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(54) **Title:** COMPOSITIONS AND METHODS FOR THE THERAPY AND DIAGNOSIS OF CANCER

(57) **Abstract:** Compositions and methods for the therapy and diagnosis of cancer are disclosed. For example, illustrative compositions comprise one or more cancer-associated antibodies, polypeptides, polynucleotides, antigen presenting cells, and the like. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly cancer.

COMPOSITIONS AND METHODS FOR THE THERAPY
AND DIAGNOSIS OF CANCER

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 61/583,033, filed January 4, 2012; U.S. Provisional Application No. 61/547,342, filed October 14, 2011; and U.S. Provisional Application No. 61/499,534, filed June 21, 2011, each of which is incorporated by reference herein in its entirety.

STATEMENT REGARDING SEQUENCE LISTING

The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is ONCF_001_03WO_ST25.txt. The text file is 88 KB, was created on June 19, 2012, and is being submitted electronically via EFS-Web.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates generally to therapy and diagnosis of cancer. The invention is more specifically related to pharmaceutical and diagnostic compositions comprising antibodies and antigen-binding fragments that specifically bind to cancer-associated proteins (*e.g.*, oncofactors). The invention further relates to pharmaceutical and diagnostic compositions comprising cancer-associated polynucleotides, polypeptides, expression vectors, host cells and the like.

Description of the Related Art

Cancer is a significant health problem throughout the world. Although advances have been made in detection and therapy of cancer, no universally successful method for prevention and/or treatment is currently available. Current therapies, which are generally based on a combination of chemotherapy, surgery and/or radiation, are relatively non-selective and continue to prove inadequate in many patients.

Chemotherapy, in particular, results in numerous side effects, in some cases so severe as to limit the dosage that can be given and thus preclude the use of potentially effective drugs. Moreover, cancers often develop resistance to chemotherapeutic drugs.

In spite of considerable research, there remain great obstacles in effective diagnosis and treatment of many human cancer types. Accordingly, there remains a need in the art for alternative and improved methods for detecting and treating such cancers. The present invention fulfills these needs and further provides other related advantages.

SUMMARY OF THE INVENTION

According to one aspect of the present invention, there is provided a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an isolated antibody or antigen-binding fragment thereof that specifically binds a sequence selected from the group consisting of IL1f5 (SEQ ID NO: 1), CCBP2 (SEQ ID NO: 2), IL1R2 (SEQ ID NO: 3), IL1RAPL1 (SEQ ID NO: 4), IL18BP (SEQ ID NO: 5), CLEC2B (SEQ ID NO: 6), C4BPA (SEQ ID NO: 7), C4BPB (SEQ ID NO: 8), SERPINI1 (SEQ ID NO: 9), IL1RAP isoform 1 (SEQ ID NO: 10), IL1RAP isoform 2 (SEQ ID NO: 11), GPR1 (SEQ ID NO: 12), GPR4 (SEQ ID NO: 13), GPR15 (SEQ ID NO: 14), GPR32 (SEQ ID NO: 15), GPR34 (SEQ ID NO: 16), GPR183 (SEQ ID NO: 17), SERPINA4 (SEQ ID NO: 18), SERPINB5 (SEQ ID NO: 19), SEMA4B (SEQ ID NO: 20), SEMA4D (SEQ ID NO: 21), CCL14 (SEQ ID NO: 22), NKTR (SEQ ID NO: 23) and SFTPD (SEQ ID NO: 24). In another aspect, an endotoxin-free pharmaceutical composition comprising a pharmaceutically acceptable carrier and an isolated antibody or antigen-binding fragment thereof that specifically binds a sequence selected from the group consisting of IL1f5 (SEQ ID NO: 1), CCBP2 (SEQ ID NO: 2), IL1R2 (SEQ ID NO: 3), IL1RAPL1 (SEQ ID NO: 4), IL18BP (SEQ ID NO: 5), CLEC2B (SEQ ID NO: 6), C4BPA (SEQ ID NO: 7), C4BPB (SEQ ID NO: 8), SERPINI1 (SEQ ID NO: 9), IL1RAP isoform 1 (SEQ ID NO: 10), IL1RAP isoform 2 (SEQ ID NO: 11), GPR1 (SEQ ID NO: 12), GPR4 (SEQ ID NO: 13), GPR15 (SEQ ID NO: 14), GPR32 (SEQ ID NO: 15), GPR34 (SEQ ID NO: 16), GPR183 (SEQ ID NO: 17), SERPINA4 (SEQ ID NO: 18), SERPINB5 (SEQ ID NO: 19), SEMA4B (SEQ ID NO: 20), SEMA4D

(SEQ ID NO: 21), CCL14 (SEQ ID NO: 22), NKTR (SEQ ID NO: 23) and SFTPD (SEQ ID NO: 24) is provided.

In a further aspect, a pharmaceutical composition formulated for intravenous injection for use in a patient having or at risk for having cancer, said composition comprising a pharmaceutically acceptable carrier and an isolated antibody or antigen-binding fragment thereof that specifically binds a sequence selected from the group consisting of IL1f5 (SEQ ID NO: 1), CCBP2 (SEQ ID NO: 2), IL1R2 (SEQ ID NO: 3), IL1RAPL1 (SEQ ID NO: 4), IL18BP (SEQ ID NO: 5), CLEC2B (SEQ ID NO: 6), C4BPA (SEQ ID NO: 7), C4BPB (SEQ ID NO: 8), SERPINI1 (SEQ ID NO: 9), IL1RAP isoform 1 (SEQ ID NO: 10), IL1RAP isoform 2 (SEQ ID NO: 11), GPR1 (SEQ ID NO: 12), GPR4 (SEQ ID NO: 13), GPR15 (SEQ ID NO: 14), GPR32 (SEQ ID NO: 15), GPR34 (SEQ ID NO: 16), GPR183 (SEQ ID NO: 17), SERPINA4 (SEQ ID NO: 18), SERPINB5 (SEQ ID NO: 19), SEMA4B (SEQ ID NO: 20), SEMA4D (SEQ ID NO: 21), CCL14 (SEQ ID NO: 22), NKTR (SEQ ID NO: 23) and SFTPD (SEQ ID NO: 24).

In a particular aspect, a composition comprises one or more antibodies or antigen binding fragments thereof, wherein each of the one or more antibodies or antigen binding fragments thereof specifically bind a sequence selected from the group consisting of IL1f5 (SEQ ID NO: 1), CCBP2 (SEQ ID NO: 2), IL1R2 (SEQ ID NO: 3), IL1RAPL1 (SEQ ID NO: 4), IL18BP (SEQ ID NO: 5), CLEC2B (SEQ ID NO: 6), C4BPA (SEQ ID NO: 7), C4BPB (SEQ ID NO: 8), SERPINI1 (SEQ ID NO: 9), IL1RAP isoform 1 (SEQ ID NO: 10), IL1RAP isoform 2 (SEQ ID NO: 11), GPR1 (SEQ ID NO: 12), GPR4 (SEQ ID NO: 13), GPR15 (SEQ ID NO: 14), GPR32 (SEQ ID NO: 15), GPR34 (SEQ ID NO: 16), GPR183 (SEQ ID NO: 17), SERPINA4 (SEQ ID NO: 18), SERPINB5 (SEQ ID NO: 19), SEMA4B (SEQ ID NO: 20), SEMA4D (SEQ ID NO: 21), CCL14 (SEQ ID NO: 22), NKTR (SEQ ID NO: 23) and SFTPD (SEQ ID NO: 24).

In a certain aspect, a composition is 95%, 96%, 97%, 98%, or 99% endotoxin free.

In a more specific embodiment of the invention, an isolated antibody or antigen-binding fragment of the invention is a monoclonal antibody or antigen-binding

fragment.

In another specific embodiment of the invention, an isolated antibody or antigen-binding fragment of the invention is a humanized antibody or antigen-binding fragment.

In still another specific embodiment, an antibody or antigen binding fragment of the invention is conjugated to a toxin, including, without limitation, a ricin toxin, abrin toxin, diphtheria toxin, cholera toxin, gelonin toxin, Pseudomonas exotoxin, Shigella toxin, and pokeweed antiviral protein.

In another specific embodiment, an antibody or antigen-binding fragment of the invention is conjugated to a radionuclide, including, without limitation, ^{90}Y , ^{123}I , ^{125}I , ^{131}I , ^{186}Re , ^{188}Re , ^{211}At and ^{212}Bi .

According to another aspect of the invention, there is provided a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an isolated polypeptide of any one of SEQ ID NOs: 1-24, or a fragment or variant thereof having at least 70%, 80%, 90% or 95% identity thereto, or an isolated polynucleotide encoding any one of the foregoing polypeptides. Of course, it will be recognized that additional components may be present in the pharmaceutical compositions of the invention, such as immunostimulants and the like. It will also be recognized, in the context of embodiments employing polynucleotides of the invention that the polynucleotides may be present, for example, in expression vectors, host cells and the like.

According to another aspect of the invention, there is provided a method for the treatment of cancer in a subject in need thereof comprising administering to the subject a pharmaceutical composition as described according to the present invention. The cancer to be treated can be essentially any cancer type with which a sequence of the invention is associated, including, without limitation, cancers of the liver, pancreas, lung, breast, bladder, kidney, and skin (*e.g.*, melanoma), as well as hematological cancers (*e.g.*, leukemia, lymphoma, *etc.*).

The present invention, in another aspect, provides methods relating to the use of an isolated antibody or antigen-binding fragment that specifically binds to a sequence set forth in any one of SEQ ID NOs: 1-24, in the manufacture of a

medicament for the treatment of cancer.

The present invention, in another aspect, provides methods relating to the use of an isolated polypeptide comprising a sequence set forth in any one of SEQ ID NOs: 1-24, or a fragment or variant thereof having at least 90% identity thereto, in the manufacture of a medicament for the treatment of cancer.

The present invention, in another aspect, provides methods relating to the use of an isolated polynucleotide encoding an amino acid sequence set forth in any one of SEQ ID NOs: 1-24, or encoding a fragment or variant of a sequence set forth in SEQ ID NOs: 1-24 having at least 90% identity thereto, in the manufacture of a medicament for the treatment of cancer.

The present invention, in another aspect, provides methods relating to the use of an oligonucleotide that is complementary to a polynucleotide encoding an amino acid sequence set forth in any one of SEQ ID NOs: 1-24, or encoding a fragment or variant of a sequence set forth in SEQ ID NOs: 1-24 having at least 90% identity thereto, in the manufacture of a medicament for the treatment of cancer. In certain embodiments, the oligonucleotide is an antisense oligonucleotide, an RNAi molecule, a ribozyme, or another inhibitory nucleic acid molecule.

According to yet another aspect of the present invention, there is provided a method for detecting the presence of a cancer in a patient, comprising the steps of: (a) obtaining a biological sample from the patient; (b) contacting the biological sample with an antibody or antigen-binding fragment that specifically binds to a polypeptide of any one of SEQ ID NOs: 1-24; (c) detecting in the sample an amount of polypeptide that binds to the antibody or antigen-binding fragment; and (d) comparing the amount of polypeptide to a predetermined cut-off value and therefrom determining the presence of a cancer in the patient.

According to still another aspect of the invention, there is provided a method for detecting the presence of a cancer in a patient, comprising the steps of: (a) obtaining a biological sample from the patient; (b) contacting the biological sample with an oligonucleotide that hybridizes to a polynucleotide, wherein the polynucleotide encodes a polypeptide of any one of SEQ ID NOs: 1-24, or encodes a fragment or variant thereof having at least 90% identity thereto; (c) detecting in the sample an

amount of a polynucleotide that hybridizes to the oligonucleotide; and (d) comparing the amount of polynucleotide that hybridizes to the oligonucleotide to a predetermined cut-off value, and therefrom determining the presence of the cancer in the patient.

In still another aspect, the present invention provides a diagnostic kit comprising at least one isolated antibody or antigen-binding fragment thereof that specifically binds to a sequence of any one of SEQ ID NOs: 1-24 and a detection reagent, wherein the detection reagent comprises a reporter group.

In still another aspect, the present invention provides a diagnostic kit comprising at least one oligonucleotide that hybridizes to a polynucleotide, wherein the polynucleotide encodes a polypeptide of any one of SEQ ID NOs: 1-24, or encodes a fragment or variant thereof having at least 90% identity thereto. In one aspect, a method is provided for treating a cancer in a patient, comprising the steps of: (a) detecting an amount of polypeptide of any one of SEQ ID NOs: 1-24 in a biological sample of a patient; (b) comparing the amount of the polypeptide to a predetermined cut-off value and therefrom determining the presence of a cancer in the patient; and (c) administering a composition according to any one of claims 1 to 14 to a subject determined to have cancer in step (b).

In a certain aspect, a method is provided for treating a cancer in a patient, comprising the steps of: (a) obtaining a biological sample from the patient; (b) contacting the biological sample with an oligonucleotide that hybridizes to a polynucleotide, wherein the polynucleotide encodes a polypeptide of any one of SEQ ID NOs: 1-24, or encodes a fragment or variant thereof having at least 90% identity thereto; (c) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide; (d) comparing the amount of polynucleotide that hybridizes to the oligonucleotide to a predetermined cut-off value, and therefrom determining the presence of the cancer in the patient; and (e) administering a composition according to any one of claims 1 to 14 to a subject determined to have cancer in step (d).

In another aspect, the cancer is selected from liver cancer, pancreatic cancer, lung cancer, breast cancer, bladder cancer, kidney cancer, skin cancer and hematological cancer. These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings.

All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the results from a representative mixed tumor lymphocyte culture (MTLC) assay. Raji B lymphoma cells were cocultured with PBMCs and treated with various concentrations of recombinant IL1f5 polypeptide.

Figure 2 shows the results from a representative MTLC assay. Raji B lymphoma cells were cocultured with PBMCs and treated with various concentrations of recombinant IL1RAP2 polypeptide.

Figure 3 shows the results from a representative MTLC assay. Raji B lymphoma cells were cocultured with PBMCs and treated with various concentrations of recombinant CCL14 polypeptide.

Figure 4 shows the results from a representative immunohistochemistry (IHC) assay. IL1f5 expression in invasive ductal carcinoma breast cancer cells is increased compared to IL1f5 expression in normal ductal epithelium, vessels, and stroma.

Figure 5 shows the results from a representative IHC assay. IL1f5 expression in adenocarcinoma colon cancer cells is increased compared to IL1f5 expression in normal colonic tissue.

Figure 6 shows the results from a representative IHC assay. IL1f5 expression in adenocarcinoma prostate cancer cells is increased compared to IL1f5 expression in normal prostate gland and stroma.

Figure 7 shows the results from a representative IHC assay. IL1f5 expression in squamous cell carcinoma, adenocarcinoma, and papillary adenocarcinoma lung cancer cells is increased compared to IL1f5 expression in normal alveolar tissue.

Figure 8 shows the results from a representative IHC assay. GPR183 expression in invasive ductal carcinoma breast cancer cells is increased compared to GPR183 expression in normal ductal epithelium, vessels, and stroma.

Figure 9 shows the results from a representative IHC assay. GPR183 expression in adenocarcinoma colon cancer cells is increased compared to GPR183 expression in normal colonic tissue.

Figure 10 shows the results from a representative IHC assay. GPR183 expression in squamous cell carcinoma and adenocarcinoma lung cancer cells is increased compared to GPR183 expression in normal alveolar tissue.

Figure 11 shows the results from a representative IHC assay. IL1RAP expression in invasive ductal carcinoma breast cancer cells is increased compared to IL1RAP expression in normal breast ductal epithelium, vessels, and stroma.

Figure 12 shows the results from a representative IHC assay. IL1RAP expression in lung cancer cells is increased compared to IL1RAP expression in normal lung alveoli.

Figure 13 shows the results from a representative IHC assay. CCL14 expression in invasive ductal carcinoma breast cancer cells is increased compared to CCL14 expression in normal breast ductal epithelium, vessels, and stroma.

Figure 14 shows the results from a representative IHC assay. CCL14 expression in prostate adenocarcinoma is increased compared to CCL14 expression in normal prostate glands and stroma.

Figure 15 shows the results from a representative IHC assay. CCL14 expression in lung cancer cells is increased compared to CCL14 expression in normal lung alveoli.

Figure 16 shows the results from a representative IHC assay. SEMA4D expression in invasive ductal carcinoma breast cancer cells is increased compared to SEMA4D expression in normal breast ductal epithelium, vessels, and stroma.

Figure 17 shows the results from a representative IHC assay. IL1R2 expression in invasive ductal carcinoma breast cancer cells is increased compared to IL1R2 expression in normal breast ductal epithelium, vessels, and stroma.

Figure 18 shows the results from a representative MTLN assay. Raji B lymphoma cells were cocultured with PBMCs and treated with various concentrations of recombinant IL1R2 polypeptide.

Figure 19 shows the results from a representative T cell proliferation assay. Raji B lymphoma cells were cocultured with PBMCs and treated with various concentrations of recombinant IL1f5 polypeptide. The effect of IL1f5 on T cell proliferation was measured.

BRIEF DESCRIPTION OF THE SEQUENCE IDENTIFIERS

SEQ ID NO:1 is an amino acid sequence of the protein IL1f5 (NP_036407.1).

SEQ ID NO:2 is an amino acid sequence of the protein CCBP2 (NP_001287.2).

SEQ ID NO:3 is an amino acid sequence of the protein IL1R2 (NP_004624.1).

SEQ ID NO:4 is an amino acid sequence of the protein IL1RAPL1 (NP_055086.1).

SEQ ID NO:5 is an amino acid sequence of the protein IL18BP (NP_766630.2).

SEQ ID NO:6 is an amino acid sequence of the protein CLEC2B (NP_005118.2).

SEQ ID NO:7 is an amino acid sequence of the protein C4BPA (NP_000706.1).

SEQ ID NO:8 is an amino acid sequence of the protein C4BPB (NP_000707.1).

SEQ ID NO:9 is an amino acid sequence of the protein SERPINI1 (NP_005016.1).

SEQ ID NO:10 is an amino acid sequence of the protein IL1RAP isoform 1 (NP_002173.1).

SEQ ID NO:11 is an amino acid sequence of the protein IL1RAP isoform 2 (NP_608273.1).

SEQ ID NO:12 is an amino acid sequence of the protein GPR1 (NP_005270.2).

SEQ ID NO:13 is an amino acid sequence of the protein GPR4
(NP_005273.1).

SEQ ID NO:14 is an amino acid sequence of the protein GPR15
(NP_005281.1).

SEQ ID NO:15 is an amino acid sequence of the protein GPR32
(NP_001497.1).

SEQ ID NO:16 is an amino acid sequence of the protein GPR34
(NP_005291.1).

SEQ ID NO:17 is an amino acid sequence of the protein GPR183
(NP_004942.1).

SEQ ID NO:18 is an amino acid sequence of the protein SERPINA4
(NP_006206.2).

SEQ ID NO:19 is an amino acid sequence of the protein SERPINB5
(NP_002630.2).

SEQ ID NO:20 is an amino acid sequence of the protein SEMA4B
(NP_064595.2).

SEQ ID NO:21 is an amino acid sequence of the protein SEMA4D
(NP_006369.3).

SEQ ID NO:22 is an amino acid sequence of the protein CCL14
(NP_116739.1).

SEQ ID NO:23 is an amino acid sequence of the protein NKTR
(NP_005376.2).

SEQ ID NO:24 is an amino acid sequence of the protein SFTPD
(NP_003010.4).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates generally to pharmaceutical compositions comprising one or more of the oncofactor antibodies, polynucleotides, polypeptides, T-cells and/or other compositions disclosed herein in pharmaceutically-acceptable carriers for administration to a cell or an animal, either alone, or in combination with one or more other modalities of therapy. More specifically, as described herein, the

polynucleotide and polypeptide sequences of the present invention represent oncofactor sequences and are important targets useful in the detection and treatment of cancer. Accordingly, illustrative aspects of the present invention include, but are not restricted to, various uses of the described oncofactor sequences and related binding agents (*e.g.*, antibodies) in the detection and/or treatment of cancer.

The practice of the present invention will employ, unless indicated specifically to the contrary, conventional methods of virology, immunology, microbiology, molecular biology and recombinant DNA techniques within the skill of the art, many of which are described below for the purpose of illustration. Such techniques are explained fully in the literature. See, *e.g.*, Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); Maniatis *et al.*, *Molecular Cloning: A Laboratory Manual* (1982); *DNA Cloning: A Practical Approach*, vol. I & II (D. Glover, ed.); *Oligonucleotide Synthesis* (N. Gait, ed., 1984); *Nucleic Acid Hybridization* (B. Hames & S. Higgins, eds., 1985); *Transcription and Translation* (B. Hames & S. Higgins, eds., 1984); *Animal Cell Culture* (R. Freshney, ed., 1986); Perbal, *A Practical Guide to Molecular Cloning* (1984).

All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

As used in this specification and the appended claims, the singular forms “a,” “an” and “the” include plural references unless the content clearly dictates otherwise.

Antibodies, Fragments Thereof and Other Binding Agents

As noted, according to one aspect, the present invention provides pharmaceutical compositions comprising binding agents, such as antibodies and antigen-binding fragments thereof, that exhibit immunological binding to a cancer-associated sequence disclosed herein (*e.g.*, oncofactors), or to a portion, variant or derivative thereof.

More specifically, in certain preferred embodiments, the pharmaceutical compositions of the invention comprise antibodies and/or antigen-binding fragments that are capable of specifically binding to a cancer-associated polypeptide sequence selected from the group consisting of IL1f5 (SEQ ID NO: 1), CCBP2 (SEQ ID NO: 2),

IL1R2 (SEQ ID NO: 3), IL1RAPL1 (SEQ ID NO: 4), IL18BP (SEQ ID NO: 5), CLEC2B (SEQ ID NO: 6), C4BPA (SEQ ID NO: 7), C4BPB (SEQ ID NO: 8), SERPINI1 (SEQ ID NO: 9), IL1RAP isoform 1 (SEQ ID NO: 10), IL1RAP isoform 2 (SEQ ID NO: 11), GPR1 (SEQ ID NO: 12), GPR4 (SEQ ID NO: 13), GPR15 (SEQ ID NO: 14), GPR32 (SEQ ID NO: 15), GPR34 (SEQ ID NO: 16), GPR183 (SEQ ID NO: 17), SERPINA4 (SEQ ID NO: 18), SERPINB5 (SEQ ID NO: 19), SEMA4B (SEQ ID NO: 20), SEMA4D (SEQ ID NO: 21), CCL14 (SEQ ID NO: 22), NKTR (SEQ ID NO: 23) and SFTPD (SEQ ID NO: 24).

An antibody, or antigen-binding fragment thereof, is said to “specifically bind,” “immunologically bind,” and/or is “immunologically reactive” to a polypeptide of the invention if it reacts at a detectable level (within, for example, an ELISA assay) with the polypeptide, and does not react detectably with unrelated polypeptides under similar conditions. Immunological binding, as used in this context, generally refers to the non-covalent interactions of the type which occur between an immunoglobulin molecule and an antigen for which the immunoglobulin is specific. The strength, or affinity of immunological binding interactions can be expressed in terms of the dissociation constant (K_d) of the interaction, wherein a smaller K_d represents a greater affinity. Immunological binding properties of selected polypeptides can be quantified using methods well known in the art. One such method entails measuring the rates of antigen-binding site/antigen complex formation and dissociation, wherein those rates depend on the concentrations of the complex partners, the affinity of the interaction, and on geometric parameters that equally influence the rate in both directions. Thus, both the “on rate constant” (K_{on}) and the “off rate constant” (K_{off}) can be determined by calculation of the concentrations and the actual rates of association and dissociation. The ratio of K_{off}/K_{on} enables cancellation of all parameters not related to affinity, and is thus equal to the dissociation constant K_d . See, generally, Davies *et al.* (1990) *Annual Rev. Biochem.* 59:439-473.

An “antigen-binding site,” or “binding portion” of an antibody refers to the part of the immunoglobulin molecule that participates in antigen binding. The antigen binding site is formed by amino acid residues of the N-terminal variable (“V”) regions of the heavy (“H”) and light (“L”) chains. Three highly divergent stretches

within the V regions of the heavy and light chains are referred to as “hypervariable regions” which are interposed between more conserved flanking stretches known as “framework regions,” or “FRs”. Thus the term “FR” refers to amino acid sequences which are naturally found between and adjacent to hypervariable regions in immunoglobulins. In an antibody molecule, the three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain are disposed relative to each other in three dimensional space to form an antigen-binding surface. The antigen-binding surface is complementary to the three-dimensional surface of a bound antigen, and the three hypervariable regions of each of the heavy and light chains are referred to as “complementarity-determining regions,” or “CDRs.”

In a preferred embodiment, a binding agent is an antibody or an antigen-binding fragment thereof. Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art. *See, e.g.,* Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In general, antibodies can be produced by cell culture techniques, including the generation of monoclonal antibodies as described herein, or via transfection of antibody genes into suitable bacterial or mammalian cell hosts, in order to allow for the production of recombinant antibodies. In one technique, an immunogen comprising the polypeptide is initially injected into any of a wide variety of mammals (*e.g.,* mice, rats, rabbits, sheep or goats). In this step, the polypeptides of this invention may serve as the immunogen without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for an antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* 6:511-519, 1976, and improvements thereto. Briefly, these methods involve

the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and their culture supernatants tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. The polypeptides of this invention may be used in the purification process in, for example, an affinity chromatography step.

A number of therapeutically useful molecules are known in the art which comprise antigen-binding sites that are capable of exhibiting immunological binding properties of an antibody molecule. The proteolytic enzyme papain preferentially cleaves IgG molecules to yield several fragments, two of which (the "F(ab)" fragments) each comprise a covalent heterodimer that includes an intact antigen-binding site. The enzyme pepsin is able to cleave IgG molecules to provide several fragments, including the "F(ab)₂" fragment which comprises both antigen-binding sites. An "Fv" fragment can be produced by preferential proteolytic cleavage of an IgM, and on rare occasions IgG or IgA immunoglobulin molecule. Fv fragments are, however, more commonly derived using recombinant techniques known in the art. The Fv fragment includes a

non-covalent $V_H::V_L$ heterodimer including an antigen-binding site which retains much of the antigen recognition and binding capabilities of the native antibody molecule.

Inbar *et al.* (1972) *Proc. Nat. Acad. Sci. USA* 69:2659-2662; Hochman *et al.* (1976) *Biochem* 15:2706-2710; and Ehrlich *et al.* (1980) *Biochem* 19:4091-4096.

A single chain Fv (“sFv”) polypeptide is a covalently linked $V_H::V_L$ heterodimer which is expressed from a gene fusion including V_H - and V_L -encoding genes linked by a peptide-encoding linker. Huston *et al.* (1988) *Proc. Nat. Acad. Sci. USA* 85(16):5879-5883. A number of methods have been described to discern chemical structures for converting the naturally aggregated—but chemically separated—light and heavy polypeptide chains from an antibody V region into an sFv molecule which will fold into a three dimensional structure substantially similar to the structure of an antigen-binding site. See, *e.g.*, U.S. Pat. Nos. 5,091,513 and 5,132,405, to Huston *et al.*; and U.S. Pat. No. 4,946,778, to Ladner *et al.*

Each of the above-described molecules includes a heavy chain and a light chain CDR set, respectively interposed between a heavy chain and a light chain FR set which provide support to the CDRs and define the spatial relationship of the CDRs relative to each other. As used herein, the term “CDR set” refers to the three hypervariable regions of a heavy or light chain V region. Proceeding from the N-terminus of a heavy or light chain, these regions are denoted as “CDR1,” “CDR2,” and “CDR3” respectively. An antigen-binding site, therefore, includes six CDRs, comprising the CDR set from each of a heavy and a light chain V region. A polypeptide comprising a single CDR, (*e.g.*, a CDR1, CDR2 or CDR3) is referred to herein as a “molecular recognition unit.” Crystallographic analysis of a number of antigen-antibody complexes has demonstrated that the amino acid residues of CDRs form extensive contact with bound antigen, wherein the most extensive antigen contact is with the heavy chain CDR3. Thus, the molecular recognition units are primarily responsible for the specificity of an antigen-binding site.

As used herein, the term “FR set” refers to the four flanking amino acid sequences which frame the CDRs of a CDR set of a heavy or light chain V region. Some FR residues may contact bound antigen; however, FRs are primarily responsible for folding the V region into the antigen-binding site, particularly the FR residues

directly adjacent to the CDRs. Within FRs, certain amino residues and certain structural features are very highly conserved. In this regard, all V region sequences contain an internal disulfide loop of around 90 amino acid residues. When the V regions fold into a binding-site, the CDRs are displayed as projecting loop motifs which form an antigen-binding surface. It is generally recognized that there are conserved structural regions of FRs which influence the folded shape of the CDR loops into certain “canonical” structures—regardless of the precise CDR amino acid sequence. Further, certain FR residues are known to participate in non-covalent interdomain contacts which stabilize the interaction of the antibody heavy and light chains.

A number of “humanized” antibody molecules comprising an antigen-binding site derived from a non-human immunoglobulin have been described, including chimeric antibodies having rodent V regions and their associated CDRs fused to human constant domains (Winter *et al.* (1991) *Nature* 349:293-299; Lobuglio *et al.* (1989) *Proc. Nat. Acad. Sci. USA* 86:4220-4224; Shaw *et al.* (1987) *J Immunol.* 138:4534-4538; and Brown *et al.* (1987) *Cancer Res.* 47:3577-3583), rodent CDRs grafted into a human supporting FR prior to fusion with an appropriate human antibody constant domain (Riechmann *et al.* (1988) *Nature* 332:323-327; Verhoeven *et al.* (1988) *Science* 239:1534-1536; and Jones *et al.* (1986) *Nature* 321:522-525), and rodent CDRs supported by recombinantly veneered rodent FRs (European Patent Publication No. 519,596, published Dec. 23, 1992). These “humanized” molecules are designed to minimize unwanted immunological response toward rodent antihuman antibody molecules which limits the duration and effectiveness of therapeutic applications of those moieties in human recipients.

As used herein, the terms “veneered FRs” and “recombinantly veneered FRs” refer to the selective replacement of FR residues from, *e.g.*, a rodent heavy or light chain V region, with human FR residues in order to provide a xenogeneic molecule comprising an antigen-binding site which retains substantially all of the native FR polypeptide folding structure. Veneering techniques are based on the understanding that the ligand binding characteristics of an antigen-binding site are determined primarily by the structure and relative disposition of the heavy and light chain CDR sets within the antigen-binding surface. Davies *et al.* (1990) *Ann. Rev. Biochem.* 59:439-

473. Thus, antigen binding specificity can be preserved in a humanized antibody only wherein the CDR structures, their interaction with each other, and their interaction with the rest of the V region domains are carefully maintained. By using veneering techniques, exterior (*e.g.*, solvent-accessible) FR residues which are readily encountered by the immune system are selectively replaced with human residues to provide a hybrid molecule that comprises either a weakly immunogenic, or substantially non-immunogenic veneered surface.

The process of veneering makes use of the available sequence data for human antibody variable domains compiled by Kabat *et al.*, in Sequences of Proteins of Immunological Interest, 4th ed., (U.S. Dept. of Health and Human Services, U.S. Government Printing Office, 1987), updates to the Kabat database, and other accessible U.S. and foreign databases (both nucleic acid and protein). Solvent accessibilities of V region amino acids can be deduced from the known three-dimensional structure for human and murine antibody fragments. There are two general steps in veneering a murine antigen-binding site. Initially, the FRs of the variable domains of an antibody molecule of interest are compared with corresponding FR sequences of human variable domains obtained from the above-identified sources. The most homologous human V regions are then compared residue by residue to corresponding murine amino acids. The residues in the murine FR which differ from the human counterpart are replaced by the residues present in the human moiety using recombinant techniques well known in the art. Residue switching is only carried out with moieties which are at least partially exposed (solvent accessible), and care is exercised in the replacement of amino acid residues which may have a significant effect on the tertiary structure of V region domains, such as proline, glycine and charged amino acids.

In this manner, the resultant “veneered” murine antigen-binding sites are thus designed to retain the murine CDR residues, the residues substantially adjacent to the CDRs, the residues identified as buried or mostly buried (solvent inaccessible), the residues believed to participate in non-covalent (*e.g.*, electrostatic and hydrophobic) contacts between heavy and light chain domains, and the residues from conserved structural regions of the FRs which are believed to influence the “canonical” tertiary structures of the CDR loops. These design criteria are then used to prepare recombinant

nucleotide sequences which combine the CDRs of both the heavy and light chain of a murine antigen-binding site into human-appearing FRs that can be used to transfect mammalian cells for the expression of recombinant human antibodies which exhibit the antigen specificity of the murine antibody molecule.

In another embodiment of the invention, monoclonal antibodies of the present invention may be coupled to one or more therapeutic agents. Suitable agents in this regard include radionuclides, differentiation inducers, drugs, toxins, and derivatives thereof. Preferred radionuclides include ^{90}Y , ^{123}I , ^{125}I , ^{131}I , ^{186}Re , ^{188}Re , ^{211}At , and ^{212}Bi . Preferred drugs include methotrexate, and pyrimidine and purine analogs. Preferred differentiation inducers include phorbol esters and butyric acid. Preferred toxins include ricin, abrin, diphtheria toxin, cholera toxin, gelonin, *Pseudomonas* exotoxin, *Shigella* toxin, and pokeweed antiviral protein.

A therapeutic agent may be coupled (*e.g.*, covalently bonded) to a suitable monoclonal antibody either directly or indirectly (*e.g.*, via a linker group). A direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulfhydryl group, on one may be capable of reacting with a carbonyl-containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (*e.g.*, a halide) on the other.

