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(71) Applicants:
- **Deutsches Krebsforschungszentrum Stiftung des öffentlichen Rechts 69120 Heidelberg (DE)**
- **Ruprecht-Karls-Universität Heidelberg 69117 Heidelberg (DE)**

(72) Inventors:
- **Schirrmacher, Volker 69177 Heidelberg (DE)**
- **Beckhove, Philipp 69120 Heidelberg (DE)**
- **Sommerfeldt, Nora 69124 Eppelheim (DE)**

(74) Representative: **Isenbruck, Günter, Dr.**
Isenbruck, Bösl, Hörschler, Wichmann, Huhn, Patentanwälte
Theodor-Heuss-Anlage 12
68165 Mannheim (DE)

(54) **Heparanase-derived peptides for vaccination of tumor patients**

(57) Disclosed is a vaccine against diseases, particularly tumor diseases, being associated with an enhanced heparanase expression and/or activity, wherein the vaccine contains a heparanase peptide, which binds to a HLA molecule.

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Description

[0001] The present invention refers to nonapeptides derived from human heparanase which are useful for the therapeutic vaccination of tumor patients as well as for generating specific immune cells for cell therapies. Furthermore, the present nonapeptides can be employed in a method to increase the immune reaction of a patient against a key enzyme in metastasis.

[0002] The successful invasion of malignant tumor cells into the basement membrane represents an important step for the generation of tumor metastases. The basement membrane and the extracellular matrix (ECM) form the barriers between different tissues. These structures contain complex macromolecules, for example type IV-collagen, laminin, heparan sulfate-proteoglycan and fibronectin. The process of tumor invasion and metastasis involves a variety of proteinases which degrade said components of the ECM and the basement membrane and, as a consequence, enable the migration of foreign cells into the surrounding affected tissue or organ.

[0003] Heparan sulfate (HS) and heparan sulfate-proteoglycans (HSPG) are present on the extracelluar surface and within the ECM. The HS-chains play a major role in cell to cell and cell to matrix interactions which are involved in various physiological and non-physiological processes. Examples of such processes are adhesion, migration, differentiation and proliferation of cells. Several molecules interact with HS and/or HSPG, like for example growth factors (e.g. FGF, PDGF; VEGF), cytokines (IL-2), extracellular matrix proteins (fibronectin, collagen), factors involved in hemostasis (heparin-cofactor II), or other molecules like e.g. lipoproteins, DNA topoisomerases and a β-amyloid proteins. Thus, it becomes evident that enzymes which modulate HS and/or HSPG may play a pivotal role in any of the above described processes.

[0004] A known HS/HSPG-modulating enzyme which has been identified in murine metastatic melanomal cells is heparanase, an endo-β-glucuronidase. Heparanase cleaves HS into characteristic fragments with a high molecular weight. This activity correlates with the metastatic potential of melanoma cells. An increased heparanase activity has also been demonstrated in other mobile, invasive cells, for example in relationship with lymphomas, mastocytomas, adenocarcinomas, leukemias and rheumatoid fibroblasts.

[0005] Based on the observations described in a prior art the problem underlying the present invention refers to the identification of new molecules that would interfere with heparanase expression and/or activity and, thus, prevent an undesired migration of cells into the neighboring tissue, like it is the case for metastases.

[0006] Heparanase-derived peptides and nucleic acids which may exhibit heparanase-inhibiting properties are known to the person skilled in the art. For example, WO 99/21975 describes an immunologically interactive molecule which is capable of binding to and/or inhibiting the catalytic activity of a heparanase polypeptide. WO-A 99/40207 discloses antagonists and inhibitors of heparanase which inhibit or eliminate the function of a heparanase polypeptide. As an example for an antagonist, an antibody against heparanase is described. As an example of an inhibitor, a small molecule inhibitor which inactivates heparanase by binding to and occupying the catalytic site, thereby making the catalytic site inaccessible to a substrate such that the biological activity of heparanase is prevented, is described. It is further illustrated in WO-A 99/40207 that such antagonists and inhibitors may be used to treat cancer, angiogenesis by preventing heparanase from functioning to breakdown extracellular matrix and release heparan sulfate from extracellular matrix and cell surface. Furthermore, DE 199 55 803 describes heparanase inhibitors which inhibit the enzymatic activity of heparanase or its expression. According to the invention, these inhibitors bind to heparanase or to heparanase coding nucleic acids in order to be useful in the treatment of disfunctions of the heart.

[0007] In none of the documents known in the state of the art peptides were identified which can be employed for vaccination even though the tumor-associated antigen heparanase is highly over-expressed on the surface of tumor cells. Tumor vaccination represents an efficient therapy method which relies on the induction of a tumor-specific immune response. Through vaccination with tumor-specific antigens the own immune system should be enabled to recognize and destroy residual tumor cells. For example, lymphomas are successfully treated with this kind of therapy from the beginning of the eighties. It is known in the art that a vaccination with tumor-specific antigens increases the frequency of tumor-specific T-cells which mediate the destruction of the tumor carrying the antigen on the cellular surface.

[0008] Only a small portion of tumor patients possesses pre-formed memory T-cells against the tumor peptides known from the prior art (for example MUC1, or Her2neu). However, it could be assumed that successful therapeutic vaccination strategies may depend on the prevalence of pre-formed peptide-specific memory T cells. A low number of memory T-cells may be the reason for a rather weak response of tumor patients to peptide vaccination described so far in the prior art.

[0009] It is therefore an object of the present invention to provide a vaccine against diseases, preferably tumor diseases, being accompanied with an increased heparanase expression, which overcome the disadvantages of the presently known vaccines namely the relatively weak induction of an immune response and the low abundance of memory T cells. The invention is based on the cognition that such a vaccine can be obtained by identifying heparanase-derived peptides which exhibit a high binding capacity to HLA-A2 (Human Leukocyte Antigen type A2), a type of so-called class I histocompatibility molecules (MHC class I). MHC class I molecules with bound peptides/antigens are

commonly presented on the surface of cells, which are then recognized and destroyed by so-called cytotoxic T-cells ($CD8^+$-cells, T_{KILLER} cells) of the immune system.

[0010] The applicant has identified characteristic nonapeptides derived from the heparanase molecule against which the majority of female patients with breast cancer possess pre-formed memory T-cells. In contrast, only for 10 % of the same female patients memory T-cells with specificity against the currently used peptides derived from MUC and Her2neu antigens could be detected. Memory T-cells stay in a resting state, until encountering the peptide-MHC complex they recognize (e.g. during a re-infection with the same antigen), whereupon they become mature $CD8^+$-cells. This indicates a particular immunological relevance and a therapeutic potential of the peptides of the present invention.

[0011] Thus, the object of the present invention is a vaccine against a disease being associated with an enhanced heparanase expression and/or activity, wherein the vaccine contains a heparanase peptide, or a functional variant thereof, which binds to a HLA molecule. In a preferred embodiment of the present invention, the disease being associated with an enhanced heparanase expression and/or activity is a metastatic tumor.

[0012] The term "HLA molecule" encompasses both MHC class I and MHC class II molecules, both of which are encoded by at least three different HLA genes.

[0013] The person skilled in the art knows three HLA genes encoding MHC class I molecules: HLA-A, HLA-B and HLA-C, all of which are included in the present invention. Preferably, the heparanase peptide binds to HLA-A-encoded molecules. Most preferred it binds to MHC class I molecules from the HLA-A2 allele, which is expressed on the cell surface of 50% of the Northern European population.

[0014] In an embodiment of the present invention, the vaccine contains at least one heparanase peptide selected from the group consisting of SEQ ID NOs: 1-505 (see also DRAWING, Table 1). Preferably, the vaccine contains at least one heparanase peptide selected from the group consisting of SEQ ID NOs: 1-187 (binding score 31 to 12). Even more preferably, the vaccine contains at least one heparanase peptide selected from the group consisting of SEQ ID NOs: 1-92 (binding score 31 to 16).

[0015] In the most preferred embodiment of the present invention, the vaccine contains at least one heparanase peptide selected from the group consisting of SEQ ID NOs: 1, 2, and 3 (binding score 31 to 28).

[0016] Another object of the present invention is a heparanase peptide, or a functional derivative thereof, that binds to HLA molecule, wherein the heparanase peptide is a nonapeptide having the sequence selected from the group consisting of heparanase peptide that binds to HLA molecule, wherein the heparanase peptide is a nonapeptide having the sequence selected from the group consisting of SEQ ID NOs:1-505, preferably SEQ ID NOs:1-187, more preferably SEQ ID NOs: 1-92 and most preferably SEQ ID NOs: 1-3.

[0017] Furthermore, the person skilled in the art is aware of three HLA genes encoding MHC class II molecules: HLA-DP, HLA-DQ and HLA-DR, all of which are included in the present invention. MHC class II molecules with bound peptides/antigens are also presented on the surface of antigen presenting cells; however, in contrast to MHC class I, these cells are then recognized by so-called helper T-cells ($CD4^+$- T cells) of the immune system. Thus, a heparanase peptide which binds to MHC class II molecules, as depicted e.g. in SEQ ID NOs: 506-980 (see also DRAWING, Table 2), induces a $CD4^+$- T cell - mediated immune response.

[0018] All three alleles of MHC class I molecules and all three alleles of MHC class II molecules are referred to hereinafter generally as "HLA" or "HLA molecules".

[0019] In the context of the present invention, a functional variant of a heparanase peptide comprises all compounds which induce an immune response according to the same effect of the heparanase peptide of the present invention.

[0020] More specifically, the functional variant can be a peptide, a fragment or derivative thereof, which differs from the heparanase peptide of the present invention in that one or more amino acids are either deleted, inserted, substituted or otherwise chemically modified (e.g. acetylated, phosphorylated, glycosylated, or myristoylated), provided that the property of the functional variant, namely the induction of T cell specific immune response by binding to HLA molecules is maintained. In this respect, the peptide can be extended or shortened on either the amino- or the carboxyterminal end or internally, or extended on one end and shortened on the other end, provided that the desired function as described is maintained.

[0021] It is also possible that the heparanase peptide of the present invention is conjugated or fused to one or more other peptides or lipids, which may confer a desired property to the heparanase peptide, e.g. for the detection or the purification of the heparanase peptide. For example, the heparanase peptide of the present invention can be fused to a so-called marker which enables the localization of the heparanase peptide in a cell or tissue. Suitable markers include "epitope tags" (like c-myc, hemagglutinin, FLAG-tag), biotin, digoxigenin, (strept-) avidin, Green Fluorescent Protein (GFP, and derivatives thereof), enzymes like horseradish peroxidase, alkaline phosphatase, beta-galactosidase, luciferase, beta-glucuronidase and beta-lactamase. Examples for fusion partners that allow for the purification of the heparanase peptide include HIS-tag and glutathion S transferase (GST).

[0022] For the present invention it can also be useful if the heparanase peptide is fused to an immunogenic carrier or moiety, which can be any macromolecule that enhances the immunogenicity of the vaccine. Examples of such immunogenic carriers include keyhole limpet hemocyanin (KLH), recombinant exoprotein A (rEPA), diphtheria protein

CRM9 and tetanus toxoid (TT).

[0023] The conjugation or fusion of the heparanase peptide to any of the modifying compounds described supra can occur by any suitable method known to the skilled artisan, either by chemical or gene technological methods. The latter requires, that a nucleic acid coding for the whole fusion construct is inserted into an expression vector and expressed as an entity.

[0024] Furthermore, in order to deliver the heparanase peptide directly to or into the target cell it can be fused to a carrier peptide that mediates the cellular uptake of the peptide. Appropriate carriers are known to the person skilled in the art and include TAT, fibroblast growth factor, galparan (transportan), poly-arginine, and Pep-1. Furthermore, the heparanase peptide may be fused to a ligand for a cell surface receptor, or a functional portion thereof, and thus internalized by receptor-mediated endocytosis.

[0025] In a further embodiment, the functional variant of the heparanase peptide also encompasses nucleic acids, DNA or RNA, which encode the heparanase peptides, or their functional peptide variants, of the present invention. There are several well-known methods of introducing nucleic acids into animal cells, any of which may be used in the present invention and which depend on the host. Typical hosts include mammalian species, such as humans, non-human primates, dogs, cats, cattle, horses, sheep, and the like. At the simplest, the nucleic acid can be directly injected into the target cell / target tissue, or by so-called microinjection into the nucleus. Other methods include fusion of the recipient cell with bacterial protoplasts containing the nucleic acid, the use of compositions like calcium chloride, rubidium chloride, lithium chloride, calcium phosphate, DEAE dextran, cationic lipids or liposomes or methods like receptor-mediated endocytosis, biolistic particle bombardment ("gene gun" method), infection with viral vectors, electroporation, and the like.

[0026] For the introduction of the heparanase peptide, respectively the nucleic acid encoding it, into the cell and its expression it can be advantageous if the nucleic acid is integrated in an expression vector. The expression vector is preferably a eukaryotic expression vector, or a retroviral vector, a plasmid, bacteriophage, or any other vector typically used in the biotechnology field. If necessary or desired, the nucleic acid encoding the heparanase peptide can be operatively linked to regulatory elements which direct the transcription and the synthesis of a translatable mRNA in pro- or eukaryotic cells. Such regulatory elements are promoters, enhancers or transcription termination signals, but can also comprise introns or similar elements, for example those, which promote or contribute to the stability and the amplification of the vector, the selection for successful delivery and/or the integration into the host's genome, like regions that promote homologous recombination at a desired site in the genome. For therapeutic purposes, the use of retroviral vectors has been proven to be most appropriate to deliver a desired nucleic acid into a target cell.

[0027] The cell to which the heparanase peptide, a functional variant thereof, or the nucleic acid encoding it, is applied to a professional antigen-presenting cell such as a B cell, a microphage or a dendritic cell, or any other cell within which the heparanase peptide can be loaded onto the HLA molecule and transported to the cell surface and presented as an antigen in order to induce the described immune response.

[0028] In particular, dendritic cells have been proven to be especially useful as vaccination "vehicles". Dendritic cells which are located in nearly all tissue types of the body incorporate a compound like heparanase peptide and migrate together with the lymph stream to the lymph node where they encounter with precursors of antigen-specific cytotoxic T cells. For the purposes of the present invention as well as for therapeutic purposes in general, dendritic cells can be generated and cultured *in vitro* by cultivating monocytes in the presence of Interleukin-4 (IL-4) and Granulocyte Macrophage Colony Stimulating Factor (GM-CSF). Alternatively, dendritic cell can be generated from $CD34^+$ haematopoietic stem cells of the periphery blood. By systematic application of growth factors, like e.g. Flt3 ligand, dendritic cells can also be expanded in the blood *in vivo* by several orders of magnitude. Isolated dendritic or other professional antigen-presenting cells can be loaded ("pulsed") with the heparanase peptide or the nucleic acid encoding it in order to enable the presentation of the heparanase peptide on the surface of these cells.

