ENHANCED AFFINITY T CELL RECEPTORS AND METHODS FOR MAKING THE SAME

CROSS REFERENCE TO RELATED APPLICATION

This application claims the benefit under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 61/642,358 filed on May 3, 2012, which application is incorporated by reference herein in its entirety.

STATEMENT REGARDING SEQUENCE LISTING

The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is 360056_412WO_SEQUENCE_LISTING.TXT. The text file is 129KB, was created on May 2, 2013 and is being submitted electronically via EFS-Web.

STATEMENT OF GOVERNMENT INTEREST

This invention was made with government support under Contract No. P01 CA 18029 awarded by National Institute of Health/National Cancer Institute. The government has certain rights in this invention.

BACKGROUND

Technical Field

The present disclosure relates to enhanced affinity T cell receptors (TCRs) and, more particularly, to using agonist selection of hematopoietic progenitor cells expressing an antigen specific TCRα to generate enhanced affinity TCRs, and to uses thereof.

Description of the Related Art

TCR gene therapy is an emerging treatment approach that can overcome many of the obstacles associated with conventional T cell adoptive immunotherapy, such as the extensive time and labor required to isolate, characterize, and expand tumor

antigen-specific T cell clones (Schmitt, Ragnarsson, & Greenberg, 2009, Hum. Gene Ther. 20:1240-1248). Further benefits of gene therapy include the ability to utilize defined populations of T cells capable of long-term persistence *in vivo* (Berger et al., 2008, J. Clin. Invest. 118:294-305; Hinrichs et al., 2009, Proc. Natl. Acad. Sci. USA 106:17469-17474). Such T cells can be transduced with genes encoding well-characterized TCRs that have a high affinity for tumor antigens, thereby increasing the likelihood of mediating an antitumor effect. Indeed, a recent report of therapy targeting advanced B cell leukemia with genetically modified T cells expressing a high affinity chimeric receptor targeting a self/tumor-antigen has highlighted the potential of using engineered high avidity T cells for the treatment of leukemia (Kalos et al., 2011, Sci. Transl. Med. 3:95ra73). However, since most tumor antigens targeted by T cell immunotherapy are over-expressed self-proteins, high affinity T cells specific for these antigens are generally subject to negative selection in the thymus. Therefore, one significant limitation of T cell based immunotherapies in general is the limited availability of T cells expressing an endogenous TCR with sufficiently high affinity for non-mutated tumor antigens.

Several strategies have been developed to enhance the affinity of TCRs intended for use in TCR gene therapy (Richman & Kranz, 2007, Biomol. Eng. 24:361-373; Udyavar et al., 2009, J. Immunol. 182:4439-4447; Zhao et al., 2007, J. Immunol. 179:5845-5854). These approaches generally entail the generation of libraries of TCR mutants that have undergone rounds of mutagenesis and subsequent screening for mutations that confer higher affinity for the target peptide/MHC ligand. Mutations are generally made in the CDR regions that are known to interact with peptide/MHC. CDR1 and CDR2 regions predominantly make contact with the MHC molecule, while the hypervariable CDR3 region primarily contacts the peptide (Wucherpfennig et al., 2010, Cold Spring Harbor Perspectives in Biology 2:a005140-a005140). Site-directed mutagenesis strategies generally target selected portions of all three of these regions, but still are not always successful in generating a higher affinity variant, and the improvements are limited to changes only in the specifically targeted regions. Moreover, mutations introduced into the MHC contact residues have the risk of potentially increasing the affinity of the TCR for MHC while decreasing the overall

specificity of the receptor for its cognate peptide. Ideally, most mutations introduced to enhance the affinity of a TCR would be restricted to the CDR3 region for this reason. However, current methodologies are limited in the capacity to generate CDR3 diversity, because site-directed mutagenesis is constrained by the original length of the CDR3 region.

Given the difficulty of isolating high affinity T cells that recognize relevant tumor associated antigens, there is a continuing need for alternative methods for generating enhanced affinity TCRs.

BRIEF SUMMARY

In one aspect, the present disclosure provides a method for generating an enhanced affinity TCR comprising: a) contacting hematopoietic progenitor cells with stromal cells and a peptide antigen, under conditions and for a time sufficient to induce differentiation of the hematopoietic progenitor cells into DN TCR$\alpha\beta^+$ thymocytes, wherein the hematopoietic progenitor cells comprise a non-endogenous nucleic acid sequence encoding a TCRα chain from a parent TCR specific for the peptide antigen, and wherein the stromal cells comprise a non-endogenous nucleic acid sequence encoding Delta-like-1 or Delta-like-4 and a nucleic acid sequence encoding an MHC molecule; b) isolating nucleic acid sequences encoding the various TCRβ chains from the DN TCR$\alpha\beta^+$ thymocytes and introducing the nucleic acid sequences encoding the TCRβ chains into cells that are capable of expressing a TCR on the cell surface and comprising the nucleic acid sequence encoding the TCRα chain from step a); and identifying enhanced affinity TCR (*e.g.*, by detecting or selecting high affinity TCR$\alpha\beta$ candidate by an MHC tetramer assay, and then measuring binding affinity as compared to a parent TCR$\alpha\beta$).

In further aspects, enhanced affinity TCRs generated by methods disclosed herein are provided, which may be cell-bound or in soluble form, and may further be codon optimized to enhance expression in T cells.

In still further aspects, enhanced affinity TCRs of the present disclosure may be used to treat a disease (such as cancer, infectious disease, or autoimmune disease) in a subject by administering a composition comprising the enhanced affinity

TCRs. In further embodiments, enhanced affinity TCRs of the instant disclosure may be used in diagnostic methods or imaging methods, including these methods used in relation to the indications or conditions identified herein.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURES 1A-D: Thymocytes from OT-1 transgenic mice were sorted for $TCR\beta^-TCR\gamma\delta^-CD4^-CD8^-CD117^+CD44^+$ DN1 and DN2 progenitor cells and cultured on OP9-DL1 cells expressing MHC Class I H-2Kb molecule for 20 days in the presence of various concentrations of ovalbumin SIINFEKL peptide (SEQ ID NO:1) as indicated. **(A, B, C)** Cultures were analyzed by flow cytometry at the timepoints indicated. **(D)** Total cellularity of each culture was determined on day 20 of culture.

FIGURE 2: $CD69^-$ DP thymocytes that have not yet gone through positive selection sorted from B6 or OT-1 transgenic mice were cultured on OP9-DL1 cells expressing MHC Class I H-2Kb molecule in the presence of ovalbumin SIINFEKL peptide (SEQ ID NO:1).

FIGURES 3A-C: B6 thymocytes were sorted for $CD4^-CD8^-CD117^+CD44^+$ DN1 and DN2 progenitor cells and transduced with the TCRα chain of the affinity enhanced WT1 specific TCR 3D clone, and cultured on OP9-DL1 cells expressing MHC Class I H-2Db molecule in the presence or absence of 1μM of WT1 peptide RMFPNAPYL (SEQ ID NO:2). **(A)** On day 16 of culture, transduced ($hCD2^+$) and untransduced ($hCD2^-$) cells were analyzed by flow cytometry. **(B)** On day 21 of OP9-DL1 culture in the presence of 1μM WT1 peptide RMFPNAPYL (SEQ ID NO:2), DN $TCR\alpha\beta^+$ cells were sorted according to the scheme indicated. **(C)** Sorted cells were lysed, DNA was isolated, and PCR was performed using a Vb10-specific forward primer and a Cb2-specific reverse primer. The Vb10 PCR product was then directionally TOPO-cloned into vector pENTR/D-TOPO, transferred to the retroviral

vector MigR1-attR using Gateway® technology, and retroviral supernatant was generated and used to transduce murine $58^{-/-}$ cells for library screening as described.

FIGURES 4A-C: The retroviral TCRβ library was used to transduce $CD8^{+}3D\alpha^{+}$ $58^{-/-}$ cells. **(A)** Transduced cells were initially sorted on GFP expression only (data not shown), followed by two additional sorts on GFP and high MHC-WT1 peptide tetramer expression as indicated. Sorted $58^{-/-}$ cells were also analyzed for staining with the non-specific, but MHC H-2Db-peptide tetramer specific for GP33 as a control for non-specific tetramer binding. **(B)** Sequence analysis of isolated TCRβ chains. **(C)** Four candidate TCRβ chains were identified by sequence analysis, and were transferred back into MigR1-attR retroviral vector. Retroviral supernatant was generated, and used to transduce $CD8^{+}3D\alpha^{+}$ $58^{-/-}$ cells.

FIGURES 5A-C: **(A)** $58^{-/-}$ cells transduced with each of the candidate TCRβ chains paired with 3Dα were stained with MHC-WT1 peptide specific tetramer, as well as several non-specific MHC H-2Db-peptide tetramers in order to assess potential peptide-independent reactivity towards MHC. **(B)** The relative affinity of the three highest affinity TCRs was determined by staining each transduced cell line with MHC-peptide tetramer followed by flow cytometry. K_D measurements were performed using six 2-fold dilutions of PE-conjugated tetramers, and apparent K_D values were determined from binding curves by non-linear regression, as the concentration of ligand that yielded half-maximal binding. **(C)** The highest affinity TCRβ chain (clone#1) was codon-optimized, and tetramer binding was compared to the original enhanced affinity 3Dαβ construct.

FIGURES 6A-B: Analysis of CD4 and CD8 expression of $TCR\beta^{+}$ thymocytes (**A**) and splenocytes (**B**) from 3D-PYYα-IRES-hCD2 and 7431α-IRES-hCD2 retrogenic mice. Vβ10 and Vβ9 expression of $TCR\beta^{+}$ thymocytes (**A**) from 3D-PYYα-IRES-hCD2 and 7431α-IRES-hCD2 retrogenic mice.

FIGURE 7: Analysis of splenocytes from retrogenic mice after 6 days of WT1 of mesothelin peptide stimulation +IL2 *in vitro*.

DETAILED DESCRIPTION

The instant disclosure provides methods and compositions for generating enhanced or high affinity TCRs, in which the TCRα chain from an antigen-specific TCR is used to select *de novo* generated TCRβ chains that pair with an antigen-specific TCRα chain during T cell development *in vitro,* to form new, enhanced affinity receptors that can advantageously drive T cell maturation independent of negative selection through a novel selection process in order to target an antigen of interest.

In one aspect, the present disclosure provides a method for generating an enhanced affinity T cell receptor (TCR) by culturing hematopoietic progenitor cells (containing a non-endogenous nucleic acid sequence encoding an antigen specific TCRα chain) with stromal cells (containing a non-endogenous nucleic acid sequence encoding Delta-like-1 or Delta-like-4 and a nucleic acid sequence encoding an MHC molecule) in the presence of a peptide antigen, which will induce differentiation of the hematopoietic progenitor cells into DN TCRαβ$^+$ thymocytes. Then, nucleic acid sequences encoding various TCRβ chains from the DN TCRαβ$^+$ thymocytes are isolated and introduced into cells that are capable of expressing a TCR on the cell surface and also express the TCRα chain noted above. Finally, an enhanced affinity TCR is identified by comparing the binding affinity of candidate TCRαβ with the parent TCRαβ.

Additionally, this disclosure provides enhanced affinity TCRs generated using such methods, as well as compositions and methods for using the enhanced affinity TCRs of the present disclosure in various therapeutic applications, including the treatment of a disease in subject (*e.g.*, cancer, infectious disease, autoimmune disease).

Prior to setting forth this disclosure in more detail, it may be helpful to an understanding thereof to provide definitions of certain terms to be used herein. Additional definitions are set forth throughout this disclosure.

In the present description, the terms "about" and "consisting essentially of" mean ±20% of the indicated range, value, or structure, unless otherwise indicated. It should be understood that the terms "a" and "an" as used herein refer to "one or more" of the enumerated components. The use of the alternative (*e.g.*, "or") should be

understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the terms "include," "have" and "comprise" are used synonymously, which terms and variants thereof are intended to be construed as non-limiting.

"T cell receptor" (TCR) refers to a molecule found on the surface of T cells (or T lymphocytes) that, in association with CD3, is generally responsible for recognizing antigens bound to major histocompatibility complex (MHC) molecules. The TCR has a disulfide-linked heterodimer of the highly variable α and β chains (also known as TCRα and TCRβ, respectively) in most T cells. In a small subset of T cells, the TCR is made up of a heterodimer of variable γ and δ chains (also known as TCRγ and TCRδ, respectively). Each chain of the TCR is a member of the immunoglobulin superfamily and possesses one N-terminal immunoglobulin variable domain, one immunoglobulin constant domain, a transmembrane region, and a short cytoplasmic tail at the C-terminal end (see Janeway et al., *Immunobiology: The Immune System in Health and Disease*, 3rd Ed., Current Biology Publications, p. 4:33, 1997). TCR as used in the present disclosure may be from various animal species, including human, mouse, rat, or other mammals. A TCR may be cell-bound or in soluble form.

TCRs and binding domains thereof of this disclosure can be "immunospecific" or capable of binding to a desired degree, including "specifically or selectively binding" a target while not significantly binding other components present in a test sample, if they bind a target molecule with an affinity or K_a (*i.e.*, an equilibrium association constant of a particular binding interaction with units of 1/M) of, for example, greater than or equal to about 10^5 M^{-1}, 10^6 M^{-1}, 10^7 M^{-1}, 10^8 M^{-1}, 10^9 M^{-1}, 10^{10} M^{-1}, 10^{11} M^{-1}, 10^{12} M^{-1}, or 10^{13} M^{-1}. "High affinity" binding domains refers to those binding domains with a K_a of at least 10^7 M^{-1}, at least 10^8 M^{-1}, at least 10^9 M^{-1}, at least 10^{10} M^{-1}, at least 10^{11} M^{-1}, at least 10^{12} M^{-1}, at least 10^{13} M^{-1}, or greater. Alternatively, affinity may be defined as an equilibrium dissociation constant (K_d) of a particular binding interaction with units of M (*e.g.*, 10^{-5} M to 10^{-13} M). Affinities of TCRs and binding domain polypeptides according to the present disclosure can be readily determined using conventional techniques (see, *e.g.*, Scatchard et al. (1949) Ann. N.Y. Acad. Sci. 51:660; and U.S. Pat. Nos. 5,283,173; 5,468,614, Biacore® analysis, or the equivalent). Therefore, "enhanced affinity T cell receptor" (enhanced affinity TCR)

refers to a selected or engineered TCR with stronger binding to a target antigen than the wild type (or parent) TCR. Enhanced affinity may be indicated by a TCR with a Ka (equilibrium association constant) for the target antigen higher than that of the wild type (also called parent or original) TCR, a TCR with a K_d (dissociation constant) for the target antigen less than that of the wild type (also called parent or original) TCR, or with an off-rate (K_{off}) for the target antigen less than that of the wild type (or parent) TCR.

"Major histocompatibility complex molecules" (MHC molecules) refer to glycoproteins that deliver peptide antigens to a cell surface. MHC class I molecules are heterodimers consisting of a membrane spanning α chain (with three α domains) and a non-covalently associated β2 microglobulin. MHC class II molecules are composed of two transmembrane glycoproteins, α and β, both of which span the membrane. Each chain has two domains. MHC class I molecules deliver peptides originating in the cytosol to the cell surface, where peptide:MHC complex is recognized by $CD8^+$ T cells. MHC class II molecules deliver peptides originating in the vesicular system to the cell surface, where they are recognized by $CD4^+$ T cells. An MHC molecule may be from various animal species, including human, mouse, rat, or other mammals.

A "hematopoietic progenitor cell" is a cell derived from hematopoietic stem cells or fetal tissue that is capable of further differentiation into mature cells types (*e.g.*, cells of the T cell lineage). In a particular embodiment, $CD24^{lo}$ Lin^- $CD117^+$ hematopoietic progenitor cells are used. As defined herein, hematopoietic progenitor cells may include embryonic stem cells, which are capable of further differentiation to cells of the T cell lineage. Hematopoietic progenitor cells may be from various animal species, including human, mouse, rat, or other mammals.

A "thymocyte progenitor cell" or "thymocyte" is a hematopoietic progenitor cell present in the thymus.

"Hematopoietic stem cells" refer to undifferentiated hematopoietic cells that are capable of essentially unlimited propagation either *in vivo* or *ex vivo* and capable of differentiation to other cell types including cells of the T cell lineage.

Hematopoietic stem cells may be isolated from, for example, fetal liver, bone marrow, and cord blood.

"Cells of T cell lineage" refer to cells that show at least one phenotypic characteristic of a T cell or a precursor or progenitor thereof that distinguishes the cells from other lymphoid cells, and cells of the erythroid or myeloid lineages. Such phenotypic characteristics can include expression of one or more proteins specific for T cells (*e.g.*, $CD8^+$), or a physiological, morphological, functional, or immunological feature specific for a T cell. For example, cells of the T cell lineage may be progenitor or precursor cells committed to the T cell lineage; $CD25^+$ immature and inactivated T cells; cells that have undergone CD4 or CD8 linage commitment; thymocyte progenitor cells that are $CD4^+CD8^+$ double positive; single positive $CD4^+$ or $CD8^+$; TCRαβ or TCR γδ; or mature and functional or activated T cells.

"Stromal cells" are connective tissue cells of any organ. In a particular embodiment, the stromal cells are bone marrow stromal cells. Examples of stromal cell lines that can be engineered to express DLL1 or DLL4 include the mouse stromal cell line MS5 (Itoh, et al., Exp. Hematol. 1989, 17:145-153) and S17, and the human stromal cell lines HGS2.11, HGS2.52, HGS.18, HGS3.30, HGS3.65, HGS.3.66, HGS3.103, and HGS3.114 (available from Human Genome Sciences Inc., MD, see US Published Application 20020001826). In a particular embodiment, OP9 cells (Kodama et al., 1994, Exp. Hematol. 22:979-984; available from RIKEN cell depository) are used. OP9 cells expressing DLL1 and DLL4 have been previously described (see, *e.g.*, Schmitt et al., 2002, Immunity:17:749-756; U.S. Patent No. 7,575,925)

"Double negative TCRαβ thymocytes" (DN TCRαβ thymocytes) refer to a population of thymocytes that do not express the CD4 and CD8 co-receptors, but do express TCRα and β chains.

"Peptide antigen" refers to an amino acid sequence, ranging from about 7 amino acids to about 25 amino acids in length that is specifically recognized by a TCR, or binding domains thereof, as an antigen, and which may be derived from or based on a fragment of a longer target biological molecule (*e.g.*, polypeptide, protein) or derivative thereof. An antigen may be expressed on a cell surface, within a cell, or as

an integral membrane protein. An antigen may be a host-derived (*e.g.*, tumor antigen, autoimmune antigen) or have an exogenous origin (*e.g.*, bacterial, viral).

"Nucleic acid sequence", or polynucleotides, may be in the form of RNA or DNA, which includes cDNA, genomic DNA, and synthetic DNA. The nucleic acid sequence may be double stranded or single stranded, and if single stranded, may be the coding strand or non-coding (anti-sense strand). A coding sequence may be identical to the coding sequence known in the art or may be a different coding sequence, which, as the result of the redundancy or degeneracy of the genetic code, or by splicing, encodes the same polypeptide.

"Non-endogenous" refers to a molecule (*e.g.*, nucleic acid sequence) that is not present in the host cell(s)/sample into which a molecule is introduced, for example, recombinantly introduced. A non-endogenous molecule may be from the same species or a different species.

Notch ligands "Delta-like-1" (DL1 or DLL1) and "Delta-like-4" (DL4 or DLL4) are homologs of the Notch Delta ligand and are members of the delta/serrate/jagged protein family. They play a role in mediating cell fate decisions during hematopoiesis and may play a role in cell-to-cell communication. Exemplary Delta-like-1 sequences include Genbank Accession No. NM_005618.3 (SEQ ID NO:3) and NP_005609.3 (SEQ ID NO:4) (*Homo sapiens* transcript and protein sequences, respectively) and Genbank Accession No. NM_007865.3 (SEQ ID NO:5) and NP_031891.2 (SEQ ID NO:6) (*Mus musculus* transcript and protein sequences, respectively). Exemplary Delta-like-4 sequences include Genbank Accession No. NM_019074.3 (SEQ ID NO:7) and NP_061947.1 (SEQ ID NO:8) (*Homo sapiens* transcript and protein sequences, respectively) and Genbank Accession No. NM_019454.3 (SEQ ID NO:9) and NP_062327.2 (SEQ ID NO:10) (*Mus musculus* transcript and protein sequences, respectively). Notch ligands are commercially available or can be produced by standard recombinant DNA techniques and purified to various degrees.

"Embryonic stem cells" or "ES cells" or "ESCs" refer to undifferentiated embryonic stem cells that have the ability to integrate into and become part of the germ line of a developing embryo. Embryonic stem cells are capable of differentiating into

hematopoietic progenitor cells. Embryonic stem cells that are suitable for use herein include cells from the J1 ES cell line, 129J ES cell line, murine stem cell line D3 (American Type Culture Collection catalog # CRL 1934), the R1 or E14K cell lines derived from 129/Sv mice, cell lines derived from Balb/c and C57Bl/6 mice, and human embryonic stem cells (*e.g.* from WiCell Research Institute, WI; or ES cell International, Melbourne, Australia).