Alternatively, it may be desirable to couple a therapeutic agent and an antibody via a linker group. A linker group can function as a spacer to distance an antibody from an agent in order to avoid interference with binding capabilities. A linker group can also serve to increase the chemical reactivity of a substituent on an agent or an antibody, and thus increase the coupling efficiency. An increase in chemical reactivity may also facilitate the use of agents, or functional groups on agents, which otherwise would not be possible.

It will be evident to those skilled in the art that a variety of bifunctional or polyfunctional reagents, both homo- and hetero-functional (such as those described in the catalog of the Pierce Chemical Co., Rockford, IL), may be employed as the linker group. Coupling may be effected, for example, through amino groups, carboxyl groups,

sulfhydryl groups or oxidized carbohydrate residues. There are numerous references describing such methodology, *e.g.*, U.S. Patent No. 4,671,958, to Rodwell *et al.*

Where a therapeutic agent is more potent when free from the antibody portion of the immunoconjugates of the present invention, it may be desirable to use a linker group which is cleavable during or upon internalization into a cell. A number of different cleavable linker groups have been described. The mechanisms for the intracellular release of an agent from these linker groups include cleavage by reduction of a disulfide bond (*e.g.*, U.S. Patent No. 4,489,710, to Spitler), by irradiation of a photolabile bond (*e.g.*, U.S. Patent No. 4,625,014, to Senter *et al.*), by hydrolysis of derivatized amino acid side chains (*e.g.*, U.S. Patent No. 4,638,045, to Kohn *et al.*), by serum complement-mediated hydrolysis (*e.g.*, U.S. Patent No. 4,671,958, to Rodwell *et al.*), and acid-catalyzed hydrolysis (*e.g.*, U.S. Patent No. 4,569,789, to Blattler *et al.*).

It may be desirable to couple more than one agent to an antibody. In one embodiment, multiple molecules of an agent are coupled to one antibody molecule. In another embodiment, more than one type of agent may be coupled to one antibody. Regardless of the particular embodiment, immunoconjugates with more than one agent may be prepared in a variety of ways. For example, more than one agent may be coupled directly to an antibody molecule, or linkers that provide multiple sites for attachment can be used. Alternatively, a carrier can be used.

A carrier may bear the agents in a variety of ways, including covalent bonding either directly or via a linker group. Suitable carriers include proteins such as albumins (*e.g.*, U.S. Patent No. 4,507,234, to Kato *et al.*), peptides and polysaccharides such as aminodextran (*e.g.*, U.S. Patent No. 4,699,784, to Shih *et al.*). A carrier may also bear an agent by noncovalent bonding or by encapsulation, such as within a liposome vesicle (*e.g.*, U.S. Patent Nos. 4,429,008 and 4,873,088). Carriers specific for radionuclide agents include radiohalogenated small molecules and chelating compounds. For example, U.S. Patent No. 4,735,792 discloses representative radiohalogenated small molecules and their synthesis. A radionuclide chelate may be formed from chelating compounds that include those containing nitrogen and sulfur atoms as the donor atoms for binding the metal, or metal oxide, radionuclide. For

example, U.S. Patent No. 4,673,562, to Davison *et al.* discloses representative chelating compounds and their synthesis.

The above antibody types and procedures for making the same are provide for purposes of illustration but not by way of limitation. It will be understood that these and many other methodologies are known and established in the art for producing and characterizing antibodies and other binding agents, and these methodologies may be used in the context of the present invention.

Polypeptide Compositions

Another aspect of the present invention provides cancer-associated polypeptides. In certain related embodiments, the polypeptides are used, for example, in the context of pharmaceutical and/or vaccine compositions for the treatment of cancer.

As used herein, the term “polypeptide” “ is used in its conventional meaning, *i.e.*, as a sequence of amino acids. The polypeptides are not limited to a specific length of the product; thus, peptides, oligopeptides, and proteins are included within the definition of polypeptide, and such terms may be used interchangeably herein unless specifically indicated otherwise. This term also does not refer to or exclude post-expression modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like, as well as other modifications known in the art, both naturally occurring and non-naturally occurring. A polypeptide may be an entire protein, or a subsequence thereof. Particular polypeptides of interest in the context of this invention are amino acid subsequences comprising epitopes, *i.e.*, antigenic determinants substantially responsible for the immunogenic properties of a polypeptide and being capable of evoking an immune response.

In certain compositions and methods of the invention, preferred polypeptides comprise those set forth in any one of SEQ ID NOs: 1-24, or a variant or fragment of any of the foregoing, such as a variant or fragment having at least 70%, 80%, 90% or 95% identity thereto.

The polypeptides of the present invention are sometimes herein referred to as cancer-associated proteins or tumor polypeptides or oncofactor polypeptides, as an indication of their cancer association, such as their increased levels of expression and/or

activity in tumor samples. Thus, in certain embodiments, the terms “oncofactor,” “tumor polypeptide,” or “cancer-associated polypeptide,” are generally used interchangeably and refer to a polypeptide sequence of the present invention, or a polynucleotide sequence encoding such a polypeptide, that is expressed and/or active in a substantial proportion of tumor samples, for example preferably greater than about 20%, more preferably greater than about 30%, and most preferably greater than about 50% or more of tumor samples tested, at a level that is at least two fold, and preferably at least five fold, greater than the level of expression in normal tissues, as determined using a representative assay provided herein. Oncofactor polypeptides of the invention having increased levels of expression and/or activity in tumor cells find particular utility both as a diagnostic marker as well as a therapeutic target, as further described below.

In certain embodiments, the polypeptides used according to the compositions and/or method of the present invention are immunogenic, *i.e.*, they react detectably within an immunoassay (such as an ELISA or T-cell stimulation assay) with antisera and/or T-cells from a patient with cancer. Screening for immunogenic activity can be performed using techniques well known to the skilled artisan. For example, such screens can be performed using methods such as those described in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In one illustrative example, a polypeptide may be immobilized on a solid support and contacted with patient sera to allow binding of antibodies within the sera to the immobilized polypeptide. Unbound sera may then be removed and bound antibodies detected using, for example, ¹²⁵I-labeled Protein A.

As would be recognized by the skilled artisan, immunogenic portions of the polypeptides disclosed herein are also encompassed by the present invention. An “immunogenic portion,” as used herein, is a fragment of an immunogenic polypeptide of the invention that itself is immunologically reactive (*i.e.*, specifically binds) with the B-cells and/or T-cell surface antigen receptors that recognize the polypeptide. Immunogenic portions may generally be identified using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3rd ed., 243-247 (Raven Press, 1993) and references cited therein. Such techniques include screening polypeptides for the ability to react with antigen-specific antibodies, antisera and/or T-cell lines or

clones. As used herein, antisera and antibodies are “antigen-specific” if they specifically bind to an antigen (*i.e.*, they react with the protein in an ELISA or other immunoassay, and do not react detectably with unrelated proteins). Such antisera and antibodies may be prepared as described herein, and using well-known techniques.

In one illustrative embodiment, an immunogenic portion of a polypeptide of the present invention is a portion that reacts with antisera and/or T-cells at a level that is not substantially less than the reactivity of the full-length polypeptide (*e.g.*, in an ELISA and/or T-cell reactivity assay). Preferably, the level of immunogenic activity of the immunogenic portion is at least about 50%, preferably at least about 70% and most preferably greater than about 90% of the immunogenicity for the full-length polypeptide. In some instances, preferred immunogenic portions will be identified that have a level of immunogenic activity greater than that of the corresponding full-length polypeptide, *e.g.*, having greater than about 100% or 150% or more immunogenic activity.

The present invention, in other embodiments, employs polypeptide fragments comprising at least about 5, 10, 15, 20, 25, 50, or 100 contiguous amino acids, or more, including all intermediate lengths, of a polypeptide compositions set forth herein, such as those set forth in SEQ ID NOs: 1-24.

In still other embodiments, the present invention employs variants of the polypeptide compositions described herein in the compositions and methods described herein. Polypeptide variants generally encompassed by the present invention will typically exhibit at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or more identity (determined as described below), along its length, to a polypeptide sequences set forth herein.

A polypeptide “variant,” as the term is used herein, is a polypeptide that typically differs from a polypeptide specifically disclosed herein in one or more substitutions, deletions, additions and/or insertions. Such variants may be naturally occurring or may be synthetically generated, for example, by modifying one or more of the above polypeptide sequences of the invention and evaluating their immunogenic activity as described herein and/or using any of a number of techniques well known in the art.

In many instances, a variant will contain conservative substitutions. A “conservative substitution” is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydrophobic nature of the polypeptide to be substantially unchanged. As described above, modifications may be made in the structure of the polynucleotides and polypeptides of the present invention and still obtain a functional molecule that encodes a variant or derivative polypeptide with desirable characteristics, *e.g.*, with immunogenic characteristics. When it is desired to alter the amino acid sequence of a polypeptide to create an equivalent, or even an improved, immunogenic variant or portion of a polypeptide of the invention, one skilled in the art will typically change one or more of the codons of the encoding DNA sequence according to Table 1.

For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence, and, of course, its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. It is thus contemplated that various changes may be made in the peptide sequences of the disclosed compositions, or corresponding DNA sequences which encode said peptides without appreciable loss of their biological utility or activity.

Table 1

Amino Acids			Codons						
Alanine	Ala	A	GCA	GCC	GCG	GCU			
Cysteine	Cys	C	UGC	UGU					
Aspartic acid	Asp	D	GAC	GAU					
Glutamic acid	Glu	E	GAA	GAG					
Phenylalanine	Phe	F	UUC	UUU					
Glycine	Gly	G	GGA	GGC	GGG	GGU			
Histidine	His	H	CAC	CAU					
Isoleucine	Ile	I	AUA	AUC	AUU				
Lysine	Lys	K	AAA	AAG					
Leucine	Leu	L	UUA	UUG	CUA	CUC	CUG	CUU	
Methionine	Met	M	AUG						
Asparagine	Asn	N	AAC	AAU					
Proline	Pro	P	CCA	CCC	CCG	CCU			
Glutamine	Gln	Q	CAA	CAG					
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGU	
Serine	Ser	S	AGC	AGU	UCA	UCC	UCG	UCU	
Threonine	Thr	T	ACA	ACC	ACG	ACU			
Valine	Val	V	GUA	GUC	GUG	GUU			
Tryptophan	Trp	W	UGG						
Tyrosine	Tyr	Y	UAC	UAU					

In making such changes, the hydrophatic index of amino acids may be considered. The importance of the hydrophatic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982, incorporated herein by reference). It is accepted that the relative hydrophatic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydrophatic index on the basis of its hydrophobicity and charge characteristics (Kyte and Doolittle, 1982). These values are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine

(+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, *i.e.*, still obtain a biological functionally equivalent protein. In making such changes, the substitution of amino acids whose hydropathic indices are within ± 2 is preferred, those within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred. It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U. S. Patent 4,554,101 (specifically incorporated herein by reference in its entirety), states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein.

As detailed in U. S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 \pm 1); glutamate (+3.0 \pm 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 \pm 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent protein. In such changes, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take various of the foregoing characteristics into consideration are well known to those

of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

In addition, any polynucleotide may be further modified to increase stability *in vivo*. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine and wybutosine, as well as acetyl-methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine and uridine.

Amino acid substitutions may further be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. A variant may also, or alternatively, contain nonconservative changes. In a preferred embodiment, variant polypeptides differ from a native sequence by substitution, deletion or addition of five amino acids or fewer. Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenicity, secondary structure and hydrophobic nature of the polypeptide.

Polypeptides may comprise a signal (or leader) sequence at the N-terminal end of the protein, which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (*e.g.*, poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

When comparing polypeptide sequences, two sequences are said to be “identical” if the sequence of amino acids in the two sequences is the same when aligned for maximum correspondence, as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A “comparison window” as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O., (1978) *A model of evolutionary change in proteins – Matrices for detecting distant relationships*. In Dayhoff, M.O. (ed.) *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J. (1990) *Unified Approach to Alignment and Phylogenesis*, pp. 626-645 *Methods in Enzymology* vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M., *CABIOS* 5:151-153 (1989); Myers, E.W. and Muller W., *CABIOS* 4:11-17 (1988); Robinson, E.D., *Comb. Theor* 11:105 (1971); Saitou, N. Nei, M., *Mol. Biol. Evol.* 4:406-425 (1987); Sneath, P.H.A. and Sokal, R.R., *Numerical Taxonomy – the Principles and Practice of Numerical Taxonomy*, Freeman Press, San Francisco, CA (1973); Wilbur, W.J. and Lipman, D.J., *Proc. Natl. Acad., Sci. USA* 80:726-730 (1983).

Alternatively, optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman, *Add. APL. Math* 2:482 (1981), by the identity alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity methods of Pearson and Lipman, *Proc. Natl. Acad. Sci. USA* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by inspection.

One preferred example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.*, *Nucl. Acids Res.* 25:3389-3402 (1977), and Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990), respectively. BLAST and BLAST 2.0 can be used, for example with the parameters described herein, to determine percent sequence identity for the polynucleotides and polypeptides of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. For amino acid sequences, a scoring matrix can be used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment.

In one preferred approach, the “percentage of sequence identity” is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polypeptide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (*i.e.*, the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

Fusion Polypeptides

Within other illustrative embodiments, an oncofactor polypeptide of the invention may be in the form of a fusion polypeptide that comprises multiple cancer-associated polypeptides as described herein, or that comprises at least one cancer-associated polypeptide as described herein and an unrelated sequence, such as a known tumor protein or other heterologous sequence of interest. A fusion partner may, for

example, assist in providing T helper epitopes (an immunological fusion partner), preferably T helper epitopes recognized by humans, or may assist in expressing the protein (an expression enhancer) at higher yields than the native recombinant protein. Certain preferred fusion partners are both immunological and expression enhancing fusion partners. Other fusion partners may be selected so as to increase the solubility of the polypeptide or to enable the polypeptide to be targeted to desired intracellular compartments. Still further fusion partners include affinity tags, which facilitate purification of the polypeptide.

Fusion polypeptides may generally be prepared using standard techniques, including chemical conjugation. Preferably, a fusion polypeptide is expressed as a recombinant polypeptide, allowing the production of increased levels, relative to a non-fused polypeptide, in an expression system. Briefly, DNA sequences encoding the polypeptide components may be assembled separately, and ligated into an appropriate expression vector. The 3' end of the DNA sequence encoding one polypeptide component is ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide component so that the reading frames of the sequences are in phase. This permits translation into a single fusion polypeptide that retains the biological activity of both component polypeptides.

A peptide linker sequence may be employed to separate the first and second polypeptide components by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion polypeptide using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea *et al.*, *Gene* 40:39-46, 1985; Murphy *et al.*, *Proc. Natl. Acad. Sci. USA* 83:8258-8262, 1986; U.S. Patent No. 4,935,233 and U.S.

Patent No. 4,751,180. The linker sequence may generally be from 1 to about 50 amino acids in length. Linker sequences are not required when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference.

The ligated DNA sequences are operably linked to suitable transcriptional or translational regulatory elements. The regulatory elements responsible for expression of DNA are located only 5' to the DNA sequence encoding the first polypeptides. Similarly, stop codons required to end translation and transcription termination signals are only present 3' to the DNA sequence encoding the second polypeptide.

Cancer-associated polypeptides (*e.g.*, oncofactors) of the invention are prepared using any of a variety of well known synthetic and/or recombinant techniques, the latter of which are further described below. Polypeptides, portions and other variants generally less than about 150 amino acids can be generated by synthetic means, using techniques well known to those of ordinary skill in the art. In one illustrative example, such polypeptides are synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. *See Merrifield, J. Am. Chem. Soc. 85:2149-2146, 1963.* Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied BioSystems Division (Foster City, CA), and may be operated according to the manufacturer's instructions.

In general, polypeptide compositions (including fusion polypeptides) of the invention are isolated. An "isolated" polypeptide is one that is removed from its original environment. For example, a naturally-occurring protein or polypeptide is isolated if it is separated from some or all of the coexisting materials in the natural system. Preferably, such polypeptides are also purified, *e.g.*, are at least about 90% pure, more preferably at least about 95% pure and most preferably at least about 99% pure.

Polynucleotide Compositions

As noted above, another aspect of the invention relates to the polynucleotides encoding the oncofactor polypeptides described herein, and their use in the context of pharmaceutical and diagnostic compositions for the treatment and detection of cancer.

The terms “DNA” and “polynucleotide” are used essentially interchangeably herein to refer to a DNA molecule that has been isolated free of total genomic DNA of a particular species. “Isolated,” as used herein, means that a polynucleotide is substantially away from other coding sequences, and that the DNA molecule does not contain large portions of unrelated coding DNA, such as large chromosomal fragments or other functional genes or polypeptide coding regions. Of course, this refers to the DNA molecule as originally isolated, and does not exclude genes or coding regions later added to the segment by the hand of man.

As will be understood by those skilled in the art, the polynucleotide compositions of this invention can include genomic sequences, extra-genomic and plasmid-encoded sequences and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides, peptides and the like. Such segments may be naturally isolated, or modified synthetically by the hand of man.

As will be also recognized by the skilled artisan, polynucleotides of the invention may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules may include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

Polynucleotides may comprise a native sequence (*i.e.*, an endogenous sequence that encodes a polypeptide/protein of the invention or a portion thereof) or may comprise a sequence that encodes a variant or derivative, preferably and immunogenic variant or derivative, of such a sequence.

In certain embodiments, the present invention employs polynucleotide variants having substantial identity to a sequence encoding a cancer-associated polypeptide sequences disclosed herein in SEQ ID NOs: 1-24, for example those comprising at least 70% sequence identity, preferably at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% or higher, sequence identity compared to a polynucleotide sequence of this invention using the methods described herein, (*e.g.*, BLAST analysis using standard parameters, as described below). One skilled in this art will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like.

Typically, polynucleotide variants will contain one or more substitutions, additions, deletions and/or insertions, preferably such that the immunogenicity of the polypeptide encoded by the variant polynucleotide is not substantially diminished relative to a polypeptide encoded by a polynucleotide sequence specifically set forth herein). The term “variants” should also be understood to encompass homologous genes of xenogenic origin.

In additional embodiments, the present invention provides polynucleotide fragments comprising or consisting of various lengths of contiguous stretches of sequence identical to or complementary to one or more of the sequences described herein. For example, polynucleotides are provided by this invention that comprise or consist of at least about 10, 15, 20, 30, 40, 50, 75, 100, 150, 200, 300, 400, 500 or 1000 or more contiguous nucleotides of a sequence encoding a polypeptide disclosed herein as well as all intermediate lengths there between. It will be readily understood that “intermediate lengths”, in this context, means any length between the quoted values, such as 16, 17, 18, 19, *etc.*; 21, 22, 23, *etc.*; 30, 31, 32, *etc.*; 50, 51, 52, 53, *etc.*; 100, 101, 102, 103, *etc.*; 150, 151, 152, 153, *etc.*; including all integers through 200-500; 500-1,000, and the like. A polynucleotide sequence as described here may be extended at one or both ends by additional nucleotides not found in the native sequence. This additional sequence may consist of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,

16, 17, 18, 19, or 20 nucleotides at either end of the disclosed sequence or at both ends of the disclosed sequence.

In another embodiment of the invention, polynucleotide compositions are provided that are capable of hybridizing under moderate to high stringency conditions to a polynucleotide sequence that encodes a polypeptide sequence provided herein, or a fragment thereof, or a complementary sequence thereof. Hybridization techniques are well known in the art of molecular biology. For purposes of illustration, suitable moderately stringent conditions for testing the hybridization of a polynucleotide of this invention with other polynucleotides include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-60°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS. One skilled in the art will understand that the stringency of hybridization can be readily manipulated, such as by altering the salt content of the hybridization solution and/or the temperature at which the hybridization is performed. For example, in another embodiment, suitable highly stringent hybridization conditions include those described above, with the exception that the temperature of hybridization is increased, *e.g.*, to 60-65°C or 65-70°C.

The polynucleotides of the present invention, or fragments thereof, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol. For example, illustrative polynucleotide segments with total lengths of about 10,000, about 5000, about 3000, about 2,000, about 1,000, about 500, about 200, about 100, about 50 base pairs in length, and the like, (including all intermediate lengths) are contemplated to be useful in many implementations of this invention.

When comparing polynucleotide sequences, two sequences are said to be “identical” if the sequence of nucleotides in the two sequences is the same when aligned for maximum correspondence, as described below. Comparisons between two

sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A “comparison window” as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins – Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J., *Unified Approach to Alignment and Phylogenesis*, pp. 626-645 (1990); *Methods in Enzymology* vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M., *CABIOS* 5:151-153 (1989); Myers, E.W. and Muller W., *CABIOS* 4:11-17 (1988); Robinson, E.D., *Comb. Theor* 11:105 (1971); Santou, N. Nes, M., *Mol. Biol. Evol.* 4:406-425 (1987); Sneath, P.H.A. and Sokal, R.R., *Numerical Taxonomy – the Principles and Practice of Numerical Taxonomy*, Freeman Press, San Francisco, CA (1973); Wilbur, W.J. and Lipman, D.J., *Proc. Natl. Acad., Sci. USA* 80:726-730 (1983).

Alternatively, optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman, *Add. APL. Math* 2:482 (1981), by the identity alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity methods of Pearson and Lipman, *Proc. Natl. Acad. Sci. USA* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by inspection.

One preferred example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.*, *Nucl. Acids Res.* 25:3389-3402

(1977), and Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990), respectively. BLAST and BLAST 2.0 can be used, for example with the parameters described herein, to determine percent sequence identity for the polynucleotides of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. In one illustrative example, cumulative scores can be calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)) alignments, (B) of 50, expectation (E) of 10, M=5, N=-4 and a comparison of both strands.

In certain embodiments, the “percentage of sequence identity” is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (*i.e.*, the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides

that vary due to differences in codon usage are specifically contemplated by the present invention. Further, alleles of the genes comprising the polynucleotide sequences provided herein are within the scope of the present invention. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides. The resulting mRNA and protein may, but need not, have an altered structure or function. Alleles may be identified using standard techniques (such as hybridization, amplification and/or database sequence comparison).

Therefore, in another embodiment of the invention, a mutagenesis approach, such as site-specific mutagenesis, is employed for the preparation of variants and/or derivatives of the polypeptides described herein. By this approach, specific modifications in a polypeptide sequence can be made through mutagenesis of the underlying polynucleotides that encode them. These techniques provides a straightforward approach to prepare and test sequence variants, for example, incorporating one or more of the foregoing considerations, by introducing one or more nucleotide sequence changes into the polynucleotide.

Site-specific mutagenesis allows the production of mutants through the use of specific oligonucleotide sequences which encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. Mutations may be employed in a selected polynucleotide sequence to improve, alter, decrease, modify, or otherwise change the properties of the polynucleotide itself, and/or alter the properties, activity, composition, stability, or primary sequence of the encoded polypeptide.

In certain embodiments of the present invention, the inventors contemplate the mutagenesis of the disclosed polynucleotide sequences to alter one or more properties of the encoded polypeptide, such as the immunogenicity of a polypeptide vaccine. The techniques of site-specific mutagenesis are well-known in the art, and are widely used to create variants of both polypeptides and polynucleotides. For example, site-specific mutagenesis is often used to alter a specific portion of a DNA molecule. In such embodiments, a primer comprising typically about 14 to about 25

nucleotides or so in length is employed, with about 5 to about 10 residues on both sides of the junction of the sequence being altered.

The preparation of sequence variants of the selected peptide-encoding DNA segments using site-directed mutagenesis provides a means of producing potentially useful species and is not meant to be limiting as there are other ways in which sequence variants of peptides and the DNA sequences encoding them may be obtained. For example, recombinant vectors encoding the desired peptide sequence may be treated with mutagenic agents, such as hydroxylamine, to obtain sequence variants. Specific details regarding these methods and protocols are found in the teachings of Maloy *et al.*, 1994; Segal, 1976; Prokop and Bajpai, 1991; Kuby, 1994; and Maniatis *et al.*, 1982, each incorporated herein by reference, for that purpose.

In other embodiments of the present invention, the polynucleotide sequences provided herein can be advantageously used as probes or primers for nucleic acid hybridization, *e.g.*, for use in diagnosis and/or monitoring of cancer in a subject. As such, it is contemplated that nucleic acid segments that comprise or consist of a sequence region of at least about a 15 nucleotide long contiguous sequence that has the same sequence as, or is complementary to, a 15 nucleotide long contiguous sequence described herein will find particular utility. Longer contiguous identical or complementary sequences, *e.g.*, those of about 20, 30, 40, 50, 100, 200, 500, 1000 (including all intermediate lengths) and even up to full length sequences will also be of use in certain embodiments.

The ability of such nucleic acid probes to specifically hybridize to a sequence of interest will enable them to be of use in detecting the presence of complementary sequences in a given sample. However, other uses are also envisioned, such as the use of the sequence information for the preparation of mutant species primers, or primers for use in preparing other genetic constructions.

Polynucleotide molecules having sequence regions consisting of contiguous nucleotide stretches of 10-14, 15-20, 30, 50, or even of 100-200 nucleotides or so (including intermediate lengths as well), identical or complementary to a polynucleotide sequence described herein, are particularly contemplated as hybridization probes for use in, *e.g.*, Southern and Northern blotting. This would allow

a gene product, or fragment thereof, to be analyzed, both in diverse cell types and also in various bacterial cells. The total size of fragment, as well as the size of the complementary stretch(es), will ultimately depend on the intended use or application of the particular nucleic acid segment. Smaller fragments will generally find use in hybridization embodiments, wherein the length of the contiguous complementary region may be varied, such as between about 15 and about 100 nucleotides, but larger contiguous complementarity stretches may be used, according to the length complementary sequences one wishes to detect.

The use of a hybridization probe of about 15-25 nucleotides in length allows the formation of a duplex molecule that is both stable and selective. Molecules having contiguous complementary sequences over stretches greater than 15 bases in length are generally preferred, though, in order to increase stability and selectivity of the hybrid, and thereby improve the quality and degree of specific hybrid molecules obtained. One will generally prefer to design nucleic acid molecules having gene-complementary stretches of 15 to 25 contiguous nucleotides, or even longer where desired.

Hybridization probes may be selected from any portion of any of the sequences described herein. All that is required is to review the sequences from about 15-25 nucleotides in length up to and including the full length sequence, that one wishes to utilize as a probe or primer. The choice of probe and primer sequences may be governed by various factors that are known and established in the art.

Small polynucleotide segments or fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, as is commonly practiced using an automated oligonucleotide synthesizer. Also, fragments may be obtained by application of nucleic acid reproduction technology, such as the PCRTM technology of U. S. Patent 4,683,202 (incorporated herein by reference), by introducing selected sequences into recombinant vectors for recombinant production, and by other recombinant DNA techniques generally known to those of skill in the art of molecular biology.

The nucleotide sequences according to the invention may be used for their ability to selectively form duplex molecules with complementary stretches of the

entire gene or gene fragments of interest. Depending on the application envisioned, one will typically desire to employ varying conditions of hybridization to achieve varying degrees of selectivity of probe towards target sequence. For applications requiring high selectivity, one will typically desire to employ relatively stringent conditions to form the hybrids, *e.g.*, one will select relatively low salt and/or high temperature conditions, such as provided by a salt concentration of from about 0.02 M to about 0.15 M salt at temperatures of from about 50°C to about 70°C. Such selective conditions tolerate little, if any, mismatch between the probe and the template or target strand, and would be particularly suitable for isolating related sequences.

Of course, for some applications, for example, where one desires to prepare mutants employing a mutant primer strand hybridized to an underlying template, less stringent (reduced stringency) hybridization conditions will typically be needed in order to allow formation of the heteroduplex. In these circumstances, one may desire to employ salt conditions such as those of from about 0.15 M to about 0.9 M salt, at temperatures ranging from about 20°C to about 55°C. Cross-hybridizing species can thereby be readily identified as positively hybridizing signals with respect to control hybridizations. In any case, it is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide, which serves to destabilize the hybrid duplex in the same manner as increased temperature. Thus, hybridization conditions can be readily manipulated, and thus will generally be a method of choice depending on the desired results.

According to another embodiment of the present invention, polynucleotide compositions comprising antisense oligonucleotides are provided for use in the compositions and methods herein. Antisense oligonucleotides have been demonstrated to be effective and targeted inhibitors of protein synthesis, and, consequently, provide a therapeutic approach by which a disease can be treated by inhibiting the synthesis of proteins that contribute to the disease. The efficacy of antisense oligonucleotides for inhibiting protein synthesis is well established. For example, the synthesis of polygalacturonase and the muscarine type 2 acetylcholine receptor are inhibited by antisense oligonucleotides directed to their respective mRNA sequences (U. S. Patent 5,739,119 and U. S. Patent 5,759,829). Further, examples of

antisense inhibition have been demonstrated with the nuclear protein cyclin, the multiple drug resistance gene (MDG1), ICAM-1, E-selectin, STK-1, striatal GABA_A receptor and human EGF (Jaskulski *et al.*, *Science* 1988 Jun 10;240(4858):1544-6; Vasanthakumar and Ahmed, *Cancer Commun.* 1989;1(4):225-32; Peris *et al.*, *Brain Res Mol Brain Res.* 1998 Jun 15;57(2):310-20; U. S. Patent 5,801,154; U.S. Patent 5,789,573; U. S. Patent 5,718,709 and U.S. Patent 5,610,288). Antisense constructs have also been described that inhibit and can be used to treat a variety of abnormal cellular proliferations, *e.g.*, cancer (U. S. Patent 5,747,470; U. S. Patent 5,591,317 and U. S. Patent 5,783,683).

Therefore, in certain embodiments, the present invention provides oligonucleotide sequences that comprise all, or a portion of, any sequence that is capable of specifically binding to polynucleotide sequence described herein, or a complement thereof. In one embodiment, the antisense oligonucleotides comprise DNA or derivatives thereof. In another embodiment, the oligonucleotides comprise RNA or derivatives thereof. In another embodiment, the oligonucleotides are modified DNAs comprising a phosphorothioated modified backbone. In another embodiment, the oligonucleotide sequences comprise peptide nucleic acids or derivatives thereof. In each case, illustrative compositions comprise a sequence region that is complementary, and more preferably substantially-complementary, and even more preferably, completely complementary to one or more portions of polynucleotides described herein. Selection of antisense compositions specific for a given gene sequence is based upon analysis of the chosen target sequence and determination of secondary structure, T_m , binding energy, and relative stability. Antisense compositions may be selected based upon their relative inability to form dimers, hairpins, or other secondary structures that would reduce or prohibit specific binding to the target mRNA in a host cell. Highly preferred target regions of the mRNA, are those which are at or near the AUG translation initiation codon, and those sequences which are substantially complementary to 5' regions of the mRNA. These secondary structure analyses and target site selection considerations can be performed, for example, using v.4 of the OLIGO primer analysis software and/or the BLASTN 2.0.5 algorithm software (Altschul *et al.*, *Nucleic Acids Res.* 1997, 25(17):3389-402).

According to another embodiment of the invention, the polynucleotide compositions described herein are used in the design and preparation of ribozyme molecules for use in compositions and methods for inhibiting the expression of the tumor polypeptides and proteins of the present invention in tumor cells. Ribozymes are RNA-protein complexes that cleave nucleic acids in a site-specific fashion. Ribozymes have specific catalytic domains that possess endonuclease activity (Kim and Cech, *Proc. Natl. Acad. Sci. USA*. 1987 Dec;84(24):8788-92; Forster and Symons, *Cell*. 1987 Apr 24;49(2):211-20). For example, a large number of ribozymes accelerate phosphoester transfer reactions with a high degree of specificity, often cleaving only one of several phosphoesters in an oligonucleotide substrate (Cech *et al.*, *Cell*. 1981 Dec;27(3 Pt 2):487-96; Michel and Westhof, *J Mol Biol*. 1990 Dec 5;216(3):585-610; Reinhold-Hurek and Shub, *Nature*. 1992 May 14;357(6374):173-6). This specificity has been attributed to the requirement that the substrate bind via specific base-pairing interactions to the internal guide sequence ("IGS") of the ribozyme prior to chemical reaction.

Enzymatic RNAs can catalyze the hydrolysis of RNA phosphodiester bonds *in trans* (and thus can cleave other RNA molecules) under physiological conditions. In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

The enzymatic nature of a ribozyme is advantageous over many technologies, such as antisense technology (where a nucleic acid molecule simply binds to a nucleic acid target to block its translation) since the concentration of ribozyme necessary to affect a therapeutic treatment is lower than that of an antisense oligonucleotide. This advantage reflects the ability of the ribozyme to act

enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can completely eliminate catalytic activity of a ribozyme. Similar mismatches in antisense molecules do not prevent their action (Woolf *et al.*, *Proc. Natl. Acad. Sci. USA*. 1992 Aug 15;89(16):7305-9). Thus, the specificity of action of a ribozyme is greater than that of an antisense oligonucleotide binding the same RNA site.