[0029] For the purpose of the present invention, dendritic or other cells carrying the heparanase peptide can be applied to a tumor patient by different methods of injection: (i) sub-/intra-cutanous, which requires migration to the lymph nodes; (ii) direct intranodal injection into a lymph node, circumventing the migration requirement; and (iii) intravenous injection.

[0030] Particularly useful to determine the frequency of heparanase peptide-specific $CD8^+$ T cells in immunised patients is the tetramer analysis. Such MHC tetramers are complexes of 4 MHC molecules which are associated with heparanase peptide and bound to a fluorochrome, e.g. phycoerythrin. The complexes bind to a distinct set of T cell receptors (TCRs) on the surface of $CD8^+$ T cells. Thus, by mixing tetramers with mononuclear cells from peripheral blood or bone marrow or whole blood of tumor patients and using flow cytometry as a detection system, a count of all T cells that are specific for heparanase is provided. The invention further includes the similar detection by using MHC dimers instead of tetramers.

[0031] The vaccine containing the heparanase peptide, a functional variant thereof, or the nucleic acid encoding it, as disclosed in the present invention can be used as a pharmaceutical. This is a further embodiment of the present invention.

[0032] The vaccine containing the heparanase peptide, a functional variant thereof, or the nucleic acid encoding it can be administered alone or in combination with one or more other active compounds which may aid to increase the immunogenicity of the vaccine. The latter can be administered before, after or simultaneously with the administration of the heparanase peptide, a functional variant thereof, or the nucleic acid encoding it. The dose of either the heparanase peptide, a functional variant thereof, or the nucleic acid encoding it or the active compound as well as the duration and the temperature of incubation can be variable and depends on the target that is to be treated.

[0033] A further object of the present invention are pharmaceutical preparations which comprise an effective dose of vaccine containing at least one heparanase peptide, a functional variant thereof, or the nucleic acid encoding it, optionally in combination with at least one active compound and a pharmaceutically acceptable carrier, i.e. one or more pharmaceutically acceptable carrier substances and/or additives.

[0034] The pharmaceutical/vaccine according to the invention can be administered orally, for example in the form of pills, tablets, lacquered tablets, sugar-coated tablets, granules, hard and soft gelatin capsules, aqueous, alcoholic or oily solutions, syrups, emulsions or suspensions, or rectally, for example in the form of suppositories. Administration can also be carried out parenterally, for example subcutaneously, intramuscularly or intravenously in the form of solutions for injection or infusion. Other suitable administration forms are, for example, percutaneous or topical administration, for example in the form of ointments, tinctures, sprays or transdermal therapeutic systems, or the inhalative administration in the form of nasal sprays or aerosol mixtures, or, for example, microcapsules, implants or rods. The preferred administration form depends, for example, on the disease to be treated and on its severity.

[0035] The preparation of the pharmaceutical compositions can be carried out in a manner known per se. To this end, the heparanase peptide, a functional variant thereof, or the nucleic acid encoding it and/or the active compound, together with one or more solid or liquid pharmaceutical carrier substances and/or additives (or auxiliary substances) and, if desired, in combination with other pharmaceutically active compounds having therapeutic or prophylactic action, are brought into a suitable administration form or dosage form which can then be used as a pharmaceutical in human or veterinary medicine.

[0036] For the production of pills, tablets, sugar-coated tablets and hard gelatin capsules it is possible to use, for example, lactose, starch, for example maize starch, or starch derivatives, talc, stearic acid or its salts, etc. Carriers for soft gelatin capsules and suppositories are, for example, fats, waxes, semisolid and liquid polyols, natural or hardened oils, etc. Suitable carriers for the preparation of solutions, for example of solutions for injection, or of emulsions or syrups are, for example, water, physiological sodium chloride solution, alcohols such as ethanol, glycerol, polyols, sucrose, invert sugar, glucose, mannitol, vegetable oils, etc. It is also possible to lyophilize the heparanase peptide, a functional variant thereof, or the nucleic acid encoding it, and/or the active compound and to use the resulting lyophilisates, for example, for preparing preparations for injection or infusion. Suitable carriers for microcapsules, implants or rods are, for example, copolymers of glycolic acid and lactic acid.

[0037] The pharmaceutical preparations can also contain additives, for example fillers, disintegrants, binders, lubricants, wetting agents, stabilizers, emulsifiers, dispersants, preservatives, sweeteners, colorants, flavorings, aromatizers, thickeners, diluents, buffer substances, solvents, solubilizers, agents for achieving a depot effect, salts for altering the osmotic pressure, coating agents or antioxidants.

[0038] The dosage of the vaccine containing the heparanase peptide, a functional variant thereof, or the nucleic acid encoding it, in combination with one or more active compounds to be administered, depends on the individual case and is, as is customary, to be adapted to the individual circumstances to achieve an optimum effect. Thus, it depends on the nature and the severity of the disorder to be treated, and also on the sex, age, weight and individual responsiveness of the human or animal to be treated, on the efficacy and duration of action of the compounds used, on whether the therapy is acute or chronic or prophylactic, or on whether other active compounds are administered in addition to the heparanase peptide, a functional variant thereof, or the nucleic acid encoding it.

[0039] The vaccine containing the heparanase peptide according to the present invention, or a functional variant thereof, respectively the medicaments containing it, can be used for the treatment of all metastatic and invasive cancer types or tumors exhibiting an increased heparanase expression and/or activity. Examples of such cancer types comprise neuroblastoma, intestine carcinoma such as rectum carcinoma, colon carcinoma, familiary adenomatous polyposis carcinoma and hereditary non-polyposis colorectal cancer, esophageal carcinoma, labial carcinoma, larynx carcinoma, hypopharynx carcinoma, tong carcinoma, salivary gland carcinoma, gastric carcinoma, adenocarcinoma, medullary thyroid carcinoma, papillary thyroid carcinoma, follicular thyroid carcinoma, anaplastic thyroid carcinoma, renal carcinoma, kidney parenchym carcinoma, ovarian carcinoma, cervix carcinoma, uterine corpus carcinoma, endometrium carcinoma, chorion carcinoma, pancreatic carcinoma, prostate carcinoma, testis carcinoma, breast carcinoma, urinary carcinoma, melanoma, brain tumors such as glioblastoma, astrocytoma, meningioma, medulloblastoma and peripheral neuroectodermal tumors, Hodgkin lymphoma, non-Hodgkin lymphoma, Burkitt lymphoma, acute lymphatic leukemia (ALL), chronic lymphatic leukemia (CLL), acute myeolid leukemia (AML), chronic myeloid leukemia (CML), adult T-cell leukemia lymphoma, hepatocellular carcinoma, gall bladder carcinoma, bronchial carcinoma, small cell lung carcinoma, non-small cell lung carcinoma, multiple myeloma, basalioma, teratoma, retinoblastoma, choroidea

melanoma, seminoma, rhabdomyosarcoma, craniopharyngeoma, osteosarcoma, chondrosarcoma, myosarcoma, liposarcoma, fibrosarcoma, Ewing sarcoma and plasmocytoma.

[0040] Examples of invasive cancer types where the use of the vaccine containing the heparanase peptide according to the present invention, respectively the medicaments containing it, is particularly advantageous include breast carcinoma, lung carcinoma, prostate carcinoma and colon carcinoma. Most preferably, the heparanase peptide is useful for the treatment of breast carcinoma.

[0041] Furthermore, the vaccine containing the heparanase peptide according to the present invention, respectively the medicaments containing it, can also be used for the treatment of all autoimmune or other inflammatory diseases which are accompanied by an increased cell migration due to an enhanced heparanase activity.

[0042] Examples of autoimmune diseases include collagen diseases such as rheumatoid arthritis, Lupus erythematodes disseminatus, Sharp syndrome, CREST syndrome (calcinosis, Raynaud syndrome, esophageal dysmotility, teleangiectasia), dermatomyositis, vasculitis (Morbus Wegener) and Sjögren syndrome, renal diseases such as Goodpasture syndrome, rapidly-progressing glomerulonephritis and membrane-proliferative glomerulonephritis type II, endocrine diseases such as type-1 diabetes, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), autoimmune parathyreoidism, pernicious anemia, gonad insufficiency, idiopathic Morbus Addison, hyperthyreosis, Hashimoto thyreoiditis and primary myxedemia, skin diseases such as Pemphigus vulgaris, bullous pemphigoid, Herpes gestationis, Epidermolysis bullosa and Erythema multiforme major, liver diseases such as primary biliary cirrhosis, autoimmune cholangitis, autoimmune hepatitis type-1, autoimmune hepatitis type-2, primary sclerosing cholangitis, neuronal diseases such as multiple sclerosis, Myastenia gravis, myasthenic Lambert-Eaton syndrome, acquired neuromyotony, Guillain-Barre syndrome (Muller-Fischer syndrome), Stiff-man syndrome, cerebellar degeneration, ataxia, opsoklonus, sensoric neuropathy and achalasia, blood diseases such as autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura (Morbus Werlhof), infectious diseases with associated autoimmune reactions such as AIDS, Malaria and Chagas disease.

[0043] In a further embodiment, the present invention refers to a diagnostic method which can be used to determine the presence and frequency of T cells which are specific for a heparanase peptide of the present invention. The method comprises the following steps:

(a) isolating mononuclear cells from the peripheral blood or bone marrow of a patient,
(b) incubating the cells with heparanase-conjugated HLA tetramers, dimers or other multimers, and
(c) measuring the number of $CD8^+$- or $CD4^+$-/tetramer double-positive T cells.

[0044] Alternatively, the method can be employed by incubating the dendritic cells of a patient or animal to be diagnosed with heparanase peptide only and by determining the frequency of heparanase peptide specific T cells with the so-called Interferon-gamma Enzyme Linked Immuno Assay (ELISpot), a technique which is known to the person skilled in the art and further described in Example 4.

[0045] Therefore, in a further aspect the invention refers to a diagnostic kit comprising at least a heparanase peptide, or a functional variant thereof, and/or the nucleic encoding it, optionally together with a HLA tetramer/dimer, and optionally together with other compounds (e.g. enzymes, chromophores, salts, buffers) which are necessary to perform an optimal measurement.

BRIEF DESCRIPTION OF THE DRWAING

Table 1

[0046] Table 1 shows 505 heparanase derived nonamers, selected from full-length amino acid sequence of human heparanase according to their capacity to bind to HLA-A2 molecules. Calculated binding score (last column) decreases from the top to the bottom.

Table 2

[0047] Table 2 shows 475 heparanase derived 15-mers, selected from full-length amino acid sequence of human heparanase according to their capacity to bind to HLA-DR molecules. Calculated binding score (last column) decreases from the top to the bottom.

[0048] The invention is further illustrated by the following examples.

EXAMPLES

Example 1: Peptides

[0049] Nonameric peptides with a potential (calculated) binding capacity to HLA-A2 molecules have been selected from the full-length amino acid sequence of human heparanase (gene bank accession no. NP_006656 and NM_006665). The search was carried out with the use of the SYFPEITHY web page (http://www.uni-tuebingen.de/uni/kxi). As examples, three peptides (heparanase p8: A L P P P L M L L), heparanase p16: L L L G P L G P L, and heparanase p183: D L I F G L N A L) have been synthesized in the laboratory of Dr. Pipkorn (German Cancer Research Centre). The peptides were dissolved in ddH_2O 10 % DMSO.

Example 2: *Generation of dendritic cells (DC) and T-lymphocytes TC)*

[0050] Mononuclear cells (MNC) from periphery blood (PB) and bone marrow (BM) were isolated via Ficoll gradients (Biocoll separating solution, Biochrom AG). MNC were washed two times with RPMI 1640, transferred to uncoated cell culture dishes, and grown for two hours at 37°C, 5% CO_2 in x-VIVO-20 media (BioWhittaker, Walkersville, Maryland) for adhesion. Adherent cells were cultivated for 7 day in x-VIVO-20 media with the addition of GM-CSF (50 µg/ml; Behringwerke, Marburg) and IL-4 (1000 U/ml; Promocell, Heidelberg). Dendritic cells (DCs) were magnetically isolated via anti-CD-3-coated and anti-CD-19-coated magnetic beads (Dynal). Non-adherent cells were cultivated for 7 days in RPMI 1640 supplemented with 8% human AB sera (Sigma), rhuIL-2 (100 U/ml; Chiron, Ratingen) and IL-4 (60 U/ml). T cells (TCs) were purified via anti-CD-56-coated, anti-CD-19-coated and anti-CD-15-coated magnetic beads.

Example 3: HLA-typing

[0051] HLA-typing of test patients was performed by staining of mononuclear cells with the hybridoma supernatant BB7.2 (mouse-anti-human-HLA-A2), and goat-anti-mouse-FITC (Immuno Research). The analysis was performed by fluorescent flow cytometry (FACSCan).

Example 4: IFN-γ enzyme-linked immuno assay (ELISpot)

[0052] The number of peptide-specific T-cells from the bone marrow (BMTCs) of female patients is determined by the ELISpot method. For this purpose, a 96-well ELISpot plate (Millipore) is coated with anti-human-IFNγ antibodies (ELISpot Kit, Mabtech) over night at 4 °C and then one hour blocked with RPMI 5 % AB sera (37°C, 5 % CO_2). 10^4 DCs, 10^5 TCs and 10 µg/ml peptide are cultivated on the IFN-γ-coated ELISpot plate for 40 hours (37°C, 5% CO_2). Supernatants are discarded and the plate is developed via the ELISpot kit (Mabtech). IFN-γ producing cells are counted with Axioplan Mikroskop (Zeiss) by using the KS ELISpot software. For negative controls, HIV or insulin peptides are used. Each group is determined in triplicate. Positive results are measured via the so-called T-test ($p < 0.05$).

Results:

[0053] (BMTCs of 15 female breast cancer patients, insulin p34 [H L V E A L Y L V] was used as negative control): 53% (8 out of 15) of the patients significantly reacted against human heparanase peptides. In particular, 20% (3/15) reacted against heparanase p8 (Hpa8), 33% (5/15) against heparanase p16 (Hpa16), and 40% (6/15) against Hpa183) (see Table 3).

