Arg Val Thr Ala Gly Lys Asp Ser Tyr Ile Ala Leu
1 5 10 15

Val Asp Lys Asn Ile Met Gly Tyr Ile Ala Ser
20 25

<210> 23
<211> 27
<212> PRT
<213> Mus musculus

<400> 23

Glu His Ile His Arg Ala Gly Gly Leu Phe Val Ala Asp Ala Ile Gln
1 5 10 15

Val Gly Phe Gly Arg Ile Gly Lys His Phe Trp
20 25

<210> 24
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 24

Gly Leu Ser Gly Leu
1 5

<210> 25
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 25

Gly Leu Gly Gly Leu
1 5

<210> 26
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Hinge region

<400> 26

Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr His Thr
1 5 10

<210> 27
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Hinge region

<400> 27

Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro
1 5 10 15

<210> 28
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 28

Gly Gly Gly Ser Ser Gly Gly Gly Ser Gly
1 5 10

<210> 29
<211> 107
<212> PRT
<213> Homo sapiens

<400> 29

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
1 5 10 15

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
20 25 30

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly Gln Pro Glu
35 40 45

Asn Asn Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe
50 55 60

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
65 70 75 80

Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn Arg Phe
85 90 95

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
100 105

<210> 30
<211> 666
<212> PRT
<213> Artificial Sequence

<220>
<223> Construct

<400> 30

Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala
1 5 10 15

Leu Cys Asn Gln Val Leu Ser Ala Pro Leu Ala Ala Asp Thr Pro Thr
20 25 30

Ala Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile
35 40 45

Ala Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Ser Val Ile
50 55 60

Phe Leu Thr Lys Arg Gly Arg Gln Val Cys Ala Asp Pro Ser Glu Glu
65 70 75 80

Trp Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala Glu Leu Lys
85 90 95

Thr Pro Leu Gly Asp Thr Thr His Thr Ile Glu Pro Lys Ser Cys Asp
100 105 110

Thr Pro Pro Pro Cys Pro Arg Cys Pro Gly Gly Gly Ser Ser Gly Gly
115 120 125

Gly Ser Gly Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
130 135 140

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
145 150 155 160

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly
165 170 175

```
Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp
            180                 185                 190

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
        195                 200                 205

Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His
    210                 215                 220

Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Gly Leu
225                 230                 235                 240

Gly Gly Leu Met His Gly Asp Thr Pro Thr Leu His Glu Tyr Met Leu
                245                 250                 255

Asp Leu Gln Pro Glu Thr Thr Asp Leu Tyr Gly Tyr Gly Gln Leu Asn
            260                 265                 270

Asp Ser Ser Glu Glu Glu Asp Glu Ile Asp Gly Pro Ala Gly Gln Ala
        275                 280                 285

Glu Pro Asp Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys
    290                 295                 300

Asp Ser Thr Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg
305                 310                 315                 320

Thr Leu Glu Asp Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile
                325                 330                 335

Cys Ser Gln Lys Pro Gly Gly Gly Ser Ser Gly Gly Gly Ser Gly Val
            340                 345                 350

Ile Leu Pro Gln Ala Pro Ser Gly Pro Ser Tyr Ala Thr Tyr Leu Gln
        355                 360                 365

Pro Ala Gln Ala Gln Met Leu Thr Pro Pro Gly Gly Gly Gly Ser Leu
    370                 375                 380

His Ser Gly Gln Asn His Leu Lys Glu Met Ala Ile Ser Val Leu Glu
385                 390                 395                 400

Ala Arg Ala Cys Ala Ala Ala Gly Gln Ser Gly Gly Gly Gly Ser Pro
                405                 410                 415

Leu Leu Pro Phe Tyr Pro Pro Asp Glu Ala Leu Glu Ile Gly Leu Glu
            420                 425                 430

Leu Asn Ser Ser Ala Leu Pro Pro Thr Glu Gly Gly Gly Gly Ser Ala
        435                 440                 445
```

```
Gly Thr Gln Cys Glu Tyr Trp Ala Ser Arg Ala Leu Asp Ser Glu His
    450                 455                 460

Ser Ile Gly Ser Met Ile Gln Leu Pro Gln Gly Gly Gly Gly Ser Ala
465                 470                 475                 480

Ala Tyr Lys Gly His His Tyr Pro Gly Pro Gly Asn Tyr Phe Trp Lys
                485                 490                 495

Cys Leu Phe Met Ser Gly Leu Ser Glu Val Gly Gly Gly Gly Ser Asp
            500                 505                 510

Thr Leu Ser Ala Met Ser Asn Pro Arg Ala Met Gln Val Leu Leu Gln
        515                 520                 525

Ile Gln Gln Gly Leu Gln Thr Leu Ala Thr Gly Gly Gly Gly Ser Asp
    530                 535                 540

Lys Pro Leu Arg Arg Asn Asn Ser Tyr Thr Ser Tyr Ile Met Ala Ile
545                 550                 555                 560

Cys Gly Met Pro Leu Asp Ser Phe Arg Ala Gly Gly Gly Gly Ser Glu
                565                 570                 575

Val Ile Gln Thr Ser Lys Tyr Tyr Met Arg Asp Val Ile Ala Ile Glu
            580                 585                 590

Ser Ala Trp Leu Leu Glu Leu Ala Pro His Gly Gly Gly Gly Ser Gly
        595                 600                 605

Tyr Ile Ser Arg Val Thr Ala Gly Lys Asp Ser Tyr Ile Ala Leu Val
    610                 615                 620

Asp Lys Asn Ile Met Gly Tyr Ile Ala Ser Gly Gly Gly Gly Ser Glu
625                 630                 635                 640

His Ile His Arg Ala Gly Gly Leu Phe Val Ala Asp Ala Ile Gln Val
                645                 650                 655

Gly Phe Gly Arg Ile Gly Lys His Phe Trp
            660                 665
```

<210> 31
<211> 442
<212> PRT
<213> Artificial Sequence

<220>
<223> Construct

<400> 31

```
Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala
1               5                   10                  15
```

```
Leu Cys Asn Gln Val Leu Ser Ala Pro Leu Ala Ala Asp Thr Pro Thr
            20                  25                  30

Ala Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile
        35                  40                  45

Ala Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Ser Val Ile
    50                  55                  60

Phe Leu Thr Lys Arg Gly Arg Gln Val Cys Ala Asp Pro Ser Glu Glu
65                  70                  75                  80

Trp Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala Glu Leu Lys
                85                  90                  95

Thr Pro Leu Gly Asp Thr Thr His Thr Ile Glu Pro Lys Ser Cys Asp
            100                 105                 110

Thr Pro Pro Pro Cys Pro Arg Cys Pro Gly Gly Gly Ser Ser Gly Gly
        115                 120                 125

Gly Ser Gly Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
    130                 135                 140

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
145                 150                 155                 160

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly
                165                 170                 175

Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp
            180                 185                 190

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
        195                 200                 205

Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His
    210                 215                 220

Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Gly Leu
225                 230                 235                 240

Gly Gly Leu Met His Gly Asp Thr Pro Thr Leu His Glu Tyr Met Leu
                245                 250                 255

Asp Leu Gln Pro Glu Thr Thr Asp Leu Tyr Gly Tyr Gly Gln Leu Asn
            260                 265                 270

Asp Ser Ser Glu Glu Glu Asp Glu Ile Asp Gly Pro Ala Gly Gln Ala
        275                 280                 285
```

```
Glu Pro Asp Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys
    290                 295                 300

Asp Ser Thr Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg
305                 310                 315                 320

Thr Leu Glu Asp Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile
                325                 330                 335

Cys Ser Gln Lys Pro Gly Gly Gly Ser Ser Gly Gly Gly Ser Gly Val
            340                 345                 350

Ile Leu Pro Gln Ala Pro Ser Gly Pro Ser Tyr Ala Thr Tyr Leu Gln
        355                 360                 365

Pro Ala Gln Ala Gln Met Leu Thr Pro Pro Gly Gly Gly Gly Ser Leu
    370                 375                 380

His Ser Gly Gln Asn His Leu Lys Glu Met Ala Ile Ser Val Leu Glu
385                 390                 395                 400

Ala Arg Ala Cys Ala Ala Ala Gly Gln Ser Gly Gly Gly Gly Ser Pro
                405                 410                 415

Leu Leu Pro Phe Tyr Pro Pro Asp Glu Ala Leu Glu Ile Gly Leu Glu
            420                 425                 430

Leu Asn Ser Ser Ala Leu Pro Pro Thr Glu
        435                 440
```

<210> 32
<211> 817
<212> PRT
<213> Artificial Sequence

<220>
<223> Construct

<400> 32

```
Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala
1               5                   10                  15

Leu Cys Asn Gln Val Leu Ser Ala Pro Leu Ala Ala Asp Thr Pro Thr
            20                  25                  30

Ala Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile
        35                  40                  45

Ala Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Ser Val Ile
    50                  55                  60
```

```
Phe Leu Thr Lys Arg Gly Arg Gln Val Cys Ala Asp Pro Ser Glu Glu
65                  70                  75                  80

Trp Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala Glu Leu Lys
                85                  90                  95

Thr Pro Leu Gly Asp Thr Thr His Thr Ile Glu Pro Lys Ser Cys Asp
            100                 105                 110

Thr Pro Pro Pro Cys Pro Arg Cys Pro Gly Gly Gly Ser Ser Gly Gly
        115                 120                 125

Gly Ser Gly Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
    130                 135                 140

Ser Arg Glu Glu Met Thr Lys Met Gln Val Ser Thr Ala Ala Leu Ala
145                 150                 155                 160

Val Leu Leu Cys Thr Met Ala Leu Cys Asn Gln Val Leu Ser Ala Pro
                165                 170                 175

Leu Ala Ala Asp Thr Pro Thr Ala Cys Cys Phe Ser Tyr Thr Ser Arg
            180                 185                 190

Gln Ile Pro Gln Asn Phe Ile Ala Asp Tyr Phe Glu Thr Ser Ser Gln
        195                 200                 205

Cys Ser Lys Pro Ser Val Ile Phe Leu Thr Lys Arg Gly Arg Gln Val
    210                 215                 220

Cys Ala Asp Pro Ser Glu Glu Trp Val Gln Lys Tyr Val Ser Asp Leu
225                 230                 235                 240

Glu Leu Ser Ala Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr His Thr
                245                 250                 255

Ile Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro
            260                 265                 270

Gly Gly Gly Ser Ser Gly Gly Gly Ser Gly Gly Gln Pro Arg Glu Pro
        275                 280                 285

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
    290                 295                 300

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
305                 310                 315                 320

Val Glu Trp Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr Asn Thr Thr
                325                 330                 335
```

```
Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
            340                 345                 350

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser
        355                 360                 365

Val Met His Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser
    370                 375                 380

Leu Ser Pro Gly Lys Gly Leu Gly Gly Leu Met His Gly Asp Thr Pro
385                 390                 395                 400

Thr Leu His Glu Tyr Met Leu Asp Leu Gln Pro Glu Thr Thr Asp Leu
                405                 410                 415

Tyr Gly Tyr Gly Gln Leu Asn Asp Ser Ser Glu Glu Glu Asp Glu Ile
            420                 425                 430

Asp Gly Pro Ala Gly Gln Ala Glu Pro Asp Arg Ala His Tyr Asn Ile
        435                 440                 445

Val Thr Phe Cys Cys Lys Cys Asp Ser Thr Leu Arg Leu Cys Val Gln
    450                 455                 460

Ser Thr His Val Asp Ile Arg Thr Leu Glu Asp Leu Leu Met Gly Thr
465                 470                 475                 480

Leu Gly Ile Val Cys Pro Ile Cys Ser Gln Lys Pro Gly Gly Gly Ser
                485                 490                 495

Ser Gly Gly Gly Ser Gly Pro Ser Lys Pro Ser Phe Gln Glu Phe Val
            500                 505                 510

Asp Trp Glu Asn Val Ser Pro Glu Leu Asn Ser Thr Asp Gln Pro Phe
        515                 520                 525

Leu Gly Gly Gly Gly Ser Arg Glu Gly Val Glu Leu Cys Pro Gly Asn
    530                 535                 540

Lys Tyr Glu Met Arg Arg His Gly Thr Thr His Ser Leu Val Ile His
545                 550                 555                 560

Asp Gly Gly Gly Gly Ser Ser His Cys His Trp Asn Asp Leu Ala Val
                565                 570                 575

Ile Pro Ala Gly Val Val His Asn Trp Asp Phe Glu Pro Arg Lys Val
            580                 585                 590

Ser Gly Gly Gly Gly Ser Gly Arg Gly His Leu Leu Gly Arg Leu Ala
        595                 600                 605
```