The enzymatic nucleic acid molecule may be formed in a hammerhead, hairpin, a hepatitis δ virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) or Neurospora VS RNA motif. Examples of hammerhead motifs are described by Rossi *et al.* *Nucleic Acids Res.* 1992 Sep 11;20(17):4559-65. Examples of hairpin motifs are described by Hampel *et al.* (Eur. Pat. Appl. Publ. No. EP 0360257), Hampel and Tritz, *Biochemistry* 1989 Jun 13;28(12):4929-33; Hampel *et al.*, *Nucleic Acids Res.* 1990 Jan 25;18(2):299-304 and U. S. Patent 5,631,359. An example of the hepatitis δ virus motif is described by Perrotta and Been, *Biochemistry.* 1992 Dec 1;31(47):11843-52; an example of the RNaseP motif is described by Guerrier-Takada *et al.*, *Cell.* 1983 Dec;35(3 Pt 2):849-57; Neurospora VS RNA ribozyme motif is described by Collins (Saville and Collins, *Cell.* 1990 May 18;61(4):685-96; Saville and Collins, *Proc. Natl. Acad. Sci. USA*, 88(19):8826-30 (Oct 1 1991); Collins and Olive, *Biochemistry* 32(11):2795-9 (Mar 23 1993); and an example of the Group I intron is described in (U. S. Patent 4,987,071). All that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule. Thus the ribozyme constructs need not be limited to specific motifs mentioned herein.

Ribozymes may be designed as described in Int. Pat. Appl. Publ. No. WO 93/23569 and Int. Pat. Appl. Publ. No. WO 94/02595, each specifically incorporated herein by reference) and synthesized to be tested *in vitro* and *in vivo*, as

described. Such ribozymes can also be optimized for delivery. While specific examples are provided, those in the art will recognize that equivalent RNA targets in other species can be utilized when necessary.

In another embodiment of the invention, peptide nucleic acids (PNAs) compositions are provided. PNA is a DNA mimic in which the nucleobases are attached to a pseudopeptide backbone (Good and Nielsen, *Antisense Nucleic Acid Drug Dev.* 1997 7(4) 431-37). PNA is able to be utilized in a number of methods that traditionally have used RNA or DNA. Often PNA sequences perform better in techniques than the corresponding RNA or DNA sequences and have utilities that are not inherent to RNA or DNA. A review of PNA including methods of making, characteristics of, and methods of using, is provided by Corey (*Trends Biotechnol* 15(6):224-9 (Jun 1997)). As such, in certain embodiments, one may prepare PNA sequences that are complementary to one or more portions of the ACE mRNA sequence, and such PNA compositions may be used to regulate, alter, decrease, or reduce the translation of ACE-specific mRNA, and thereby alter the level of ACE activity in a host cell to which such PNA compositions have been administered.

PNAs have 2-aminoethyl-glycine linkages replacing the normal phosphodiester backbone of DNA (Nielsen *et al.*, *Science* 254(5037):1497-500 (Dec 6 1991); Hanvey *et al.*, *Science* 258(5087):1481-5 (Nov 27 1992); Hyrup and Nielsen, *Bioorg. Med. Chem.* 4(1):5-23 (Jan 1996). This chemistry has three important consequences: firstly, in contrast to DNA or phosphorothioate oligonucleotides, PNAs are neutral molecules; secondly, PNAs are achiral, which avoids the need to develop a stereoselective synthesis; and thirdly, PNA synthesis uses standard Boc or Fmoc protocols for solid-phase peptide synthesis, although other methods, including a modified Merrifield method, have been used.

PNA monomers or ready-made oligomers are commercially available from PerSeptive Biosystems (Framingham, MA). PNA syntheses by either Boc or Fmoc protocols are straightforward using manual or automated protocols (Norton *et al.*, *Bioorg. Med. Chem.* 3(4):437-45 (Apr 1995)). The manual protocol lends itself to the production of chemically modified PNAs or the simultaneous synthesis of families of closely related PNAs.

As with peptide synthesis, the success of a particular PNA synthesis will depend on the properties of the chosen sequence. For example, while in theory PNAs can incorporate any combination of nucleotide bases, the presence of adjacent purines can lead to deletions of one or more residues in the product. In expectation of this difficulty, it is suggested that, in producing PNAs with adjacent purines, one should repeat the coupling of residues likely to be added inefficiently. This should be followed by the purification of PNAs by reverse-phase high-pressure liquid chromatography, providing yields and purity of product similar to those observed during the synthesis of peptides.

Modifications of PNAs for a given application may be accomplished by coupling amino acids during solid-phase synthesis or by attaching compounds that contain a carboxylic acid group to the exposed N-terminal amine. Alternatively, PNAs can be modified after synthesis by coupling to an introduced lysine or cysteine. The ease with which PNAs can be modified facilitates optimization for better solubility or for specific functional requirements. Once synthesized, the identity of PNAs and their derivatives can be confirmed by mass spectrometry. Several studies have made and utilized modifications of PNAs (for example, Norton *et al.*, *Bioorg Med Chem* 3(4):437-45 (Apr 1995); Petersen *et al.*, *J Pept Sci* 1(3):175-83 (May-Jun 1995); Orum *et al.*, *Biotechniques* 19(3):472-80 (Sep 1995); Footer *et al.*, *Biochemistry*. 1996 Aug 20;35(33):10673-9; Griffith *et al.*, *Nucleic Acids Res* 23(15):3003-8 (Aug 11 1995); Pardridge *et al.*, *Proc. Natl. Acad. Sci. USA*. 92(12):5592-6 (Jun 6 1995); Boffa *et al.*, *Proc. Natl. Acad. Sci. USA*. 92(6):1901-5 (Mar 14 1995); Gambacorti-Passerini *et al.*, *Blood* 88(4):1411-7 (Aug 15 1996); Armitage *et al.*, *Proc. Natl. Acad. Sci. USA*. 94(23):12320-5 (Nov 11 1997); Seeger *et al.*, *Biotechniques* 23(3):512-7 (Sep 1997)). U.S. Patent No. 5,700,922 discusses PNA-DNA-PNA chimeric molecules and their uses in diagnostics, modulating protein in organisms, and treatment of conditions susceptible to therapeutics.

Methods of characterizing the antisense binding properties of PNAs are discussed in Rose (*Anal Chem* 65(24):3545-9 (Dec 15 1993) and Jensen *et al.* (*Biochemistry*. 1997 Apr 22;36(16):5072-7). Rose uses capillary gel electrophoresis to determine binding of PNAs to their complementary oligonucleotide, measuring the

relative binding kinetics and stoichiometry. Similar types of measurements were made by Jensen *et al.* using BIAcore™ technology.

Other applications of PNAs that have been described and will be apparent to the skilled artisan include use in DNA strand invasion, antisense inhibition, mutational analysis, enhancers of transcription, nucleic acid purification, isolation of transcriptionally active genes, blocking of transcription factor binding, genome cleavage, biosensors, *in situ* hybridization, and the like.

Polynucleotide Identification, Characterization and Expression

Polynucleotides (and polypeptide) compositions of the present invention may be identified, prepared and/or manipulated using any of a variety of well established techniques (see generally, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989, and other like references).

Many template dependent processes are available to amplify a target sequence of interest present in a sample. One of the best known amplification methods is the polymerase chain reaction (PCR™) which is described in detail in U.S. Patent Nos. 4,683,195, 4,683,202 and 4,800,159, each of which is incorporated herein by reference in its entirety. Briefly, in PCR™, two primer sequences are prepared which are complementary to regions on opposite complementary strands of the target sequence. An excess of deoxynucleoside triphosphates is added to a reaction mixture along with a DNA polymerase (*e.g.*, *Taq* polymerase). If the target sequence is present in a sample, the primers will bind to the target and the polymerase will cause the primers to be extended along the target sequence by adding on nucleotides. By raising and lowering the temperature of the reaction mixture, the extended primers will dissociate from the target to form reaction products, excess primers will bind to the target and to the reaction product and the process is repeated. Preferably reverse transcription and PCR™ amplification procedure may be performed in order to quantify the amount of mRNA amplified. Polymerase chain reaction methodologies are well known in the art.

Any of a number of other template dependent processes, many of which are variations of the PCR™ amplification technique, are readily known and available in

the art. Illustratively, some such methods include the ligase chain reaction (referred to as LCR), described, for example, in Eur. Pat. Appl. Publ. No. 320,308 and U.S. Patent No. 4,883,750; Qbeta Replicase, described in PCT Intl. Pat. Appl. Publ. No. PCT/US87/00880; Strand Displacement Amplification (SDA) and Repair Chain Reaction (RCR). Still other amplification methods are described in Great Britain Pat. Appl. No. 2 202 328, and in PCT Intl. Pat. Appl. Publ. No. PCT/US89/01025. Other nucleic acid amplification procedures include transcription-based amplification systems (TAS) (PCT Intl. Pat. Appl. Publ. No. WO 88/10315), including nucleic acid sequence based amplification (NASBA) and 3SR. Eur. Pat. Appl. Publ. No. 329,822 describes a nucleic acid amplification process involving cyclically synthesizing single-stranded RNA ("ssRNA"), ssDNA, and double-stranded DNA (dsDNA). PCT Intl. Pat. Appl. Publ. No. WO 89/06700 describes a nucleic acid sequence amplification scheme based on the hybridization of a promoter/primer sequence to a target single-stranded DNA ("ssDNA") followed by transcription of many RNA copies of the sequence. Other amplification methods such as "RACE" (Frohman, 1990), and "one-sided PCR" (Ohara, 1989) are also well-known to those of skill in the art.

An amplified portion of a polynucleotide of the present invention may be used to isolate a full length gene from a suitable library (*e.g.*, a tumor cDNA library) using well known techniques. Within such techniques, a library (cDNA or genomic) is screened using one or more polynucleotide probes or primers suitable for amplification. Preferably, a library is size-selected to include larger molecules. Random primed libraries may also be preferred for identifying 5' and upstream regions of genes. Genomic libraries are preferred for obtaining introns and extending 5' sequences.

For hybridization techniques, a partial sequence may be labeled (*e.g.*, by nick-translation or end-labeling with ³²P) using well known techniques. A bacterial or bacteriophage library is then generally screened by hybridizing filters containing denatured bacterial colonies (or lawns containing phage plaques) with the labeled probe (*see Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989*). Hybridizing colonies or plaques are selected and expanded, and the DNA is isolated for further analysis. cDNA clones may be analyzed to determine the amount of additional sequence by, for example, PCR

using a primer from the partial sequence and a primer from the vector. Restriction maps and partial sequences may be generated to identify one or more overlapping clones. The complete sequence may then be determined using standard techniques, which may involve generating a series of deletion clones. The resulting overlapping sequences can then be assembled into a single contiguous sequence. A full length cDNA molecule can be generated by ligating suitable fragments, using well known techniques.

As will be understood by those of skill in the art, it may be advantageous in some instances to produce polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce a recombinant RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

Sequences encoding a desired polypeptide may be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers, M. H. *et al.* (1980) *Nucl. Acids Res. Symp. Ser.* 215-223, Horn, T. *et al.* (1980) *Nucl. Acids Res. Symp. Ser.* 225-232). Alternatively, the protein itself may be produced using chemical methods to synthesize the amino acid sequence of a polypeptide, or a portion thereof. For example, peptide synthesis can be performed using various solid-phase techniques (Roberge, J. Y. *et al.* (1995) *Science* 269:202-204) and automated synthesis may be achieved, for example, using the ABI 431A Peptide Synthesizer (Perkin Elmer, Palo Alto, CA).

A newly synthesized peptide may be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, T. (1983) *Proteins, Structures and Molecular Principles*, WH Freeman and Co., New York, N.Y.) or other comparable techniques available in the art. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure). Additionally, the amino acid sequence of a polypeptide, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

In order to express a desired polypeptide, the nucleotide sequences encoding the polypeptide, or functional equivalents, may be inserted into an appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding a polypeptide of interest and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described, for example, in Sambrook, J. *et al.* (1989) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Press, Plainview, N.Y., and Ausubel, F. M. *et al.* (1989) *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, N.Y.

A variety of expression vector/host systems may be utilized to contain and express polynucleotide sequences. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (*e.g.*, baculovirus); plant cell systems transformed with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmids); or animal cell systems.

The “control elements” or “regulatory sequences” present in an expression vector are those non-translated regions of the vector—enhancers, promoters,

5' and 3' untranslated regions—which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the pBLUESCRIPT phagemid (Stratagene, La Jolla, Calif.) or pSPORT1 plasmid (Gibco BRL, Gaithersburg, MD) and the like may be used. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are generally preferred. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding a polypeptide, vectors based on SV40 or EBV may be advantageously used with an appropriate selectable marker.

In mammalian host cells, a number of viral-based expression systems are generally available. For example, in cases where an adenovirus is used as an expression vector, sequences encoding a polypeptide of interest may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing the polypeptide in infected host cells (Logan, J. and Shenk, T. (1984) *Proc. Natl. Acad. Sci.* 81:3655-3659). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be used to achieve more efficient translation of sequences encoding a polypeptide of interest. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding the polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers which are

appropriate for the particular cell system which is used, such as those described in the literature (Scharf, D. *et al.* (1994) *Results Probl. Cell Differ.* 20:125-162).

In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding and/or function. Different host cells such as CHO, COS, HeLa, MDCK, HEK293, and WI38, which have specific cellular machinery and characteristic mechanisms for such post-translational activities, may be chosen to ensure the correct modification and processing of the foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is generally preferred. For example, cell lines which stably express a polynucleotide of interest may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler, M. *et al.* (1977) *Cell* 11:223-32) and adenine phosphoribosyltransferase (Lowy, I. *et al.* (1990) *Cell* 22:817-23) genes which can be employed in tk.sup.- or aprt.sup.- cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler, M. *et al.* (1980) *Proc. Natl. Acad. Sci.* 77:3567-70); npt, which confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin, F. *et al.* (1981) *J. Mol. Biol.* 150:1-14); and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, *supra*).

Additional selectable genes have been described, for example, *trpB*, which allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine (Hartman, S. C. and R. C. Mulligan (1988) *Proc. Natl. Acad. Sci.* 85:8047-51). The use of visible markers has gained popularity with such markers as anthocyanins, beta-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes, C. A. *et al.* (1995) *Methods Mol. Biol.* 55:121-131).

Alternatively, host cells that contain and express a desired polynucleotide sequence may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include, for example, membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein.

A variety of protocols for detecting and measuring the expression of polynucleotide-encoded products, using either polyclonal or monoclonal antibodies specific for the product are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on a given polypeptide may be preferred for some applications, but a competitive binding assay may also be employed. These and other assays are described, among other places, in Hampton, R. *et al.* (1990; *Serological Methods, a Laboratory Manual*, APS Press, St Paul, Minn.) and Maddox, D. E. *et al.* (1983; *J. Exp. Med.* 158:1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides include oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, the sequences, or any portions thereof may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA

probes *in vitro* by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits. Suitable reporter molecules or labels, which may be used include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with a polynucleotide sequence of interest may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides of the invention may be designed to contain signal sequences which direct secretion of the encoded polypeptide through a prokaryotic or eukaryotic cell membrane. Other recombinant constructions may be used to join sequences encoding a polypeptide of interest to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). The inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen, San Diego, Calif.) between the purification domain and the encoded polypeptide may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a polypeptide of interest and a nucleic acid encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography) as described in Porath, J. *et al.* (1992, *Prot. Exp. Purif.* 3:263-281) while the enterokinase cleavage site provides a means for purifying the desired polypeptide from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D. J. *et al.* (1993; *DNA Cell Biol.* 12:441-453).

In addition to recombinant production methods, polypeptides of the invention, and fragments thereof, may be produced by direct peptide synthesis using

solid-phase techniques (Merrifield J. (1963) *J. Am. Chem. Soc.* 85:2149-2154). Protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Alternatively, various fragments may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

T Cell Compositions

The present invention, in another aspect, provides T cells specific for a cancer-associated polypeptide disclosed herein (*e.g.*, oncofactor), or for a variant or derivative or fragment thereof. Such cells may generally be prepared *in vitro* or *ex vivo*, using standard procedures. For example, T cells may be isolated from bone marrow, peripheral blood, or a fraction of bone marrow or peripheral blood of a patient, using a commercially available cell separation system, such as the Isolex™ System, available from Nexell Therapeutics, Inc. (Irvine, CA; see also U.S. Patent No. 5,240,856; U.S. Patent No. 5,215,926; WO 89/06280; WO 91/16116 and WO 92/07243). Alternatively, T cells may be derived from related or unrelated humans, non-human mammals, cell lines or cultures.

T cells may be stimulated with a cancer-associated polypeptide, polynucleotide encoding a polypeptide and/or an antigen presenting cell (APC) that expresses such a polypeptide. Such stimulation is performed under conditions and for a time sufficient to permit the generation of T cells that are specific for the polypeptide of interest. Preferably, a tumor polypeptide or polynucleotide of the invention is present within a delivery vehicle, such as a microsphere, to facilitate the generation of specific T cells.

T cells are considered to be specific for a polypeptide of the present invention if the T cells specifically proliferate, secrete cytokines or kill target cells coated with the polypeptide or expressing a gene encoding the polypeptide. T cell specificity may be evaluated using any of a variety of standard techniques. For example, within a chromium release assay or proliferation assay, a stimulation index of more than two fold increase in lysis and/or proliferation, compared to negative controls,

indicates T cell specificity. Such assays may be performed, for example, as described in Chen *et al.*, *Cancer Res.* 54:1065-1070, 1994. Alternatively, detection of the proliferation of T cells may be accomplished by a variety of known techniques. For example, T cell proliferation can be detected by measuring an increased rate of DNA synthesis (*e.g.*, by pulse-labeling cultures of T cells with tritiated thymidine and measuring the amount of tritiated thymidine incorporated into DNA). Contact with a tumor polypeptide (100 ng/ml–100 µg/ml, preferably 200 ng/ml–25 µg/ml) for 3-7 days will typically result in at least a two fold increase in proliferation of the T cells. Contact as described above for 2-3 hours should result in activation of the T cells, as measured using standard cytokine assays in which a two fold increase in the level of cytokine release (*e.g.*, TNF or IFN- γ) is indicative of T cell activation (*see* Coligan *et al.*, *Current Protocols in Immunology*, vol. 1, Wiley Interscience (Greene 1998)). T cells that have been activated in response to a tumor polypeptide, polynucleotide or polypeptide-expressing APC may be CD4⁺ and/or CD8⁺. Tumor polypeptide-specific T cells may be expanded using standard techniques. Within certain embodiments, the T cells are derived from a patient, a related donor or an unrelated donor, and are administered to the patient following stimulation and expansion.

For therapeutic purposes, CD4⁺ or CD8⁺ T cells that proliferate in response to a tumor polypeptide, polynucleotide or APC can be expanded in number either *in vitro* or *in vivo*. Proliferation of such T cells *in vitro* may be accomplished in a variety of ways. For example, the T cells can be re-exposed to a tumor polypeptide, or a short peptide corresponding to an immunogenic portion of such a polypeptide, with or without the addition of T cell growth factors, such as interleukin-2, and/or stimulator cells that synthesize a tumor polypeptide. Alternatively, one or more T cells that proliferate in the presence of the tumor polypeptide can be expanded in number by cloning. Methods for cloning cells are well known in the art, and include limiting dilution.

The T cell receptor (TCR) consists of 2 different, highly variable polypeptide chains, termed the T-cell receptor α and β chains, that are linked by a disulfide bond (Janeway, Travers, Walport. *Immunobiology*. Fourth Ed., 148-159. Elsevier Science Ltd/Garland Publishing. 1999). The α/β heterodimer complexes with

the invariant CD3 chains at the cell membrane. This complex recognizes specific antigenic peptides bound to MHC molecules. The enormous diversity of TCR specificities is generated much like immunoglobulin diversity, through somatic gene rearrangement. The β chain genes contain over 50 variable (V), 2 diversity (D), over 10 joining (J) segments, and 2 constant region segments (C). The α chain genes contain over 70 V segments, and over 60 J segments but no D segments, as well as one C segment. During T cell development in the thymus, the D to J gene rearrangement of the β chain occurs, followed by the V gene segment rearrangement to the DJ. This functional VDJ β exon is transcribed and spliced to join to a C β . For the α chain, a V α gene segment rearranges to a J α gene segment to create the functional exon that is then transcribed and spliced to the C α . Diversity is further increased during the recombination process by the random addition of P and N-nucleotides between the V, D, and J segments of the β chain and between the V and J segments in the α chain (Janeway, Travers, Walport. Immunobiology. Fourth Ed., 98 and 150. Elsevier Science Ltd/Garland Publishing. 1999).

The present invention, in another aspect, provides TCRs specific for a cancer-associated polypeptide disclosed herein, or for a variant or derivative thereof. In accordance with the present invention, polynucleotide and amino acid sequences are provided for the V-J or V-D-J junctional regions or parts thereof for the alpha and beta chains of the T-cell receptor which recognize tumor polypeptides described herein. In general, this aspect of the invention relates to T-cell receptors which recognize or bind tumor polypeptides presented in the context of MHC. In a preferred embodiment the tumor antigens recognized by the T-cell receptors comprise a polypeptide of the present invention. For example, cDNA encoding a TCR specific for a tumor peptide can be isolated from T cells specific for a tumor polypeptide using standard molecular biological and recombinant DNA techniques.

This invention further includes the T-cell receptors or analogs thereof having substantially the same function or activity as the T-cell receptors of this invention which recognize or bind tumor polypeptides. Such receptors include, but are not limited to, a fragment of the receptor, or a substitution, addition or deletion mutant of a T-cell receptor provided herein. This invention also encompasses polypeptides or

peptides that are substantially homologous to the T-cell receptors provided herein or that retain substantially the same activity. The term “analog” includes any protein or polypeptide having an amino acid residue sequence substantially identical to the T-cell receptors provided herein in which one or more residues, preferably no more than 5 residues, more preferably no more than 25 residues have been conservatively substituted with a functionally similar residue and which displays the functional aspects of the T-cell receptor as described herein.

The present invention further provides for suitable mammalian host cells, for example, non-specific T-cells, that are transfected with a polynucleotide encoding TCRs specific for a polypeptide described herein, thereby rendering the host cell specific for the polypeptide. The α and β chains of the TCR may be contained on separate expression vectors or alternatively, on a single expression vector that also contains an internal ribosome entry site (IRES) for cap-independent translation of the gene downstream of the IRES. Said host cells expressing TCRs specific for the polypeptide may be used, for example, for adoptive immunotherapy of cancer as discussed further below.

In further aspects of the present invention, cloned TCRs specific for a polypeptide recited herein may be used in a kit for the diagnosis of cancer. For example, the nucleic acid sequence or portions thereof, of tumor-specific TCRs can be used as probes or primers for the detection of expression of the rearranged genes encoding the specific TCR in a biological sample. In this respect, the present invention further provides for an assay for detecting messenger RNA or DNA encoding the TCR specific for a polypeptide.

Pharmaceutical & Other Compositions

Pharmaceutical compositions of the invention generally comprise one or more of the cancer-associated antibodies, polynucleotides, polypeptides, T-cells, or TCR compositions disclosed herein in pharmaceutically-acceptable carriers for administration to a cell or an animal, either alone, or in combination with one or more other modalities of therapy.

It will be understood that, if desired, a composition as disclosed herein may be administered in combination with other agents as well, such as, *e.g.*, other

proteins or polypeptides or various pharmaceutically-active agents. In fact, there is virtually no limit to other components that may also be included, given that the additional agents do not cause a significant adverse effect upon contact with the target cells or host tissues. The compositions may thus be delivered along with various other agents as required in the particular instance. Such compositions may be purified from host cells or other biological sources, or alternatively may be chemically synthesized as described herein. Likewise, such compositions may further comprise substituted or derivatized RNA or DNA compositions.

Therefore, in another aspect of the present invention, pharmaceutical compositions are provided comprising one or more of the polynucleotide, polypeptide, antibody, TCR, and/or T-cell compositions described herein in combination with a physiologically acceptable carrier.

In one embodiment, the invention contemplates, in part, compositions comprising one or more antibodies to a cancer-associated polypeptide or oncofactor disclosed herein. In particular embodiments of the invention, the composition comprises one or more purified oncofactor antibodies and the composition is substantially free from endotoxin, has little or no aggregate formation, and the purified isolated polypeptide of the composition is soluble in a therapeutically acceptable formulation.

In one embodiment, the invention contemplates compositions comprising at least fragments thereof having at least one purified isolated antibody to an oncofactor wherein the antibody and composition are substantially free from endotoxin, wherein the p antibody has little or no aggregate formation, wherein the antibody is soluble in a therapeutically acceptable formulation and wherein the composition is substantially free of mammalian proinflammatory agents.

Endotoxins are toxins associated with certain bacteria, typically gram-negative bacteria, although endotoxins may be found in gram-positive bacteria, such as *Listeria monocytogenes*. The most prevalent endotoxins are lipopolysaccharides (LPS) or lipooligosaccharides (LOS) found in the outer membrane of various Gram-negative bacteria, and which represent a central pathogenic feature in the ability of these bacteria to cause disease. Small amounts of endotoxin in humans can produce fever, a lowering

of the blood pressure, and activation of inflammation and coagulation, among other adverse physiological effects. Therefore, it is often desirable to remove most or all traces of endotoxin from drugs and drug product containers, because even small amounts may cause adverse effects in humans. Producing formulations that are endotoxin-free can require special equipment, expert artisans, and can be significantly more expensive than making formulations that are not endotoxin-free.

Endotoxins can be detected using routine techniques known in the art. For example, the Limulus Amebocyte Lysate assay, which utilizes blood from the horseshoe crab, is a very sensitive assay for detecting presence of endotoxin. In this test, very low levels of LPS can cause detectable coagulation of the limulus lysate due a powerful enzymatic cascade that amplifies this reaction. Endotoxins can also be quantitated by enzyme-linked immunosorbent assay (ELISA). As used herein, the term "endotoxin free" refers to compositions that contain at most trace amounts (*i.e.*, amounts having no adverse physiological effects to a subject) of endotoxin, and preferably undetectable amounts of endotoxin. In one embodiment, the term "endotoxin free" refers to a composition that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% endotoxin free. In one embodiment, the term "endotoxin free" refers to endotoxin levels or an endotoxin profile that may be less than about 0.001, 0.005, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.08, 0.09, 0.1, 0.5, 1.0, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, or 10 endotoxin units (EU)/ml or EU/mg. Typically, 1 ng lipopolysaccharide (LPS) corresponds to about 1-10 EU.

To be substantially endotoxin free, endotoxin levels or endotoxin profile may be less than about 0.001, 0.005, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.08, 0.09, 0.1, 0.5, 1.0, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, or 10 EU/ml.

In certain embodiments, the invention contemplates, in part, an oncofactor antibody comprising an endotoxin profile of less than about 50 EU/mg, less than about 30 EU/mg, less than about 25 EU/mg, less than about 20 EU/mg, less than about 15 EU/mg, less than about 10 EU/mg, less than about 8 EU/mg, less than about 7 EU/mg, less than about 6 EU/mg, less than about 5 EU/mg, less than about 4 EU/mg, less than about 3 EU/mg, less than about 2 EU/mg, less than about 1.5 EU/mg, less than about 1.4 EU/mg, less than about 1.3 EU/mg, less than about 1.2 EU/mg, less than

about 1.1 EU/mg, less than about 1.0 EU/mg, less than about 0.9 EU/mg, less than about 0.8 EU/mg, less than about 0.7 EU/mg, less than about 0.6 EU/mg, less than about 0.5 EU/mg, less than about 0.4 EU/mg, less than about 0.3 EU/mg, less than about 0.2 EU/mg, less than about 0.1 EU/mg or less endotoxin units per mg of purified CT-1 polypeptide. The endotoxin levels or profile may be assessed at room temperature (20°C-25°C) or at body temperature (37°C).

The invention additionally provides oncofactor antibodies having improved stability as compared to existing antibodies. Stability can generally be defined as the propensity of the molecule to remain in its folded and active state. Naturally occurring molecules are usually of limited stability as their metabolism, and often their fast metabolism, is a key characteristic of their intrinsic mechanism of action in the body.

Usually, a stable protein in its folded and native structure cannot be degraded by proteases or other mechanisms. It is due to two key off pathways from the stable state by which proteins are usually eliminated in the body. These two are unfolding and aggregation. They are usually linked. Unfolding is the pathway of reverting the folded active molecule into a less folded state. Aggregation is the result of misfolding such that the molecule irreversibly turns into a non-active state. Both unfolding and aggregation significantly increase the protein's susceptibility to proteolytic or other digestion. The present invention provides a modified folding and unfolding pathway of an oncofactor antibody such that the resulting entity is more stable than an oncofactor antibody that is not produced by the methods of the invention.

In particular embodiments, the invention provides an antibody composition having increased stability against insoluble protein aggregate formation. "Protein aggregate" or "protein aggregation" is used herein to refer to protein that is no longer in solution. While protein aggregate can refer to agglomeration or oligomerization of two or more individual protein molecules, it is not limited to such a definition. Protein aggregates, as used in the art, can be soluble or insoluble; however, for the purposes of particular embodiments of the invention, protein aggregates are usually considered to be insoluble, unless otherwise specifically noted. Insoluble aggregates whose formation should be prevented in the process according to the

invention are essentially understood as protein aggregates having a size of at least 1 μm but can also be in the range above 10 μm . The particles can be determined by suitable particle counting methods using commercial particle counting instruments such as, for example, the particle counting instrument AccuSizer 700 from PSS (Particle Sizing Systems, USA) or a Pacific Scientific HIAC Royco liquid particle counting system, model 9703, equipped with a LD400 laser counter. According to the USP (US-Pharmacopoeia) a maximum of 6000 particles in the range above 10 μm and a maximum of 600 particles in the range above 25 μm are allowed per injected dose of a pharmaceutical preparation. This can be achieved according to the invention to provide for therapeutic compositions of oncofactor antibodies.

In a particular embodiments, the a composition comprising one or more oncofactor antibodies has increased stability against aggregate formation induced by one or more freeze/thaw cycles, agitation stress, or one or more outside physical or chemical stresses including non-limiting examples of heat stress, chemical stress (*e.g.*, pH, low/high salt, and the like), fluid stress (*e.g.*, compression stresses, such as those caused by fluid movement through constricted openings). As used herein “agitation stress” is taken to mean any physical movement applied to the composition either passively or actively. Non-limiting examples of agitation stresses, include bumping, dropping, shaking, swirling, vortexing, decanting, injecting, withdrawing (as into a syringe from a containing or vessel), and the like. The compositions of the invention are particularly stabilized with respect to the forces of shipping and transportation.

Oncofactor antibodies having improved stability may retain 90% residual activity at a temperature that is 2-10 degrees higher than existing antibodies. The percentage of residual (*i.e.*, folded, active) protein may be measured by routine biochemical techniques such as HPLC, SDS PAGE or by activity assays such as binding assays or eliciting a response from cells.

In particular embodiments, the present invention contemplates compositions comprising cancer-associated antibodies, wherein the antibodies are stable for at least 6 hours, at least 7 hours, at least 8 hours, at least 9 hours, at least 10 hours, at least 11 hours, at least 12 hours, at least 15 hours, at least 18 hours, at least 21

hours, at least 24 hours, at least 48 hours, or more, at about 37°C compared to existing antibodies that are not formulated according to the methods of the present invention.

The term “solubility” refers to the property of an agent provided herein to dissolve in a liquid solvent and form a homogeneous solution. Solubility is typically expressed as a concentration, either by mass of solute per unit volume of solvent (g of solute per kg of solvent, g per dL (100 mL), mg/mL, etc.), molarity, molality, mole fraction or other similar descriptions of concentration. The maximum equilibrium amount of solute that can dissolve per amount of solvent is the solubility of that solute in that solvent under the specified conditions, including temperature, pressure, pH, and the nature of the solvent. In certain embodiments, solubility is measured at physiological pH. In certain embodiments, solubility is measured in water or a physiological buffer such as PBS. In certain embodiments, solubility is measured in a biological fluid (solvent) such as blood or serum. In certain embodiments, the temperature can be about room temperature (*e.g.*, about 20, 21, 22, 23, 24, 25°C) or about body temperature (37°C). In certain embodiments, an agent such as a CT-1 polypeptide of the invention has a solubility of at least about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, or 30 mg/mL at room temperature (20°C -25°C) or at 37°C.

Protein characteristics including purity, solubility and degree of aggregation can be assessed using protein-based analytical assays and methods. Protein purity can be assessed a number of ways. For instance, purity can be assessed based on primary structure, higher order structure, size, charge, hydrophobicity, and glycosylation. Examples of methods for assessing primary structure include N- and C-terminal sequencing and peptide-mapping (see, *e.g.*, Allen et al., *Biologicals*. 24:255-275, 1996)). Examples of methods for assessing higher order structure include circular dichroism (see, *e.g.*, Kelly et al., *Biochim Biophys Acta*. 1751:119-139, 2005), fluorescent spectroscopy (see, *e.g.*, Meagher et al., *J. Biol. Chem.* 273:23283-89, 1998), FT-IR, amide hydrogen-deuterium exchange kinetics, differential scanning calorimetry, NMR spectroscopy, immunoreactivity with conformationally sensitive antibodies. Higher order structure can also be assessed as a function of a variety of parameters such as pH, temperature, or added salts.

Examples of methods for assessing protein characteristics such as size include analytical ultracentrifugation and size exclusion HPLC (SEC-HPLC, or alternatively, HPLC-SEC), and exemplary methods for measuring charge include ion-exchange chromatography and isoelectric focusing. Hydrophobicity can be assessed, for example, by reverse-phase HPLC and hydrophobic interaction chromatography HPLC. Glycosylation can affect pharmacokinetics (*e.g.*, clearance), conformation or stability, receptor binding, and protein function, and can be assessed, for example, by mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy.