Table 3:

Patient	Hpa p8		Hpa p16		Hpa p183	
	p < 0,05	frequency	p < 0,05	frequency	p < 0,05	frequency
503	0,019	**1 : 4100**	0,012	**1 : 3600**	0,008	**1 : 3500**
505	-		-		-	
512	-		-		-	
579	-		0,038	**1 : 1700**	0,043	**1 : 1600**
581	-		-		0,023	**1 : 3000**
595	-		-		-	

Table 3: (continued)

Patient	Hpa p8		Hpa p16		Hpa p183	
	p < 0,05	frequency	p < 0,05	frequency	p < 0,05	frequency
590	-		0,023	**1 : 1200**	-	
639	0,008	**1 : 650**	0,039	**1: 580**	0,017	**1 : 660**
662	-		-		-	
696	-		-		-	
704	0,025	**1 : 2700**	-		-	
753	-		-		0,037	**1 : 12500**
756	-		-		-	
771	-		-		-	
790	-		0,032	**1 : 3200**	0,045	**1 : 6000**

Example 5: Cytotoxicity assay

[0054] The cytotoxic activity of peptide-specific T-cells is measured with a cytotoxicity assay (Chrome-51 Release Assay).
Isolated DCs and TCs are co-cultivated at a ratio ranging from 1:10 to 1:40 in RPMI supplemented with 8% AB sera and 20 U/1 rhuIL-2 (recombinant human interleukin-2) for 7 days. At a day 0, (heparanase p8, heparanase p16, heparanase p183) at a concentration of 10 μg/ml are added. 5 x 10^5 target cells, MCF-7 cells (human breast epithelial cancer cells, mock- and hpahu-treated) are incubated with 200 μci radioactive chrome-51 for 90 minutes. Chromated targets and pre-stimulated TCs are titrated in triplicate and incubated for 4 hours at 37°C and 5% CO_2. The supernatant is transferred to scintillation tubes and measured in a gamma-counter for 50 sec/tube.

Example 6: Tetramer staining

[0055] Phycoerythrin (PE-)conjugated tetramer complexes consisting of HLA-A2 and either heparanase p8, heparanase p16 or HIV (S L Y N T V A T L) peptides are obtained from the NIAID facility (Bethesda, Maryland).
10^6 of each BM-MNC and PB-MNC are blocked with 5% endobulin (immunoglobulin G), incubated with tetramers on ice for 45 min and then stained with CD8-FITC (Becton Dickinson). Dead cells are identified by propidium iodide. The number of T-cells which are double positive for CD8 and tetramer are determined by flow cytometry.

Results:

[0056] HLA-A2 peptide staining of 2 examined patients (MaCa numbers Table 4) revealed enriched fractions of CD8-positive T cells with specificity for i) heparanase-derived peptide Hpa.8-17/ALPPPLMLL (see % values of CD8-positive T cells), and for ii) heparanase-derived peptide Hpa.16-23/LLLGPLGPL (see % values of CD8-positive T cells). The staining of HLA-A2/HIV-peptide complexes as negative controls resulted in significantly lower values (0.1 and 0.01 respectively). (see table 4)

Table 4

MaCa		Tetramer HIV	Tetramer Hpa 8	Tetramer Hpa 16
889	PBTC	-	-	-
	BMTC	0.01 %	0.19 %	0.11 %
923	PBTC	0.09 %	0.55 %	3.05 %
	BMTC	0.06 %	0.34 %	5.7 %
959	PBTC	0.07 %	0.07 %	0.52 %
	BMTC	0.1 %	0.08 %	0.73 %
961	PBTC	0.1 %	0.05 %	0.14 %

Table 4 (continued)