Ala Ile Val Gly Lys Gln Val Leu Leu Gly Arg Lys Val Val Val Val
610 615 620

Arg Gly Gly Gly Gly Ser Phe Arg Arg Lys Ala Phe Leu His Trp Tyr
625 630 635 640

Thr Gly Glu Ala Met Asp Glu Met Glu Phe Thr Glu Ala Glu Ser Asn
645 650 655

Met Gly Gly Gly Gly Ser Val Val Asp Arg Asn Pro Gln Phe Leu Asp
660 665 670

Pro Val Leu Ala Tyr Leu Met Lys Gly Leu Cys Glu Lys Pro Leu Ala
675 680 685

Ser Gly Gly Gly Gly Ser Ser Ser Pro Asp Glu Val Ala Leu Val Glu
690 695 700

Gly Val Gln Ser Leu Gly Phe Thr Tyr Leu Arg Leu Lys Asp Asn Tyr
705 710 715 720

Met Gly Gly Gly Gly Ser Glu Phe Lys His Ile Lys Ala Phe Asp Arg
725 730 735

Thr Phe Ala Asn Asn Pro Gly Pro Met Val Val Phe Ala Thr Pro Gly
740 745 750

Met Gly Gly Gly Gly Ser Ser Thr Ala Asn Tyr Asn Thr Ser His Leu
755 760 765

Asn Asn Asp Val Trp Gln Ile Phe Glu Asn Pro Val Asp Trp Lys Glu
770 775 780

Lys Gly Gly Gly Gly Ser Asp Ser Gly Ser Pro Phe Pro Ala Ala Val
785 790 795 800

Ile Leu Arg Asp Ala Leu His Met Ala Arg Gly Leu Lys Tyr Leu His
805 810 815

Gln

<210> 33
<211> 442
<212> PRT
<213> Artificial Sequence

<220>
<223> Construct

<400> 33

Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala
1 5 10 15

```
Leu Cys Asn Gln Val Leu Ser Ala Pro Leu Ala Ala Asp Thr Pro Thr
            20                  25                  30

Ala Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile
        35                  40                  45

Ala Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Ser Val Ile
    50                  55                  60

Phe Leu Thr Lys Arg Gly Arg Gln Val Cys Ala Asp Pro Ser Glu Glu
65                  70                  75                  80

Trp Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala Glu Leu Lys
                85                  90                  95

Thr Pro Leu Gly Asp Thr Thr His Thr Ile Glu Pro Lys Ser Cys Asp
            100                 105                 110

Thr Pro Pro Pro Cys Pro Arg Cys Pro Gly Gly Gly Ser Ser Gly Gly
        115                 120                 125

Gly Ser Gly Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
    130                 135                 140

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
145                 150                 155                 160

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly
                165                 170                 175

Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp
            180                 185                 190

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
        195                 200                 205

Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His
    210                 215                 220

Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Gly Leu
225                 230                 235                 240

Gly Gly Leu Met His Gly Asp Thr Pro Thr Leu His Glu Tyr Met Leu
                245                 250                 255

Asp Leu Gln Pro Glu Thr Thr Asp Leu Tyr Gly Tyr Gly Gln Leu Asn
            260                 265                 270

Asp Ser Ser Glu Glu Glu Asp Glu Ile Asp Gly Pro Ala Gly Gln Ala
        275                 280                 285
```

Glu Pro Asp Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys
290 295 300

Asp Ser Thr Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg
305 310 315 320

Thr Leu Glu Asp Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile
325 330 335

Cys Ser Gln Lys Pro Gly Gly Gly Ser Ser Gly Gly Gly Ser Gly Pro
340 345 350

Ser Lys Pro Ser Phe Gln Glu Phe Val Asp Trp Glu Asn Val Ser Pro
355 360 365

Glu Leu Asn Ser Thr Asp Gln Pro Phe Leu Gly Gly Gly Gly Ser Arg
370 375 380

Glu Gly Val Glu Leu Cys Pro Gly Asn Lys Tyr Glu Met Arg Arg His
385 390 395 400

Gly Thr Thr His Ser Leu Val Ile His Asp Gly Gly Gly Gly Ser Ser
405 410 415

His Cys His Trp Asn Asp Leu Ala Val Ile Pro Ala Gly Val Val His
420 425 430

Asn Trp Asp Phe Glu Pro Arg Lys Val Ser
435 440

<210> 34
<211> 19
<212> PRT
<213> Artificial Sequence

<220>
<223> Signal peptide

<400> 34

Met Asn Phe Gly Leu Arg Leu Ile Phe Leu Val Leu Thr Leu Lys Gly
1 5 10 15

Val Gln Cys

<210> 35
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Signal peptide

<400> 35

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1 5 10 15

Ala Val Phe Val Ser Pro
20

<210> 36
<211> 27
<212> PRT
<213> Mus musculus

<400> 36

Ala Asn Phe Glu Ser Gly Lys His Lys Tyr Arg Gln Thr Ala Met Phe
1 5 10 15

Thr Ala Thr Met Pro Pro Ala Val Glu Arg Leu
20 25

<210> 37
<211> 711
<212> PRT
<213> Artificial Sequence

<220>
<223> Construct

<400> 37

Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala
1 5 10 15

Leu Cys Asn Gln Val Leu Ser Ala Pro Leu Ala Ala Asp Thr Pro Thr
20 25 30

Ala Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile
35 40 45

Ala Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Ser Val Ile
50 55 60

Phe Leu Thr Lys Arg Gly Arg Gln Val Cys Ala Asp Pro Ser Glu Glu
65 70 75 80

Trp Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala Glu Leu Lys
85 90 95

Thr Pro Leu Gly Asp Thr Thr His Thr Ile Glu Pro Lys Ser Cys Asp
100 105 110

Thr Pro Pro Pro Cys Pro Arg Cys Pro Gly Gly Gly Ser Ser Gly Gly
115 120 125

Gly Ser Gly Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro

130 135 140

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
145 150 155 160

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly
165 170 175

Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp
180 185 190

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
195 200 205

Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His
210 215 220

Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Gly Leu
225 230 235 240

Gly Gly Leu Met His Gly Asp Thr Pro Thr Leu His Glu Tyr Met Leu
245 250 255

Asp Leu Gln Pro Glu Thr Thr Asp Leu Tyr Gly Tyr Gly Gln Leu Asn
260 265 270

Asp Ser Ser Glu Glu Glu Asp Glu Ile Asp Gly Pro Ala Gly Gln Ala
275 280 285

Glu Pro Asp Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys
290 295 300

Asp Ser Thr Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg
305 310 315 320

Thr Leu Glu Asp Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile
325 330 335

Cys Ser Gln Lys Pro Gly Gly Gly Ser Ser Gly Gly Gly Ser Gly Pro
340 345 350

Ser Lys Pro Ser Phe Gln Glu Phe Val Asp Trp Glu Asn Val Ser Pro
355 360 365

Glu Leu Asn Ser Thr Asp Gln Pro Phe Leu Gly Gly Gly Gly Ser Gly
370 375 380

Gly Gly Gly Ser Arg Glu Gly Val Glu Leu Cys Pro Gly Asn Lys Tyr
385 390 395 400

Glu Met Arg Arg His Gly Thr Thr His Ser Leu Val Ile His Asp Gly

405 410 415

Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser His Cys His Trp Asn Asp
420 425 430

Leu Ala Val Ile Pro Ala Gly Val Val His Asn Trp Asp Phe Glu Pro
435 440 445

Arg Lys Val Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Arg
450 455 460

Gly His Leu Leu Gly Arg Leu Ala Ala Ile Val Gly Lys Gln Val Leu
465 470 475 480

Leu Gly Arg Lys Val Val Val Arg Gly Gly Gly Gly Ser Gly Gly
485 490 495

Gly Gly Ser Phe Arg Arg Lys Ala Phe Leu His Trp Tyr Thr Gly Glu
500 505 510

Ala Met Asp Glu Met Glu Phe Thr Glu Ala Glu Ser Asn Met Gly Gly
515 520 525

Gly Gly Ser Gly Gly Gly Gly Ser Val Val Asp Arg Asn Pro Gln Phe
530 535 540

Leu Asp Pro Val Leu Ala Tyr Leu Met Lys Gly Leu Cys Glu Lys Pro
545 550 555 560

Leu Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Ser Pro
565 570 575

Asp Glu Val Ala Leu Val Glu Gly Val Gln Ser Leu Gly Phe Thr Tyr
580 585 590

Leu Arg Leu Lys Asp Asn Tyr Met Gly Gly Gly Gly Ser Gly Gly Gly
595 600 605

Gly Ser Glu Phe Lys His Ile Lys Ala Phe Asp Arg Thr Phe Ala Asn
610 615 620

Asn Pro Gly Pro Met Val Val Phe Ala Thr Pro Gly Met Gly Gly Gly
625 630 635 640

Gly Ser Gly Gly Gly Gly Ser Ser Thr Ala Asn Tyr Asn Thr Ser His
645 650 655

Leu Asn Asn Asp Val Trp Gln Ile Phe Glu Asn Pro Val Asp Trp Lys
660 665 670

Glu Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ser Gly Ser

675 680 685

Pro Phe Pro Ala Ala Val Ile Leu Arg Asp Ala Leu His Met Ala Arg
690 695 700

Gly Leu Lys Tyr Leu His Gln
705 710

<210> 38
<211> 452
<212> PRT
<213> Artificial Sequence

<220>
<223> Construct

<400> 38

Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala
1 5 10 15

Leu Cys Asn Gln Val Leu Ser Ala Pro Leu Ala Ala Asp Thr Pro Thr
20 25 30

Ala Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile
35 40 45

Ala Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Ser Val Ile
50 55 60

Phe Leu Thr Lys Arg Gly Arg Gln Val Cys Ala Asp Pro Ser Glu Glu
65 70 75 80

Trp Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala Glu Leu Lys
85 90 95

Thr Pro Leu Gly Asp Thr Thr His Thr Ile Glu Pro Lys Ser Cys Asp
100 105 110

Thr Pro Pro Pro Cys Pro Arg Cys Pro Gly Gly Gly Ser Ser Gly Gly
115 120 125

Gly Ser Gly Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
130 135 140

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
145 150 155 160

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly
165 170 175

Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp
180 185 190

```
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
        195                 200                 205

Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His
    210                 215                 220

Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Gly Leu
225                 230                 235                 240

Gly Gly Leu Met His Gly Asp Thr Pro Thr Leu His Glu Tyr Met Leu
                245                 250                 255

Asp Leu Gln Pro Glu Thr Thr Asp Leu Tyr Gly Tyr Gly Gln Leu Asn
            260                 265                 270

Asp Ser Ser Glu Glu Glu Asp Glu Ile Asp Gly Pro Ala Gly Gln Ala
        275                 280                 285

Glu Pro Asp Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys
    290                 295                 300

Asp Ser Thr Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg
305                 310                 315                 320

Thr Leu Glu Asp Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile
                325                 330                 335

Cys Ser Gln Lys Pro Gly Gly Gly Ser Ser Gly Gly Gly Ser Gly Pro
            340                 345                 350

Ser Lys Pro Ser Phe Gln Glu Phe Val Asp Trp Glu Asn Val Ser Pro
        355                 360                 365

Glu Leu Asn Ser Thr Asp Gln Pro Phe Leu Gly Gly Gly Gly Ser Gly
    370                 375                 380

Gly Gly Gly Ser Arg Glu Gly Val Glu Leu Cys Pro Gly Asn Lys Tyr
385                 390                 395                 400

Glu Met Arg Arg His Gly Thr Thr His Ser Leu Val Ile His Asp Gly
                405                 410                 415

Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser His Cys His Trp Asn Asp
            420                 425                 430

Leu Ala Val Ile Pro Ala Gly Val Val His Asn Trp Asp Phe Glu Pro
        435                 440                 445

Arg Lys Val Ser
    450
```