"WT1" refers to Wilm's tumor 1, a transcription factor that contains four zinc-finger motifs at the C-terminus and a proline/glutamine-rich DNA binding domain at the N-terminus. WT1 has an essential role in the normal development of the urogential system and is mutated in a small subset of patients with Wilm's tumors. High expression of WT1 has been observed in various cancers, including, breast cancer, ovarian cancer, acute leukemias, vascular neoplasms, melanomas, colon cancer, lung cancer, thyroid cancer, bone and soft tissue sarcoma, and esophageal cancer. Alternative splicing has been noted for WT1. Exemplary WT1 sequences include Genbank Accession Nos: NM_000378.4 (SEQ ID NO:11) (human transcript), NP_000369.3 (SEQ ID NO:12) (human protein); NM_024424.3 (SEQ ID NO:13) (human transcript), NP_077742.2 (SEQ ID NO:14) (human protein); NM_024426.4 (SEQ ID NO:15) (human transcript), NP_077744.3 (SEQ ID NO:16); NM_001198552.1 (SEQ ID NO:17), NP_001185481.1 (SEQ ID NO:18) (human protein); NM_001198551.1 (SEQ ID NO:19) (human transcript), NP_001185480.1 (SEQ ID NO:20) (human protein); NM_144783.2 (SEQ ID NO:21) (mouse transcript), and NP_659032.3 (SEQ ID NO:22) (mouse protein).

"Mesothelin" (MSLN) refers to a gene that encodes a precursor protein that is cleaved into two products, megakaryocyte potentiating factor and mesothelin. Megakaryocyte potentiation factor functions as a cytokine that can stimulate colony formation in bone marrow megakaryocytes. Mesothelian is a glycosylphosphatidylinositol-anchored cell-surface protein that may function as a cell adhesion protein. This protein is overexpressed in epithelial mesotheliomas, ovarian cancers and in specific squamous cell carcinomas. Alternative splicing results in multiple transcript variants. Exemplary mesothelin sequences include Genbank Accession Nos: NM_001177355.1 (SEQ ID NO:23), NP_001170826.1 (SEQ ID

NO:24) (human transcript and pre-protein sequences, respectively); NM_005823.5 (SEQ ID NO:25), NP_005814.2 (SEQ ID NO:26)(human transcript and pre-protein sequences, respectively); NM_013404.4 (SEQ ID NO:27), NP_037536.2 (SEQ ID NO:28) (human transcript and pre-protein sequences, respectively); NM_018857.1 (SEQ ID NO:29), NP_061345.1 (SEQ ID NO:30) (mouse transcript and precursor protein sequences, respectively).

"MHC-peptide tetramer staining" refers to an assay used to detect antigen-specific T cells, which features a tetramer of MHC molecules, each comprising an identical peptide having an amino acid sequence that is cognate (*e.g.*, identical or related to) at least one antigen, wherein the complex is capable of binding T cells specific for the cognate antigen. Each of the MHC molecules may be tagged with a biotin molecule. Biotinylated MHC/peptides are tetramerized by the addition of streptavidin, which is typically fluorescently labeled. The tetramer may be detected by flow cytometry via the fluorescent label. In certain embodiments, an MHC-peptide tetramer assay is used to detect or select high affinity TCRs of the instant disclosure.

Methods for Generating Enhanced Affinity TCRs

By way of background, during T cell development in the thymus, progenitor thymocytes are subjected to a number of TCR-mediated checkpoints. The first of these is termed β-selection, and occurs at double negative 3 (DN3) stage of murine T cell development. DN3 cells that produce a successful rearrangement at the *Tcrb* gene locus can express TCRβ protein at the cell surface paired with the invariant pre-Tα protein. This receptor is called the Pre-TCR, and it signals in a ligand-independent fashion to promote proliferation, differentiation of αβ lineage cells to the CD4/CD8 double positive (DP) stage, and rearrangement at the *Tcra* gene locus (Boehmer et al., 1999, Curr. Opin. Immunol. 11:135-142). While the TCRα locus is inactive and closed to TCR gene rearrangements prior to β-selection, both the TCRγ and -δ loci also undergo rearrangements at the DN3 stage of development, and successful rearrangements at both these loci results in the expression of a mature γδ-TCR that can provide signals that drive differentiation towards the γδ T cell lineage – γδ T cells do not differentiate through a DP stage during development, and generally remain DN or CD8αα+. The αβ/γδ cell fate decision is determined by the strength of

the TCR signal at this stage of development, as the developing T cell distinguishes between a pre-TCR signal and a γδ TCR signal by the stronger signal associated with the mature γδ TCR (Pennington, Silva-Santos, & Hayday, 2005, Curr. Opin. Immunol. 17:108-115). Interestingly, many αβ TCR transgenic mice have a large population of mature $CD24^-$ TCRαβ positive CD4/CD8 double negative (DN) cells in the thymus, which have been shown to represent "γδ wanna-be" cells that develop as a result of the stronger signal from the mature αβ transgenic TCR at the β-selection checkpoint (Egawa et al., 2000, PLOS One 3:1512).

Disclosed herein is a method for generating enhanced affinity TCRs, wherein ectopic expression of an antigen-specific TCRα chain prior to β-selection allows the development of T cells expressing a high affinity TCR for the same antigen when differentiated in the presence of the cognate antigen during *in vitro* T cell differentiation. Using this method, T cells expressing high affinity receptors by-pass negative selection by adopting a DN $TCR\alpha\beta^+$ lineage fate in response to agonist signals at the DN3 stage of T cell development.

In certain embodiments, the present disclosure provides a method for generating an enhanced affinity TCR comprising: a) contacting hematopoietic progenitor cells with stromal cells and a peptide antigen, under conditions and for a time sufficient to induce differentiation of hematopoietic progenitor cells into DN $TCR\alpha\beta^+$ thymocytes, wherein the hematopoietic progenitor cells comprise a non-endogenous nucleic acid sequence encoding a TCRα chain from a parent TCR specific for the peptide antigen, and wherein the stromal cells comprise a non-endogenous nucleic acid sequence encoding Delta-like-1 or Delta-like-4 and a nucleic acid sequence encoding an MHC molecule; b) isolating nucleic acid sequences encoding the various TCRβ chains from the DN $TCR\alpha\beta^+$ thymocytes and introducing the nucleic acid sequences encoding the TCRβ chains into cells that are capable of expressing a TCR on the cell surface and comprise the nucleic acid sequence encoding the TCRα chain from step a); and identifying the enhanced affinity TCR (*e.g.*, by detecting or selecting high affinity TCRαβ candidates by an MHC tetramer assay, and then measuring binding affinity as compared to a parent TCRαβ).

In certain embodiments, hematopoietic progenitor cells comprise thymocyte progenitor cells or embryonic stem cells. In other embodiments, hematopoietic progenitor cells are derived from fetal liver tissue. In other embodiments, hematopoietic progenitor cells comprise hematopoietic stem cells that are derived or originate from bone marrow, cord blood, or peripheral blood. In yet other embodiments, hematopoietic progenitor cells are derived from human, mouse, rat, or other mammals. In a particular embodiment, $CD24^{lo}$ Lin^- $CD117^+$ thymocyte progenitor cells are used.

The hematopoietic progenitor cells have been modified to comprise a non-endogenous nucleic acid sequence encoding a TCRα chain from a parent TCR specific for the peptide antigen. In a specific embodiment, the TCRβ chain is also isolated from the parent TCR. Cloning of TCRα and β chains may be performed using standard molecular biology techniques that are known in the art. Methods for cloning TCR chains are known in the art (*see, e.g.*, Walchli et al., 2011, PLoS ONE 6:e27930; Birkholz et al., 2009, J. Immunol. Methods 346:45-54; Kurokawa et al, 2001, Clin. Exp. Immunol. 123:340-345).

A "stromal cell" is a connective tissue cell of any organ. Stromal cells that may be used according to the invention include human and mouse stromal cells. Examples of stromal cell lines that can be engineered to express DL1 or DL4 include the mouse stromal cell line MS5 (Itoh, et al., Exp. Hematol. 1989, 17:145-153) and S17, and the human stromal cell lines HGS2.11, HGS2.52, HGS.18, HGS3.30, HGS3.65, HGS.3.66, HGS3.103, and HGS3.114 (available from Human Genome Sciences Inc., MD, see US Published Application 20020001826). In certain embodiments, stromal cells are bone marrow stromal cells. In further embodiments, OP9 cells are used.

In certain embodiments, stromal cells comprise non-endogenous nucleic acid sequences encoding DL1, such as human DL1. Exemplary Delta-like-1 sequences include Genbank Accession No. NM_005618.3 (SEQ ID NO:3) and NP_005609.3 (SEQ ID NO:4) (*Homo sapiens* transcript and protein sequences, respectively) and Genbank Accession No. NM_007865.3 (SEQ ID NO:5) and NP_031891.2 (SEQ ID NO:6) (*Mus musculus* transcript and protein sequences, respectively). In certain

embodiments, stromal cells comprise non-endogenous nucleic acid sequences encoding DL4, such as human DL4. Exemplary Delta-like-4 sequences include Genbank Accession No. NM_019074.3 (SEQ ID NO:7) and NP_061947.1 (SEQ ID NO:8) (*Homo sapiens* transcript and protein sequences, respectively) and Genbank Accession No. NM_019454.3 (SEQ ID NO:9) and NP_062327.2 (SEQ ID NO:10) (*Mus musculus* transcript and protein sequences, respectively). Notch ligands are commercially available or can be produced by standard recombinant DNA techniques and purified to various degrees.

In still further embodiments, stromal cells are OP9 cells or a derivative thereof expressing DL1, such as human DL1. OP9 cells expressing DL1 and DL4 have been previously described (Schmitt et al., 2002, Immunity 17:749-756; U.S. Patent No. 7,575,925).

In certain embodiments, stromal cells also comprise a nucleic acid sequence encoding an MHC molecule. In particular embodiments, stromal cells comprise a nucleic acid sequence encoding an MHC Class I molecule, and may optionally also comprise a nucleic acid sequence encoding a β2 microglobulin. The MHC Class I and β2 microglobulin molecules may be derived from human, mouse, rat, or other mammalian species MHC Class I molecules, whose genes and protein sequences are known in the art. In other embodiments, the stromal cells comprise a nucleic acid sequence encoding an MHC Class II molecule. The MHC Class II molecule may be derived from human, mouse, rat, or other mammalian species MHC molecules, whose genes and protein sequences are known in the art.

A given T cell will recognize a peptide antigen only when it is bound to a host cell's MHC molecule (MHC-restricted antigen recognition). A parent TCR with specificity for a known peptide antigen is selected for enhancement of the TCR affinity using the disclosed methods. Therefore, an MHC molecule that binds the particular peptide antigen is also selected and expressed in the stromal cells to allow MHC-restricted antigen recognition in the disclosed *in vitro* system. Methods for identifying an MHC molecule that binds a peptide antigen are known in the art (see, *e.g.*, Akatsuka et al., 2002, Tissue Antigens 59:502-511). In certain embodiments, an MHC molecule comprises HLA-A2 and beta-2 microglobulin, preferably of human origin, which can

bind to, for example, the WT1 peptide RMFPNAPYL (SEQ ID NO:2). In other embodiments, an MHC molecule comprises mouse H-2D^b, which can bind to, for example, the WT1 peptide RMFPNAPYL or various mesothelin peptides as disclosed in Fig. 3A of Hung et al., 2007, Gene Therapy 14:921-929, or H-2K^b which can bind to, for example, various mesothelin peptides as disclosed in Fig. 3A of Hung et al. Potential H-2D^b restricted mesothelin epitopes disclosed in Hung et al. include: ISKANVDVL (SEQ ID NO:42), GQKMNAQAI (SEQ ID NO:43), SAFQNVSGL (SEQ ID NO:44), and LLGPNIVDL (SEQ ID NO:45). Potential H-2Kb restricted mesothelin epitopes disclosed in Hung et al. include: EIPFTYEQL (SEQ ID NO:46) and GIPNGYLVL (SEQ ID NO:47).

A peptide antigen used in the disclosed methods refers to a peptide sequence of an antigen, or target biological molecule (*e.g.*, a polypeptide, protein), to which the parent TCR specifically binds. A peptide sequence may be derived from an antigen that is expressed on the cell surface, within a cell, or that is an integral membrane protein. The antigen may be a host-derived antigen (*e.g.*, a tumor/cancer antigen, and autoimmune antigen), or an exogenous antigen (*e.g.*, viral, bacterial, protozoan antigen). A tumor or cancer antigen may be derived from various cancers, such as those noted herein. In some embodiments, a cancer antigen comprises a leukemia antigen. In certain embodiments, a peptide antigen is derived from Wilm's tumor 1 (WT1), such as a WT1 peptide comprising the amino acid sequence RMFPNAPYL (SEQ ID NO:2). In other embodiments, a peptide antigen is derived from mesothelin, such as mesothelin peptides disclosed in Fig. 3A of Hung et al., 2007, Gene Therapy 14:921-929. In some embodiments, the mesothelin peptide comprises the amino acid sequence GQKMNAQAI (SEQ ID NO:31). In other embodiments, the mesothelin peptide comprises an amino acid sequence comprising ISKANVDVL (SEQ ID NO:42), GQKMNAQAI (SEQ ID NO:43), SAFQNVSGL (SEQ ID NO:44), and LLGPNIVDL (SEQ ID NO:45), EIPFTYEQL (SEQ ID NO:46), or GIPNGYLVL (SEQ ID NO:47). Autoimmune antigens are antigens that are recognized by autoreactive TCRs specific for self-antigens, with the ensuing immune effector functions causing autoimmune disease, exacerbating autoimmune disease, contributing to progression of autoimmune disease, causing or worsening symptoms associated with

autoimmune disease. For example, autoreactive TCRs specific for a collagen peptide may be useful for suppressive gene therapy of Tregs in rheumatoid arthritis. Autoimmune antigens may also be antigens located on other immune cells that cause autoimmune disease or mediate symptoms of autoimmune disease (*e.g.*, B cells that produce autoantibodies). For example, CD20 peptide antigens may be useful for generating enhanced affinity TCRs that target B cells involved in or associated with rheumatoid arthritis. A peptide antigen may be added to a culture system to hematopoietic progenitor cells and stromal cells as described herein. Alternatively, stromal cells comprising a nucleic acid sequence encoding a peptide antigen of interest may be used to express such antigen in the cell culture. Without wishing to be bound by theory, a peptide antigen, whether added as an exogenous peptide antigen to the culture system or expressed by stromal cells, forms a complexe with a MHC molecule expressed by the stromal cells to form an MHC-peptide antigen complex. MHC-peptide antigen complex allows for MHC-restricted peptide antigen recognition by TCRs in the culture system. In certain embodiments, OP9 cells are transduced with a nucleic acid sequence to express the WT1 antigen peptide RMFPNAPYL (SEQ ID NO:2). In other embodiments, OP9 cells are transduced with a nucleic acid sequence to express the mesothelin antigen peptide GQKMNAQAI (SEQ ID NO:31).

Peptides that bind to MHC class I molecules are generally from about 7 to about 10 amino acids in length. Peptides that bind to MHC class II molecules are variable in length, usually about 10-25 amino acids long. In certain embodiments, the parent TCR's peptide antigen specificity is known. In other embodiments, the parent TCR's peptide antigen specificity needs to be determined using methods known in the art (Borras et al., 2002, J. Immunol. Methods 267:79-97; Hiemstra et al., 2000, Cur. Opin. Immunol. 12:80-4). For example, if the target antigen of a parent TCR is known, though not the specific peptide sequence, peptide libraries derived from the target antigen polypeptide sequence may be used for screening and identifying the specific peptide antigen for the parent TCR.

A "vector" is a nucleic acid molecule that is capable of transporting another nucleic acid. Vectors may be, for example, plasmids, cosmids, viruses, or phage. An "expression vector" is a vector that is capable of directing the expression of a

protein encoded by one or more genes carried by the vector when it is present in the appropriate environment.

"Retroviruses" are viruses having an RNA genome. "Gammaretrovirus" refers to a genus of the retroviridae family. Exemplary gammaretroviruses include, but are not limited to, mouse stem cell virus, murine leukemia virus, feline leukemia virus, feline sarcoma virus, and avian reticuloendotheliosis viruses.

"Lentivirus" refers to a genus of retroviruses that are capable of infecting dividing and non-dividing cells. Several examples of lentiviruses include HIV (human immunodeficiency virus: including HIV type 1, and HIV type 2); equine infectious anemia virus; feline immunodeficiency virus (FIV); bovine immune deficiency virus (BIV); and simian immunodeficiency virus (SIV).

A vector that encodes a core virus is also known as a "viral vector." There are a large number of available viral vectors that are suitable for use with the invention, including those identified for human gene therapy applications, such as those described by Pfeifer and Verma (Pfeifer, A. and I. M. Verma. 2001. Ann. Rev. Genomics Hum. Genet. 2:177-211). Suitable viral vectors include vectors based on RNA viruses, such as retrovirus-derived vectors, *e.g.*, Moloney murine leukemia virus (MLV)-derived vectors, and include more complex retrovirus-derived vectors, *e.g.*, lentivirus-derived vectors. HIV-1-derived vectors belong to this category. Other examples include lentivirus vectors derived from HIV-2, FIV, equine infectious anemia virus, SIV, and maedi/visna virus. Methods of using retroviral and lentiviral viral vectors and packaging cells for transducing mammalian target cells with viral particles containing TCRs transgenes are well known in the art and have been previous described, for example, in U.S. Patent 8,119,772; Walchli et al., 2011, PLoS One 6:327930; Zhao et al., J. Immunol., 2005, 174:4415-4423; Engels et al., 2003, Hum. Gene Ther. 14:1155-68; Frecha et al., 2010, Mol. Ther. 18:1748-57; Verhoeyen et al., 2009, Methods Mol. Biol. 506:97-114. Retroviral and lentiviral vector constructs and expression systems are also commercially available.

In a specific embodiment, a viral vector is used to introduce the non-endogenous nucleic acid sequence encoding TCRα chain specific for the peptide antigen into the hematopoietic progenitor cells. In another embodiment a viral vector is

used to introduce non-endogenous nucleic acid sequence encoding DL1 or DL4 and a nucleic acid sequence encoding an MHC molecule into stromal cells. The viral vector may be a retroviral vector or a lentiviral vector. The viral vector may also include a nucleic acid sequence encoding a marker for transduction. Transduction markers for viral vectors are known in the art and include selection markers, which may confer drug resistance, or detectable markers, such as fluorescent markers or cell surface proteins that can be detected by methods such as flow cytometry. In a particular embodiment, the viral vector further comprises a gene marker for transduction comprising green fluorescent protein or the extracellular domain of human CD2. Where the viral vector genome comprises more than one nucleic acid sequence to be expressed in the host cell as separate transcripts, the viral vector may also comprise additional sequence between the two (or more) transcripts allowing bicistronic or multicistronic expression. Examples of such sequences used in viral vectors include internal ribosome entry sites (IRES), furin cleavage sites, viral 2A peptide.

Other vectors also can be used for polynucleotide delivery including DNA viral vectors, including, for example adenovirus-based vectors and adeno-associated virus (AAV)-based vectors; vectors derived from herpes simplex viruses (HSVs), including amplicon vectors, replication-defective HSV and attenuated HSV (Krisky et al., 1998, Gene Ther. 5: 1517-30).

Other vectors that have recently been developed for gene therapy uses can also be used with the methods of this disclosure. Such vectors include those derived from baculoviruses and alpha-viruses. (Jolly D J. 1999. Emerging viral vectors. pp 209-40 in Friedmann T. ed. 1999. The development of human gene therapy. New York: Cold Spring Harbor Lab).

The hematopoietic progenitor cells are cultured with stromal cells comprising a nucleic acid sequence encoding a non-endogenous DL1 or DL4 and a nucleic acid sequence encoding a MHC molecule under conditions and for a time sufficient to induce differentiation of hematopoietic progenitor cells into DN TCR$\alpha\beta^+$ thymocytes. In certain embodiments, the hematopoietic progenitor cells are cultured in a 6 cm or 10 cm tissue culture-treated dish. The concentration of hematopoietic progenitor cells in the culture can be between 1-10^9, or $1x10^2$ to $1x10^6$, or $1x10^3$ to

1x 10^4. In some embodiments, hematopoietic progenitor cells (about 1-5 x 10^4 cells) are cultured on a monolayer of OP9 cells expressing DL1.

One or more cytokines that promote commitment and differentiation of hematopoietic progenitor cells may also be added to the culture. The cytokines may be derived from human or other species. The concentration of a cytokine in culture can range from about 1 ng/ml to about 50 ng/ml. Representative examples of cytokines that may be used include: all members of the FGF family, including FGF-4 and FGF-2; Flt-3-ligand, stem cell factor (SCF), thrombopoietin (TPO), and IL-7. Cytokines may be used in combination with a glycosaminoglycan, such as heparin sulfate. Cytokines are commercially available or can be produced by recombinant DNA techniques and purified to various degrees. Some cytokines may be purified from culture media of cell lines by standard biochemical techniques.

The hematopoietic progenitor cells may be cultured in culture medium comprising conditioned medium, non-conditioned medium, or embryonic stem cell medium. Examples of suitable conditioned medium include IMDM, DMEM, or αMEM, conditioned with embryonic fibroblast cells (*e.g.*, human embryonic fibroblast cells), or equivalent medium. Examples of suitable non-conditioned medium include Iscove's Modified Delbucco's Medium (IDMD), DMEM, or αMEM, or equivalent medium. The culture medium may comprise serum (*e.g.*, bovine serum, fetal bovine serum, calf bovine serum, horse serum, human serum, or an artificial serum substitute) or it may be serum free.

Culture conditions entail culturing the hematopoietic progenitor cells for a sufficient time to induce differentiation of hematopoietic progenitor cells into DN TCR$\alpha\beta^+$ thymocytes. The cells are maintained in culture generally for about 4-5 days, preferably about 5 to 20 days. It will be appreciate that the cells may be maintained for the appropriate amount of time required to achieve a desired result, *i.e.*, desired cellular composition. For example, to generate a cellular composition comprising primarily immature and inactivated T cells, the cells may be maintained in culture for about 5 to 20 days. Cells may be maintained in culture for 20 to 30 days to generate a cellular composition comprising primarily mature T cells. Non-adherent cells may also be collected from culture at various time points, such as from about several days to about

25 days. Culture methods for hematopoietic stem cells on stromal cells lines have been previously described (U.S. Patent #7,575,925; Schmitt et al., 2004, Nat. Immunol. 5:410-417; Schmitt et al., 2002, Immunity 17:749-756).