MaCa		Tetramer HIV	Tetramer Hpa 8	Tetramer Hpa 16
	BMTC	0.05 %	0 %	0.33 %

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Leu Leu Arg Lys Ser Thr Phe Lys Asn Ala Lys Leu Tyr Gly Pro
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Arg Lys Thr Ala Lys Met Leu Lys Ser Phe Leu Lys Ala Gly Gly
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Thr Ala Thr Arg Glu Asp Phe Leu Asn Pro Asp Val Leu Asp Ile
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Leu Leu Phe Lys Lys Leu Val Gly Thr Lys Val Leu Met Ala Ser
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Pro Arg Tyr Lys Glu Gly Asp Leu Thr Leu Tyr Ala Ile Asn Leu
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Gly Asp Leu Thr Leu Tyr Ala Ile Asn Leu His Asn Val Thr Lys
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Leu Arg Leu Pro Tyr Pro Phe Ser Asn Lys Gln Val Asp Lys Tyr
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Pro Leu Gly Pro His Gly Leu Leu Ser Lys Ser Val Gln Leu Asn
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Phe Ser Tyr Ser Phe Phe Val Ile Arg Asn Ala Lys Val Ala Ala
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Gln Lys Lys Phe Lys Asn Ser Thr Tyr Ser Arg Ser Ser Val Asp
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Asn Ser Thr Tyr Ser Arg Ser Ser Val Asp Val Leu Tyr Thr Phe
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Leu Tyr Thr Phe Ala Asn Cys Ser Gly Leu Asp Leu Ile Phe Gly
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Leu Leu Asp Tyr Cys Ser Ser Lys Gly Tyr Asn Ile Ser Trp Glu
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Ser Lys Gly Tyr Asn Ile Ser Trp Glu Leu Gly Asn Glu Pro Asn
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Asn Ile Ser Trp Glu Leu Gly Asn Glu Pro Asn Ser Phe Leu Lys
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Gly Glu Asp Phe Ile Gln Leu His Lys Leu Leu Arg Lys Ser Thr
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Lys Ser Thr Phe Lys Asn Ala Lys Leu Tyr Gly Pro Asp Val Gly
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Ala Lys Leu Tyr Gly Pro Asp Val Gly Gln Pro Arg Arg Lys Thr
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His His Tyr Tyr Leu Asn Gly Arg Thr Ala Thr Arg Glu Asp Phe
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Arg Glu Asp Phe Leu Asn Pro Asp Val Leu Asp Ile Phe Ile Ser
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Gln Val Phe Phe Gly Ala Gly Asn Tyr His Leu Val Asp Glu Asn
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Ala Gly Asn Tyr His Leu Val Asp Glu Asn Phe Asp Pro Leu Pro
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Leu Pro Asp Tyr Trp Leu Ser Leu Leu Phe Lys Lys Leu Val Gly
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Leu His Lys Leu Leu Arg Lys Ser Thr Phe Lys Asn Ala Lys Leu
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Leu Met Leu Leu Leu Leu Gly Pro Leu Gly Pro Leu Ser Pro Gly
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Met Leu Leu Leu Leu Gly Pro Leu Gly Pro Leu Ser Pro Gly Ala
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Pro Gly Ala Leu Pro Arg Pro Ala Gln Ala Gln Asp Val Val Asp
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Gln Asp Val Val Asp Leu Asp Phe Phe Thr Gln Glu Pro Leu His
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Pro Leu His Leu Val Ser Pro Ser Phe Leu Ser Val Thr Ile Asp
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Leu His Leu Val Ser Pro Ser Phe Leu Ser Val Thr Ile Asp Ala
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Phe Leu Ile Leu Leu Gly Ser Pro Lys Leu Arg Thr Leu Ala Arg
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Leu Ile Leu Leu Gly Ser Pro Lys Leu Arg Thr Leu Ala Arg Gly
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Leu Arg Thr Leu Ala Arg Gly Leu Ser Pro Ala Tyr Leu Arg Phe
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Ala Arg Gly Leu Ser Pro Ala Tyr Leu Arg Phe Gly Gly Thr Lys
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Pro Ala Tyr Leu Arg Phe Gly Gly Thr Lys Thr Asp Phe Leu Ile
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Asp Phe Leu Ile Phe Asp Pro Lys Lys Glu Ser Thr Phe Glu Glu
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Gln Ser Gln Val Asn Gln Asp Ile Cys Lys Tyr Gly Ser Ile Pro
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Asn Gln Asp Ile Cys Lys Tyr Gly Ser Ile Pro Pro Asp Val Glu
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Glu Glu Lys Leu Arg Leu Glu Trp Pro Tyr Gln Glu Gln Leu Leu
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Ser Val Asp Val Leu Tyr Thr Phe Ala Asn Cys Ser Gly Leu Asp
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Leu Asp Leu Ile Phe Gly Leu Asn Ala Leu Leu Arg Thr Ala Asp
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Gln Leu Leu Leu Asp Tyr Cys Ser Ser Lys Gly Tyr Asn Ile Ser
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Gly Tyr Asn Ile Ser Trp Glu Leu Gly Asn Glu Pro Asn Ser Phe
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Lys Ala Asp Ile Phe Ile Asn Gly Ser Gln Leu Gly Glu Asp Phe
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His Lys Leu Leu Arg Lys Ser Thr Phe Lys Asn Ala Lys Leu Tyr
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Ala Lys Met Leu Lys Ser Phe Leu Lys Ala Gly Gly Glu Val Ile
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Gly Gly Glu Val Ile Asp Ser Val Thr Trp His His Tyr Tyr Leu
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Gly Glu Val Ile Asp Ser Val Thr Trp His His Tyr Tyr Leu Asn
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Ile Asp Ser Val Thr Trp His His Tyr Tyr Leu Asn Gly Arg Thr
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Glu Asp Phe Leu Asn Pro Asp Val Leu Asp Ile Phe Ile Ser Ser
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Val Gln Lys Val Phe Gln Val Val Glu Ser Thr Arg Pro Gly Lys
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Phe Gln Val Val Glu Ser Thr Arg Pro Gly Lys Lys Val Trp Leu
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Lys Val Trp Leu Gly Glu Thr Ser Ser Ala Tyr Gly Gly Gly Ala
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Gly Ala Pro Leu Leu Ser Asp Thr Phe Ala Ala Gly Phe Met Trp
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Phe Met Trp Leu Asp Lys Leu Gly Leu Ser Ala Arg Met Gly Ile
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Glu Val Val Met Arg Gln Val Phe Phe Gly Ala Gly Asn Tyr His
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Phe Lys Lys Leu Val Gly Thr Lys Val Leu Met Ala Ser Val Gln
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Lys Val Leu Met Ala Ser Val Gln Gly Ser Lys Arg Arg Lys Leu
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Lys Leu Arg Val Tyr Leu His Cys Thr Asn Thr Asp Asn Pro Arg
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Arg Val Tyr Leu His Cys Thr Asn Thr Asp Asn Pro Arg Tyr Lys
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Ala Ile Asn Leu His Asn Val Thr Lys Tyr Leu Arg Leu Pro Tyr
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Leu His Asn Val Thr Lys Tyr Leu Arg Leu Pro Tyr Pro Phe Ser
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Thr Lys Tyr Leu Arg Leu Pro Tyr Pro Phe Ser Asn Lys Gln Val
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Asn Lys Gln Val Asp Lys Tyr Leu Leu Arg Pro Leu Gly Pro His
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Leu Arg Pro Leu Gly Pro His Gly Leu Leu Ser Lys Ser Val Gln
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His Gly Leu Leu Ser Lys Ser Val Gln Leu Asn Gly Leu Thr Leu
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Ser Lys Ser Val Gln Leu Asn Gly Leu Thr Leu Lys Met Val Asp
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Leu Lys Met Val Asp Asp Gln Thr Leu Pro Pro Leu Met Glu Lys
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Gly Ser Ser Leu Gly Leu Pro Ala Phe Ser Tyr Ser Phe Phe Val
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Ser Leu Gly Leu Pro Ala Phe Ser Tyr Ser Phe Phe Val Ile Arg
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Gly Pro Leu Ser Pro Gly Ala Leu Pro Arg Pro Ala Gln Ala Gln
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Gly Ala Leu Pro Arg Pro Ala Gln Ala Gln Asp Val Val Asp Leu
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Ala Leu Pro Arg Pro Ala Gln Ala Gln Asp Val Val Asp Leu Asp
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Gln Ala Gln Asp Val Val Asp Leu Asp Phe Phe Thr Gln Glu Pro
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Asp Val Val Asp Leu Asp Phe Phe Thr Gln Glu Pro Leu His Leu
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Asp Phe Phe Thr Gln Glu Pro Leu His Leu Val Ser Pro Ser Phe
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Val Ser Pro Ser Phe Leu Ser Val Thr Ile Asp Ala Asn Leu Ala
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Ser Phe Leu Ser Val Thr Ile Asp Ala Asn Leu Ala Thr Asp Pro
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Ala Thr Asp Pro Arg Phe Leu Ile Leu Leu Gly Ser Pro Lys Leu
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Arg Thr Leu Ala Arg Gly Leu Ser Pro Ala Tyr Leu Arg Phe Gly
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Gly Leu Ser Pro Ala Tyr Leu Arg Phe Gly Gly Thr Lys Thr Asp
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Gly Thr Lys Thr Asp Phe Leu Ile Phe Asp Pro Lys Lys Glu Ser
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Ile Phe Asp Pro Lys Lys Glu Ser Thr Phe Glu Glu Arg Ser Tyr
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Lys Lys Glu Ser Thr Phe Glu Glu Arg Ser Tyr Trp Gln Ser Gln
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Ser Gln Val Asn Gln Asp Ile Cys Lys Tyr Gly Ser Ile Pro Pro
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Ser Ile Pro Pro Asp Val Glu Glu Lys Leu Arg Leu Glu Trp Pro
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Val Glu Glu Lys Leu Arg Leu Glu Trp Pro Tyr Gln Glu Gln Leu
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Pro Tyr Gln Glu Gln Leu Leu Leu Arg Glu His Tyr Gln Lys Lys
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Tyr Gln Glu Gln Leu Leu Leu Arg Glu His Tyr Gln Lys Lys Phe
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Lys Lys Phe Lys Asn Ser Thr Tyr Ser Arg Ser Ser Val Asp Val
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Lys Phe Lys Asn Ser Thr Tyr Ser Arg Ser Ser Val Asp Val Leu
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Thr Tyr Ser Arg Ser Ser Val Asp Val Leu Tyr Thr Phe Ala Asn
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Ser Arg Ser Ser Val Asp Val Leu Tyr Thr Phe Ala Asn Cys Ser
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Ala Asn Cys Ser Gly Leu Asp Leu Ile Phe Gly Leu Asn Ala Leu
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Asn Cys Ser Gly Leu Asp Leu Ile Phe Gly Leu Asn Ala Leu Leu
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Leu Arg Thr Ala Asp Leu Gln Trp Asn Ser Ser Asn Ala Gln Leu
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Arg Thr Ala Asp Leu Gln Trp Asn Ser Ser Asn Ala Gln Leu Leu
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Ala Asp Leu Gln Trp Asn Ser Ser Asn Ala Gln Leu Leu Leu Asp
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Ser Ser Asn Ala Gln Leu Leu Leu Asp Tyr Cys Ser Ser Lys Gly
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Ser Asn Ala Gln Leu Leu Leu Asp Tyr Cys Ser Ser Lys Gly Tyr
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Cys Ser Ser Lys Gly Tyr Asn Ile Ser Trp Glu Leu Gly Asn Glu
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Trp Glu Leu Gly Asn Glu Pro Asn Ser Phe Leu Lys Lys Ala Asp
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Asn Glu Pro Asn Ser Phe Leu Lys Lys Ala Asp Ile Phe Ile Asn
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Phe Leu Lys Lys Ala Asp Ile Phe Ile Asn Gly Ser Gln Leu Gly
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Lys Lys Ala Asp Ile Phe Ile Asn Gly Ser Gln Leu Gly Glu Asp
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Phe Ile Asn Gly Ser Gln Leu Gly Glu Asp Phe Ile Gln Leu His
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Asn Gly Ser Gln Leu Gly Glu Asp Phe Ile Gln Leu His Lys Leu
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Gln Leu Gly Glu Asp Phe Ile Gln Leu His Lys Leu Leu Arg Lys
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Thr Phe Lys Asn Ala Lys Leu Tyr Gly Pro Asp Val Gly Gln Pro
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Lys Leu Tyr Gly Pro Asp Val Gly Gln Pro Arg Arg Lys Thr Ala
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Tyr Gly Pro Asp Val Gly Gln Pro Arg Arg Lys Thr Ala Lys Met
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Gln Pro Arg Arg Lys Thr Ala Lys Met Leu Lys Ser Phe Leu Lys
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Phe Leu Lys Ala Gly Gly Glu Val Ile Asp Ser Val Thr Trp His
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Leu Lys Ala Gly Gly Glu Val Ile Asp Ser Val Thr Trp His His
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Lys Ala Gly Gly Glu Val Ile Asp Ser Val Thr Trp His His Tyr
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Val Ile Asp Ser Val Thr Trp His His Tyr Tyr Leu Asn Gly Arg
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Val Thr Trp His His Tyr Tyr Leu Asn Gly Arg Thr Ala Thr Arg
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Thr Trp His His Tyr Tyr Leu Asn Gly Arg Thr Ala Thr Arg Glu
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Tyr Leu Asn Gly Arg Thr Ala Thr Arg Glu Asp Phe Leu Asn Pro
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Ala Thr Arg Glu Asp Phe Leu Asn Pro Asp Val Leu Asp Ile Phe
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Asp Phe Leu Asn Pro Asp Val Leu Asp Ile Phe Ile Ser Ser Val
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Phe Leu Asn Pro Asp Val Leu Asp Ile Phe Ile Ser Ser Val Gln
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Ser Ser Val Gln Lys Val Phe Gln Val Val Glu Ser Thr Arg Pro
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Lys Val Phe Gln Val Val Glu Ser Thr Arg Pro Gly Lys Lys Val
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Thr Arg Pro Gly Lys Lys Val Trp Leu Gly Glu Thr Ser Ser Ala
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Pro Gly Lys Lys Val Trp Leu Gly Glu Thr Ser Ser Ala Tyr Gly
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Leu Gly Glu Thr Ser Ser Ala Tyr Gly Gly Gly Ala Pro Leu Leu
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Tyr Gly Gly Gly Ala Pro Leu Leu Ser Asp Thr Phe Ala Ala Gly
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Gly Gly Gly Ala Pro Leu Leu Ser Asp Thr Phe Ala Ala Gly Phe
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Gly Gly Ala Pro Leu Leu Ser Asp Thr Phe Ala Ala Gly Phe Met
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Pro Leu Leu Ser Asp Thr Phe Ala Ala Gly Phe Met Trp Leu Asp
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Leu Ser Asp Thr Phe Ala Ala Gly Phe Met Trp Leu Asp Lys Leu
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Asp Thr Phe Ala Ala Gly Phe Met Trp Leu Asp Lys Leu Gly Leu
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Met Trp Leu Asp Lys Leu Gly Leu Ser Ala Arg Met Gly Ile Glu
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Ala Arg Met Gly Ile Glu Val Val Met Arg Gln Val Phe Phe Gly
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Val Val Met Arg Gln Val Phe Phe Gly Ala Gly Asn Tyr His Leu
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Gly Asn Tyr His Leu Val Asp Glu Asn Phe Asp Pro Leu Pro Asp
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Asn Phe Asp Pro Leu Pro Asp Tyr Trp Leu Ser Leu Leu Phe Lys
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Pro Leu Pro Asp Tyr Trp Leu Ser Leu Leu Phe Lys Lys Leu Val
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Lys Leu Val Gly Thr Lys Val Leu Met Ala Ser Val Gln Gly Ser
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Leu Val Gly Thr Lys Val Leu Met Ala Ser Val Gln Gly Ser Lys
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Lys Arg Arg Lys Leu Arg Val Tyr Leu His Cys Thr Asn Thr Asp
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Arg Lys Leu Arg Val Tyr Leu His Cys Thr Asn Thr Asp Asn Pro
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Leu His Cys Thr Asn Thr Asp Asn Pro Arg Tyr Lys Glu Gly Asp
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Asp Asn Pro Arg Tyr Lys Glu Gly Asp Leu Thr Leu Tyr Ala Ile
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Arg Tyr Lys Glu Gly Asp Leu Thr Leu Tyr Ala Ile Asn Leu His
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Lys Glu Gly Asp Leu Thr Leu Tyr Ala Ile Asn Leu His Asn Val
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Tyr Ala Ile Asn Leu His Asn Val Thr Lys Tyr Leu Arg Leu Pro
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Leu Pro Tyr Pro Phe Ser Asn Lys Gln Val Asp Lys Tyr Leu Leu
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Pro Phe Ser Asn Lys Gln Val Asp Lys Tyr Leu Leu Arg Pro Leu
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Lys Gln Val Asp Lys Tyr Leu Leu Arg Pro Leu Gly Pro His Gly
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Tyr Leu Leu Arg Pro Leu Gly Pro His Gly Leu Leu Ser Lys Ser
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Leu Gly Pro His Gly Leu Leu Ser Lys Ser Val Gln Leu Asn Gly
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Gly Pro His Gly Leu Leu Ser Lys Ser Val Gln Leu Asn Gly Leu
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Gly Leu Leu Ser Lys Ser Val Gln Leu Asn Gly Leu Thr Leu Lys
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Leu Leu Ser Lys Ser Val Gln Leu Asn Gly Leu Thr Leu Lys Met
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Leu Ser Lys Ser Val Gln Leu Asn Gly Leu Thr Leu Lys Met Val
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Lys Ser Val Gln Leu Asn Gly Leu Thr Leu Lys Met Val Asp Asp
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Leu Thr Leu Lys Met Val Asp Asp Gln Thr Leu Pro Pro Leu Met
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Pro Leu Met Glu Lys Pro Leu Arg Pro Gly Ser Ser Leu Gly Leu
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Ser Ser Leu Gly Leu Pro Ala Phe Ser Tyr Ser Phe Phe Val Ile
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Leu Gly Leu Pro Ala Phe Ser Tyr Ser Phe Phe Val Ile Arg Asn
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Pro Ala Phe Ser Tyr Ser Phe Phe Val Ile Arg Asn Ala Lys Val
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Glu Ser Thr Phe Glu Glu Arg Ser Tyr Trp Gln Ser Gln Val Asn
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Arg Glu His Tyr Gln Lys Lys Phe Lys Asn Ser Thr Tyr Ser Arg
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Pro Asn Ser Phe Leu Lys Lys Ala Asp Ile Phe Ile Asn Gly Ser
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Gly Phe Met Trp Leu Asp Lys Leu Gly Leu Ser Ala Arg Met Gly
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Tyr Ser Phe Phe Val Ile Arg Asn Ala Lys Val Ala Ala Cys Ile
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Glu Arg Ser Tyr Trp Gln Ser Gln Val Asn Gln Asp Ile Cys Lys
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Glu Trp Pro Tyr Gln Glu Gln Leu Leu Leu Arg Glu His Tyr Gln
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Ser Val Thr Trp His His Tyr Tyr Leu Asn Gly Arg Thr Ala Thr
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Ser Asp Thr Phe Ala Ala Gly Phe Met Trp Leu Asp Lys Leu Gly
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Asn Pro Arg Tyr Lys Glu Gly Asp Leu Thr Leu Tyr Ala Ile Asn
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Arg Leu Pro Tyr Pro Phe Ser Asn Lys Gln Val Asp Lys Tyr Leu
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Leu Pro Ala Phe Ser Tyr Ser Phe Phe Val Ile Arg Asn Ala Lys
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Pro Pro Asp Val Glu Glu Lys Leu Arg Leu Glu Trp Pro Tyr Gln
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Lys Leu Gly Leu Ser Ala Arg Met Gly Ile Glu Val Val Met Arg
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Trp Leu Ser Leu Leu Phe Lys Lys Leu Val Gly Thr Lys Val Leu
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Leu Ser Leu Leu Phe Lys Lys Leu Val Gly Thr Lys Val Leu Met
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Lys Lys Leu Val Gly Thr Lys Val Leu Met Ala Ser Val Gln Gly
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Pro His Gly Leu Leu Ser Lys Ser Val Gln Leu Asn Gly Leu Thr
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Leu Asn Gly Leu Thr Leu Lys Met Val Asp Asp Gln Thr Leu Pro
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Leu Gly Pro Leu Ser Pro Gly Ala Leu Pro Arg Pro Ala Gln Ala
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Ser Val Thr Ile Asp Ala Asn Leu Ala Thr Asp Pro Arg Phe Leu
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Arg Phe Leu Ile Leu Leu Gly Ser Pro Lys Leu Arg Thr Leu Ala
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Leu Asn Ala Leu Leu Arg Thr Ala Asp Leu Gln Trp Asn Ser Ser
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Asn Ala Lys Leu Tyr Gly Pro Asp Val Gly Gln Pro Arg Arg Lys
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Thr Ala Lys Met Leu Lys Ser Phe Leu Lys Ala Gly Gly Glu Val
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Lys Ser Phe Leu Lys Ala Gly Gly Glu Val Ile Asp Ser Val Thr
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Ala Pro Leu Leu Ser Asp Thr Phe Ala Ala Gly Phe Met Trp Leu
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Met Arg Gln Val Phe Phe Gly Ala Gly Asn Tyr His Leu Val Asp
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Tyr His Leu Val Asp Glu Asn Phe Asp Pro Leu Pro Asp Tyr Trp
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Glu Gly Asp Leu Thr Leu Tyr Ala Ile Asn Leu His Asn Val Thr
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Leu Tyr Ala Ile Asn Leu His Asn Val Thr Lys Tyr Leu Arg Leu
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Asp Lys Tyr Leu Leu Arg Pro Leu Gly Pro His Gly Leu Leu Ser
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Ile Leu Leu Gly Ser Pro Lys Leu Arg Thr Leu Ala Arg Gly Leu
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His Tyr Gln Lys Lys Phe Lys Asn Ser Thr Tyr Ser Arg Ser Ser
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Lys Asn Ser Thr Tyr Ser Arg Ser Ser Val Asp Val Leu Tyr Thr
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Gln Leu His Lys Leu Leu Arg Lys Ser Thr Phe Lys Asn Ala Lys
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Asp Val Gly Gln Pro Arg Arg Lys Thr Ala Lys Met Leu Lys Ser
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Gln Gly Ser Lys Arg Arg Lys Leu Arg Val Tyr Leu His Cys Thr
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Arg Ser Lys Pro Ala Leu Pro Pro Pro Leu Met Leu Leu Leu Leu
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Ser Lys Pro Ala Leu Pro Pro Pro Leu Met Leu Leu Leu Leu Gly
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Leu Pro Pro Pro Leu Met Leu Leu Leu Leu Gly Pro Leu Gly Pro
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Leu Ser Pro Gly Ala Leu Pro Arg Pro Ala Gln Ala Gln Asp Val
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Leu Pro Arg Pro Ala Gln Ala Gln Asp Val Val Asp Leu Asp Phe
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Pro Lys Leu Arg Thr Leu Ala Arg Gly Leu Ser Pro Ala Tyr Leu
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Arg Gly Leu Ser Pro Ala Tyr Leu Arg Phe Gly Gly Thr Lys Thr
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Gly Gly Thr Lys Thr Asp Phe Leu Ile Phe Asp Pro Lys Lys Glu
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Asp Pro Lys Lys Glu Ser Thr Phe Glu Glu Arg Ser Tyr Trp Gln
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Pro Lys Lys Glu Ser Thr Phe Glu Glu Arg Ser Tyr Trp Gln Ser
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Gly Ser Ile Pro Pro Asp Val Glu Glu Lys Leu Arg Leu Glu Trp
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Ile Pro Pro Asp Val Glu Glu Lys Leu Arg Leu Glu Trp Pro Tyr
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Pro Asp Val Glu Glu Lys Leu Arg Leu Glu Trp Pro Tyr Gln Glu
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Glu Lys Leu Arg Leu Glu Trp Pro Tyr Gln Glu Gln Leu Leu Leu
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Trp Pro Tyr Gln Glu Gln Leu Leu Leu Arg Glu His Tyr Gln Lys
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Leu Leu Leu Arg Glu His Tyr Gln Lys Lys Phe Lys Asn Ser Thr
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Leu Leu Arg Glu His Tyr Gln Lys Lys Phe Lys Asn Ser Thr Tyr
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Tyr Gln Lys Lys Phe Lys Asn Ser Thr Tyr Ser Arg Ser Ser Val
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Phe Lys Asn Ser Thr Tyr Ser Arg Ser Ser Val Asp Val Leu Tyr
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Tyr Ser Arg Ser Ser Val Asp Val Leu Tyr Thr Phe Ala Asn Cys
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Val Leu Tyr Thr Phe Ala Asn Cys Ser Gly Leu Asp Leu Ile Phe
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Tyr Thr Phe Ala Asn Cys Ser Gly Leu Asp Leu Ile Phe Gly Leu
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Phe Ala Asn Cys Ser Gly Leu Asp Leu Ile Phe Gly Leu Asn Ala
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Gln Trp Asn Ser Ser Asn Ala Gln Leu Leu Leu Asp Tyr Cys Ser
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Asn Ser Ser Asn Ala Gln Leu Leu Leu Asp Tyr Cys Ser Ser Lys
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Leu Leu Leu Asp Tyr Cys Ser Ser Lys Gly Tyr Asn Ile Ser Trp
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Asp Tyr Cys Ser Ser Lys Gly Tyr Asn Ile Ser Trp Glu Leu Gly
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Ser Ser Lys Gly Tyr Asn Ile Ser Trp Glu Leu Gly Asn Glu Pro
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Glu Leu Gly Asn Glu Pro Asn Ser Phe Leu Lys Lys Ala Asp Ile
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Leu Gly Asn Glu Pro Asn Ser Phe Leu Lys Lys Ala Asp Ile Phe
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Gly Asn Glu Pro Asn Ser Phe Leu Lys Lys Ala Asp Ile Phe Ile
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Ser Phe Leu Lys Lys Ala Asp Ile Phe Ile Asn Gly Ser Gln Leu
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Ile Asn Gly Ser Gln Leu Gly Glu Asp Phe Ile Gln Leu His Lys
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Ile Gln Leu His Lys Leu Leu Arg Lys Ser Thr Phe Lys Asn Ala
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Lys Leu Leu Arg Lys Ser Thr Phe Lys Asn Ala Lys Leu Tyr Gly
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Lys Asn Ala Lys Leu Tyr Gly Pro Asp Val Gly Gln Pro Arg Arg
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Leu Tyr Gly Pro Asp Val Gly Gln Pro Arg Arg Lys Thr Ala Lys
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Gly Gln Pro Arg Arg Lys Thr Ala Lys Met Leu Lys Ser Phe Leu
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Arg Arg Lys Thr Ala Lys Met Leu Lys Ser Phe Leu Lys Ala Gly
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Lys Met Leu Lys Ser Phe Leu Lys Ala Gly Gly Glu Val Ile Asp
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Ser Phe Leu Lys Ala Gly Gly Glu Val Ile Asp Ser Val Thr Trp
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Ala Gly Gly Glu Val Ile Asp Ser Val Thr Trp His His Tyr Tyr
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Glu Val Ile Asp Ser Val Thr Trp His His Tyr Tyr Leu Asn Gly
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Tyr Tyr Leu Asn Gly Arg Thr Ala Thr Arg Glu Asp Phe Leu Asn
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Leu Asn Gly Arg Thr Ala Thr Arg Glu Asp Phe Leu Asn Pro Asp
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Gly Arg Thr Ala Thr Arg Glu Asp Phe Leu Asn Pro Asp Val Leu
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Arg Thr Ala Thr Arg Glu Asp Phe Leu Asn Pro Asp Val Leu Asp
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Ser Val Gln Lys Val Phe Gln Val Val Glu Ser Thr Arg Pro Gly
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Val Glu Ser Thr Arg Pro Gly Lys Lys Val Trp Leu Gly Glu Thr
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Arg Pro Gly Lys Lys Val Trp Leu Gly Glu Thr Ser Ser Ala Tyr
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Glu Thr Ser Ser Ala Tyr Gly Gly Gly Ala Pro Leu Leu Ser Asp
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Thr Ser Ser Ala Tyr Gly Gly Gly Ala Pro Leu Leu Ser Asp Thr
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Ser Ala Tyr Gly Gly Gly Ala Pro Leu Leu Ser Asp Thr Phe Ala
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Ala Tyr Gly Gly Gly Ala Pro Leu Leu Ser Asp Thr Phe Ala Ala
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Leu Leu Ser Asp Thr Phe Ala Ala Gly Phe Met Trp Leu Asp Lys
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Thr Phe Ala Ala Gly Phe Met Trp Leu Asp Lys Leu Gly Leu Ser
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Trp Leu Asp Lys Leu Gly Leu Ser Ala Arg Met Gly Ile Glu Val
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Asp Lys Leu Gly Leu Ser Ala Arg Met Gly Ile Glu Val Val Met
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Leu Gly Leu Ser Ala Arg Met Gly Ile Glu Val Val Met Arg Gln
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Gly Leu Ser Ala Arg Met Gly Ile Glu Val Val Met Arg Gln Val
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Leu Ser Ala Arg Met Gly Ile Glu Val Val Met Arg Gln Val Phe
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Met Gly Ile Glu Val Val Met Arg Gln Val Phe Phe Gly Ala Gly
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Val Met Arg Gln Val Phe Phe Gly Ala Gly Asn Tyr His Leu Val
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Val Phe Phe Gly Ala Gly Asn Tyr His Leu Val Asp Glu Asn Phe
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Phe Gly Ala Gly Asn Tyr His Leu Val Asp Glu Asn Phe Asp Pro
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Gly Ala Gly Asn Tyr His Leu Val Asp Glu Asn Phe Asp Pro Leu
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His Leu Val Asp Glu Asn Phe Asp Pro Leu Pro Asp Tyr Trp Leu
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Leu Val Asp Glu Asn Phe Asp Pro Leu Pro Asp Tyr Trp Leu Ser
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Glu Asn Phe Asp Pro Leu Pro Asp Tyr Trp Leu Ser Leu Leu Phe
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Asp Pro Leu Pro Asp Tyr Trp Leu Ser Leu Leu Phe Lys Lys Leu
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Tyr Trp Leu Ser Leu Leu Phe Lys Lys Leu Val Gly Thr Lys Val
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Leu Phe Lys Lys Leu Val Gly Thr Lys Val Leu Met Ala Ser Val
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Val Gly Thr Lys Val Leu Met Ala Ser Val Gln Gly Ser Lys Arg
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Val Leu Met Ala Ser Val Gln Gly Ser Lys Arg Arg Lys Leu Arg
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Leu Met Ala Ser Val Gln Gly Ser Lys Arg Arg Lys Leu Arg Val
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Gly Ser Lys Arg Arg Lys Leu Arg Val Tyr Leu His Cys Thr Asn
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Tyr Leu His Cys Thr Asn Thr Asp Asn Pro Arg Tyr Lys Glu Gly
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His Cys Thr Asn Thr Asp Asn Pro Arg Tyr Lys Glu Gly Asp Leu
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Asn Thr Asp Asn Pro Arg Tyr Lys Glu Gly Asp Leu Thr Leu Tyr
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Tyr Lys Glu Gly Asp Leu Thr Leu Tyr Ala Ile Asn Leu His Asn
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Ile Asn Leu His Asn Val Thr Lys Tyr Leu Arg Leu Pro Tyr Pro
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His Asn Val Thr Lys Tyr Leu Arg Leu Pro Tyr Pro Phe Ser Asn
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Lys Tyr Leu Arg Leu Pro Tyr Pro Phe Ser Asn Lys Gln Val Asp
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Tyr Pro Phe Ser Asn Lys Gln Val Asp Lys Tyr Leu Leu Arg Pro
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Phe Ser Asn Lys Gln Val Asp Lys Tyr Leu Leu Arg Pro Leu Gly
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Gln Val Asp Lys Tyr Leu Leu Arg Pro Leu Gly Pro His Gly Leu
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Leu Leu Arg Pro Leu Gly Pro His Gly Leu Leu Ser Lys Ser Val
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Arg Pro Leu Gly Pro His Gly Leu Leu Ser Lys Ser Val Gln Leu
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Gln Leu Asn Gly Leu Thr Leu Lys Met Val Asp Asp Gln Thr Leu
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Asn Gly Leu Thr Leu Lys Met Val Asp Asp Gln Thr Leu Pro Pro
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Lys Met Val Asp Asp Gln Thr Leu Pro Pro Leu Met Glu Lys Pro
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Met Val Asp Asp Gln Thr Leu Pro Pro Leu Met Glu Lys Pro Leu
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Val Asp Asp Gln Thr Leu Pro Pro Leu Met Glu Lys Pro Leu Arg
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Thr Leu Pro Pro Leu Met Glu Lys Pro Leu Arg Pro Gly Ser Ser
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Met Glu Lys Pro Leu Arg Pro Gly Ser Ser Leu Gly Leu Pro Ala
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Pro Leu Arg Pro Gly Ser Ser Leu Gly Leu Pro Ala Phe Ser Tyr
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Leu Arg Pro Gly Ser Ser Leu Gly Leu Pro Ala Phe Ser Tyr Ser
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Arg Pro Gly Ser Ser Leu Gly Leu Pro Ala Phe Ser Tyr Ser Phe
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Pro Gly Ser Ser Leu Gly Leu Pro Ala Phe Ser Tyr Ser Phe Phe
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Gly Leu Pro Ala Phe Ser Tyr Ser Phe Phe Val Ile Arg Asn Ala
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Phe Leu Ile Phe Asp Pro Lys Lys Glu Ser Thr Phe Glu Glu Arg
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Pro Tyr Pro Phe Ser Asn Lys Gln Val Asp Lys Tyr Leu Leu Arg
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Val Asp Lys Tyr Leu Leu Arg Pro Leu Gly Pro His Gly Leu Leu
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His Tyr Tyr Leu Asn Gly Arg Thr Ala Thr Arg Glu Asp Phe Leu
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Gly Ile Glu Val Val Met Arg Gln Val Phe Phe Gly Ala Gly Asn
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Leu Pro Pro Leu Met Glu Lys Pro Leu Arg Pro Gly Ser Ser Leu
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Ser Pro Gly Ala Leu Pro Arg Pro Ala Gln Ala Gln Asp Val Val
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Asn Leu Ala Thr Asp Pro Arg Phe Leu Ile Leu Leu Gly Ser Pro
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Claims