<210> 39
<211> 666
<212> PRT
<213> Artificial Sequence

<220>
<223> Construct

<400> 39

```
Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala
1               5                   10                  15

Leu Cys Asn Gln Val Leu Ser Ala Pro Leu Ala Ala Asp Thr Pro Thr
            20                  25                  30

Ala Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile
        35                  40                  45

Ala Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Ser Val Ile
    50                  55                  60

Phe Leu Thr Lys Arg Gly Arg Gln Val Cys Ala Asp Pro Ser Glu Glu
65                  70                  75                  80

Trp Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala Glu Leu Lys
                85                  90                  95

Thr Pro Leu Gly Asp Thr Thr His Thr Ile Glu Pro Lys Ser Cys Asp
            100                 105                 110

Thr Pro Pro Pro Cys Pro Arg Cys Pro Gly Gly Gly Ser Ser Gly Gly
        115                 120                 125

Gly Ser Gly Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
    130                 135                 140

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
145                 150                 155                 160

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly
                165                 170                 175

Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp
            180                 185                 190

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
        195                 200                 205

Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His
    210                 215                 220

Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Gly Leu
```

225 230 235 240

Gly Gly Leu Met His Gly Asp Thr Pro Thr Leu His Glu Tyr Met Leu
245 250 255

Asp Leu Gln Pro Glu Thr Thr Asp Leu Tyr Gly Tyr Gly Gln Leu Asn
260 265 270

Asp Ser Ser Glu Glu Glu Asp Glu Ile Asp Gly Pro Ala Gly Gln Ala
275 280 285

Glu Pro Asp Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys
290 295 300

Asp Ser Thr Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg
305 310 315 320

Thr Leu Glu Asp Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile
325 330 335

Cys Ser Gln Lys Pro Gly Gly Gly Ser Ser Gly Gly Gly Ser Gly Ser
340 345 350

Thr Ala Asn Tyr Asn Thr Ser His Leu Asn Asn Asp Val Trp Gln Ile
355 360 365

Phe Glu Asn Pro Val Asp Trp Lys Glu Lys Gly Gly Gly Gly Ser Phe
370 375 380

Arg Arg Lys Ala Phe Leu His Trp Tyr Thr Gly Glu Ala Met Asp Glu
385 390 395 400

Met Glu Phe Thr Glu Ala Glu Ser Asn Met Gly Gly Gly Gly Ser Pro
405 410 415

Ser Lys Pro Ser Phe Gln Glu Phe Val Asp Trp Glu Asn Val Ser Pro
420 425 430

Glu Leu Asn Ser Thr Asp Gln Pro Phe Leu Gly Gly Gly Gly Ser Gly
435 440 445

Arg Gly His Leu Leu Gly Arg Leu Ala Ala Ile Val Gly Lys Gln Val
450 455 460

Leu Leu Gly Arg Lys Val Val Val Val Arg Gly Gly Gly Gly Ser Val
465 470 475 480

Val Asp Arg Asn Pro Gln Phe Leu Asp Pro Val Leu Ala Tyr Leu Met
485 490 495

Lys Gly Leu Cys Glu Lys Pro Leu Ala Ser Gly Gly Gly Gly Ser Glu

500 505 510

Phe Lys His Ile Lys Ala Phe Asp Arg Thr Phe Ala Asn Asn Pro Gly
515 520 525

Pro Met Val Val Phe Ala Thr Pro Gly Met Gly Gly Gly Gly Ser Asp
530 535 540

Ser Gly Ser Pro Phe Pro Ala Ala Val Ile Leu Arg Asp Ala Leu His
545 550 555 560

Met Ala Arg Gly Leu Lys Tyr Leu His Gln Gly Gly Gly Gly Ser Ser
565 570 575

His Cys His Trp Asn Asp Leu Ala Val Ile Pro Ala Gly Val Val His
580 585 590

Asn Trp Asp Phe Glu Pro Arg Lys Val Ser Gly Gly Gly Gly Ser Ser
595 600 605

Ser Pro Asp Glu Val Ala Leu Val Glu Gly Val Gln Ser Leu Gly Phe
610 615 620

Thr Tyr Leu Arg Leu Lys Asp Asn Tyr Met Gly Gly Gly Gly Ser Arg
625 630 635 640

Glu Gly Val Glu Leu Cys Pro Gly Asn Lys Tyr Glu Met Arg Arg His
645 650 655

Gly Thr Thr His Ser Leu Val Ile His Asp
660 665

<210> 40
<211> 442
<212> PRT
<213> Artificial Sequence

<220>
<223> Construct

<400> 40

Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala
1 5 10 15

Leu Cys Asn Gln Val Leu Ser Ala Pro Leu Ala Ala Asp Thr Pro Thr
20 25 30

Ala Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile
35 40 45

Ala Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Ser Val Ile
50 55 60

```
Phe Leu Thr Lys Arg Gly Arg Gln Val Cys Ala Asp Pro Ser Glu Glu
65                  70                  75                  80

Trp Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala Glu Leu Lys
                85                  90                  95

Thr Pro Leu Gly Asp Thr Thr His Thr Ile Glu Pro Lys Ser Cys Asp
            100                 105                 110

Thr Pro Pro Pro Cys Pro Arg Cys Pro Gly Gly Gly Ser Ser Gly Gly
        115                 120                 125

Gly Ser Gly Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
    130                 135                 140

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
145                 150                 155                 160

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly
                165                 170                 175

Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp
            180                 185                 190

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
        195                 200                 205

Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His
    210                 215                 220

Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Gly Leu
225                 230                 235                 240

Gly Gly Leu Met His Gly Asp Thr Pro Thr Leu His Glu Tyr Met Leu
                245                 250                 255

Asp Leu Gln Pro Glu Thr Thr Asp Leu Tyr Gly Tyr Gly Gln Leu Asn
            260                 265                 270

Asp Ser Ser Glu Glu Glu Asp Glu Ile Asp Gly Pro Ala Gly Gln Ala
        275                 280                 285

Glu Pro Asp Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys
    290                 295                 300

Asp Ser Thr Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg
305                 310                 315                 320

Thr Leu Glu Asp Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile
                325                 330                 335
```

```
Cys Ser Gln Lys Pro Gly Gly Gly Ser Ser Gly Gly Gly Ser Gly Pro
            340                 345                 350

Ser Lys Pro Ser Phe Gln Glu Phe Val Asp Trp Glu Asn Val Ser Pro
        355                 360                 365

Glu Leu Asn Ser Thr Asp Gln Pro Phe Leu Gly Gly Gly Gly Ser Glu
    370                 375                 380

Phe Lys His Ile Lys Ala Phe Asp Arg Thr Phe Ala Asn Asn Pro Gly
385                 390                 395                 400

Pro Met Val Val Phe Ala Thr Pro Gly Met Gly Gly Gly Gly Ser Ser
                405                 410                 415

His Cys His Trp Asn Asp Leu Ala Val Ile Pro Ala Gly Val Val His
            420                 425                 430

Asn Trp Asp Phe Glu Pro Arg Lys Val Ser
        435                 440


<210>  41
<211>  442
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Construct

<400>  41

Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala
1               5                   10                  15

Leu Cys Asn Gln Val Leu Ser Ala Pro Leu Ala Ala Asp Thr Pro Thr
            20                  25                  30

Ala Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile
        35                  40                  45

Ala Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Ser Val Ile
    50                  55                  60

Phe Leu Thr Lys Arg Gly Arg Gln Val Cys Ala Asp Pro Ser Glu Glu
65                  70                  75                  80

Trp Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala Glu Leu Lys
                85                  90                  95

Thr Pro Leu Gly Asp Thr Thr His Thr Ile Glu Pro Lys Ser Cys Asp
            100                 105                 110

Thr Pro Pro Pro Cys Pro Arg Cys Pro Gly Gly Gly Ser Ser Gly Gly
```

115 120 125

Gly Ser Gly Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
130 135 140

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
145 150 155 160

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly
165 170 175

Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp
180 185 190

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
195 200 205

Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His
210 215 220

Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Gly Leu
225 230 235 240

Gly Gly Leu Met His Gly Asp Thr Pro Thr Leu His Glu Tyr Met Leu
245 250 255

Asp Leu Gln Pro Glu Thr Thr Asp Leu Tyr Gly Tyr Gly Gln Leu Asn
260 265 270

Asp Ser Ser Glu Glu Glu Asp Glu Ile Asp Gly Pro Ala Gly Gln Ala
275 280 285

Glu Pro Asp Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys
290 295 300

Asp Ser Thr Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg
305 310 315 320

Thr Leu Glu Asp Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile
325 330 335

