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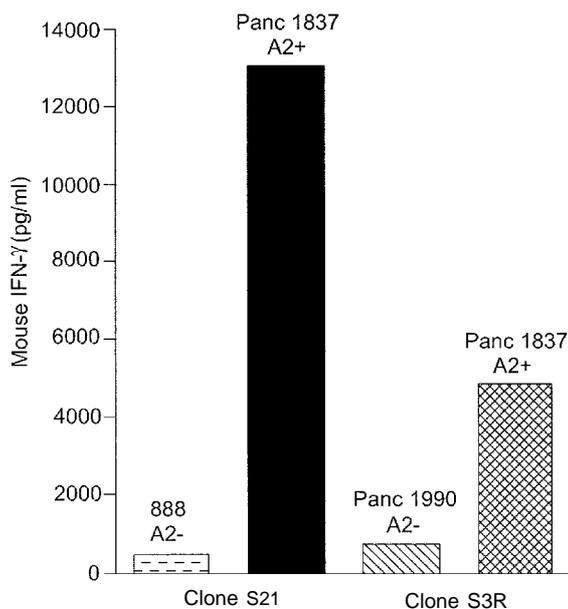
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[Continued on next page]

(54) Title: SURVIVIN SPECIFIC T CELL RECEPTOR FOR TREATING CANCER

(57) Abstract: Embodiments of the invention provide an isolated or purified T cell receptor (TCR) comprising one or more amino acid sequences encoded by one or more of SEQ ID NOS: 1-8, an isolated or purified polypeptide comprising an amino acid sequence encoded by any of SEQ ID NOS: 1-8, related polypeptides and proteins, as well as related nucleic acids, recombinant expression vectors, host cells, populations of cells, antibodies, or antigen binding portions thereof, and pharmaceutical compositions. Further embodiments of the invention provide a method of treating cancer in a host and a method of detecting the presence of cancer in a host.

Figure 7



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SURVIVIN SPECIFIC T CELL RECEPTOR FOR TREATING CANCER

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This patent application claims the benefit of U.S. Provisional Patent Application No. 61/140,338, filed December 23, 2008, which is incorporated by reference.

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0002] Incorporated by reference in its entirety herein is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: One 17,774 Byte ASCII (Text) file named "705832ST25.txt" created on December 21, 2009.

BACKGROUND OF THE INVENTION

[0003] Cytotoxic T lymphocytes (CTLs) are the major effectors of cell-based antitumor immunity and can mediate tumor regression via recognition of antigens in the context of class I MHC molecules on tumor cells. Survivin, an inhibitor of apoptosis protein (IAP), is a tumor-associated antigen that is widely expressed in nearly all human cancer cells and undetectable in most normal tissues. In transformed cells, survivin functions to antagonize apoptosis, promote tumor-associated angiogenesis, and promote resistance to chemotherapy and radiation.

[0004] There continues to exist a need to develop treatments of cancer, including treatments based on survivin recognition.

BRIEF SUMMARY OF THE INVENTION

[0005] An embodiment of the invention provides an isolated or purified T cell receptor (TCR) comprising one or more amino acid sequences encoded by one or more of SEQ ID NOS: 1-8. Another embodiment of the invention provides an isolated or purified polypeptide comprising an amino acid sequence encoded by any of SEQ ID NOS: 1-8.

[0006] The invention also provides related polypeptides and proteins, as well as related nucleic acids, recombinant expression vectors, host cells, and populations of cells. Further provided by the invention are antibodies, or an antigen binding portion thereof, and pharmaceutical compositions relating to the TCRs of the invention.

[0007] A further embodiment of the invention provides a method of treating cancer in a host, comprising administering to the host the TCRs of the invention in an amount effective to treat cancer in the host. A method of detecting the presence of cancer in a host is further provided by an embodiment of the invention. The inventive method of detecting the presence of cancer in a host comprises (i) contacting a sample comprising one or more cells from the host with any of the inventive TCRs, polypeptides, proteins, nucleic acids, recombinant expression vectors, host cells, populations of cells, or antibodies, or antigen binding portions thereof, described herein, thereby forming a complex, and (ii) detecting the complex, wherein detection of the complex is indicative of the presence of cancer in the host.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] Figure 1 is a bar graph showing the number of interferon- γ (IFN- γ) producing cells per million splenocytes upon incubation of the splenocytes with Survivin 95 (95), Survivin 96N (96N), Survivin 96M (96M), and E7 (control) peptides. The splenocytes were harvested from mice vaccinated with Survivin 95.

[0009] Figure 2 is a bar graph showing the number of IFN- γ producing cells per million splenocytes upon incubation of the splenocytes with Survivin 95 (95), Survivin 96N (96N), Survivin 96M (96M), and E7 (control) peptides. The splenocytes were harvested from mice vaccinated with Survivin 96N.

[0010] Figure 3 is a bar graph showing the number of IFN- γ producing cells per million splenocytes upon incubation of the splenocytes with Survivin 95 (95), Survivin 96N (96N), Survivin 96M (96M), and E7 (control) peptides. The splenocytes were harvested from mice vaccinated with Survivin 96M.

[0011] Figure 4 is a bar graph showing the number of IFN- γ producing cells per million lymph node cells upon incubation of the lymph node cells with Survivin 95 (95), Survivin 96N (96N), Survivin 96M (96M), and E7 (control) peptides. The lymph node cells were harvested from mice vaccinated with Survivin 96M.

[0012] Figure 5 is a bar graph showing the number of IFN- γ producing cells per million splenocytes. The splenocytes were harvested from mice vaccinated with Survivin 96M, restimulated with T2 cells pulsed with Survivin 96M, and then incubated with two concentrations of Survivin 96N (96N) and Survivin 45 (Surv45; control) peptides.

[0013] Figure 6 is a bar graph showing the concentration of IFN- γ produced from bulk splenocytes. The splenocytes were harvested from mice vaccinated with Survivin 96M, restimulated twice with T2 cells pulsed with Survivin 96M at the listed concentrations, and then incubated with T2 cells pulsed with Survivin 88 (Surv88; control), Survivin 96M (Surv96M), or Survivin 96N (Surv96N).

[0014] Figure 7 is a bar graph showing the concentration of IFN- γ produced when selected clones of CTLs of Surv96M-vaccinated mice were co-cultured with tumor (A2+) or control (A2-) cells.

[0015] Figure 8 is a bar graph showing the concentration of IFN- γ produced when additional selected clones of CTLs of Surv96M-vaccinated mice were co-cultured with tumor (A2+) or control (A2-) cells.

[0016] Figure 9 is a bar graph showing the concentration of IFN- γ produced when survivin TCR-transduced peripheral blood lymphocytes (PBLs) were co-cultured with HLA-A2+ and HLA-A2- pediatric sarcoma cell lines.

[0017] Figure 10 is a bar graph showing the concentration of IFN- γ produced when survivin TCR-transduced PBLs were co-cultured with T2 cells pulsed with different concentrations of survivin peptide 96N or control peptides (E7 and CMV).

DETAILED DESCRIPTION OF THE INVENTION

[0018] An embodiment of the invention provides an isolated or purified T cell receptor (TCR) comprising one or more amino acid sequences encoded by one or more of SEQ ID NOS: 1-8. Another embodiment of the invention provides an isolated or purified polypeptide comprising an amino acid sequence encoded by any of SEQ ID NOS: 1-8. The TCRs of the invention are reactive against survivin.

[0019] Survivin is an Inhibitor of Apoptosis Protein (IAP) that is sharply differentially expressed in many adult and pediatric cancers. Antibody staining for survivin is strong in both embryonic and alveolar rhabdomyosarcoma but undetectable in normal muscle. CTLs reactive to endogenous A2-restricted survivin peptides Survivin95-104 (Surv95: ELTLGEFLKL (SEQ ID NO: 9)), Survivin96-104 (Surv96N: LTLGEFLKL (SEQ ID NO: 10)), and modified Survivin96-104 (Surv96M: LMLGEFLKL (SEQ ID NO: 11)) are associated with tumor reactivity *in vitro* and *in vivo*. The full-length human survivin amino acid sequence is:

MGAPTLPPAWQPFLKDHRISTFKNWPFLGCACTPERMAEAGFIHCPTENEPDLAQC
FFCFKELEGWEPDDDDPIEEHKKHSSGCAFLSVKKQFEELTLGEFLKLD RERAKNKIAK
ETNKKKEFEETVKKVRRRAIEQLAAMD (SEQ ID NO: 12). The mouse sequence of
Surv95 is: ELTVSEFLKL (SEQ ID NO: 13). Although there is evidence for cytotoxic T cell
responses against survivin in patients, tolerance to self antigens can be a limiting factor in
generating highly avid T cells capable of killing tumor cells. An alternate approach involves
peptide immunization of HLA-A2 transgenic mice that express the human class I HLA-A2
molecule, as shown in the Examples below, which may be able to generate more highly avid
TCRs to human antigens.

[0020] The TCR proteins of the invention can comprise an α and a β chain. For example,
the protein of the invention can comprise a first polypeptide chain comprising the amino acid
sequence of SEQ ID NO: 1 (an α chain) and a second polypeptide chain comprising the
amino acid sequence of SEQ ID NO: 2 (a β chain). The protein of the invention can, for
example, comprise a first polypeptide chain comprising the amino acid sequence of SEQ ID
NO: 3 (an α chain) and a second polypeptide chain comprising the amino acid sequence of
SEQ ID NO: 4 (a β chain). The protein of the invention can, for example, comprise a first
polypeptide chain comprising the amino acid sequence of SEQ ID NO: 5 (an α chain) and a
second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 6 (a β chain).
The protein of the invention can, for example, comprise a first polypeptide chain comprising
the amino acid sequence of SEQ ID NO: 7 (an α chain) and a second polypeptide chain
comprising the amino acid sequence of SEQ ID NO: 4 (a β chain). The protein of the
invention can, for example, comprise a first polypeptide chain comprising the amino acid
sequence of SEQ ID NO: 8 (an α chain) and a second polypeptide chain comprising the
amino acid sequence of SEQ ID NO: 6 (a β chain). Also, the proteins of the invention may
comprise other α/β combinations, such as SEQ ID NO: 1/SEQ ID NO: 4, SEQ ID NO:
1/SEQ ID NO: 6, SEQ ID NO: 3/SEQ ID NO: 2, SEQ ID NO: 3/SEQ ID NO: 6, SEQ ID NO:
5/SEQ ID NO: 2, SEQ ID NO: 5/SEQ ID NO: 4, SEQ ID NO: 7/SEQ ID NO: 2, SEQ ID NO:
7/SEQ ID NO: 6, SEQ ID NO: 8/SEQ ID NO: 2, SEQ ID NO: 8/SEQ ID NO: 4.
Alternatively, if, for example, the protein comprises a single polypeptide chain comprising
SEQ ID NO: 3 and SEQ ID NO: 4, or if the first and/or second polypeptide chain(s) of the
protein further comprise(s) other amino acid sequences, e.g., an amino acid sequence
encoding an immunoglobulin or a portion thereof, then the inventive protein can be a fusion

protein. In this regard, the invention also provides a fusion protein comprising at least one of the inventive polypeptides described herein along with at least one other polypeptide. The other polypeptide can exist as a separate polypeptide of the fusion protein, or can exist as a polypeptide, which is expressed in frame (in tandem) with one of the inventive polypeptides described herein. The other polypeptide can encode any peptidic or proteinaceous molecule, or a portion thereof, including, but not limited to an immunoglobulin, CD3, CD4, CD8, an MHC molecule, a CD1 molecule, e.g., CD1a, CD1b, CD1c, CD1d, etc.

[0021] The invention also provides related polypeptides and proteins, as well as related nucleic acids, recombinant expression vectors, host cells, and populations of cells. Further provided by the invention are antibodies, or an antigen binding portion thereof, and pharmaceutical compositions relating to the TCRs of the invention.

[0022] A further embodiment of the invention provides a method of treating cancer in a host, comprising administering to the host the TCRs of the invention in an amount effective to treat cancer in the host by administering to the host any of the inventive TCRs, polypeptides, proteins, nucleic acids, recombinant expression vectors, host cells, populations of cells, or antibodies, or antigen binding portions thereof, described herein. A method of detecting the presence of cancer in a host is further provided by an embodiment of the invention. The inventive method of detecting the presence of cancer in a host comprises (i) contacting a sample comprising one or more cells from the host with any of the inventive TCRs, polypeptides, proteins, nucleic acids, recombinant expression vectors, host cells, populations of cells, or antibodies, or antigen binding portions thereof, described herein, thereby forming a complex, and (ii) detecting the complex, wherein detection of the complex is indicative of the presence of cancer in the host.

[0023] Another embodiment of the invention provides for use of an effective amount of an isolated or purified TCR comprising one or more amino acid sequences encoded by one or more of SEQ ID NOS: 1-8 in the manufacture of a medicament for treating cancer in a host. Other embodiments of the invention provide for use of an effective amount of an isolated or purified TCR comprising a first polypeptide comprising an amino acid sequence encoded by SEQ ID NO: 1 and a second polypeptide comprising an amino acid sequence encoded by SEQ ID NO: 2, an isolated or purified TCR comprising a first polypeptide comprising an amino acid sequence encoded by SEQ ID NO: 3 and a second polypeptide comprising an amino acid sequence encoded by SEQ ID NO: 4, an isolated or purified TCR comprising a first polypeptide comprising an amino acid sequence encoded by SEQ ID NO: 5 and a second

polypeptide comprising an amino acid sequence encoded by SEQ ID NO: 6, an isolated or purified TCR comprising a first polypeptide comprising an amino acid sequence encoded by SEQ ID NO: 7 and a second polypeptide comprising an amino acid sequence encoded by SEQ ID NO: 4, and/or an isolated or purified TCR comprising a first polypeptide comprising an amino acid sequence encoded by SEQ ID NO: 8 and a second polypeptide comprising an amino acid sequence encoded by SEQ ID NO: 6 in the manufacture of a medicament for treating cancer in a host. The invention further provides for use of an effective amount of an isolated or purified polypeptide comprising an amino acid sequence encoded by any of SEQ ID NOS: 1-8 in the manufacture of a medicament for treating cancer in a host. Another embodiment of the invention provides for use of an effective amount of a nucleic acid comprising any of SEQ ID NOS: 1-8, an effective amount of a nucleic acid encoding any of the amino acid sequences described herein, an effective amount of any of the recombinant vectors described herein, and/or an effective amount of the population of cells described herein in the manufacture of a medicament for treating cancer in a host.