Differentiation of hematopoietic progenitor cells into DN TCRαβ+ thymocytes may be detected and these cells isolated using standard flow cytometry methods. One or more cell sorts may be employed to isolate the DN TCRαβ+ thymocytes. For example, a first cell sort may identify hematopoietic progenitor cells expressing the transduction marker (*i.e.*, marker for TCRα expression). In certain embodiments, a transduction marker is the extracellular domain of human CD2. In further embodiments, transduction marker positive cells may be subjected to a second cell sort to screen for cells that are $CD4^-$ and $CD8^-$. A third cell sort on the DN cells may screen for cells expressing TCRβ. It will be apparent to one skilled in the art that a subset of these sorts, or single or multiple cell sorts can be designed using different combinations of cell surface or transduction markers, in order to identify the desired subpopulation of DN TCRαβ+ thymocytes. Methods for sorting DN TCRαβ+ cells are known in the art (U.S. 7,575,925 and Schmitt et al., 2002, Immunity:17:749-756).

The nucleic acid sequences encoding the various TCRβ chains from the DN $TCR\alpha\beta^+$ thymocytes are isolated and introduced into T cells comprising the nucleic acid sequence encoding the TCRα chain from the parent TCR. As discussed herein, methods of cloning TCRβ chains from cells are well known in the art and have been previously described. In certain embodiments, once the nucleic acid sequences encoding the candidate TCRβ chains have been isolated from the DN $TCR\alpha\beta^+$ thymocytes, the nucleic acid sequences may be subjected to a further selection process whereby the TCRβ chains with the same V_β gene used by the parent TCRβ chain are selected for introduction into T cells. Parent V_β gene containing TCRβ chain may be identified within the sorted cell population using V_β gene specific primers for PCR. One concern associated with enhancing the affinity of antigen-specific TCRs *in vitro* is that some modifications might increase the affinity of the receptor for MHC only, rather than peptide/MHC, thereby increasing the likelihood that the TCR will be autoreactive. Restricting the candidate TCRβ chains to those containing the parent V_β gene increases the likelihood of retaining the TCR CDR1 and CDR2 domains that contact the MHC,

and limiting variability to CDR3. As previously discussed, viral vectors, such as retroviral vectors and lentiviral vectors, are suitable for introducing the nucleic acid sequences encoding the various TCRβ chains and/or the parent TCRα into T cells. In some embodiments, the viral vector further comprises a gene marker for transduction (*e.g.* green fluorescent protein).

Cells that are capable of expressing a TCR on the cell surface are used for transformation or transduction with the nucleic acid sequences encoding the various TCRβ chains from the DN TCRαβ$^+$ thymocytes. Cells that are capable of expressing a TCR on the cell surface express a CD3 molecule. "CD3" is a multi-protein complex of six chains that are stably associated with a TCR on the cell surface. In mammals, the complex comprises a CD3γ chain, a CDδ chain, two CD3ε, and a homodimer of CD3ζ chains. The CD3γ, CD3δ, and CD3ε are highly related cell surface proteins of the immunoglobulin superfamily containing a single immunoglobulin domain. The transmembrane regions of CD3γ, CD3δ, and CD3ε are negatively charged, which is a characteristic that allows these chains to associate with the positively charged TCR chains. The cytoplasmic domains of the CD3γ, CD3δ, and CD3ε chains contain immunoreceptor tyrosine-based activation motifs (ITAMs) that allow them to associate with cytosolic protein tyrosine kinases following receptor stimulation and thereby signal to the cell interior. CD3 proteins are required for cell-surface expression of the TCR (*see* Janeway et al., *Immunobiology: The Immune System in Health and Disease*, 3rd Ed., Current Biology Publications, p. 4:39, 1997).

In some embodiments, cells that are capable of expressing a TCR on the cell surface are T cells, including primary cells or cell lines derived from human, mouse, rat, or other mammals. If obtained from a mammal, a T cell can be obtained from numerous sources, including blood, bone marrow, lymph node, thymus, or other tissues or fluids. A T cell may be enriched or purified. T cell lines are well known in the art, some of which are described in Sandberg et al., 2000, Leukemia 21:230-237. In certain embodiments, T cells which lack endogenous expression of TCRα and β chains are used. Such T cells may naturally lack endogenous expression of TCRα and β chains or may have been modified to block expression (*e.g.*, T cells from a transgenic mouse that does not express TCR α and β chains or a cell line that has been manipulated to

inhibit expression of TCR α and β chains). In certain embodiments, 58 $\alpha^-\beta^-$ cells, a murine T cell line that lacks endogenous TCRα and TCRβ chains, is used (Letourneur and Malissen, 1989, Eur. J. Immunol. 19:2269-74). In other embodiments, H9 T cell line is used (Catalog # HTB-176, ATCC, Manassas, VA). In certain embodiments, cells that capable of expressing a TCR on the cell surface are not T cells or cells of a T cell lineage, but cells that have been modified to express CD3, enabling cell surface expression of a TCR (*e.g.*, 293 cells or 3T3 cells). Cell surface expression of TCRs on cells that are not of a T cell lineage has been previously described (Szymczak et al., 2004, Nat. Biotechnol. 22:589-594).

To identify a potential enhanced affinity TCR, once cells that are capable of expressing a TCR on the cell surface that also express the parent TCRα chain have been transformed or transduced with a library of candidate TCRβ chains, antigen-specific cells are sorted or identified using MHC-peptide tetramer staining. MHC-peptide tetramer staining features a tetramer of MHC molecules, each comprising an identical peptide having an amino acid sequence that is cognate (*e.g.*, identical or related to) at least one antigen, wherein the complex is capable of binding T cells specific for the cognate antigen. Each of the MHC molecules may be tagged with a biotin molecule. Biotinylated MHC/peptides are tetramerized by the addition of streptavidin, which is typically fluorescently labeled. The tetramer may be detected by flow cytometry via the fluorescent label. MHC-peptide tetramer staining methods for detecting antigen specific T cells are well known in the art (*e.g.*, Altman et al, 1996, Science 274:94-96; Kalergis et al., 2000, J. Immunol. Methods 234:61-70; Xu and Screaton, 2002, J. Immunol. Methods 268:21-8; James et al., J. Vis. Exp.25:1167). In certain embodiments, the MHC-peptide tetramer comprises MHC Class I molecules. In other embodiments, the MHC-peptide tetramer comprises MHC Class II molecules. In further embodiments, the same peptide antigen used the culture step of the disclosed method is the same as the peptide incorporated into the MHC-peptide tetramer. In other embodiments, the MHC molecule expressed by the stromal cells in the culture step of the disclosed method is the same as an MHC molecule in the MHC-peptide tetramer. MHC-peptide tetramer stained cells may be sorted by flow cytometry one or more times. A first sort may select for transduced cells expressing a detectable transduction

marker (*e.g.*, green fluorescent protein). The transduction positive cells may also be sorted one or more times for cells that express the same Vβ chain as the parent TCR. It will be apparent to one skilled in the art that a subset of these sorts, or single or multiple cell sorts can be designed using different combinations of cell surface or transduction markers, in order to identify the desired subpopulation of cells.

An enhanced affinity TCR is identified by comparing the binding affinity of a candidate TCRαβ with the parent TCRαβ. Antigen-specific T cells may then be cloned and sequenced using standard molecular biology techniques. Candidate TCRβ clones may then be used to transduce T cells comprising the parent TCRα chain and MHC-peptide tetramer staining may be used to compare staining levels with the parent TCRαβ, as previously described. Increased staining observed with a candidate TCRβ may be indicative of enhanced affinity as compared with the parent TCRαβ. However, if the parent TCRαβ was codon-optimized for increased expression in the T cell, direct comparison of tetramer staining levels with the candidate TCRβ may not be possible. Candidate TCRβ chains may also be codon optimized for direct comparison with the parent TCRβ

A candidate TCRαβ has enhanced affinity compared to a parent TCRαβ if it has stronger binding to the peptide antigen than the parent TCRαβ. Enhanced affinity may be indicated by a TCR with a K_a (equilibrium association constant) for the target antigen higher than that of the parent TCR, a TCR with a K_D (dissociation constant) for the target antigen less than that of the parent TCR, or with an off-rate (K_{off}) for the target antigen less than that of the wild type (or parent) TCR. Methods of measuring TCR binding affinity have been previously described (*e.g.*, Laugel et al., 2007, J. Biol. Chem. 282:23799-23810; Garcia et al., 2001, Proc. Natl. Acad. Sci. USA 98:6818-6823).

Enhanced Affinity TCRs and Compositions

In another aspect, enhanced affinity TCRs generated by methods disclosed herein are provided. An enhanced affinity TCR may be cell-bound (*e.g.*, expressed on the surface of a mature T cell) or in soluble form. In certain

embodiments, enhanced affinity TCRs may be codon optimized to enhance expression in T cells (Scholten et al., 2006, Clin. Immunol. 119:135-145).

In other embodiments, enhanced affinity TCRs may also be a component of a fusion protein, which may further comprise a cytotoxic component (*e.g.*, chemotherapeutic drugs such as vindesine, antifolates; bacterial toxins, ricin, anti-virals), which is useful for specific killing or disabling of a cancer cell or infected cell or a detectable component (*e.g.*, biotin, fluorescent moiety, radionuclide), which is useful for imaging cancer cells, infected cells, or tissues under autoimmune attack.

The present disclosure also provides pharmaceutical compositions comprising an enhanced affinity TCR generated by the methods disclosed herein and a pharmaceutically acceptable carrier, diluents, or excipient. Suitable excipients include water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof.

Applications

Enhanced affinity TCRs generated by the methods of the present disclosure may be used to treat a disease (such as cancer, infectious disease, or autoimmune disease) in a subject by administering a composition comprising the enhanced affinity TCRs.

Diseases that may be treated with enhance affinity TCR therapy include cancer, infectious diseases (viral, bacterial, protozoan infections), and autoimmune diseases. TCR gene therapy is a promising treatment for various types of cancer (Morgan et al., 2006, Science 314:126-129; reviewed in Schmitt et al, 2009, Human Gene Therapy; reviewed in June, 2007, J. Clin. Invest. 117:1466-1476) and infectious disease (Kitchen et al., 2009, PLoS One 4:38208; Rossi et al., 2007, Nat. Biotechnol. 25:1444-54; Zhang et al., PLoS Pathog. 6:e1001018; Luo et al., 2011, J. Mol. Med. 89:903-913). Immunosuppressive gene therapy for autoimmune diseases using regulatory T cells comprising autoreactive TCRs is also an emerging treatment (Fujio et al., 2006, J. Immunol. 177:8140-8147; Brusko et al., 2008, Immunol. Rev. 223:371-390).

A wide variety of cancers, including solid tumors and leukemias are amenable to the compositions and methods disclosed herein. Types of cancer that may

be treated include: adenocarcinoma of the breast, prostate, and colon; all forms of bronchogenic carcinoma of the lung; myeloid; melanoma; hepatoma; neuroblastoma; papilloma; apudoma; choristoma; branchioma; malignant carcinoid syndrome; carcinoid heart disease; and carcinoma (*e.g.*, Walker, basal cell, basosquamous, Brown-Pearce, ductal, Ehrlich tumor, Krebs 2, merkel cell, mucinous, non-small cell lung, oat cell, papillary, scirrhous, bronchiolar, bronchogenic, squamous cell, and transitional cell). Additional types of cancers that may be treated include: histiocytic disorders; leukemia; histiocytosis malignant; Hodgkin's disease; immunoproliferative small; non-Hodgkin's lymphoma; plasmacytoma; reticuloendotheliosis; melanoma; chondroblastoma; chondroma; chondrosarcoma; fibroma; fibrosarcoma; giant cell tumors; histiocytoma; lipoma; liposarcoma; mesothelioma; myxoma; myxosarcoma; osteoma; osteosarcoma; chordoma; craniopharyngioma; dysgerminoma; hamartoma; mesenchymoma; mesonephroma; myosarcoma; ameloblastoma; cementoma; odontoma; teratoma; thymoma; trophoblastic tumor. Further, the following types of cancers are also contemplated as amenable to treatment: adenoma; cholangioma; cholesteatoma; cyclindroma; cystadenocarcinoma; cystadenoma; granulosa cell tumor; gynandroblastoma; hepatoma; hidradenoma; islet cell tumor; Leydig cell tumor; papilloma; sertoli cell tumor; theca cell tumor; leimyoma; leiomyosarcoma; myoblastoma; myomma; myosarcoma; rhabdomyoma; rhabdomyosarcoma; ependymoma; ganglioneuroma; glioma; medulloblastoma; meningioma; neurilemmoma; neuroblastoma; neuroepithelioma; neurofibroma; neuroma; paraganglioma; paraganglioma nonchromaffin. The types of cancers that may be treated also include: angiokeratoma; angiolymphoid hyperplasia with eosinophilia; angioma sclerosing; angiomatosis; glomangioma; hemangioendothelioma; hemangioma; hemangiopericytoma; hemangiosarcoma; lymphangioma; lymphangiomyoma; lymphangiosarcoma; pinealoma; carcinosarcoma; chondrosarcoma; cystosarcoma phyllodes; fibrosarcoma; hemangiosarcoma; leiomyosarcoma; leukosarcoma; liposarcoma; lymphangiosarcoma; myosarcoma; myxosarcoma; ovarian carcinoma; rhabdomyosarcoma; sarcoma; neoplasms; nerofibromatosis; and cervical dysplasia.

Exemplifying the variety of hyperproliferative disorders amenable to enhanced TCR therapy are B-cell cancers, including B-cell lymphomas (such as various

forms of Hodgkin's disease, non-Hodgkins lymphoma (NHL) or central nervous system lymphomas), leukemias (such as acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), Hairy cell leukemia and chronic myoblastic leukemia) and myelomas (such as multiple myeloma). Additional B cell cancers include small lymphocytic lymphoma, B-cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, splenic marginal zone lymphoma, plasma cell myeloma, solitary plasmacytoma of bone, extraosseous plasmacytoma, extra-nodal marginal zone B-cell lymphoma of mucosa-associated (MALT) lymphoid tissue, nodal marginal zone B-cell lymphoma, follicular lymphoma, mantle cell lymphoma, diffuse large B-cell lymphoma, mediastinal (thymic) large B-cell lymphoma, intravascular large B-cell lymphoma, primary effusion lymphoma, Burkitt's lymphoma/leukemia, B-cell proliferations of uncertain malignant potential, lymphomatoid granulomatosis, and post-transplant lymphoproliferative disorder.

Autoimmune diseases include: arthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, polychondritis, psoriatic arthritis, psoriasis, dermatitis, polymyositis/dermatomyositis, inclusion body myositis, inflammatory myositis, toxic epidermal necrolysis, systemic scleroderma and sclerosis, CREST syndrome, responses associated with inflammatory bowel disease, Crohn's disease, ulcerative colitis, respiratory distress syndrome, adult respiratory distress syndrome (ARDS), meningitis, encephalitis, uveitis, colitis, glomerulonephritis, allergic conditions, eczema, asthma, conditions involving infiltration of T cells and chronic inflammatory responses, atherosclerosis, autoimmune myocarditis, leukocyte adhesion deficiency, systemic lupus erythematosus (SLE), subacute cutaneous lupus erythematosus, discoid lupus, lupus myelitis, lupus cerebritis, juvenile onset diabetes, multiple sclerosis, allergic encephalomyelitis, neuromyelitis optica, rheumatic fever, Sydenham's chorea, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, tuberculosis, sarcoidosis, granulomatosis including Wegener's granulomatosis and Churg-Strauss disease, agranulocytosis, vasculitis (including hypersensitivity vasculitis/angiitis, ANCA and rheumatoid vasculitis), aplastic anemia, Diamond Blackfan anemia, immune hemolytic anemia including autoimmune hemolytic anemia (AIHA), pernicious anemia, pure red

cell aplasia (PRCA), Factor VIII deficiency, hemophilia A, autoimmune neutropenia, pancytopenia, leukopenia, diseases involving leukocyte diapedesis, central nervous system (CNS) inflammatory disorders, multiple organ injury syndrome, myasthenia gravis, antigen-antibody complex mediated diseases, anti-glomerular basement membrane disease, anti-phospholipid antibody syndrome, allergic neuritis, Behcet disease, Castleman's syndrome, Goodpasture's syndrome, Lambert-Eaton Myasthenic Syndrome, Reynaud's syndrome, Sjorgen's syndrome, Stevens-Johnson syndrome, solid organ transplant rejection, graft versus host disease (GVHD), bullous pemphigoid, pemphigus, autoimmune polyendocrinopathies, seronegative spondyloarthropathies, Reiter's disease, stiff-man syndrome, giant cell arteritis, immune complex nephritis, IgA nephropathy, IgM polyneuropathies or IgM mediated neuropathy, idiopathic thrombocytopenic purpura (ITP), thrombotic throbocytopenic purpura (TTP), Henoch-Schonlein purpura, autoimmune thrombocytopenia, autoimmune disease of the testis and ovary including autoimmune orchitis and oophoritis, primary hypothyroidism; autoimmune endocrine diseases including autoimmune thyroiditis, chronic thyroiditis (Hashimoto's Thyroiditis), subacute thyroiditis, idiopathic hypothyroidism, Addison's disease, Grave's disease, autoimmune polyglandular syndromes (or polyglandular endocrinopathy syndromes), Type I diabetes also referred to as insulin-dependent diabetes mellitus (IDDM) and Sheehan's syndrome; autoimmune hepatitis, lymphoid interstitial pneumonitis (HIV), bronchiolitis obliterans (non-transplant) vs NSIP, Guillain-BarréSyndrome, large vessel vasculitis (including polymyalgia rheumatica and giant cell (Takayasu's) arteritis), medium vessel vasculitis (including Kawasaki's disease and polyarteritis nodosa), polyarteritis nodosa (PAN) ankylosing spondylitis, Berger's disease (IgA nephropathy), rapidly progressive glomerulonephritis, primary biliary cirrhosis, Celiac sprue (gluten enteropathy), cryoglobulinemia, cryoglobulinemia associated with hepatitis, amyotrophic lateral sclerosis (ALS), coronary artery disease, familial Mediterranean fever, microscopic polyangiitis, Cogan's syndrome, Whiskott-Aldrich syndrome and thromboangiitis obliterans.

In a particular embodiments, a method of treating a subject with the enhanced affinity TCRs generated by the methods disclosed herein include a subject

with acute myelocytic leukemia, acute lymphocytic leukemia, or chronic myelocytic leukemia.

Infectious diseases include those associated with infectious agents and include any of a variety of bacteria (*e.g.*, pathogenic *E. coli, S. typhimurium, P. aeruginosa, B. anthracis, C. botulinum, C. difficile, C. perfringens, H. pylori, V. cholerae, Listeria spp., Rickettsia spp., Chlamydia spp.*, and the like), mycobacteria, and parasites (including any known parasitic member of the Protozoa). Infectious viruses include eukaryotic viruses (e.g., adenovirus, bunyavirus, herpesvirus, papovavirus, paramyxovirus, picornavirus, rhabdovirus (e.g., Rabies), orthomyxovirus (e.g., influenza), poxvirus (e.g., Vaccinia), reovirus, retroviruses, lentiviruses (*e.g.*, HIV), flaviviruses (*e.g.*, HCV) and the like). In certain embodiments, infection with cytosolic pathogens whose antigens are processed and displayed with MHC Class I molecules, are treated with the enhanced affinity TCRs of the invention.

The enhanced affinity TCRs may be administered to a subject in cell-bound form (*i.e.*, gene therapy of target cell population (mature T cells (*e.g.*, $CD8^-$ T cells) or other cells of T cell lineage)). In a particular embodiment, the cells of T cell lineage comprising enhanced affinity TCRs administered to the subject are autologous cells. In another embodiment, the enhanced affinity TCRs may be administered to a subject in soluble form. Soluble TCRs are known in the art (*see, e.g.*, Molloy et al., 2005, Curr. Opin. Pharmacol. 5:438-443; U.S. Patent #6,759,243).

"Treat" and "treatment" refer to medical management of a disease, disorder, or condition of a subject (*i.e.*, individual who may be a human or non-human mammal (*e.g.*, primate, mouse, rat)). In general, an appropriate dose and treatment regimen provide the herein described enhanced affinity TCRs, and optionally, an adjuvant, in an amount sufficient to provide therapeutic or prophylactic benefit. Therapeutic and prophylactic benefits include improved clinical outcome; lessening or alleviation of symptoms associated with the disease; decreased occurrence of symptoms; improved quality of life; longer disease-free status; diminishment of extent of disease, stabilization of disease state; delay of disease progression; remission; survival; or prolonging survival.

Pharmaceutical compositions including the enhanced affinity receptors may be administered in a manner appropriate to the disease or condition to be treated (or prevented) as determined by persons skilled in the medical art. An appropriate dose, suitable duration, and frequency of administration of the compositions will be determined by such factors as the condition of the patient, size, type and severity of the disease, particular form of the active ingredient, and the method of administration.

In further embodiments, enhanced affinity TCRs of the instant disclosure may be used in diagnostic methods or imaging methods, including these methods used in relation to the indications or conditions identified herein.