Cys Ser Gln Lys Pro Gly Gly Gly Ser Ser Gly Gly Gly Ser Gly Pro
340 345 350

Ser Lys Pro Ser Phe Gln Glu Phe Val Asp Trp Glu Asn Val Ser Pro
355 360 365

Glu Leu Asn Ser Thr Asp Gln Pro Phe Leu Gly Gly Gly Gly Ser Ser
370 375 380

His Cys His Trp Asn Asp Leu Ala Val Ile Pro Ala Gly Val Val His

385 390 395 400

Asn Trp Asp Phe Glu Pro Arg Lys Val Ser Gly Gly Gly Gly Ser Arg
405 410 415

Glu Gly Val Glu Leu Cys Pro Gly Asn Lys Tyr Glu Met Arg Arg His
420 425 430

Gly Thr Thr His Ser Leu Val Ile His Asp
435 440

<210> 42
<211> 666
<212> PRT
<213> Artificial Sequence

<220>
<223> Construct

<400> 42

Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala
1 5 10 15

Leu Cys Asn Gln Val Leu Ser Ala Pro Leu Ala Ala Asp Thr Pro Thr
20 25 30

Ala Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile
35 40 45

Ala Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Ser Val Ile
50 55 60

Phe Leu Thr Lys Arg Gly Arg Gln Val Cys Ala Asp Pro Ser Glu Glu
65 70 75 80

Trp Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala Glu Leu Lys
85 90 95

Thr Pro Leu Gly Asp Thr Thr His Thr Ile Glu Pro Lys Ser Cys Asp
100 105 110

Thr Pro Pro Pro Cys Pro Arg Cys Pro Gly Gly Gly Ser Ser Gly Gly
115 120 125

Gly Ser Gly Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
130 135 140

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
145 150 155 160

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly
165 170 175

```
Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp
            180                 185                 190

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
        195                 200                 205

Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His
    210                 215                 220

Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Gly Leu
225                 230                 235                 240

Gly Gly Leu Met His Gly Asp Thr Pro Thr Leu His Glu Tyr Met Leu
                245                 250                 255

Asp Leu Gln Pro Glu Thr Thr Asp Leu Tyr Gly Tyr Gly Gln Leu Asn
            260                 265                 270

Asp Ser Ser Glu Glu Glu Asp Glu Ile Asp Gly Pro Ala Gly Gln Ala
        275                 280                 285

Glu Pro Asp Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys
    290                 295                 300

Asp Ser Thr Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg
305                 310                 315                 320

Thr Leu Glu Asp Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile
                325                 330                 335

Cys Ser Gln Lys Pro Gly Gly Gly Ser Ser Gly Gly Gly Ser Gly Pro
            340                 345                 350

Ser Lys Pro Ser Phe Gln Glu Phe Val Asp Trp Glu Asn Val Ser Pro
        355                 360                 365

Glu Leu Asn Ser Thr Asp Gln Pro Phe Leu Gly Gly Gly Gly Ser Arg
    370                 375                 380

Glu Gly Val Glu Leu Cys Pro Gly Asn Lys Tyr Glu Met Arg Arg His
385                 390                 395                 400

Gly Thr Thr His Ser Leu Val Ile His Asp Gly Gly Gly Gly Ser Ser
                405                 410                 415

His Cys His Trp Asn Asp Leu Ala Val Ile Pro Ala Gly Val Val His
            420                 425                 430

Asn Trp Asp Phe Glu Pro Arg Lys Val Ser Gly Gly Gly Gly Ser Gly
        435                 440                 445
```

```
Arg Gly His Leu Leu Gly Arg Leu Ala Ala Ile Val Gly Lys Gln Val
    450                 455                 460

Leu Leu Gly Arg Lys Val Val Val Val Arg Gly Gly Gly Gly Ser Ala
465                 470                 475                 480

Asn Phe Glu Ser Gly Lys His Lys Tyr Arg Gln Thr Ala Met Phe Thr
                485                 490                 495

Ala Thr Met Pro Pro Ala Val Glu Arg Leu Gly Gly Gly Gly Ser Val
            500                 505                 510

Val Asp Arg Asn Pro Gln Phe Leu Asp Pro Val Leu Ala Tyr Leu Met
        515                 520                 525

Lys Gly Leu Cys Glu Lys Pro Leu Ala Ser Gly Gly Gly Gly Ser Ser
    530                 535                 540

Ser Pro Asp Glu Val Ala Leu Val Glu Gly Val Gln Ser Leu Gly Phe
545                 550                 555                 560

Thr Tyr Leu Arg Leu Lys Asp Asn Tyr Met Gly Gly Gly Gly Ser Glu
                565                 570                 575

Phe Lys His Ile Lys Ala Phe Asp Arg Thr Phe Ala Asn Asn Pro Gly
            580                 585                 590

Pro Met Val Val Phe Ala Thr Pro Gly Met Gly Gly Gly Gly Ser Ser
        595                 600                 605

Thr Ala Asn Tyr Asn Thr Ser His Leu Asn Asn Asp Val Trp Gln Ile
    610                 615                 620

Phe Glu Asn Pro Val Asp Trp Lys Glu Lys Gly Gly Gly Gly Ser Asp
625                 630                 635                 640

Ser Gly Ser Pro Phe Pro Ala Ala Val Ile Leu Arg Asp Ala Leu His
                645                 650                 655

Met Ala Arg Gly Leu Lys Tyr Leu His Gln
            660                 665


<210>  43
<211>  986
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Construct

<400>  43

Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala
```

```
1               5                   10                  15

Leu Cys Asn Gln Val Leu Ser Ala Pro Leu Ala Ala Asp Thr Pro Thr
            20                  25                  30

Ala Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile
        35                  40                  45

Ala Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Ser Val Ile
    50                  55                  60

Phe Leu Thr Lys Arg Gly Arg Gln Val Cys Ala Asp Pro Ser Glu Glu
65                  70                  75                  80

Trp Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala Glu Leu Lys
                85                  90                  95

Thr Pro Leu Gly Asp Thr Thr His Thr Ile Glu Pro Lys Ser Cys Asp
            100                 105                 110

Thr Pro Pro Pro Cys Pro Arg Cys Pro Gly Gly Gly Ser Ser Gly Gly
        115                 120                 125

Gly Ser Gly Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
    130                 135                 140

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
145                 150                 155                 160

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly
                165                 170                 175

Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp
            180                 185                 190

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
        195                 200                 205

Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His
    210                 215                 220

Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Gly Leu
225                 230                 235                 240

Gly Gly Leu Met His Gly Asp Thr Pro Thr Leu His Glu Tyr Met Leu
                245                 250                 255

Asp Leu Gln Pro Glu Thr Thr Asp Leu Tyr Gly Tyr Gly Gln Leu Asn
            260                 265                 270

Asp Ser Ser Glu Glu Glu Asp Glu Ile Asp Gly Pro Ala Gly Gln Ala
```

275 280 285

Glu Pro Asp Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys
290 295 300

Asp Ser Thr Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg
305 310 315 320

Thr Leu Glu Asp Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile
325 330 335