[0024] The host referred to herein can be any host. The host may be a mammal. As used herein, the term "mammal" refers to any mammal, including, but not limited to, mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits. The mammals may be from the order Carnivora, including Felines (cats) and Canines (dogs). The mammals may be from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perssodactyla, including Equines (horses). The mammals may be of the order Primates, Ceboids, or Simoids (monkeys) or of the order Anthropoids (humans and apes). The mammal may be a human.

[0025] The phrases "have antigen specificity" and "elicit antigen-specific response" as used herein means that the TCR can specifically bind to and immunologically recognize an antigen, such that binding of the TCR to the antigen elicits an immune response.

[0026] The term "cancer antigen" as used herein refers to any molecule (e.g., protein, peptide, lipid, carbohydrate, etc.) solely or predominantly expressed or over-expressed by a tumor cell or cancer cell, such that the antigen is associated with the tumor or cancer. An example of a cancer antigen is survivin. The cancer antigen additionally can be expressed by normal, non-tumor, or non-cancerous cells. However, in such a situation, the expression of the cancer antigen by normal, non-tumor, or non-cancerous cells is not as robust as the expression by tumor or cancer cells. In this regard, the tumor or cancer cells can over-express the antigen or express the antigen at a significantly higher level, as compared to the

expression of the antigen by normal, non-tumor, or non-cancerous cells. Also, the cancer antigen additionally can be expressed by cells of a different state of development or maturation. For instance, the cancer antigen can be additionally expressed by cells of the embryonic or fetal stage, which cells are not normally found in an adult host. Alternatively, the cancer antigen additionally can be expressed by stem cells or precursor cells, which cells are not normally found in an adult host. Another group of cancer antigens are represented by the differentiation antigens that are expressed in only a limited set of tissues in the adult, such as the melanocytes differentiation antigens, whose expression is limited to normal melanocytes. Although it is not known why these molecules elicit immune responses, the limited expression pattern of these proteins may allow these molecules to be recognized by the immune system.

[0027] The cancer antigen can be an antigen expressed by any cell of any cancer or tumor, including the cancers and tumors described herein. Alternatively, the cancer antigen may be a cancer antigen (e.g., may be characteristic) of more than one type of cancer or tumor. For example, the cancer antigen may be expressed by both breast and prostate cancer cells and not expressed at all by normal, non-tumor, or non-cancer cells. In an embodiment of the invention, the cancer antigen is a pancreatic cancer antigen.

[0028] Included in the scope of the invention are functional variants of the inventive TCRs, polypeptides, and proteins described herein. The term "functional variant" as used herein refers to a TCR, polypeptide, or protein having substantial or significant sequence identity or similarity to a parent TCR, polypeptide, or protein, which functional variant retains the biological activity of the TCR, polypeptide, or protein of which it is a variant. Functional variants encompass, for example, those variants of the TCR, polypeptide, or protein described herein (the parent TCR, polypeptide, or protein) that retain the ability to recognize target cells to a similar extent, the same extent, or to a higher extent, as the parent TCR, polypeptide, or protein. In reference to the parent TCR, polypeptide, or protein, the functional variant can, for instance, be at least about 30%, 50%, 75%, 80%, 90%, 98% or more identical in amino acid sequence to the parent TCR, polypeptide, or protein.

[0029] The functional variant can, for example, comprise the amino acid sequence of the parent TCR, polypeptide, or protein with at least one conservative amino acid substitution. Alternatively or additionally, the functional variants can comprise the amino acid sequence of the parent TCR, polypeptide, or protein with at least one non-conservative amino acid substitution. In this case, it is preferable for the non-conservative amino acid substitution to

not interfere with or inhibit the biological activity of the functional variant. The non-conservative amino acid substitution may enhance the biological activity of the functional variant, such that the biological activity of the functional variant is increased as compared to the parent TCR, polypeptide, or protein.

[0030] The TCRs of the invention can comprise one or more immature TCR chains comprising a leader sequence or one or more mature chains in which the leader sequence has been cleaved off. As one of ordinary skill in the art appreciates, the leader sequence of a TCR chain comprises the amino acids at the N-terminus which together serve as a signal to transport the TCR to the plasma membrane and which amino acids are cleaved off to yield the mature form of the TCR.

[0031] The TCR, polypeptide, or protein can consist essentially of the specified amino acid sequence or sequences described herein, such that other components e.g., other amino acids, do not materially change the biological activity of the functional variant.

[0032] The TCRs, polypeptides, and proteins of the invention (including functional portions and functional variants) can be of any length, i.e., can comprise any number of amino acids, provided that the TCRs, polypeptides, or proteins (or functional portions or functional variants thereof) retain their biological activity, e.g., the ability to specifically bind to antigen, detect diseased cells in a host, or treat or prevent disease in a host, etc. For example, the polypeptide can be about 50 to about 5000 amino acids long, such as 50, 70, 75, 100, 125, 150, 175, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more amino acids in length. In this regard, the polypeptides of the invention also include oligopeptides.

[0033] The TCRs, polypeptides, and proteins of the invention (including functional portions and functional variants) of the invention can comprise synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids are known in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, α -amino n-decanoic acid, homoserine, S-acetylaminoethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4-aminophenylalanine, 4-nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β -phenylserine β -hydroxyphenylalanine, phenylglycine, α -naphthylalanine, cyclohexylalanine, cyclohexylglycine, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, α -aminocyclopentane carboxylic acid, α -aminocyclohexane carboxylic acid, α -aminocycloheptane carboxylic acid, α -(2-amino-2-norbornane)-carboxylic acid, α,γ -

diaminobutyric acid, α,β -diaminopropionic acid, homophenylalanine, and α -tert-butylglycine.

[0034] The TCRs, polypeptides, and proteins of the invention (including functional portions and functional variants) can be, for example, glycosylated, amidated, carboxylated, phosphorylated, esterified, N-acylated, cyclized via, e.g., a disulfide bridge, or converted into an acid addition salt and/or optionally dimerized or polymerized, or conjugated.

[0035] When the TCRs, polypeptides, and proteins of the invention (including functional portions and functional variants) are in the form of a salt, the polypeptides may be in the form of a pharmaceutically acceptable salt. Suitable pharmaceutically acceptable acid addition salts include those derived from mineral acids, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric, and sulphuric acids, and organic acids, such as tartaric, acetic, citric, malic, lactic, fumaric, benzoic, glycolic, gluconic, succinic, and arylsulphonic acids, for example, p-toluenesulphonic acid.

[0036] The TCRs, polypeptides, and/or proteins of the invention (including functional portions and functional variants thereof) can be obtained by methods known in the art. Suitable methods of *de novo* synthesizing polypeptides and proteins are described in references, such as Chan et al., *Fmoc Solid Phase Peptide Synthesis*, Oxford University Press, Oxford, United Kingdom, 2005; *Peptide and Protein Drug Analysis*, ed. Reid, R., Marcel Dekker, Inc., 2000; *Epitope Mapping*, ed. Westwood et al., Oxford University Press, Oxford, United Kingdom, 2000; and U.S. Patent No. 5,449,752. Also, polypeptides and proteins can be recombinantly produced using the nucleic acids described herein using standard recombinant methods. See, for instance, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 3rd ed., Cold Spring Harbor Press, Cold Spring Harbor, NY 2001; and Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing Associates and John Wiley & Sons, NY, 1994. Further, some of the TCRs, polypeptides, and proteins of the invention (including functional portions and functional variants thereof) can be isolated and/or purified from a source, such as a plant, a bacterium, an insect, a mammal, e.g., a rat, a human, etc. Methods of isolation and purification are well-known in the art. Alternatively, the TCRs, polypeptides, and/or proteins described herein (including functional portions and functional variants thereof) can be commercially synthesized by companies, such as Synpep (Dublin, CA), Peptide Technologies Corp. (Gaithersburg, MD), and Multiple Peptide Systems (San Diego, CA). In this respect, the inventive TCRs, polypeptides, and proteins can be synthetic, recombinant, isolated, and/or purified.

[0037] The protein of the invention can be a recombinant antibody comprising at least one of the inventive polypeptides described herein. As used herein, "recombinant antibody" refers to a recombinant (e.g., genetically engineered) protein comprising at least one of the polypeptides of the invention and a polypeptide chain of an antibody, or a portion thereof. The polypeptide of an antibody, or portion thereof, can be a heavy chain, a light chain, a variable or constant region of a heavy or light chain, a single chain variable fragment (scFv), or an Fc, Fab, or F(ab')₂ fragment of an antibody, etc. The polypeptide chain of an antibody, or portion thereof, can exist as a separate polypeptide of the recombinant antibody. Alternatively, the polypeptide chain of an antibody, or portion thereof, can exist as a polypeptide, which is expressed in frame (in tandem) with the polypeptide of the invention. The polypeptide of an antibody, or portion thereof, can be a polypeptide of any antibody or any antibody fragment, including any of the antibodies and antibody fragments described herein.

[0038] The invention further provides an antibody, or antigen binding portion thereof, which specifically binds to an epitope of the TCR of the invention. The antibody can be any type of immunoglobulin that is known in the art. For instance, the antibody can be of any isotype, e.g., IgA, IgD, IgE, IgG, IgM, etc. The antibody can be monoclonal or polyclonal. The antibody can be a naturally-occurring antibody, e.g., an antibody isolated and/or purified from a mammal, e.g., mouse, rabbit, goat, horse, chicken, hamster, human, etc. Alternatively, the antibody can be a genetically-engineered antibody, e.g., a humanized antibody or a chimeric antibody. The antibody can be in monomeric or polymeric form. Also, the antibody can have any level of affinity or avidity for the functional portion of the inventive TCR.

[0039] Methods of testing antibodies for the ability to bind to any functional portion of the inventive TCR are known in the art and include any antibody-antigen binding assay, such as, for example, radioimmunoassay (RIA), ELISA, Western blot, immunoprecipitation, and competitive inhibition assays (see, e.g., Janeway et al., *infra*, and U.S. Patent Application Publication No. 2002/0197266 A1).

[0040] Suitable methods of making antibodies are known in the art. For instance, standard hybridoma methods are described in, e.g., Köhler and Milstein, *Eur. J. Immunol.*, 5, 511-519 (1976), Harlow and Lane (eds.), *Antibodies: A Laboratory Manual*, CSH Press (1988), and CA. Janeway et al. (eds.), *Immunobiology*, 5th Ed., Garland Publishing, New York, NY (2001)). Alternatively, other methods, such as EBV-hybridoma methods (Haskard

and Archer, *J. Immunol. Methods*, 74(2), 361-67 (1984), and Roder et al., *Methods Enzymol.*, 121, 140-67 (1986)), and bacteriophage vector expression systems (see, e.g., Huse et al., *Science*, 246, 1275-81 (1989)) are known in the art. Further, methods of producing antibodies in non-human animals are described in, e.g., U.S. Patents 5,545,806, 5,569,825, and 5,714,352, and U.S. Patent Application Publication No. 2002/0197266 A1).

[0041] Phage display furthermore can be used to generate the antibody of the invention. In this regard, phage libraries encoding antigen-binding variable (V) domains of antibodies can be generated using standard molecular biology and recombinant DNA techniques (see, e.g., Sambrook et al. (eds.), *Molecular Cloning, A Laboratory Manual*, 3rd Edition, Cold Spring Harbor Laboratory Press, New York (2001)). Phage encoding a variable region with the desired specificity are selected for specific binding to the desired antigen, and a complete or partial antibody is reconstituted comprising the selected variable domain. Nucleic acid sequences encoding the reconstituted antibody are introduced into a suitable cell line, such as a myeloma cell used for hybridoma production, such that antibodies having the characteristics of monoclonal antibodies are secreted by the cell (see, e.g., Janeway et al., *supra*, Huse et al., *supra*, and U.S. Patent 6,265,150).

[0042] Antibodies can be produced by transgenic mice that are transgenic for specific heavy and light chain immunoglobulin genes. Such methods are known in the art and described in, for example U.S. Patents 5,545,806 and 5,569,825, and Janeway et al., *supra*.

[0043] Methods for generating humanized antibodies are well known in the art and are described in detail in, for example, Janeway et al., *supra*, U.S. Patents 5,225,539, 5,585,089 and 5,693,761, European Patent No. 0239400 B1, and United Kingdom Patent No. 2188638. Humanized antibodies can also be generated using the antibody resurfacing technology described in U.S. Patent 5,639,641 and Pedersen et al., *J. Mol. Biol.*, 235, 959-973 (1994).

[0044] The invention also provides antigen binding portions of any of the antibodies described herein. The antigen binding portion can be any portion that has at least one antigen binding site, such as Fab, F(ab')₂, dsFv, sFv, diabodies, and triabodies.

[0045] A single-chain variable region fragment (sFv) antibody fragment, which consists of a truncated Fab fragment comprising the variable (V) domain of an antibody heavy chain linked to a V domain of a light antibody chain via a synthetic peptide, can be generated using routine recombinant DNA technology techniques (see, e.g., Janeway et al., *supra*). Similarly, disulfide-stabilized variable region fragments (dsFv) can be prepared by recombinant DNA technology (see, e.g., Reiter et al., *Protein Engineering*, 7, 697-704 (1994)). Antibody

fragments of the invention, however, are not limited to these exemplary types of antibody fragments.

[0046] Also, the antibody, or antigen binding portion thereof, can be modified to comprise a detectable label, such as, for instance, a radioisotope, a fluorophore (e.g., fluorescein isothiocyanate (FITC), phycoerythrin (PE)), an enzyme (e.g., alkaline phosphatase, horseradish peroxidase), and element particles (e.g., gold particles).

[0047] Further provided by the invention is a nucleic acid comprising a nucleotide sequence encoding any of the TCRs, polypeptides, or proteins described herein (including functional portions and functional variants thereof).