EXAMPLES

The following examples demonstrate that, as provided by the instant disclosure, for example, TCR transgenic thymocytes efficiently differentiate into a "γδ like" $CD4^-CD8^-CD24^-TCR\beta^+$ lineage when exposed to their cognate antigen in OP9-DL1 cultures. Furthermore, progenitor thymocytes expressing only the TCRα chain from a T cell clone specific for the tumor antigen WT1 can also differentiate into this mature TCRαβ+ lineage in OP9-DL1 culture. A library of TCRβ chains was generated from a population of DN TCRαβ+ cells sorted from these cultures, and screened for WT1 MHC tetramer reactivity when paired with the antigen-specific TCRα chain. Using this approach, several TCRβ chains were identified that can pair with an antigen-specific TCRα chain to generate TCRs with up to 10-fold higher affinity for WT1 peptide as compared to the original TCR.

Example 1: Engagement of peptide agonist during differentiation on OP9-DL1 cells can drive differentiation of mature TCRαβ+ DN cells from T cell progenitors purified from TCR transgenic mice.

Agonist signals through an αβ TCR prior to β-selection results in the differentiation of "γδ like" double negative (DN) $TCR\alpha\beta^+$ cells during T cell development *in vivo,* and TCR cross-linking at the DN3 stage leads to the

differentiation of a similar lineage during *in vitro* T cell differentiation on OP9-DL1 cells. In order to determine whether progenitor T cells from TCR transgenic mice could also differentiate into a DN TCR$\alpha\beta^+$ lineage in response to cognate peptide antigen at the DN3 stage, TCR$\alpha\beta^-$CD4$^-$CD8$^-$CD117$^+$CD44$^+$ DN1 and DN2 progenitor thymocytes were sorted from transgenic OT-1 mice (express TCR specific for ovalbumin peptide sequence SIINFEKL (SEQ ID NO:1) presented on MHC Class I H-2K^b; Stock #003831, Jackson Laboratory, ME; *see also* Hogquist et al., 1994, Cell 76:17-27) and cultured with OP9-DL1 cells (Schmitt et al., 2002, Immunity 17:749-756; U.S. Patent No. 7,575,925) transduced to express the mouse MHC Class I molecule H-2K^b, either in the absence of peptide, or with increasing concentrations of ovalbumin-specific peptide (SEQ ID NO:1) for 20 days and analyzed at various time points by flow cytometry. In the absence of peptide, double positive (DP) T cells could be detected by day 16, and constituted a major fraction of the culture by day 20 (Fig. 1A). However, the development or survival of DP T cells was diminished by even very low concentrations of peptide (0.0001μM), and DP were completely absent from cultures containing 0.01μM or more of peptide (Fig. 1A), demonstrating that DP cells are negatively selected by strong agonist signaling in OP9-DL1 cultures.

In order to determine whether increasingly strong agonist signals drive the development of TCR$\alpha\beta^+$ DN cells, the DN population was analyzed for expression of CD24, a maturation marker that is expressed at high levels on all immature progenitor T cell populations, and TCRβ. The majority of cells were found to express high levels of CD24 and to lack TCRβ expression at day 5 (Fig. 1B), but by day 16, a majority of DN cells from all culture conditions expressed TCRβ, although a substantially greater number of CD24$^-$ cells were observed from cultures that contained 0.01μM or more of peptide (38.2% and 31.4% TCR$^+$CD24$^-$ cells in cultures containing 0.01 and 1.0μM of peptide, respectively, compared to 6.9% TCR$^+$CD24$^-$ in the no peptide culture) (Fig. 1B). By day 20, ~60% of all DN cells were TCRβ$^+$CD24$^-$ from cultures containing 0.01μM or 1.0μM peptide, while in cultures that received no peptide or a low concentration (0.0001μM) of peptide, only ~20% of DNs were TCRβ$^+$CD24$^-$, and close to 50% were TCRβ$^-$ (Fig. 1B, 1C). Furthermore, when the level of TCR surface expression is compared between the different culture conditions, the TCRβ$^+$

cells that developed in response to high levels of peptide expressed higher levels of TCRβ on the cell surface (Fig. 1C). Without wishing to be bound by theory, it is possible that the development of some TCRαβ$^+$ DN cells in cultures without added peptide is due to cross-reactivity with other peptide-MHC ligands in the OP9-DL1 culture system. To confirm that the TCRαβ$^+$ DN cells observed in these cultures did not develop through a DP stage, CD69$^-$ DP cells that have not yet been positively selected were sorted from B6 or OT-1 thymus and cultured in the presence or absence of ovalbumin SIINFEKL peptide (SEQ ID NO:1). B6 DP cells were unaffected by the presence of SIINFEKL peptide (SEQ ID NO:1), but when OT-1 DP thymocytes were cultured on OP9-DL1 cells in the presence SIINFEKL (SEQ ID NO:1), all the hallmarks of negative selection were observed, including a massive loss of cellularity and co-receptor down-modulation (Fig. 2). Importantly, the DN cells observed in these cultures were uniformly TCR negative (Fig. 2).

These data indicate that engagement of a peptide agonist during differentiation on OP9-DL1 cells can drive the differentiation of mature TCRαβ$^+$ DN cells from T cell progenitors purified from TCR transgenic mice.

Example 2: A transgenic TCRα chain pairs with endogenous TCRβ chains to drive the development of DN CD24$^-$ TCRαβ$^+$ "γδ wanna-be" cells in the OP9-DL1 culture system

To determine whether the expression of only a TCRα chain prior to β-selection should also result in the lineage diversion of DN3 T cell progenitors that express an endogenous TCRβ chain that pairs with the introduced TCRα chain capable of engaging a peptide-MHC ligand in the OP9-DL1 culture system above a certain affinity threshold, CD4$^-$CD8$^-$CD117$^+$CD44$^+$ DN1 and DN2 progenitor thymocytes were sorted from B6 mice and transduced with a TCRα chain from the Wilm's tumor antigen (WT1) specific T cell clone 3D that had previously been identified as an affinity enhanced variant isolated from a saturation mutagenesis library of the CDR3 region of the 3Dα. The 3Dα expression construct contains an intra-ribosomal entry sequence motif, followed by the extracellular domain of human CD2 (Genbank Accession Nos. NM_001767.3 (SEQ ID NO:48) and NP_001758.2 (SEQ ID NO:49) (transcript and

protein sequences for full length CD2, respectively)) (IRES-hCD2) as a marker transduction. Transduced progenitor thymocytes were cultured in the presence or absence of 1.0μM of the MHC Class I H-2D^b restricted WT1 peptide RMFPNAPYL (SEQ ID NO:2) for 14 days, and then analyzed by flow cytometry. DN cells within the hCD2 negative fraction contained few TCRαβ$^+$ cells, regardless of the presence of peptide in the culture conditions. In contrast, the hCD2 positive fraction (which expressed the 3Dα gene) from cultures that did not receive peptide contained 6.8% TCRβ$^+$ cells, and the number of TCRαβ$^+$ cells increased to 16.6% when 1.0μM WT1 peptide was added (Fig. 3A). These data indicate that a significant population of TCRαβ$^+$ DN cells can develop from early progenitor thymocytes that ectopically express a TCRα chain prior to β-selection. Furthermore, the fact that this population of TCRαβ$^+$ DN cells increases when cognate peptide (for the introduced TCRα chain) is present suggests that a substantial fraction of these cells developed in response to WT1 antigen-specific signals.

Taken together, these data indicate that the TCRαβ$^+$ DN population could potentially contain cells that express a TCRβ chain that can pair with the introduced 3Dα to form a TCR with a higher affinity for the MHC-WT1 peptide tetramer than the original enhanced affinity receptor, and significantly higher than could be isolated from the normal T cell repertoire.

Therefore, 3Dα-transduced CD4$^-$CD8$^-$CD117$^+$CD44$^+$ DN1 and DN2 progenitor thymocytes were differentiated on OP9-DL1 cells expressing mouse MHC Class 1 H-2D^b and also transduced to express WT1. Non-adherent cells were collected at for several days up to day 21 and sorted for hCD2$^+$CD4$^-$CD8$^-$TCRβ$^+$ cells into TRIzol reagent (Invitrogen) (Fig. 3B). Cell sorts from individual days were pooled; RNA was purified, and cDNA was generated. The parent 3D TCR uses the Vb10 variable region. In order to retain the TCR CDR1 and CDR2 domains that contact MHC, the candidate TCRβ chains were restricted to those containing this variable region. Therefore, Vβ10-containing TCRβ chains within the sorted cell population were isolated by PCR using a Vβ10 specific forward primer, and a Cβ2 specific reverse primer (Fig. 3C). The Vb10-specific forward primer was designed to contain a CACC sequence allowing for directional TOPO-cloning into the pENTR^{TM}/D-TOPO® vector

(Invitrogen), followed by transfer using Gateway® technology for recombination (Invitrogen) into the retroviral vector MigR1-attR (a version of the MigR1 vector (Pear et al.,1998, Blood 92:3780-3792) that has been modified to contain attR sites and the ccdB gene for Gateway® cloning). The MigR1-TCRβ library was used to transduce PlatE retroviral packaging cells (Morita et al., 2000, Gene Therapy 7:1063-1066; Cell Biolabs, Inc.) to generate retroviral supernatant, which was then used to retrovirally transduce 58 $\alpha^-\beta^-$ cells, a murine T cell line that lacks endogenous TCRα and TCRβ chains, ($58^{-/-}$) (Letourneur and Malissen, 1989, Eur. J. Immunol. 19:2269-74).

Retroviral TCRβ library supernatant was titrated, and a dilution that resulted in less than 20% transduced cells following transduction was used in order to ensure that most cells contained only one retroviral integration. Transduced cells were sorted first for GFP positive cells, and then resorted two more times on $V\beta10^+$ cells that also had high levels of MHC-WT1 peptide tetramer staining (Fig. 4A). Following the second sort, cells were analyzed for staining with an unrelated, but MHC H-$2D^b$-peptide tetramer specific for GP33, in order to assess whether MHC-WT1 peptide tetramer positive cells were binding in a peptide-independent manner to MHC residues (Fig. 4A).

Following the third sort for MHC-WT1 peptide tetramer high, library-transduced $58^{-/-}$ cells, the sorted cells were expanded, lysed, and the DNA was isolated. Retroviral inserts were recovered by PCR using MigR1-attR vector specific primers, designed to include AttB Gateway® cloning sites from the vector. Using a two-step approach, inserts were cloned first into the pDONR™ vector (Invitrogen) using Gateway® recombination cloning technology, and then back into MigR1-attR. Individual bacterial colonies were picked from the recombinational cloning reaction and sequenced. Following sequence analysis of >30 clones, the four most prevalent TCRβ chains were identified for further analysis. Interestingly, several of the clones had CDR3β sequences that shared multiple conserved residues with the original 3Dβ chain (Fig. 4B). One of the clones (Clone#1) was found to be almost identical to the original 3Dβ, except for a P108Q substitution and a G112S substitution (Fig. 4B). The four candidate TCRβ chains were retrovirally transduced into $3D\alpha^+58^{-/-}$ cells and analyzed by flow cytometry (Fig. 4C). All four candidate clones bound MHC-WT1 peptide

tetramer when transduced into $3D\alpha^{+}58^{-/-}$ cells, although clone#4 bound MHC-WT1 peptide tetramer at significantly lower levels than the others and was not analyzed further. The parent 3Dβ chain had previously been codon-optimized, and therefore expressed higher levels of TCR at the cell surface, precluding direct comparison of tetramer staining levels between 3Dβ and the isolated clones.

In order to more directly assess the relative affinity of each of the TCRβ chains for MHC-WT1 peptide tetramer, $3D\alpha^{+}58^{-/-}$ cells transduced with 3Dα, and each of the candidate TCRβ chains were stained with six 2-fold serial dilutions of MHC-WT1 peptide tetramer and MFI values were fit to a saturation binding curve by non-linear regression, as the concentration of ligand that yielded half-maximal binding (Fig. 5A). The apparent affinities of all three candidate TCRβ chains, when paired with 3Dα, were found to be higher than the parent 3Dβ, and Clone#1 had ~10 fold higher affinity (Fig. 5A). Therefore, in order directly compare tetramer staining of 3Dα paired with Clone#1 versus the parent 3Dβ, Clone#1 was codon-optimized such that the only sequence differences between the original 3Dβ and Clone#1 were in the CDR3 region. Both constructs were transduced into $58^{-/-}$ cells and assessed by flow cytometry for MHC-WT1 peptide tetramer staining. When Clone#1 was codon-optimized, it was found to bind tetramer at a higher level than the original 3Dβ as expected (Fig. 5B).

One concern associated with enhancing the affinity of antigen-specific TCRs *in vitro* is that some modifications might increase the affinity of the receptor for MHC only, rather than peptide/MHC, thereby increasing the likelihood that the TCR will be autoreactive. This risk was minimized by restricting the TCRβ library to TCRβ chains that share the same variable domain (Vb10) in order to restrict variablility to CDR3. In order to determine whether any of the candidate TCRβ chains conferred an increased propensity to bind MHC H-$2D^{b}$ molecule in a peptide-independent manner, transduced $58^{-/-}$ cells were stained with a panel of MHC H-$2D^{b}$ tetramers (peptides: WT1, GP33, E4, MESN, SQV). All three candidate TCRβ chains were stained by the MHC-WT1 peptide tetramer at high levels when paired with 3Dα, similar to the original 3Dβ (Fig. 5C). When stained with four other MHC H-$2D^{b}$-peptide tetramers, all three TCRβ chains were uniformly negative for tetramer staining, suggesting that the

increase in affinity observed for these receptors is not the result of an increased affinity for MHC alone (Fig. 5C).

Example 3: Generation of high affinity WT1-specific T cells by ectopic expression of an antigen-specific TCRα chain during early human T cell development *in vitro*.

The Wilm's tumor (WT1) antigen is expressed at abnormally high levels on the surface of leukemia cells. HLA A2/WT1-specific T cell clones have been screened for clones with high specific activity. The TCRα and TCRβ chains from the C4 clone, which was determined to have the highest affinity for WT1, were isolated. A lentiviral vector comprising the C4 TCR and that confers high-level expression is subject of a TCR gene therapy clinical trial scheduled for 2012. In order to further enhance the affinity of the C4 TCR for the WT1 antigen, the *in vitro* differentiation system described in the previous examples is used with human cord blood progenitor cells expressing the C4 TCRα chain.

Generation of WT1-specific T cells:

A variant of the OP9-DL1 cell line described in Example 1, which expressed the human Class I MHC molecule HLA-A2 (Genbank Accession Nos. U18930.1 (SEQ ID NO:50) and AAA87076.1 (SEQ ID NO:51), transcript and protein sequences, respectively) and human Class I MHC β2 microglobulin (β2M) molecule (Genbank Accession Nos. NM_004048.2 (SEQ ID NO:52) and NP_004039.1 (SEQ ID NO:53), transcript and protein sequences, respectively) was generated. The TCRα chain of the C4 TCR clone is stably transduced into cord blood-derived hematopoietic progenitor cells by retroviral transduction, using a retroviral vector that also encodes green fluorescent protein (GFP) as a transduction marker. Progenitor cells expressing GFP are sorted by flow cytometry and cultured on OP9-DL1-A2/β2M stroma cells in the presence or absence of WT1 peptide RMFPNAPYL (SEQ ID NO:2). Human hematopoietic progenitor cells readily proliferate and differentiate in OP9-DL1 culture to a stage of human T cell development characterized by the phenotype $CD34^+CD1a^+CD4^+$ (La Motte-Mohs et al., 2005, Blood 105:1431-1439), at which point they are undergoing TCR gene rearrangements at the β, γ, and δ loci (Spits, 2002, Nat. Rev. Immunol. 2:760-772). It is hypothesized that, like their murine counterparts,

TCRα-expressing human T cell progenitors that produce an in-frame rearrangement at the TCRβ locus will adapt one of two cell fates: those expressing a TCRβ chain that does not pair well with the transgenic TCRα, or that pairs with the transgenic TCRα but does not receive a strong signal through this αβTCR, will differentiate to the DP stage in response to signaling though the pre-TCR; on the other hand, those that generate a TCRβ chain that can pair with the transgenic TCRα and receive a sufficiently strong signal through this mature αβTCR will be signaled to differentiate towards a DN TCRαβ+ γδ-like lineage. Since DP cells only survive for ~3-4 days without a positive selection signal, and since efficient positive selection does not occur in OP9-DL1 cultures, the vast majority of cells that do not receive an agonist signal through the αβ TCR will be eliminated from the culture, allowing γδ-like cells that develop due to early αβ TCR signaling to accumulate.

Isolation of candidate TCRβ chains:

At various points of the culture, non-adherent cells that have a DN TCRαβ+ γδ-like phenotype and are WT1 peptide/A2 MHC-tetramer positive are collected by cell sorting. It may not be possible to detect WT1 tetramer positive cells, as the continued presence of antigen in the cultures may result in TCR down-modulation that could decrease tetramer staining below detection. Furthermore, since these cells are likely not to express CD8αβ, high affinity receptors that are not CD8-independent are undetectable by tetramer staining. Therefore, it may be necessary to screen the TCRβ chains from all DN TCRαβ+ cells that emerge in the culture (see below). It may also be desirable to restrict candidate T cells to those that use the same Vβ segment utilized by the original C4 TCRβ chain (Vβ17), in order to retain the CDR1 and CDR2 MHC contacts of the parent C4 TCR.

Following cell sorting, the endogenous TCRβ chains are cloned by purifying total RNA, performing full-length RACE RT-PCR with C-β1 or C-β2 primers, and cloning the PCR products into the pENTR™/D-TOPO® vector (Invitrogen), which allows directional TOPO-cloning and incorporates attL sites that allow rapid and efficient transfer to the retroviral vector Mig-attR (a variant of MigR1 (Pear et al.,1998, Blood 92:3780-3792) that contains attR sites for insertion of gene of interest) using Invitrogen's Gateway® technology recombination system. The products

of the recombination reaction are electroporated into high efficiency bacteria, and colonies are scraped together and maxiprepped to generate a retroviral library of potentially WT1-reactive TCRβ chains.

Screening of high affinity WT1-specific TCRs:

TCRβ chains that can pair with the C4 TCRα chain to form a high affinity WT1-specific TCR are identified by transducing the TCRβ library into the human T cell line H9 (Catalog # HTB-176, ATCC, Manassas, VA) that has been transduced to express the C4 TCRα chain (H9-C4α). Transduced cells are sorted by flow cytometry for high levels of MHC-WT1 peptide tetramer staining and retroviral inserts will be amplified by PCR from the sorted population. Candidate TCRβ chains are identified by TOPO-cloning of the PCR product followed by sequence analysis. The selected TCRβ chains and the parental C4α are transduced into H9-C4α cells and the relative affinities for the MHC-WT1 peptide tetramer will be calculated by staining transduced cells with serial 2-fold dilutions of PE-conjugated tetramers (as described in Example 2). Affinity values are determined by fitting the MFI for each dilution to a binding curve by non-linear regression and KD defined as tetramer concentration yielding half-maximal binding. TCRβ chains that can pair with C4 TCRα to generate a TCR with higher affinity by MHC-peptide tetramer staining than the wildtype C4 receptor are further characterized for safety and efficacy.

Example 4: Characterization of the efficacy and safety of candidate high affinity TCRs using an in vivo mouse model of WT1-targeted TCR gene therapy.

Enhanced affinity human WT1-specific TCRs that are identified as in Example 3 are tested for safety and efficacy in an HLA-A2 transgenic mouse model of WT1 targeted gene therapy.

Assessing enhanced TCRs for off-target activity:

Promiscuous activation of high affinity TCRs are assessed by measuring cytokine production by TCR-transduced T cells in response to a panel of A2 expressing target cells in the presence or absence of WT1 peptide. TCRs that exhibit off-target recognition of WT1 negative target cells compared to the parent C4 TCR are not advanced for further study.

Enhanced affinity TCRs activity on normal tissue *in vivo*:

WT1 expression in normal tissue is similar in both mouse and man, and the WT1 peptide recognized by the C4 TCR is identical in mice and known to be processed and presented by mouse cells (Gaiger et al., 2000, Blood 96:1480-9). HLA-A2 transgenic mice have been used to test for recognition of normal tissues by T cells expressing human high affinity WT1-specific TCRs (Kuball et al., 2009, J. Exp. Med. 206:463-475).

In order to evaluate the safety of enhanced affinity TCRs generated *in vitro* as disclosed in the previous example, $CD8^+$ T cells from B6.A2/D^b mice, which express a transgene encoding α1 and α2 domains of A2 fused to α3 of D^b (for binding mouse CD8) (Newberg et al., 1996, J. Immunol. 156:2473-2480), are transduced to expressed candidate enhanced affinity TCRs. The TCRs are modified prior to transduction to contain mouse rather than human Cα and Cβ domains, which increases expression in mouse T cells (Pouw et al., 2007, J. Gene Med. 9:561-570). About 4-6 weeks following transfer of TCR-transduced T cells into mice, tissues known to naturally express WT1 (*e.g.*, lungs and kidney) are analyzed by histology for evidence of T cell infiltration and tissue damage, and bone marrow is assessed by flow cytometry for depletion of WT1-expression hematopoietic progenitor cells.

Correlation of enhanced affinity with improved target recognition and function:

There is evidence that an affinity threshold may exist for TCRs, above which further enhancements will not increase T cell function and may actually decrease antigen sensitivity (Schmid et al., 2010, J. Immunol. 184:4936-46). Therefore, the response of high affinity TCR-transduced $CD8^+$ T cells to target cells pulsed with limiting peptide concentrations are compared with T cells expressing the parent C4 TCR. Cytokine production (IFNγ/IL-2) and proliferation, as well as lytic activity, are analyzed. TCRs exhibiting increased affinity and enhanced function are advanced for further study and for potential use in TCR gene therapy trials.

Example 5: Generation of high affinity WT1-specific T cells in vivo.