Cys Ser Gln Lys Pro Gly Gly Gly Ser Ser Gly Gly Gly Ser Gly Pro
340 345 350

Ser Lys Pro Ser Phe Gln Glu Phe Val Asp Trp Glu Asn Val Ser Pro
355 360 365

Glu Leu Asn Ser Thr Asp Gln Pro Phe Leu Gly Gly Gly Gly Ser Arg
370 375 380

Glu Gly Val Glu Leu Cys Pro Gly Asn Lys Tyr Glu Met Arg Arg His
385 390 395 400

Gly Thr Thr His Ser Leu Val Ile His Asp Gly Gly Gly Gly Ser Ser
405 410 415

His Cys His Trp Asn Asp Leu Ala Val Ile Pro Ala Gly Val Val His
420 425 430

Asn Trp Asp Phe Glu Pro Arg Lys Val Ser Gly Gly Gly Gly Ser Gly
435 440 445

Arg Gly His Leu Leu Gly Arg Leu Ala Ala Ile Val Gly Lys Gln Val
450 455 460

Leu Leu Gly Arg Lys Val Val Val Val Arg Gly Gly Gly Gly Ser Ala
465 470 475 480

Asn Phe Glu Ser Gly Lys His Lys Tyr Arg Gln Thr Ala Met Phe Thr
485 490 495

Ala Thr Met Pro Pro Ala Val Glu Arg Leu Gly Gly Gly Gly Ser Val
500 505 510

Val Asp Arg Asn Pro Gln Phe Leu Asp Pro Val Leu Ala Tyr Leu Met
515 520 525

Lys Gly Leu Cys Glu Lys Pro Leu Ala Ser Gly Gly Gly Gly Ser Ser
530 535 540

Ser Pro Asp Glu Val Ala Leu Val Glu Gly Val Gln Ser Leu Gly Phe

545 550 555 560

Thr Tyr Leu Arg Leu Lys Asp Asn Tyr Met Gly Gly Gly Gly Ser Glu
565 570 575

Phe Lys His Ile Lys Ala Phe Asp Arg Thr Phe Ala Asn Asn Pro Gly
580 585 590

Pro Met Val Val Phe Ala Thr Pro Gly Met Gly Gly Gly Gly Ser Ser
595 600 605

Thr Ala Asn Tyr Asn Thr Ser His Leu Asn Asn Asp Val Trp Gln Ile
610 615 620

Phe Glu Asn Pro Val Asp Trp Lys Glu Lys Gly Gly Gly Gly Ser Asp
625 630 635 640

Ser Gly Ser Pro Phe Pro Ala Ala Val Ile Leu Arg Asp Ala Leu His
645 650 655

Met Ala Arg Gly Leu Lys Tyr Leu His Gln Gly Gly Gly Gly Ser Pro
660 665 670

Ser Lys Pro Ser Phe Gln Glu Phe Val Asp Trp Glu Asn Val Ser Pro
675 680 685

Glu Leu Asn Ser Thr Asp Gln Pro Phe Leu Gly Gly Gly Gly Ser Arg
690 695 700

Glu Gly Val Glu Leu Cys Pro Gly Asn Lys Tyr Glu Met Arg Arg His
705 710 715 720

Gly Thr Thr His Ser Leu Val Ile His Asp Gly Gly Gly Gly Ser Ser
725 730 735

His Cys His Trp Asn Asp Leu Ala Val Ile Pro Ala Gly Val Val His
740 745 750

Asn Trp Asp Phe Glu Pro Arg Lys Val Ser Gly Gly Gly Gly Ser Gly
755 760 765

Arg Gly His Leu Leu Gly Arg Leu Ala Ala Ile Val Gly Lys Gln Val
770 775 780

Leu Leu Gly Arg Lys Val Val Val Val Arg Gly Gly Gly Gly Ser Ala
785 790 795 800

Asn Phe Glu Ser Gly Lys His Lys Tyr Arg Gln Thr Ala Met Phe Thr
805 810 815

Ala Thr Met Pro Pro Ala Val Glu Arg Leu Gly Gly Gly Gly Ser Val

820 825 830

Val Asp Arg Asn Pro Gln Phe Leu Asp Pro Val Leu Ala Tyr Leu Met
835 840 845

Lys Gly Leu Cys Glu Lys Pro Leu Ala Ser Gly Gly Gly Gly Ser Ser
850 855 860

Ser Pro Asp Glu Val Ala Leu Val Glu Gly Val Gln Ser Leu Gly Phe
865 870 875 880

Thr Tyr Leu Arg Leu Lys Asp Asn Tyr Met Gly Gly Gly Gly Ser Glu
885 890 895

Phe Lys His Ile Lys Ala Phe Asp Arg Thr Phe Ala Asn Asn Pro Gly
900 905 910

Pro Met Val Val Phe Ala Thr Pro Gly Met Gly Gly Gly Gly Ser Ser
915 920 925

Thr Ala Asn Tyr Asn Thr Ser His Leu Asn Asn Asp Val Trp Gln Ile
930 935 940

Phe Glu Asn Pro Val Asp Trp Lys Glu Lys Gly Gly Gly Gly Ser Asp
945 950 955 960

Ser Gly Ser Pro Phe Pro Ala Ala Val Ile Leu Arg Asp Ala Leu His
965 970 975

Met Ala Arg Gly Leu Lys Tyr Leu His Gln
980 985

<210> 44
<211> 666
<212> PRT
<213> Artificial Sequence

<220>
<223> Construct

<400> 44

Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala
1 5 10 15

Leu Cys Asn Gln Val Leu Ser Ala Pro Leu Ala Ala Asp Thr Pro Thr
20 25 30

Ala Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile
35 40 45

Ala Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Ser Val Ile
50 55 60

```
Phe Leu Thr Lys Arg Gly Arg Gln Val Cys Ala Asp Pro Ser Glu Glu
65                  70                  75                  80

Trp Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala Glu Leu Lys
                85                  90                  95

Thr Pro Leu Gly Asp Thr Thr His Thr Ile Glu Pro Lys Ser Cys Asp
            100                 105                 110

Thr Pro Pro Pro Cys Pro Arg Cys Pro Gly Gly Gly Ser Ser Gly Gly
        115                 120                 125

Gly Ser Gly Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
    130                 135                 140

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
145                 150                 155                 160

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly
                165                 170                 175

Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp
            180                 185                 190

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
        195                 200                 205

Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His
    210                 215                 220

Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Gly Leu
225                 230                 235                 240

Gly Gly Leu Met His Gly Asp Thr Pro Thr Leu His Glu Tyr Met Leu
                245                 250                 255

Asp Leu Gln Pro Glu Thr Thr Asp Leu Tyr Gly Tyr Gly Gln Leu Asn
            260                 265                 270

Asp Ser Ser Glu Glu Glu Asp Glu Ile Asp Gly Pro Ala Gly Gln Ala
        275                 280                 285

Glu Pro Asp Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys
    290                 295                 300

Asp Ser Thr Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg
305                 310                 315                 320

Thr Leu Glu Asp Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile
                325                 330                 335
```

```
Cys Ser Gln Lys Pro Gly Gly Gly Ser Ser Gly Gly Gly Ser Gly Ser
            340                 345                 350

His Cys His Trp Asn Asp Leu Ala Val Ile Pro Ala Gly Val Val His
        355                 360                 365

Asn Trp Asp Phe Glu Pro Arg Lys Val Ser Gly Gly Gly Gly Ser Gly
    370                 375                 380

Arg Gly His Leu Leu Gly Arg Leu Ala Ala Ile Val Gly Lys Gln Val
385                 390                 395                 400

Leu Leu Gly Arg Lys Val Val Val Val Arg Gly Gly Gly Gly Ser Ser
                405                 410                 415

Ser Pro Asp Glu Val Ala Leu Val Glu Gly Val Gln Ser Leu Gly Phe
            420                 425                 430

Thr Tyr Leu Arg Leu Lys Asp Asn Tyr Met Gly Gly Gly Gly Ser Ser
        435                 440                 445

Thr Ala Asn Tyr Asn Thr Ser His Leu Asn Asn Asp Val Trp Gln Ile
    450                 455                 460

Phe Glu Asn Pro Val Asp Trp Lys Glu Lys Gly Gly Gly Gly Ser Asp
465                 470                 475                 480

Ser Gly Ser Pro Phe Pro Ala Ala Val Ile Leu Arg Asp Ala Leu His
                485                 490                 495

Met Ala Arg Gly Leu Lys Tyr Leu His Gln Gly Gly Gly Gly Ser Ser
            500                 505                 510

His Cys His Trp Asn Asp Leu Ala Val Ile Pro Ala Gly Val Val His
        515                 520                 525

Asn Trp Asp Phe Glu Pro Arg Lys Val Ser Gly Gly Gly Gly Ser Gly
    530                 535                 540

Arg Gly His Leu Leu Gly Arg Leu Ala Ala Ile Val Gly Lys Gln Val
545                 550                 555                 560

Leu Leu Gly Arg Lys Val Val Val Val Arg Gly Gly Gly Gly Ser Ser
                565                 570                 575

Ser Pro Asp Glu Val Ala Leu Val Glu Gly Val Gln Ser Leu Gly Phe
            580                 585                 590

Thr Tyr Leu Arg Leu Lys Asp Asn Tyr Met Gly Gly Gly Gly Ser Ser
        595                 600                 605
```

```
Thr Ala Asn Tyr Asn Thr Ser His Leu Asn Asn Asp Val Trp Gln Ile
    610                 615                 620

Phe Glu Asn Pro Val Asp Trp Lys Glu Lys Gly Gly Gly Gly Ser Asp
625                 630                 635                 640

Ser Gly Ser Pro Phe Pro Ala Ala Val Ile Leu Arg Asp Ala Leu His
                645                 650                 655

Met Ala Arg Gly Leu Lys Tyr Leu His Gln
            660                 665
```

<210> 45
<211> 826
<212> PRT
<213> Artificial Sequence

<220>
<223> Construct

<400> 45

```
Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala
1               5                   10                  15

Leu Cys Asn Gln Val Leu Ser Ala Pro Leu Ala Ala Asp Thr Pro Thr
            20                  25                  30

Ala Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile
        35                  40                  45

Ala Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Ser Val Ile
    50                  55                  60

Phe Leu Thr Lys Arg Gly Arg Gln Val Cys Ala Asp Pro Ser Glu Glu
65                  70                  75                  80

Trp Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala Glu Leu Lys
                85                  90                  95

Thr Pro Leu Gly Asp Thr Thr His Thr Ile Glu Pro Lys Ser Cys Asp
            100                 105                 110

Thr Pro Pro Pro Cys Pro Arg Cys Pro Gly Gly Gly Ser Ser Gly Gly
        115                 120                 125

Gly Ser Gly Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
    130                 135                 140

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
145                 150                 155                 160

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly
```

```
                165                 170                 175

Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp
            180                 185                 190

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
        195                 200                 205

Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His
    210                 215                 220

Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Gly Leu
225                 230                 235                 240

Gly Gly Leu Met His Gly Asp Thr Pro Thr Leu His Glu Tyr Met Leu
                245                 250                 255

Asp Leu Gln Pro Glu Thr Thr Asp Leu Tyr Gly Tyr Gly Gln Leu Asn
            260                 265                 270

Asp Ser Ser Glu Glu Glu Asp Glu Ile Asp Gly Pro Ala Gly Gln Ala
        275                 280                 285

Glu Pro Asp Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys
    290                 295                 300

Asp Ser Thr Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg
305                 310                 315                 320

Thr Leu Glu Asp Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile
                325                 330                 335

Cys Ser Gln Lys Pro Gly Gly Gly Ser Ser Gly Gly Gly Ser Gly Ser
            340                 345                 350

His Cys His Trp Asn Asp Leu Ala Val Ile Pro Ala Gly Val Val His
        355                 360                 365

Asn Trp Asp Phe Glu Pro Arg Lys Val Ser Gly Gly Gly Gly Ser Gly
    370                 375                 380

Arg Gly His Leu Leu Gly Arg Leu Ala Ala Ile Val Gly Lys Gln Val
385                 390                 395                 400

Leu Leu Gly Arg Lys Val Val Val Val Arg Gly Gly Gly Gly Ser Ser
                405                 410                 415

Ser Pro Asp Glu Val Ala Leu Val Glu Gly Val Gln Ser Leu Gly Phe
            420                 425                 430

Thr Tyr Leu Arg Leu Lys Asp Asn Tyr Met Gly Gly Gly Gly Ser Ser
```