[0048] By "nucleic acid" as used herein includes "polynucleotide," "oligonucleotide," and "nucleic acid molecule," and generally means a polymer of DNA or RNA, which can be single-stranded or double-stranded, synthesized or obtained (e.g., isolated and/or purified) from natural sources, which can contain natural, non-natural or altered nucleotides, and which can contain a natural, non-natural or altered internucleotide linkage, such as a phosphoroamidate linkage or a phosphorothioate linkage, instead of the phosphodiester found between the nucleotides of an unmodified oligonucleotide. It is generally preferred that the nucleic acid does not comprise any insertions, deletions, inversions, and/or substitutions. However, it may be suitable in some instances, as discussed herein, for the nucleic acid to comprise one or more insertions, deletions, inversions, and/or substitutions.

[0049] The nucleic acids of the invention may be recombinant. As used herein, the term "recombinant" refers to (i) molecules that are constructed outside living cells by joining natural or synthetic nucleic acid segments to nucleic acid molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above. For purposes herein, the replication can be *in vitro* replication or *in vivo* replication.

[0050] A recombinant nucleic acid may be one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques, such as those described in Sambrook et al., *supra*.

[0051] The nucleic acids can be constructed based on chemical synthesis and/or enzymatic ligation reactions using procedures known in the art. See, for example, Sambrook et al., *supra*, and Ausubel et al., *supra*. For example, a nucleic acid can be chemically

synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed upon hybridization (e.g., phosphorothioate derivatives and acridine substituted nucleotides). Examples of modified nucleotides that can be used to generate the nucleic acids include, but are not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-substituted adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, 3-(3-amino-3-N-2-carboxypropyl) uracil, and 2,6-diaminopurine. Alternatively, one or more of the nucleic acids of the invention can be purchased from companies, such as Macromolecular Resources (Fort Collins, CO) and Synthegen (Houston, TX).

[0052] The nucleic acid can comprise any isolated or purified nucleotide sequence which encodes any of the TCRs, polypeptides, or proteins, or functional portions or functional variants thereof. Alternatively, the nucleotide sequence can comprise a nucleotide sequence which is degenerate to any of the sequences or a combination of degenerate sequences.

[0053] The invention also provides an isolated or purified nucleic acid comprising a nucleotide sequence which is complementary to the nucleotide sequence of any of the nucleic acids described herein or a nucleotide sequence which hybridizes under stringent conditions to the nucleotide sequence of any of the nucleic acids described herein.

[0054] The nucleotide sequence which hybridizes under stringent conditions may hybridize under high stringency conditions. By "high stringency conditions" is meant that the nucleotide sequence specifically hybridizes to a target sequence (the nucleotide sequence of any of the nucleic acids described herein) in an amount that is detectably stronger than non-specific hybridization. High stringency conditions include conditions which would distinguish a polynucleotide with an exact complementary sequence, or one containing only a few scattered mismatches from a random sequence that happened to have a few small regions (e.g., 3-10 bases) that matched the nucleotide sequence. Such small regions of

complementarity are more easily melted than a full-length complement of 14-17 or more bases, and high stringency hybridization makes them easily distinguishable. Relatively high stringency conditions would include, for example, low salt and/or high temperature conditions, such as provided by about 0.02-0.1 M NaCl or the equivalent, at temperatures of about 50-70 °C. Such high stringency conditions tolerate little, if any, mismatch between the nucleotide sequence and the template or target strand, and are particularly suitable for detecting expression of any of the inventive TCRs. It is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide.

[0055] The nucleic acids of the invention can be incorporated into a recombinant expression vector. In this regard, the invention provides recombinant expression vectors comprising any of the nucleic acids of the invention. For purposes herein, the term "recombinant expression vector" means a genetically-modified oligonucleotide or polynucleotide construct that permits the expression of an mRNA, protein, polypeptide, or peptide by a host cell, when the construct comprises a nucleotide sequence encoding the mRNA, protein, polypeptide, or peptide, and the vector is contacted with the cell under conditions sufficient to have the mRNA, protein, polypeptide, or peptide expressed within the cell. The vectors of the invention are not naturally-occurring as a whole. However, parts of the vectors can be naturally-occurring. The inventive recombinant expression vectors can comprise any type of nucleotides, including, but not limited to DNA and RNA, which can be single-stranded or double-stranded, synthesized or obtained in part from natural sources, and which can contain natural, non-natural or altered nucleotides. The recombinant expression vectors can comprise naturally-occurring, non-naturally-occurring internucleotide linkages, or both types of linkages. Preferably, the non-naturally occurring or altered nucleotides or internucleotide linkages does not hinder the transcription or replication of the vector.

[0056] The recombinant expression vector of the invention can be any suitable recombinant expression vector, and can be used to transform or transfect any suitable host. Suitable vectors include those designed for propagation and expansion or for expression or both, such as plasmids and viruses. The vector can be selected from the group consisting of the pUC series (Fermentas Life Sciences, Glen Burnie, MD), the pBluescript series (Stratagene, LaJolla, CA), the pET series (Novagen, Madison, WI), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), and the pEX series (Clontech, Palo Alto, CA). Bacteriophage vectors, such as λ GT10, λ GT11, λ ZapII (Stratagene), λ EMBL4, and λ NMI 149, also can be used. Examples of plant expression vectors include pBIOL1, pBHO1.2,

pBI101.3, pBI121 and pBIN19 (Clontech). Examples of animal expression vectors include pEUK-Cl, pMAM and pMAMneo (Clontech). The recombinant expression vector may be a viral vector, e.g., a retroviral vector.

[0057] The recombinant expression vectors of the invention can be prepared using standard recombinant DNA techniques described in, for example, Sambrook et al., supra, and Ausubel et al., supra. Constructs of expression vectors, which are circular or linear, can be prepared to contain a replication system functional in a prokaryotic or eukaryotic host cell. Replication systems can be derived, e.g., from CoIE1, 2 μ plasmid, λ , SV40, bovine papilloma virus, and the like.

[0058] The recombinant expression vector may comprise regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type of host (e.g., bacterium, fungus, plant, or animal) into which the vector is to be introduced, as appropriate, and taking into consideration whether the vector is DNA- or RNA-based.

[0059] The recombinant expression vector can include one or more marker genes, which allow for selection of transformed or transfected hosts. Marker genes include biocide resistance, e.g., resistance to antibiotics, heavy metals, etc., complementation in an auxotrophic host to provide prototrophy, and the like. Suitable marker genes for the inventive expression vectors include, for instance, neomycin/G418 resistance genes, hygromycin resistance genes, histidinol resistance genes, tetracycline resistance genes, and ampicillin resistance genes.

[0060] The recombinant expression vector can comprise a native or normative promoter operably linked to the nucleotide sequence encoding the TCR, polypeptide, or protein (including functional portions and functional variants thereof), or to the nucleotide sequence which is complementary to or which hybridizes to the nucleotide sequence encoding the TCR, polypeptide, or protein. The selection of promoters, e.g., strong, weak, inducible, tissue-specific and developmental-specific, is within the ordinary skill of the artisan. Similarly, the combining of a nucleotide sequence with a promoter is also within the skill of the artisan. The promoter can be a non-viral promoter or a viral promoter, e.g., a cytomegalovirus (CMV) promoter, an SV40 promoter, an RSV promoter, and a promoter found in the long-terminal repeat of the murine stem cell virus.

[0061] The inventive recombinant expression vectors can be designed for either transient expression, for stable expression, or for both. Also, the recombinant expression vectors can be made for constitutive expression or for inducible expression.

[0062] Further, the recombinant expression vectors can be made to include a suicide gene. As used herein, the term "suicide gene" refers to a gene that causes the cell expressing the suicide gene to die. The suicide gene can be a gene that confers sensitivity to an agent, e.g., a drug, upon the cell in which the gene is expressed, and causes the cell to die when the cell is contacted with or exposed to the agent. Suicide genes are known in the art (see, for example, *Suicide Gene Therapy: Methods and Reviews*, Springer, Caroline J. (Cancer Research UK Centre for Cancer Therapeutics at the Institute of Cancer Research, Sutton, Surrey, UK), Humana Press, 2004) and include, for example, the Herpes Simplex Virus (HSV) thymidine kinase (TK) gene, cytosine deaminase, purine nucleoside phosphorylase, and nitroreductase.

[0063] Included in the scope of the invention are conjugates, e.g., bioconjugates, comprising any of the inventive TCRs, polypeptides, or proteins (including any of the functional portions or variants thereof), nucleic acids, recombinant expression vectors, host cells, populations of host cells, or antibodies, or antigen binding portions thereof. Conjugates, as well as methods of synthesizing conjugates in general, are known in the art (See, for instance, Hudecz, F., *Methods Mol. Biol.* 298: 209-223 (2005) and Kirin et al., *Inorg Chem.* 44(15): 5405-5415 (2005)).

[0064] The invention further provides a host cell comprising any of the recombinant expression vectors described herein. As used herein, the term "host cell" refers to any type of cell that can contain the inventive recombinant expression vector. The host cell can be a eukaryotic cell, e.g., plant, animal, fungi, or algae, or can be a prokaryotic cell, e.g., bacteria or protozoa. The host cell can be a cultured cell or a primary cell, i.e., isolated directly from an organism, e.g., a human. The host cell can be an adherent cell or a suspended cell, i.e., a cell that grows in suspension. Suitable host cells are known in the art and include, for instance, DH5 α *E. coli* cells, Chinese hamster ovarian cells, monkey VERO cells, COS cells, HEK293 cells, and the like. For purposes of amplifying or replicating the recombinant expression vector, the host cell may be a prokaryotic cell, e.g., a DH5 α cell. For purposes of producing a recombinant TCR, polypeptide, or protein, the host cell may be a mammalian cell. The host cell may be a human cell. While the host cell can be of any cell type, can originate from any type of tissue, and can be of any developmental stage, the host cell may be a peripheral blood lymphocyte (PBL). The host cell may be a T cell.

[0065] For purposes herein, the T cell can be any T cell, such as a cultured T cell, e.g., a primary T cell, or a T cell from a cultured T cell line, e.g., Jurkat, SupT1, etc., or a T cell

obtained from a mammal. If obtained from a mammal, the T cell can be obtained from numerous sources, including but not limited to blood, bone marrow, lymph node, the thymus, or other tissues or fluids. T cells can also be enriched for or purified. The T cell may be a human T cell. The T cell may be a T cell isolated from a human. The T cell can be any type of T cell and can be of any developmental stage, including but not limited to, CD4+/CD8+ double positive T cells, CD4+ helper T cells, e.g., Th1 and Th2 cells, CD8+ T cells (e.g., cytotoxic T cells), peripheral blood mononuclear cells (PBMCs), peripheral blood leukocytes (PBLs), tumor infiltrating cells (TILs), memory T cells, naive T cells, and the like. The T cell may be a CD8+ T cell or a CD4+ T cell.

[0066] Also provided by the invention is a population of cells comprising at least one host cell described herein. The population of cells can be a heterogeneous population comprising the host cell comprising any of the recombinant expression vectors described, in addition to at least one other cell, e.g., a host cell (e.g., a T cell), which does not comprise any of the recombinant expression vectors, or a cell other than a T cell, e.g., a B cell, a macrophage, a neutrophil, an erythrocyte, a hepatocyte, an endothelial cell, an epithelial cells, a muscle cell, a brain cell, etc. Alternatively, the population of cells can be a substantially homogeneous population, in which the population comprises mainly of host cells (e.g., consisting essentially of) comprising the recombinant expression vector. The population also can be a clonal population of cells, in which all cells of the population are clones of a single host cell comprising a recombinant expression vector, such that all cells of the population comprise the recombinant expression vector. In one embodiment of the invention, the population of cells is a clonal population comprising host cells comprising a recombinant expression vector as described herein.

[0067] The inventive TCRs, polypeptides, proteins (including functional portions and variants thereof), nucleic acids, recombinant expression vectors, host cells (including populations thereof), and antibodies (including antigen binding portions thereof), all of which are collectively referred to as "inventive TCR materials" hereinafter, can be isolated and/or purified. The term "isolated" as used herein means having been removed from its natural environment. The term "purified" or "isolated" does not require absolute purity or isolation; rather, it is intended as a relative term. Thus, for example, a purified (or isolated) protein preparation is one in which the protein is more pure than the protein in its natural environment within a cell. Such proteins may be produced, for example, by standard purification techniques, or by recombinant expression. In some embodiments, a preparation

of a protein is purified such that the protein represents at least 50%, for example at least 70%, of the total protein content of the preparation. For example, the purity can be at least about 50%, can be greater than about 60%, about 70% or about 80%, or can be about 100%.

[0068] The inventive TCR materials can be formulated into a composition, such as a pharmaceutical composition. In this regard, the invention provides a pharmaceutical composition comprising any of the TCRs, polypeptides, proteins, functional portions, functional variants, nucleic acids, expression vectors, host cells (including populations thereof), and antibodies (including antigen binding portions thereof), and a pharmaceutically acceptable carrier. The inventive pharmaceutical compositions containing any of the inventive TCR materials can comprise more than one inventive TCR material, e.g., a polypeptide and a nucleic acid, or two or more different TCRs. Alternatively, the pharmaceutical composition can comprise an inventive TCR material in combination with another pharmaceutically active agents or drugs, such as a chemotherapeutic agents, e.g., asparaginase, busulfan, carboplatin, cisplatin, daunorubicin, doxorubicin, fluorouracil, gemcitabine, hydroxyurea, methotrexate, paclitaxel, rituximab, vinblastine, vincristine, etc.

[0069] With respect to pharmaceutical compositions, the pharmaceutically acceptable carrier can be any of those conventionally used and is limited only by chemico-physical considerations, such as solubility and lack of reactivity with the active(s), and by the route of administration. The pharmaceutically acceptable carriers described herein, for example, vehicles, adjuvants, excipients, and diluents, are well-known to those skilled in the art and are readily available to the public. It is preferred that the pharmaceutically acceptable carrier be one which is chemically inert to the active agent(s) and one which has no detrimental side effects or toxicity under the conditions of use.

[0070] The choice of carrier will be determined in part by the particular inventive TCR material, as well as by the particular method used to administer the inventive TCR material. Accordingly, there are a variety of suitable formulations of the pharmaceutical composition of the invention.

[0071] Preservatives may be used. Suitable preservatives may include, for example, methylparaben, propylparaben, sodium benzoate, and benzalkonium chloride. A mixture of two or more preservatives optionally may be used. The preservative or mixtures thereof are typically present in an amount of about 0.0001% to about 2% by weight of the total composition.