An *in vivo* mouse model (TCRα retrogenic mice) was used to determine whether $TCR\beta^+$ double negative (DN) cells can develop in the thymus. Retrogenic

(retrovirally transduced) mice allow for rapid generation, compared with transgenic methods, of mice expressing a specific TCR transgene. Methods of making retrogenic mice are known in the art (*see, e.g.*, Holst et al., 2006, Nat. Protoc. 1:406-417; Holst et al., 2006, Nat. Methods 3:191-197; Bettini et al., 2012, Immunology 136:265-272). Briefly, hematopoietic progenitor/stem cells were purified from the bone marrow of B6 mice and transduced to express the TCRα chain from either the high affinity WT1 specific 3D-PYY TCR or the low affinity mesothelin specific TCR 7431. The 3D-PYY TCR is a higher affinity TCR engineered from the 3D TCR, identified using a T cell display system and selection with WT1/D^b Ig DimerX (BD Biosciences) (Stone et al., 2011, J. Immunol. 186:5193-5200; Chervin et al., 2008, J. Immunol. Methods 339:175-184). The retroviral constructs comprising the 3D-PYY TCRα or 7431α transgenes also include the extracellular domain of human CD2 as a transduction marker, with an IRES between the two transgenes. Transduced bone-marrow derived progenitors were transferred into lethally irradiated B6 host mice to generate bone marrow chimeras expressing the introduced TCRα chains. Six weeks after *in vivo* transfer of the TCRα-transduced bone marrow cells, mice were sacrificed. Cells from the thymus and spleen were analyzed for CD4 and CD8 expression by flow cytometry (Figures 6A, 6B). Analysis of CD4 and CD8 expression by $TCR\beta^+$ cells in the thymus (Figure 6A) shows that a large population of double negative $TCR\beta^+$ cells can be detected *in vivo* in the transduced thymocytes that ectopically express a TCRα chain early in development, and that this population is more pronounced in mice expressing a TCRα from a high affinity TCR (*e.g.*, 3D-PYYα). DN $TCR\beta^+$ thymocytes from 3D-PYYα and 7431α retrogenic mice were also analyzed for expression of Vβ10 and Vβ9, respectively (Figure 6A). These data show that the DN $TCR\beta^+$ population is enriched for cells that utilize the same Vβ gene segment as the original antigen specific TCR. Taken together, these data support the hypothesis that the DN TCRβ+ cells develop in response to relatively strong TCR signaling resulting from cognate interactions with the target antigen (*i.e.*, WT1 or Mesothelin) expressed in the thymus. Analysis of CD4 and CD8 expression of TCRβ+ retrogenic splenocytes shows that these DN TCRβ+ cells are also present in the periphery of retrogenic mice (Figure 6B).

Splenocytes from 3D-PYYα and 7431α retrogenic mice were stimulated with WT1 peptide and Mesothelin peptide, repectively, and cultured *in vitro* in the presence of IL-2 for 6 days. IL-2 was added to the culture in order to potentially expand antigen specific cells so they could be detected by tetramer staining. Cultures were analyzed for CD4 and CD8 expression by flow cytometry within the TCRβ+ gate, as well as for expression of the parental TCR Vβ gene (Figure 7). Again, enrichment for the parental Vβ gene family is observed, especially for the high affinity 3D-PYY. Cultured T cells were also analyzed for the presence of antigen-specific T cells by staining with WT1 or Mesothelin peptide/MHC tetramers (Figure 7). These data show that, especially for the high affinity 3D-PYYα retrogenic mice, a significant number of antigen specific T cells are present in these cultures. The fact that the tetramer positive cells are found within the TCRα-transduced ($hCD2^+$) population indicates that these cells developed as a result of the early expression of the TCRα chain. This demonstrates that the DN TCRβ+ cells that develop in these mice actually do contain high affinity antigen specific T cells. Since these are DN cells, they don't have the contribution of CD8 to help with tetramer binding – these TCRs are then "$CD8^-$ independent" – CD8-independent tetramer binding requires a high affinity TCR.

The various embodiments described above can be combined to provide further embodiments. All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, applications and publications to provide yet further embodiments.

These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible

embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

CLAIMS

What is claimed is:

1. A method for generating an enhanced affinity T cell receptor (TCR), comprising:

a. contacting hematopoietic progenitor cells with stromal cells and a peptide antigen, under conditions and for a time sufficient to induce differentiation of hematopoietic progenitor cells into DN TCR$\alpha\beta^+$ thymocytes,

b. isolating nucleic acid sequences encoding the various TCRβ chains from the DN TCR$\alpha\beta^+$ thymocytes and introducing the nucleic acid sequences encoding the TCRβ chains into cells that are capable of expressing a TCR on the cell surface and comprise the nucleic acid sequence encoding the TCRα chain from step (a); and

c. identifying enhanced affinity TCR,

wherein the hematopoietic progenitor cells comprise a non-endogenous nucleic acid sequence encoding a TCRα chain from a parent TCR specific for the peptide antigen, and

wherein the stromal cells comprise a non-endogenous nucleic acid sequence encoding Delta-like-1 or Delta-like-4 and a nucleic acid sequence encoding an MHC molecule.

2. The method of claim 1, wherein the TCRβ chain is isolated from the parent TCR.

3. The method of claim 1, wherein the hematopoietic progenitor cells comprise thymocyte progenitor cells or embryonic stem cells.

4. The method of claim 1, wherein the hematopoietic progenitor cells comprise hematopoietic stem cells derived from bone marrow or cord blood.

5. The method of claim 1, wherein a viral vector is used to introduce the non-endogenous nucleic acid sequence encoding the TCRα chain specific for the peptide antigen into the hematopoeitic progenitor cells.

6. The method of claim 5, wherein the viral vector is a retroviral vector.

7. The method of claim 5, wherein the viral vector is a lentiviral vector.

8. The method of claim 5, wherein the viral vector further comprises a gene marker for transduction.

9. The method of claim 8, wherein the gene marker for transduction comprises green fluorescent protein or the extracellular domain of human CD2.

10. The method of claim 1, wherein the stromal cells express Delta-like-1.

11. The method of claim 1, wherein the stromal cells are derived from OP9.

12. The method of claim 1, wherein the method further comprises selecting the cells that are capable of expressing a TCR on the cell surface resulting from step (b) with MHC-peptide tetramer staining.

13. The method of claim 12, wherein the cells that are capable of expressing a TCR on the cell surface resulting from step (b) are selected with MHC-peptide tetramer staining multiple times.

14. The method of claim 1, wherein a viral vector is used to introduce the nucleic acid sequences encoding the various TCRβ chains from step (b) into the cells that are capable of expressing TCR on the cell surface.

15. The method of claim 14, wherein the viral vector is a retroviral vector.

16. The method of claim 14, wherein the viral vector is a lentiviral vector.

17. The method of claim 14, wherein the viral vector further comprises a gene marker for transduction.

18. The method of claim 17, wherein the gene marker for transduction comprises green fluorescent protein.

19. The method of claim 1, wherein the cells that are capable of expressing a TCR on the cell surface are derived from $TCR\alpha^-/\beta^-$ 58 T cell hybridoma.

20. The method of claim 1, wherein the enhanced affinity TCR is a human TCR.

21. The method of claim 1, wherein the MHC molecule comprises a Class I MHC molecule or a Class II MHC molecule.

22. The method of claim 21, wherein the MHC molecule comprises HLA-A2 and human beta-2-microglobulin (β2M).

23. The method of claim 1, wherein the peptide antigen is selected from the group consisting of: a viral antigen, a bacterial antigen, a cancer antigen, and an autoimmune antigen.

24. The method of claim 23, wherein the peptide antigen is a WT1 peptide antigen or a mesothelin peptide antigen.

25. The method of claim 24, wherein the WT1 peptide antigen comprises an amino acid sequence RMFPNAPYL (SEQ ID NO:2).

26. The method of claim 24, wherein the mesothelin peptide antigen comprises an amino acid sequence GQKMNAQAI (SEQ ID NO:31).

27. The method of claim 1, wherein the peptide antigen is added to the hematopoietic progenitor cells and stromal cells in culture.

28. The method of claim 1, wherein the stromal cells comprise a nucleic acid sequence encoding the peptide antigen.

29. The method of claim 1, wherein isolating the nucleic acid sequences encoding the various TCRβ chains from the DN TCRαβ$^-$ thymocytes further comprises selecting TCRβ chains with the same V_β gene as the parent TCRβ chain prior to introducing the selected TCRβ chains into cells capable of expressing a TCR on the cell surface.

30. An enhanced affinity TCR generated by the method of claim 1.

31. A fusion protein comprising an enhanced affinity TCR generated by the method of claim 1 and a cytotoxic or detectable component.

32. An enhanced affinity TCR generated by the method of claim 23 or 24.

33. A pharmaceutical composition, comprising an enhanced affinity TCR generated by the method of claim 1, and a pharmaceutically acceptable carrier, diluent, or excipient.

34. A method of treating a disease in a subject comprising administering an enhanced affinity TCR generated by the method of claim 1.

35. The method of claim 34, wherein the disease is selected from the group consisting of viral infection, bacterial infection, cancer, and autoimmune disease.

36. The method of claim 34, wherein the subject is human.

37. The method of claim 34, wherein the enhanced TCR is administered to the subject as a soluble TCR.

38. The method of claim 34, wherein the subject is administered T cells comprising the enhanced affinity TCR.

39. The method of claim 38, wherein the T cells comprise regulatory T cells.

40. The method of claim 38, wherein the T cells comprise $CD8^+$ T cells or $CD4^+$ T cell.

41. The method of claim 38, wherein the T cells are autologous T cells.

SEQUENCE LISTING

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<120> ENHANCED AFFINITY T CELL RECEPTORS AND METHODS FOR MAKING THE SAME

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<212> DNA
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<212> PRT
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Val Asn Lys Lys Gly Leu Leu Gly Asn Arg Asn Cys Cys Arg Gly Gly
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Ala Gly Pro Pro Pro Cys Ala Cys Arg Thr Phe Phe Arg Val Cys Leu
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Lys His Tyr Gln Ala Ser Val Ser Pro Glu Pro Pro Cys Thr Tyr Gly
65                  70                  75                  80
Ser Ala Val Thr Pro Val Leu Gly Val Asp Ser Phe Ser Leu Pro Asp
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Gly Gly Gly Ala Asp Ser Ala Phe Ser Asn Pro Ile Arg Phe Pro Phe
            100                 105                 110
Gly Phe Thr Trp Pro Gly Thr Phe Ser Leu Ile Ile Glu Ala Leu His
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Ser Arg Leu Ala Thr Gln Arg His Leu Thr Val Gly Glu Glu Trp Ser
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Arg Pro Arg Asp Asp Ala Phe Gly His Phe Thr Cys Gly Glu Arg Gly
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Glu Lys Val Cys Asn Pro Gly Trp Lys Gly Pro Tyr Cys Thr Glu Pro
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Ile Cys Leu Pro Gly Cys Asp Glu Gln His Gly Phe Cys Asp Lys Pro
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Ala Thr Gln Ile Lys Asn Thr Asn Lys Lys Ala Asp Phe His Gly Asp
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His Ser Ala Asp Lys Asn Gly Phe Lys Ala Arg Tyr Pro Ala Val Asp
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Ala His Ser Lys Arg Asp Thr Lys Cys Gln Pro Gln Gly Ser Ser Gly
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Glu Glu Lys Gly Thr Pro Thr Thr Leu Arg Gly Gly Glu Ala Ser Glu
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Ala Val Thr Pro Val Leu Gly Val Asp Ser Phe Ser Leu Pro Asp Gly
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Ala Gly Ile Asp Pro Ala Phe Ser Asn Pro Ile Arg Phe Pro Phe Gly
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Phe Thr Trp Pro Gly Thr Phe Ser Leu Ile Ile Glu Ala Leu His Thr
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Arg Leu Thr Thr Gln Arg His Leu Thr Val Gly Glu Glu Trp Ser Gln
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Asp Leu His Ser Ser Gly Arg Thr Asp Leu Arg Tyr Ser Tyr Arg Phe
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Val Cys Asp Glu His Tyr Tyr Gly Glu Gly Cys Ser Val Phe Cys Arg
180 185 190
Pro Arg Asp Asp Ala Phe Gly His Phe Thr Cys Gly Asp Arg Gly Glu
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Lys Met Cys Asp Pro Gly Trp Lys Gly Gln Tyr Cys Thr Asp Pro Ile
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Cys Leu Pro Gly Cys Asp Asp Gln His Gly Tyr Cys Asp Lys Pro Gly
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Glu Cys Lys Cys Arg Val Gly Trp Gln Gly Arg Tyr Cys Asp Glu Cys
245 250 255
Ile Arg Tyr Pro Gly Cys Leu His Gly Thr Cys Gln Gln Pro Trp Gln
260 265 270
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290 295 300
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305 310 315 320
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325 330 335
Lys Asn Gly Ala Ser Cys Thr Asp Leu Glu Asp Ser Phe Ser Cys Thr
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Ala Lys Cys Val Asp Leu Gly Asn Ser Tyr Leu Cys Arg Cys Gln Ala
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<212> DNA
<213> Homo sapiens

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<210> 8
<211> 685
<212> PRT
<213> Homo sapiens

<400> 8

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            20                  25                  30
Gln Leu Gln Glu Phe Ile Asn Glu Arg Gly Val Leu Ala Ser Gly Arg
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Pro Cys Glu Pro Gly Cys Arg Thr Phe Phe Arg Val Cys Leu Lys His
    50                  55                  60
Phe Gln Ala Val Val Ser Pro Gly Pro Cys Thr Phe Gly Thr Val Ser
65                  70                  75                  80
Thr Pro Val Leu Gly Thr Asn Ser Phe Ala Val Arg Asp Asp Ser Ser
                85                  90                  95
Gly Gly Gly Arg Asn Pro Leu Gln Leu Pro Phe Asn Phe Thr Trp Pro
            100                 105                 110
Gly Thr Phe Ser Leu Ile Ile Glu Ala Trp His Ala Pro Gly Asp Asp
        115                 120                 125
Leu Arg Pro Glu Ala Leu Pro Pro Asp Ala Leu Ile Ser Lys Ile Ala
    130                 135                 140
Ile Gln Gly Ser Leu Ala Val Gly Gln Asn Trp Leu Leu Asp Glu Gln
145                 150                 155                 160
Thr Ser Thr Leu Thr Arg Leu Arg Tyr Ser Tyr Arg Val Ile Cys Ser
                165                 170                 175
Asp Asn Tyr Tyr Gly Asp Asn Cys Ser Arg Leu Cys Lys Lys Arg Asn
            180                 185                 190
Asp His Phe Gly His Tyr Val Cys Gln Pro Asp Gly Asn Leu Ser Cys
        195                 200                 205
Leu Pro Gly Trp Thr Gly Glu Tyr Cys Gln Gln Pro Ile Cys Leu Ser
    210                 215                 220
Gly Cys His Glu Gln Asn Gly Tyr Cys Ser Lys Pro Ala Glu Cys Leu
225                 230                 235                 240
Cys Arg Pro Gly Trp Gln Gly Arg Leu Cys Asn Glu Cys Ile Pro His
                245                 250                 255
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Asn Gly Cys Arg His Gly Thr Cys Ser Thr Pro Trp Gln Cys Thr Cys
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Thr His His Ser Pro Cys Lys Asn Gly Ala Thr Cys Ser Asn Ser Gly
    290                 295                 300
Gln Arg Ser Tyr Thr Cys Thr Cys Arg Pro Gly Tyr Thr Gly Val Asp
305                 310                 315                 320
Cys Glu Leu Glu Leu Ser Glu Cys Asp Ser Asn Pro Cys Arg Asn Gly
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Gly Ser Cys Lys Asp Gln Glu Asp Gly Tyr His Cys Leu Cys Pro Pro
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Gly Tyr Tyr Gly Leu His Cys Glu His Ser Thr Leu Ser Cys Ala Asp
        355                 360                 365
Ser Pro Cys Phe Asn Gly Gly Ser Cys Arg Glu Arg Asn Gln Gly Ala
    370                 375                 380
Asn Tyr Ala Cys Glu Cys Pro Pro Asn Phe Thr Gly Ser Asn Cys Glu
385                 390                 395                 400
Lys Lys Val Asp Arg Cys Thr Ser Asn Pro Cys Ala Asn Gly Gly Gln
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Cys Leu Asn Arg Gly Pro Ser Arg Met Cys Arg Cys Arg Pro Gly Phe
            420                 425                 430
Thr Gly Thr Tyr Cys Glu Leu His Val Ser Asp Cys Ala Arg Asn Pro
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Cys Ala His Gly Gly Thr Cys His Asp Leu Glu Asn Gly Leu Met Cys
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Thr Cys Pro Ala Gly Phe Ser Gly Arg Arg Cys Glu Val Arg Thr Ser
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Ile Asp Ala Cys Ala Ser Ser Pro Cys Phe Asn Arg Ala Thr Cys Tyr
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Thr Asp Leu Ser Thr Asp Thr Phe Val Cys Asn Cys Pro Tyr Gly Phe
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Val Gly Ser Arg Cys Glu Phe Pro Val Gly Leu Pro Pro Ser Phe Pro
        515                 520                 525
Trp Val Ala Val Ser Leu Gly Val Gly Leu Ala Val Leu Leu Val Leu
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Leu Gly Met Val Ala Val Ala Val Arg Gln Leu Arg Leu Arg Arg Pro
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Asp Asp Gly Ser Arg Glu Ala Met Asn Asn Leu Ser Asp Phe Gln Lys
                565                 570                 575
Asp Asn Leu Ile Pro Ala Ala Gln Leu Lys Asn Thr Asn Gln Lys Lys
            580                 585                 590
Glu Leu Glu Val Asp Cys Gly Leu Asp Lys Ser Asn Cys Gly Lys Gln
        595                 600                 605
Gln Asn His Thr Leu Asp Tyr Asn Leu Ala Pro Gly Pro Leu Gly Arg
    610                 615                 620
Gly Thr Met Pro Gly Lys Phe Pro His Ser Asp Lys Ser Leu Gly Glu
625                 630                 635                 640
Lys Ala Pro Leu Arg Leu His Ser Glu Lys Pro Glu Cys Arg Ile Ser
                645                 650                 655
Ala Ile Cys Ser Pro Arg Asp Ser Met Tyr Gln Ser Val Cys Leu Ile
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Ser Glu Glu Arg Asn Glu Cys Val Ile Ala Thr Glu Val
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<210> 9
<211> 3451
<212> DNA
<213> Mus musculus

<400> 9

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ctcgagaccc taggatttgc tccaggacac gtacttagag cagccaccgc ccagtcgccc     180
tcacctggat tacctaccga ggcatcgagc agcggagttt ttgagaaggc gacaagggag     240
cagcgtcccg aggggaatca gcttttcagg aactcggctg gcagacggga cttgcgggag     300
agcgacatcc ctaacaagca gattcggagt cccggagtgg agaggacacc ccaagggatg     360
acgcctgcgt cccggagcgc ctgtcgctgg gcgctactgc tgctggcggt actgtggccg     420
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tgccttaagc acttccaggc aaccttctcc gagggaccct gcacctttgg caatgtctcc     600
acgccggtat tgggcaccaa ctccttcgtc gtcagggaca agaatagcgg cagtggtcgc     660
aaccctctgc agttgccctt caatttcacc tggccgggaa ccttctcact caacatccaa     720
gcttggcaca caccgggaga cgacctgcgg ccagagactt cgccaggaaa ctctctcatc     780
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aatgacaccc tcaccagact gagctactct taccgggtca tctgcagtga caactactat     900
ggagagagct gttctcgcct atgcaagaag cgcgatgacc acttcggaca ttatgagtgc     960
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<210> 10
<211> 686
<212> PRT
<213> Mus musculus