435 440 445

Thr Ala Asn Tyr Asn Thr Ser His Leu Asn Asn Asp Val Trp Gln Ile
450 455 460

Phe Glu Asn Pro Val Asp Trp Lys Glu Lys Gly Gly Gly Gly Ser Asp
465 470 475 480

Ser Gly Ser Pro Phe Pro Ala Ala Val Ile Leu Arg Asp Ala Leu His
485 490 495

Met Ala Arg Gly Leu Lys Tyr Leu His Gln Gly Gly Gly Gly Ser Ser
500 505 510

His Cys His Trp Asn Asp Leu Ala Val Ile Pro Ala Gly Val Val His
515 520 525

Asn Trp Asp Phe Glu Pro Arg Lys Val Ser Gly Gly Gly Gly Ser Gly
530 535 540

Arg Gly His Leu Leu Gly Arg Leu Ala Ala Ile Val Gly Lys Gln Val
545 550 555 560

Leu Leu Gly Arg Lys Val Val Val Val Arg Gly Gly Gly Gly Ser Ser
565 570 575

Ser Pro Asp Glu Val Ala Leu Val Glu Gly Val Gln Ser Leu Gly Phe
580 585 590

Thr Tyr Leu Arg Leu Lys Asp Asn Tyr Met Gly Gly Gly Gly Ser Ser
595 600 605

Thr Ala Asn Tyr Asn Thr Ser His Leu Asn Asn Asp Val Trp Gln Ile
610 615 620

Phe Glu Asn Pro Val Asp Trp Lys Glu Lys Gly Gly Gly Gly Ser Asp
625 630 635 640

Ser Gly Ser Pro Phe Pro Ala Ala Val Ile Leu Arg Asp Ala Leu His
645 650 655

Met Ala Arg Gly Leu Lys Tyr Leu His Gln Gly Gly Gly Gly Ser Ser
660 665 670

His Cys His Trp Asn Asp Leu Ala Val Ile Pro Ala Gly Val Val His
675 680 685

Asn Trp Asp Phe Glu Pro Arg Lys Val Ser Gly Gly Gly Gly Ser Gly
690 695 700

Arg Gly His Leu Leu Gly Arg Leu Ala Ala Ile Val Gly Lys Gln Val

705 710 715 720

Leu Leu Gly Arg Lys Val Val Val Val Arg Gly Gly Gly Gly Ser Ser
725 730 735

Ser Pro Asp Glu Val Ala Leu Val Glu Gly Val Gln Ser Leu Gly Phe
740 745 750

Thr Tyr Leu Arg Leu Lys Asp Asn Tyr Met Gly Gly Gly Gly Ser Ser
755 760 765

Thr Ala Asn Tyr Asn Thr Ser His Leu Asn Asn Asp Val Trp Gln Ile
770 775 780

Phe Glu Asn Pro Val Asp Trp Lys Glu Lys Gly Gly Gly Gly Ser Asp
785 790 795 800

Ser Gly Ser Pro Phe Pro Ala Ala Val Ile Leu Arg Asp Ala Leu His
805 810 815

Met Ala Arg Gly Leu Lys Tyr Leu His Gln
820 825

<210> 46
<211> 896
<212> PRT
<213> Artificial Sequence

<220>
<223> VB construct

<400> 46

Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala
1 5 10 15

Leu Cys Asn Gln Val Leu Ser Ala Pro Leu Ala Ala Asp Thr Pro Thr
20 25 30

Ala Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile
35 40 45

Ala Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Ser Val Ile
50 55 60

Phe Leu Thr Lys Arg Gly Arg Gln Val Cys Ala Asp Pro Ser Glu Glu
65 70 75 80

Trp Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala Glu Leu Lys
85 90 95

Thr Pro Leu Gly Asp Thr Thr His Thr Ile Glu Pro Lys Ser Cys Asp
100 105 110

```
Thr Pro Pro Pro Cys Pro Arg Cys Pro Gly Gly Gly Ser Ser Gly Gly
        115                 120                 125

Gly Ser Gly Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
    130                 135                 140

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
145                 150                 155                 160

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly
                165                 170                 175

Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp
            180                 185                 190

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
        195                 200                 205

Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His
    210                 215                 220

Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Gly Leu
225                 230                 235                 240

Gly Gly Leu Met His Gly Asp Thr Pro Thr Leu His Glu Tyr Met Leu
                245                 250                 255

Asp Leu Gln Pro Glu Thr Thr Asp Leu Tyr Gly Tyr Gly Gln Leu Asn
            260                 265                 270

Asp Ser Ser Glu Glu Glu Asp Glu Ile Asp Gly Pro Ala Gly Gln Ala
        275                 280                 285

Glu Pro Asp Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys
    290                 295                 300

Asp Ser Thr Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg
305                 310                 315                 320

Thr Leu Glu Asp Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile
                325                 330                 335

Cys Ser Gln Lys Pro Gly Gly Gly Ser Ser Gly Gly Gly Ser Gly Pro
            340                 345                 350

Ser Lys Pro Ser Phe Gln Glu Phe Val Asp Trp Glu Asn Val Ser Pro
        355                 360                 365

Glu Leu Asn Ser Thr Asp Gln Pro Phe Leu Gly Gly Gly Gly Ser Gly
    370                 375                 380
```

```
Gly Gly Gly Ser Arg Glu Gly Val Glu Leu Cys Pro Gly Asn Lys Tyr
385                 390                 395                 400

Glu Met Arg Arg His Gly Thr Thr His Ser Leu Val Ile His Asp Gly
                405                 410                 415

Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser His Cys His Trp Asn Asp
            420                 425                 430

Leu Ala Val Ile Pro Ala Gly Val Val His Asn Trp Asp Phe Glu Pro
        435                 440                 445

Arg Lys Val Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Arg
    450                 455                 460

Gly His Leu Leu Gly Arg Leu Ala Ala Ile Val Gly Lys Gln Val Leu
465                 470                 475                 480

Leu Gly Arg Lys Val Val Val Val Arg Gly Gly Gly Gly Ser Gly Gly
                485                 490                 495

Gly Gly Ser Phe Arg Arg Lys Ala Phe Leu His Trp Tyr Thr Gly Glu
            500                 505                 510

Ala Met Asp Glu Met Glu Phe Thr Glu Ala Glu Ser Asn Met Gly Gly
        515                 520                 525

Gly Gly Ser Gly Gly Gly Gly Ser Val Val Asp Arg Asn Pro Gln Phe
    530                 535                 540

Leu Asp Pro Val Leu Ala Tyr Leu Met Lys Gly Leu Cys Glu Lys Pro
545                 550                 555                 560

Leu Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Ser Pro
                565                 570                 575

Asp Glu Val Ala Leu Val Glu Gly Val Gln Ser Leu Gly Phe Thr Tyr
            580                 585                 590

Leu Arg Leu Lys Asp Asn Tyr Met Gly Gly Gly Gly Ser Gly Gly Gly
        595                 600                 605

Gly Ser Glu Phe Lys His Ile Lys Ala Phe Asp Arg Thr Phe Ala Asn
    610                 615                 620

Asn Pro Gly Pro Met Val Val Phe Ala Thr Pro Gly Met Gly Gly Gly
625                 630                 635                 640

Gly Ser Gly Gly Gly Gly Ser Ser Thr Ala Asn Tyr Asn Thr Ser His
                645                 650                 655
```

```
Leu Asn Asn Asp Val Trp Gln Ile Phe Glu Asn Pro Val Asp Trp Lys
            660                 665                 670

Glu Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ser Gly Ser
        675                 680                 685

Pro Phe Pro Ala Ala Val Ile Leu Arg Asp Ala Leu His Met Ala Arg
    690                 695                 700

Gly Leu Lys Tyr Leu His Gln Gly Gly Gly Gly Ser Gly Gly Gly Gly
705                 710                 715                 720

Ser Ala Asn Phe Glu Ser Gly Lys His Lys Tyr Arg Gln Thr Ala Met
                725                 730                 735

Phe Thr Ala Thr Met Pro Pro Ala Val Glu Arg Leu Gly Gly Gly Gly
            740                 745                 750

Ser Gly Gly Gly Gly Ser Asn His Ser Gly Leu Val Thr Phe Gln Ala
        755                 760                 765

Phe Ile Asp Val Met Ser Arg Glu Thr Thr Asp Thr Asp Thr Ala Asp
    770                 775                 780

Gln Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Cys Gly Thr Ala Phe
785                 790                 795                 800

Phe Ile Asn Phe Ile Ala Ile Tyr His His Ala Ser Arg Ala Ile Pro
                805                 810                 815

Phe Gly Thr Met Val Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
            820                 825                 830

Phe Val Val Lys Ala Tyr Leu Pro Val Asn Glu Ser Phe Ala Phe Thr
        835                 840                 845

Ala Asp Leu Arg Ser Asn Thr Gly Gly Gln Ala Gly Gly Gly Gly Ser
    850                 855                 860

Gly Gly Gly Gly Ser Thr Pro Pro Pro Glu Glu Ala Met Pro Phe Glu
865                 870                 875                 880

Phe Asn Gly Pro Ala Gln Gly Asp His Ser Gln Pro Pro Leu Gln Val
                885                 890                 895
```

<210> 47
<211> 1081
<212> PRT
<213> Artificial Sequence

<220>
<223> VB vector

<400> 47

```
Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala
1               5                  10                  15

Leu Cys Asn Gln Val Leu Ser Ala Pro Leu Ala Ala Asp Thr Pro Thr
            20                  25                  30

Ala Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile
        35                  40                  45

Ala Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Ser Val Ile
    50                  55                  60

Phe Leu Thr Lys Arg Gly Arg Gln Val Cys Ala Asp Pro Ser Glu Glu
65                  70                  75                  80

Trp Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala Glu Leu Lys
                85                  90                  95

Thr Pro Leu Gly Asp Thr Thr His Thr Ile Glu Pro Lys Ser Cys Asp
            100                 105                 110

Thr Pro Pro Pro Cys Pro Arg Cys Pro Gly Gly Gly Ser Ser Gly Gly
        115                 120                 125

Gly Ser Gly Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
    130                 135                 140

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
145                 150                 155                 160

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly
                165                 170                 175

Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp
            180                 185                 190

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
        195                 200                 205

Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His
    210                 215                 220

Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Gly Leu
225                 230                 235                 240

Gly Gly Leu Met His Gly Asp Thr Pro Thr Leu His Glu Tyr Met Leu
                245                 250                 255

Asp Leu Gln Pro Glu Thr Thr Asp Leu Tyr Gly Tyr Gly Gln Leu Asn
```

260 265 270

Asp Ser Ser Glu Glu Glu Asp Glu Ile Asp Gly Pro Ala Gly Gln Ala
275 280 285

Glu Pro Asp Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys
290 295 300

Asp Ser Thr Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg
305 310 315 320

Thr Leu Glu Asp Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile
325 330 335

Cys Ser Gln Lys Pro Gly Gly Gly Ser Ser Gly Gly Gly Ser Gly Pro
340 345 350

Ser Lys Pro Ser Phe Gln Glu Phe Val Asp Trp Glu Asn Val Ser Pro
355 360 365

Glu Leu Asn Ser Thr Asp Gln Pro Phe Leu Gly Gly Gly Gly Ser Gly
370 375 380

Gly Gly Gly Ser Arg Glu Gly Val Glu Leu Cys Pro Gly Asn Lys Tyr
385 390 395 400

Glu Met Arg Arg His Gly Thr Thr His Ser Leu Val Ile His Asp Gly
405 410 415

Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser His Cys His Trp Asn Asp
420 425 430

Leu Ala Val Ile Pro Ala Gly Val Val His Asn Trp Asp Phe Glu Pro
435 440 445

Arg Lys Val Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Arg
450 455 460

Gly His Leu Leu Gly Arg Leu Ala Ala Ile Val Gly Lys Gln Val Leu
465 470 475 480

Leu Gly Arg Lys Val Val Val Val Arg Gly Gly Gly Gly Ser Gly Gly
485 490 495

Gly Gly Ser Phe Arg Arg Lys Ala Phe Leu His Trp Tyr Thr Gly Glu
500 505 510

Ala Met Asp Glu Met Glu Phe Thr Glu Ala Glu Ser Asn Met Gly Gly
515 520 525

Gly Gly Ser Gly Gly Gly Gly Ser Val Val Asp Arg Asn Pro Gln Phe

530 535 540

Leu Asp Pro Val Leu Ala Tyr Leu Met Lys Gly Leu Cys Glu Lys Pro
545 550 555 560

Leu Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Ser Pro
565 570 575

Asp Glu Val Ala Leu Val Glu Gly Val Gln Ser Leu Gly Phe Thr Tyr
580 585 590

Leu Arg Leu Lys Asp Asn Tyr Met Gly Gly Gly Gly Ser Gly Gly Gly
595 600 605

Gly Ser Glu Phe Lys His Ile Lys Ala Phe Asp Arg Thr Phe Ala Asn
610 615 620

Asn Pro Gly Pro Met Val Val Phe Ala Thr Pro Gly Met Gly Gly Gly
625 630 635 640

Gly Ser Gly Gly Gly Gly Ser Ser Thr Ala Asn Tyr Asn Thr Ser His
645 650 655

Leu Asn Asn Asp Val Trp Gln Ile Phe Glu Asn Pro Val Asp Trp Lys
660 665 670

Glu Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ser Gly Ser
675 680 685

Pro Phe Pro Ala Ala Val Ile Leu Arg Asp Ala Leu His Met Ala Arg
690 695 700

Gly Leu Lys Tyr Leu His Gln Gly Gly Gly Gly Ser Gly Gly Gly Gly
705 710 715 720

Ser Ala Asn Phe Glu Ser Gly Lys His Lys Tyr Arg Gln Thr Ala Met
725 730 735

Phe Thr Ala Thr Met Pro Pro Ala Val Glu Arg Leu Gly Gly Gly Gly
740 745 750

Ser Gly Gly Gly Gly Ser Asn His Ser Gly Leu Val Thr Phe Gln Ala
755 760 765

Phe Ile Asp Val Met Ser Arg Glu Thr Thr Asp Thr Asp Thr Ala Asp
770 775 780

Gln Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Cys Gly Thr Ala Phe
785 790 795 800

Phe Ile Asn Phe Ile Ala Ile Tyr His His Ala Ser Arg Ala Ile Pro

805 810 815

Phe Gly Thr Met Val Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
820 825 830

Phe Val Val Lys Ala Tyr Leu Pro Val Asn Glu Ser Phe Ala Phe Thr
835 840 845

Ala Asp Leu Arg Ser Asn Thr Gly Gly Gln Ala Gly Gly Gly Gly Ser
850 855 860

Gly Gly Gly Gly Ser Thr Pro Pro Pro Glu Glu Ala Met Pro Phe Glu
865 870 875 880

Phe Asn Gly Pro Ala Gln Gly Asp His Ser Gln Pro Pro Leu Gln Val
885 890 895

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Pro Lys Pro Asp Phe Ser
900 905 910

Gln Leu Gln Arg Asn Ile Leu Pro Ser Asn Pro Arg Val Thr Arg Phe
915 920 925

His Ile Asn Trp Asp Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ile
930 935 940

Pro Ser Gly Thr Thr Ile Leu Asn Cys Phe His Asp Val Leu Ser Gly
945 950 955 960

Lys Leu Ser Gly Gly Ser Pro Gly Val Pro Gly Gly Gly Gly Ser Gly
965 970 975

Gly Gly Gly Ser Gly Phe Ser Gln Pro Leu Arg Arg Leu Val Leu His
980 985 990

Val Val Ser Ala Ala Gln Ala Glu Arg Leu Ala Arg Ala Glu Glu Gly
995 1000 1005

Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Cys Arg Ile Thr Ser
1010 1015 1020