Certain embodiments include the use of SEC-HPLC to assess protein characteristics such as purity, size (*e.g.*, size homogeneity) or degree of aggregation, and/or to purify proteins, among other uses. SEC, also including gel-filtration chromatography (GFC) and gel-permeation chromatography (GPC), refers to a chromatographic method in which molecules in solution are separated in a porous material based on their size, or more specifically their hydrodynamic volume, diffusion coefficient, and/or surface properties. The process is generally used to separate biological molecules, and to determine molecular weights and molecular weight distributions of polymers. Typically, a biological or protein sample (such as a protein extract produced according to the protein expression methods provided herein and known in the art) is loaded into a selected size-exclusion column with a defined stationary phase (the porous material), preferably a phase that does not interact with the proteins in the sample. In certain aspects, the stationary phase is composed of inert particles packed into a dense three-dimensional matrix within a glass or steel column. The mobile phase can be pure water, an aqueous buffer, an organic solvent, or a mixture thereof. The stationary-phase particles typically have small pores and/or channels which only allow molecules below a certain size to enter. Large particles are therefore excluded from these pores and channels, and their limited interaction with the stationary phase leads them to elute as a “totally-excluded” peak at the beginning of the experiment. Smaller molecules, which can fit into the pores, are removed from the flowing mobile phase, and the time they spend immobilized in the stationary-phase pores depends, in part, on how far into the pores they penetrate. Their removal from the mobile phase flow causes them to take longer to elute from the column and results in a

separation between the particles based on differences in their size. A given size exclusion column has a range of molecular weights that can be separated. Overall, molecules larger than the upper limit will not be trapped by the stationary phase, molecules smaller than the lower limit will completely enter the solid phase and elute as a single band, and molecules within the range will elute at different rates, defined by their properties such as hydrodynamic volume. For examples of these methods in practice with pharmaceutical proteins, see Bruner *et al.*, *Journal of Pharmaceutical and Biomedical Analysis*. 15: 1929-1935, 1997.

Protein purity for clinical applications is also discussed, for example, by Anicetti *et al.* (*Trends in Biotechnology*. 7:342-349, 1989). More recent techniques for analyzing protein purity include, without limitation, the LabChip GXII, an automated platform for rapid analysis of proteins and nucleic acids, which provides high throughput analysis of titer, sizing, and purity analysis of proteins. In certain non-limiting embodiments, clinical grade proteins such as protein fragments and antibodies can be obtained by utilizing a combination of chromatographic materials in at least two orthogonal steps, among other methods (*see, e.g.*, *Therapeutic Proteins: Methods and Protocols*. Vol. 308, Eds., Smales and James, Humana Press Inc., 2005).

In certain embodiments, compositions comprising one or more oncofactor antibody have a purity of at least about 90%, with respect to the antibody and as measured according to routine techniques in the art. In certain embodiments, such as diagnostic compositions or certain therapeutic compositions, the antibody compositions of the invention have a purity of at least about 95%. In specific embodiments, such as therapeutic or pharmaceutical compositions, the antibody compositions of the invention have a purity of at least about 97% or 98% or 99%. In other embodiments, such as when being used as reference or research reagents, antibody of the invention can be of lesser purity, and may have a purity of at least about 70%, 75%, 80%, or 85%. Purity can be measured overall or in relation to selected components, such as other proteins, *e.g.*, purity on a protein basis.

Protein solubility assays are also included. Such assays can be utilized, for example, to determine optimal growth and purification conditions for recombinant production, to optimize the choice of buffer(s), and to optimize the choice of antibodies.

Solubility or aggregation can be evaluated according to a variety of parameters, including temperature, pH, salts, and the presence or absence of other additives. Examples of solubility screening assays include, without limitation, microplate-based methods of measuring protein solubility using turbidity or other measure as an end point, high-throughput assays for analysis of the solubility of purified recombinant proteins (see, e.g., Stenvall *et al.*, *Biochim Biophys Acta*. 1752:6-10, 2005), assays that use structural complementation of a genetic marker protein to monitor and measure protein folding and solubility in vivo (see, e.g., Wigley *et al.*, *Nature Biotechnology*. 19:131-136, 2001), and electrochemical screening of recombinant protein solubility in *Escherichia coli* using scanning electrochemical microscopy (SECM) (see, e.g., Nagamine *et al.*, *Biotechnology and Bioengineering*. 96:1008-1013, 2006), among others.

In particular embodiments, a human therapeutic composition is provided, comprising a modified polypeptide of the invention or fragment thereof as described elsewhere herein and a pharmacokinetic (PK) modulator. As used herein, the term “pharmacokinetic modulator” generally refers to an antibody modification that increases the pharmacokinetic parameters of the antibody, including, without limitation, half-life, solubility, stability, activity compared to an antibody that lacks the PK modulator. In one embodiment, the PK modulator comprises a biocompatible polymer conjugated to the antibody, including for example, polyethylene glycol (PEG).

In certain preferred embodiments, the pharmaceutical compositions of the invention comprise isolated antibodies or antigen-binding fragments thereof that specifically bind at least one cancer-associated polypeptide of the present invention, such as a cancer-associated polypeptide of any one of SEQ ID NOs: 1-24.

In certain other embodiments, the pharmaceutical compositions of the invention may comprise immunogenic polynucleotides and/or polypeptides compositions of the invention for use in prophylactic or therapeutic vaccine applications. Vaccine preparation is generally described in, for example, M.F. Powell and M.J. Newman, eds., “Vaccine Design (the subunit and adjuvant approach),” Plenum Press (NY, 1995). Generally, such compositions will comprise one or more polynucleotide

and/or polypeptide compositions of the present invention in combination with one or more immunostimulants.

In one embodiment, a pharmaceutical composition comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more oncofactor antibodies or antigen binding fragments thereof. The antibodies may be directed to the same or different oncofactors.

In still other embodiments, the pharmaceutical compositions of the invention may comprise polynucleotides (*e.g.*, antisense, ribozyme, RNAi or siRNA sequences) that are effective for inhibiting the expression of one or more cancer-associated polynucleotide sequences of the invention.

It will be apparent that any of the pharmaceutical compositions described herein can contain pharmaceutically acceptable salts of the polynucleotides and polypeptides of the invention. Such salts can be prepared, for example, from pharmaceutically acceptable non-toxic bases, including organic bases (*e.g.*, salts of primary, secondary and tertiary amines and basic amino acids) and inorganic bases (*e.g.*, sodium, potassium, lithium, ammonium, calcium and magnesium salts).

In other embodiments, illustrative immunogenic compositions, *e.g.*, vaccine compositions, of the present invention comprise DNA encoding one or more of the cancer-associated polypeptides as described above, such that the polypeptide is generated *in situ*. As noted above, the polynucleotide may be administered within any of a variety of delivery systems known to those of ordinary skill in the art. Indeed, numerous gene delivery techniques are well known in the art, such as those described by Rolland, *Crit. Rev. Therap. Drug Carrier Systems* 15:143-198, 1998, and references cited therein. Appropriate polynucleotide expression systems will, of course, contain the necessary regulatory DNA regulatory sequences for expression in a patient (such as a suitable promoter and terminating signal). Alternatively, bacterial delivery systems may involve the administration of a bacterium (such as *Bacillus-Calmette-Guerrin*) that expresses an immunogenic portion of the polypeptide on its cell surface or secretes such an epitope.

Therefore, in certain embodiments, polynucleotides encoding immunogenic polypeptides described herein are introduced into suitable mammalian host cells for expression using any of a number of known viral-based systems. In one

illustrative embodiment, retroviruses provide a convenient and effective platform for gene delivery systems. A selected nucleotide sequence encoding a polypeptide of the present invention can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to a subject. A number of illustrative retroviral systems have been described (*e.g.*, U.S. Pat. No. 5,219,740; Miller and Rosman (1989) *BioTechniques* 7:980-990; Miller, A. D. (1990) *Human Gene Therapy* 1:5-14; Scarpa *et al.* (1991) *Virology* 180:849-852; Burns *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:8033-8037; and Boris-Lawrie and Temin (1993) *Cur. Opin. Genet. Develop.* 3:102-109.

In addition, a number of illustrative adenovirus-based systems have also been described. Unlike retroviruses which integrate into the host genome, adenoviruses persist extrachromosomally thus minimizing the risks associated with insertional mutagenesis (Haj-Ahmad and Graham (1986) *J. Virol.* 57:267-274; Bett *et al.* (1993) *J. Virol.* 67:5911-5921; Mittereder *et al.* (1994) *Human Gene Therapy* 5:717-729; Seth *et al.* (1994) *J. Virol.* 68:933-940; Barr *et al.* (1994) *Gene Therapy* 1:51-58; Berkner, K. L. (1988) *BioTechniques* 6:616-629; and Rich *et al.* (1993) *Human Gene Therapy* 4:461-476).

Various adeno-associated virus (AAV) vector systems have also been developed for polynucleotide delivery. AAV vectors can be readily constructed using techniques well known in the art. *See, e.g.*, U.S. Pat. Nos. 5,173,414 and 5,139,941; International Publication Nos. WO 92/01070 and WO 93/03769; Lebkowski *et al.* (1988) *Molec. Cell. Biol.* 8:3988-3996; Vincent *et al.* (1990) *Vaccines 90* (Cold Spring Harbor Laboratory Press); Carter, B. J. (1992) *Current Opinion in Biotechnology* 3:533-539; Muzyczka, N. (1992) *Current Topics in Microbiol. and Immunol.* 158:97-129; Kotin, R. M. (1994) *Human Gene Therapy* 5:793-801; Shelling and Smith (1994) *Gene Therapy* 1:165-169; and Zhou *et al.* (1994) *J. Exp. Med.* 179:1867-1875.

Additional viral vectors useful for delivering the polynucleotides encoding polypeptides of the present invention by gene transfer include those derived from the pox family of viruses, such as vaccinia virus and avian poxvirus. By way of example, vaccinia virus recombinants expressing the novel molecules can be constructed as follows. The DNA encoding a polypeptide is first inserted into an

appropriate vector so that it is adjacent to a vaccinia promoter and flanking vaccinia DNA sequences, such as the sequence encoding thymidine kinase (TK). This vector is then used to transfect cells which are simultaneously infected with vaccinia.

Homologous recombination serves to insert the vaccinia promoter plus the gene encoding the polypeptide of interest into the viral genome. The resulting TK.sup.(-) recombinant can be selected by culturing the cells in the presence of 5-bromodeoxyuridine and picking viral plaques resistant thereto.

A vaccinia-based infection/transfection system can be conveniently used to provide for inducible, transient expression or coexpression of one or more polypeptides described herein in host cells of an organism. In this particular system, cells are first infected *in vitro* with a vaccinia virus recombinant that encodes the bacteriophage T7 RNA polymerase. This polymerase displays exquisite specificity in that it only transcribes templates bearing T7 promoters. Following infection, cells are transfected with the polynucleotide or polynucleotides of interest, driven by a T7 promoter. The polymerase expressed in the cytoplasm from the vaccinia virus recombinant transcribes the transfected DNA into RNA which is then translated into polypeptide by the host translational machinery. The method provides for high level, transient, cytoplasmic production of large quantities of RNA and its translation products. See, *e.g.*, Elroy-Stein and Moss, *Proc. Natl. Acad. Sci. USA* 87:6743-6747 (1990); Fuerst *et al.*, *Proc. Natl. Acad. Sci. USA* 83:8122-8126 (1986).

Alternatively, avipoxviruses, such as the fowlpox and canarypox viruses, can also be used to deliver the coding sequences of interest. Recombinant avipox viruses, expressing immunogens from mammalian pathogens, are known to confer protective immunity when administered to non-avian species. The use of an Avipox vector is particularly desirable in human and other mammalian species since members of the Avipox genus can only productively replicate in susceptible avian species and therefore are not infective in mammalian cells. Methods for producing recombinant Avipoxviruses are known in the art and employ genetic recombination, as described above with respect to the production of vaccinia viruses. See, *e.g.*, WO 91/12882; WO 89/03429; and WO 92/03545.

Any of a number of alphavirus vectors can also be used for delivery of polynucleotide compositions of the present invention, such as those vectors described in U.S. Patent Nos. 5,843,723; 6,015,686; 6,008,035 and 6,015,694. Certain vectors based on Venezuelan Equine Encephalitis (VEE) can also be used, illustrative examples of which can be found in U.S. Patent Nos. 5,505,947 and 5,643,576.

Moreover, molecular conjugate vectors, such as the adenovirus chimeric vectors described in Michael *et al.* *J. Biol. Chem.* 268:6866-6869 (1993) and Wagner *et al.*, *Proc. Natl. Acad. Sci. USA* 89:6099-6103 (1992), can also be used for gene delivery under the invention.

Additional illustrative information on these and other known viral-based delivery systems can be found, for example, in Fisher-Hoch *et al.*, *Proc. Natl. Acad. Sci. USA* 86:317-321, 1989; Flexner *et al.*, *Ann. N.Y. Acad. Sci.* 569:86-103, 1989; Flexner *et al.*, *Vaccine* 8:17-21, 1990; U.S. Patent Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973; U.S. Patent No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805; Berkner, *Biotechniques* 6:616-627, 1988; Rosenfeld *et al.*, *Science* 252:431-434, 1991; Kolls *et al.*, *Proc. Natl. Acad. Sci. USA* 91:215-219, 1994; Kass-Eisler *et al.*, *Proc. Natl. Acad. Sci. USA* 90:11498-11502, 1993; Guzman *et al.*, *Circulation* 88:2838-2848, 1993; and Guzman *et al.*, *Cir. Res.* 73:1202-1207, 1993.

In certain embodiments, a polynucleotide may be integrated into the genome of a target cell. This integration may be in the specific location and orientation *via* homologous recombination (gene replacement) or it may be integrated in a random, non-specific location (gene augmentation). In yet further embodiments, the polynucleotide may be stably maintained in the cell as a separate, episomal segment of DNA. Such polynucleotide segments or "episomes" encode sequences sufficient to permit maintenance and replication independent of or in synchronization with the host cell cycle. The manner in which the expression construct is delivered to a cell and where in the cell the polynucleotide remains is dependent on the type of expression construct employed.

In another embodiment of the invention, a polynucleotide is administered/delivered as "naked" DNA, for example as described in Ulmer *et al.*, *Science* 259:1745-1749, 1993 and reviewed by Cohen, *Science* 259:1691-1692, 1993.

The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

In still another embodiment, a composition of the present invention can be delivered via a particle bombardment approach, many of which have been described. In one illustrative example, gas-driven particle acceleration can be achieved with devices such as those manufactured by Powderject Pharmaceuticals PLC (Oxford, UK) and Powderject Vaccines Inc. (Madison, WI), some examples of which are described in U.S. Patent Nos. 5,846,796; 6,010,478; 5,865,796; 5,584,807; and EP Patent No. 0500 799. This approach offers a needle-free delivery approach wherein a dry powder formulation of microscopic particles, such as polynucleotide or polypeptide particles, are accelerated to high speed within a helium gas jet generated by a hand held device, propelling the particles into a target tissue of interest.

In a related embodiment, other devices and methods that may be useful for gas-driven needle-less injection of compositions of the present invention include those provided by Bioject, Inc. (Portland, OR), some examples of which are described in U.S. Patent Nos. 4,790,824; 5,064,413; 5,312,335; 5,383,851; 5,399,163; 5,520,639 and 5,993,412.

According to another embodiment, the pharmaceutical compositions described herein will comprise one or more immunostimulants in addition to the immunogenic polynucleotide, polypeptide, antibody, T-cell, TCR, and/or APC compositions of this invention. An immunostimulant refers to essentially any substance that enhances or potentiates an immune response (antibody and/or cell-mediated) to an exogenous antigen. One preferred type of immunostimulant comprises an adjuvant. Many adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, *Bordetella pertussis* or *Mycobacterium tuberculosis* derived proteins. Certain adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ); AS-2 (SmithKline Beecham, Philadelphia, PA); aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine;

acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF, interleukin-2, -7, -12, and other like growth factors, may also be used as adjuvants.

Certain preferred adjuvants for eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A, together with an aluminum salt. MPL[®] adjuvants are available from Corixa Corporation (Seattle, WA; *see*, for example, US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555, WO 99/33488 and U.S. Patent Nos. 6,008,200 and 5,856,462. Immunostimulatory DNA sequences are also described, for example, by Sato *et al.*, *Science* 273:352, 1996. Another preferred adjuvant comprises a saponin, such as Quil A, or derivatives thereof, including QS21 and QS7 (Aquila Biopharmaceuticals Inc., Framingham, MA); Escin; Digitonin; or *Gypsophila* or *Chenopodium quinoa* saponins. Other preferred formulations include more than one saponin in the adjuvant combinations of the present invention, for example combinations of at least two of the following group comprising QS21, QS7, Quil A, β -escin, or digitonin.

Alternatively the saponin formulations may be combined with vaccine vehicles composed of chitosan or other polycationic polymers, polylactide and polylactide-co-glycolide particles, poly-N-acetyl glucosamine-based polymer matrix, particles composed of polysaccharides or chemically modified polysaccharides, liposomes and lipid-based particles, particles composed of glycerol monoesters, *etc.* The saponins may also be formulated in the presence of cholesterol to form particulate structures such as liposomes or ISCOMs. Furthermore, the saponins may be formulated together with a polyoxyethylene ether or ester, in either a non-particulate solution or suspension, or in a particulate structure such as a paucilamellar liposome or ISCOM. The saponins may also be formulated with excipients such as Carbopol^R to increase

viscosity, or may be formulated in a dry powder form with a powder excipient such as lactose.

In one embodiment, the adjuvant system includes the combination of a monophosphoryl lipid A and a saponin derivative, such as the combination of QS21 and 3D-MPL[®] adjuvant, as described in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol, as described in WO 96/33739. Other preferred formulations comprise an oil-in-water emulsion and tocopherol. Another particularly preferred adjuvant formulation employing QS21, 3D-MPL[®] adjuvant and tocopherol in an oil-in-water emulsion is described in WO 95/17210.

Another enhanced adjuvant system involves the combination of a CpG-containing oligonucleotide and a saponin derivative particularly the combination of CpG and QS21 is disclosed in WO 00/09159. Preferably the formulation additionally comprises an oil in water emulsion and tocopherol.

Additional illustrative adjuvants for use in the pharmaceutical compositions of the invention include Montanide ISA 720 (Seppic, France), SAF (Chiron, California, United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series of adjuvants (*e.g.*, SBAS-2 or SBAS-4, available from SmithKline Beecham, Rixensart, Belgium), Detox (Enhanzyn[®]) (Corixa, Hamilton, MT), RC-529 (Corixa, Hamilton, MT) and other aminoalkyl glucosaminide 4-phosphates (AGPs), such as those described in pending U.S. Patent Application Serial Nos. 08/853,826 and 09/074,720, the disclosures of which are incorporated herein by reference in their entireties, and polyoxyethylene ether adjuvants such as those described in WO 99/52549A1.

Other preferred adjuvants include adjuvant molecules of the general formula



wherein, n is 1-50, A is a bond or $-\text{C}(\text{O})-$, R is C_{1-50} alkyl or Phenyl C_{1-50} alkyl.

One embodiment of the present invention consists of a vaccine formulation comprising a polyoxyethylene ether of general formula (I), wherein n is between 1 and 50, preferably 4-24, most preferably 9; the R component is C_{1-50} , preferably $\text{C}_4\text{-C}_{20}$ alkyl and most preferably C_{12} alkyl, and A is a bond. The concentration of the polyoxyethylene ethers should be in the range 0.1-20%, preferably

from 0.1-10%, and most preferably in the range 0.1-1%. Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether, polyoxyethylene-9-stearyl ether, polyoxyethylene-8-stearyl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether. Poxoxyethylene ethers such as polyoxyethylene lauryl ether are described in the Merck index (12th edition: entry 7717). These adjuvant molecules are described in WO 99/52549.

The polyoxyethylene ether according to the general formula (I) above may, if desired, be combined with another adjuvant. For example, a preferred adjuvant combination is preferably with CpG as described in the pending UK patent application GB 9820956.2.

According to another embodiment of this invention, an immunogenic composition described herein is delivered to a host via antigen presenting cells (APCs), such as dendritic cells, macrophages, B cells, monocytes and other cells that may be engineered to be efficient APCs. Such cells may, but need not, be genetically modified to increase the capacity for presenting the antigen, to improve activation and/or maintenance of the T cell response, to have anti-tumor effects *per se* and/or to be immunologically compatible with the receiver (*i.e.*, matched HLA haplotype). APCs may generally be isolated from any of a variety of biological fluids and organs, including tumor and peritumoral tissues, and may be autologous, allogeneic, syngeneic or xenogeneic cells.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will typically vary depending on the mode of administration. Compositions of the present invention may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, mucosal, intravenous, intracranial, intraperitoneal, subcutaneous and intramuscular administration.

Carriers for use within such pharmaceutical compositions are biocompatible, and may also be biodegradable. In certain embodiments, the formulation preferably provides a relatively constant level of active component release. In other embodiments, however, a more rapid rate of release immediately upon

administration may be desired. The formulation of such compositions is well within the level of ordinary skill in the art using known techniques. Illustrative carriers useful in this regard include microparticles of poly(lactide-co-glycolide), polyacrylate, latex, starch, cellulose, dextran and the like. Other illustrative delayed-release carriers include supramolecular biovectors, which comprise a non-liquid hydrophilic core (*e.g.*, a cross-linked polysaccharide or oligosaccharide) and, optionally, an external layer comprising an amphiphilic compound, such as a phospholipid (*see e.g.*, U.S. Patent No. 5,151,254 and PCT applications WO 94/20078, WO/94/23701 and WO 96/06638). The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

In another illustrative embodiment, biodegradable microspheres (*e.g.*, polylactate polyglycolate) are employed as carriers for the compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268; 5,075,109; 5,928,647; 5,811,128; 5,820,883; 5,853,763; 5,814,344, 5,407,609 and 5,942,252. Modified hepatitis B core protein carrier systems, such as described in WO/99 40934, and references cited therein, will also be useful for many applications. Another illustrative carrier/delivery system employs a carrier comprising particulate-protein complexes, such as those described in U.S. Patent No. 5,928,647, which are capable of inducing a class I-restricted cytotoxic T lymphocyte responses in a host.

In another illustrative embodiment, calcium phosphate core particles are employed as carriers, vaccine adjuvants, or as controlled release matrices for the compositions of this invention. Exemplary calcium phosphate particles are disclosed, for example, in published patent application No. WO/0046147.

The pharmaceutical compositions of the invention will often further comprise one or more buffers (*e.g.*, neutral buffered saline or phosphate buffered saline), carbohydrates (*e.g.*, glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, bacteriostats, chelating agents such as EDTA or glutathione, adjuvants (*e.g.*, aluminum hydroxide), solutes that render the formulation isotonic, hypotonic or weakly hypertonic with the blood of a

recipient, suspending agents, thickening agents and/or preservatives. Alternatively, compositions of the present invention may be formulated as a lyophilizate.

The pharmaceutical compositions described herein may be presented in unit-dose or multi-dose containers, such as sealed ampoules or vials. Such containers are typically sealed in such a way to preserve the sterility and stability of the formulation until use. In general, formulations may be stored as suspensions, solutions or emulsions in oily or aqueous vehicles. Alternatively, a pharmaceutical composition may be stored in a freeze-dried condition requiring only the addition of a sterile liquid carrier immediately prior to use.

The development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including *e.g.*, oral, parenteral, intravenous, intranasal, and intramuscular administration and formulation, is well known in the art, some of which are briefly discussed below for general purposes of illustration.

In certain applications, the pharmaceutical compositions disclosed herein may be delivered *via* oral administration to an animal. As such, these compositions may be formulated with an inert diluent or with an assimilable edible carrier, or they may be enclosed in hard- or soft-shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet.

The active compounds may even be incorporated with excipients and used in the form of ingestible tablets, buccal tables, troches, capsules, elixirs, suspensions, syrups, wafers, and the like (see, for example, Mathiowitz *et al.*, Nature 1997 Mar 27;386(6623):410-4; Hwang *et al.*, Crit Rev Ther Drug Carrier Syst 1998;15(3):243-84; U. S. Patent 5,641,515; U. S. Patent 5,580,579 and U. S. Patent 5,792,451). Tablets, troches, pills, capsules and the like may also contain any of a variety of additional components, for example, a binder, such as gum tragacanth, acacia, cornstarch, or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose or saccharin may be added or a flavoring agent, such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to

materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar, or both. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compounds may be incorporated into sustained-release preparation and formulations.

Typically, these formulations will contain at least about 0.1% of the active compound or more, although the percentage of the active ingredient(s) may, of course, be varied and may conveniently be between about 1 or 2% and about 60% or 70% or more of the weight or volume of the total formulation. Naturally, the amount of active compound(s) in each therapeutically useful composition may be prepared in such a way that a suitable dosage will be obtained in any given unit dose of the compound. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

In certain circumstances it will be desirable to deliver the pharmaceutical compositions disclosed herein parenterally, intravenously, intramuscularly, or even intraperitoneally. Such approaches are well known to the skilled artisan, some of which are further described, for example, in U. S. Patent 5,543,158; U. S. Patent 5,641,515 and U. S. Patent 5,399,363. In certain embodiments, solutions of the active compounds as free base or pharmacologically acceptable salts may be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations generally will contain a preservative to prevent the growth of microorganisms.

Illustrative pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (for example, see U. S. Patent 5,466,468).

In one embodiment, for parenteral administration in an aqueous solution, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, a sterile aqueous medium that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. Moreover, for human administration, preparations will of course preferably meet sterility, pyrogenicity, and the general safety and purity standards as required by FDA Office of Biologics standards.

In another embodiment of the invention, the compositions disclosed herein may be formulated in a neutral or salt form. Illustrative pharmaceutically-acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective.

The carriers can further comprise any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions. The phrase

“pharmaceutically-acceptable” refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human.

In certain embodiments, the pharmaceutical compositions may be delivered by intranasal sprays, inhalation, and/or other aerosol delivery vehicles. Methods for delivering genes, nucleic acids, and peptide compositions directly to the lungs *via* nasal aerosol sprays has been described, *e.g.*, in U. S. Patent 5,756,353 and U. S. Patent 5,804,212. Likewise, the delivery of drugs using intranasal microparticle resins (Takenaga *et al.*, *J Controlled Release* 1998 Mar 2;52(1-2):81-7) and lysophosphatidyl-glycerol compounds (U. S. Patent 5,725,871) are also well-known in the pharmaceutical arts. Likewise, illustrative transmucosal drug delivery in the form of a polytetrafluoroethylene support matrix is described in U. S. Patent 5,780,045.

In certain embodiments, liposomes, nanocapsules, microparticles, lipid particles, vesicles, and the like, are used for the introduction of the compositions of the present invention into suitable host cells/organisms. In particular, the compositions of the present invention may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, or a nanoparticle or the like. Alternatively, compositions of the present invention can be bound, either covalently or non-covalently, to the surface of such carrier vehicles.

The formation and use of liposome and liposome-like preparations as potential drug carriers is generally known to those of skill in the art (see for example, Lasic, *Trends Biotechnol* 1998 Jul;16(7):307-21; Takakura, *Nippon Rinsho* 1998 Mar;56(3):691-5; Chandran *et al.*, *Indian J Exp Biol.* 1997 Aug;35(8):801-9; Margalit, *Crit Rev Ther Drug Carrier Syst.* 1995;12(2-3):233-61; U.S. Patent 5,567,434; U.S. Patent 5,552,157; U.S. Patent 5,565,213; U.S. Patent 5,738,868 and U.S. Patent 5,795,587, each specifically incorporated herein by reference in its entirety).

Liposomes have been used successfully with a number of cell types that are normally difficult to transfect by other procedures, including T cell suspensions, primary hepatocyte cultures and PC 12 cells (Renneisen *et al.*, *J Biol Chem.* 1990 Sep 25;265(27):16337-42; Muller *et al.*, *DNA Cell Biol.* 1990 Apr;9(3):221-9). In addition, liposomes are free of the DNA length constraints that are typical of viral-based delivery systems. Liposomes have been used effectively to introduce genes, various drugs,

radiotherapeutic agents, enzymes, viruses, transcription factors, allosteric effectors and the like, into a variety of cultured cell lines and animals. Furthermore, the use of liposomes does not appear to be associated with autoimmune responses or unacceptable toxicity after systemic delivery.

In certain embodiments, liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs)).

Alternatively, in other embodiments, the invention provides for pharmaceutically-acceptable nanocapsule formulations of the compositions of the present invention. Nanocapsules can generally entrap compounds in a stable and reproducible way (see, for example, Quintanar-Guerrero *et al.*, Drug Dev Ind Pharm. 1998 Dec;24(12):1113-28). To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μm) may be designed using polymers able to be degraded *in vivo*. Such particles can be made as described, for example, by Couvreur *et al.*, Crit Rev Ther Drug Carrier Syst. 1988;5(1):1-20; zur Muhlen *et al.*, Eur J Pharm Biopharm. 1998 Mar;45(2):149-55; Zambaux *et al.* J Controlled Release. 1998 Jan 2;50(1-3):31-40; and U. S. Patent 5,145,684.

Cancer Therapeutic Methods

Immunologic approaches to cancer therapy are based on the recognition that cancer cells can often evade the body's defenses against aberrant or foreign cells and molecules, and that these defenses might be therapeutically stimulated to regain the lost ground, *e.g.*, pgs. 623-648 in Klein, Immunology (Wiley-Interscience, New York, 1982). Numerous recent observations that various immune effectors can directly or indirectly inhibit growth of tumors has led to renewed interest in this approach to cancer therapy, *e.g.*, Jager, *et al.*, Oncology 2001;60(1):1-7; Renner, *et al.*, Ann Hematol 2000 Dec;79(12):651-9.

Four basic cell types whose function has been associated with antitumor cell immunity and the elimination of tumor cells from the body are: i) B-lymphocytes which secrete immunoglobulins into the blood plasma for identifying and labeling the nonself invader cells; ii) monocytes which secrete the complement proteins that are responsible for lysing and processing the immunoglobulin-coated target invader cells;

iii) natural killer lymphocytes having two mechanisms for the destruction of tumor cells, antibody-dependent cellular cytotoxicity and natural killing; and iv) T-lymphocytes possessing antigen-specific receptors and having the capacity to recognize a tumor cell carrying complementary marker molecules (Schreiber, H., 1989, in *Fundamental Immunology* (ed). W. E. Paul, pp. 923-955).

Cancer immunotherapy generally focuses on inducing humoral immune responses, cellular immune responses, or both. Moreover, it is well established that induction of CD4⁺ T helper cells is necessary in order to secondarily induce either antibodies or cytotoxic CD8⁺ T cells. Polypeptide antigens that are selective or ideally specific for cancer cells offer a powerful approach for inducing immune responses against cancer, and are an important aspect of the present invention.

Therefore, in further aspects of the present invention, the pharmaceutical compositions described herein may be administered to a subject in need thereof, such as a subject afflicted with or prone to develop cancer. Within such methods, the pharmaceutical compositions described herein are administered to a patient, typically a warm-blooded animal, preferably a human.

Pharmaceutical compositions and vaccines of the invention may be administered prior to or following surgical removal of primary tumors and/or treatment such as administration of radiotherapy or conventional chemotherapeutic drugs. As discussed above, administration of the pharmaceutical compositions may be by any suitable method, including administration by intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal, intradermal, anal, vaginal, topical and oral routes.

The cancer type to be treated according to the methods of the invention can be essentially any type with which a polypeptide of the invention is associated. In certain illustrative embodiments, for example, the cancer type to be treated using a compositions of the present invention is liver cancer, pancreatic cancer, lung cancer, breast cancer, bladder cancer, kidney cancer, skin cancer and hematological cancer.

Within certain embodiments, immunotherapy may be active immunotherapy, in which treatment relies on the *in vivo* stimulation of the endogenous host immune system to react against tumors with the administration of immune

response-modifying agents (such as polypeptides and polynucleotides as provided herein).

Within certain other embodiments, immunotherapy may be passive immunotherapy, in which treatment involves the delivery of agents with established tumor-immune reactivity (such as antibodies or effector cells) that can directly or indirectly mediate antitumor effects and does not necessarily depend on an intact host immune system.

Examples of effector cells include T cells as discussed above, T lymphocytes (such as CD8⁺ cytotoxic T lymphocytes and CD4⁺ T-helper tumor-infiltrating lymphocytes), killer cells (such as Natural Killer cells and lymphokine-activated killer cells), B cells and antigen-presenting cells (such as dendritic cells and macrophages) expressing a polypeptide provided herein. T cell receptors and antibody receptors specific for the polypeptides recited herein may be cloned, expressed and transferred into other vectors or effector cells for adoptive immunotherapy. The polypeptides provided herein may also be used to generate antibodies or anti-idiotypic antibodies (*e.g.*, as described above and in U.S. Patent No. 4,918,164) for passive immunotherapy using standard methodologies.

Monoclonal antibodies may be labeled with any of a variety of labels for desired selective usages in detection, diagnostic assays or therapeutic applications (as described in U.S. Patent Nos. 6,090,365; 6,015,542; 5,843,398; 5,595,721; and 4,708,930, hereby incorporated by reference in their entirety as if each was incorporated individually). In each case, the binding of the labelled monoclonal antibody to the determinant site of the antigen will signal detection or delivery of a particular therapeutic agent to the antigenic determinant on the non-normal cell. A further object of this invention is to provide the specific monoclonal antibody suitably labelled for achieving such desired selective usages thereof.