[0072] Suitable buffering agents may include, for example, citric acid, sodium citrate, phosphoric acid, potassium phosphate, and various other acids and salts. A mixture of two or more buffering agents optionally may be used. The buffering agent or mixtures thereof are typically present in an amount of about 0.001% to about 4% by weight of the total composition.

[0073] The concentration of inventive TCR material in the pharmaceutical formulations can vary, e.g., from less than about 1%, usually at or at least about 10%, to as much as 20% to 50% or more by weight, and can be selected primarily by fluid volumes, and viscosities, in accordance with the particular mode of administration selected.

[0074] Methods for preparing administrable (e.g., parenterally administrable) compositions are known or apparent to those skilled in the art and are described in more detail in, for example, Remington: The Science and Practice of Pharmacy, Lippincott Williams & Wilkins; 21st ed. (May 1, 2005).

[0075] An "effective amount" or "an amount effective to treat" refers to a dose that is adequate to prevent or treat cancer in an individual. Amounts effective for a therapeutic or prophylactic use will depend on, for example, the stage and severity of the disease or disorder being treated, the age, weight, and general state of health of the patient, and the judgment of the prescribing physician. The size of the dose will also be determined by the active selected, method of administration, timing and frequency of administration as well as the existence, nature, and extent of any adverse side-effects that might accompany the administration of a particular active and the desired physiological effect. It will be appreciated by one of skill in the art that various diseases or disorders could require prolonged treatment involving multiple administrations, perhaps using the inventive TCR materials in each or various rounds of administration.

[0076] The following formulations for oral, aerosol, parenteral (e.g., subcutaneous, intravenous, intraarterial, intramuscular, intradermal, interperitoneal, and intrathecal), and rectal administration are merely exemplary and are in no way limiting. More than one route can be used to administer the inventive TCR materials, and in certain instances, a particular route can provide a more immediate and more effective response than another route.

[0077] Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the inventive TCR material dissolved in diluents, such as water, saline, or orange juice; (b) capsules, sachets, tablets, lozenges, and troches, each containing a predetermined amount of the active ingredient, as solids or granules; (c)

powders; (d) suspensions in an appropriate liquid; and (e) suitable emulsions. Liquid formulations may include diluents, such as water and alcohols, for example, ethanol, benzyl alcohol, and the polyethylene alcohols, either with or without the addition of a pharmaceutically acceptable surfactant. Capsule forms can be of the ordinary hard or soft shelled gelatin type containing, for example, surfactants, lubricants, and inert fillers, such as lactose, sucrose, calcium phosphate, and corn starch. Tablet forms can include one or more of lactose, sucrose, mannitol, corn starch, potato starch, alginic acid, microcrystalline cellulose, acacia, gelatin, guar gum, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, calcium stearate, zinc stearate, stearic acid, and other excipients, colorants, diluents, buffering agents, disintegrating agents, moistening agents, preservatives, flavoring agents, and other pharmacologically compatible excipients. Lozenge forms can comprise the inventive TCR material in a flavor, usually sucrose and acacia or tragacanth, as well as pastilles comprising the inventive TCR material in an inert base, such as gelatin and glycerin, or sucrose and acacia, emulsions, gels, and the like containing, in addition to, such excipients as are known in the art.

[0078] Formulations suitable for parenteral administration include aqueous and non aqueous isotonic sterile injection solutions, which can contain anti oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The inventive TCR material can be administered in a physiologically acceptable diluent in a pharmaceutical carrier, such as a sterile liquid or mixture of liquids, including water, saline, aqueous dextrose and related sugar solutions, an alcohol, such as ethanol or hexadecyl alcohol, a glycol, such as propylene glycol or polyethylene glycol, dimethylsulfoxide, glycerol, ketals such as 2,2-dimethyl-1,3-dioxolane-4-methanol, ethers, poly(ethyleneglycol) 400, oils, fatty acids, fatty acid esters or glycerides, or acetylated fatty acid glycerides with or without the addition of a pharmaceutically acceptable surfactant, such as a soap or a detergent, suspending agent, such as pectin, carbomers, methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose, or emulsifying agents and other pharmaceutical adjuvants.

[0079] Oils, which can be used in parenteral formulations include petroleum, animal, vegetable, or synthetic oils. Specific examples of oils include peanut, soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral

formulations include oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters.

[0080] Suitable soaps for use in parenteral formulations include, for example, fatty alkali metal, ammonium, and triethanolamine salts, and suitable detergents include (a) cationic detergents such as, for example, dimethyl dialkyl ammonium halides, and alkyl pyridinium halides, (b) anionic detergents such as, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c) nonionic detergents such as, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylenepolypropylene copolymers, (d) amphoteric detergents such as, for example, alkyl- β -aminopropionates, and 2-alkyl-imidazoline quaternary ammonium salts, and (e) mixtures thereof.

[0081] The parenteral formulations will typically contain from about 0.5% to about 25% by weight of the inventive TCR material in solution. Preservatives and buffers may be used. In order to minimize or eliminate irritation at the site of injection, such compositions may contain one or more nonionic surfactants, for example, having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulations will typically range from about 5% to about 15% by weight. Suitable surfactants include polyethylene glycol sorbitan fatty acid esters, such as sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol. The parenteral formulations can be presented in unit-dose or multi-dose sealed containers, such as ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

[0082] Injectable formulations are in accordance with the invention. The requirements for effective pharmaceutical carriers for injectable compositions are well-known to those of ordinary skill in the art (see, e.g., *Pharmaceutics and Pharmacy Practice*, J.B. Lippincott Company, Philadelphia, PA, Banker and Chalmers, eds., pages 238-250 (1982), and *ASHP Handbook on Injectable Drugs*, Toissel, 4th ed., pages 622-630 (1986)).

[0083] Topical formulations, including those that are useful for transdermal drug release, are well known to those of skill in the art and are suitable in the context of embodiments of the invention for application to skin.

[0084] The inventive TCR material, alone or in combination with other suitable components, can be made into aerosol formulations to be administered via inhalation. These aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. They also may be formulated as pharmaceuticals for non pressured preparations, such as in a nebulizer or an atomizer. Such spray formulations also may be used to spray mucosa.

[0085] Additionally, the inventive TCR materials, or compositions comprising such inventive TCR materials, can be made into suppositories by mixing with a variety of bases, such as emulsifying bases or water-soluble bases. Formulations suitable for vaginal administration can be presented as pessaries, tampons, creams, gels, pastes, foams, or spray formulas containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate.

[0086] For purposes of the invention, the amount or dose of the inventive TCR material administered should be sufficient to effect, e.g., a therapeutic or prophylactic response, in the subject or animal over a reasonable time frame. For example, the dose of the inventive TCR material should be sufficient to bind to antigen, or detect, treat or prevent disease in a period of from about 2 hours or longer, e.g., 12 to 24 or more hours, from the time of administration. In certain embodiments, the time period could be even longer. The dose will be determined by the efficacy of the particular inventive TCR material and the condition of the animal (e.g., human), as well as the body weight of the animal (e.g., human) to be treated.

[0087] The dose of the inventive TCR material also will be determined by the existence, nature and extent of any adverse side effects that might accompany the administration of a particular inventive TCR material. Typically, the attending physician will decide the dosage of the inventive TCR material with which to treat each individual patient, taking into consideration a variety of factors, such as age, body weight, general health, diet, sex, inventive TCR material to be administered, route of administration, and the severity of the condition being treated. By way of example and not intending to limit the invention, the dose of the inventive TCR material can be about 0.001 to about 1000 mg/kg body weight of the subject being treated/day, from about 0.01 to about 10 mg/kg body weight/day, about 0.01 mg to about 1 mg/kg body weight/day.

[0088] Many assays for determining an administered dose are known in the art. For purposes of the invention, an assay, which comprises comparing the extent to which target cells are lysed or IFN- γ is secreted by T cells expressing the inventive TCR, polypeptide, or

protein upon administration of a given dose of such T cells to a mammal among a set of mammals of which is each given a different dose of the T cells, could be used to determine a starting dose to be administered to a mammal. The extent to which target cells are lysed or IFN- γ is secreted upon administration of a certain dose can be assayed by methods known in the art.

[0089] When the inventive TCR materials are administered with one or more additional therapeutic agents, one or more additional therapeutic agents can be coadministered to the mammal. By "coadministering" is meant administering one or more additional therapeutic agents and the inventive TCR materials sufficiently close in time such that the inventive TCR materials can enhance the effect of one or more additional therapeutic agents. In this regard, the inventive TCR materials can be administered first and the one or more additional therapeutic agents can be administered second, or vice versa. Alternatively, the inventive TCR materials and the one or more additional therapeutic agents can be administered simultaneously.

[0090] For purposes of the inventive methods, wherein host cells or populations of cells are administered to the host, the cells can be cells that are allogeneic or autologous to the host. The cells may be autologous to the host.

[0091] It is contemplated that the inventive pharmaceutical compositions, TCRs, polypeptides, proteins, nucleic acids, recombinant expression vectors, host cells, or populations of cells can be used in methods of treating or preventing a disease in a host. Without being bound to a particular theory, the inventive TCRs have biological activity, e.g., ability to recognize antigen, such that the TCR (or related inventive polypeptide or protein) when expressed by a cell is able to mediate an immune response against the cell expressing the antigen for which the TCR is specific. In this regard, the invention provides a method of treating or preventing a disease in a host, comprising administering to the host any of the pharmaceutical compositions in an amount effective to treat or prevent the disease in the host.

[0092] With respect to the inventive methods, the cancer can be any cancer in which survivin is expressed, including any of acute lymphocytic cancer, acute myeloid leukemia, alveolar rhabdomyosarcoma, bone cancer, brain cancer, breast cancer, cancer of the anus, anal canal, or anorectum, cancer of the eye, cancer of the intrahepatic bile duct, cancer of the joints, cancer of the neck, gallbladder, or pleura, cancer of the nose, nasal cavity, or middle ear, cancer of the oral cavity, cancer of the vulva, chronic lymphocytic leukemia, chronic myeloid cancer, colon cancer, esophageal cancer, cervical cancer, gastrointestinal carcinoid

tumor. Hodgkin lymphoma, hypopharynx cancer, kidney cancer, larynx cancer, liver cancer, lung cancer, malignant mesothelioma, melanoma, multiple myeloma, nasopharynx cancer, non-Hodgkin lymphoma, ovarian cancer, pancreatic cancer, peritoneum, omentum, and mesentery cancer, pharynx cancer, prostate cancer, rectal cancer, renal cancer (e.g., renal cell carcinoma (RCC)), small intestine cancer, soft tissue cancer, stomach cancer, testicular cancer, thyroid cancer, ureter cancer, and urinary bladder cancer. The cancer may be melanoma.

[0093] The terms "treat," and "prevent" as well as words stemming therefrom, as used herein, do not necessarily imply 100% or complete treatment or prevention. Rather, there are varying degrees of treatment or prevention of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the inventive methods can provide any amount of any level of treatment or prevention of cancer in a mammal.

Furthermore, the treatment or prevention provided by the inventive method can include treatment or prevention of one or more conditions or symptoms of the disease, e.g., cancer, being treated or prevented. Also, for purposes herein, "prevention" can encompass delaying the onset of the disease, or a symptom or condition thereof.

[0094] Methods of testing a TCR for the ability to recognize target cells and for antigen specificity are known in the art. For instance, Clay et al., *J. Immunol.*, 163: 507-513 (1999), teaches methods of measuring the release of cytokines (e.g., interferon- γ , granulocyte/monocyte colony stimulating factor (GM-CSF), tumor necrosis factor α (TNF- α) or interleukin 2 (IL-2)). In addition, TCR function can be evaluated by measurement of cellular cytotoxicity, as described in Zhao et al., *J. Immunol.*, 174: 4415-4423 (2005). Methods of testing a TCR for the ability to recognize target cells and for antigen specificity are described herein in the Examples section.

[0095] A biopsy is the removal of tissue and/or cells from an individual. Such removal may be to collect tissue and/or cells from the individual in order to perform experimentation on the removed tissue and/or cells. This experimentation may include experiments to determine if the individual has and/or is suffering from a certain condition or disease-state. The condition or disease may be, e.g., cancer.

[0096] With respect to the inventive method of detecting a diseased cell in a host, the sample comprising cells of the host can be a sample comprising whole cells, lysates thereof, or a fraction of the whole cell lysates, e.g., a nuclear or cytoplasmic fraction, a whole protein

fraction, or a nucleic acid fraction. If the sample comprises whole cells, the cells can be any cells of the host, e.g., the cells of any organ or tissue, including blood cells.

[0097] For purposes of the inventive detecting method, the contacting can take place *in vitro* or *in vivo* with respect to the host. The contacting may be *in vitro*.

[0098] Also, detection of the complex can occur through any number of ways known in the art. For instance, the inventive TCRs, polypeptides, proteins, nucleic acids, recombinant expression vectors, host cells, populations of cells, or antibodies, or antigen binding portions thereof, described herein, can be labeled with a detectable label such as, for instance, a radioisotope, a fluorophore (e.g., fluorescein isothiocyanate (FITC), phycoerythrin (PE)), an enzyme (e.g., alkaline phosphatase, horseradish peroxidase), and/or element particles (e.g., gold particles).

[0099] In addition to the aforescribed pharmaceutical compositions, the inventive TCR materials can be formulated as inclusion complexes, such as cyclodextrin inclusion complexes, or liposomes. Liposomes can serve to target the inventive TCR materials to a particular tissue. Liposomes also can be used to increase the half-life of the inventive TCR materials. Many methods are available for preparing liposomes, as described in, for example, Szoka et al, *Ann. Rev. Biophys. Bioeng.*, 9, 467 (1980) and U.S. Patents 4,235,871, 4,501,728, 4,837,028, and 5,019,369.

[0100] The delivery systems useful in the context of embodiments of the invention may include time-released, delayed release, and sustained release delivery systems such that the delivery of the inventive composition occurs prior to, and with sufficient time to cause, sensitization of the site to be treated. The inventive composition can be used in conjunction with other therapeutic agents or therapies. Such systems can avoid repeated administrations of the inventive composition, thereby increasing convenience to the subject and the physician, and may be particularly suitable for certain composition embodiments of the invention.