Asn Phe Val Ile Pro Ser Glu Tyr Trp Val Glu Glu Lys Glu Glu
1025 1030 1035

Lys Gln Lys Leu Ile Gln Gly Gly Gly Gly Ser Gly Gly Gly Gly
1040 1045 1050

Ser Asn Ile Glu Gly Ile Asp Lys Leu Thr Gln Leu Lys Lys Pro
1055 1060 1065

Phe Leu Val Asn Asn Lys Ile Asn Lys Ile Glu Asn Ile

1070 1075 1080

<210> 48
<211> 896
<212> PRT
<213> Artificial Sequence

<220>
<223> VB construct

<400> 48

Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala
1 5 10 15

Leu Cys Asn Gln Val Leu Ser Ala Pro Leu Ala Ala Asp Thr Pro Thr
20 25 30

Ala Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile
35 40 45

Ala Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Ser Val Ile
50 55 60

Phe Leu Thr Lys Arg Gly Arg Gln Val Cys Ala Asp Pro Ser Glu Glu
65 70 75 80

Trp Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala Glu Leu Lys
85 90 95

Thr Pro Leu Gly Asp Thr Thr His Thr Ile Glu Pro Lys Ser Cys Asp
100 105 110

Thr Pro Pro Pro Cys Pro Arg Cys Pro Gly Gly Gly Ser Ser Gly Gly
115 120 125

Gly Ser Gly Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
130 135 140

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
145 150 155 160

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly
165 170 175

Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp
180 185 190

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
195 200 205

Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His
210 215 220

```
Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Gly Leu
225                 230                 235                 240

Gly Gly Leu Met His Gly Asp Thr Pro Thr Leu His Glu Tyr Met Leu
                245                 250                 255

Asp Leu Gln Pro Glu Thr Thr Asp Leu Tyr Gly Tyr Gly Gln Leu Asn
            260                 265                 270

Asp Ser Ser Glu Glu Glu Asp Glu Ile Asp Gly Pro Ala Gly Gln Ala
        275                 280                 285

Glu Pro Asp Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys
    290                 295                 300

Asp Ser Thr Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg
305                 310                 315                 320

Thr Leu Glu Asp Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile
                325                 330                 335

Cys Ser Gln Lys Pro Gly Gly Gly Ser Ser Gly Gly Gly Ser Gly Val
            340                 345                 350

Ile Leu Pro Gln Ala Pro Ser Gly Pro Ser Tyr Ala Thr Tyr Leu Gln
        355                 360                 365

Pro Ala Gln Ala Gln Met Leu Thr Pro Pro Gly Gly Gly Gly Ser Gly
    370                 375                 380

Gly Gly Gly Ser Leu His Ser Gly Gln Asn His Leu Lys Glu Met Ala
385                 390                 395                 400

Ile Ser Val Leu Glu Ala Arg Ala Cys Ala Ala Ala Gly Gln Ser Gly
                405                 410                 415

Gly Gly Gly Ser Gly Gly Gly Gly Ser Pro Leu Leu Pro Phe Tyr Pro
            420                 425                 430

Pro Asp Glu Ala Leu Glu Ile Gly Leu Glu Leu Asn Ser Ser Ala Leu
        435                 440                 445

Pro Pro Thr Glu Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Gly
    450                 455                 460

Thr Gln Cys Glu Tyr Trp Ala Ser Arg Ala Leu Asp Ser Glu His Ser
465                 470                 475                 480

Ile Gly Ser Met Ile Gln Leu Pro Gln Gly Gly Gly Gly Ser Gly Gly
                485                 490                 495
```

```
Gly Gly Ser Ala Ala Tyr Lys Gly His His Tyr Pro Gly Pro Gly Asn
            500                 505                 510

Tyr Phe Trp Lys Cys Leu Phe Met Ser Gly Leu Ser Glu Val Gly Gly
        515                 520                 525

Gly Gly Ser Gly Gly Gly Gly Ser Asp Thr Leu Ser Ala Met Ser Asn
    530                 535                 540

Pro Arg Ala Met Gln Val Leu Leu Gln Ile Gln Gln Gly Leu Gln Thr
545                 550                 555                 560

Leu Ala Thr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Lys Pro
                565                 570                 575

Leu Arg Arg Asn Asn Ser Tyr Thr Ser Tyr Ile Met Ala Ile Cys Gly
            580                 585                 590

Met Pro Leu Asp Ser Phe Arg Ala Gly Gly Gly Gly Ser Gly Gly Gly
        595                 600                 605

Gly Ser Glu Val Ile Gln Thr Ser Lys Tyr Tyr Met Arg Asp Val Ile
    610                 615                 620

Ala Ile Glu Ser Ala Trp Leu Leu Glu Leu Ala Pro His Gly Gly Gly
625                 630                 635                 640

Gly Ser Gly Gly Gly Gly Ser Gly Tyr Ile Ser Arg Val Thr Ala Gly
                645                 650                 655

Lys Asp Ser Tyr Ile Ala Leu Val Asp Lys Asn Ile Met Gly Tyr Ile
            660                 665                 670

Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu His Ile His
        675                 680                 685

Arg Ala Gly Gly Leu Phe Val Ala Asp Ala Ile Gln Val Gly Phe Gly
    690                 695                 700

Arg Ile Gly Lys His Phe Trp Gly Gly Gly Gly Ser Gly Gly Gly Gly
705                 710                 715                 720

Ser Gln Ala Ile Val Arg Gly Cys Ser Met Pro Gly Pro Trp Arg Ser
                725                 730                 735

Gly Arg Leu Leu Val Ser Arg Arg Trp Ser Val Glu Gly Gly Gly Gly
            740                 745                 750

Ser Gly Gly Gly Gly Ser Asp Gly Gln Leu Glu Leu Leu Ala Gln Gly
        755                 760                 765
```

```
Ala Leu Asp Asn Ala Leu Ser Ser Met Gly Ala Leu His Ala Leu Arg
    770                 775                 780

Pro Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser His Asp Ser Arg
785                 790                 795                 800

Lys Ser Thr Ser Phe Met Ser Val Asn Pro Ser Lys Glu Ile Lys Ile
                805                 810                 815

Val Ser Ala Val Arg Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
            820                 825                 830

His Thr Pro Ser Ser Tyr Ile Glu Thr Leu Pro Lys Ala Ile Lys Arg
        835                 840                 845

Arg Ile Asn Ala Leu Lys Gln Leu Gln Val Arg Gly Gly Gly Gly Ser
    850                 855                 860

Gly Gly Gly Gly Ser Met Lys Ala Phe Ile Phe Lys Tyr Ser Ala Lys
865                 870                 875                 880

Thr Gly Phe Thr Lys Leu Ile Asp Ala Ser Arg Val Ser Glu Thr Glu
                885                 890                 895
```

<210> 49
<211> 1081
<212> PRT
<213> Artificial Sequence

<220>
<223> VB construct

<400> 49

```
Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala
1               5                   10                  15

Leu Cys Asn Gln Val Leu Ser Ala Pro Leu Ala Ala Asp Thr Pro Thr
            20                  25                  30

Ala Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile
        35                  40                  45

Ala Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Ser Val Ile
    50                  55                  60

Phe Leu Thr Lys Arg Gly Arg Gln Val Cys Ala Asp Pro Ser Glu Glu
65                  70                  75                  80

Trp Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala Glu Leu Lys
                85                  90                  95

Thr Pro Leu Gly Asp Thr Thr His Thr Ile Glu Pro Lys Ser Cys Asp
```

```
            100                 105                 110

Thr Pro Pro Pro Cys Pro Arg Cys Pro Gly Gly Gly Ser Ser Gly Gly
        115                 120                 125

Gly Ser Gly Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
    130                 135                 140

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
145                 150                 155                 160

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly
                165                 170                 175

Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp
            180                 185                 190

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
        195                 200                 205

Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His
    210                 215                 220

Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Gly Leu
225                 230                 235                 240

Gly Gly Leu Met His Gly Asp Thr Pro Thr Leu His Glu Tyr Met Leu
                245                 250                 255

Asp Leu Gln Pro Glu Thr Thr Asp Leu Tyr Gly Tyr Gly Gln Leu Asn
            260                 265                 270

Asp Ser Ser Glu Glu Glu Asp Glu Ile Asp Gly Pro Ala Gly Gln Ala
        275                 280                 285

Glu Pro Asp Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys
    290                 295                 300

Asp Ser Thr Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg
305                 310                 315                 320

Thr Leu Glu Asp Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile
                325                 330                 335

Cys Ser Gln Lys Pro Gly Gly Gly Ser Ser Gly Gly Gly Ser Gly Val
            340                 345                 350

Ile Leu Pro Gln Ala Pro Ser Gly Pro Ser Tyr Ala Thr Tyr Leu Gln
        355                 360                 365

Pro Ala Gln Ala Gln Met Leu Thr Pro Pro Gly Gly Gly Gly Ser Gly
```

370 375 380

Gly Gly Gly Ser Leu His Ser Gly Gln Asn His Leu Lys Glu Met Ala
385 390 395 400

Ile Ser Val Leu Glu Ala Arg Ala Cys Ala Ala Ala Gly Gln Ser Gly
405 410 415

Gly Gly Gly Ser Gly Gly Gly Gly Ser Pro Leu Leu Pro Phe Tyr Pro
420 425 430

Pro Asp Glu Ala Leu Glu Ile Gly Leu Glu Leu Asn Ser Ser Ala Leu
435 440 445

Pro Pro Thr Glu Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Gly
450 455 460

Thr Gln Cys Glu Tyr Trp Ala Ser Arg Ala Leu Asp Ser Glu His Ser
465 470 475 480

Ile Gly Ser Met Ile Gln Leu Pro Gln Gly Gly Gly Gly Ser Gly Gly
485 490 495

Gly Gly Ser Ala Ala Tyr Lys Gly His His Tyr Pro Gly Pro Gly Asn
500 505 510

Tyr Phe Trp Lys Cys Leu Phe Met Ser Gly Leu Ser Glu Val Gly Gly
515 520 525

Gly Gly Ser Gly Gly Gly Gly Ser Asp Thr Leu Ser Ala Met Ser Asn
530 535 540

Pro Arg Ala Met Gln Val Leu Leu Gln Ile Gln Gln Gly Leu Gln Thr
545 550 555 560

Leu Ala Thr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Lys Pro
565 570 575

Leu Arg Arg Asn Asn Ser Tyr Thr Ser Tyr Ile Met Ala Ile Cys Gly
580 585 590

Met Pro Leu Asp Ser Phe Arg Ala Gly Gly Gly Gly Ser Gly Gly Gly
595 600 605

Gly Ser Glu Val Ile Gln Thr Ser Lys Tyr Tyr Met Arg Asp Val Ile
610 615 620

Ala Ile Glu Ser Ala Trp Leu Leu Glu Leu Ala Pro His Gly Gly Gly
625 630 635 640

Gly Ser Gly Gly Gly Gly Ser Gly Tyr Ile Ser Arg Val Thr Ala Gly

645 650 655

Lys Asp Ser Tyr Ile Ala Leu Val Asp Lys Asn Ile Met Gly Tyr Ile
660 665 670

Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu His Ile His
675 680 685

Arg Ala Gly Gly Leu Phe Val Ala Asp Ala Ile Gln Val Gly Phe Gly
690 695 700

Arg Ile Gly Lys His Phe Trp Gly Gly Gly Gly Ser Gly Gly Gly Gly
705 710 715 720

Ser Gln Ala Ile Val Arg Gly Cys Ser Met Pro Gly Pro Trp Arg Ser
725 730 735

Gly Arg Leu Leu Val Ser Arg Arg Trp Ser Val Glu Gly Gly Gly Gly
740 745 750

Ser Gly Gly Gly Gly Ser Asp Gly Gln Leu Glu Leu Leu Ala Gln Gly
755 760 765

Ala Leu Asp Asn Ala Leu Ser Ser Met Gly Ala Leu His Ala Leu Arg
770 775 780

Pro Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser His Asp Ser Arg
785 790 795 800