1. A vaccine against a disease being associated with an enhanced heparanase expression and/or activity, wherein the vaccine contains a heparanase peptide, or a functional variant thereof, which binds to a HLA molecule.

2. A vaccine according to claim 1, wherein the HLA molecule is HLA-A2.

3. A vaccine according to claim 1 or 2, wherein the heparanase peptide is selected from the group consisting of SEQ ID NOs: 1-505.

4. A vaccine according to any of claims 1 to 3, wherein the heparanase peptide is selected from the group consisting of SEQ ID NOs: 1-92.

5. A vaccine according to any of claims 1 to 4, wherein the heparanase peptide is selected from the group consisting of SEQ ID NOs: 1, 2, and 3.

6. A vaccine according to any of claims 1 to 5, wherein the functional variant is a nucleic acid coding for heparanase peptide according to SEQ ID NOs: 1, 2, or 3.

7. A vaccine according to claim 6, wherein the nucleic acid is inserted into an expression vector.

8. A vaccine according to any of claims 1 to 7, wherein said vaccine is suitable to be delivered into a cell.

9. A cell containing a vaccine according to any of claims 1 to 8.

10. The use of a heparanase peptide selected from the group consisting of SEQ ID NOs: 1-505, or a functional derivative thereof, which binds to a HLA molecule, for the manufacture of a medicament which induces an immune response.

11. The use of a heparanase peptide selected from the group consisting of SEQ ID NOs: 1-505, or a functional derivative thereof, which binds to a HLA molecule, for the manufacture of a medicament for the treatment of metastatic tumors.

12. The use according to claim 11, wherein the metastatic tumor is selected from the group consisting of neuroblastoma, rectum carcinoma, colon carcinoma, familiary adenomatous polyposis carcinoma and hereditary non-polyposis colorectal cancer, esophageal carcinoma, labial carcinoma, larynx carcinoma, hypopharynx carcinoma, tong carcinoma, salivary gland carcinoma, gastric carcinoma, adenocarcinoma, medullary thyroid carcinoma, papillary thyroid carcinoma, follicular thyroid carcinoma, anaplastic thyroid carcinoma, renal carcinoma, kidney parenchym carcinoma, ovarian carcinoma, cervix carcinoma, uterine corpus carcinoma, endometrium carcinoma, chorion carcinoma, pancreatic carcinoma, prostate carcinoma, testis carcinoma, breast carcinoma, urinary carcinoma, melanoma, glioblastoma, astrocytoma, meningioma, medulloblastoma and peripheral neuroectodermal tumors, Hodgkin lymphoma, non-Hodgkin lymphoma, Burkitt lymphoma, acute lymphatic leukemia (ALL), chronic lymphatic leukemia (CLL), acute myeolid leukemia (AML), chronic myeloid leukemia (CML), adult T-cell leukemia lymphoma, hepatocellular carcinoma, gall bladder carcinoma, bronchial carcinoma, small cell lung carcinoma, non-small cell lung carcinoma, multiple myeloma, basalioma, teratoma, retinoblastoma, choroidea melanoma, seminoma, rhabdomyosarcoma, craniopharyngeoma, osteosarcoma, chondrosarcoma, myosarcoma, liposarcoma, fibrosarcoma, Ewing sarcoma and plasmocytoma.

13. The use according to claim 11 or 12, wherein the metastatic tumor is selected from the group consisting of breast carcinoma, lung carcinoma, prostate carcinoma and colon carcinoma.

14. The use according to any of claims 11 to 13, wherein the metastatic tumor is breast carcinoma.

15. A medicament for the treatment of metastatic carcinoma, containing at least a vaccine according to any of claims 1 to 8, optionally in combination with a pharmaceutically acceptable carrier.

16. A diagnostic method for the determination of the presence and frequency of T cells which are specific for a heparanase peptide according to SEQ ID NOs: 1, 2 and/or 3, the method comprising

(a) isolating mononuclear cells from the periphery blood of bone marrow of a patient,
(b) incubating the cells with heparanase-conjugated HLA tetramers or dimers, and
(c) measuring the number of $CD8^+$- or $CD4^+$-/tetramer double-positive cells.

17. A diagnostic kit containing at least one heparanase peptide, or a functional variant thereof, optionally together with a HLA tetramer or dimer, and optionally together with other compounds.

18. A heparanase peptide that binds to HLA molecule, wherein the heparanase peptide is a nonapeptide having the sequence selected from the group consisting of SEQ ID NOs: 1-505, or a functional derivative thereof.

19. A heparanase peptide according to claim 18, wherein the heparanase peptide is a nonapeptide having the sequence selected from the group consisting of SEQ ID NOs: 1 -3, or a functional derivative thereof.