[0101] Many types of release delivery systems are available and known to those of ordinary skill in the art. They include polymer base systems such as poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Patent 5,075,109. Delivery systems also include non-polymer systems that are lipids including sterols such as cholesterol, cholesterol esters, and fatty acids or neutral fats such as mono-di-and tri-glycerides; hydrogel release systems; sylastic systems;

peptide based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include, but are not limited to: (a) erosional systems in which the active composition is contained in a form within a matrix such as those described in U.S. Patents 4,452,775, 4,667,014, 4,748,034, and 5,239,660 and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Patents 3,832,253 and 3,854,480. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

[0102] A number of transfection techniques are generally known in the art (see, e.g., Graham et al., *Virology*, 52: 456-467 (1973); Sambrook et al., *supra*; Davis et al., *Basic Methods in Molecular Biology*, Elsevier (1986); and Chu et al., *Gene*, 13: 97 (1981)). Transfection methods include calcium phosphate co precipitation (see, e.g., Graham et al., *supra*), direct micro injection into cultured cells (see, e.g., Capecchi, *Cell*, 22: 479-488 (1980)), electroporation (see, e.g., Shigekawa et al., *BioTechniques*, 6: 742-751 (1988)), liposome mediated gene transfer (see, e.g., Mannino et al., *BioTechniques*, 6: 682-690 (1988)), lipid mediated transduction (see, e.g., Feigner et al., *Proc. Natl. Acad. Sci. USA*, 84: 7413-7417 (1987)), and nucleic acid delivery using high velocity microprojectiles (see, e.g., Klein et al., *Nature*, 327: 70-73 (1987)).

[0103] One of ordinary skill in the art will readily appreciate that the inventive TCR materials of the invention can be modified in any number of ways, such that the therapeutic or prophylactic efficacy of the inventive TCR materials is increased through the modification. For instance, the inventive TCR materials can be conjugated either directly or indirectly through a linker to a targeting moiety. The practice of conjugating compounds, e.g., inventive TCR materials, to targeting moieties is known in the art. See, for instance, Wadwa et al., *J. Drug Targeting* 3: 111 (1995) and U.S. Patent No. 5,087,616. The term "targeting moiety" as used herein, refers to any molecule or agent that specifically recognizes and binds to a cell-surface receptor, such that the targeting moiety directs the delivery of the inventive TCR materials to a population of cells on which surface the receptor is expressed. Targeting moieties include, but are not limited to, antibodies, or fragments thereof, peptides, hormones, growth factors, cytokines, and any other natural or non-natural ligands, which bind to cell surface receptors (e.g., Epithelial Growth Factor Receptor (EGFR), T-cell receptor (TCR), B-cell receptor (BCR), CD28, Platelet-derived Growth Factor Receptor (PDGF), nicotinic acetylcholine receptor (nAChR), etc.). The term "linker" as used herein, refers to any agent

or molecule that bridges the inventive TCR materials to the targeting moiety. One of ordinary skill in the art recognizes that sites on the inventive TCR materials, which are not necessary for the function of the inventive TCR materials, are ideal sites for attaching a linker and/or a targeting moiety, provided that the linker and/or targeting moiety, once attached to the inventive TCR materials, do(es) not interfere with the function of the inventive TCR materials, i.e., the ability to bind to antigen, or to detect, treat, or prevent disease.

[0104] Alternatively, the inventive TCR materials can be modified into a depot form, such that the manner in which the inventive TCR materials is released into the body to which it is administered is controlled with respect to time and location within the body (see, for example, U.S. Patent No. 4,450,150). Depot forms of inventive TCR materials can be, for example, an implantable composition comprising the inventive TCR materials and a porous or non-porous material, such as a polymer, wherein the inventive TCR materials is encapsulated by or diffused throughout the material and/or degradation of the non-porous material. The depot is then implanted into the desired location within the body and the inventive TCR materials are released from the implant at a predetermined rate.

[0105] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

[0106] This example demonstrates survivin 96M peptide elicits an antigen-specific response, showing cross-reactivity to survivin 95 and survivin 96N, in HLA-A2 transgenic mice.

[0107] HLA-A2 transgenic mice (C57BL/6-Tg(HLA-A2.1)lEng/J transgenic mice; Jackson Laboratories, Bar Harbor, ME) were immunized with a vaccine consisting of 100 µg of cognate peptide (Survivin 95, 96N, or 96M (SEQ ID NOS: 9, 10, 11, respectively) purchased from New England Peptide, Gardner, MA) and 120 µg of a CD4+ helper peptide (Hepatitis B viral core 128-140, provided by Dr. Jay Berzofsky, National Cancer Institute, Bethesda, MD) emulsified in Incomplete Freund's Adjuvant (Sigma-Aldrich, St. Louis, MO) on day 0 and boosted with an identical vaccine on day 7. Animals were sacrificed on day 14 and spleens and lymph nodes were harvested. Splenocytes from animals vaccinated with Surv95, 96N, and 96M were incubated (in duplicate) overnight with 10 µM of Surv95, Surv96N, Surv96M (SEQ ID NOS: 9, 10, and 11, respectively), or E7 irrelevant/background

peptide (RAHYNIVTF (SEQ ID NO: 14); purchased from BACHEM Americas, Torrance, CA) as control. IFN- γ production was measured via ELISPOT (R&D Systems, Minneapolis, MN).

[0108] The splenocytes of two mice vaccinated with Surv95 did not show responses above background (Figure 1). The splenocytes of three mice vaccinated with Surv96N did not show responses above background (Figure 2). The splenocytes of three mice vaccinated with Surv96M did show responses above background for Surv95, Surv96N, and Surv96M (Figure 3). The lymph nodes of mice vaccinated with Surv96M also showed responses above background (Figure 4).

[0109] These data demonstrate survivin 96M peptide elicits an antigen-specific response, showing cross-reactivity to survivin 95 and survivin 96N, in HLA-A2 transgenic mice.

EXAMPLE 2

[0110] This example further demonstrates survivin 96M peptide elicits an antigen-specific response, showing cross-reactivity to survivin 96N, in HLA-A2 transgenic mice.

[0111] Three additional A2-Tg mice received the 96M vaccine of Example 1 on day 0 and 9. Splenocytes were harvested for ELISPOT and restimulated on day 20 with Surv96M *in vitro* using T2 cells pulsed with 0.01, 0.1, or 1 $\mu\text{g/ml}$ of Surv96M peptide. Effector cells were incubated overnight in the presence of 10 μM or 1 μM of Surv96N or with 10 μM Surv45 (HCPTENEPDL (SEQ ID NO: 15) purchased from New England Peptide, Gardner, MA) as an irrelevant control.

[0112] Mice immunized with Surv96M showed reactivity to Surv96N over control Surv45 (Figure 5).

[0113] These data further demonstrate survivin 96M peptide elicits an antigen-specific response, showing cross-reactivity to survivin 96N, in HLA-A2 transgenic mice.

EXAMPLE 3

[0114] This example demonstrates *in vitro* restimulation of bulk splenocytes from Surv96M-vaccinated mice generates CTLs that selectively produce IFN- γ upon survivin-peptide encounter.

[0115] Splenocytes from mice in Example 2 were restimulated *in vitro* using T2 cells pulsed with 0.01, 0.1, or 1 $\mu\text{g/ml}$ of Surv96M peptide. Following two restimulations, bulk

cells were tested in ELISA (Pierce, Rockford, IL) against target T2 cells pulsed with 1µg/ml Surv88 (control; LSVKKQFEEL (SEQ ID NO: 16) purchased from BACHEM Americas, Torrance, CA), Surv96M, or Surv96N. Mouse IFN γ production (pg/ml) was determined.

[0116] Bulk splenocytes respond to cognate peptide but not control peptide (Figure 6).

[0117] *In vitro* restimulation of bulk splenocytes from Surv96M-vaccinated mice generated CTLs that selectively produce IFN- γ upon survivin-peptide encounter.

EXAMPLE 4

[0118] This example further demonstrates *in vitro* restimulation of bulk splenocytes from Surv96M-vaccinated mice generates CTLs that selectively produce IFN- γ upon survivin-peptide encounter.

[0119] Splenocytes from mice in Example 2 were restimulated *in vitro* using T2 cells pulsed with 0.01, 0.1, or 1 µg/ml of Surv96M peptide. Following three restimulations *in vitro*, bulk splenocytes were tested in ELISA (Pierce, Rockford, IL) against irrelevant (Surv88) and cognate peptides (Surv 96N and Surv95) at 1µg/ml.

[0120] Bulk splenocytes respond to cognate peptide but not control peptide (Tables IA-1C).

[0121] Tables IA, IB, and 1C: Values are shown in pg/ml. The detection limit was 3,000 pg/ml. Higher values were determined through dilution and extrapolation using particular samples. There were 20,000 cells/well for the targets (T2 cells pulsed with peptide) as well as for the effectors (mouse splenocytes).

Table IA

| | Mouse 1 0.01 ug/ml bulk well | Mouse 2 0.01 ug/ml bulk well | Mouse 3 0.01 ug/ml bulk well |
|--|------------------------------------|------------------------------------|------------------------------------|
| T2's pulsed with Surv88 control (1 ug/ml) | 1723.315 | 2379.085 | 6237.71 |
| T2's pulsed with Surv96N (1ug/ml) | 19672.085 | 352869.41 | 9316.825 |
| T2's pulsed with Surv95 (1 ug/ml) | 6866.69 | 28141.35 | 11018.98 |

Table 1B

| | Mouse 1 0.1 ug/ml bulk well | Mouse 2 0.1 ug/ml bulk well | Mouse 3 0.1 ug/ml bulk well |
|--|-----------------------------------|-----------------------------------|-----------------------------------|
| T2's pulsed with Surv88 control (1 ug/ml) | 2562.53 | 4509.2 | 1241.855 |
| T2's pulsed with Surv96N (1ug/ml) | 105145.6 | > 50000 | 6247.365 |
| T2's pulsed with Surv95 (1 ug/ml) | 68001.25 | 36702.155 | 1860.925 |

Table 1C

| | Mouse 1 1 ug/ml bulk well | Mouse 2 1 ug/ml bulk well | Mouse 3 1 ug/ml bulk well |
|--|---------------------------------|---------------------------------|---------------------------------|
| T2's pulsed with Surv88 control (1 ug/ml) | 3121.65 | 3627.305 | 1760.395 |
| T2's pulsed with Surv96N (1ug/ml) | 34807.595 | 75810.605 | 5757.685 |
| T2's pulsed with Surv95 (1 ug/ml) | 52846.005 | 16727.12 | 5939.515 |

[0122] *In vitro* restimulation of bulk splenocytes from Surv96M-vaccinated mice generated CTLs that selectively produce IFN- γ upon survivin-peptide encounter.

EXAMPLE 5

[0123] This example demonstrates that limiting dilution cloning of CTLs generated from bulk splenocytes of Surv96M-vaccinated mice generates T cell clones with specific reactivity against Survivin96-104 peptide.

[0124] Splenocytes from mice in Example 2 were restimulated *in vitro* using T2 cells pulsed with 0.01, 0.1, or 1 $\mu\text{g/ml}$ of Surv96M peptide. After three restimulations *in vitro*, effector cells from Mouse 2 of Example 4 were plated at 10 cells/well for limiting dilution cloning along with 50,000 C57B1/6 feeder cells and 50,000 peptide-pulsed T2 cells/well for the three peptide concentrations (0.01 $\mu\text{g/ml}$, 0.1 $\mu\text{g/ml}$, and 1 $\mu\text{g/ml}$). For the three conditions, 600 wells were plated in 96-well plates. The growth positive clones were expanded into 48-well plates.

[0125] Table 2 shows the number of growth positive wells in the 96-well experiments.

[0126] Table 2

| Peptide Concentration | No. Growth Positive Wells |
|----------------------------|---------------------------|
| 0.01 $\mu\text{g/ml}$ bulk | 11 |
| 0.1 $\mu\text{g/ml}$ bulk | 92 |
| 1 $\mu\text{g/ml}$ bulk | 33 |

[0127] From the clones that were expanded into a 48-well plate, 66 expanded well. Sixteen of these 66 representative clones are displayed in Table 3. The table represents mouse IFN- γ production (pg/ml) measured with ELISA (pierce, Rockford, IL). Clones showed low background IFN- γ levels and robust responses against T2's pulsed with either Surv96M or Surv96N. These CTLs were CD8+.

[0128] Table 3: Values are shown in pg/ml. The detection limit was 3,000 pg/ml. Higher values were determined through dilution and extrapolation using particular samples.

| T2 Targets 20,000 cells/well | | | | | |
|------------------------------|------------------------------|-----------------------------------|-------------------------------------|-------------------------------------|-------------------------------|
| Clone Name | Background (Effectors Alone) | T2's pulsed with Surv88 (1 ug/ml) | T2's pulsed with Surv 96M (1 ug/ml) | T2's pulsed with Surv 96N (1 ug/ml) | Bulk Culture Source (Mouse 2) |
| S1 I | 502 | 476 | > 10000 | > 10000 | 0.1 |
| S1 J | 50 | 42 | > 10000 | > 10000 | 0.1 |
| S1 L | 99 | 77 | > 10000 | > 10000 | 0.1 |
| S1 P | 69 | 66 | > 10000 | > 10000 | 0.1 |
| S2 A | 250 | 368 | > 10000 | > 10000 | 0.1 |
| S2 B | 486 | 701 | > 10000 | > 10000 | 0.1 |
| S2 E | 91 | 132 | > 10000 | > 10000 | 0.1 |
| S2 G | 178 | 212 | > 10000 | > 10000 | 0.1 |
| S2 I | 769 | 771 | 9607 | > 10000 | 0.1 |
| S2 K | 446 | 509 | > 10000 | > 10000 | 0.1 |
| S2 R | 221 | 225 | 147052 | > 10000 | 0.1 |
| S3 L | 411 | 404 | 36619 | 7016 | 0.1 |
| S3 R | 1814 | 1757 | 409777 | > 10000 | 0.1 |
| S7 A | 733 | 641 | > 10000 | > 10000 | 0.01 |
| S8 D | 173 | 143 | 31784 | 2129 | 1 |
| S8 O | 2265 | 1750 | 28544 | 46838 | 1 |

[0129] These data show that limiting dilution cloning of CTLs generated from bulk splenocytes of Surv96M-vaccinated mice generated T cell clones with specific reactivity against Survivin96-104 peptide.