Routes and frequency of administration of the therapeutic compositions described herein, as well as dosage, will vary from individual to individual, and may be readily established using standard techniques. In general, the pharmaceutical compositions and vaccines may be administered by injection (*e.g.*, intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (*e.g.*, by aspiration) or orally.

Preferably, between 1 and 10 doses may be administered over a 52 week period. Preferably, 6 doses are administered, at intervals of 1 month, and booster vaccinations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an amount of a compound that, when administered as described above, is capable of promoting an anti-tumor immune response, and is at least 10-50% above the basal (*i.e.*, untreated) level. Such response can be monitored by measuring the anti-tumor antibodies in a patient or by vaccine-dependent generation of cytolytic effector cells capable of killing the patient's tumor cells *in vitro*. Such vaccines should also be capable of causing an immune response that leads to an improved clinical outcome (*e.g.*, more frequent remissions, complete or partial or longer disease-free survival) in vaccinated patients as compared to non-vaccinated patients. In general, for pharmaceutical compositions and vaccines comprising one or more polypeptides, the amount of each polypeptide present in a dose ranges from about 25 µg to 5 mg per kg of host. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

In general, an appropriate dosage and treatment regimen provides the active compound(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Such a response can be monitored by establishing an improved clinical outcome (*e.g.*, more frequent remissions, complete or partial, or longer disease-free survival) in treated patients as compared to non-treated patients. Increases in preexisting immune responses to a tumor protein generally correlate with an improved clinical outcome. Such immune responses may generally be evaluated using standard proliferation, cytotoxicity or cytokine assays, which may be performed using samples obtained from a patient before and after treatment.

Cancer Detection and Diagnostic Compositions, Methods and Kits

The cancer-associated sequences and binding agents of the present invention can also be used in the context of cancer diagnostic compositions, methods and kits.

In general, a cancer may be detected in a patient based on the presence of one or more cancer-associated polypeptides and/or polynucleotides encoding such polypeptides in a biological sample (for example, blood, sera, sputum urine and/or

tumor biopsies) obtained from the patient. In other words, such proteins may be used as markers to indicate the presence or absence of a cancer.

In some embodiments, polynucleotide primers and probes may be used to detect the level of mRNA encoding a cancer-associated protein, which is also indicative of the presence or absence of a cancer. In general, a cancer-associated sequence may be present at a level that is at least two-fold, preferably three-fold, and more preferably five-fold or higher in tumor tissue than in normal tissue of the same type from which the tumor arose. Expression levels of a particular tumor sequence in tissue types different from that in which the tumor arose are irrelevant in certain diagnostic embodiments since the presence of tumor cells can be confirmed by observation of predetermined differential expression levels, *e.g.*, 2-fold, 5-fold, etc, in tumor tissue to expression levels in normal tissue of the same type.

Other differential expression patterns can be utilized advantageously for diagnostic purposes. For example, in one aspect of the invention, overexpression of a tumor sequence in tumor tissue and normal tissue of the same type, but not in other normal tissue types, *e.g.*, PBMCs, can be exploited diagnostically. In this case, the presence of metastatic tumor cells, for example in a sample taken from the circulation or some other tissue site different from that in which the tumor arose, can be identified and/or confirmed by detecting expression of the tumor sequence in the sample, for example using RT-PCR analysis. In many instances, it will be desired to enrich for tumor cells in the sample of interest, *e.g.*, PBMCs, using cell capture or other like techniques.

There are a variety of assay formats known to those of ordinary skill in the art for using a binding agent to detect polypeptide markers in a sample. *See, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In general, the presence or absence of a cancer in a patient may be determined by (a) contacting a biological sample obtained from a patient with a binding agent; (b) detecting in the sample a level of polypeptide that binds to the binding agent; and (c) comparing the level of polypeptide with a predetermined cut-off value.

In an illustrative embodiment, the assay involves the use of binding agent immobilized on a solid support to bind to and remove the polypeptide from the

remainder of the sample. The bound polypeptide may then be detected using a detection reagent that contains a reporter group and specifically binds to the binding agent/polypeptide complex. Such detection reagents may comprise, for example, a binding agent that specifically binds to the polypeptide or an antibody or other agent that specifically binds to the binding agent, such as an anti-immunoglobulin, protein G, protein A or a lectin. Alternatively, a competitive assay may be utilized, in which a polypeptide is labeled with a reporter group and allowed to bind to the immobilized binding agent after incubation of the binding agent with the sample. The extent to which components of the sample inhibit the binding of the labeled polypeptide to the binding agent is indicative of the reactivity of the sample with the immobilized binding agent. Suitable polypeptides for use within such assays include full length cancer-associated proteins and polypeptide portions thereof to which the binding agent binds, as described above.

The solid support may be any material known to those of ordinary skill in the art to which the tumor protein may be attached. For example, the solid support may be a test well in a microtiter plate or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681. The binding agent may be immobilized on the solid support using a variety of techniques known to those of skill in the art, which are amply described in the patent and scientific literature. In the context of the present invention, the term "immobilization" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the agent and functional groups on the support or may be a linkage by way of a cross-linking agent). Immobilization by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the binding agent, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and about 1 day. In general, contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of binding agent ranging from about 10 ng to about

10 µg, and preferably about 100 ng to about 1 µg, is sufficient to immobilize an adequate amount of binding agent.

Covalent attachment of binding agent to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the binding agent. For example, the binding agent may be covalently attached to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the binding partner (*see, e.g.*, Pierce Immunotechnology Catalog and Handbook, 1991, at A12-A13).

In certain embodiments, the assay is a two-antibody sandwich assay. This assay may be performed by first contacting an antibody that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that polypeptides within the sample are allowed to bind to the immobilized antibody. Unbound sample is then removed from the immobilized polypeptide-antibody complexes and a detection reagent (preferably a second antibody capable of binding to a different site on the polypeptide) containing a reporter group is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the specific reporter group.

More specifically, once the antibody is immobilized on the support as described above, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin or Tween 20™ (Sigma Chemical Co., St. Louis, MO). The immobilized antibody is then incubated with the sample, and polypeptide is allowed to bind to the antibody. The sample may be diluted with a suitable diluent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact time (*i.e.*, incubation time) is a period of time that is sufficient to detect the presence of polypeptide within a sample obtained from an individual with cancer at least about 95% of that achieved at equilibrium between bound and unbound polypeptide. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of

time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20™. The second antibody, which contains a reporter group, may then be added to the solid support. Preferred reporter groups include those groups recited above.

The detection reagent is then incubated with the immobilized antibody-polypeptide complex for an amount of time sufficient to detect the bound polypeptide. An appropriate amount of time may generally be determined by assaying the level of binding that occurs over a period of time. Unbound detection reagent is then removed and bound detection reagent is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of a cancer, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. In one embodiment, the cut-off value for the detection of a cancer is the average mean signal obtained when the immobilized antibody is incubated with samples from patients without the cancer. In general, a sample generating a signal that is three standard deviations above the predetermined cut-off value is considered positive for the cancer. In an alternate embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett *et al.*, *Clinical Epidemiology: A Basic Science for Clinical Medicine*, Little Brown and Co., 1985, p. 106-7. Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (*i.e.*, sensitivity) and false positive rates (100%-specificity) that correspond to each possible

cut-off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (*i.e.*, the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for a cancer.

In a related embodiment, the assay is performed in a flow-through or strip test format, wherein the binding agent is immobilized on a membrane, such as nitrocellulose. In the flow-through test, polypeptides within the sample bind to the immobilized binding agent as the sample passes through the membrane. A second, labeled binding agent then binds to the binding agent-polypeptide complex as a solution containing the second binding agent flows through the membrane. The detection of bound second binding agent may then be performed as described above. In the strip test format, one end of the membrane to which binding agent is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a region containing second binding agent and to the area of immobilized binding agent. Concentration of second binding agent at the area of immobilized antibody indicates the presence of a cancer. Typically, the concentration of second binding agent at that site generates a pattern, such as a line, that can be read visually. The absence of such a pattern indicates a negative result. In general, the amount of binding agent immobilized on the membrane is selected to generate a visually discernible pattern when the biological sample contains a level of polypeptide that would be sufficient to generate a positive signal in the two-antibody sandwich assay, in the format discussed above. Preferred binding agents for use in such assays are antibodies and antigen-binding fragments thereof. Preferably, the amount of antibody immobilized on the membrane ranges from about 25 ng to about 1 μ g, and more preferably from about 50 ng to about 500 ng. Such tests can typically be performed with a very small amount of biological sample.

Of course, numerous other assay protocols exist that are suitable for use with the tumor proteins or binding agents of the present invention. The above descriptions are intended to be exemplary only. For example, it will be apparent to those of ordinary skill in the art that the above protocols may be readily modified to use tumor polypeptides to detect antibodies that bind to such polypeptides in a biological sample. The detection of such tumor protein specific antibodies may correlate with the presence of a cancer.

A cancer may also, or alternatively, be detected based on the presence of T cells that specifically react with a cancer-associated protein of the invention in a biological sample. Within certain methods, a biological sample comprising CD4⁺ and/or CD8⁺ T cells isolated from a patient is incubated with a tumor polypeptide, a polynucleotide encoding such a polypeptide and/or an APC that expresses at least an immunogenic portion of such a polypeptide, and the presence or absence of specific activation of the T cells is detected. Suitable biological samples include, but are not limited to, isolated T cells. For example, T cells may be isolated from a patient by routine techniques (such as by Ficoll/Hypaque density gradient centrifugation of peripheral blood lymphocytes). T cells may be incubated *in vitro* for 2-9 days (typically 4 days) at 37°C with polypeptide (*e.g.*, 5-25 µg/ml). It may be desirable to incubate another aliquot of a T cell sample in the absence of tumor polypeptide to serve as a control. For CD4⁺ T cells, activation is preferably detected by evaluating proliferation of the T cells. For CD8⁺ T cells, activation is preferably detected by evaluating cytolytic activity. A level of proliferation that is at least two fold greater and/or a level of cytolytic activity that is at least 20% greater than in disease-free patients indicates the presence of a cancer in the patient.

As noted above, a cancer may also, or alternatively, be detected based on the level of mRNA encoding a cancer-associated protein of the present invention in a biological sample. For example, at least two oligonucleotide primers may be employed in a polymerase chain reaction (PCR) based assay to amplify a portion of a tumor cDNA derived from a biological sample, wherein at least one of the oligonucleotide primers is specific for (*i.e.*, hybridizes to) a polynucleotide encoding the tumor protein.

The amplified cDNA is then separated and detected using techniques well known in the art, such as gel electrophoresis.

Similarly, oligonucleotide probes that specifically hybridize to a polynucleotide encoding a cancer-associated protein may be used in a hybridization assay to detect the presence of polynucleotide encoding the cancer-associated protein in a biological sample.

To permit hybridization under assay conditions, oligonucleotide primers and probes should comprise an oligonucleotide sequence that has at least about 60%, preferably at least about 75% and more preferably at least about 90%, identity to a portion of a polynucleotide encoding a cancer-associated protein of the invention that is at least 10 nucleotides, and preferably at least 20 nucleotides, in length. Preferably, oligonucleotide primers and/or probes hybridize to a polynucleotide encoding a polypeptide described herein under moderately stringent conditions, as defined above. Oligonucleotide primers and/or probes which may be usefully employed in the diagnostic methods described herein preferably are at least 10-40 nucleotides in length. In one embodiment, the oligonucleotide primers comprise at least 10 contiguous nucleotides, more preferably at least 15 contiguous nucleotides, of a DNA molecule having a sequence as disclosed herein. Techniques for both PCR based assays and hybridization assays are well known in the art (*see, for example, Mullis et al., Cold Spring Harbor Symp. Quant. Biol., 51:263, 1987; Erlich ed., PCR Technology, Stockton Press, NY, 1989*).

One assay employs RT-PCR, in which PCR is applied in conjunction with reverse transcription. Typically, RNA is extracted from a biological sample, such as biopsy tissue, and is reverse transcribed to produce cDNA molecules. PCR amplification using at least one specific primer generates a cDNA molecule, which may be separated and visualized using, for example, gel electrophoresis. Amplification may be performed on biological samples taken from a test patient and from an individual who is not afflicted with a cancer. The amplification reaction may be performed on several dilutions of cDNA spanning two orders of magnitude. A two-fold or greater increase in expression in several dilutions of the test patient sample as compared to the same dilutions of the non-cancerous sample is typically considered positive.

In another aspect of the present invention, cell capture technologies may be used in conjunction, with, for example, real-time PCR to provide a more sensitive tool for detection of metastatic cells expressing tumor antigens. Detection of cancer cells in biological samples, *e.g.*, bone marrow samples, peripheral blood, and small needle aspiration samples is desirable for diagnosis and prognosis in cancer patients.

Immunomagnetic beads coated with specific monoclonal antibodies to surface cell markers, or tetrameric antibody complexes, may be used to first enrich or positively select cancer cells in a sample. Various commercially available kits may be used, including Dynabeads® Epithelial Enrich (DynaL Biotech, Oslo, Norway), StemSep™ (StemCell Technologies, Inc., Vancouver, BC), and RosetteSep (StemCell Technologies). A skilled artisan will recognize that other methodologies and kits may also be used to enrich or positively select desired cell populations. Dynabeads® Epithelial Enrich contains magnetic beads coated with mAbs specific for two glycoprotein membrane antigens expressed on normal and neoplastic epithelial tissues. The coated beads may be added to a sample and the sample then applied to a magnet, thereby capturing the cells bound to the beads. The unwanted cells are washed away and the magnetically isolated cells eluted from the beads and used in further analyses.

RosetteSep can be used to enrich cells directly from a blood sample and consists of a cocktail of tetrameric antibodies that targets a variety of unwanted cells and crosslinks them to glycophorin A on red blood cells (RBC) present in the sample, forming rosettes. When centrifuged over Ficoll, targeted cells pellet along with the free RBC. The combination of antibodies in the depletion cocktail determines which cells will be removed and consequently which cells will be recovered. Antibodies that are available include, but are not limited to: CD2, CD3, CD4, CD5, CD8, CD10, CD11b, CD14, CD15, CD16, CD19, CD20, CD24, CD25, CD29, CD33, CD34, CD36, CD38, CD41, CD45, CD45RA, CD45RO, CD56, CD66B, CD66e, HLA-DR, IgE, and TCR $\alpha\beta$.

Additionally, it is contemplated in the present invention that mAbs specific for tumor antigens can be generated and used in a similar manner. For example, mAbs that bind to tumor-specific cell surface antigens may be conjugated to magnetic beads, or formulated in a tetrameric antibody complex, and used to enrich or

positively select metastatic tumor cells from a sample. Once a sample is enriched or positively selected, cells may be lysed and RNA isolated. RNA may then be subjected to RT-PCR analysis using tumor-specific primers in a real-time PCR assay as described herein. One skilled in the art will recognize that enriched or selected populations of cells may be analyzed by other methods (*e.g.*, *in situ* hybridization or flow cytometry).

In another embodiment, the compositions described herein may be used as markers for the progression of cancer. In this embodiment, assays as described above for the diagnosis of a cancer may be performed over time, and the change in the level of reactive polypeptide(s) or polynucleotide(s) evaluated. For example, the assays may be performed every 24-72 hours for a period of 6 months to 1 year, and thereafter performed as needed. In general, a cancer is progressing in those patients in whom the level of polypeptide or polynucleotide detected increases over time. In contrast, the cancer is not progressing when the level of reactive polypeptide or polynucleotide either remains constant or decreases with time.

Certain *in vivo* diagnostic assays may be performed directly on a tumor. One such assay involves contacting tumor cells with a binding agent. The bound binding agent may then be detected directly or indirectly via a reporter group. Such binding agents may also be used in histological applications. Alternatively, polynucleotide probes may be used within such applications.

As noted above, to improve sensitivity, multiple tumor protein markers may be assayed within a given sample. It will be apparent that binding agents specific for different proteins provided herein may be combined within a single assay. Further, multiple primers or probes may be used concurrently. The selection of tumor protein markers may be based on routine experiments to determine combinations that results in optimal sensitivity. In addition, or alternatively, assays for tumor proteins provided herein may be combined with assays for other known tumor antigens.

The present invention further provides kits for use within any of the above diagnostic methods. Such kits typically comprise two or more components necessary for performing a diagnostic assay. Components may be compounds, reagents, containers and/or equipment. For example, one container within a kit may contain a monoclonal antibody or fragment thereof that specifically binds to a tumor

protein. Such antibodies or fragments may be provided attached to a support material, as described above. One or more additional containers may enclose elements, such as reagents or buffers, to be used in the assay. Such kits may also, or alternatively, contain a detection reagent as described above that contains a reporter group suitable for direct or indirect detection of antibody binding.

Alternatively, a kit may be designed to detect the level of mRNA encoding a tumor protein in a biological sample. Such kits generally comprise at least one oligonucleotide probe or primer, as described above, that hybridizes to a polynucleotide encoding a tumor protein. Such an oligonucleotide may be used, for example, within a PCR or hybridization assay. Additional components that may be present within such kits include a second oligonucleotide and/or a diagnostic reagent or container to facilitate the detection of a polynucleotide encoding a tumor protein.

Screening Assays for Drug Candidates

Screening assays for drug candidates may be designed to identify compounds that bind to or complex with the cancer-associated polypeptides of the invention, or otherwise interfere with the interaction of the polypeptides with other cellular proteins. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates. Small molecules contemplated include synthetic organic or inorganic compounds, including peptides, preferably soluble peptides, (poly)peptide-immunoglobulin fusions, and, in particular, antibodies including, without limitation, poly- and monoclonal antibodies and antibody fragments, single-chain antibodies, anti-idiotypic antibodies, and chimeric or humanized versions of such antibodies or fragments, as well as human antibodies and antibody fragments. The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays and cell based assays, which are well characterized in the art. All assays are common in that they call for contacting the drug candidate with a polypeptide of the invention under conditions and for a time sufficient to allow these two components to interact.

In binding assays, the interaction is binding and the complex formed can be isolated or detected in the reaction mixture. In a particular embodiment, the

polypeptide of the invention or the drug candidate is immobilized on a solid phase, *e.g.*, on a microtiter plate, by covalent or non-covalent attachments. Non-covalent attachment generally is accomplished by coating the solid surface with a solution of the polypeptide and drying. Alternatively, an immobilized antibody, *e.g.*, a monoclonal antibody, specific for the polypeptide to be immobilized can be used to anchor it to a solid surface. The assay is performed by adding the non-immobilized component, which may be labeled by a detectable label, to the immobilized component, *e.g.*, the coated surface containing the anchored component. When the reaction is complete, the non-reacted components are removed, *e.g.*, by washing, and complexes anchored on the solid surface are detected. When the originally non-immobilized component carries a detectable label, the detection of label immobilized on the surface indicates that complexing occurred. Where the originally non-immobilized component does not carry a label, complexing can be detected, for example, by using a labelled antibody specifically binding the immobilized complex.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1

GENERATION AND CHARACTERIZATION OF ANTIBODIES AGAINST ONCOFACTORS

As noted, the present invention relates, in certain aspects, to pharmaceutical compositions comprising isolated antibodies and antigen-binding fragments thereof that specifically bind an oncofactor sequence selected from the group consisting of IL1f5 (SEQ ID NO: 1), CCBP2 (SEQ ID NO: 2), IL1R2 (SEQ ID NO: 3), IL1RAPL1 (SEQ ID NO: 4), IL18BP (SEQ ID NO: 5), CLEC2B (SEQ ID NO: 6), C4BPA (SEQ ID NO: 7), C4BPB (SEQ ID NO: 8), SERPIN1 (SEQ ID NO: 9), IL1RAP isoform 1 (SEQ ID NO: 10), IL1RAP isoform 2 (SEQ ID NO: 11), GPR1 (SEQ ID NO: 12), GPR4 (SEQ ID NO: 13), GPR15 (SEQ ID NO: 14), GPR32 (SEQ ID NO: 15), GPR34 (SEQ ID NO: 16), GPR183 (SEQ ID NO: 17), SERPINA4 (SEQ ID NO: 18), SERPINB5 (SEQ ID NO: 19), SEMA4B (SEQ ID NO: 20), SEMA4D

(SEQ ID NO: 21), CCL14 (SEQ ID NO: 22), NKTR (SEQ ID NO: 23) and SFTPD (SEQ ID NO: 24). Such compositions can be made and used according to the general disclosure herein and further in view of the illustrative examples set out below.

a. Characterization of expression in cell lines

Baseline expression of oncofactor sequences is characterized by RT-PCR in representative cell lines, including cell lines: IM-9 (B cell lymphoma), 4T1 (breast cancer carcinoma), C1498 (acute myeloid leukemia), and TRAMP-C2 (prostate carcinoma).

Real-time PCR (*see Gibson et al., Genome Research 6:995-1001, 1996; Heid et al., Genome Research 6:986-994, 1996*) is a technique that evaluates the level of PCR product accumulation during amplification. This technique permits quantitative evaluation of mRNA levels in multiple samples. Briefly, in one illustrative approach, mRNA is extracted from tumor and normal tissue and cDNA is prepared using standard techniques. Real-time PCR is performed, for example, using a Perkin Elmer/Applied Biosystems (Foster City, CA) 7700 Prism instrument. Matching primers and fluorescent probes are designed for genes of interest using, for example, the primer express program provided by Perkin Elmer/Applied Biosystems (Foster City, CA). Optimal concentrations of primers and probes are initially determined by those of ordinary skill in the art, and control (*e.g.*, β -actin) primers and probes are obtained commercially from, for example, Perkin Elmer/Applied Biosystems (Foster City, CA). To quantitate the amount of specific RNA in a sample, a standard curve is typically generated using a plasmid containing the gene of interest. Standard curves are generated using the Ct values determined in the real-time PCR, which are related to the initial cDNA concentration used in the assay. Standard dilutions ranging from 10^{-10} to 10^{-6} copies of the gene of interest are generally sufficient. In addition, a standard curve is generated for the control sequence. This permits standardization of initial RNA content of a tissue sample to the amount of control for comparison purposes.

An alternative real-time PCR procedure can be carried out as follows: The first-strand cDNA to be used in the quantitative real-time PCR is synthesized from 20 μ g of total RNA that is first treated with DNase I (*e.g.*, Amplification Grade, Gibco BRL Life Technology, Gaithersburg, MD), using Superscript Reverse Transcriptase

(RT) (e.g., Gibco BRL Life Technology, Gaithersburg, MD). Real-time PCR is performed, for example, with a GeneAmp™ 5700 sequence detection system (PE Biosystems, Foster City, CA). The 5700 system uses SYBR™ green, a fluorescent dye that only intercalates into double stranded DNA, and a set of gene-specific forward and reverse primers. The increase in fluorescence is monitored during the whole amplification process. The optimal concentration of primers is determined using a checkerboard approach and a pool of cDNAs from tumors is used in this process. The PCR reaction is performed in 25µl volumes that include 2.5µl of SYBR green buffer, 2µl of cDNA template and 2.5µl each of the forward and reverse primers for the gene of interest. The cDNAs used for RT reactions are diluted approximately 1:10 for each gene of interest and 1:100 for the β-actin control. In order to quantitate the amount of specific cDNA (and hence initial mRNA) in the sample, a standard curve is generated for each run using the plasmid DNA containing the gene of interest. Standard curves are generated using the Ct values determined in the real-time PCR which are related to the initial cDNA concentration used in the assay. Standard dilution ranging from 20-2x10⁶ copies of the gene of interest are used for this purpose. In addition, a standard curve is generated for β-actin ranging from 200fg-2000fg. This enables standardization of the initial RNA content of a tissue sample to the amount of β-actin for comparison purposes. The mean copy number for each group of tissues tested is normalized to a constant amount of β-actin, allowing the evaluation of the over-expression levels seen with each of the genes.

b. Generation of cell lines that vary by expression

For cell lines that are baseline low/negative, oncofactor polypeptides are ectopically overexpressed by cloning the coding sequence into a suitable vector, such as the retrovirally expression vector pMXs-IP, and using that vector to transduce the cell lines. Positively transduced cell lines are selected for with puromycin, as the vector encodes a puromycin resistance cassette, and overexpression is confirmed by RT-PCR.

For cell lines that are baseline positive, cancer associated polypeptide expression is knocked down with RNAi. shRNA constructs that target the oncofactor sequence are cloned into the HuSH vector (Origene, Rockville, MD) and delivered to

target cells via shRNA transduction. Knockdown of the cancer associated sequences is confirmed by RT-PCR.

c. Characterization of effects on cell proliferation and morphology

Cell lines which are positive or negative for expression of the oncofactor sequence(s) are plated at equal density and counted every day for seven days. Simultaneously, cells are observed for morphological changes that vary with oncofactor protein expression.

d. Characterization of antibody binding

Antibodies targeting the oncofactor protein sequences are characterized for binding by western blotting. Lysates of cells that are positive or negative for expression are run on SDS-PAGE gel and blotted with the antibody. In cases where the oncofactor protein is a secreted protein, supernatants from cells expressing the protein are collected, proteins are precipitated with trichloroacetic acid, run on SDS-PAGE, and western blotted with the antibody. In cases where the protein is bound to the cell surface, antibody binding may also be confirmed by flow cytometry. Cells which are positive for target expression are stained with the antibody, and then counterstained with a relevant secondary antibody (which is specific to the constant region of the primary antibody, and which is conjugated to a fluorescent protein. Antibody binding is then visualized in the fluorescence channel of the cytometer.

e. Antibody dependent cell cytotoxicity (ADCC) assays

Target cell lines which are positive or negative for oncofactor sequence expression will be loaded with Calcein-AM reagent (Beckton Dickinson, Sparks, MD) which is cleaved by intracellular esterases and retained within the cell cytosol. Cells are incubated with antibody specific to the oncofactor protein or an isotype control. Cells are then mixed in varying ratios with human PBMCs from a healthy adult volunteer which have been isolated by Ficoll gradient. Following 4 hours incubation, supernatants are collected, and time resolved fluorescence is measured as a readout for cell lysis, and thus ADCC.

f. Complement dependent cytotoxicity (CDC) assay

Target cell lines which are positive or negative for oncofactor protein expression will be loaded with Calcein AM reagent which is cleaved by intracellular

esterases and retained within the cell cytosol. Cells are incubated with antibody specific to the oncofactor protein or an isotype control. Cells are then mixed with varying dilutions of fresh human serum from a healthy adult volunteer. Following 4 hours of incubation, supernatants are collected and time resolved fluorescence is measured as a readout for cell lysis, and thus CDC.

g. Mixed lymphocyte reactions

Raji B lymphoma cells which are positive or negative for oncofactor protein expression will be treated with mitomycin-C to inhibit proliferation and activation. Raji B cells are then incubated with peripheral blood mononuclear cells (PBMCs) isolated by Ficoll density gradient from whole blood of adult healthy volunteers. The mixed cell cultures are incubated together for various lengths of time and in varying cell ratios.

Cytokine analysis: Supernatants from the mixed cultures are collected and cytokine release by the bulk mixed lymphocyte cultures is assessed by commercially available ELISA reagents. Cytokines examined include IL-1 β , IL-2, IL-4, IL-6, IL-10, IFN γ , TNF, and TGF β . Additionally, cytokines released by specific cell populations will be assessed by intracellular cytokine staining in conjunction with lineage marker staining and assessed by flow cytometry. Specific cell populations will be defined as Th1 T cells: CD3+, CD4+, Tbet+, CD8-, CD19-, CD11b-; Th2 T cells: CD3+, CD4+, GATA3+, CD19-, CD11b-, Treg T cells: CD3+, CD4+, Foxp3+, CD19-, CD11b-; Th17 T cells: CD3+, CD4+, CD8-, ROR γ T+, CD19-, CD11b-; CD8 T cells: CD3+, CD4-, CD8+, CD19-, CD11b-, B cells: CD3-, CD19+, CD11b-, dendritic cells: CD3-, CD19-, CD11b+, CD11c+; macrophages: CD3-, CD19-, CD11b+, CD11c-; NK cells: asialo-GM1+, CD3-, CD19-, and CD11b-.

Activation marker expression: Additionally, at the same time points, cells are collected from the mixed cultures, stained for lineage markers and evaluated for upregulation of immune activation markers by flow cytometry. Activation markers include (for T cells): CD25, CD44, CD69, and CD154; (for antigen presenting cells including B cells): CD40, CD80, CD86, MHCII; and (for NK cells): CD69 and CD161.

Proliferation of responding PBMCs: PBMC populations are assessed by staining the PBMCs with CFSE prior to incubation with the Raji B cells. At various

time points after coculture, proliferation of the PBMCs is assessed by dilution of CFSE in specific populations, as delineated by surface markers. Stained PBMCs that are not cocultured with Raji B cells are used as a negative control.

Cytotoxic activity assay: Raji B cells positive or negative for oncofactor protein expression are incubated with PBMCs for six days. Subsequently, PBMC/ Raji B coculture are added to fresh Raji B cells that have been loaded with Calcein-AM reagent. Following 4 hours incubation, supernatants are collected and time resolved fluorescence is measured as a readout for lysis of the Raji B cells.

This is similar to the ADCC assays, but for these assays, the oncofactor protein-expressing Raji B cells are permitted to activate the PBMCs for several days in the absence of antibody, where in the ADCC assay, fresh PBMCs are added to oncofactor protein-expressing cells (Raji B, 4T1, C1498, or TRAMP-C2) together with antibody and cell lysis is measured after only hours.

Mixed lymphocyte reactions with antibody: Assays of the mixed lymphocyte reactions are repeated in the presence of antibody which specifically binds to the oncofactor protein. The ability of the antibody to reverse the oncofactor protein-mediated phenotype is assessed.

h. Characterization of expression in primary tumors

Antibodies that bind to an oncofactor protein by western blot (as described above) are used to characterize oncofactor protein expression on primary human tumor. Core samples from a spectrum of tumors (including but not limited to liver, pancreas, breast, lung, bladder, prostate) and matched normal tissue are stained with the antibody. The degree of antibody staining is assessed, preferably by a licensed pathologist.

EXAMPLE 2

ACTIVATION OF PBMCs WITH ONCOFACTORS

INCREASES SPECIFIC LYSIS OF TUMOR CELLS

Tumor cell lysis methods

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll Hypaque gradient separation from whole blood obtained by venous puncture from a

healthy donor. Raji B lymphoma cells were treated with mitomycin C to cross link DNA and prevent replication. A mixed tumor lymphocyte culture (MTLC) was performed; PBMCs were cocultured with Raji cells for 6 days at a ratio of 10:1 with recombinant oncofactors at various concentrations (see Table 2). PBMCs were activated during the 6 day coculture period to specifically recognize the Raji cells and the PBMC CD8+ T lymphocyte population was stimulated to specifically lyse the Raji cells.

On day 6, fresh Raji cells were labeled with the fluorescent calcein dye, Calcein AM, and incubated with the preactivated PBMCs for 4.5 hours. CD8+ T cell lysis of the Raji cells was detected by Calcein AM release into the supernatant, which was quantified using a fluorometer. Specific lysis was calculated as a ratio of Calcein AM release from the mixed tumor lymphocyte cultures versus Calcein AM release from Raji cells treated with Triton X-100 to achieve total lysis, after a correction for spontaneous Calcein AM release.

$$\text{Specific Lysis} = (\text{MTLC-PBMC alone}) / (\text{Triton-PBMC alone})$$

To control for spontaneous release of Calcein AM, Raji cells were loaded with Calcein AM but were not incubated with PBMCs for the 4.5 hour lysis phase. To control for nonspecific lysis, PBMCs were cultured for an initial 6 days both without Raji cells, and, in parallel, with phytohaemagglutinin (PHA), which induces nonspecific activation. These PBMCs were added to calcein loaded Raji cells for the 4.5 hour lysis phase.

Table 2

Oncofactor	Recombinant oncofactor concentration
IL1f5	0.1 µg/ml, 1 µg/ml, 3 µg/ml
IL1RAP2	0.2 µg/ml, 2 µg/ml, 5 µg/ml
CCL14	0.5 µg/ml, 3 µg/ml, 10 µg/ml
IL1R2	0.5 µg/ml, 1 µg/ml, 3 µg/ml

Results of IL1f5 Tumor Cell Lysis Experiments

Figure 1 shows that MTLC comprising PBMCs cultured with Raji B lymphoma cells and recombinant IL1f5 polypeptide exhibit a dose dependent reduction

of Raji B lymphoma cell lysis compared to MTLC cultured without IL1f5. These results indicate that IL1f5 is capable of inhibiting immune responses. To control for nonspecific lysis, PBMCs were cultured for the initial 6 days with IL1f5 in the absence of Raji B lymphoma cells, and then added to calcein loaded Raji cells for the 4.5 hour lysis period.

Results of IL1RAP2 Tumor Cell Lysis Experiments

Figure 2 shows that MTLC comprising PBMCs cultured with Raji B lymphoma cells and recombinant IL1RAP2 polypeptide exhibit a dose dependent reduction of Raji B lymphoma cell lysis compared to MTLC cultured without IL1RAP2. These results indicate that IL1RAP2 is capable of inhibiting immune responses.

Results of CCL14 Tumor Cell Lysis Experiments

Figure 3 shows that MTLC comprising PBMCs cultured with Raji B lymphoma cells and recombinant CCL14 polypeptide exhibit a dose dependent reduction of lysis of Raji B lymphoma cells compared to MTLC cultured without CCL14. These results indicate that CCL14 is capable of inhibiting immune responses.

Results of IL1R2 Tumor Cell Lysis Experiments

Figure 18 shows that MTLC comprising PBMCs cultured with Raji B lymphoma cells and recombinant IL1R2 polypeptide exhibit a dose dependent reduction of lysis of Raji B lymphoma cells compared to MTLC cultured without IL1R2. These results indicate that IL1R2 is capable of inhibiting immune responses.