<400> 10
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            20                  25                  30
Leu Arg Leu Gln Glu Phe Val Asn Gln Arg Gly Met Leu Ala Asn Gly
        35                  40                  45
Gln Ser Cys Glu Pro Gly Cys Arg Thr Phe Phe Arg Ile Cys Leu Lys
    50                  55                  60
His Phe Gln Ala Thr Phe Ser Glu Gly Pro Cys Thr Phe Gly Asn Val
65                  70                  75                  80
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Ser Thr Pro Val Leu Gly Thr Asn Ser Phe Val Val Arg Asp Lys Asn
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Ser Gly Ser Gly Arg Asn Pro Leu Gln Leu Pro Phe Asn Phe Thr Trp
            100                 105                 110
Pro Gly Thr Phe Ser Leu Asn Ile Gln Ala Trp His Thr Pro Gly Asp
        115                 120                 125
Asp Leu Arg Pro Glu Thr Ser Pro Gly Asn Ser Leu Ile Ser Gln Ile
    130                 135                 140
Ile Ile Gln Gly Ser Leu Ala Val Gly Lys Ile Trp Arg Thr Asp Glu
145                 150                 155                 160
Gln Asn Asp Thr Leu Thr Arg Leu Ser Tyr Ser Tyr Arg Val Ile Cys
                165                 170                 175
Ser Asp Asn Tyr Tyr Gly Glu Ser Cys Ser Arg Leu Cys Lys Lys Arg
            180                 185                 190
Asp Asp His Phe Gly His Tyr Glu Cys Gln Pro Asp Gly Ser Leu Ser
        195                 200                 205
Cys Leu Pro Gly Trp Thr Gly Lys Tyr Cys Asp Gln Pro Ile Cys Leu
    210                 215                 220
Ser Gly Cys His Glu Gln Asn Gly Tyr Cys Ser Lys Pro Asp Glu Cys
225                 230                 235                 240
Ile Cys Arg Pro Gly Trp Gln Gly Arg Leu Cys Asn Glu Cys Ile Pro
                245                 250                 255
His Asn Gly Cys Arg His Gly Thr Cys Ser Ile Pro Trp Gln Cys Ala
            260                 265                 270
Cys Asp Glu Gly Trp Gly Gly Leu Phe Cys Asp Gln Asp Leu Asn Tyr
        275                 280                 285
Cys Thr His His Ser Pro Cys Lys Asn Gly Ser Thr Cys Ser Asn Ser
    290                 295                 300
Gly Pro Lys Gly Tyr Thr Cys Thr Cys Leu Pro Gly Tyr Thr Gly Glu
305                 310                 315                 320
His Cys Glu Leu Gly Leu Ser Lys Cys Ala Ser Asn Pro Cys Arg Asn
                325                 330                 335
Gly Gly Ser Cys Lys Asp Gln Glu Asn Ser Tyr His Cys Leu Cys Pro
            340                 345                 350
Pro Gly Tyr Tyr Gly Gln His Cys Glu His Ser Thr Leu Thr Cys Ala
        355                 360                 365
Asp Ser Pro Cys Phe Asn Gly Gly Ser Cys Arg Glu Arg Asn Gln Gly
    370                 375                 380
Ser Ser Tyr Ala Cys Glu Cys Pro Pro Asn Phe Thr Gly Ser Asn Cys
385                 390                 395                 400
Glu Lys Lys Val Asp Arg Cys Thr Ser Asn Pro Cys Ala Asn Gly Gly
                405                 410                 415
Gln Cys Gln Asn Arg Gly Pro Ser Arg Thr Cys Arg Cys Arg Pro Gly
            420                 425                 430
Phe Thr Gly Thr His Cys Glu Leu His Ile Ser Asp Cys Ala Arg Ser
        435                 440                 445
Pro Cys Ala His Gly Gly Thr Cys His Asp Leu Glu Asn Gly Pro Val
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Cys Thr Cys Pro Ala Gly Phe Ser Gly Arg Arg Cys Glu Val Arg Ile
465                 470                 475                 480
Thr His Asp Ala Cys Ala Ser Gly Pro Cys Phe Asn Gly Ala Thr Cys
                485                 490                 495
Tyr Thr Gly Leu Ser Pro Asn Asn Phe Val Cys Asn Cys Pro Tyr Gly
            500                 505                 510
Phe Val Gly Ser Arg Cys Glu Phe Pro Val Gly Leu Pro Pro Ser Phe
        515                 520                 525
Pro Trp Val Ala Val Ser Leu Gly Val Gly Leu Val Val Leu Leu Val
    530                 535                 540
Leu Leu Val Met Val Val Val Ala Val Arg Gln Leu Arg Leu Arg Arg
545                 550                 555                 560
Pro Asp Asp Glu Ser Arg Glu Ala Met Asn Asn Leu Ser Asp Phe Gln
                565                 570                 575
Lys Asp Asn Leu Ile Pro Ala Ala Gln Leu Lys Asn Thr Asn Gln Lys
            580                 585                 590
Lys Glu Leu Glu Val Asp Cys Gly Leu Asp Lys Ser Asn Cys Gly Lys
        595                 600                 605
Leu Gln Asn His Thr Leu Asp Tyr Asn Leu Ala Pro Gly Leu Leu Gly
    610                 615                 620
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Arg Gly Gly Met Pro Gly Lys Tyr Pro His Ser Asp Lys Ser Leu Gly
625 630 635 640
Glu Lys Val Pro Leu Arg Leu His Ser Glu Lys Pro Glu Cys Arg Ile
645 650 655
Ser Ala Ile Cys Ser Pro Arg Asp Ser Met Tyr Gln Ser Val Cys Leu
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Ile Ser Glu Glu Arg Asn Glu Cys Val Ile Ala Thr Glu Val
675 680 685

<210> 11
<211> 2977
<212> DNA
<213> Homo sapiens

<400> 11
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cagagccggg acggcagccc aggcgcccgg gccccgccgt ctcctcgccg cgatcctgga 180
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<210> 12
<211> 497

<212> PRT
<213> Homo sapiens

<400> 12
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Thr Leu Arg Ser Gly Pro Gly Cys Leu Gln Gln Pro Glu Gln Gln Gly
            20   25   30
Val Arg Asp Pro Gly Gly Ile Trp Ala Lys Leu Gly Ala Ala Glu Ala
        35   40   45
Ser Ala Glu Arg Leu Gln Gly Arg Arg Ser Arg Gly Ala Ser Gly Ser
    50   55   60
Glu Pro Gln Gln Met Gly Ser Asp Val Arg Asp Leu Asn Ala Leu Leu
65   70   75   80
Pro Ala Val Pro Ser Leu Gly Gly Gly Gly Gly Cys Ala Leu Pro Val
                85   90   95
Ser Gly Ala Ala Gln Trp Ala Pro Val Leu Asp Phe Ala Pro Pro Gly
            100   105   110
Ala Ser Ala Tyr Gly Ser Leu Gly Gly Pro Ala Pro Pro Pro Ala Pro
        115   120   125
Pro Pro Pro Pro Pro Pro Pro Pro His Ser Phe Ile Lys Gln Glu Pro
    130   135   140
Ser Trp Gly Gly Ala Glu Pro His Glu Glu Gln Cys Leu Ser Ala Phe
145   150   155   160
Thr Val His Phe Ser Gly Gln Phe Thr Gly Thr Ala Gly Ala Cys Arg
                165   170   175
Tyr Gly Pro Phe Gly Pro Pro Pro Pro Ser Gln Ala Ser Ser Gly Gln
            180   185   190
Ala Arg Met Phe Pro Asn Ala Pro Tyr Leu Pro Ser Cys Leu Glu Ser
        195   200   205
Gln Pro Ala Ile Arg Asn Gln Gly Tyr Ser Thr Val Thr Phe Asp Gly
    210   215   220
Thr Pro Ser Tyr Gly His Thr Pro Ser His His Ala Ala Gln Phe Pro
225   230   235   240
Asn His Ser Phe Lys His Glu Asp Pro Met Gly Gln Gln Gly Ser Leu
                245   250   255
Gly Glu Gln Gln Tyr Ser Val Pro Pro Pro Val Tyr Gly Cys His Thr
            260   265   270
Pro Thr Asp Ser Cys Thr Gly Ser Gln Ala Leu Leu Leu Arg Thr Pro
        275   280   285
Tyr Ser Ser Asp Asn Leu Tyr Gln Met Thr Ser Gln Leu Glu Cys Met
    290   295   300
Thr Trp Asn Gln Met Asn Leu Gly Ala Thr Leu Lys Gly His Ser Thr
305   310   315   320
Gly Tyr Glu Ser Asp Asn His Thr Thr Pro Ile Leu Cys Gly Ala Gln
                325   330   335
Tyr Arg Ile His Thr His Gly Val Phe Arg Gly Ile Gln Asp Val Arg
            340   345   350
Arg Val Pro Gly Val Ala Pro Thr Leu Val Arg Ser Ala Ser Glu Thr
        355   360   365
Ser Glu Lys Arg Pro Phe Met Cys Ala Tyr Pro Gly Cys Asn Lys Arg
    370   375   380
Tyr Phe Lys Leu Ser His Leu Gln Met His Ser Arg Lys His Thr Gly
385   390   395   400
Glu Lys Pro Tyr Gln Cys Asp Phe Lys Asp Cys Glu Arg Arg Phe Ser
                405   410   415
Arg Ser Asp Gln Leu Lys Arg His Gln Arg Arg His Thr Gly Val Lys
            420   425   430
Pro Phe Gln Cys Lys Thr Cys Gln Arg Lys Phe Ser Arg Ser Asp His
        435   440   445
Leu Lys Thr His Thr Arg Thr His Thr Gly Glu Lys Pro Phe Ser Cys
    450   455   460
Arg Trp Pro Ser Cys Gln Lys Lys Phe Ala Arg Ser Asp Glu Leu Val
465   470   475   480
Arg His His Asn Met His Gln Arg Asn Met Thr Lys Leu Gln Leu Ala
                485   490   495
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<210> 13
<211> 3028
<212> DNA
<213> Homo sapiens

<400> 13
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<210> 14
<211> 514
<212> PRT
<213> Homo sapiens

<400> 14
Met Gln Asp Pro Ala Ser Thr Cys Val Pro Glu Pro Ala Ser Gln His
 1   5   10   15
Thr Leu Arg Ser Gly Pro Gly Cys Leu Gln Gln Pro Glu Gln Gln Gly

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Val Arg Asp Pro Gly Gly Ile Trp Ala Lys Leu Gly Ala Ala Glu Ala
        35                  40                  45
Ser Ala Glu Arg Leu Gln Gly Arg Arg Ser Arg Gly Ala Ser Gly Ser
    50                  55                  60
Glu Pro Gln Gln Met Gly Ser Asp Val Arg Asp Leu Asn Ala Leu Leu
65                  70                  75                  80
Pro Ala Val Pro Ser Leu Gly Gly Gly Gly Gly Cys Ala Leu Pro Val
                85                  90                  95
Ser Gly Ala Ala Gln Trp Ala Pro Val Leu Asp Phe Ala Pro Pro Gly
            100                 105                 110
Ala Ser Ala Tyr Gly Ser Leu Gly Gly Pro Ala Pro Pro Pro Ala Pro
        115                 120                 125
Pro Pro Pro Pro Pro Pro Pro Pro His Ser Phe Ile Lys Gln Glu Pro
    130                 135                 140
Ser Trp Gly Gly Ala Glu Pro His Glu Glu Gln Cys Leu Ser Ala Phe
145                 150                 155                 160
Thr Val His Phe Ser Gly Gln Phe Thr Gly Thr Ala Gly Ala Cys Arg
                165                 170                 175
Tyr Gly Pro Phe Gly Pro Pro Pro Pro Ser Gln Ala Ser Ser Gly Gln
            180                 185                 190
Ala Arg Met Phe Pro Asn Ala Pro Tyr Leu Pro Ser Cys Leu Glu Ser
        195                 200                 205
Gln Pro Ala Ile Arg Asn Gln Gly Tyr Ser Thr Val Thr Phe Asp Gly
    210                 215                 220
Thr Pro Ser Tyr Gly His Thr Pro Ser His His Ala Ala Gln Phe Pro
225                 230                 235                 240
Asn His Ser Phe Lys His Glu Asp Pro Met Gly Gln Gln Gly Ser Leu
                245                 250                 255
Gly Glu Gln Gln Tyr Ser Val Pro Pro Pro Val Tyr Gly Cys His Thr
            260                 265                 270
Pro Thr Asp Ser Cys Thr Gly Ser Gln Ala Leu Leu Leu Arg Thr Pro
        275                 280                 285
Tyr Ser Ser Asp Asn Leu Tyr Gln Met Thr Ser Gln Leu Glu Cys Met
    290                 295                 300
Thr Trp Asn Gln Met Asn Leu Gly Ala Thr Leu Lys Gly Val Ala Ala
305                 310                 315                 320
Gly Ser Ser Ser Ser Val Lys Trp Thr Glu Gly Gln Ser Asn His Ser
                325                 330                 335
Thr Gly Tyr Glu Ser Asp Asn His Thr Thr Pro Ile Leu Cys Gly Ala
            340                 345                 350
Gln Tyr Arg Ile His Thr His Gly Val Phe Arg Gly Ile Gln Asp Val
        355                 360                 365
Arg Arg Val Pro Gly Val Ala Pro Thr Leu Val Arg Ser Ala Ser Glu
    370                 375                 380
Thr Ser Glu Lys Arg Pro Phe Met Cys Ala Tyr Pro Gly Cys Asn Lys
385                 390                 395                 400
Arg Tyr Phe Lys Leu Ser His Leu Gln Met His Ser Arg Lys His Thr
                405                 410                 415
Gly Glu Lys Pro Tyr Gln Cys Asp Phe Lys Asp Cys Glu Arg Arg Phe
            420                 425                 430
Ser Arg Ser Asp Gln Leu Lys Arg His Gln Arg Arg His Thr Gly Val
        435                 440                 445
Lys Pro Phe Gln Cys Lys Thr Cys Gln Arg Lys Phe Ser Arg Ser Asp
    450                 455                 460
His Leu Lys Thr His Thr Arg Thr His Thr Gly Glu Lys Pro Phe Ser
465                 470                 475                 480
Cys Arg Trp Pro Ser Cys Gln Lys Lys Phe Ala Arg Ser Asp Glu Leu
                485                 490                 495
Val Arg His His Asn Met His Gln Arg Asn Met Thr Lys Leu Gln Leu
            500                 505                 510
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<210> 15
<211> 3037
<212> DNA
<213> Homo sapiens

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cagagccggg acggcagccc aggcgcccgg gccccgccgt ctcctcgccg cgatcctgga 180
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<210> 16
<211> 517
<212> PRT
<213> Homo sapiens

<400> 16
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Thr Leu Arg Ser Gly Pro Gly Cys Leu Gln Gln Pro Glu Gln Gln Gly
            20   25   30
Val Arg Asp Pro Gly Gly Ile Trp Ala Lys Leu Gly Ala Ala Glu Ala
        35   40   45
Ser Ala Glu Arg Leu Gln Gly Arg Arg Ser Arg Gly Ala Ser Gly Ser
    50   55   60

Glu Pro Gln Gln Met Gly Ser Asp Val Arg Asp Leu Asn Ala Leu Leu
65 70 75 80
Pro Ala Val Pro Ser Leu Gly Gly Gly Gly Gly Cys Ala Leu Pro Val
85 90 95
Ser Gly Ala Ala Gln Trp Ala Pro Val Leu Asp Phe Ala Pro Pro Gly
100 105 110
Ala Ser Ala Tyr Gly Ser Leu Gly Gly Pro Ala Pro Pro Pro Ala Pro
115 120 125
Pro Pro Pro Pro Pro Pro Pro Pro His Ser Phe Ile Lys Gln Glu Pro
130 135 140
Ser Trp Gly Gly Ala Glu Pro His Glu Glu Gln Cys Leu Ser Ala Phe
145 150 155 160
Thr Val His Phe Ser Gly Gln Phe Thr Gly Thr Ala Gly Ala Cys Arg
165 170 175
Tyr Gly Pro Phe Gly Pro Pro Pro Pro Ser Gln Ala Ser Ser Gly Gln
180 185 190
Ala Arg Met Phe Pro Asn Ala Pro Tyr Leu Pro Ser Cys Leu Glu Ser
195 200 205
Gln Pro Ala Ile Arg Asn Gln Gly Tyr Ser Thr Val Thr Phe Asp Gly
210 215 220
Thr Pro Ser Tyr Gly His Thr Pro Ser His His Ala Ala Gln Phe Pro
225 230 235 240
Asn His Ser Phe Lys His Glu Asp Pro Met Gly Gln Gln Gly Ser Leu
245 250 255
Gly Glu Gln Gln Tyr Ser Val Pro Pro Pro Val Tyr Gly Cys His Thr
260 265 270
Pro Thr Asp Ser Cys Thr Gly Ser Gln Ala Leu Leu Leu Arg Thr Pro
275 280 285
Tyr Ser Ser Asp Asn Leu Tyr Gln Met Thr Ser Gln Leu Glu Cys Met
290 295 300
Thr Trp Asn Gln Met Asn Leu Gly Ala Thr Leu Lys Gly Val Ala Ala
305 310 315 320
Gly Ser Ser Ser Ser Val Lys Trp Thr Glu Gly Gln Ser Asn His Ser
325 330 335
Thr Gly Tyr Glu Ser Asp Asn His Thr Thr Pro Ile Leu Cys Gly Ala
340 345 350
Gln Tyr Arg Ile His Thr His Gly Val Phe Arg Gly Ile Gln Asp Val
355 360 365
Arg Arg Val Pro Gly Val Ala Pro Thr Leu Val Arg Ser Ala Ser Glu
370 375 380
Thr Ser Glu Lys Arg Pro Phe Met Cys Ala Tyr Pro Gly Cys Asn Lys
385 390 395 400
Arg Tyr Phe Lys Leu Ser His Leu Gln Met His Ser Arg Lys His Thr
405 410 415
Gly Glu Lys Pro Tyr Gln Cys Asp Phe Lys Asp Cys Glu Arg Arg Phe
420 425 430
Ser Arg Ser Asp Gln Leu Lys Arg His Gln Arg Arg His Thr Gly Val
435 440 445
Lys Pro Phe Gln Cys Lys Thr Cys Gln Arg Lys Phe Ser Arg Ser Asp
450 455 460
His Leu Lys Thr His Thr Arg Thr His Thr Gly Lys Thr Ser Glu Lys
465 470 475 480
Pro Phe Ser Cys Arg Trp Pro Ser Cys Gln Lys Lys Phe Ala Arg Ser
485 490 495
Asp Glu Leu Val Arg His His Asn Met His Gln Arg Asn Met Thr Lys
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Leu Gln Leu Ala Leu
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<210> 17
<211> 2438
<212> DNA
<213> Homo sapiens

<400> 17
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gggcatcctt gggcccgagt tctggggtgc ggacggacgt ctcgagagtg ggtgccgcga 180

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gccaggcttt gctgctgagg acgccctaca gcagtgacaa tttataccaa atgacatccc     540
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gctgcaataa gagatatttt aagctgtccc acttacagat gcacagcagg aagcacactg     840
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tctacaagta tatgtgaaaa aaatgagact tactgggtga ggaaatccat tgtttaaaga    2160
tggtcgtgtg tgtgtgtgtg tgtgtgtgtg tgtgtgtgtt gtgttgtgtt ttgtttttta    2220
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<210> 18
<211> 288
<212> PRT
<213> Homo sapiens

<400> 18

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            20                  25                  30
Lys His Glu Asp Pro Met Gly Gln Gln Gly Ser Leu Gly Glu Gln Gln
        35                  40                  45
Tyr Ser Val Pro Pro Pro Val Tyr Gly Cys His Thr Pro Thr Asp Ser
    50                  55                  60
Cys Thr Gly Ser Gln Ala Leu Leu Leu Arg Thr Pro Tyr Ser Ser Asp
65                  70                  75                  80
Asn Leu Tyr Gln Met Thr Ser Gln Leu Glu Cys Met Thr Trp Asn Gln
                85                  90                  95
Met Asn Leu Gly Ala Thr Leu Lys Gly His Ser Thr Gly Tyr Glu Ser
            100                 105                 110
Asp Asn His Thr Thr Pro Ile Leu Cys Gly Ala Gln Tyr Arg Ile His
        115                 120                 125
Thr His Gly Val Phe Arg Gly Ile Gln Asp Val Arg Arg Val Pro Gly
    130                 135                 140
Val Ala Pro Thr Leu Val Arg Ser Ala Ser Glu Thr Ser Glu Lys Arg
145                 150                 155                 160
Pro Phe Met Cys Ala Tyr Pro Gly Cys Asn Lys Arg Tyr Phe Lys Leu
                165                 170                 175
Ser His Leu Gln Met His Ser Arg Lys His Thr Gly Glu Lys Pro Tyr
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            180                 185                 190
Gln Cys Asp Phe Lys Asp Cys Glu Arg Arg Phe Ser Arg Ser Asp Gln
        195                 200                 205
Leu Lys Arg His Gln Arg Arg His Thr Gly Val Lys Pro Phe Gln Cys
    210                 215                 220
Lys Thr Cys Gln Arg Lys Phe Ser Arg Ser Asp His Leu Lys Thr His
225                 230                 235                 240
Thr Arg Thr His Thr Gly Lys Thr Ser Glu Lys Pro Phe Ser Cys Arg
                245                 250                 255
Trp Pro Ser Cys Gln Lys Lys Phe Ala Arg Ser Asp Glu Leu Val Arg
            260                 265                 270
His His Asn Met His Gln Arg Asn Met Thr Lys Leu Gln Leu Ala Leu
        275                 280                 285
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<210> 19
<211> 2480
<212> DNA
<213> Homo sapiens

<400> 19

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<210> 20
<211> 302
<212> PRT
<213> Homo sapiens

<400> 20

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            20                  25                  30
Lys His Glu Asp Pro Met Gly Gln Gln Gly Ser Leu Gly Glu Gln Gln
        35                  40                  45
Tyr Ser Val Pro Pro Pro Val Tyr Gly Cys His Thr Pro Thr Asp Ser
    50                  55                  60
Cys Thr Gly Ser Gln Ala Leu Leu Leu Arg Thr Pro Tyr Ser Ser Asp
65                  70                  75                  80
Asn Leu Tyr Gln Met Thr Ser Gln Leu Glu Cys Met Thr Trp Asn Gln
                85                  90                  95
Met Asn Leu Gly Ala Thr Leu Lys Gly Val Ala Ala Gly Ser Ser Ser
            100                 105                 110
Ser Val Lys Trp Thr Glu Gly Gln Ser Asn His Ser Thr Gly Tyr Glu
        115                 120                 125
Ser Asp Asn His Thr Thr Pro Ile Leu Cys Gly Ala Gln Tyr Arg Ile
    130                 135                 140
His Thr His Gly Val Phe Arg Gly Ile Gln Asp Val Arg Arg Val Pro
145                 150                 155                 160
Gly Val Ala Pro Thr Leu Val Arg Ser Ala Ser Glu Thr Ser Glu Lys
                165                 170                 175
Arg Pro Phe Met Cys Ala Tyr Pro Gly Cys Asn Lys Arg Tyr Phe Lys
            180                 185                 190
Leu Ser His Leu Gln Met His Ser Arg Lys His Thr Gly Glu Lys Pro
        195                 200                 205
Tyr Gln Cys Asp Phe Lys Asp Cys Glu Arg Arg Phe Ser Arg Ser Asp
    210                 215                 220
Gln Leu Lys Arg His Gln Arg Arg His Thr Gly Val Lys Pro Phe Gln
225                 230                 235                 240
Cys Lys Thr Cys Gln Arg Lys Phe Ser Arg Ser Asp His Leu Lys Thr
                245                 250                 255
His Thr Arg Thr His Thr Gly Glu Lys Pro Phe Ser Cys Arg Trp Pro
            260                 265                 270
Ser Cys Gln Lys Lys Phe Ala Arg Ser Asp Glu Leu Val Arg His His
        275                 280                 285
Asn Met His Gln Arg Asn Met Thr Lys Leu Gln Leu Ala Leu
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<210> 21
<211> 3092
<212> DNA
<213> Mus musculus