EXAMPLE 6

[0130] This example demonstrates that the CTL clones of CTLs generated from bulk splenocytes of Surv96M-vaccinated mice bind Surv96M and Surv96N tetramers.

[0131] The S2E, S3L, and S8D clones of Example 5 were costained with CD8 tetramer (BD Bioscience, San Jose, CA) and Surv96M tetramer (Prolimmune, Bradenton, FL) or irrelevant/control tetramer (CYPI 90; Prolimmune). The S8O clone of Example 5 was costained with CD8 and Surv96N tetramer or irrelevant/control tetramer (CYPI 90). Stained cells were analyzed on a FACSCaliber (BD Bioscience) and data were analyzed using Flowjo software.

[0132] The experiments showed that the S2E, S3L, and S8D CTL clones bind to Surv96M tetramer, and the S8O CTL clone binds to Surv96N tetramer, but neither bind to control.

[0133] These data show that CTL clones of CTLs generated from bulk splenocytes of Surv96M-vaccinated mice bind Surv96M and Surv96N tetramers.

EXAMPLE 7

[0134] This example demonstrates that CTL clones of CTLs generated from bulk splenocytes of Surv96M-vaccinated mice show reactivity against a human pancreatic cancer cell line.

[0135] CTL clones of Example 5 were tested against Pane 1837 A2+ tumor cells or Pane 1990 A2- or 888 A2- control cells. Clones and targets were co-cultured for 36 hours and ELISA (pierce, Rockford, IL) was used to measure mouse IFN- γ .

[0136] In two separate assays, the CTL clones recognized the pancreatic cancer cell line 1837 A2+ but not the A2- control (Figure 7). A third co-culture was performed and three new clones showed reactivity against Pane 1837 A2+ but not control; clone S2I again showed reactivity (Figure 8).

[0137] CTL clones of CTLs generated from bulk splenocytes of Surv96M-vaccinated mice show reactivity against a human pancreatic cancer cell line.

EXAMPLE 8

[0138] This example demonstrates the determination of the sequences of CTL clones of CTLs generated from bulk splenocytes of Surv96M-vaccinated mice.

[0139] Total RNAs were extracted from CTL clones of Example 5 using RNeasy mini kit (Qiagen, Valencia, CA). RACE-ready cDNAs were synthesized using Smart RACE Ready cDNA kit (Clontech, Mountain View, CA). 5' RACE fragment of TCR alpha and beta chains were amplified using SMART oligo and primers from constant region of TCR alpha and beta chains (Clontech). The PCR fragments were cloned into PCR4-Topo vector (Invitrogen, Carlsbad, CA) and sequenced.

[0140] The SEQ ID NOS: 1-8 were determined using the methods described.

[0141] The determination of the sequences of CTL clones of CTLs generated from bulk splenocytes of Surv96M-vaccinated mice was shown in this example.

EXAMPLE 9

[0142] This example demonstrates production of cells transduced with survivin construct TCRs and the reactivity of the TCRs against cancer cell lines.

[0143] The sequences for TCRs of reactive CTLs from mice vaccinated with survivin 96M were cloned and inserted into a retrovirus vector (pMSGV1) incorporating 2A linker

peptides between the coding sequences of the α - and β - chains of the TCRs. The TCR sequences were TCR2 (SEQ ID NO: 17) and TCR3 (SEQ ID NO: 18), which are the GSGP2A constructs. TCR2 contains the α chain of SEQ ID NO: 7, the 2A linker of SEQ ID NO: 19, and the β chain of SEQ ID NO: 4. TCR3 contains the α chain of SEQ ID NO: 8, the 2A linker of SEQ ID NO: 19, and the β chain of SEQ ID NO: 6. Retroviral supernatants were produced by transient transfection of 293-GP cells with the vectors. The supernatants were used to transduce PBL cells. Seven days post-transduction, the PBLs were stained using standard FACS staining methods, first gated against CD8 (using an antibody purchased from BD Biosciences, San Jose, CA), and stained for human survivin 96M tetramer (using an antibody purchased from Beckman Coulter, Brea, CA) and CMV. Expression was analyzed via flow cytometry. Mock transductions were performed using vector without any construct inserted. The percentage of mouse TCRV β positive cells was used to indicate transduction efficiency. Both TCR2 and TCR3 showed expression of the survivin tetramer and a high transduction efficiency, whereas the mock transduction and control (CMV) showed no expression.

[0144] A CDI 07a degranulation assay was also performed. Seven to ten days after TCR transduction, PBMCs transduced with the GSGP2A construct vectors were incubated with targets (peptide-pulsed T2 cells or SB tumor cell line) at an effector-to-target (E:T) ratio of 1:2 for 4 hours at 37°C in 5% CO₂. Subsequently, the cells were stained with PE-coupled CD 107a mAb and APC-Cy7-coupled CD8 mAb (BD Biosciences) to measure degranulation. Survivin 96M-TCR-transduced PBLs showed antigen-specific degranulation and killing activity.

[0145] In another experiment, PBLs were transduced with supernatant from retroviral constructs with TCRs generated against surv96M. Fourteen days post-transduction, the PBL were co-cultured with HLA-A2+ and HLA-A2- pediatric sarcoma cell lines (Figure 9) and T2 cells pulsed with different concentrations of survivin 96N or control peptides (E7 and CMV) (Figure 10). Antigen-specific IFN- γ secretion was determined by ELISA. Figure 10 shows that there was little reactivity against T2 cells that were unpulsed or against T2 cells pulsed with the control peptides but substantial reactivity with T2 cells pulsed with the survivin peptide with diminishing reactivity as peptide concentration diminished. Transduction of survivin specific TCR retrovirus constructs into human PBL yielded up to 70% TCR redirected lymphocytes.

[0146] Production of cells transduced with survivin construct TCRs and their reactivity against cancer cell lines were illustrated in this Example.

[0147] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0148] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0149] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

CLAIM(S):

1. An isolated or purified T cell receptor (TCR) comprising one or more amino acid sequences encoded by one or more of SEQ ID NOS: 1-8.
2. The isolated or purified TCR of claim 1, comprising a first polypeptide comprising an amino acid sequence encoded by SEQ ID NO: 1 and a second polypeptide comprising an amino acid sequence encoded by SEQ ID NO: 2.
3. The isolated or purified TCR of claim 1, comprising a first polypeptide comprising an amino acid sequence encoded by SEQ ID NO: 3 and a second polypeptide comprising an amino acid sequence encoded by SEQ ID NO: 4.
4. The isolated or purified TCR of claim 1, comprising a first polypeptide comprising an amino acid sequence encoded by SEQ ID NO: 5 and a second polypeptide comprising an amino acid sequence encoded by SEQ ID NO: 6.
5. The isolated or purified TCR of claim 1, comprising a first polypeptide comprising an amino acid sequence encoded by SEQ ID NO: 7 and a second polypeptide comprising an amino acid sequence encoded by SEQ ID NO: 4.
6. The isolated or purified TCR of claim 1, comprising a first polypeptide comprising an amino acid sequence encoded by SEQ ID NO: 8 and a second polypeptide comprising an amino acid sequence encoded by SEQ ID NO: 6.
7. The isolated or purified TCR of any of claims 1-6, further comprising a linker sequence between the first and second polypeptides.
8. The isolated or purified TCR of claim 7, wherein the linker sequence is SEQ ID NO: 19.
9. An isolated or purified polypeptide comprising an amino acid sequence encoded by any of SEQ ID NOS: 1-8.
10. An isolated or purified nucleic acid comprising a nucleotide sequence encoding the TCR of any of claims 1-8 or the polypeptide of claim 9.

11. The isolated or purified nucleic acid of claim 10, wherein the nucleotide sequence comprises one or more of SEQ ID NOS: 1-8.
12. The isolated or purified nucleic acid of claim 10 or 11, wherein the nucleotide sequence comprises SEQ ID NO: 1 and SEQ ID NO: 2.
13. The isolated or purified nucleic acid of claim 10 or 11, wherein the nucleotide sequence comprises SEQ ID NO: 3 and SEQ ID NO: 4.
14. The isolated or purified nucleic acid of claim 10 or 11, wherein the nucleotide sequence comprises SEQ ID NO: 5 and SEQ ID NO: 6.
15. The isolated or purified nucleic acid of claim 10 or 11, wherein the nucleotide sequence comprises SEQ ID NO: 7 and SEQ ID NO: 4.
16. The isolated or purified nucleic acid of claim 10 or 11, wherein the nucleotide sequence comprises SEQ ID NO: 8 and SEQ ID NO: 6.
17. A recombinant vector comprising the nucleic acid of any of claims 10-16.
18. The recombinant vector of claim 17, which is a viral vector.
19. The recombinant vector of claim 18, wherein the viral vector is a retroviral vector.
20. A host cell comprising the recombinant expression vector of any of claims 17-19.
21. The host cell of claim 20, wherein the cell is a peripheral blood lymphocyte (PBL) or a peripheral blood mononuclear cell (PBMC).
22. The host cell of claim 21, wherein the PBL or PMBC is a CD8⁺ T cell or a CD4⁺ T cell.
23. A population of cells comprising at least one cell of any of claims 20-22.
24. An antibody, or antigen binding portion thereof, which specifically binds to a TCR of any of claims 1-8.

25. A composition comprising the TCR of any of claims 1-8 and a pharmaceutically acceptable carrier.
26. A composition comprising the nucleic acid of any of claims 10-16 and a pharmaceutically acceptable carrier.
27. A composition comprising the recombinant vector of any of claims 17-19 and a pharmaceutically acceptable carrier.
28. Use of an effective amount of the TCR of any of claims 1-8 in the manufacture of a medicament for treating cancer in a host.
29. Use of an effective amount of the nucleic acid of any of claims 10-16 in the manufacture of a medicament for treating cancer in a host.
30. Use of an effective amount of the recombinant vector of any of claims 17-19 in the manufacture of a medicament for treating cancer in a host.
31. Use of an effective amount of the population of cells of claim 23 in the manufacture of a medicament for treating cancer in a host.
32. A method of detecting the presence of cancer in a host, comprising:
- (i) contacting a sample comprising one or more cells from the host with the TCR of any of claims 1 to 8, the polypeptide of any of claim 9, the nucleic acid of any of claims 10 to 16, the recombinant expression vector of claim 17 to 19, the host cell of any of claims 20 to 22, the population of cells of claim 23, or the antibody, or antigen binding portion thereof, of claim 24, thereby forming a complex, and
 - (ii) detecting the complex,
- wherein detection of the complex is indicative of the presence of cancer in the host.