TABLE 1

HLA-A*0201 nonamers			
Number	Pos	1 2 3 4 5 6 7 8 9	score
1	16	L L L G P L G P L	31
2	8	A L P P P L M L L	29
3	183	D L I F G L N A L	28
4	315	V L D I F I S S V	28
5	13	L M L L L L G P L	27
6	72	L I L L G S P K L	27
7	310	F L N P D V L D I	27
8	184	L I F G L N A L L	26
9	487	G L L S K S V Q L	26
10	408	L L F K K L V G T	25
11	1	M L L R S K P A L	24
12	44	F T Q E P L H L V	23
13	79	K L R T L A R G L	23
14	187	G L N A L L R T A	23
15	346	S A Y G G G A P L	23
16	494	Q L N G L T L K M	23
17	64	N L A T D P R F L	22
18	82	T L A R G L S P A	22
19	241	Q L G E D F I Q L	22
20	363	F M W L D K L G L	22
21	372	S A R M G I E V V	22
22	400	P L P D Y W L S L	22
23	405	W L S L L F K K L	22
24	51	L V S P S F L S V	21
25	75	L G S P K L R T L	21
26	180	S G L D L I F G L	21
27	299	Y L N G R T A T R	21
28	430	K L R V Y L H C T	21
29	456	N L H N V T K Y L	21
30	33	A Q A Q D V V D L	20
31	189	N A L L R T A D L	20
32	282	F L K A G G E V I	20
33	285	A G G E V I D S V	20
34	318	I F I S S V Q K V	20
35	66	A T D P R F L I L	19
36	91	Y L R F G G T K T	19
37	361	A G F M W L D K L	19
38	412	K L V G T K V L M	19
39	415	G T K V L M A S V	19
40	449	D L T L Y A I N L	19
41	452	L Y A I N L H N V	19
42	478	Y L L R P L G P H	19
43	492	S V Q L N G L T L	19
44	15	L L L L G P L G P	18
45	74	L L G S P K L R T	18
46	131	S I P P D V E E K	18
47	174	Y T F A N C S G L	18
48	206	L L L D Y C S S K	18
49	229	F L K K A D I F I	18
50	234	D I F I N G S Q L	18

51	262	KLYGPDVGQ	18
52	277	KMLKSFLKA	18
53	365	WLDKLGLSA	18
54	376	GIEVVMRQV	18
55	393	LVDENFDPL	18
56	490	SKSVQLNGL	18
57	7	PALPPPLML	17
58	176	FANCSGLDL	17
59	248	QLHKLLRKS	17
60	321	SSVQKVFQV	17
61	347	AYGGGAPLL	17
62	418	VLMASVQGS	17
63	9	LPPPLMLLL	16
64	17	LLGPLGPLS	16
65	20	PLGPLSPGA	16
66	56	FLSVTIDAN	16
67	60	TIDANLATD	16
68	142	LEWPYQEQL	16
69	151	LLREHYQKK	16
70	165	YSRSSVDVL	16
71	177	ANCSGLDLI	16
72	222	LGNEPNSFL	16
73	252	LLRKSTFKN	16
74	281	SFLKAGGEV	16
75	292	SVTWHHYYL	16
76	303	RTATREDFL	16
77	322	SVQKVFQVV	16
78	325	KVFQVVEST	16
79	353	PLLSDTFAA	16
80	354	LLSDTFAAG	16
81	371	LSARMGIEV	16
82	386	FGAGNYHLV	16
83	411	KKLVGTKVL	16
84	413	LVGTKVLMA	16
85	444	RYKEGDLTL	16
86	479	LLRPLGPHG	16
87	480	LRPLGPHGL	16
88	488	LLSKSVQLN	16
89	503	VDDQTLPPL	16
90	514	KPLRPGSSL	16
91	516	LRPGSSLGL	16
92	533	VIRNAKVAA	16
93	2	LLRSKPALP	15
94	12	PLMLLLLGP	15
95	27	GALPRPAQA	15
96	65	LATDPRFLI	15
97	67	TDPRFLILL	15
98	73	ILLGSPKLR	15
99	132	IPPDVEEKL	15
100	150	LLLREHYQK	15
101	244	EDFIQLHKL	15
102	260	NAKLYGPDV	15
103	333	TRPGKKVWL	15
104	401	LPDYWLSLL	15
105	407	SLLFKKLVG	15
106	454	AINLHNVTK	15

107	481	R**P**L G P H G L **L**	15
108	495	L **N** G L T L K M **V**	15
109	500	L **K** M V D D Q T **L**	15
110	506	Q **T** L P P L M E **K**	15
111	507	T **L** P P L M E K **P**	15
112	521	S **L** G L P A F S **Y**	15
113	523	G **L** P A F S Y S **F**	15
114	531	F **F** V I R N A K **V**	15
115	5	S **K** P A L P P P **L**	14
116	14	M **L** L L L G P L **G**	14
117	28	A **L** P R P A Q A **Q**	14
118	49	L **H** L V S P S F **L**	14
119	53	S **P** S F L S V T **I**	14
120	57	L **S** V T I D A N **L**	14
121	59	V **T** I D A N L A **T**	14
122	71	F **L** I L L G S P **K**	14
123	84	A **R** G L S P A Y **L**	14
124	86	G **L** S P A Y L R **F**	14
125	94	F **G** G T K T D F **L**	14
126	102	L I F D P K K E **S**	14
127	172	V **L** Y T F A N C **S**	14
128	190	A **L** L R T A D L **Q**	14
129	205	Q **L** L L D Y C S **S**	14
130	214	K **G** Y N I S W E **L**	14
131	236	F **I** N G S Q L G **E**	14
132	251	K **L** L R K S T F **K**	14
133	255	K **S** T F K N A K **L**	14
134	275	T **A** K M L K S F **L**	14
135	278	M **L** K S F L K A **G**	14
136	350	G **G** A P L L S D **T**	14
137	358	T **F** A A G F M W **L**	14
138	374	R **M** G I E V V M **R**	14
139	380	V **M** R Q V F F G **A**	14
140	385	F **F** G A G N Y H **L**	14
141	406	L **S** L L F K K L **V**	14
142	419	L **M** A S V Q G S **K**	14
143	423	V **Q** G S K R R K **L**	14
144	427	K **R** R K L R V Y **L**	14
145	453	Y **A** I N L H N V **T**	14
146	463	Y **L** R L P Y P F **S**	14
147	475	V **D** K Y L L R P **L**	14
148	511	L **M** E K P L R P **G**	14
149	21	L **G** P L S P G A **L**	13
150	40	D **L** D F F T Q E **P**	13
151	101	F **L** I F D P K K **E**	13
152	124	Q **D** I C K Y G S **I**	13
153	149	Q **L** L L R E H Y **Q**	13
154	191	L **L** R T A D L Q **W**	13
155	196	D **L** Q W N S S N **A**	13
156	198	Q **W** N S S N A Q **L**	13
157	207	L **L** D Y C S S K **G**	13
158	339	V **W** L G E T S S **A**	13
159	340	W **L** G E T S S A **Y**	13
160	369	L **G** L S A R M G **I**	13
161	398	F **D** P L P D Y W **L**	13
162	497	G **L** T L K M V D **D**	13

163	499	TLKMVDDQT	13
164	525	PAFSYSFFV	13
165	10	PPPLMLLLL	12
166	23	PLSPGALPR	12
167	31	RPAQAQDVV	12
168	41	LDFFTQEPL	12
169	128	KYGSIPPDV	12
170	139	KLRLEWPYQ	12
171	141	RLEWPYQEQ	12
172	144	WPYQEQLLL	12
173	164	TYSRSSVDV	12
174	186	FGLNALLRT	12
175	210	YCSSKGYNI	12
176	217	NISWELGNE	12
177	227	NSFLKKADI	12
178	232	KADIFINGS	12
179	274	KTAKMLKSF	12
180	410	FKKLVGTKV	12
181	434	YLHCTNTDN	12
182	450	LTLYAINLH	12
183	451	TLYAINLHN	12
184	458	HNVTKYLRL	12
185	498	LTLKMVDDQ	12
186	510	PLMEKPLRP	12
187	535	RNAKVAACI	12
188	43	FFTQEPLHL	11
189	50	HLVSPSFLS	11
190	83	LARGLSPAY	11
191	103	IFDPKKEST	11
192	121	QVNQDICKY	11
193	169	SVDVLYTFA	11
194	181	GLDLIFGLN	11
195	200	NSSNAQLLL	11
196	239	GSQLGEDFI	11
197	245	DFIQLHKLL	11
198	270	QPRRKTAKM	11
199	284	KAGGEVIDS	11
200	289	VIDSVTWHH	11
201	298	YYLNGRTAT	11
202	307	REDFLNPDV	11
203	368	KLGLSARMG	11
204	370	GLSARMGIE	11
205	373	ARMGIEVVM	11
206	392	HLVDENFDP	11
207	404	YWLSLLFKK	11
208	425	GSKRRKLRV	11
209	447	EGDLTLYAI	11
210	472	NKQVDKYLL	11
211	483	LGPHGLLSK	11
212	508	LPPLMEKPL	11
213	515	PLRPGSSLG	11
214	534	IRNAKVAAC	11
215	19	GPLGPLSPG	10
216	48	PLHLVSPSF	10
217	52	VSPSFLSVT	10
218	55	SFLSVTIDA	10

219	58	S**V**TIDANL**A**	10
220	92	L**R**FGGTKT**D**	10
221	130	G**S**IPPDVE**E**	10
222	134	P**D**VEEKLR**L**	10
223	162	N**S**TYSRSS**V**	10
224	168	S**S**VDVLYT**F**	10
225	182	L**D**LIFGLN**A**	10
226	199	W**N**SSNAQL**L**	10
227	202	S**N**AQLLLD**Y**	10
228	221	E**L**GNEPNS**F**	10
229	246	F**I**QLHKLL**R**	10
230	247	I**Q**LHKLLR**K**	10
231	267	D**V**GQPRRK**T**	10
232	283	L**K**AGGEVI**D**	10
233	317	D**I**FISSVQ**K**	10
234	336	G**K**KVWLGE**T**	10
235	338	K**V**WLGETS**S**	10
236	359	F**A**AGFMWL**D**	10
237	367	D**K**LGLSAR**M**	10
238	379	V**V**MRQVFF**G**	10
239	442	N**P**RYKEGD**L**	10
240	446	K**E**GDLTLY**A**	10
241	455	I**N**LHNVTK**Y**	10
242	465	R**L**PYPFSN**K**	10
243	471	S**N**KQVDKY**L**	10
244	474	Q**V**DKYLLR**P**	10
245	482	P**L**GPHGLL**S**	10
246	484	G**P**HGLLSK**S**	10
247	501	K**M**VDDQTL**P**	10
248	529	Y**S**FFVIRN**A**	10
249	532	F**V**IRNAKV**A**	10
250	6	K**P**ALPPPL**M**	9
251	24	L**S**PGALPR**P**	9
252	34	Q**A**QDVVDL**D**	9
253	37	D**V**VDLDFF**T**	9
254	70	R**F**LILLGS**P**	9
255	81	R**T**LARGLS**P**	9
256	98	K**T**DFLIFD**P**	9
257	110	S**T**FEERSY**W**	9
258	135	D**V**EEKLRL**E**	9
259	156	Y**Q**KKFKNS**T**	9
260	167	R**S**SVDVLY**T**	9
261	203	N**A**QLLLDY**C**	9
262	218	I**S**WELGNE**P**	9
263	258	F**K**NAKLYG**P**	9
264	305	A**T**REDFLN**P**	9
265	312	N**P**DVLDIF**I**	9
266	332	S**T**RPGKKV**W**	9
267	349	G**G**GAPLLS**D**	9
268	389	G**N**YHLVDE**N**	9
269	417	K**V**LMASVQ**G**	9
270	432	R**V**YLHCTN**T**	9
271	464	L**R**LPYPFS**N**	9
272	466	L**P**YPFSNK**Q**	9
273	485	P**H**GLLSKS**V**	9
274	493	V**Q**LNGLTL**K**	9

275	519	GSSLGLPAF	9
276	520	SSLGLPAFS	9
277	526	AFSYSFFVI	9
278	46	QEPLHLVSP	8
279	95	GGTKTDFLI	8
280	118	WQSQVNQDI	8
281	125	DICKYGSIP	8
282	127	CKYGSIPPD	8
283	193	RTADLQWNS	8
284	201	SSNAQLLLD	8
285	225	EPNSFLKKA	8
286	237	INGSQLGED	8
287	263	LYGPDVGQP	8
288	268	VGQPRRKTA	8
289	288	EVIDSVTWH	8
290	308	EDFLNPDVL	8
291	314	DVLDIFISS	8
292	319	FISSVQKVF	8
293	328	QVVESTRPG	8
294	331	ESTRPGKKV	8
295	351	GAPLLSDTF	8
296	352	APLLSDTFA	8
297	364	MWLDKLGLS	8
298	366	LDKLGLSAR	8
299	375	MGIEVVMRQ	8
300	388	AGNYHLVDE	8
301	409	LFKKLVGTK	8
302	422	SVQGSKRRK	8
303	433	VYLHCTNTD	8
304	439	NTDNPRYKE	8
305	459	NVTKYLRLP	8
306	470	FSNKQVDKY	8
307	502	MVDDQTLPP	8
308	18	LGPLGPLSP	7
309	25	SPGALPRPA	7
310	30	PRPAQAQDV	7
311	38	VVDLDFFTQ	7
312	87	LSPAYLRFG	7
313	89	PAYLRFGGT	7
314	90	AYLRFGGTK	7
315	117	YWQSQVNQD	7
316	140	LRLEWPYQE	7
317	146	YQEQLLLRE	7
318	147	QEQLLLREH	7
319	163	STYSRSSVD	7
320	194	TADLQWNSS	7
321	242	LGEDFIQLH	7
322	249	LHKLLRKST	7
323	293	VTWHHYYLN	7
324	297	HYYLNGRTA	7
325	311	LNPDVLDIF	7
326	324	QKVFQVVES	7
327	387	GAGNYHLVD	7
328	426	SKRRKLRVY	7
329	445	YKEGDLTLY	7
330	4	RSKPALPPP	6

331	22	GPLSPGALP	6
332	29	LPRPAQAQD	6
333	62	DANLATDPR	6
334	76	GSPKLRTLA	6
335	114	ERSYWQSQV	6
336	143	EWPYQEQLL	6
337	171	DVLYTFANC	6
338	188	LNALLRTAD	6
339	195	ADLQWNSSN	6
340	204	AQLLLDYCS	6
341	212	SSKGYNISW	6
342	216	YNISWELGN	6
343	235	IFINGSQLG	6
344	240	SQLGEDFIQ	6
345	256	STFKNAKLY	6
346	271	PRRKTAKML	6
347	295	WHHYYLNGR	6
348	306	TREDFLNPD	6
349	329	VVESTRPGK	6
350	344	TSSAYGGGA	6
351	345	SSAYGGGAP	6
352	355	LSDTFAAGF	6
353	360	AAGFMWLDK	6
354	383	QVFFGAGNY	6
355	420	MASVQGSKR	6
356	437	CTNTDNPRY	6
357	448	GDLTLYAIN	6
358	461	TKYLRLPYP	6
359	491	KSVQLNGLT	6
360	522	LGLPAFSYS	6
361	528	SYSFFVIRN	6
362	11	PPLMLLLLG	5
363	39	VDLDFFTQE	5
364	61	IDANLATDP	5
365	63	ANLATDPRF	5
366	77	SPKLRTLAR	5
367	78	PKLRTLARG	5
368	88	SPAYLRFGG	5
369	96	GTKTDFLIF	5
370	97	TKTDFLIFD	5
371	100	DFLIFDPKK	5
372	116	SYWQSQVNQ	5
373	159	KFKNSTYSR	5
374	170	VDVLYTFAN	5
375	192	LRTADLQWN	5
376	197	LQWNSSNAQ	5
377	213	SKGYNISWE	5
378	220	WELGNEPNS	5
379	228	SFLKKADIF	5
380	230	LKKADIFIN	5
381	233	ADIFINGSQ	5
382	261	AKLYGPDVG	5
383	279	LKSFLKAGG	5
384	287	GEVIDSVTW	5
385	296	HHYYLNGRT	5
386	334	RPGKKVWLG	5