EXAMPLE 3

ONCOFACTOR IMMUNOHISTOCHEMISTRY

Immunohistochemistry Methods

Expression of individual oncofactors was evaluated in tumor and matched normal control tissue by immunohistochemistry staining. Antibodies specific to individual oncofactors was tested at various titrations to identify dilutions or concentrations that would result in minimal background and maximal signal detection. Antibody specificity was verified by staining a negative cell line, *e.g.*, HEK293T cells,

which do not express an individual oncofactor, as well as a positive cell line transiently transfected with an oncofactor, *e.g.*, HEK293T cells transfected with IL1f5 or GPR183. Oncofactor expression in the transfected HEK293T cells was verified by qPCR and western blot. Final antibody staining dilutions or concentrations are indicated in Table 3.

The primary antibodies used are indicated in Table 3. The principal detection system consisted of a Vector anti-rabbit secondary (BA-1000) and a Vector ABC-AP kit (AK-5000) with a Vector Red substrate kit (SK-5100), which produced a fuchsia-colored deposit. The negative control consisted of performing the entire immunohistochemical procedure on adjacent sections in the absence of primary antibody. Tissues were also stained with positive control antibodies (CD31 and vimentin) to ensure that tissue antigens were preserved and accessible for immunohistochemical analysis. The slides were interpreted by a pathologist and each antibody was evaluated for the presence of specific signal and level of background. Staining intensity was recorded on a 0-4 scale (0=negative, 1=blush, 2=faint, 3=moderate, 4=strong). Stained slides were imaged with a DVC 1310C digital camera coupled to a Nikon microscope. Images were stored as TIFF files with Adobe Photoshop.

Biological samples stained consisted of 40 tumor and 10 normal control tissues from each of five cancers: breast, lung, colon, pancreas, and prostate. Samples were mounted as a tissue microarray consisting of 1mm core samples from tumor biopsy along with 10 normal control tissues from the same organ.

Table 3

Oncofactor	Primary Antibody	Final Antibody concentration/dilution
IL1f5	Sigma Aldrich #HPA034542	Final dilution of 1:50
GPR183	Lifespan #LS-A46	Final Concentration of 10 µg/mL
IL1RAP	Genetex #GTX-104513	Final Concentration of 20 µg/mL
CCL14	Santa Cruz #28388	Final Concentration of 2 µg/mL

SEMA4D	Sigma Aldrich #HPA015662	Final dilution of 1:100
IL1R2	Sigma Aldrich #HPA027598	Final Dilution of 1:50

Results of IL1f5 IHC

Increased IL1f5 antibody staining was observed in tumor samples from breast, colon, lung, and prostate cancer compared to controls. Representative images are shown in Figures 4-7. IL1f5 staining was most intense in breast cancers, but was also intense in colon, lung, and prostate cancers. Furthermore, comparison of average pathology scores indicated that breast tumor samples with a higher clinical grade (indicative of more divergence from normal cell morphology) correlate with IL1f5 overexpression. The average score from Grade 3 cancers was 3.45, whereas Grade 2 cancers scored 2.31 and noncancerous tissue scored 1.12.

Results of GPR183 IHC

Increased GPR183 antibody staining was observed in tumor samples from breast, colon, and lung cancer compared to controls. Representative images are shown in Figures 8-10. GPR183 staining was most intense in breast cancers but was also intense in colon cancers and lung cancers. Furthermore, comparison of average pathology scores indicated that breast tumor samples with a higher clinical grade (indicative of more divergence from normal cell morphology) correlate with GPR183 overexpression. The average score from Grade 3 cancers was 2.32, whereas Grade 2 cancers scored 1.65 and noncancerous tissue scored 1.17.

Results of IL1RAP IHC

Increased IL1RAP antibody staining was observed in tumor samples from breast and lung cancer compared to controls. Representative images are shown in Figures 11-12. IL1f5 staining was most intense in breast cancers, but was also intense in lung cancers. The average IHC scores for malignant breast cells was 3.44, compared to 1.22 for noncancerous cells. The average IHC scores for lung tumors was 2.89, compared to 1.88 for noncancerous cells.

Results of CCL14 IHC

Increased CCL14 antibody staining was observed in tumor samples from breast, prostate, and lung cancer compared to controls. Representative images are shown in Figures 13-15. CCL14 staining was most intense in breast cancers, but was also intense in prostate, and lung cancers. The average IHC scores for malignant breast cells was 2.61, compared to 1.25 for noncancerous cells. The average IHC scores for malignant prostate cells was 1.51, compared to 0.19 for noncancerous cells. The average IHC scores for lung tumors was 1.86, compared to 0.60 for noncancerous cells.

Results of SEMA4D IHC

Increased SEMA4D antibody staining was observed in tumor samples from breast cancer compared to controls. Representative images are shown in Figure 16. SEMA4D staining was intense in breast cancers. The average IHC scores for malignant breast cells was 2.92, compared to 2.29 for noncancerous cells.

Results of IL1R2 IHC

Increased IL1R2 antibody staining was observed in tumor samples from breast cancer compared to controls. Representative images are shown in Figure 17. IL1R2 staining was intense in breast cancers. The average IHC scores for malignant breast cells was 2.25, compared to 1.5 for noncancerous cells.

EXAMPLE 4

T CELL PROLIFERATION IN THE PRESENCE OF ONCOFACTORS

Methods

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood obtained by venous puncture from a healthy donor by Ficoll Hypaque gradient separation and labeled with carboxyfluorescein diacetate (CFSE). Raji B lymphoma cells were treated with mitomycin C to cross link DNA and prevent replication. A mixed tumor lymphocyte culture (MTLC) was then set up by coculturing PBMCs and Rajis for 6 days at a ratio of 10:1 with varying concentrations of recombinant IL1f5. During this time, T lymphocytes from the PBMC population are activated and clonally divide.

CFSE is a membrane impermeant dye. The amount of CFSE per cell will be reduced by half with each successive cell division, and the number of cell divisions can be tracked by monitoring the intensity of CFSE staining by flow cytometry. T cells were identified by positive staining with anti-CD3-PE (E Bioscience anti-CD3-PE Catalog# 17-0036-42). The number of cell division was analyzed by comparing the intensity of CFSE staining to resting T cells. T cells were defined as fully divided when the level of CFSE staining was less than the limit of detection. Data was collected on a Accuri C6 flow cytometer and analyzed with FCS Express. To control for nonspecific activation by IL1f5, PBMCs were cultured with IL1f5 in the absence of Raji cells.

Results of IL1f5 IHC

MTLC comprising PBMCs cultured with Raji B lymphoma cells and recombinant IL1f5 polypeptide resulted in a dose dependent reduction of T cell proliferation compared to MTLC cultured without IL1f5 and PBMC controls. These results (Figure 19) indicate that IL1f5 is capable of inhibiting immune responses by decreasing T cell proliferation.

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

What is Claimed:

1. An isolated antibody or antigen-binding fragment that specifically binds to a sequence set forth in any one of SEQ ID NOs: 1-24, for use in the treatment of cancer.
2. An isolated polypeptide comprising a sequence set forth in any one of SEQ ID NOs: 1-24, or a fragment or variant thereof having at least 90% identity thereto, for use in the treatment of cancer.
3. An isolated polynucleotide encoding an amino acid sequence set forth in any one of SEQ ID NOs: 1-24, or encoding a fragment or variant of a sequence set forth in SEQ ID NOs: 1-24 having at least 90% identity thereto, for use in the treatment of cancer.
4. An oligonucleotide that is complementary to a polynucleotide encoding an amino acid sequence set forth in any one of SEQ ID NOs: 1-24, or encoding a fragment or variant of a sequence set forth in SEQ ID NOs: 1-24 having at least 90% identity thereto, for use in the treatment of cancer.
5. An endotoxin-free pharmaceutical composition comprising a pharmaceutically acceptable carrier and an isolated antibody or antigen-binding fragment thereof that specifically binds a sequence selected from the group consisting of IL1f5 (SEQ ID NO: 1), CCBP2 (SEQ ID NO: 2), IL1R2 (SEQ ID NO: 3), IL1RAPL1 (SEQ ID NO: 4), IL18BP (SEQ ID NO: 5), CLEC2B (SEQ ID NO: 6), C4BPA (SEQ ID NO: 7), C4BPB (SEQ ID NO: 8), SERPIN1 (SEQ ID NO: 9), IL1RAP isoform 1 (SEQ ID NO: 10), IL1RAP isoform 2 (SEQ ID NO: 11), GPR1 (SEQ ID NO: 12), GPR4 (SEQ ID NO: 13), GPR15 (SEQ ID NO: 14), GPR32 (SEQ ID NO: 15), GPR34 (SEQ ID NO: 16), GPR183 (SEQ ID NO: 17), SERPINA4 (SEQ ID NO: 18), SERPINB5 (SEQ ID NO: 19), SEMA4B (SEQ ID NO: 20), SEMA4D

(SEQ ID NO: 21), CCL14 (SEQ ID NO: 22), NKTR (SEQ ID NO: 23) and SFTPD (SEQ ID NO: 24).

6. A pharmaceutical composition formulated for intravenous injection for use in a patient having or at risk for having cancer, said composition comprising a pharmaceutically acceptable carrier and an isolated antibody or antigen-binding fragment thereof that specifically binds a sequence selected from the group consisting of IL1f5 (SEQ ID NO: 1), CCBP2 (SEQ ID NO: 2), IL1R2 (SEQ ID NO: 3), IL1RAPL1 (SEQ ID NO: 4), IL18BP (SEQ ID NO: 5), CLEC2B (SEQ ID NO: 6), C4BPA (SEQ ID NO: 7), C4BPB (SEQ ID NO: 8), SERPINI1 (SEQ ID NO: 9), IL1RAP isoform 1 (SEQ ID NO: 10), IL1RAP isoform 2 (SEQ ID NO: 11), GPR1 (SEQ ID NO: 12), GPR4 (SEQ ID NO: 13), GPR15 (SEQ ID NO: 14), GPR32 (SEQ ID NO: 15), GPR34 (SEQ ID NO: 16), GPR183 (SEQ ID NO: 17), SERPINA4 (SEQ ID NO: 18), SERPINB5 (SEQ ID NO: 19), SEMA4B (SEQ ID NO: 20), SEMA4D (SEQ ID NO: 21), CCL14 (SEQ ID NO: 22), NKTR (SEQ ID NO: 23) and SFTPD (SEQ ID NO: 24).

7. The composition of claim 5 or claim 6, wherein the composition comprises one or more antibodies or antigen-binding fragments thereof.

8. The composition of any one of claims 5 to 7, wherein the composition comprises one or more antibodies or antigen binding fragments thereof, wherein each of the one or more antibodies or antigen binding fragments thereof specifically bind a sequence selected from the group consisting of IL1f5 (SEQ ID NO: 1), CCBP2 (SEQ ID NO: 2), IL1R2 (SEQ ID NO: 3), IL1RAPL1 (SEQ ID NO: 4), IL18BP (SEQ ID NO: 5), CLEC2B (SEQ ID NO: 6), C4BPA (SEQ ID NO: 7), C4BPB (SEQ ID NO: 8), SERPINI1 (SEQ ID NO: 9), IL1RAP isoform 1 (SEQ ID NO: 10), IL1RAP isoform 2 (SEQ ID NO: 11), GPR1 (SEQ ID NO: 12), GPR4 (SEQ ID NO: 13), GPR15 (SEQ ID NO: 14), GPR32 (SEQ ID NO: 15), GPR34 (SEQ ID NO: 16), GPR183 (SEQ ID NO: 17), SERPINA4 (SEQ ID NO: 18), SERPINB5 (SEQ ID NO:

19), SEMA4B (SEQ ID NO: 20), SEMA4D (SEQ ID NO: 21), CCL14 (SEQ ID NO: 22), NKTR (SEQ ID NO: 23) and SFTPD (SEQ ID NO: 24).

9. The composition of claim 5, wherein the composition is 95% endotoxin free.

10. The composition of claim 5, wherein the composition is 98% endotoxin free.

11. The composition of claim 5 or claim 6, wherein the isolated antibody or antigen-binding fragment is a monoclonal antibody or antigen-binding fragment.

12. The composition of claim 5 or claim 6, wherein the isolated antibody or antigen-binding fragment is a humanized antibody or antigen-binding fragment.

13. The composition of claim 5 or claim 6, wherein the antibody or antigen binding fragment is conjugated to a toxin.

14. The composition of claim 13, wherein the toxin is selected from the group consisting of a ricin toxin, abrin toxin, diphtheria toxin, cholera toxin, gelonin toxin, Pseudomonas exotoxin, Shigella toxin, and pokeweed antiviral protein.

15. The composition of claim 5 or claim 6, wherein the antibody or antigen binding fragment is a monoclonal antibody or antigen binding fragment conjugated to a radionuclide.

16. The composition of claim 15 wherein the radionuclide is selected from the group consisting of ^{90}Y , ^{123}I , ^{125}I , ^{131}I , ^{186}Re , ^{188}Re , ^{211}At and ^{212}Bi .

17. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and one or more isolated polypeptide of any one of SEQ ID NOs: 1-24, or a fragment or variant thereof having at least 90% identity thereto, or an isolated polynucleotide encoding any one of the foregoing polypeptides.

18. The pharmaceutical compositions of claim 17, wherein the composition further comprises an immunostimulant.

19. A method for the treatment of cancer in a subject in need thereof comprising administering to the subject a pharmaceutical composition of any one of claims 1-14.

20. The method of claim 19 wherein the cancer is selected from liver cancer, pancreatic cancer, lung cancer, breast cancer, bladder cancer, kidney cancer, skin cancer and hematological cancer.

21. A method for detecting the presence of a cancer in a patient, comprising the steps of:

- (a) obtaining a biological sample from the patient;
- (b) contacting the biological sample with an antibody or antigen-binding fragment that specifically binds to a polypeptide of any one of SEQ ID NOs: 1-24;
- (c) detecting in the sample an amount of polypeptide that binds to the antibody or antigen-binding fragment; and
- (d) comparing the amount of polypeptide to a predetermined cut-off value and therefrom determining the presence of a cancer in the patient.

22. The method of claim 21 wherein the cancer is selected from liver cancer, pancreatic cancer, lung cancer, breast cancer, bladder cancer, kidney cancer, skin cancer and hematological cancer.

23. A method for detecting the presence of a cancer in a patient, comprising the steps of:
- (a) obtaining a biological sample from the patient;
 - (b) contacting the biological sample with an oligonucleotide that hybridizes to a polynucleotide, wherein the polynucleotide encodes a polypeptide of any one of SEQ ID NOs: 1-24, or encodes a fragment or variant thereof having at least 90% identity thereto;
 - (c) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide; and
 - (d) comparing the amount of polynucleotide that hybridizes to the oligonucleotide to a predetermined cut-off value, and therefrom determining the presence of the cancer in the patient.
24. The method of claim 23, wherein the cancer is selected from liver cancer, pancreatic cancer, lung cancer, breast cancer, bladder cancer, kidney cancer, skin cancer and hematological cancer.
25. A diagnostic kit comprising at least one isolated antibody or antigen-binding fragment thereof that specifically binds to a sequence of any one of SEQ ID NOs: 1-24 and a detection reagent, wherein the detection reagent comprises a reporter group.
26. A diagnostic kit comprising at least one oligonucleotide that hybridizes to a polynucleotide, wherein the polynucleotide encodes a polypeptide of any one of SEQ ID NOs: 1-24, or encodes a fragment or variant thereof having at least 90% identity thereto.
27. A method for treating a cancer in a patient, comprising the steps of:
- (a) detecting an amount of polypeptide of any one of SEQ ID NOs: 1-24 in a biological sample of a patient;

- (b) comparing the amount of the polypeptide to a predetermined cut-off value and therefrom determining the presence of a cancer in the patient; and
- (c) administering a composition according to any one of claims 1 to 14 to a subject determined to have cancer in step (b).

28. The method of claim 27, wherein the cancer is selected from liver cancer, pancreatic cancer, lung cancer, breast cancer, bladder cancer, kidney cancer, skin cancer and hematological cancer.

29. A method for treating a cancer in a patient, comprising the steps of:

- (a) obtaining a biological sample from the patient;
- (b) contacting the biological sample with an oligonucleotide that hybridizes to a polynucleotide, wherein the polynucleotide encodes a polypeptide of any one of SEQ ID NOs: 1-24, or encodes a fragment or variant thereof having at least 90% identity thereto;
- (c) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide;
- (d) comparing the amount of polynucleotide that hybridizes to the oligonucleotide to a predetermined cut-off value, and therefrom determining the presence of the cancer in the patient; and
- (e) administering a composition according to any one of claims 1 to 14 to a subject determined to have cancer in step (d).

30. The method of claim 29, wherein the cancer is selected from liver cancer, pancreatic cancer, lung cancer, breast cancer, bladder cancer, kidney cancer, skin cancer and hematological cancer.

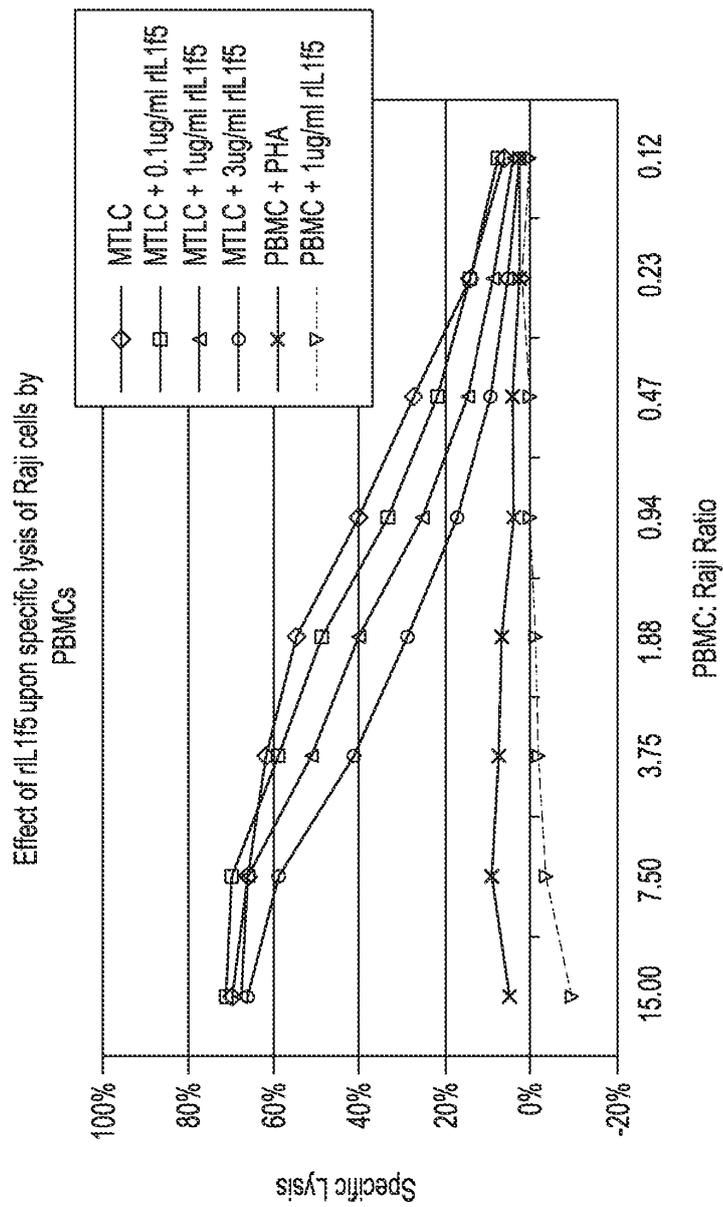


FIG. 1

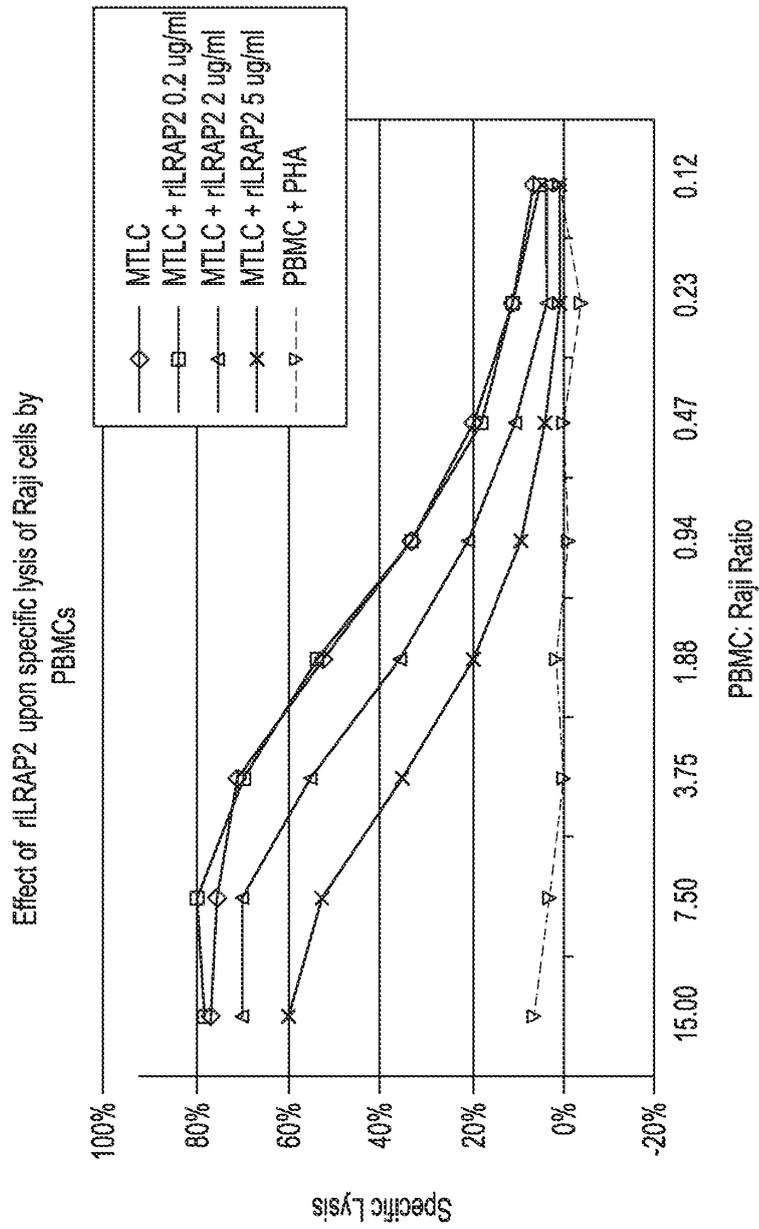


FIG. 2

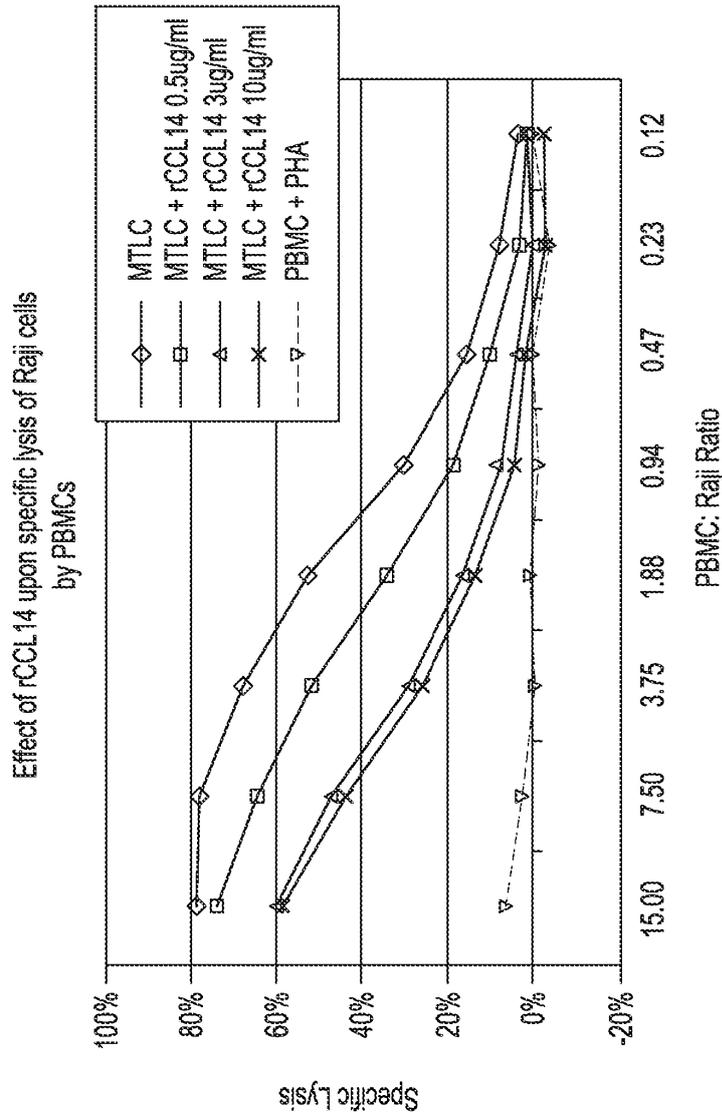
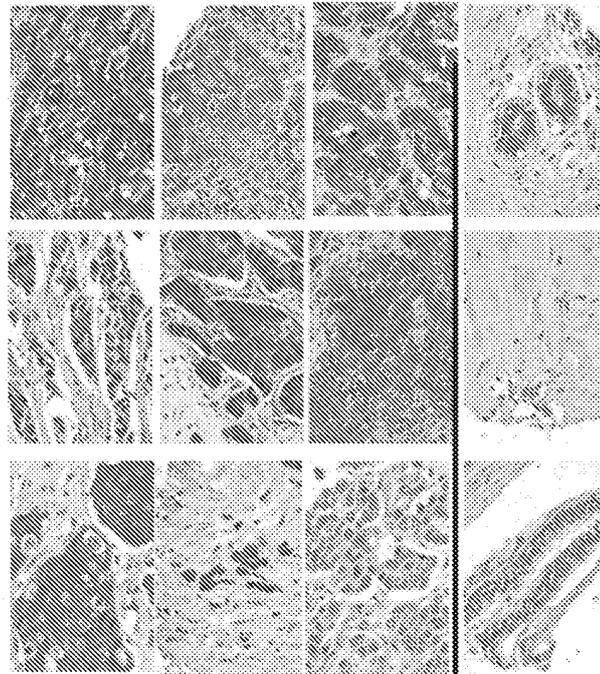


FIG. 3

Breast Cancer - IL1f5



Invasive Ductal Carcinoma

Normal Ductal Epithelium,
Vessels and Stroma

FIG. 4

Colon Cancer - IL1f5

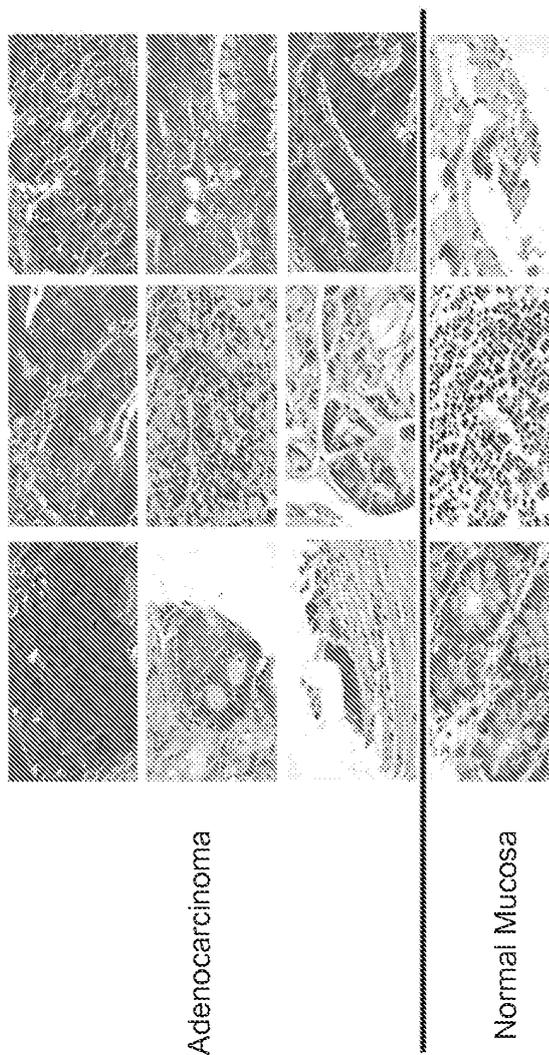
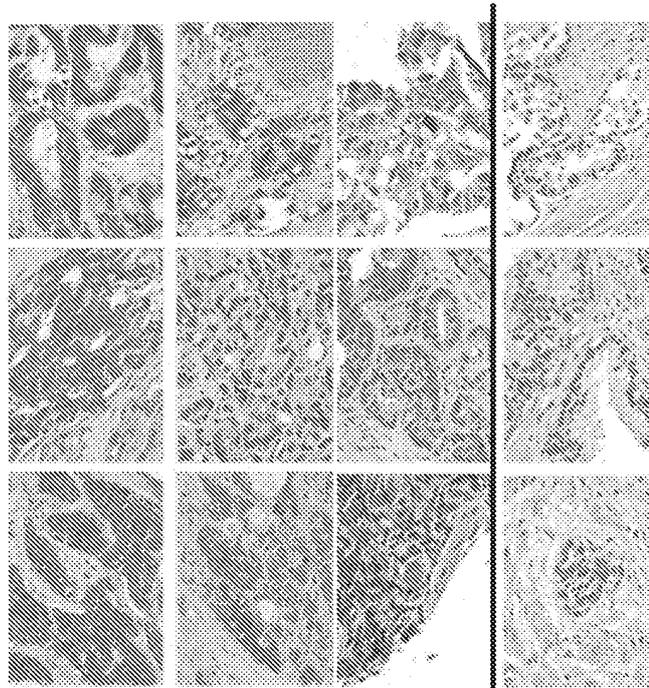


FIG. 5

Prostate Cancer - IL1f5

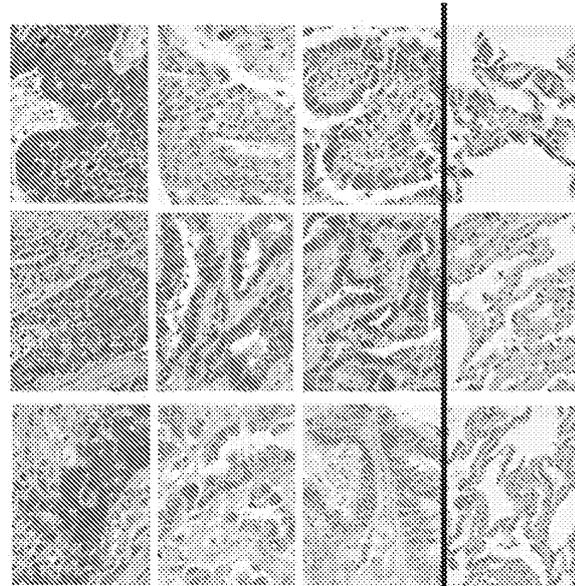


Adenocarcinoma

Normal Glands
and Stroma

FIG. 6

Lung Cancer - IL1f5



Squamous Cell Carcinoma

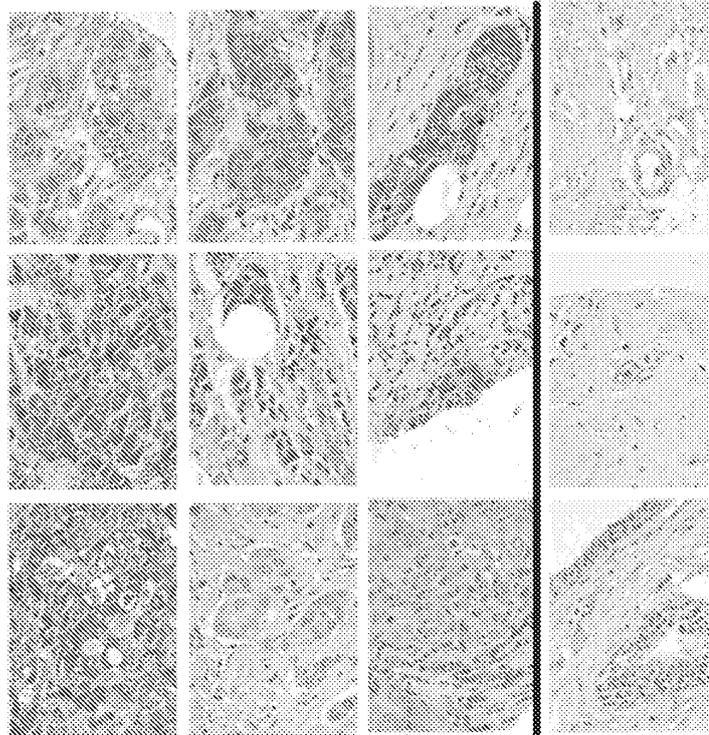
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Papillary Adenocarcinoma