Figure 1

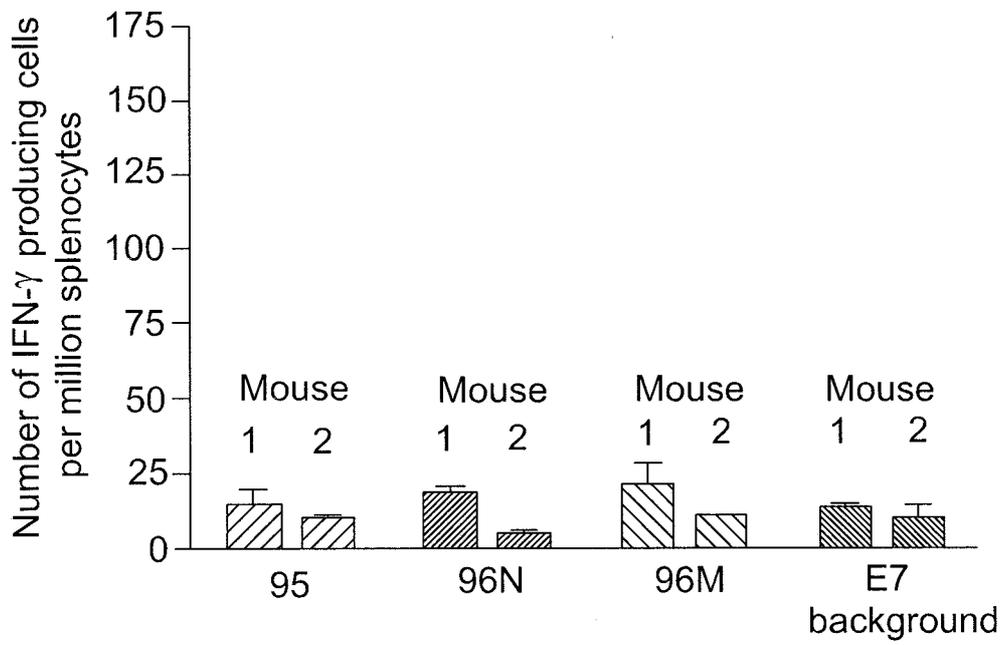


Figure 2

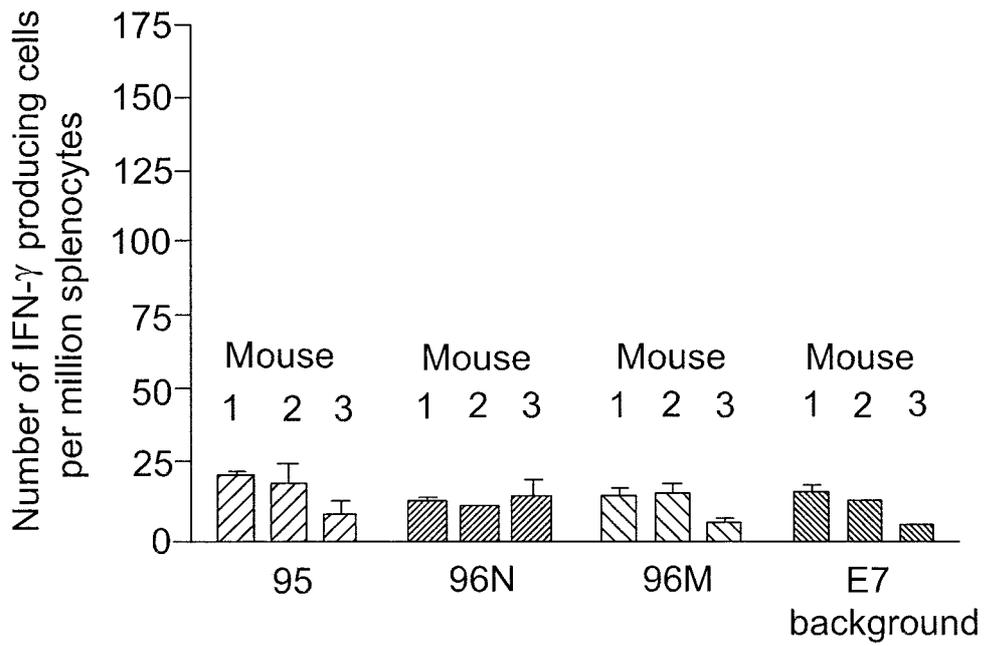


Figure 3

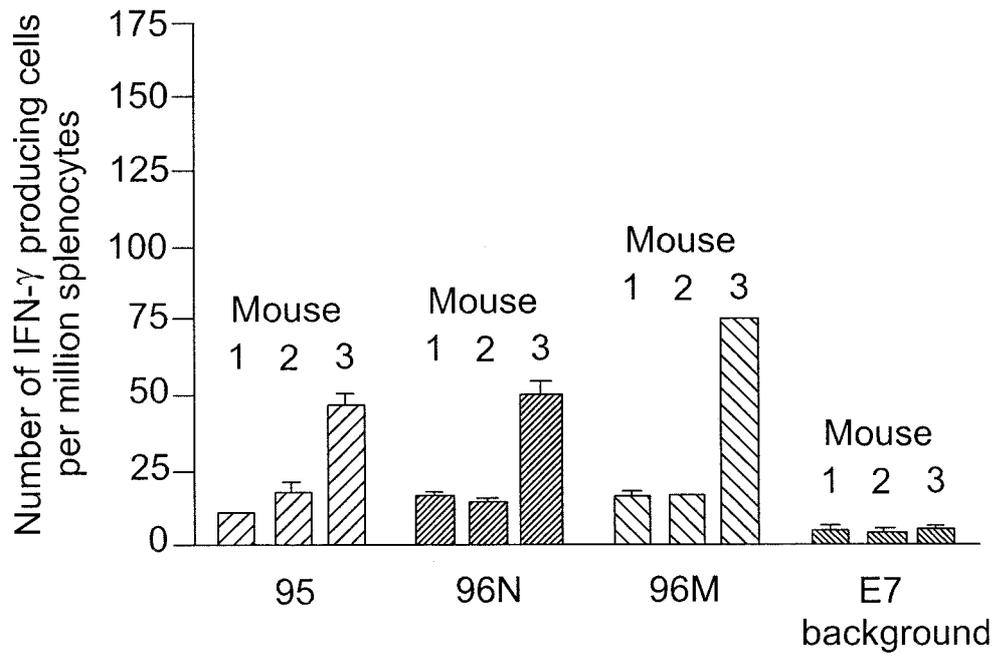


Figure 4

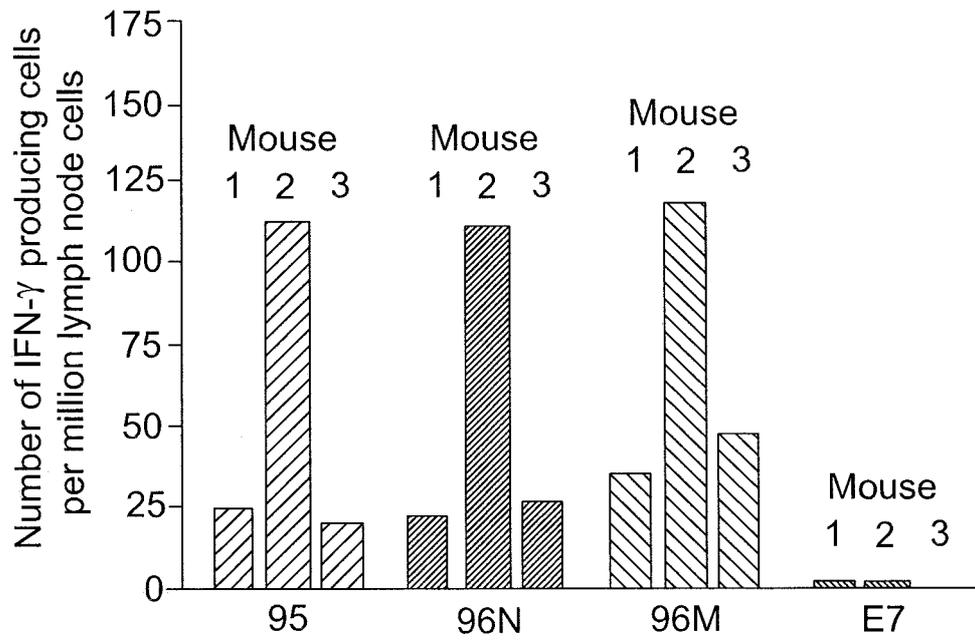


Figure 5

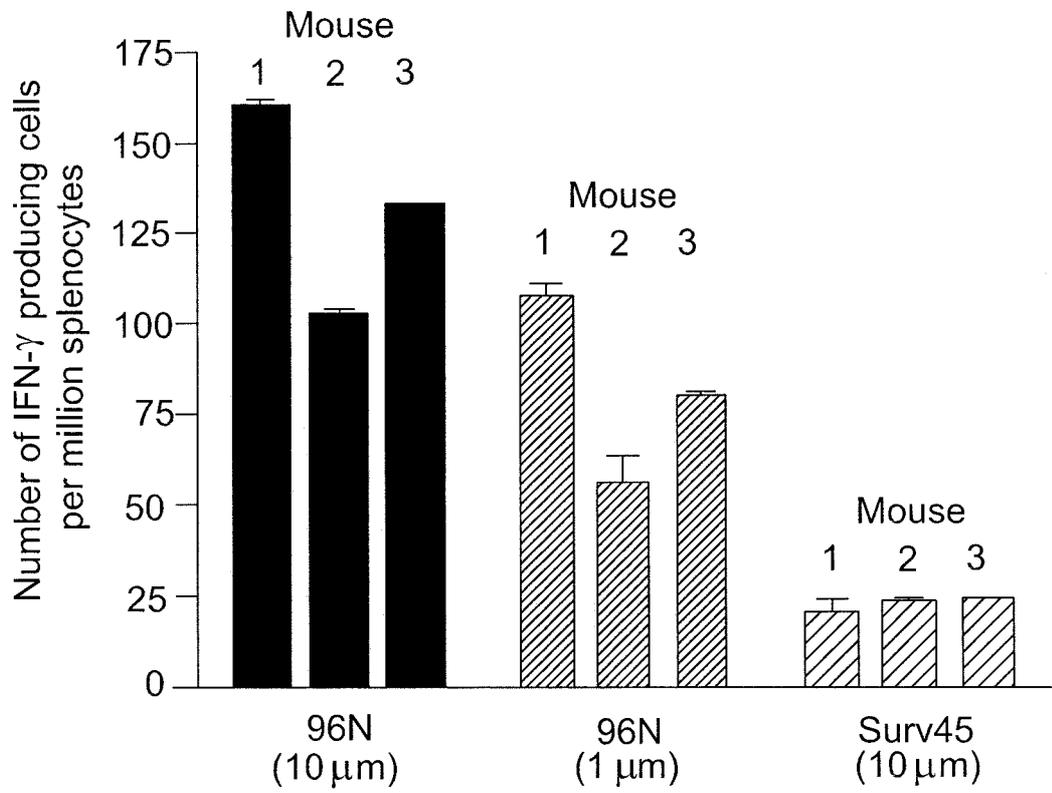


Figure 6

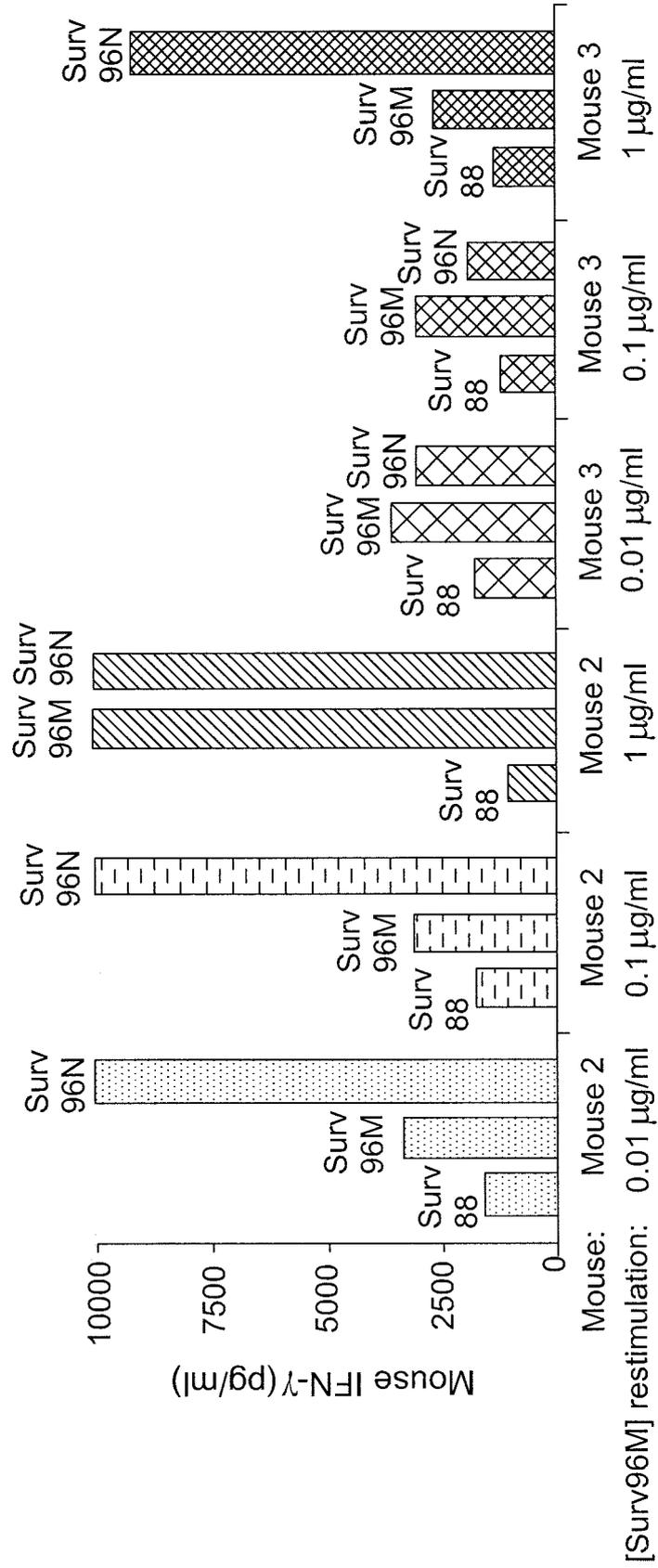


Figure 7

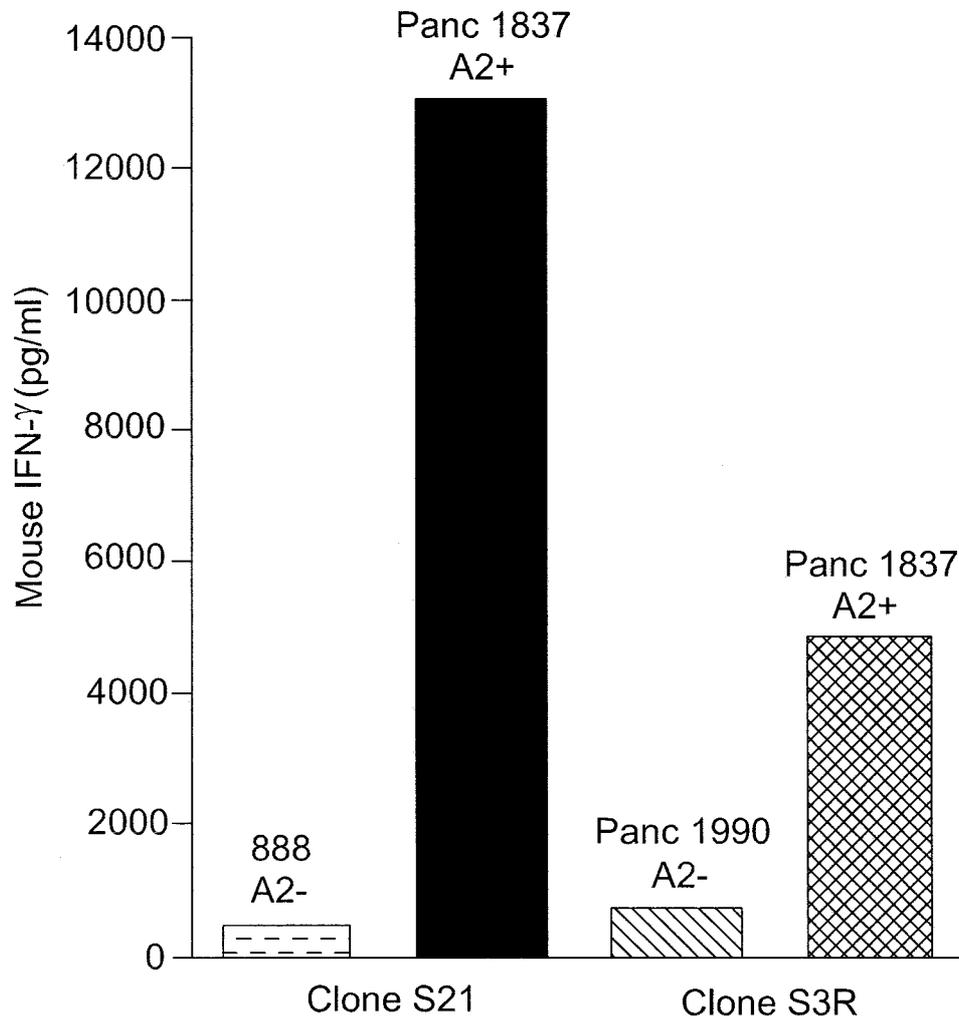
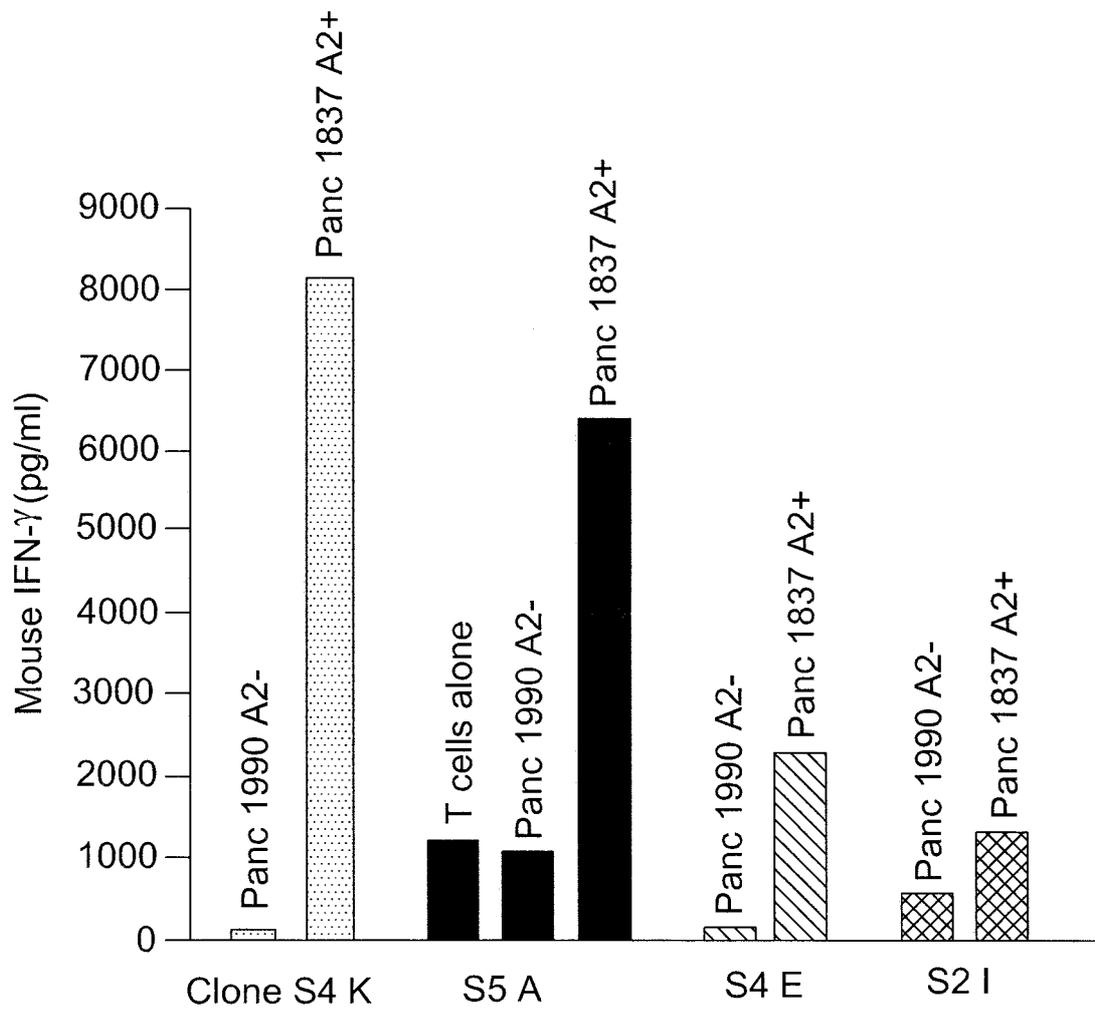
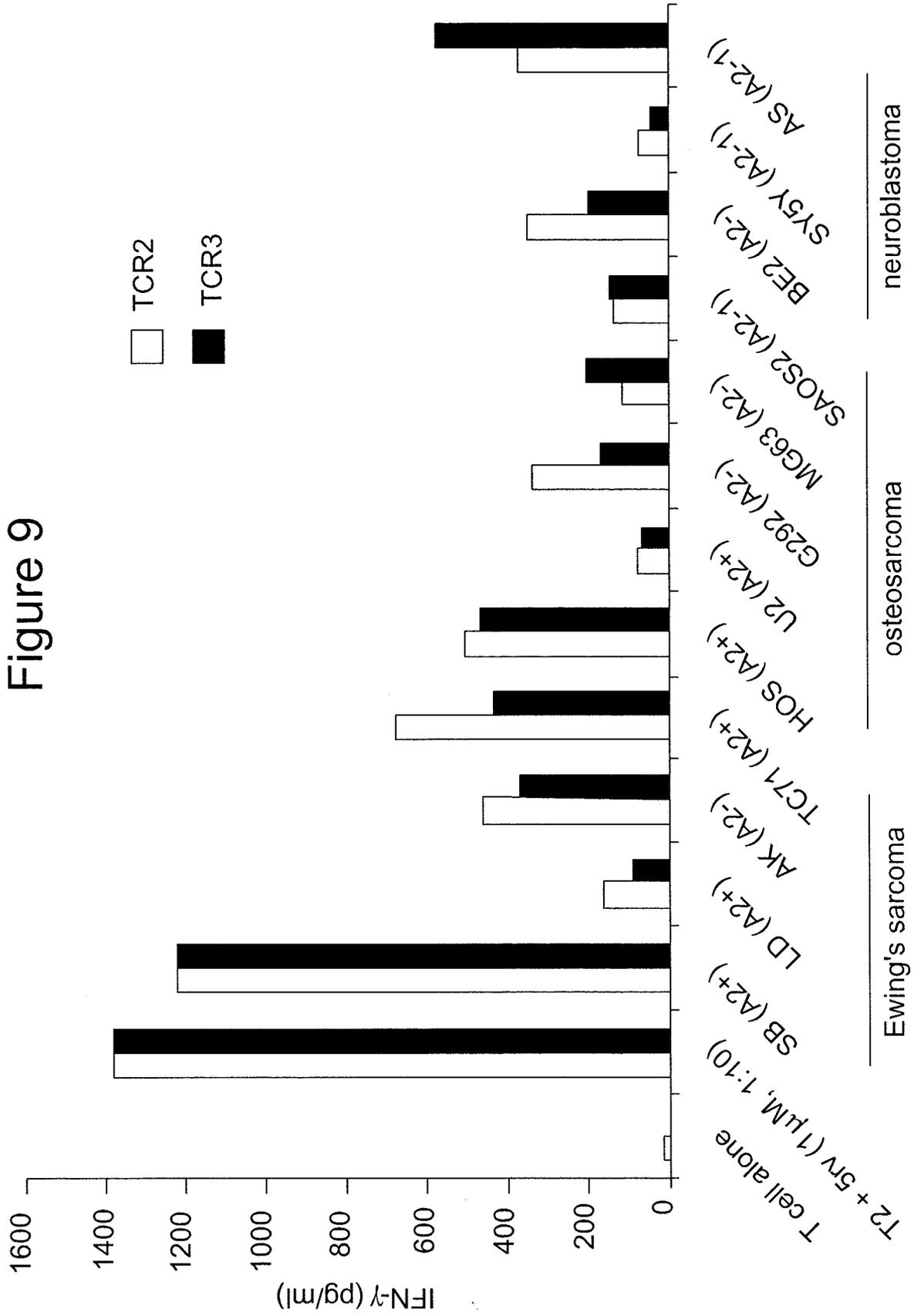


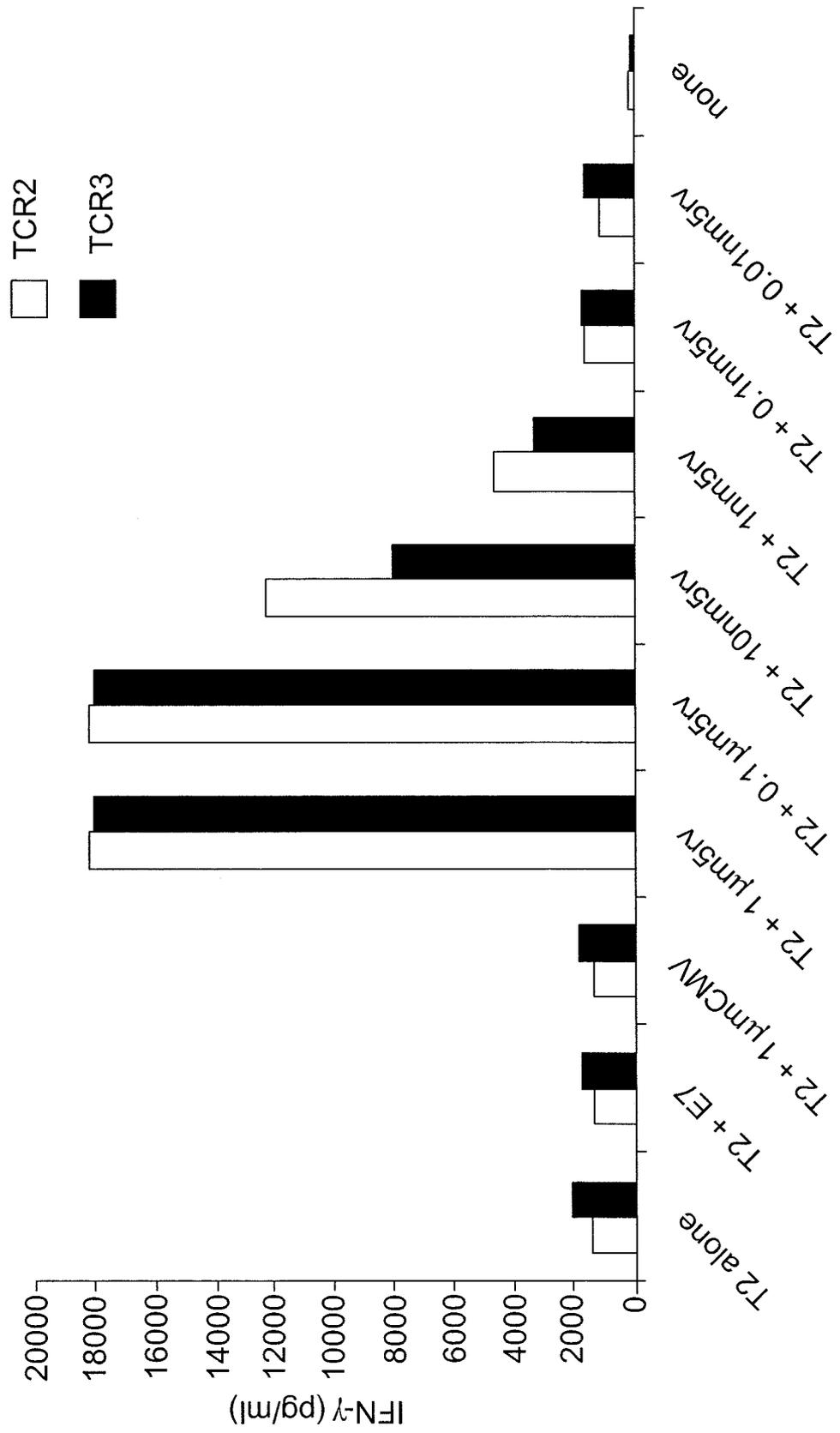
Figure 8





10/10

Figure 10



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2009/069248

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N15/12 C07K14/705 C12N15/63 C12N5/10 C07K16/28
A61K38/17 A61K31/713 G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal , Sequence Search, BIOSIS, EMBASE, MEDLINE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No |
|------------|--|----------------------|
| Y | <p>CASATI C ET AL: "The apoptosis inhibitor protein survivin induces tumor-specific CD8+ and CD4+ T cells in colorectal cancer patients"</p> <p>CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD., US, vol . 63, no. 15, 1 August 2003 (2003-08-01), pages 4507-4515, XP002385073</p> <p>ISSN: 0008-5472</p> <p>abstract</p> <p>page 4507., right-hand column., paragraph 3</p> <p>page 4511., right-hand column, paragraph 2</p> <p>page 4512., left-hand column, paragraph 1</p> <p>page 4513., right-hand column, paragraph 3</p> <p>page 4515., left-hand column, paragraphs 3,4</p> <p style="text-align: center;">-----</p> <p style="text-align: center;">-/-</p> | 1-32 |

Further documents are listed in the continuation of Box C

See patent family annex

Special categories of cited documents

- 'A' document defining the general state of the art which is not considered to be of particular relevance
- 'E' earlier document but published on or after the international filing date
- 'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- 'O' document referring to an oral disclosure, use, exhibition or other means
- 'P' document published prior to the international filing date but later than the priority date claimed

- 'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- 'X' document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- 'Y' document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- '&' document member of the same patent family