387	348	YGGGAPLL**S**	5
388	384	VFFGAGNY**H**	5
389	391	Y**H**LVDENF**D**	5
390	396	E**N**FDPLPD**Y**	5
391	403	D**Y**WLSLLF**K**	5
392	414	V**G**TKVLMA**S**	5
393	460	V**T**KYLRLP**Y**	5
394	462	K**Y**LRLPYP**F**	5
395	467	P**Y**PFSNKQ**V**	5
396	477	K**Y**LLRPLG**P**	5
397	504	D**D**QTLPPL**M**	5
398	527	F**S**YSFFVI**R**	5
399	530	S**F**FVIRNA**K**	5
400	3	L**R**SKPALP**P**	4
401	36	Q**D**VVDLDF**F**	4
402	47	E**P**LHLVSP**S**	4
403	68	D**P**RFLILL**G**	4
404	69	P**R**FLILLG**S**	4
405	85	R**G**LSPAYL**R**	4
406	104	F**D**PKKESTF	4
407	136	V**E**EKLRLE**W**	4
408	145	P**Y**QEQLLL**R**	4
409	161	K**N**STYSRS**S**	4
410	166	S**R**SSVDVL**Y**	4
411	178	N**C**SGLDLI**F**	4
412	179	C**S**GLDLIF**G**	4
413	231	K**K**ADIFIN**G**	4
414	250	H**K**LLRKST**F**	4
415	253	L**R**KSTFKN**A**	4
416	259	K**N**AKLYGP**D**	4
417	265	G**P**DVGQPR**R**	4
418	276	A**K**MLKSFL**K**	4
419	280	K**S**FLKAGG**E**	4
420	286	G**G**EVIDSV**T**	4
421	301	N**G**RTATRE**D**	4
422	309	D**F**LNPDVL**D**	4
423	320	I**S**SVQKVF**Q**	4
424	337	K**K**VWLGET**S**	4
425	356	S**D**TFAAGF**M**	4
426	357	D**T**FAAGFM**W**	4
427	428	R**R**KLRVYL**H**	4
428	431	L**R**VYLHCT**N**	4
429	435	L**H**CTNTDN**P**	4
430	440	T**D**NPRYKE**G**	4
431	457	L**H**NVTKYL**R**	4
432	473	K**Q**VDKYLL**R**	4
433	512	M**E**KPLRPG**S**	4
434	517	R**P**GSSLGL**P**	4
435	32	P**A**QAQDVV**D**	3
436	35	A**Q**DVVDLD**F**	3
437	45	T**Q**EPLHLV**S**	3
438	129	Y**G**SIPPDV**E**	3
439	152	L**R**EHYQKK**F**	3
440	185	I**F**GLNALL**R**	3
441	208	L**D**YCSSKG**Y**	3
442	211	C**S**SKGYNI**S**	3

443	219	SWELGNEPN	3
444	224	NEPNSFLKK	3
445	254	RKSTFKNAK	3
446	264	YGPDVGQPR	3
447	273	RKTAKMLKS	3
448	290	IDSVTWHHY	3
449	304	TATREDFLN	3
450	316	LDIFISSVQ	3
451	323	VQKVFQVVE	3
452	326	VFQVVESTR	3
453	330	VESTRPGKK	3
454	341	LGETSSAYG	3
455	378	EVVMRQVFF	3
456	382	RQVFFGAGN	3
457	397	NFDPLPDYW	3
458	399	DPLPDYWLS	3
459	421	ASVQGSKRR	3
460	429	RKLRVYLHC	3
461	468	YPFSNKQVD	3
462	496	NGLTLKMVD	3
463	518	PGSSLGLPA	3
464	524	LPAFSYSFF	3
465	26	PGALPRPAQ	2
466	54	PSFLSVTID	2
467	93	RFGGTKTDF	2
468	106	PKKESTFEE	2
469	107	KKESTFEER	2
470	108	KESTFEERS	2
471	111	TFEERSYWQ	2
472	120	SQVNQDICK	2
473	123	NQDICKYGS	2
474	126	ICKYGSIPP	2
475	158	KKFKNSTYS	2
476	160	FKNSTYSRS	2
477	173	LYTFANCSG	2
478	175	TFANCSGLD	2
479	215	GYNISWELG	2
480	223	GNEPNSFLK	2
481	269	GQPRRKTAK	2
482	294	TWHHYYLNG	2
483	300	LNGRTATRE	2
484	342	GETSSAYGG	2
485	343	ETSSAYGGG	2
486	362	GFMWLDKLG	2
487	377	IEVVMRQVF	2
488	381	MRQVFFGAG	2
489	394	VDENFDPLP	2
490	443	PRYKEGDLT	2
491	476	DKYLLRPLG	2
492	486	HGLLSKSVQ	2
493	489	LSKSVQLNG	2
494	42	DFFTQEPLH	1
495	80	LRTLARGLS	1
496	99	TDFLIFDPK	1
497	115	RSYWQSQVN	1
498	122	VNQDICKYG	1

499	154	E **H** Y Q K K F K **N**	1
500	157	Q **K** K F K N S T **Y**	1
501	238	N **G** S Q L G E D **F**	1
502	243	G **E** D F I Q L H **K**	1
503	313	P **D** V L D I F I **S**	1
504	327	F **Q** V V E S T R **P**	1
505	441	D **N** P R Y K E G **D**	1

TABLE 2

HLA-DRB1*0401 (DR4Dw4) 15 - mers			
Number	Pos	1 2 3 4 5 6 7 8 9 0 1 2 3 4 5	score
1	382	RQVFFGAGNYHLVDE	28
2	528	SYSFFVIRNAKVAAC	28
3	38	VVDLDFFTQEPLHLV	26
4	56	FLSVTIDANLATDPR	26
5	62	DANLATDPRFLILLG	26
6	69	PRFLILLGSPKLRTL	26
7	77	SPKLRTLARGLSPAY	26
8	167	RSSVDVLYTFANCSG	26
9	219	SWELGNEPNSFLKKA	26
10	246	FIQLHKLLRKSTFKN	26
11	313	PDVLDIFISSVQKVF	26
12	41	LDFFTQEPLHLVSPS	22
13	53	SPSFLSVTIDANLAT	22
14	68	DPRFLILLGSPKLRT	22
15	88	SPAYLRFGGTKTDFL	22
16	91	YLRFGGTKTDFLIFD	22
17	115	RSYWQSQVNQDICKY	22
18	141	RLEWPYQEQLLLREH	22
19	171	DVLYTFANCSGLDLI	22
20	279	LKSFLKAGGEVIDSV	22
21	295	WHHYYLNGRTATRED	22
22	324	QKVFQVVESTRPGKK	22
23	337	KKVWLGETSSAYGGG	22
24	360	AAGFMWLDKLGLSAR	22
25	395	DENFDPLPDYWLSLL	22
26	402	PDYWLSLLFKKLVGT	22
27	407	SLLFKKLVGTKVLMA	22
28	431	LRVYLHCTNTDNPRY	22
29	450	LTLYAINLHNVTKYL	22
30	460	VTKYLRLPYPFSNKQ	22
31	526	AFSYSFFVIRNAKVA	22
32	10	PPPLMLLLLGPLGPL	20
33	18	LGPLGPLSPGALPRP	20
34	35	AQDVVDLDFFTQEPL	20
35	46	QEPLHLVSPSFLSVT	20
36	99	TDFLIFDPKKESTFE	20
37	129	YGSIPPDVEEKLRLE	20
38	139	KLRLEWPYQEQLLLR	20
39	148	EQLLLREHYQKKFKN	20
40	170	VDVLYTFANCSGLDL	20
41	179	CSGLDLIFGLNALLR	20
42	181	GLDLIFGLNALLRTA	20
43	185	IFGLNALLRTADLQW	20
44	189	NALLRTADLQWNSSN	20
45	194	TADLQWNSSNAQLLL	20
46	203	NAQLLLDYCSSKGYN	20
47	227	NSFLKKADIFINGSQ	20

48	265	GPDVGQPRRKTAKML	20
49	312	NPDVLDIFISSVQKV	20
50	317	DIFISSVQKVFQVVE	20
51	320	ISSVQKVFQVVESTR	20
52	326	VFQVVESTRPGKKVW	20
53	336	GKKVWLGETSSAYGG	20
54	361	AGFMWLDKLGLSARM	20
55	366	LDKLGLSARMGIEVV	20
56	372	SARMGIEVVMRQVFF	20
57	374	RMGIEVVMRQVFFGA	20
58	390	NYHLVDENFDPLPDY	20
59	403	DYWLSLLFKKLVGTK	20
60	415	GTKVLMASVQGSKRR	20
61	416	TKVLMASVQGSKRRK	20
62	420	MASVQGSKRRKLRVY	20
63	428	RRKLRVYLHCTNTDN	20
64	449	DLTLYAINLHNVTKY	20
65	463	YLRLPYPFSNKQVDK	20
66	477	KYLLRPLGPHGLLSK	20
67	492	SVQLNGLTLKMVDDQ	20
68	497	GLTLKMVDDQTLPPL	20
69	499	TLKMVDDQTLPPLME	20
70	505	DQTLPPLMEKPLRPG	20
71	509	PPLMEKPLRPGSSLG	20
72	513	EKPLRPGSSLGLPAF	20
73	23	PLSPGALPRPAQAQD	18
74	50	HLVSPSFLSVTIDAN	18
75	108	KESTFEERSYWQSQV	18
76	186	FGLNALLRTADLQWN	18
77	190	ALLRTADLQWNSSNA	18
78	216	YNISWELGNEPNSFL	18
79	230	LKKADIFINGSQLGE	18
80	240	SQLGEDFIQLHKLLR	18
81	252	LLRKSTFKNAKLYGP	18
82	273	RKTAKMLKSFLKAGG	18
83	304	TATREDFLNPDVLDI	18
84	314	DVLDIFISSVQKVFQ	18
85	408	LLFKKLVGTKVLMAS	18
86	443	PRYKEGDLTLYAINL	18
87	448	GDLTLYAINLHNVTK	18
88	451	TLYAINLHNVTKYLR	18
89	464	LRLPYPFSNKQVDKY	18
90	482	PLGPHGLLSKSVQLN	18
91	527	FSYSFFVIRNAKVAA	18
92	40	DLDFFTQEPLHLVSP	16
93	98	KTDFLIFDPKKESTF	16
94	126	ICKYGSIPPDVEEKL	16
95	157	QKKFKNSTYSRSSVD	16
96	162	NSTYSRSSVDVLYTF	16
97	173	LYTFANCSGLDLIFG	16
98	183	DLIFGLNALLRTADL	16
99	196	DLQWNSSNAQLLLDY	16
100	207	LLDYCSSKGYNISWE	16
101	213	SKGYNISWELGNEPN	16
102	217	NISWELGNEPNSFLK	16
103	233	ADIFINGSQLGEDFI	16

104	243	**G**ED**F**I**QL**H**K**LLRKST	16
105	255	**K**ST**F**K**NA**K**L**YGPDVG	16
106	261	**A**KLYG**PD**VG**Q**PRRKT	16
107	296	**H**HYY**L**N**G**RT**A**TREDF	16
108	307	**R**EDFL**NP**D**V**LDIFIS	16
109	316	**L**DIF**IS**S**V**Q**K**VFQVV	16
110	345	**S**SAYG**G**G**A**P**L**LSDTF	16
111	383	**Q**VFF**G**A**G**N**Y**HLVDEN	16
112	388	**A**GNYHL**V**DE**N**FDPLP	16
113	401	**L**PDY**W**L**S**LLFKKLVG	16
114	147	**Q**EQLL**L**R**E**HYQKKFK	15
115	249	**L**HKL**L**R**K**S**T**FKNAKL	15
116	6	**K**PALP**P**P**L**M**L**LLLGP	14
117	11	**P**PL**M**L**L**LL**G**PLGPLS	14
118	12	**P**LM**L**LL**L**G**P**LGPLSP	14
119	13	**L**ML**L**LL**G**P**L**GPLSPG	14
120	14	**M**LLLG**P**L**G**PLSPGA	14
121	15	**L**LLLG**P**L**G**PLSPGAL	14
122	26	**P**GAL**P**R**P**A**Q**AQDVVD	14
123	36	**Q**DV**V**DL**D**F**F**TQEPLH	14
124	48	**P**LHLV**S**P**S**FLSVTID	14
125	49	**L**HL**V**S**P**S**F**LSVTIDA	14
126	54	**P**SFLSV**T**I**D**ANLATD	14
127	71	**F**LI**L**L**G**S**P**K**L**RTLAR	14
128	72	**L**IL**L**G**S**P**K**L**R**TLARG	14
129	80	**L**RTLA**R**G**L**S**P**AYLRF	14
130	84	**A**RGLS**P**A**Y**L**R**FGGTK	14
131	89	**P**AYL**R**F**G**G**T**KTDFLI	14
132	100	**D**FLIF**D**P**K**K**E**STFEE	14
133	119	**Q**SQ**V**N**Q**D**I**CKYGSIP	14
134	123	**N**QDIC**K**Y**G**S**I**PPDVE	14
135	137	**E**EKLRL**E**W**P**YQEQLL	14
136	149	**Q**LLLRE**H**Y**Q**KKFKNS	14
137	169	**S**VDV**L**Y**T**F**A**NCSGLD	14
138	182	LDLIF**G**L**N**ALLRTAD	14
139	204	**A**QLLL**D**Y**C**S**S**KGYNI	14
140	205	**Q**LLLD**Y**C**S**S**K**GYNIS	14
141	215	**G**YNIS**W**E**L**G**N**EPNSF	14
142	232	**K**ADIF**I**N**G**S**Q**LGEDF	14
143	239	**G**SQLG**E**D**F**IQLHKLL	14
144	244	**E**DFIQ**L**H**K**LLRKSTF	14
145	250	**H**KLLR**K**S**T**FKNAKLY	14
146	276	**A**KMLK**S**F**L**K**A**GGEVI	14
147	286	**G**GE**V**I**D**S**V**T**W**HHYYL	14
148	287	**G**EVID**S**V**T**W**H**HYYLN	14
149	290	IDS**V**T**W**H**H**YYLNGRT	14
150	308	**E**DFLN**P**D**V**LDIFISS	14
151	315	**V**LDIF**I**S**S**V**Q**KVFQV	14
152	323	**V**QK**V**F**Q**V**V**E**S**TRPGK	14
153	327	FQV**V**E**S**T**R**P**G**KKVWL	14
154	338	**K**VWLGE**T**S**S**AYGGGA	14
155	351	**G**APLL**S**D**T**FAAGFMW	14
156	363	FMWLD**K**L**G**LSARMGI	14
157	377	IEV**V**M**R**Q**V**F**F**GAGNY	14
158	378	EVV**M**R**Q**V**F**F**G**AGNYH	14
159	398	**F**DPLP**D**Y**W**L**S**LLFKK	14