Normal Alveoli

FIG. 7

Breast Cancer - GPR183

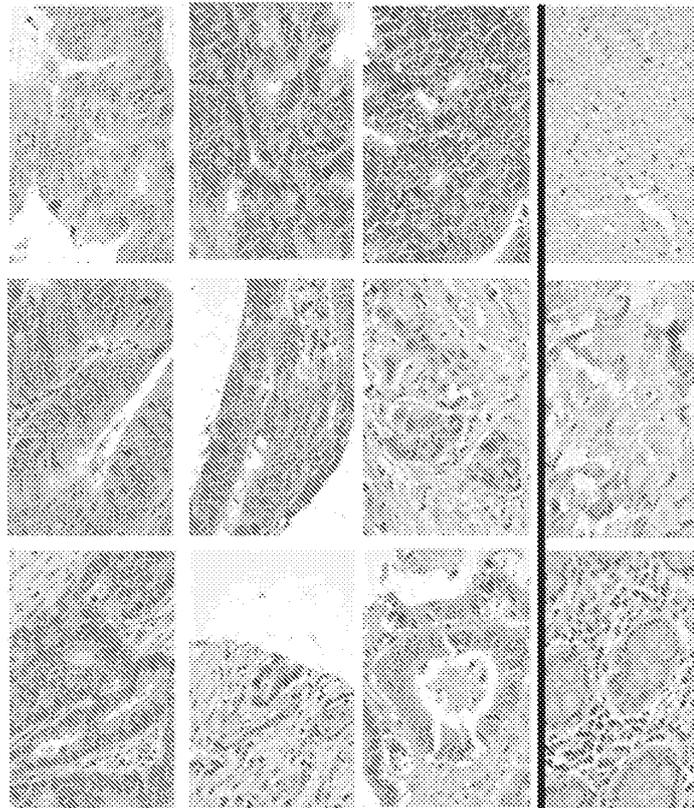


Invasive Ductal Carcinoma

Normal Ductal Epithelium,
Vessels, and Stroma

FIG. 8

Colon Cancer - GPR183



Adenocarcinoma

Normal Colonic
Tissue

FIG. 9

Lung Cancer - GPR183

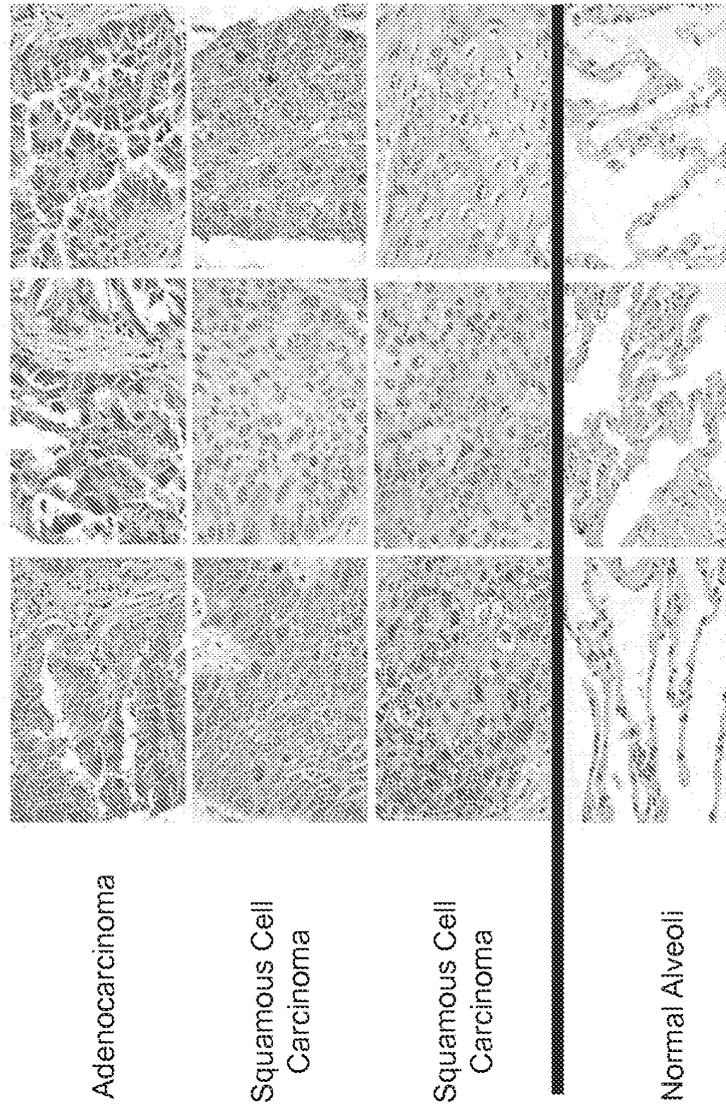
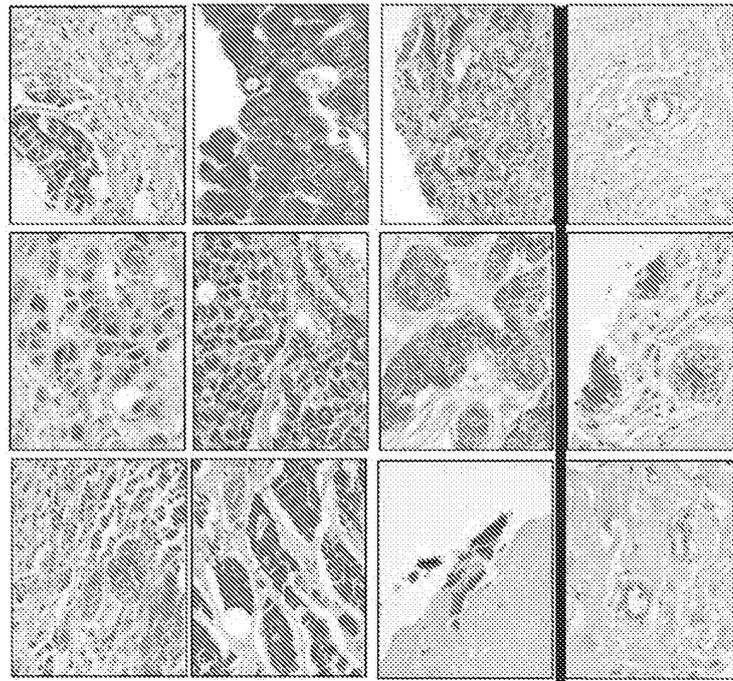


FIG. 10

Breast Cancer - IL1RAP

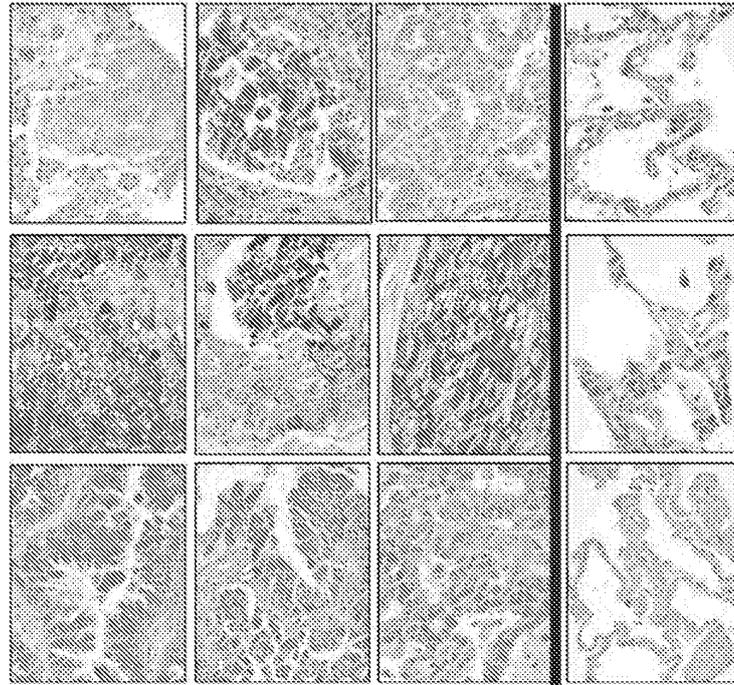


Invasive Ductal Carcinoma

Normal Ductal Epithelium,
Vessels, and Stroma

FIG. 11

Lung Cancer - IL1RAP

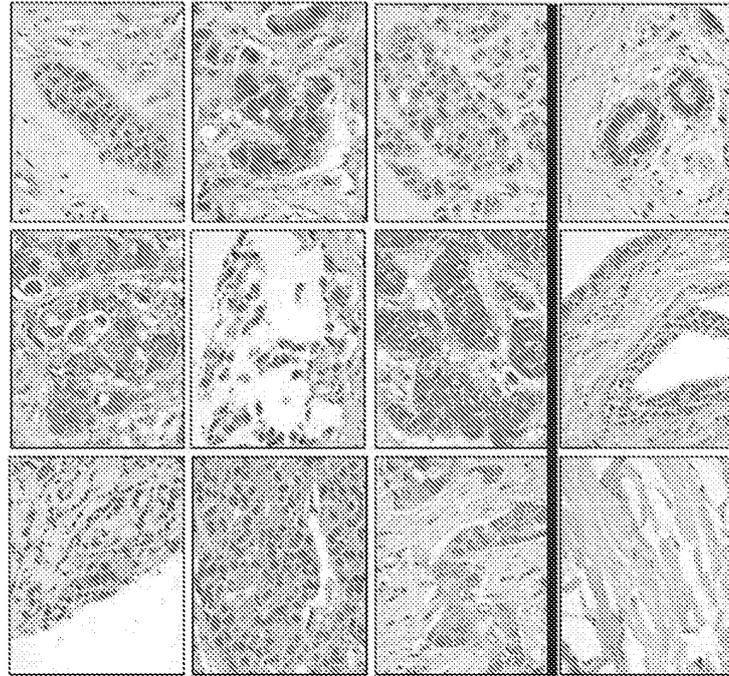


Lung Cancer

Normal Alveoli

FIG. 12

Breast Cancer - CCL14

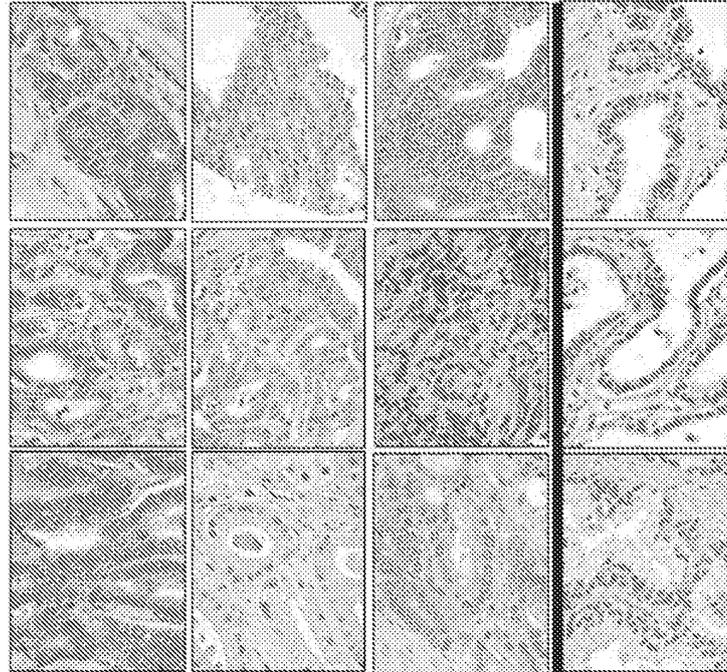


Invasive Ductal Carcinoma

Normal Ductal Epithelium,
Vessels, and Stroma

FIG. 13

Prostate Cancer - CCL14

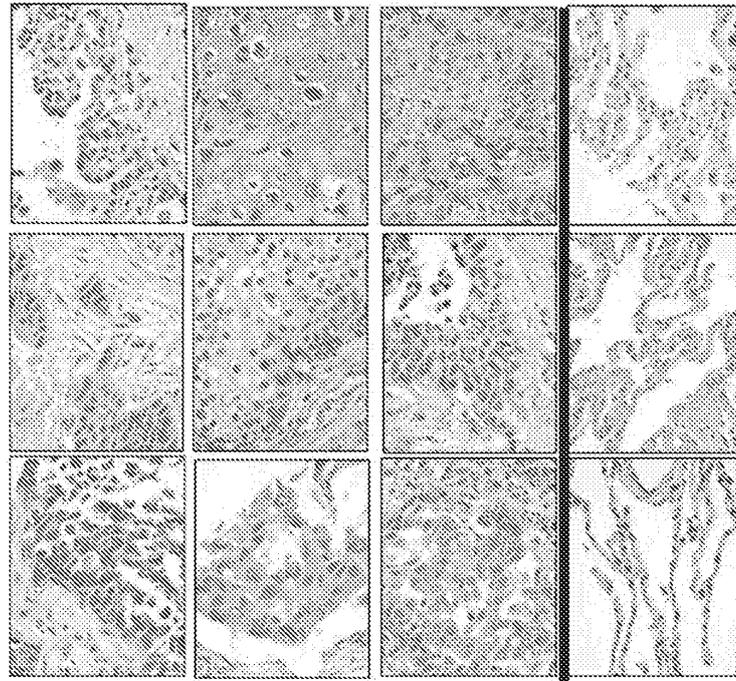


Adenocarcinoma

Normal Glands and
Stroma

FIG. 14

Lung Cancer - CCL14

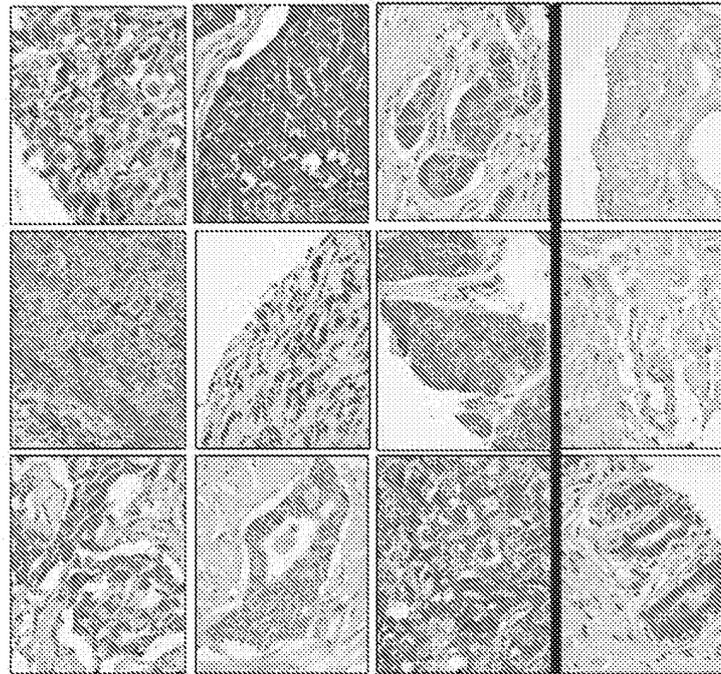


Lung Cancer

Normal Alveoli

FIG. 15

Breast Cancer - SEMA4D

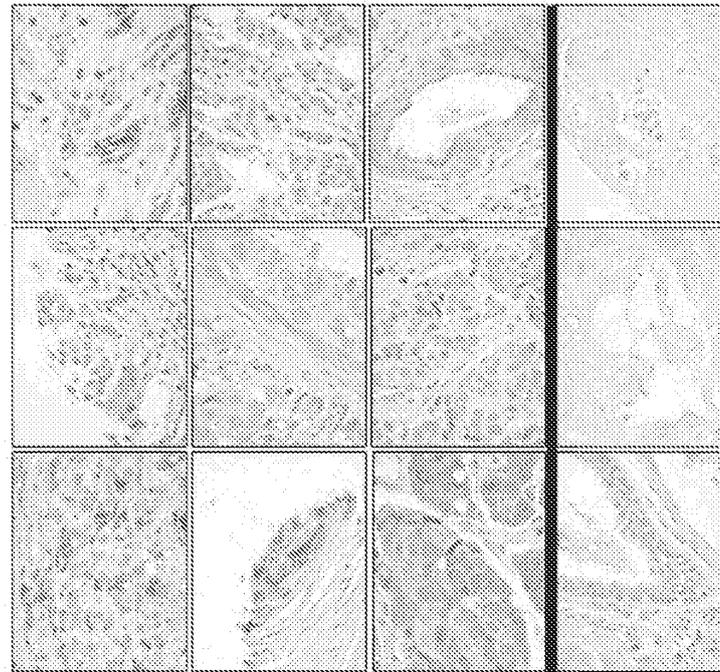


Invasive Ductal Carcinoma

Normal Ductal Epithelium,
Vessels, and Stroma

FIG. 16

Breast Cancer - IL1R2



Invasive Ductal Carcinoma

Normal Ductal Epithelium,
Vessels, and Stroma

FIG. 17

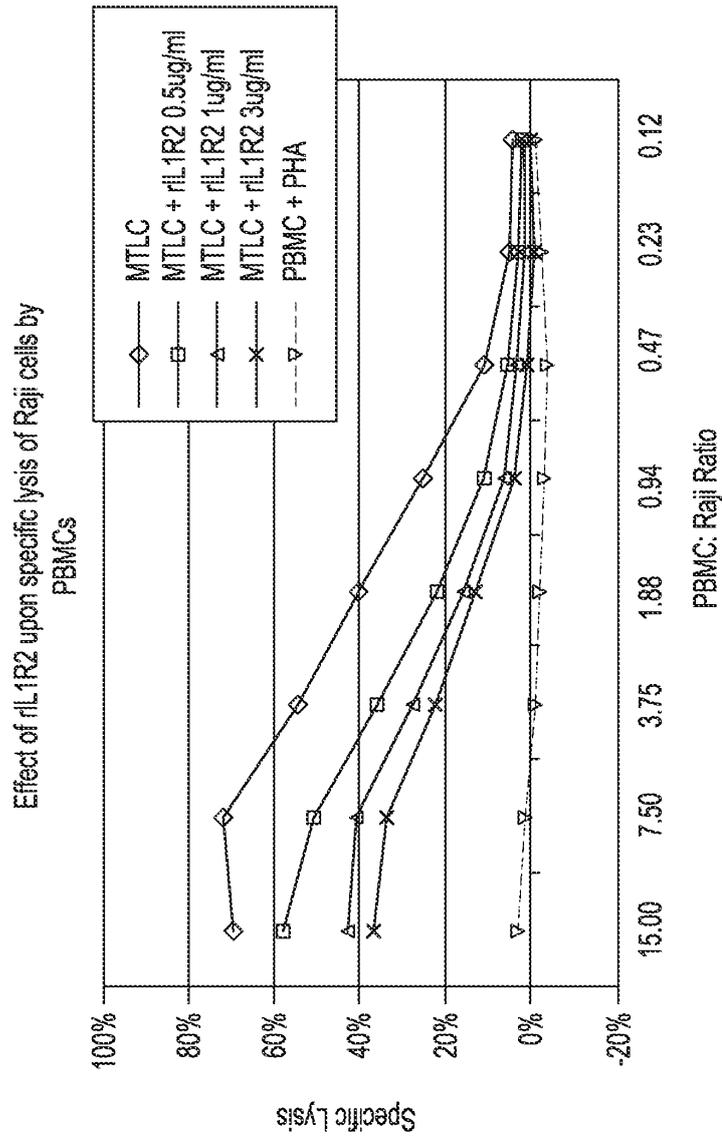


FIG. 18

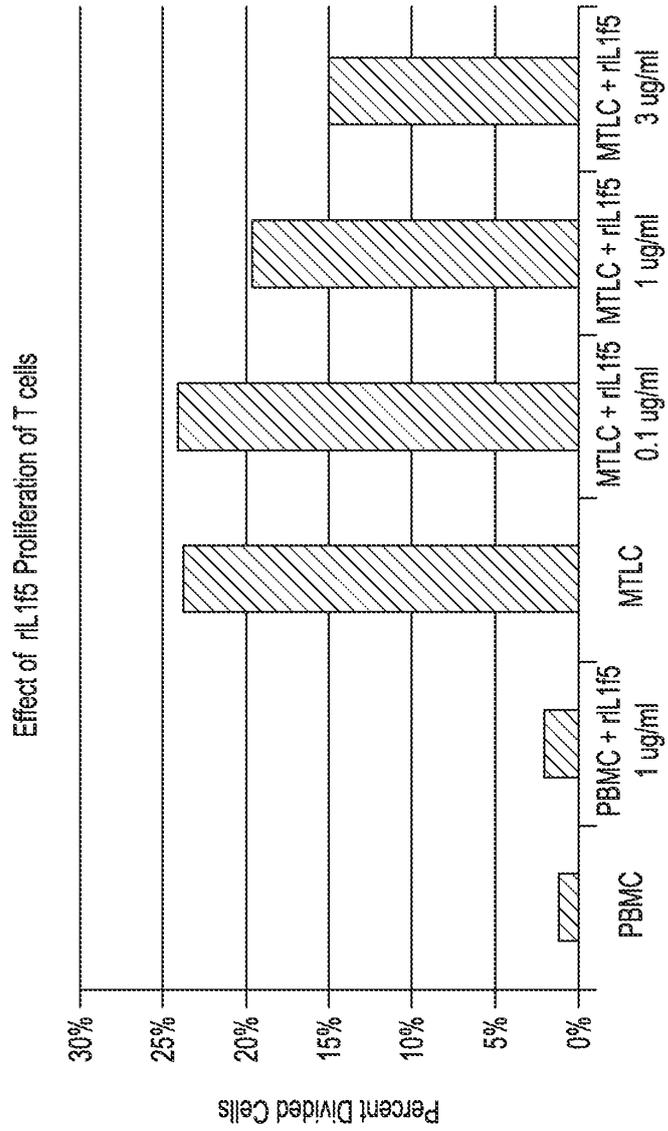


FIG. 19

SEQUENCE LISTING

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Warren, Sarah Ellen
Weissman, Carl

<120> COMPOSITIONS AND METHODS FOR THE
THERAPY AND DIAGNOSIS OF CANCER

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 <212> PRT
 <213> Homo sapiens

<400> 5
 Met Thr Met Arg His Asn Trp Thr Pro Asp Leu Ser Pro Leu Trp Val
 1 5 10 15
 Leu Leu Leu Cys Ala His Val Val Thr Leu Leu Val Arg Ala Thr Pro

ONCF_001_03WO_ST25.TXT

Val Ser Gln 20 Thr Thr Thr Ala Ala Thr Ala Ser Val Arg 30 Thr Lys
 35 40 45
 Asp Pro Cys Pro Ser Gln Pro Pro Val Phe Pro Ala Ala Lys Gln Cys
 50 55 60
 Pro Ala Leu Glu Val Thr Trp Pro Glu Val Glu Val Pro Leu Asn Gly
 65 70 75
 Thr Leu Ser Leu Ser Cys Val Ala Cys Ser Arg Phe Pro Asn Phe Ser
 85 90 95
 Ile Leu Tyr Trp Leu Gly Asn Gly Ser Phe Ile Glu His Leu Pro Gly
 100 105 110
 Arg Leu Trp Glu Gly Ser Thr Ser Arg Glu Arg Gly Ser Thr Gly Thr
 115 120 125
 Gln Leu Cys Lys Ala Leu Val Leu Glu Gln Leu Thr Pro Ala Leu His
 130 135 140
 Ser Thr Asn Phe Ser Cys Val Leu Val Asp Pro Glu Gln Val Val Gln
 145 150 155 160
 Arg His Val Val Leu Ala Gln Leu Trp Ala Gly Leu Arg Ala Thr Leu
 165 170 175
 Pro Pro Thr Gln Glu Ala Leu Pro Ser Ser His Ser Ser Pro Gln Gln
 180 185 190
 Gln Gly

<210> 6
 <211> 149
 <212> PRT
 <213> Homo sapiens

<400> 6
 Met Met Thr Lys His Lys Lys Cys Phe Ile Ile Val Gly Val Leu Ile
 1 5 10 15
 Thr Thr Asn Ile Ile Thr Leu Ile Val Lys Leu Thr Arg Asp Ser Gln
 20 25 30
 Ser Leu Cys Pro Tyr Asp Trp Ile Gly Phe Gln Asn Lys Cys Tyr Tyr
 35 40 45
 Phe Ser Lys Glu Glu Gly Asp Trp Asn Ser Ser Lys Tyr Asn Cys Ser
 50 55 60
 Thr Gln His Ala Asp Leu Thr Ile Ile Asp Asn Ile Glu Glu Met Asn
 65 70 75 80
 Phe Leu Arg Arg Tyr Lys Cys Ser Ser Asp His Trp Ile Gly Leu Lys
 85 90 95
 Met Ala Lys Asn Arg Thr Gly Gln Trp Val Asp Gly Ala Thr Phe Thr
 100 105 110
 Lys Ser Phe Gly Met Arg Gly Ser Glu Gly Cys Ala Tyr Leu Ser Asp
 115 120 125
 Asp Gly Ala Ala Thr Ala Arg Cys Tyr Thr Glu Arg Lys Trp Ile Cys
 130 135 140
 Arg Lys Arg Ile His
 145

<210> 7
 <211> 597
 <212> PRT
 <213> Homo sapiens

<400> 7
 Met His Pro Pro Lys Thr Pro Ser Gly Ala Leu His Arg Lys Arg Lys
 1 5 10 15
 Met Ala Ala Trp Pro Phe Ser Arg Leu Trp Lys Val Ser Asp Pro Ile
 20 25 30
 Leu Phe Gln Met Thr Leu Ile Ala Ala Leu Leu Pro Ala Val Leu Gly
 35 40 45
 Asn Cys Gly Pro Pro Pro Thr Leu Ser Phe Ala Ala Pro Met Asp Ile
 50 55 60
 Thr Leu Thr Glu Thr Arg Phe Lys Thr Gly Thr Thr Leu Lys Tyr Thr

ONCF_001_03wo_ST25.TXT

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

<210> 8
 <211> 252
 <212> PRT
 <213> Homo sapiens

<400> 8
 Met Phe Phe Trp Cys Ala Cys Cys Leu Met Val Ala Trp Arg Val Ser
 1 5 10 15
 Ala Ser Asp Ala Glu His Cys Pro Glu Leu Pro Pro Val Asp Asn Ser
 20 25 30
 Ile Phe Val Ala Lys Glu Val Glu Gly Gln Ile Leu Gly Thr Tyr Val
 35 40 45
 Cys Ile Lys Gly Tyr His Leu Val Gly Lys Lys Thr Leu Phe Cys Asn
 50 55 60
 Ala Ser Lys Glu Trp Asp Asn Thr Thr Thr Glu Cys Arg Leu Gly His
 65 70 75 80
 Cys Pro Asp Pro Val Leu Val Asn Gly Glu Phe Ser Ser Ser Gly Pro
 85 90 95
 Val Asn Val Ser Asp Lys Ile Thr Phe Met Cys Asn Asp His Tyr Ile
 100 105 110
 Leu Lys Gly Ser Asn Arg Ser Gln Cys Leu Glu Asp His Thr Trp Ala
 115 120 125
 Pro Pro Phe Pro Ile Cys Lys Ser Arg Asp Cys Asp Pro Pro Gly Asn
 130 135 140
 Pro Val His Gly Tyr Phe Glu Gly Asn Asn Phe Thr Leu Gly Ser Thr
 145 150 155 160
 Ile Ser Tyr Tyr Cys Glu Asp Arg Tyr Tyr Leu Val Gly Val Gln Glu
 165 170 175
 Gln Gln Cys Val Asp Gly Glu Trp Ser Ser Ala Leu Pro Val Cys Lys
 180 185 190
 Leu Ile Gln Glu Ala Pro Lys Pro Glu Cys Glu Lys Ala Leu Leu Ala
 195 200 205
 Phe Gln Glu Ser Lys Asn Leu Cys Glu Ala Met Glu Asn Phe Met Gln
 210 215 220
 Gln Leu Lys Glu Ser Gly Met Thr Met Glu Glu Leu Lys Tyr Ser Leu
 225 230 235 240
 Glu Leu Lys Lys Ala Glu Leu Lys Ala Lys Leu Leu
 245 250

<210> 9
 <211> 410
 <212> PRT
 <213> Homo sapiens

<400> 9
 Met Ala Phe Leu Gly Leu Phe Ser Leu Leu Val Leu Gln Ser Met Ala
 1 5 10 15
 Thr Gly Ala Thr Phe Pro Glu Glu Ala Ile Ala Asp Leu Ser Val Asn
 20 25 30
 Met Tyr Asn Arg Leu Arg Ala Thr Gly Glu Asp Glu Asn Ile Leu Phe
 35 40 45
 Ser Pro Leu Ser Ile Ala Leu Ala Met Gly Met Met Glu Leu Gly Ala
 50 55 60
 Gln Gly Ser Thr Gln Lys Glu Ile Arg His Ser Met Gly Tyr Asp Ser
 65 70 75 80
 Leu Lys Asn Gly Glu Glu Phe Ser Phe Leu Lys Glu Phe Ser Asn Met
 85 90 95
 Val Thr Ala Lys Glu Ser Gln Tyr Val Met Lys Ile Ala Asn Ser Leu
 100 105 110
 Phe Val Gln Asn Gly Phe His Val Asn Glu Glu Phe Leu Gln Met Met
 115 120 125
 Lys Lys Tyr Phe Asn Ala Ala Val Asn His Val Asp Phe Ser Gln Asn
 130 135 140
 Val Ala Val Ala Asn Tyr Ile Asn Lys Trp Val Glu Asn Asn Thr Asn
 145 150 155 160
 Asn Leu Val Lys Asp Leu Val Ser Pro Arg Asp Phe Asp Ala Ala Thr
 165 170 175

ONCF_001_03WO_ST25.TXT

Tyr Leu Ala Leu Ile Asn Ala Val Tyr Phe Lys Gly Asn Trp Lys Ser
 180 185
 Gln Phe Arg Pro Glu Asn Thr Arg Thr Phe Ser Phe Thr Lys Asp Asp
 195 200 205
 Glu Ser Glu Val Gln Ile Pro Met Met Tyr Gln Gln Gly Glu Phe Tyr
 210 215 220
 Tyr Gly Glu Phe Ser Asp Gly Ser Asn Glu Ala Gly Gly Ile Tyr Gln
 225 230 235
 Val Leu Glu Ile Pro Tyr Glu Gly Asp Glu Ile Ser Met Met Leu Val
 245 250 255
 Leu Ser Arg Gln Glu Val Pro Leu Ala Thr Leu Glu Pro Leu Val Lys
 260 265 270
 Ala Gln Leu Val Glu Glu Trp Ala Asn Ser Val Lys Lys Gln Lys Val
 275 280 285
 Glu Val Tyr Leu Pro Arg Phe Thr Val Glu Gln Glu Ile Asp Leu Lys
 290 295 300
 Asp Val Leu Lys Ala Leu Gly Ile Thr Glu Ile Phe Ile Lys Asp Ala
 305 310 315 320
 Asn Leu Thr Gly Leu Ser Asp Asn Lys Glu Ile Phe Leu Ser Lys Ala
 325 330 335
 Ile His Lys Ser Phe Leu Glu Val Asn Glu Glu Gly Ser Glu Ala Ala
 340 345 350
 Ala Val Ser Gly Met Ile Ala Ile Ser Arg Met Ala Val Leu Tyr Pro
 355 360 365
 Gln Val Ile Val Asp His Pro Phe Phe Phe Leu Ile Arg Asn Arg Arg
 370 375 380
 Thr Gly Thr Ile Leu Phe Met Gly Arg Val Met His Pro Glu Thr Met
 385 390 395 400
 Asn Thr Ser Gly His Asp Phe Glu Glu Leu
 405 410

<210> 10
 <211> 570
 <212> PRT
 <213> Homo sapiens

<400> 10
 Met Thr Leu Leu Trp Cys Val Val Ser Leu Tyr Phe Tyr Gly Ile Leu
 1 5 10 15
 Gln Ser Asp Ala Ser Glu Arg Cys Asp Asp Trp Gly Leu Asp Thr Met
 20 25 30
 Arg Gln Ile Gln Val Phe Glu Asp Glu Pro Ala Arg Ile Lys Cys Pro
 35 40 45
 Leu Phe Glu His Phe Leu Lys Phe Asn Tyr Ser Thr Ala His Ser Ala
 50 55 60
 Gly Leu Thr Leu Ile Trp Tyr Trp Thr Arg Gln Asp Arg Asp Leu Glu
 65 70 75 80
 Glu Pro Ile Asn Phe Arg Leu Pro Glu Asn Arg Ile Ser Lys Glu Lys
 85 90 95
 Asp Val Leu Trp Phe Arg Pro Thr Leu Leu Asn Asp Thr Gly Asn Tyr
 100 105 110
 Thr Cys Met Leu Arg Asn Thr Thr Tyr Cys Ser Lys Val Ala Phe Pro
 115 120 125
 Leu Glu Val Val Gln Lys Asp Ser Cys Phe Asn Ser Pro Met Lys Leu
 130 135 140
 Pro Val His Lys Leu Tyr Ile Glu Tyr Gly Ile Gln Arg Ile Thr Cys
 145 150 155 160
 Pro Asn Val Asp Gly Tyr Phe Pro Ser Ser Val Lys Pro Thr Ile Thr
 165 170 175
 Trp Tyr Met Gly Cys Tyr Lys Ile Gln Asn Phe Asn Asn Val Ile Pro
 180 185 190
 Glu Gly Met Asn Leu Ser Phe Leu Ile Ala Leu Ile Ser Asn Asn Gly
 195 200 205
 Asn Tyr Thr Cys Val Val Thr Tyr Pro Glu Asn Gly Arg Thr Phe His
 210 215 220
 Leu Thr Arg Thr Leu Thr Val Lys Val Val Gly Ser Pro Lys Asn Ala
 225 230 235 240