Date of the actual completion of the international search

24 February 2010

Date of mailing of the international search report

09/03/2010

Name and mailing address of the ISA/

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Fax (+31-70) 340-3016

Authorized officer

Tudor, Mark

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2 0 0 9/0 6 924 8

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

- 1 With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of
- a type of material
- a sequence listing
- table(s) related to the sequence listing
- b format of material
- on paper
- in electronic form
- c time of filing/furnishing
- contained in the international application as filed
- filed together with the international application in electronic form
- furnished subsequently to this Authority for the purpose of search
- 2 In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished
- 3 Additional comments

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2009/069248

| C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT | | |
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| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| Y | <p>DATABASE UniProt [Online] 20 February 2007 (2007-02-20), "SubName: Full=Cytotoxic Tcell receptor; Flags: Precursor;" XP002570247 retrieved from EBI accession no. UNIPROT:A2NTU7 Database accession no. A2NTU7 abstract -& SAITO H ET AL: "A 3RD REARRANGED AND EXPRESSED GENE IN A CLONE OF CYTOTOXIC T LYMPHOCYTES" NATURE (LONDON), vol. 312, no. 5989, 1984, pages 36-40, XP002570245 ISSN: 0028-0836</p> <p style="text-align: center;">-----</p> | 1-32 |
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V-

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2009/069248

| C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT | | |
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| Y | <p>XING ZHENG ET AL: "Essential role of survivin, an inhibitor of apoptosis protein, in T cell development, maturation, and homeostasis." JOURNAL OF EXPERIMENTAL MEDICINE, vol. 199, no. 1, 5 January 2004 (2004-01-05), pages 69-80, XP002570246 ISSN: 0022-1007 page 69 - page 80</p> <p style="text-align: center;">-----</p> | 1-32 |

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Information on patent family members

International application No

PCT/US2009/069248

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