<400> 21

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tccgggcgcc tcggcttacg ggtcgctggg cggtcccgcg cctcctcccg ctccgccgcc     660
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acacgactgt ggatctacat ccgacttcca agacagcaca cctgattgac tgcatcctat    1980
caggtttgcc ggaaggagtc ggtgctccgc ccacttttga ttaactcaca ggcctgaaaa    2040
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<210> 22
<211> 517
<212> PRT
<213> Mus musculus

<400> 22

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            20                  25                  30
Pro Glu Gln Pro Gly Asp Arg Gly Pro Arg Ser Ala Trp Ala Lys Ser
        35                  40                  45
Ser Ala Glu Asn Pro Gln Asp Arg Arg Ser Gly Glu Pro Ser Ala Ser
    50                  55                  60
Glu Pro His Leu Met Gly Ser Asp Val Arg Asp Leu Asn Ala Leu Leu
65                  70                  75                  80
Pro Ala Val Ser Ser Leu Gly Gly Gly Gly Gly Gly Cys Gly Leu Pro
                85                  90                  95
Val Ser Gly Ala Ala Gln Trp Ala Pro Val Leu Asp Phe Ala Pro Pro
            100                 105                 110
Gly Ala Ser Ala Tyr Gly Ser Leu Gly Gly Pro Ala Pro Pro Pro Ala
        115                 120                 125
Pro Pro Pro Pro Pro Pro Pro Pro His Ser Phe Ile Lys Gln Glu Pro
    130                 135                 140
Ser Trp Gly Gly Ala Glu Pro His Glu Glu Gln Cys Leu Ser Ala Phe
145                 150                 155                 160
Thr Leu His Phe Ser Gly Gln Phe Thr Gly Thr Ala Gly Ala Cys Arg
                165                 170                 175
Tyr Gly Pro Phe Gly Pro Pro Pro Pro Ser Gln Ala Ser Ser Gly Gln
            180                 185                 190
Ala Arg Met Phe Pro Asn Ala Pro Tyr Leu Pro Ser Cys Leu Glu Ser
        195                 200                 205
Gln Pro Thr Ile Arg Asn Gln Gly Tyr Ser Thr Val Thr Phe Asp Gly
    210                 215                 220
Ala Pro Ser Tyr Gly His Thr Pro Ser His His Ala Ala Gln Phe Pro
225                 230                 235                 240
Asn His Ser Phe Lys His Glu Asp Pro Met Gly Gln Gln Gly Ser Leu
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                245                 250                 255
Gly Glu Gln Gln Tyr Ser Val Pro Pro Pro Val Tyr Gly Cys His Thr
            260                 265                 270
Pro Thr Asp Ser Cys Thr Gly Ser Gln Ala Leu Leu Leu Arg Thr Pro
        275                 280                 285
Tyr Ser Ser Asp Asn Leu Tyr Gln Met Thr Ser Gln Leu Glu Cys Met
    290                 295                 300
Thr Trp Asn Gln Met Asn Leu Gly Ala Thr Leu Lys Gly Met Ala Ala
305                 310                 315                 320
Gly Ser Ser Ser Ser Val Lys Trp Thr Glu Gly Gln Ser Asn His Gly
                325                 330                 335
Thr Gly Tyr Glu Ser Glu Asn His Thr Ala Pro Ile Leu Cys Gly Ala
            340                 345                 350
Gln Tyr Arg Ile His Thr His Gly Val Phe Arg Gly Ile Gln Asp Val
        355                 360                 365
Arg Arg Val Ser Gly Val Ala Pro Thr Leu Val Arg Ser Ala Ser Glu
    370                 375                 380
Thr Ser Glu Lys Arg Pro Phe Met Cys Ala Tyr Pro Gly Cys Asn Lys
385                 390                 395                 400
Arg Tyr Phe Lys Leu Ser His Leu Gln Met His Ser Arg Lys His Thr
                405                 410                 415
Gly Glu Lys Pro Tyr Gln Cys Asp Phe Lys Asp Cys Glu Arg Arg Phe
            420                 425                 430
Ser Arg Ser Asp Gln Leu Lys Arg His Gln Arg Arg His Thr Gly Val
        435                 440                 445
Lys Pro Phe Gln Cys Lys Thr Cys Gln Arg Lys Phe Ser Arg Ser Asp
    450                 455                 460
His Leu Lys Thr His Thr Arg Thr His Thr Gly Lys Thr Ser Glu Lys
465                 470                 475                 480
Pro Phe Ser Cys Arg Trp His Ser Cys Gln Lys Lys Phe Ala Arg Ser
                485                 490                 495
Asp Glu Leu Val Arg His His Asn Met His Gln Arg Asn Met Thr Lys
            500                 505                 510
Leu Gln Leu Ala Leu
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<210> 23
<211> 2126
<212> DNA
<213> Homo sapiens

<400> 23

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agacacgtaa aaaaaaaaaa aaaaaa 2126

<210> 24
<211> 622
<212> PRT
<213> Homo sapiens

<400> 24
Met Ala Leu Pro Thr Ala Arg Pro Leu Leu Gly Ser Cys Gly Thr Pro
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Ala Leu Gly Ser Leu Leu Phe Leu Leu Phe Ser Leu Gly Trp Val Gln
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Pro Ser Arg Thr Leu Ala Gly Glu Thr Gly Gln Glu Ala Ala Pro Leu
        35   40   45
Asp Gly Val Leu Ala Asn Pro Pro Asn Ile Ser Ser Leu Ser Pro Arg
    50   55   60
Gln Leu Leu Gly Phe Pro Cys Ala Glu Val Ser Gly Leu Ser Thr Glu
65   70   75   80
Arg Val Arg Glu Leu Ala Val Ala Leu Ala Gln Lys Asn Val Lys Leu
                85   90   95
Ser Thr Glu Gln Leu Arg Cys Leu Ala His Arg Leu Ser Glu Pro Pro
            100   105   110
Glu Asp Leu Asp Ala Leu Pro Leu Asp Leu Leu Leu Phe Leu Asn Pro
        115   120   125
Asp Ala Phe Ser Gly Pro Gln Ala Cys Thr Arg Phe Phe Ser Arg Ile
    130   135   140
Thr Lys Ala Asn Val Asp Leu Leu Pro Arg Gly Ala Pro Glu Arg Gln
145   150   155   160
Arg Leu Leu Pro Ala Ala Leu Ala Cys Trp Gly Val Arg Gly Ser Leu
                165   170   175
Leu Ser Glu Ala Asp Val Arg Ala Leu Gly Gly Leu Ala Cys Asp Leu
            180   185   190
Pro Gly Arg Phe Val Ala Glu Ser Ala Glu Val Leu Leu Pro Arg Leu
        195   200   205
Val Ser Cys Pro Gly Pro Leu Asp Gln Asp Gln Gln Glu Ala Ala Arg
    210   215   220
Ala Ala Leu Gln Gly Gly Gly Pro Pro Tyr Gly Pro Pro Ser Thr Trp
225   230   235   240
Ser Val Ser Thr Met Asp Ala Leu Arg Gly Leu Leu Pro Val Leu Gly
                245   250   255
Gln Pro Ile Ile Arg Ser Ile Pro Gln Gly Ile Val Ala Ala Trp Arg
            260   265   270
Gln Arg Ser Ser Arg Asp Pro Ser Trp Arg Gln Pro Glu Arg Thr Ile
        275   280   285
Leu Arg Pro Arg Phe Arg Arg Glu Val Glu Lys Thr Ala Cys Pro Ser
    290   295   300
Gly Lys Lys Ala Arg Glu Ile Asp Glu Ser Leu Ile Phe Tyr Lys Lys
305   310   315   320
Trp Glu Leu Glu Ala Cys Val Asp Ala Ala Leu Leu Ala Thr Gln Met
                325   330   335
Asp Arg Val Asn Ala Ile Pro Phe Thr Tyr Glu Gln Leu Asp Val Leu
            340   345   350
Lys His Lys Leu Asp Glu Leu Tyr Pro Gln Gly Tyr Pro Glu Ser Val
        355   360   365
Ile Gln His Leu Gly Tyr Leu Phe Leu Lys Met Ser Pro Glu Asp Ile
    370   375   380
Arg Lys Trp Asn Val Thr Ser Leu Glu Thr Leu Lys Ala Leu Leu Glu
385   390   395   400
Val Asn Lys Gly His Glu Met Ser Pro Gln Val Ala Thr Leu Ile Asp

405 410 415
Arg Phe Val Lys Gly Arg Gly Gln Leu Asp Lys Asp Thr Leu Asp Thr
420 425 430
Leu Thr Ala Phe Tyr Pro Gly Tyr Leu Cys Ser Leu Ser Pro Glu Glu
435 440 445
Leu Ser Ser Val Pro Pro Ser Ser Ile Trp Ala Val Arg Pro Gln Asp
450 455 460
Leu Asp Thr Cys Asp Pro Arg Gln Leu Asp Val Leu Tyr Pro Lys Ala
465 470 475 480
Arg Leu Ala Phe Gln Asn Met Asn Gly Ser Glu Tyr Phe Val Lys Ile
485 490 495
Gln Ser Phe Leu Gly Gly Ala Pro Thr Glu Asp Leu Lys Ala Leu Ser
500 505 510
Gln Gln Asn Val Ser Met Asp Leu Ala Thr Phe Met Lys Leu Arg Thr
515 520 525
Asp Ala Val Leu Pro Leu Thr Val Ala Glu Val Gln Lys Leu Leu Gly
530 535 540
Pro His Val Glu Gly Leu Lys Ala Glu Glu Arg His Arg Pro Val Arg
545 550 555 560
Asp Trp Ile Leu Arg Gln Arg Gln Asp Asp Leu Asp Thr Leu Gly Leu
565 570 575
Gly Leu Gln Gly Gly Ile Pro Asn Gly Tyr Leu Val Leu Asp Leu Ser
580 585 590
Met Gln Glu Ala Leu Ser Gly Thr Pro Cys Leu Leu Gly Pro Gly Pro
595 600 605
Val Leu Thr Val Leu Ala Leu Leu Leu Ala Ser Thr Leu Ala
610 615 620

<210> 25
<211> 2187
<212> DNA
<213> Homo sapiens

<400> 25
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cagacacgta aaaaaaaaaa aaaaaaa 2187

<210> 26
<211> 622
<212> PRT
<213> Homo sapiens

<400> 26
Met Ala Leu Pro Thr Ala Arg Pro Leu Leu Gly Ser Cys Gly Thr Pro
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Ala Leu Gly Ser Leu Leu Phe Leu Leu Phe Ser Leu Gly Trp Val Gln
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Pro Ser Arg Thr Leu Ala Gly Glu Thr Gly Gln Glu Ala Ala Pro Leu
35 40 45
Asp Gly Val Leu Ala Asn Pro Pro Asn Ile Ser Ser Leu Ser Pro Arg
50 55 60
Gln Leu Leu Gly Phe Pro Cys Ala Glu Val Ser Gly Leu Ser Thr Glu
65 70 75 80
Arg Val Arg Glu Leu Ala Val Ala Leu Ala Gln Lys Asn Val Lys Leu
85 90 95
Ser Thr Glu Gln Leu Arg Cys Leu Ala His Arg Leu Ser Glu Pro Pro
100 105 110
Glu Asp Leu Asp Ala Leu Pro Leu Asp Leu Leu Leu Phe Leu Asn Pro
115 120 125
Asp Ala Phe Ser Gly Pro Gln Ala Cys Thr Arg Phe Phe Ser Arg Ile
130 135 140
Thr Lys Ala Asn Val Asp Leu Leu Pro Arg Gly Ala Pro Glu Arg Gln
145 150 155 160
Arg Leu Leu Pro Ala Ala Leu Ala Cys Trp Gly Val Arg Gly Ser Leu
165 170 175
Leu Ser Glu Ala Asp Val Arg Ala Leu Gly Gly Leu Ala Cys Asp Leu
180 185 190
Pro Gly Arg Phe Val Ala Glu Ser Ala Glu Val Leu Leu Pro Arg Leu
195 200 205
Val Ser Cys Pro Gly Pro Leu Asp Gln Asp Gln Gln Glu Ala Ala Arg
210 215 220
Ala Ala Leu Gln Gly Gly Gly Pro Pro Tyr Gly Pro Pro Ser Thr Trp
225 230 235 240
Ser Val Ser Thr Met Asp Ala Leu Arg Gly Leu Leu Pro Val Leu Gly
245 250 255
Gln Pro Ile Ile Arg Ser Ile Pro Gln Gly Ile Val Ala Ala Trp Arg
260 265 270
Gln Arg Ser Ser Arg Asp Pro Ser Trp Arg Gln Pro Glu Arg Thr Ile
275 280 285
Leu Arg Pro Arg Phe Arg Arg Glu Val Glu Lys Thr Ala Cys Pro Ser
290 295 300
Gly Lys Lys Ala Arg Glu Ile Asp Glu Ser Leu Ile Phe Tyr Lys Lys
305 310 315 320
Trp Glu Leu Glu Ala Cys Val Asp Ala Ala Leu Leu Ala Thr Gln Met
325 330 335
Asp Arg Val Asn Ala Ile Pro Phe Thr Tyr Glu Gln Leu Asp Val Leu
340 345 350
Lys His Lys Leu Asp Glu Leu Tyr Pro Gln Gly Tyr Pro Glu Ser Val
355 360 365
Ile Gln His Leu Gly Tyr Leu Phe Leu Lys Met Ser Pro Glu Asp Ile
370 375 380
Arg Lys Trp Asn Val Thr Ser Leu Glu Thr Leu Lys Ala Leu Leu Glu
385 390 395 400
Val Asn Lys Gly His Glu Met Ser Pro Gln Val Ala Thr Leu Ile Asp
405 410 415
Arg Phe Val Lys Gly Arg Gly Gln Leu Asp Lys Asp Thr Leu Asp Thr
420 425 430
Leu Thr Ala Phe Tyr Pro Gly Tyr Leu Cys Ser Leu Ser Pro Glu Glu
435 440 445
Leu Ser Ser Val Pro Pro Ser Ser Ile Trp Ala Val Arg Pro Gln Asp
450 455 460

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Leu Asp Thr Cys Asp Pro Arg Gln Leu Asp Val Leu Tyr Pro Lys Ala
465                 470                 475                 480
Arg Leu Ala Phe Gln Asn Met Asn Gly Ser Glu Tyr Phe Val Lys Ile
                485                 490                 495
Gln Ser Phe Leu Gly Gly Ala Pro Thr Glu Asp Leu Lys Ala Leu Ser
            500                 505                 510
Gln Gln Asn Val Ser Met Asp Leu Ala Thr Phe Met Lys Leu Arg Thr
        515                 520                 525
Asp Ala Val Leu Pro Leu Thr Val Ala Glu Val Gln Lys Leu Leu Gly
    530                 535                 540
Pro His Val Glu Gly Leu Lys Ala Glu Glu Arg His Arg Pro Val Arg
545                 550                 555                 560
Asp Trp Ile Leu Arg Gln Arg Gln Asp Asp Leu Asp Thr Leu Gly Leu
                565                 570                 575
Gly Leu Gln Gly Gly Ile Pro Asn Gly Tyr Leu Val Leu Asp Leu Ser
            580                 585                 590
Met Gln Glu Ala Leu Ser Gly Thr Pro Cys Leu Leu Gly Pro Gly Pro
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Val Leu Thr Val Leu Ala Leu Leu Leu Ala Ser Thr Leu Ala
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<210> 27
<211> 2154
<212> DNA
<213> Homo sapiens

<400> 27

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ccaacggctc gacccctgtt ggggtcctgt gggacccccg ccctcggcag cctcctgttc     180
ctgctcttca gcctcggatg ggtgcagccc tcgaggaccc tggctggaga gacagggcag     240
gaggctgcgc ccctggacgg agtcctggcc aacccaccta acatttccag cctctccccc     300
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<210> 28
<211> 630
<212> PRT
<213> Homo sapiens

<400> 28
Met Ala Leu Pro Thr Ala Arg Pro Leu Leu Gly Ser Cys Gly Thr Pro
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Ala Leu Gly Ser Leu Leu Phe Leu Leu Phe Ser Leu Gly Trp Val Gln
            20   25   30
Pro Ser Arg Thr Leu Ala Gly Glu Thr Gly Gln Glu Ala Ala Pro Leu
        35   40   45
Asp Gly Val Leu Ala Asn Pro Pro Asn Ile Ser Ser Leu Ser Pro Arg
    50   55   60
Gln Leu Leu Gly Phe Pro Cys Ala Glu Val Ser Gly Leu Ser Thr Glu
65   70   75   80
Arg Val Arg Glu Leu Ala Val Ala Leu Ala Gln Lys Asn Val Lys Leu
                85   90   95
Ser Thr Glu Gln Leu Arg Cys Leu Ala His Arg Leu Ser Glu Pro Pro
            100   105   110
Glu Asp Leu Asp Ala Leu Pro Leu Asp Leu Leu Leu Phe Leu Asn Pro
        115   120   125
Asp Ala Phe Ser Gly Pro Gln Ala Cys Thr Arg Phe Phe Ser Arg Ile
    130   135   140
Thr Lys Ala Asn Val Asp Leu Leu Pro Arg Gly Ala Pro Glu Arg Gln
145   150   155   160
Arg Leu Leu Pro Ala Ala Leu Ala Cys Trp Gly Val Arg Gly Ser Leu
                165   170   175
Leu Ser Glu Ala Asp Val Arg Ala Leu Gly Gly Leu Ala Cys Asp Leu
            180   185   190
Pro Gly Arg Phe Val Ala Glu Ser Ala Glu Val Leu Leu Pro Arg Leu
        195   200   205
Val Ser Cys Pro Gly Pro Leu Asp Gln Asp Gln Gln Glu Ala Ala Arg
    210   215   220
Ala Ala Leu Gln Gly Gly Gly Pro Pro Tyr Gly Pro Pro Ser Thr Trp
225   230   235   240
Ser Val Ser Thr Met Asp Ala Leu Arg Gly Leu Leu Pro Val Leu Gly
                245   250   255
Gln Pro Ile Ile Arg Ser Ile Pro Gln Gly Ile Val Ala Ala Trp Arg
            260   265   270
Gln Arg Ser Ser Arg Asp Pro Ser Trp Arg Gln Pro Glu Arg Thr Ile
        275   280   285
Leu Arg Pro Arg Phe Arg Arg Glu Val Glu Lys Thr Ala Cys Pro Ser
    290   295   300
Gly Lys Lys Ala Arg Glu Ile Asp Glu Ser Leu Ile Phe Tyr Lys Lys
305   310   315   320
Trp Glu Leu Glu Ala Cys Val Asp Ala Ala Leu Leu Ala Thr Gln Met
                325   330   335
Asp Arg Val Asn Ala Ile Pro Phe Thr Tyr Glu Gln Leu Asp Val Leu
            340   345   350
Lys His Lys Leu Asp Glu Leu Tyr Pro Gln Gly Tyr Pro Glu Ser Val
        355   360   365
Ile Gln His Leu Gly Tyr Leu Phe Leu Lys Met Ser Pro Glu Asp Ile
    370   375   380
Arg Lys Trp Asn Val Thr Ser Leu Glu Thr Leu Lys Ala Leu Leu Glu
385   390   395   400
Val Asn Lys Gly His Glu Met Ser Pro Gln Ala Pro Arg Arg Pro Leu
                405   410   415
Pro Gln Val Ala Thr Leu Ile Asp Arg Phe Val Lys Gly Arg Gly Gln
            420   425   430
Leu Asp Lys Asp Thr Leu Asp Thr Leu Thr Ala Phe Tyr Pro Gly Tyr
        435   440   445
Leu Cys Ser Leu Ser Pro Glu Glu Leu Ser Ser Val Pro Pro Ser Ser
    450   455   460
Ile Trp Ala Val Arg Pro Gln Asp Leu Asp Thr Cys Asp Pro Arg Gln
465   470   475   480
Leu Asp Val Leu Tyr Pro Lys Ala Arg Leu Ala Phe Gln Asn Met Asn
                485   490   495
Gly Ser Glu Tyr Phe Val Lys Ile Gln Ser Phe Leu Gly Gly Ala Pro
            500   505   510
Thr Glu Asp Leu Lys Ala Leu Ser Gln Gln Asn Val Ser Met Asp Leu
        515   520   525

Ala Thr Phe Met Lys Leu Arg Thr Asp Ala Val Leu Pro Leu Thr Val
530 535 540
Ala Glu Val Gln Lys Leu Leu Gly Pro His Val Glu Gly Leu Lys Ala
545 550 555 560
Glu Glu Arg His Arg Pro Val Arg Asp Trp Ile Leu Arg Gln Arg Gln
565 570 575
Asp Asp Leu Asp Thr Leu Gly Leu Gly Leu Gln Gly Gly Ile Pro Asn
580 585 590
Gly Tyr Leu Val Leu Asp Leu Ser Met Gln Glu Ala Leu Ser Gly Thr
595 600 605
Pro Cys Leu Leu Gly Pro Gly Pro Val Leu Thr Val Leu Ala Leu Leu
610 615 620
Leu Ala Ser Thr Leu Ala
625 630