160	410	FKKLVGTKVLMASVQ	14
161	417	KVLMASVQGSKRRKL	14
162	430	KLRVYLHCTNTDNPR	14
163	432	RVYLHCTNTDNPRYK	14
164	454	AINLHNVTKYLRLPY	14
165	457	LHNVTKYLRLPYPFS	14
166	461	TKYLRLPYPFSNKQV	14
167	472	NKQVDKYLLRPLGPH	14
168	480	LRPLGPHGLLSKSVQ	14
169	486	HGLLSKSVQLNGLTL	14
170	490	SKSVQLNGLTLKMVD	14
171	500	LKMVDDQTLPPLMEK	14
172	519	GSSLGLPAFSYSFFV	14
173	521	SLGLPAFSYSFFVIR	14
174	2	LLRSKPALPPPLMLL	12
175	3	LRSKPALPPPLMLLL	12
176	7	PALPPPLMLLLLGPL	12
177	17	LLGPLGPLSPGALPR	12
178	22	GPLSPGALPRPAQAQ	12
179	27	GALPRPAQAQDVVDL	12
180	28	ALPRPAQAQDVVDLD	12
181	34	QAQDVVDLDFFTQEP	12
182	37	DVVDLDFFTQEPLHL	12
183	42	DFFTQEPLHLVSPSF	12
184	45	TQEPLHLVSPSFLSV	12
185	47	EPLHLVSPSFLSVTI	12
186	52	VSPSFLSVTIDANLA	12
187	55	SFLSVTIDANLATDP	12
188	59	VTIDANLATDPRFLI	12
189	66	ATDPRFLILLGSPKL	12
190	74	LLGSPKLRTLARGLS	12
191	81	RTLARGLSPAYLRFG	12
192	86	GLSPAYLRFGGTKTD	12
193	96	GTKTDFLIFDPKKES	12
194	97	TKTDFLIFDPKKEST	12
195	103	IFDPKKESTFEERSY	12
196	107	KKESTFEERSYWQSQ	12
197	111	TFEERSYWQSQVNQD	12
198	112	FEERSYWQSQVNQDI	12
199	113	EERSYWQSQVNQDIC	12
200	116	SYWQSQVNQDICKYG	12
201	120	SQVNQDICKYGSIPP	12
202	131	SIPPDVEEKLRLEWP	12
203	136	VEEKLRLEWPYQEQL	12
204	145	PYQEQLLLREHYQKK	12
205	146	YQEQLLLREHYQKKF	12
206	154	EHYQKKFKNSTYSRS	12
207	158	KKFKNSTYSRSSVDV	12
208	159	KFKNSTYSRSSVDVL	12
209	164	TYSRSSVDVLYTFAN	12
210	166	SRSSVDVLYTFANCS	12
211	177	ANCSGLDLIFGLNAL	12
212	178	NCSGLDLIFGLNALL	12
213	180	SGLDLIFGLNALLRT	12
214	184	LIFGLNALLRTADLQ	12
215	191	LLRTADLQWNSSNAQ	12

216	192	LRTADLQWNSSNAQL	12
217	193	RTADLQWNSSNAQLL	12
218	195	ADLQWNSSNAQLLLD	12
219	197	LQWNSSNAQLLLDYC	12
220	201	SSNAQLLLDYCSSKG	12
221	202	SNAQLLLDYCSSKGY	12
222	211	CSSKGYNISWELGNE	12
223	220	WELGNEPNSFLKKAD	12
224	224	NEPNSFLKKADIFIN	12
225	229	FLKKADIFINGSQLG	12
226	231	KKADIFINGSQLGED	12
227	236	FINGSQLGEDFIQLH	12
228	238	NGSQLGEDFIQLHKL	12
229	241	QLGEDFIQLHKLLRK	12
230	242	LGEDFIQLHKLLRKS	12
231	257	TFKNAKLYGPDVGQP	12
232	262	KLYGPDVGQPRRKTA	12
233	264	YGPDVGQPRRKTAKM	12
234	270	QPRRKTAKMLKSFLK	12
235	282	FLKAGGEVIDSVTWH	12
236	283	LKAGGEVIDSVTWHH	12
237	284	KAGGEVIDSVTWHHY	12
238	289	VIDSVTWHHYYLNGR	12
239	293	VTWHHYYLNGRTATR	12
240	294	TWHHYYLNGRTATRE	12
241	299	YLNGRTATREDFLNP	12
242	305	ATREDFLNPDVLDIF	12
243	309	DFLNPDVLDIFISSV	12
244	310	FLNPDVLDIFISSVQ	12
245	311	LNPDVLDIFISSVQK	12
246	321	SSVQKVFQVVESTRP	12
247	325	KVFQVVESTRPGKKV	12
248	333	TRPGKKVWLGETSSA	12
249	335	PGKKVWLGETSSAYG	12
250	341	LGETSSAYGGGAPLL	12
251	348	YGGGAPLLSDTFAAG	12
252	349	GGGAPLLSDTFAAGF	12
253	350	GGAPLLSDTFAAGFM	12
254	353	PLLSDTFAAGFMWLD	12
255	355	LSDTFAAGFMWLDKL	12
256	357	DTFAAGFMWLDKLGL	12
257	364	MWLDKLGLSARMGIE	12
258	373	ARMGIEVVMRQVFFG	12
259	379	VVMRQVFFGAGNYHL	12
260	389	GNYHLVDENFDPLPD	12
261	397	NFDPLPDYWLSLLFK	12
262	400	PLPDYWLSLLFKKLV	12
263	412	KLVGTKVLMASVQGS	12
264	413	LVGTKVLMASVQGSK	12
265	427	KRRKLRVYLHCTNTD	12
266	429	RKLRVYLHCTNTDNP	12
267	435	LHCTNTDNPRYKEGD	12
268	441	DNPRYKEGDLTLYAI	12
269	444	RYKEGDLTLYAINLH	12
270	446	KEGDLTLYAINLHNV	12
271	453	YAINLHNVTKYLRLP	12

272	466	LPYPFSNKQVDKYLL	12
273	469	PFSNKQVDKYLLRPL	12
274	473	KQVDKYLLRPLGPHG	12
275	478	YLLRPLGPHGLLSKS	12
276	483	LGPHGLLSKSVQLNG	12
277	484	GPHGLLSKSVQLNGL	12
278	487	GLLSKSVQLNGLTLK	12
279	488	LLSKSVQLNGLTLKM	12
280	489	LSKSVQLNGLTLKMV	12
281	491	KSVQLNGLTLKMVDD	12
282	498	LTLKMVDDQTLPPLM	12
283	510	PLMEKPLRPGSSLGL	12
284	520	SSLGLPAFSYSFFVI	12
285	522	LGLPAFSYSFFVIRN	12
286	525	PAFSYSFFVIRNAKV	12
287	109	ESTFEERSYWQSQVN	11
288	153	REHYQKKFKNSTYSR	11
289	226	PNSFLKKADIFINGS	11
290	362	GFMWLDKLGLSARMG	11
291	529	YSFFVIRNAKVAACI	11
292	114	ERSYWQSQVNQDICK	10
293	143	EWPYQEQLLLREHYQ	10
294	292	SVTWHHYYLNGRTAT	10
295	356	SDTFAAGFMWLDKLG	10
296	442	NPRYKEGDLTLYAIN	10
297	465	RLPYPFSNKQVDKYL	10
298	524	LPAFSYSFFVIRNAK	10
299	133	PPDVEEKLRLEWPYQ	9
300	368	KLGLSARMGIEVVMR	9
301	405	WLSLLFKKLVGTKVL	9
302	406	LSLLFKKLVGTKVLM	9
303	411	KKLVGTKVLMASVQG	9
304	485	PHGLLSKSVQLNGLT	9
305	495	LNGLTLKMVDDQTLP	9
306	21	LGPLSPGALPRPAQA	8
307	58	SVTIDANLATDPRFL	8
308	70	RFLILLGSPKLRTLA	8
309	188	LNALLRTADLQWNSS	8
310	234	DIFINGSQLGEDFIQ	8
311	260	NAKLYGPDVGQPRRK	8
312	275	TAKMLKSFLKAGGEV	8
313	280	KSFLKAGGEVIDSVT	8
314	352	APLLSDTFAAGFMWL	8
315	381	MRQVFFGAGNYHLVD	8
316	391	YHLVDENFDPLPDYW	8
317	447	EGDLTLYAINLHNVT	8
318	452	LYAINLHNVTKYLRL	8
319	476	DKYLLRPLGPHGLLS	8
320	73	ILLGSPKLRTLARGL	7
321	155	HYQKKFKNSTYSRSS	7
322	161	KNSTYSRSSVDVLYT	7
323	248	QLHKLLRKSTFKNAK	7
324	267	DVGQPRRKTAKMLKS	7
325	424	QGSKRRKLRVYLHCT	7
326	4	RSKPALPPPLMLLLL	6
327	5	SKPALPPPLMLLLLG	6

328	9	LPPPLMLLLLGPLGP	6
329	24	LSPGALPRPAQAQDV	6
330	29	LPRPAQAQDVVDLDF	6
331	30	PRPAQAQDVVDLDFF	6
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European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 03 01 1038

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	WO 03/006645 A (IMCLONE SYSTEMS INC ;KUSSIE PAUL (US); BOHLEN PETER (US); HICKLIN) 23 January 2003 (2003-01-23)	1,2,9,15	C12N9/24 C12N5/00 A61K39/00
Y	* paragraphs [0029] - [0033] * * paragraph [0035] * * paragraph [0037] * * paragraphs [0047], [0049], [0052] * * claims 1,2,6,10,11,19 *	1-19	G01N33/53 A61P35/00
X	WO 01/21814 A (MERCK PATENT GMBH ;DUECKER KLAUS (DE); SIRRENBERG CHRISTIAN (DE)) 29 March 2001 (2001-03-29)	1,2,9,15	
Y	* page 3, line 34 - page 4, line 23 * * page 12, lines 8-15 * * page 15, lines 16-22 * * page 16, lines 8-26 * * page 17, lines 1-5 *	1-19	

-/--

TECHNICAL FIELDS SEARCHED (Int.Cl.7)

C12N
G01N
A61K

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
Munich	24 November 2003	Lechner, O

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C07)

European Patent Office

INCOMPLETE SEARCH
SHEET C

Application Number
EP 03 01 1038

Although claim(s) 16 is directed to a diagnostic method practised on the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number EP 03 01 1038

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
D,Y	RAMMENSEE HANS-GEORG ET AL: "SYFPEITHI: Database for MHC ligands and peptide motifs." IMMUNOGENETICS, vol. 50, no. 3-4, November 1999 (1999-11), pages 213-219, XP002254433 ISSN: 0093-7711 * the whole document *	1-19	
A	WO 00/52178 A (HADASIT MED RES SERVICE ;PECKER IRIS (IL); FEINSTEIN ELENA (IL); F) 8 September 2000 (2000-09-08) * page 2 * * page 18, line 36 - page 19, line 19 * * page 37, lines 35-37 * * page 38, lines 17-37 * * page 40, lines 26-28 *	1-19	TECHNICAL FIELDS SEARCHED (Int.Cl.7)
A	MOINGEON P: "Cancer vaccines" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 19, no. 11-12, 8 December 2001 (2001-12-08), pages 1305-1326, XP004313943 ISSN: 0264-410X * the whole document *	1-19	
A	VLODAVSKY ISRAEL ET AL: "Mammalian heparanase: involvement in cancer metastasis, angiogenesis and normal development." SEMINARS IN CANCER BIOLOGY. UNITED STATES APR 2002, vol. 12, no. 2, April 2002 (2002-04), pages 121-129, XP002254432 ISSN: 1044-579X * the whole document *	1-19	
	-/--		

EPO FORM 1503 03.82 (P04C10)

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 03 01 1038

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	KRAUSA P ET AL: "HLA-A2 polymorphism and immune functions" EUROPEAN JOURNAL OF IMMUNOGENETICS, vol. 23, no. 4, 1996, pages 261-274, XP009020307 ISSN: 0960-7420 * abstract * -----	1-19	TECHNICAL FIELDS SEARCHED (Int.Cl.7)

EPO FORM 1503 03.82 (P04C10)

European Patent Office

Application Number
EP 03 01 1038

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s):

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:

☒ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:

1-19 (all partially)

European Patent Office

LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 03 01 1038

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. claims: 1-19 (all partially)

Use of the heparanase peptide LLLGPLGPL (Seq ID No1) as a vaccine for treating metastatic tumors and in diagnostic methods for the determination of heparanase-specific T cells in patients.

---

2. claims: 1-19 (all partially)

Use of the heparanase peptide ALPPPLMLL (Seq ID No2) as a vaccine for treating metastatic tumors and in diagnostic methods for the determination of heparanase-specific T cells in patients.

---

3. claims: 1-19 (all partially)

Use of the heparanase peptide DLIFGLNAL (Seq ID No3) as a vaccine for treating metastatic tumors and in diagnostic methods for the determination of heparanase-specific T cells in patients.

---

4. claims: 1-19 (all partially)

Group of inventions 5-506:
Use of the heparanase peptide according to Seq ID No 4 - 505 as a vaccine for treating metastatic tumors and in diagnostic methods for the determination of heparanase-specific T cells in patients.

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 03 01 1038

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

24-11-2003

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

专利名称(译)	用于接种肿瘤患者的乙酰肝素酶衍生肽		
公开(公告)号	EP1479764A1	公开(公告)日	2004-11-24
申请号	EP2003011038	申请日	2003-05-19
[标]申请(专利权)人(译)	德国癌症研究公共权益基金会		
申请(专利权)人(译)	DEUTSCHES KREBSFORSCHUNGSZENTRUM STIFTUNG DESÖFFENTLICHENRECHTS 鲁普雷希特 - 卡尔斯 - 海德堡大学		
当前申请(专利权)人(译)	DEUTSCHES KREBSFORSCHUNGSZENTRUM STIFTUNG DESÖFFENTLICHENRECHTS 鲁普雷希特 - 卡尔斯 - 海德堡大学		
[标]发明人	SCHIRRMACHER VOLKER BECKHOVE PHILIPP SOMMERFELDT NORA		
发明人	SCHIRRMACHER, VOLKER BECKHOVE, PHILIPP SOMMERFELDT, NORA		
IPC分类号	A61K39/00 A61P35/00 C12N9/24 G01N33/574 C12N5/00 G01N33/53		
CPC分类号	A61K39/0005 A61K39/0008 A61K39/0011 A61K2039/5154 A61K2039/5158 A61P35/00 A61P35/04 C12N9/2402 C12Y302/01166 G01N33/574 G01N2333/924 A61K39/001154		
外部链接	Espacenet		

摘要(译)

本发明公开了一种抗增加乙酰肝素酶表达和/或活性的疾病，特别是肿瘤疾病的疫苗，其中疫苗含有与HLA分子结合的乙酰肝素酶肽。

<110> Deutsches Krebsforschungszentrum

<120> Heparanase-derived peptides for vaccination of tumor patients

<130> DK62190EP

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<170> PatentIn version 3.1

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