Lys Ser Thr Ser Phe Met Ser Val Asn Pro Ser Lys Glu Ile Lys Ile
805 810 815

Val Ser Ala Val Arg Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
820 825 830

His Thr Pro Ser Ser Tyr Ile Glu Thr Leu Pro Lys Ala Ile Lys Arg
835 840 845

Arg Ile Asn Ala Leu Lys Gln Leu Gln Val Arg Gly Gly Gly Gly Ser
850 855 860

Gly Gly Gly Gly Ser Met Lys Ala Phe Ile Phe Lys Tyr Ser Ala Lys
865 870 875 880

Thr Gly Phe Thr Lys Leu Ile Asp Ala Ser Arg Val Ser Glu Thr Glu
885 890 895

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Gly Asp Pro Cys Leu
900 905 910

Arg Ser Ser Asp Cys Ile Asp Glu Phe Cys Cys Ala Arg His Phe Trp

915 920 925

Thr Lys Ile Cys Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Trp
930 935 940

Lys Gly Gly Pro Val Lys Ile Asp Pro Leu Ala Leu Met Gln Ala Ile
945 950 955 960

Glu Arg Tyr Leu Val Val Arg Gly Tyr Gly Gly Gly Gly Gly Ser Gly
965 970 975

Gly Gly Gly Ser Val Thr Ser Ile Pro Ser Val Ser Asn Ala Leu Asn
980 985 990

Trp Lys Glu Phe Ser Phe Ile Gln Ser Thr Leu Gly Tyr Val Ala Gly
995 1000 1005

Gly Gly Gly Ser Gly Gly Gly Gly Ser Tyr Arg Gly Ala Asn Leu
1010 1015 1020

His Leu Glu Glu Thr Leu Ala Gly Phe Trp Ala Arg Leu Leu Glu
1025 1030 1035

Arg Leu Phe Lys Gln Leu Gly Gly Gly Gly Ser Gly Gly Gly Gly
1040 1045 1050

Ser Lys Thr Thr Leu Ser His Thr Gln Asp Ser Ser Gln Ser Leu
1055 1060 1065

Gln Ser Ser Ser Asp Ser Ser Lys Ser Ser Arg Cys Ser
1070 1075 1080

<210> 50
<211> 27
<212> PRT
<213> Mus musculus

<400> 50

Gln Ala Ile Val Arg Gly Cys Ser Met Pro Gly Pro Trp Arg Ser Gly
1 5 10 15

Arg Leu Leu Val Ser Arg Arg Trp Ser Val Glu
20 25

<210> 51
<211> 27
<212> PRT
<213> Mus musculus

<400> 51

Asp Gly Gln Leu Glu Leu Leu Ala Gln Gly Ala Leu Asp Asn Ala Leu
1 5 10 15

Ser Ser Met Gly Ala Leu His Ala Leu Arg Pro
20 25

<210> 52
<211> 27
<212> PRT
<213> Mus musculus

<400> 52

Ser His Asp Ser Arg Lys Ser Thr Ser Phe Met Ser Val Asn Pro Ser
1 5 10 15

Lys Glu Ile Lys Ile Val Ser Ala Val Arg Arg
20 25

<210> 53
<211> 27
<212> PRT
<213> Mus musculus

<400> 53

His Thr Pro Ser Ser Tyr Ile Glu Thr Leu Pro Lys Ala Ile Lys Arg
1 5 10 15

Arg Ile Asn Ala Leu Lys Gln Leu Gln Val Arg
20 25

<210> 54
<211> 27
<212> PRT
<213> Mus musculus

<400> 54

Met Lys Ala Phe Ile Phe Lys Tyr Ser Ala Lys Thr Gly Phe Thr Lys
1 5 10 15

Leu Ile Asp Ala Ser Arg Val Ser Glu Thr Glu
20 25

<210> 55
<211> 27
<212> PRT
<213> Mus musculus

<400> 55

Glu Gly Asp Pro Cys Leu Arg Ser Ser Asp Cys Ile Asp Glu Phe Cys
1 5 10 15

Cys Ala Arg His Phe Trp Thr Lys Ile Cys Lys
20 25

<210> 56
<211> 27
<212> PRT

<213> Mus musculus

<400> 56

Trp Lys Gly Gly Pro Val Lys Ile Asp Pro Leu Ala Leu Met Gln Ala
1 5 10 15

Ile Glu Arg Tyr Leu Val Val Arg Gly Tyr Gly
20 25

<210> 57
<211> 27
<212> PRT
<213> Mus musculus

<400> 57

Val Thr Ser Ile Pro Ser Val Ser Asn Ala Leu Asn Trp Lys Glu Phe
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Ser Phe Ile Gln Ser Thr Leu Gly Tyr Val Ala
20 25

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Tyr Arg Gly Ala Asn Leu His Leu Glu Glu Thr Leu Ala Gly Phe Trp
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Ala Arg Leu Leu Glu Arg Leu Phe Lys Gln Leu
20 25

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<400> 59

Lys Thr Thr Leu Ser His Thr Gln Asp Ser Ser Gln Ser Leu Gln Ser
1 5 10 15

Ser Ser Asp Ser Ser Lys Ser Ser Arg Cys Ser
20 25

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Asn His Ser Gly Leu Val Thr Phe Gln Ala Phe Ile Asp Val Met Ser
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Arg Glu Thr Thr Asp Thr Asp Thr Ala Asp Gln
20 25

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<400> 61

Cys Gly Thr Ala Phe Phe Ile Asn Phe Ile Ala Ile Tyr His His Ala
1 5 10 15

Ser Arg Ala Ile Pro Phe Gly Thr Met Val Ala
20 25

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Phe Val Val Lys Ala Tyr Leu Pro Val Asn Glu Ser Phe Ala Phe Thr
1 5 10 15

Ala Asp Leu Arg Ser Asn Thr Gly Gly Gln Ala
20 25

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<400> 63

Thr Pro Pro Pro Glu Glu Ala Met Pro Phe Glu Phe Asn Gly Pro Ala
1 5 10 15

Gln Gly Asp His Ser Gln Pro Pro Leu Gln Val
20 25

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Pro Lys Pro Asp Phe Ser Gln Leu Gln Arg Asn Ile Leu Pro Ser Asn
1 5 10 15

Pro Arg Val Thr Arg Phe His Ile Asn Trp Asp
20 25

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Ile Pro Ser Gly Thr Thr Ile Leu Asn Cys Phe His Asp Val Leu Ser
1 5 10 15

Gly Lys Leu Ser Gly Gly Ser Pro Gly Val Pro
20 25

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<400> 66

Gly Phe Ser Gln Pro Leu Arg Arg Leu Val Leu His Val Val Ser Ala
1 5 10 15

Ala Gln Ala Glu Arg Leu Ala Arg Ala Glu Glu
20 25

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<211> 27
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<400> 67

Glu Cys Arg Ile Thr Ser Asn Phe Val Ile Pro Ser Glu Tyr Trp Val
1 5 10 15

Glu Glu Lys Glu Glu Lys Gln Lys Leu Ile Gln
20 25

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<400> 68

Asn Ile Glu Gly Ile Asp Lys Leu Thr Gln Leu Lys Lys Pro Phe Leu
1 5 10 15

Val Asn Asn Lys Ile Asn Lys Ile Glu Asn Ile
20 25

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<400> 69

Gly Gly Gly Ser Ser
1 5

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<400> 70

Gly Gly Gly Ser Gly
1   5

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Gly Gly Gly Gly Ser
1   5

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Leu Gly Gly Gly Ser
1   5

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Gly Leu Gly Gly Ser
1   5

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Gly Gly Leu Gly Ser
1 5

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Gly Gly Gly Leu Ser
1 5

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Gly Gly Gly Gly Leu
1 5

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Leu Gly Gly Ser Gly
1 5

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Gly Leu Gly Ser Gly
1 5

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Gly Gly Leu Ser Gly
1 5

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Gly Gly Gly Leu Gly
1 5

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Gly Gly Gly Ser Leu
1 5

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Leu Gly Gly Ser Ser
1 5

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Gly Leu Gly Ser Ser
1 5

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Gly Gly Leu Ser Ser
1 5

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Gly Gly Gly Leu Ser
1 5

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Gly Gly Gly Ser Leu
1 5

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Leu Gly Leu Gly Ser
1 5

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Gly Leu Gly Leu Ser
1 5

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Gly Leu Leu Gly Ser
1   5

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Leu Gly Gly Leu Ser
1   5

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Gly Leu Gly Gly Leu
1   5

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Leu Gly Leu Ser Gly
1   5

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Gly Leu Leu Ser Gly
1   5

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<211> 5

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Gly Gly Leu Ser Leu
1 5

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Gly Gly Leu Leu Gly
1 5

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<211> 5
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Gly Leu Gly Ser Leu
1 5

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Leu Gly Leu Ser Ser
1 5

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Gly Leu Gly Leu Ser
1 5

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Gly Gly Leu Leu Ser
1 5

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Gly Leu Gly Ser Leu
1 5

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Gly Leu Gly Ser Leu
1 5

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Leu Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10

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Gly Leu Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10

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Gly Gly Leu Gly Ser Gly Gly Gly Gly Ser
1 5 10

<210> 105
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Gly Gly Gly Leu Ser Gly Gly Gly Gly Ser
1 5 10

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Gly Gly Gly Gly Leu Gly Gly Gly Gly Ser
1 5 10

<210> 107
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Leu Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10

<210> 108
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<400> 108

eolf-seql
Gly Leu Gly Ser Gly Gly Gly Gly Ser Gly
1   5   10

<210> 109
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Gly Gly Leu Ser Gly Gly Gly Gly Ser Gly
1   5   10

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Gly Gly Gly Leu Gly Gly Gly Gly Ser Gly
1   5   10

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Gly Gly Gly Ser Leu Gly Gly Gly Ser Gly
1   5   10

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Gly Gly Gly Ser Leu Gly Gly Gly Ser Gly
1   5   10

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Gly Leu Gly Ser Ser Gly Gly Gly Ser Ser
1 5 10

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Gly Gly Leu Ser Ser Gly Gly Gly Ser Ser
1 5 10

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1 5 10

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Gly Gly Gly Ser Leu Gly Gly Gly Ser Ser
1 5 10

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Leu Gly Gly Gly Ser Leu Gly Gly Gly Ser
1 5 10

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Gly Leu Gly Gly Ser Gly Leu Gly Gly Ser
1 5 10

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1 5 10

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1 5 10

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Gly Gly Gly Gly Leu Gly Gly Gly Gly Leu
1 5 10

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Leu Gly Gly Ser Gly Leu Gly Gly Ser Gly
1 5 10

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Gly Leu Gly Ser Gly Gly Leu Gly Ser Gly
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Gly Gly Leu Ser Gly Gly Gly Leu Ser Gly
1 5 10

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Gly Gly Gly Leu Gly Gly Gly Gly Leu Gly
1 5 10

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Gly Gly Gly Ser Leu Gly Gly Gly Ser Leu
1 5 10

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