<210> 29
<211> 2135
<212> DNA
<213> Mus musculus

<400> 29
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ggagtgggta ccaggggtca ttgccaaagt ttgaggactc ttgaactcaa taaacagtgg 2100
catatgctcc cttgaaaaaa aaaaaaaaaa aaaaa 2135

<210> 30
<211> 625
<212> PRT
<213> Mus musculus

<400> 30
Met Ala Leu Pro Thr Ala Arg Pro Leu Leu Gly Ser Cys Gly Ser Pro
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Ile Cys Ser Arg Ser Phe Leu Leu Leu Leu Leu Ser Leu Gly Trp Ile
20 25 30

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Pro Arg Leu Gln Thr Gln Thr Thr Lys Thr Ser Gln Glu Ala Thr Leu
        35                  40                  45
Leu His Ala Val Asn Gly Ala Ala Asp Phe Ala Ser Leu Pro Thr Gly
    50                  55                  60
Leu Phe Leu Gly Leu Thr Cys Glu Glu Val Ser Asp Leu Ser Met Glu
65                  70                  75                  80
Gln Ala Lys Gly Leu Ala Met Ala Val Arg Gln Lys Asn Ile Thr Leu
                85                  90                  95
Arg Gly His Gln Leu Arg Cys Leu Ala Arg Arg Leu Pro Arg His Leu
            100                 105                 110
Thr Asp Glu Glu Leu Asn Ala Leu Pro Leu Asp Leu Leu Leu Phe Leu
        115                 120                 125
Asn Pro Ala Met Phe Pro Gly Gln Gln Ala Cys Ala His Phe Phe Ser
    130                 135                 140
Leu Ile Ser Lys Ala Asn Val Asp Val Leu Pro Arg Arg Ser Leu Glu
145                 150                 155                 160
Arg Gln Arg Leu Leu Met Glu Ala Leu Lys Cys Gln Gly Val Tyr Gly
                165                 170                 175
Phe Gln Val Ser Glu Ala Asp Val Arg Ala Leu Gly Gly Leu Ala Cys
            180                 185                 190
Asp Leu Pro Gly Lys Phe Val Ala Arg Ser Ser Glu Val Leu Leu Pro
        195                 200                 205
Trp Leu Ala Gly Cys Gln Gly Pro Leu Asp Gln Ser Gln Glu Lys Ala
    210                 215                 220
Val Arg Glu Val Leu Arg Ser Gly Arg Thr Gln Tyr Gly Pro Pro Ser
225                 230                 235                 240
Lys Trp Ser Val Ser Thr Leu Asp Ala Leu Gln Ser Leu Val Ala Val
                245                 250                 255
Leu Asp Glu Ser Ile Val Gln Ser Ile Pro Lys Asp Val Lys Ala Glu
            260                 265                 270
Trp Leu Gln His Ile Ser Arg Asp Pro Ser Arg Leu Gly Ser Lys Leu
        275                 280                 285
Thr Val Ile His Pro Arg Phe Arg Arg Asp Ala Glu Gln Lys Ala Cys
    290                 295                 300
Pro Pro Gly Lys Glu Pro Tyr Lys Val Asp Glu Asp Leu Ile Phe Tyr
305                 310                 315                 320
Gln Asn Trp Glu Leu Glu Ala Cys Val Asp Gly Thr Met Leu Ala Arg
                325                 330                 335
Gln Met Asp Leu Val Asn Glu Ile Pro Phe Thr Tyr Glu Gln Leu Ser
            340                 345                 350
Ile Phe Lys His Lys Leu Asp Lys Thr Tyr Pro Gln Gly Tyr Pro Glu
        355                 360                 365
Ser Leu Ile Gln Gln Leu Gly His Phe Phe Arg Tyr Val Ser Pro Glu
    370                 375                 380
Asp Ile His Gln Trp Asn Val Thr Ser Pro Asp Thr Val Lys Thr Leu
385                 390                 395                 400
Leu Lys Val Ser Lys Gly Gln Lys Met Asn Ala Gln Ala Ile Ala Leu
                405                 410                 415
Val Ala Cys Tyr Leu Arg Gly Gly Gly Gln Leu Asp Glu Asp Met Val
            420                 425                 430
Lys Ala Leu Gly Asp Ile Pro Leu Ser Tyr Leu Cys Asp Phe Ser Pro
        435                 440                 445
Gln Asp Leu His Ser Val Pro Ser Ser Val Met Trp Leu Val Gly Pro
    450                 455                 460
Gln Asp Leu Asp Lys Cys Ser Gln Arg His Leu Gly Leu Leu Tyr Gln
465                 470                 475                 480
Lys Ala Cys Ser Ala Phe Gln Asn Val Ser Gly Leu Glu Tyr Phe Glu
                485                 490                 495
Lys Ile Lys Thr Phe Leu Gly Gly Ala Ser Val Lys Asp Leu Arg Ala
            500                 505                 510
Leu Ser Gln His Asn Val Ser Met Asp Ile Ala Thr Phe Lys Arg Leu
        515                 520                 525
Gln Val Asp Ser Leu Val Gly Leu Ser Val Ala Glu Val Gln Lys Leu
    530                 535                 540
Leu Gly Pro Asn Ile Val Asp Leu Lys Thr Glu Glu Asp Lys Ser Pro
545                 550                 555                 560
Val Arg Asp Trp Leu Phe Arg Gln His Gln Lys Asp Leu Asp Arg Leu
                565                 570                 575
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Gly Leu Gly Leu Gln Gly Gly Ile Pro Asn Gly Tyr Leu Val Leu Asp
580 585 590
Phe Asn Val Arg Glu Ala Phe Ser Ser Arg Ala Ser Leu Leu Gly Pro
595 600 605
Gly Phe Val Leu Ile Trp Ile Pro Ala Leu Leu Pro Ala Leu Arg Leu
610 615 620
Ser
625

<210> 31
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic mesothelin peptide

<400> 31
Gly Gln Lys Met Asn Ala Gln Ala Ile
1 5

<210> 32
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> 3D beta peptide clone from Mus musculus

<400> 32
Cys Ala Ser Ser Pro Gly Leu Gly Gly Ser Tyr Glu Gln Tyr Phe
1 5 10 15

<210> 33
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> 3D beta nucleotide clone from Mus musculus

<400> 33
tgtgccagca gccctggact ggggggatcc tatgaacagt acttc 45

<210> 34
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> V beta 10 clone #1 peptide clone from Mus musculus

<400> 34
Cys Ala Ser Ser Gln Gly Leu Gly Ser Ser Tyr Glu Gln Tyr Phe
1 5 10 15

<210> 35
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> V beta 10 clone #1 nucleotide clone from Mus

musculus

<400> 35
tgtgccagca gccagggact ggggagctcc tatgaacagt acttc 45

<210> 36
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> V beta 10 clone #2 peptide from Mus musculus

<400> 36
Cys Ala Ser Ser Tyr Ile Leu Gly Ala Tyr Glu Gln Tyr Phe
1 5 10

<210> 37
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> V beta 10 clone #2 nucleotide from Mus musculus

<400> 37
tgtgccagca gctatatact gggggcctat gaacagtact tc 42

<210> 38
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> V beta 10 clone #3 peptide from Mus musculus

<400> 38
Cys Ala Ser Ser Ser Trp Thr Val Tyr Glu Gln Tyr Phe
1 5 10

<210> 39
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> V beta 10 clone #3 nucleotide from Mus musculus

<400> 39
tgtgccagca gctcctggac agtctatgaa cagtacttc 39

<210> 40
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> V beta 10 clone #4 peptide from Mus musculus

<400> 40
Cys Ala Ser Ser Trp Thr Gly Ala Asn Thr Gly Gln Leu Tyr Phe
1 5 10 15

<210> 41
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> V beta 10 clone #4 nucleotide from Mus musculus

<400> 41
tgtgccagca gctggacagg ggcaaacacc gggcagctct acttt 45

<210> 42
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic mesothelin peptide

<400> 42
Ile Ser Lys Ala Asn Val Asp Val Leu
1 5

<210> 43
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic mesothelin peptide

<400> 43
Gly Gln Lys Met Asn Ala Gln Ala Ile
1 5

<210> 44
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic mesothelin peptide

<400> 44
Ser Ala Phe Gln Asn Val Ser Gly Leu
1 5

<210> 45
<211> 9
<212> PRT
<213> Artificial Sequence

<220>

<223> synthetic mesothelin peptide

<400> 45
Leu Leu Gly Pro Asn Ile Val Asp Leu
 1   5

<210> 46
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic mesothelin peptide

<400> 46
Glu Ile Pro Phe Thr Tyr Glu Gln Leu
 1   5

<210> 47
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic mesothelin peptide

<400> 47
Gly Ile Pro Asn Gly Tyr Leu Val Leu
 1   5

<210> 48
<211> 1595
<212> DNA
<213> Homo sapiens

<400> 48
agaatcaaaa gaggaaacca acccctaaga tgagctttcc atgtaaattt gtagccagct 60
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tggaaacctg gggtgccttg ggtcaggaca tcaacttgga cattcctagt tttcaaatga 180
gtgatgatat tgacgatata aaatgggaaa aaacttcaga caagaaaaag attgcacaat 240
tcagaaaaga gaaagagact ttcaaggaaa aagatacata taagctattt aaaaatggaa 300
ctctgaaaat taagcatctg aagaccgatg atcaggatat ctacaaggta tcaatatatg 360
atacaaaagg aaaaaatgtg ttggaaaaaa tatttgattt gaagattcaa gagagggtct 420
caaaaccaaa gatctcctgg acttgtatca acacaaccct gacctgtgag gtaatgaatg 480
gaactgaccc cgaattaaac ctgtatcaag atgggaaaca tctaaaactt tctcagaggg 540
tcatcacaca caagtggacc accagcctga gtgcaaaatt caagtgcaca gcagggaaca 600
aagtcagcaa ggaatccagt gtcgagcctg tcagctgtcc agagaaaggt ctggacatct 660
atctcatcat tggcatatgt ggaggaggca gcctcttgat ggtctttgtg gcactgctcg 720
ttttctatat caccaaaagg aaaaaacaga ggagtcggag aaatgatgag gagctggaga 780
caagagccca cagagtagct actgaagaaa ggggccggaa gccccaccaa attccagctt 840
caaccctca gaatccagca acttcccaac atcctcctcc accacctggt catcgttccc 900
aggcacctag tcatcgtccc ccgcctcctg gacaccgtgt tcagcaccag cctcagaaga 960
ggcctcctgc tccgtcgggc acacaagttc accagcagaa aggcccgccc ctccccagac 1020
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attaaaaaag atagaaactg tctttttcaa taaaaagcac tgtggatttc tgccctcctg 1140
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tttttggtct cctcagagag ctccatcaca ccagtaagga gaagcaatat aagtgtgatt 1320
gcaagaatgg tagaggaccg agcacagaaa tcttagagat ttcttgtccc ctctcaggtc 1380
atgtgtagat gcgataaatc aagtgattgg tgtgcctggg tctcactaca agcagcctat 1440
ctgcttaaga gactctggag tttcttatgt gccctggtgg acacttgccc accatcctgt 1500
gagtaaaagt gaaataaaag ctttgactag aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1560

aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaa 1595

<210> 49
<211> 351
<212> PRT
<213> Homo sapiens

<400> 49
Met Ser Phe Pro Cys Lys Phe Val Ala Ser Phe Leu Leu Ile Phe Asn
1 5 10 15
Val Ser Ser Lys Gly Ala Val Ser Lys Glu Ile Thr Asn Ala Leu Glu
20 25 30
Thr Trp Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp Ile Pro Ser Phe
35 40 45
Gln Met Ser Asp Asp Ile Asp Asp Ile Lys Trp Glu Lys Thr Ser Asp
50 55 60
Lys Lys Lys Ile Ala Gln Phe Arg Lys Glu Lys Glu Thr Phe Lys Glu
65 70 75 80
Lys Asp Thr Tyr Lys Leu Phe Lys Asn Gly Thr Leu Lys Ile Lys His
85 90 95
Leu Lys Thr Asp Asp Gln Asp Ile Tyr Lys Val Ser Ile Tyr Asp Thr
100 105 110
Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Asp Leu Lys Ile Gln Glu
115 120 125
Arg Val Ser Lys Pro Lys Ile Ser Trp Thr Cys Ile Asn Thr Thr Leu
130 135 140
Thr Cys Glu Val Met Asn Gly Thr Asp Pro Glu Leu Asn Leu Tyr Gln
145 150 155 160
Asp Gly Lys His Leu Lys Leu Ser Gln Arg Val Ile Thr His Lys Trp
165 170 175
Thr Thr Ser Leu Ser Ala Lys Phe Lys Cys Thr Ala Gly Asn Lys Val
180 185 190
Ser Lys Glu Ser Ser Val Glu Pro Val Ser Cys Pro Glu Lys Gly Leu
195 200 205
Asp Ile Tyr Leu Ile Ile Gly Ile Cys Gly Gly Gly Ser Leu Leu Met
210 215 220
Val Phe Val Ala Leu Leu Val Phe Tyr Ile Thr Lys Arg Lys Lys Gln
225 230 235 240
Arg Ser Arg Arg Asn Asp Glu Glu Leu Glu Thr Arg Ala His Arg Val
245 250 255
Ala Thr Glu Glu Arg Gly Arg Lys Pro His Gln Ile Pro Ala Ser Thr
260 265 270
Pro Gln Asn Pro Ala Thr Ser Gln His Pro Pro Pro Pro Pro Gly His
275 280 285
Arg Ser Gln Ala Pro Ser His Arg Pro Pro Pro Pro Gly His Arg Val
290 295 300
Gln His Gln Pro Gln Lys Arg Pro Pro Ala Pro Ser Gly Thr Gln Val
305 310 315 320
His Gln Gln Lys Gly Pro Pro Leu Pro Arg Pro Arg Val Gln Pro Lys
325 330 335
Pro Pro His Gly Ala Ala Glu Asn Ser Leu Ser Pro Ser Ser Asn
340 345 350

<210> 50
<211> 1098
<212> DNA
<213> Homo sapiens

<400> 50
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cagacctggg cgggctctca ctccatgagg tatttcttca catccgtgtc ccggcccggc 120
cgcggggagc cccgcttcat cgcagtgggc tacgtggacg acacgcagtt cgtgcggttc 180
gacagcgacg ccgcgagcca gaggatggag ccgcgggcgc cgtggataga gcaggagggt 240
ccggagtatt gggacgggga gacacggaaa gtgaaggccc actcacagac tcaccgagtg 300
gacctgggga ccctgcgcgg ctactacaac cagagcgagg ccggttctca caccctccag 360
atgatgtttg gctgcgacgt ggggtcggac tggcgcttcc tccgcgggta ccaccagtac 420
gcctacgacg gcaaggatta catcgccctg aaagaggacc tgcgctcttg gaccgcggcg 480
gacatggcag ctcagaccac caagcacaag tgggaggcgg cccatgtggc ggagcagttg 540

agagcctacc tggagggcac gtgcgtggag tggctccgca gatacctgga gaacgggaag 600
gagacgctgc agcgcacgga cgcccccaaa acgcatatga ctcaccacgc tgtctctgac 660
catgaagcca ccctgaggtg ctgggccctg agcttctacc ctgcggagat cacactgacc 720
tggcagcggg atggggagga ccagacccag gacacggagc tcgtggagac caggcctgca 780
ggggatggaa ccttccagaa gtgggcggct gtggtggtgc cttctggaca ggagcagaga 840
tacacctgcc atgtgcagca tgagggtttg cccaagcccc tcaccctgag atgggagccg 900
tcttcccagc ccaccatccc catcgtgggc atcattgctg gcctggttct ctttggagct 960
gtgatcactg gagctgtggt cgctgctgtg atgtggagga ggaagagctc agatagaaaa 1020
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acagcttgta aagtgtga 1098

<210> 51
<211> 365
<212> PRT
<213> Homo sapiens

<400> 51
Met Ala Val Met Ala Pro Arg Thr Leu Val Leu Leu Leu Ser Gly Ala
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Leu Ala Leu Thr Gln Thr Trp Ala Gly Ser His Ser Met Arg Tyr Phe
            20   25   30
Phe Thr Ser Val Ser Arg Pro Gly Arg Gly Glu Pro Arg Phe Ile Ala
        35   40   45
Val Gly Tyr Val Asp Asp Thr Gln Phe Val Arg Phe Asp Ser Asp Ala
    50   55   60
Ala Ser Gln Arg Met Glu Pro Arg Ala Pro Trp Ile Glu Gln Glu Gly
65   70   75   80
Pro Glu Tyr Trp Asp Gly Glu Thr Arg Lys Val Lys Ala His Ser Gln
                85   90   95
Thr His Arg Val Asp Leu Gly Thr Leu Arg Gly Tyr Tyr Asn Gln Ser
            100   105   110
Glu Ala Gly Ser His Thr Leu Gln Met Met Phe Gly Cys Asp Val Gly
        115   120   125
Ser Asp Trp Arg Phe Leu Arg Gly Tyr His Gln Tyr Ala Tyr Asp Gly
    130   135   140
Lys Asp Tyr Ile Ala Leu Lys Glu Asp Leu Arg Ser Trp Thr Ala Ala
145   150   155   160
Asp Met Ala Ala Gln Thr Thr Lys His Lys Trp Glu Ala Ala His Val
                165   170   175
Ala Glu Gln Leu Arg Ala Tyr Leu Glu Gly Thr Cys Val Glu Trp Leu
            180   185   190
Arg Arg Tyr Leu Glu Asn Gly Lys Glu Thr Leu Gln Arg Thr Asp Ala
        195   200   205
Pro Lys Thr His Met Thr His His Ala Val Ser Asp His Glu Ala Thr
    210   215   220
Leu Arg Cys Trp Ala Leu Ser Phe Tyr Pro Ala Glu Ile Thr Leu Thr
225   230   235   240
Trp Gln Arg Asp Gly Glu Asp Gln Thr Gln Asp Thr Glu Leu Val Glu
                245   250   255
Thr Arg Pro Ala Gly Asp Gly Thr Phe Gln Lys Trp Ala Ala Val Val
            260   265   270
Val Pro Ser Gly Gln Glu Gln Arg Tyr Thr Cys His Val Gln His Glu
        275   280   285
Gly Leu Pro Lys Pro Leu Thr Leu Arg Trp Glu Pro Ser Ser Gln Pro
    290   295   300
Thr Ile Pro Ile Val Gly Ile Ile Ala Gly Leu Val Leu Phe Gly Ala
305   310   315   320
Val Ile Thr Gly Ala Val Val Ala Ala Val Met Trp Arg Arg Lys Ser
                325   330   335
Ser Asp Arg Lys Gly Gly Ser Tyr Ser Gln Ala Ala Ser Ser Asp Ser
            340   345   350
Ala Gln Gly Ser Asp Val Ser Leu Thr Ala Cys Lys Val
        355   360   365

<210> 52
<211> 987
<212> DNA
<213> Homo sapiens

<400> 52
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atgtctcgct ccgtggcctt agctgtgctc gcgctactct ctctttctgg cctggaggct 120
atccagcgta ctccaaagat tcaggtttac tcacgtcatc cagcagagaa tggaaagtca 180
aatttcctga attgctatgt gtctgggttt catccatccg acattgaagt tgacttactg 240
aagaatggag agagaattga aaaagtggag cattcagact tgtctttcag caaggactgg 300
tctttctatc tcttgtacta cactgaattc acccccactg aaaaagatga gtatgcctgc 360
cgtgtgaacc atgtgacttt gtcacagccc aagatagtta agtgggatcg agacatgtaa 420
gcagcatcat ggaggtttga agatgccgca tttggattgg atgaattcca aattctgctt 480
gcttgctttt taatattgat atgcttatac acttacactt tatgcacaaa atgtagggtt 540
ataataatgt taacatggac atgatcttct ttataattct actttgagtg ctgtctccat 600
gtttgatgta tctgagcagg ttgctccaca ggtagctcta ggagggctgg caacttagag 660
gtggggagca gagaattctc ttatccaaca tcaacatctt ggtcagattt gaactcttca 720
atctcttgca ctcaaagctt gttaagatag ttaagcgtgc ataagttaac ttccaattta 780
catactctgc ttagaatttg ggggaaaatt tagaaatata attgacagga ttattggaaa 840
tttgttataa tgaatgaaac attttgtcat ataagattca tatttacttc ttatacattt 900
gataaagtaa ggcatggttg tggttaatct ggtttatttt tgttccacaa gttaaataaa 960
tcataaaact tgatgtgtta tctctta 987

<210> 53
<211> 119
<212> PRT
<213> Homo sapiens

<400> 53
Met Ser Arg Ser Val Ala Leu Ala Val Leu Ala Leu Leu Ser Leu Ser
1 5 10 15
Gly Leu Glu Ala Ile Gln Arg Thr Pro Lys Ile Gln Val Tyr Ser Arg
20 25 30
His Pro Ala Glu Asn Gly Lys Ser Asn Phe Leu Asn Cys Tyr Val Ser
35 40 45
Gly Phe His Pro Ser Asp Ile Glu Val Asp Leu Leu Lys Asn Gly Glu
50 55 60
Arg Ile Glu Lys Val Glu His Ser Asp Leu Ser Phe Ser Lys Asp Trp
65 70 75 80
Ser Phe Tyr Leu Leu Tyr Tyr Thr Glu Phe Thr Pro Thr Glu Lys Asp
85 90 95
Glu Tyr Ala Cys Arg Val Asn His Val Thr Leu Ser Gln Pro Lys Ile
100 105 110
Val Lys Trp Asp Arg Asp Met
115