ONCF_001_03WO_ST25.TXT

Val Pro Pro Val Ile His Ser Pro Asn Asp His Val Val Tyr Glu Lys
 245 250
 Glu Pro Gly Glu Glu Leu Leu Ile Pro Cys Thr Val Tyr Phe Ser Phe
 260 265
 Leu Met Asp Ser Arg Asn Glu Val Trp Trp Thr Ile Asp Gly Lys Lys
 275 280
 Pro Asp Asp Ile Thr Ile Asp Val Thr Ile Asn Glu Ser Ile Ser His
 290 295
 Ser Arg Thr Glu Asp Glu Thr Arg Thr Gln Ile Leu Ser Ile Lys Lys
 305 310 315 320
 Val Thr Ser Glu Asp Leu Lys Arg Ser Tyr Val Cys His Ala Arg Ser
 325 330 335
 Ala Lys Gly Glu Val Ala Lys Ala Ala Lys Val Lys Gln Lys Val Pro
 340 345 350
 Ala Pro Arg Tyr Thr Val Glu Leu Ala Cys Gly Phe Gly Ala Thr Val
 355 360 365
 Leu Leu Val Val Ile Leu Ile Val Val Tyr His Val Tyr Trp Leu Glu
 370 375 380
 Met Val Leu Phe Tyr Arg Ala His Phe Gly Thr Asp Glu Thr Ile Leu
 385 390 395 400
 Asp Gly Lys Glu Tyr Asp Ile Tyr Val Ser Tyr Ala Arg Asn Ala Glu
 405 410 415
 Glu Glu Glu Phe Val Leu Leu Thr Leu Arg Gly Val Leu Glu Asn Glu
 420 425 430
 Phe Gly Tyr Lys Leu Cys Ile Phe Asp Arg Asp Ser Leu Pro Gly Gly
 435 440 445
 Ile Val Thr Asp Glu Thr Leu Ser Phe Ile Gln Lys Ser Arg Arg Leu
 450 455 460
 Leu Val Val Leu Ser Pro Asn Tyr Val Leu Gln Gly Thr Gln Ala Leu
 465 470 475 480
 Leu Glu Leu Lys Ala Gly Leu Glu Asn Met Ala Ser Arg Gly Asn Ile
 485 490 495
 Asn Val Ile Leu Val Gln Tyr Lys Ala Val Lys Glu Thr Lys Val Lys
 500 505 510
 Glu Leu Lys Arg Ala Lys Thr Val Leu Thr Val Ile Lys Trp Lys Gly
 515 520 525
 Glu Lys Ser Lys Tyr Pro Gln Gly Arg Phe Trp Lys Gln Leu Gln Val
 530 535 540
 Ala Met Pro Val Lys Lys Ser Pro Arg Arg Ser Ser Ser Asp Glu Gln
 545 550 555 560
 Gly Leu Ser Tyr Ser Ser Leu Lys Asn Val 570

<210> 11
 <211> 356
 <212> PRT
 <213> Homo sapiens

<400> 11
 Met Thr Leu Leu Trp Cys Val Val Ser Leu Tyr Phe Tyr Gly Ile Leu
 1 5 10 15
 Gln Ser Asp Ala Ser Glu Arg Cys Asp Asp Trp Gly Leu Asp Thr Met
 20 25 30
 Arg Gln Ile Gln Val Phe Glu Asp Glu Pro Ala Arg Ile Lys Cys Pro
 35 40 45
 Leu Phe Glu His Phe Leu Lys Phe Asn Tyr Ser Thr Ala His Ser Ala
 50 55 60
 Gly Leu Thr Leu Ile Trp Tyr Trp Thr Arg Gln Asp Arg Asp Leu Glu
 65 70 75 80
 Glu Pro Ile Asn Phe Arg Leu Pro Glu Asn Arg Ile Ser Lys Glu Lys
 85 90 95
 Asp Val Leu Trp Phe Arg Pro Thr Leu Leu Asn Asp Thr Gly Asn Tyr
 100 105 110
 Thr Cys Met Leu Arg Asn Thr Thr Tyr Cys Ser Lys Val Ala Phe Pro
 115 120 125
 Leu Glu Val Val Gln Lys Asp Ser Cys Phe Asn Ser Pro Met Lys Leu
 130 135 140
 Pro Val His Lys Leu Tyr Ile Glu Tyr Gly Ile Gln Arg Ile Thr Cys

ONCF_001_03wo_ST25.TXT

145	Pro	Asn	Val	Asp	Gly	150	Tyr	Phe	Pro	Ser	Ser	Val	Lys	Pro	Thr	Ile	160	Thr
					165						170						175	
	Trp	Tyr	Met	Gly	Cys	Tyr	Lys	Ile	Gln	Asn	Phe	Asn	Asn	Val	Ile	Pro		
				180					185					190				
	Glu	Gly	Met	Asn	Leu	Ser	Phe	Leu	Ile	Ala	Leu	Ile	Ser	Asn	Asn	Gly		
			195					200					205					
	Asn	Tyr	Thr	Cys	Val	Val	Thr	Tyr	Pro	Glu	Asn	Gly	Arg	Thr	Phe	His		
		210					215					220						
	Leu	Thr	Arg	Thr	Leu	Thr	Val	Lys	Val	Val	Gly	Ser	Pro	Lys	Asn	Ala		
		225				230					235					240		
	Val	Pro	Pro	Val	Ile	His	Ser	Pro	Asn	Asp	His	Val	Val	Tyr	Glu	Lys		
				245						250					255			
	Glu	Pro	Gly	Glu	Glu	Leu	Leu	Ile	Pro	Cys	Thr	Val	Tyr	Phe	Ser	Phe		
				260					265					270				
	Leu	Met	Asp	Ser	Arg	Asn	Glu	Val	Trp	Trp	Thr	Ile	Asp	Gly	Lys	Lys		
			275					280					285					
	Pro	Asp	Asp	Ile	Thr	Ile	Asp	Val	Thr	Ile	Asn	Glu	Ser	Ile	Ser	His		
		290					295					300						
	Ser	Arg	Thr	Glu	Asp	Glu	Thr	Arg	Thr	Gln	Ile	Leu	Ser	Ile	Lys	Lys		
		305				310					315				320			
	Val	Thr	Ser	Glu	Asp	Leu	Lys	Arg	Ser	Tyr	Val	Cys	His	Ala	Arg	Ser		
				325						330					335			
	Ala	Lys	Gly	Glu	Val	Ala	Lys	Ala	Ala	Lys	Val	Lys	Gln	Lys	Gly	Asn		
				340				345						350				
	Arg	Cys	Gly	Gln														
			355															

<210> 12
 <211> 355
 <212> PRT
 <213> Homo sapiens

<400> 12

Met	Glu	Asp	Leu	Glu	Glu	Thr	Leu	Phe	Glu	Glu	Phe	Glu	Asn	Tyr	Ser
1				5					10					15	
Tyr	Asp	Leu	Asp	Tyr	Tyr	Ser	Leu	Glu	Ser	Asp	Leu	Glu	Glu	Lys	Val
			20					25					30		
Gln	Leu	Gly	Val	Val	His	Trp	Val	Ser	Leu	Val	Leu	Tyr	Cys	Leu	Ala
		35					40					45			
Phe	Val	Leu	Gly	Ile	Pro	Gly	Asn	Ala	Ile	Val	Ile	Trp	Phe	Thr	Gly
		50				55					60				
Phe	Lys	Trp	Lys	Lys	Thr	Val	Thr	Thr	Leu	Trp	Phe	Leu	Asn	Leu	Ala
65					70					75					80
Ile	Ala	Asp	Phe	Ile	Phe	Leu	Leu	Phe	Leu	Pro	Leu	Tyr	Ile	Ser	Tyr
			85					90						95	
Val	Ala	Met	Asn	Phe	His	Trp	Pro	Phe	Gly	Ile	Trp	Leu	Cys	Lys	Ala
			100					105					110		
Asn	Ser	Phe	Thr	Ala	Gln	Leu	Asn	Met	Phe	Ala	Ser	Val	Phe	Phe	Leu
		115					120					125			
Thr	Val	Ile	Ser	Leu	Asp	His	Tyr	Ile	His	Leu	Ile	His	Pro	Val	Leu
		130				135					140				
Ser	His	Arg	His	Arg	Thr	Leu	Lys	Asn	Ser	Leu	Ile	Val	Ile	Ile	Phe
		145				150				155					160
Ile	Trp	Leu	Leu	Ala	Ser	Leu	Ile	Gly	Gly	Pro	Ala	Leu	Tyr	Phe	Arg
				165				170						175	
Asp	Thr	Val	Glu	Phe	Asn	Asn	His	Thr	Leu	Cys	Tyr	Asn	Asn	Phe	Gln
			180					185					190		
Lys	His	Asp	Pro	Asp	Leu	Thr	Leu	Ile	Arg	His	His	Val	Leu	Thr	Trp
		195					200					205			
Val	Lys	Phe	Ile	Ile	Gly	Tyr	Leu	Phe	Pro	Leu	Leu	Thr	Met	Ser	Ile
		210				215					220				
Cys	Tyr	Leu	Cys	Leu	Ile	Phe	Lys	Val	Lys	Lys	Arg	Ser	Ile	Leu	Ile
		225			230				235						240
Ser	Ser	Arg	His	Phe	Trp	Thr	Ile	Leu	Val	Val	Val	Val	Ala	Phe	Val
				245					250					255	
Val	Cys	Trp	Thr	Pro	Tyr	His	Leu	Phe	Ser	Ile	Trp	Glu	Leu	Thr	Ile

ONCF_001_03WO_ST25.TXT

260
 His His Asn Ser Tyr Ser His His Val Met Gln Ala Gly Ile Pro Leu
 275
 Ser Thr Gly Leu Ala Phe Leu Asn Ser Cys Leu Asn Pro Ile Leu Tyr
 290
 Val Leu Ile Ser Lys Lys Phe Gln Ala Arg Phe Arg Ser Ser Val Ala
 305
 Glu Ile Leu Lys Tyr Thr Leu Trp Glu Val Ser Cys Ser Gly Thr Val
 325
 Ser Glu Gln Leu Arg Asn Ser Glu Thr Lys Asn Leu Cys Leu Leu Glu
 340
 Thr Ala Gln
 355

<210> 13
 <211> 362
 <212> PRT
 <213> Homo sapiens

<400> 13
 Met Gly Asn His Thr Trp Glu Gly Cys His Val Asp Ser Arg Val Asp
 1 5 10 15
 His Leu Phe Pro Pro Ser Leu Tyr Ile Phe Val Ile Gly Val Gly Leu
 20 25 30
 Pro Thr Asn Cys Leu Ala Leu Trp Ala Ala Tyr Arg Gln Val Gln Gln
 35 40 45
 Arg Asn Glu Leu Gly Val Tyr Leu Met Asn Leu Ser Ile Ala Asp Leu
 50 55 60
 Leu Tyr Ile Cys Thr Leu Pro Leu Trp Val Asp Tyr Phe Leu His His
 65 70 75 80
 Asp Asn Trp Ile His Gly Pro Gly Ser Cys Lys Leu Phe Gly Phe Ile
 85 90 95
 Phe Tyr Thr Asn Ile Tyr Ile Ser Ile Ala Phe Leu Cys Cys Ile Ser
 100 105 110
 Val Asp Arg Tyr Leu Ala Val Ala His Pro Leu Arg Phe Ala Arg Leu
 115 120 125
 Arg Arg Val Lys Thr Ala Val Ala Val Ser Ser Val Val Trp Ala Thr
 130 135 140
 Glu Leu Gly Ala Asn Ser Ala Pro Leu Phe His Asp Glu Leu Phe Arg
 145 150 155 160
 Asp Arg Tyr Asn His Thr Phe Cys Phe Glu Lys Phe Pro Met Glu Gly
 165 170 175
 Trp Val Ala Trp Met Asn Leu Tyr Arg Val Phe Val Gly Phe Leu Phe
 180 185 190
 Pro Trp Ala Leu Met Leu Leu Ser Tyr Arg Gly Ile Leu Arg Ala Val
 195 200 205
 Arg Gly Ser Val Ser Thr Glu Arg Gln Glu Lys Ala Lys Ile Lys Arg
 210 215 220
 Leu Ala Leu Ser Leu Ile Ala Ile Val Leu Val Cys Phe Ala Pro Tyr
 225 230 235 240
 His Val Leu Leu Leu Ser Arg Ser Ala Ile Tyr Leu Gly Arg Pro Trp
 245 250 255
 Asp Cys Gly Phe Glu Glu Arg Val Phe Ser Ala Tyr His Ser Ser Leu
 260 265 270
 Ala Phe Thr Ser Leu Asn Cys Val Ala Asp Pro Ile Leu Tyr Cys Leu
 275 280 285
 Val Asn Glu Gly Ala Arg Ser Asp Val Ala Lys Ala Leu His Asn Leu
 290 295 300
 Leu Arg Phe Leu Ala Ser Asp Lys Pro Gln Glu Met Ala Asn Ala Ser
 305 310 315 320
 Leu Thr Leu Glu Thr Pro Leu Thr Ser Lys Arg Asn Ser Thr Ala Lys
 325 330 335
 Ala Met Thr Gly Ser Trp Ala Ala Thr Pro Pro Ser Gln Gly Asp Gln
 340 345 350
 Val Gln Leu Lys Met Leu Pro Pro Ala Gln
 355 360

ONCF_001_03WO_ST25.TXT

<210> 14
 <211> 360
 <212> PRT
 <213> Homo sapiens

<400> 14
 Met Asp Pro Glu Glu Thr Ser Val Tyr Leu Asp Tyr Tyr Tyr Ala Thr
 1 5 10 15
 Ser Pro Asn Ser Asp Ile Arg Glu Thr His Ser His Val Pro Tyr Thr
 20 25 30
 Ser Val Phe Leu Pro Val Phe Tyr Thr Ala Val Phe Leu Thr Gly Val
 35 40 45
 Leu Gly Asn Leu Val Leu Met Gly Ala Leu His Phe Lys Pro Gly Ser
 50 55 60
 Arg Arg Leu Ile Asp Ile Phe Ile Ile Asn Leu Ala Ala Ser Asp Phe
 65 70 75 80
 Ile Phe Leu Val Thr Leu Pro Leu Trp Val Asp Lys Glu Ala Ser Leu
 85 90 95
 Gly Leu Trp Arg Thr Gly Ser Phe Leu Cys Lys Gly Ser Ser Tyr Met
 100 105 110
 Ile Ser Val Asn Met His Cys Ser Val Leu Leu Leu Thr Cys Met Ser
 115 120 125
 Val Asp Arg Tyr Leu Ala Ile Val Trp Pro Val Val Ser Arg Lys Phe
 130 135 140
 Arg Arg Thr Asp Cys Ala Tyr Val Val Cys Ala Ser Ile Trp Phe Ile
 145 150 155 160
 Ser Cys Leu Leu Gly Leu Pro Thr Leu Leu Ser Arg Glu Leu Thr Leu
 165 170 175
 Ile Asp Asp Lys Pro Tyr Cys Ala Glu Lys Lys Ala Thr Pro Ile Lys
 180 185 190
 Leu Ile Trp Ser Leu Val Ala Leu Ile Phe Thr Phe Phe Val Pro Leu
 195 200 205
 Leu Ser Ile Val Thr Cys Tyr Cys Cys Ile Ala Arg Lys Leu Cys Ala
 210 215 220
 His Tyr Gln Gln Ser Gly Lys His Asn Lys Lys Leu Lys Lys Ser Ile
 225 230 235 240
 Lys Ile Ile Phe Ile Val Val Ala Ala Phe Leu Val Ser Trp Leu Pro
 245 250 255
 Phe Asn Thr Phe Lys Phe Leu Ala Ile Val Ser Gly Leu Arg Gln Glu
 260 265 270
 His Tyr Leu Pro Ser Ala Ile Leu Gln Leu Gly Met Glu Val Ser Gly
 275 280 285
 Pro Leu Ala Phe Ala Asn Ser Cys Val Asn Pro Phe Ile Tyr Tyr Ile
 290 295 300
 Phe Asp Ser Tyr Ile Arg Arg Ala Ile Val His Cys Leu Cys Pro Cys
 305 310 315 320
 Leu Lys Asn Tyr Asp Phe Gly Ser Ser Thr Glu Thr Ser Asp Ser His
 325 330 335
 Leu Thr Lys Ala Leu Ser Thr Phe Ile His Ala Glu Asp Phe Ala Arg
 340 345 350
 Arg Arg Lys Arg Ser Val Ser Leu
 355 360

<210> 15
 <211> 356
 <212> PRT
 <213> Homo sapiens

<400> 15
 Met Asn Gly Val Ser Glu Gly Thr Arg Gly Cys Ser Asp Arg Gln Pro
 1 5 10 15
 Gly Val Leu Thr Arg Asp Arg Ser Cys Ser Arg Lys Met Asn Ser Ser
 20 25 30
 Gly Cys Leu Ser Glu Glu Val Gly Ser Leu Arg Pro Leu Thr Val Val
 35 40 45
 Ile Leu Ser Ala Ser Ile Val Val Gly Val Leu Gly Asn Gly Leu Val

ONCF_001_03WO_ST25.TXT

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

<210> 16
 <211> 381
 <212> PRT
 <213> Homo sapiens

<400> 16
 Met Arg Ser His Thr Ile Thr Met Thr Thr Thr Ser Val Ser Ser Trp
 1 5 10 15
 Pro Tyr Ser Ser His Arg Met Arg Phe Ile Thr Asn His Ser Asp Gln
 20 25 30
 Pro Pro Gln Asn Phe Ser Ala Thr Pro Asn Val Thr Thr Cys Pro Met
 35 40 45
 Asp Glu Lys Leu Leu Ser Thr Val Leu Thr Thr Ser Tyr Ser Val Ile
 50 55 60
 Phe Ile Val Gly Leu Val Gly Asn Ile Ile Ala Leu Tyr Val Phe Leu
 65 70 75 80
 Gly Ile His Arg Lys Arg Asn Ser Ile Gln Ile Tyr Leu Leu Asn Val
 85 90 95
 Ala Ile Ala Asp Leu Leu Leu Ile Phe Cys Leu Pro Phe Arg Ile Met
 100 105 110
 Tyr His Ile Asn Gln Asn Lys Trp Thr Leu Gly Val Ile Leu Cys Lys
 115 120 125
 Val Val Gly Thr Leu Phe Tyr Met Asn Met Tyr Ile Ser Ile Ile Leu
 130 135 140
 Leu Gly Phe Ile Ser Leu Asp Arg Tyr Ile Lys Ile Asn Arg Ser Ile
 145 150 155 160
 Gln Gln Arg Lys Ala Ile Thr Thr Lys Gln Ser Ile Tyr Val Cys Cys

ONCF_001_03WO_ST25.TXT

Ile	Val	Trp	Met	165	Leu	Ala	Leu	Gly	Gly	170	Phe	Leu	Thr	Met	Ile	175	Ile	Leu	
Thr	Leu	Lys	Lys	180	Gly	Gly	His	Asn	Ser	185	Thr	Met	Cys	Phe	190	His	Tyr	Arg	
Asp	Lys	His	Asn	195	Ala	Lys	Gly	Glu	Ala	200	Ile	Phe	Asn	Phe	205	Ile	Leu	Val	
Val	Met	Phe	Trp	210	Leu	Ile	Phe	Leu	Leu	215	Ile	Ile	Leu	Ser	Tyr	Ile	Lys	240	
Ile	Gly	Lys	Asn	225	Leu	Leu	Arg	Ile	Ser	230	Lys	Arg	Arg	Ser	Lys	Phe	Pro	255	
Asn	Ser	Gly	Lys	245	Tyr	Ala	Thr	Thr	Ala	250	Arg	Asn	Ser	Phe	Ile	Val	Leu	270	
Ile	Ile	Phe	Thr	260	Ile	Cys	Phe	Val	Pro	265	Tyr	His	Ala	Phe	Arg	Phe	Ile	285	
Tyr	Ile	Ser	Ser	275	Gln	Leu	Asn	Val	Ser	280	Ser	Ser	Cys	Tyr	Trp	Lys	Glu	Ile	
Val	His	Lys	Thr	290	Asn	Glu	Ile	Met	Leu	295	Val	Leu	Val	Leu	Ser	Ser	Phe	Asn	320
Cys	Leu	Asp	Pro	305	Val	Met	Tyr	Phe	Leu	310	Met	Ser	Ser	Asn	Ile	Arg	Lys	335	
Ile	Met	Cys	Gln	325	Leu	Leu	Phe	Arg	Arg	330	Phe	Gln	Gly	Glu	Pro	Ser	Arg	350	
Ser	Glu	Ser	Thr	340	Ser	Glu	Phe	Lys	Pro	345	Gly	Tyr	Ser	Leu	His	Asp	Thr	365	
Ser	Val	Ala	Val	355	Lys	Ile	Gln	Ser	Ser	360	Ser	Ser	Lys	Ser	Thr			380	
				370						375									

<210> 17
 <211> 361
 <212> PRT
 <213> Homo sapiens

<400> 17

Met	Asp	Ile	Gln	Met	Ala	Asn	Asn	Phe	Thr	Pro	Pro	Ser	Ala	Thr	Pro			
1				5					10					15				
Gln	Gly	Asn	Asp	Cys	Asp	Leu	Tyr	Ala	His	His	Ser	Thr	Ala	Arg	Ile			
			20					25					30					
Val	Met	Pro	Leu	His	Tyr	Ser	Leu	Val	Phe	Ile	Ile	Gly	Leu	Val	Gly			
			35				40					45						
Asn	Leu	Leu	Ala	Leu	Val	Val	Ile	Val	Gln	Asn	Arg	Lys	Lys	Ile	Asn			
			50			55				60								
Ser	Thr	Thr	Leu	Tyr	Ser	Thr	Asn	Leu	Val	Ile	Ser	Asp	Ile	Leu	Phe			
65				70					75					80				
Thr	Thr	Ala	Leu	Pro	Thr	Arg	Ile	Ala	Tyr	Tyr	Ala	Met	Gly	Phe	Asp			
				85				90					95					
Trp	Arg	Ile	Gly	Asp	Ala	Leu	Cys	Arg	Ile	Thr	Ala	Leu	Val	Phe	Tyr			
			100					105					110					
Ile	Asn	Thr	Tyr	Ala	Gly	Val	Asn	Phe	Met	Thr	Cys	Leu	Ser	Ile	Asp			
			115				120					125						
Arg	Phe	Ile	Ala	Val	Val	His	Pro	Leu	Arg	Tyr	Asn	Lys	Ile	Lys	Arg			
			130			135					140							
Ile	Glu	His	Ala	Lys	Gly	Val	Cys	Ile	Phe	Val	Trp	Ile	Leu	Val	Phe			
145				150						155					160			
Ala	Gln	Thr	Leu	Pro	Leu	Leu	Ile	Asn	Pro	Met	Ser	Lys	Gln	Glu	Ala			
				165					170					175				
Glu	Arg	Ile	Thr	Cys	Met	Glu	Tyr	Pro	Asn	Phe	Glu	Glu	Thr	Lys	Ser			
			180					185					190					
Leu	Pro	Trp	Ile	Leu	Leu	Gly	Ala	Cys	Phe	Ile	Gly	Tyr	Val	Leu	Pro			
			195				200					205						
Leu	Ile	Ile	Ile	Leu	Ile	Cys	Tyr	Ser	Gln	Ile	Cys	Cys	Lys	Leu	Phe			
						215					220							
Arg	Thr	Ala	Lys	Gln	Asn	Pro	Leu	Thr	Glu	Lys	Ser	Gly	Val	Asn	Lys			
225				230						235				240				
Lys	Ala	Leu	Asn	Thr	Ile	Ile	Leu	Ile	Ile	Val	Val	Phe	Val	Leu	Cys			
				245						250				255				
Phe	Thr	Pro	Tyr	His	Val	Ala	Ile	Ile	Gln	His	Met	Ile	Lys	Lys	Leu			

ONCF_001_03WO_ST25.TXT

Arg Phe Ser 260 Asn Phe Leu Glu Cys 265 Ser Gln Arg His Ser 270 Phe Gln Ile
 Ser Leu His 275 Phe Thr Val Cys 280 Leu Met Asn Phe Asn Cys 285 Cys Met Asp
 Pro Phe Ile Tyr Phe Phe Ala Cys Lys Gly Tyr Lys Arg Lys Val Met 320
 305 Arg Met Leu Lys Arg Gln Val Ser Val Ser Ile Ser Ser Ala Val Lys
 Ser Ala Pro Glu Glu Asn Ser Arg Glu Met Thr Glu Thr Gln Met Met 335
 Ile His Ser 340 Lys Ser Ser Asn Gly Lys 345 350 355 360

<210> 18
 <211> 427
 <212> PRT
 <213> Homo sapiens

<400> 18
 Met His Leu Ile Asp Tyr Leu Leu Leu Leu Leu Val Gly Leu Leu Ala
 1 Ser His Gly Gln Leu His Val Glu His Asp Gly Glu Ser Cys Ser
 Asn Ser Ser 20 His Gln Gln Ile Leu 25 Glu Thr Gly Glu Gly Ser Pro Ser
 Leu Lys Ile Ala Pro Ala Asn Ala Asp Phe Ala Phe Arg Phe Tyr Tyr
 50 Ile Ala Ser Glu Thr Pro Gly Lys Asn Ile Phe Phe Ser Pro Leu
 65 Ser Ile Ser Ala Ala Tyr Ala Met Leu Ser 90 Leu Gly Ala Cys Ser His
 Ser Arg Ser Gln Ile Leu Glu Gly Leu Gly Phe Asn Leu Thr Glu Leu
 100 Ser Glu Ser Asp Val His Arg Gly Phe Gln His Leu Leu His Thr Leu
 115 Asn Leu Pro Gly His Gly Leu Glu Thr Arg Val Gly Ser Ala Leu Phe
 130 Leu Ser His Asn Leu Lys Phe Leu Ala Lys Phe Leu Asn Asp Thr Met
 145 Ala Val Tyr Glu Ala Lys Leu Phe His Thr Asn Phe Tyr Asp Thr Val
 Gly Thr Ile Gln Leu Ile Asn Asp His Val Lys Lys Glu Thr Arg Gly
 180 Lys Ile Val Asp Leu Val Ser Glu Leu Lys Lys Asp Val Leu Met Val
 195 Leu Val Asn Tyr Ile Tyr Phe Lys Ala Leu Trp Glu Lys Pro Phe Ile
 210 Ser Ser Arg Thr Thr Pro Lys Asp Phe Tyr Val Asp Glu Asn Thr Thr
 225 Val Arg Val Pro Met Met Leu Gln Asp Gln Glu His His Trp Tyr Leu
 245 His Asp Arg Tyr Leu Pro Cys Ser Val Leu Arg Met Asp Tyr Lys Gly
 260 Asp Ala Thr Val Phe Phe Ile Leu Pro Asn Gln Gly Lys Met Arg Glu
 275 Ile Glu Glu Val Leu Thr Pro Glu Met Leu Met Arg Trp Asn Asn Leu
 290 Leu Arg Lys Arg Asn Phe Tyr Lys Lys Leu Glu Leu His Leu Pro Lys
 305 Phe Ser Ile Ser Gly Ser Tyr Val Leu Asp Gln Ile Leu Pro Arg Leu
 325 Gly Phe Thr Asp Leu Phe Ser Lys Trp Ala Asp Leu Ser Gly Ile Thr
 340 Lys Gln Gln Lys Leu Glu Ala Ser Lys Ser Phe His Lys Ala Thr Leu
 355 Asp Val Asp Glu Ala Gly Thr Glu Ala Ala Ala Ala Thr Ser Phe Ala

ONCF_001_03WO_ST25.TXT

370
 Ile Lys Phe Phe Ser Ala 375 Gln Thr Asn Arg His 380 Ile Leu Arg Phe Asn
 385 390 Val Val Ile Phe Ser Thr Ser Thr Gln Ser Val Leu
 Arg Pro Phe Leu Val 405 Val Val Asp Pro Thr Lys Pro
 Phe Leu Gly Lys Val 420 Val Asp Pro Thr Lys Pro
 415

<210> 19
 <211> 375
 <212> PRT
 <213> Homo sapiens

<400> 19
 Met Asp Ala Leu Gln Leu Ala Asn Ser Ala Phe Ala Val Asp Leu Phe
 1 5 10 15
 Lys Gln Leu Cys Glu Lys Glu Pro Leu Gly Asn Val Leu Phe Ser Pro
 20 25 30
 Ile Cys Leu Ser Thr Ser Leu Ser Leu Ala Gln Val Gly Ala Lys Gly
 35 40 45
 Asp Thr Ala Asn Glu Ile Gly Gln Val Leu His Phe Glu Asn Val Lys
 50 55 60
 Asp Val Pro Phe Gly Phe Gln Thr Val Thr Ser Asp Val Asn Lys Leu
 65 70 75 80
 Ser Ser Phe Tyr Ser Leu Lys Leu Ile Lys Arg Leu Tyr Val Asp Lys
 85 90 95
 Ser Leu Asn Leu Ser Thr Glu Phe Ile Ser Ser Thr Lys Arg Pro Tyr
 100 105 110
 Ala Lys Glu Leu Glu Thr Val Asp Phe Lys Asp Lys Leu Glu Glu Thr
 115 120 125
 Lys Gly Gln Ile Asn Asn Ser Ile Lys Asp Leu Thr Asp Gly His Phe
 130 135 140
 Glu Asn Ile Leu Ala Asp Asn Ser Val Asn Asp Gln Thr Lys Ile Leu
 145 150 155 160
 Val Val Asn Ala Ala Tyr Phe Val Gly Lys Trp Met Lys Lys Phe Ser
 165 170 175
 Glu Ser Glu Thr Lys Glu Cys Pro Phe Arg Val Asn Lys Thr Asp Thr
 180 185 190
 Lys Pro Val Gln Met Met Asn Met Glu Ala Thr Phe Cys Met Gly Asn
 195 200 205
 Ile Asp Ser Ile Asn Cys Lys Ile Ile Glu Leu Pro Phe Gln Asn Lys
 210 215 220
 His Leu Ser Met Phe Ile Leu Leu Pro Lys Asp Val Glu Asp Glu Ser
 225 230 235 240
 Thr Gly Leu Glu Lys Ile Glu Lys Gln Leu Asn Ser Glu Ser Leu Ser
 245 250 255
 Gln Trp Thr Asn Pro Ser Thr Met Ala Asn Ala Lys Val Lys Leu Ser
 260 265 270
 Ile Pro Lys Phe Lys Val Glu Lys Met Ile Asp Pro Lys Ala Cys Leu
 275 280 285
 Glu Asn Leu Gly Leu Lys His Ile Phe Ser Glu Asp Thr Ser Asp Phe
 290 295 300
 Ser Gly Met Ser Glu Thr Lys Gly Val Ala Leu Ser Asn Val Ile His
 305 310 315 320
 Lys Val Cys Leu Glu Ile Thr Glu Asp Gly Gly Asp Ser Ile Glu Val
 325 330 335
 Pro Gly Ala Arg Ile Leu Gln His Lys Asp Glu Leu Asn Ala Asp His
 340 345 350
 Pro Phe Ile Tyr Ile Ile Arg His Asn Lys Thr Arg Asn Ile Ile Phe
 355 360 365
 Phe Gly Lys Phe Cys Ser Pro
 370 375

<210> 20
 <211> 837
 <212> PRT

<213> Homo sapiens

<400> 20

Met Leu Arg Thr Ala Met Gly Leu Arg Ser Trp Leu Ala Ala Pro Trp
 1 5 10 15
 Gly Ala Leu Pro Pro Arg Pro Pro Leu Leu Leu Leu Leu Leu
 20 25 30
 Leu Leu Leu Gln Pro Pro Pro Pro Thr Trp Ala Leu Ser Pro Arg Ile
 35 40 45
 Ser Leu Pro Leu Gly Ser Glu Glu Arg Pro Phe Leu Arg Phe Glu Ala
 50 55 60
 Glu His Ile Ser Asn Tyr Thr Ala Leu Leu Leu Ser Arg Asp Gly Arg
 65 70 75 80
 Thr Leu Tyr Val Gly Ala Arg Glu Ala Leu Phe Ala Leu Ser Ser Asn
 85 90 95
 Leu Ser Phe Leu Pro Gly Gly Glu Tyr Gln Glu Leu Leu Trp Gly Ala
 100 105 110
 Asp Ala Glu Lys Lys Gln Gln Cys Ser Phe Lys Gly Lys Asp Pro Gln
 115 120 125
 Arg Asp Cys Gln Asn Tyr Ile Lys Ile Leu Leu Pro Leu Ser Gly Ser
 130 135 140
 His Leu Phe Thr Cys Gly Thr Ala Ala Phe Ser Pro Met Cys Thr Tyr
 145 150 155 160
 Ile Asn Met Glu Asn Phe Thr Leu Ala Arg Asp Glu Lys Gly Asn Val
 165 170 175
 Leu Leu Glu Asp Gly Lys Gly Arg Cys Pro Phe Asp Pro Asn Phe Lys
 180 185 190
 Ser Thr Ala Leu Val Val Asp Gly Glu Leu Tyr Thr Gly Thr Val Ser
 195 200 205
 Ser Phe Gln Gly Asn Asp Pro Ala Ile Ser Arg Ser Gln Ser Leu Arg
 210 215 220
 Pro Thr Lys Thr Glu Ser Ser Leu Asn Trp Leu Gln Asp Pro Ala Phe
 225 230 235 240
 Val Ala Ser Ala Tyr Ile Pro Glu Ser Leu Gly Ser Leu Gln Gly Asp
 245 250 255
 Asp Asp Lys Ile Tyr Phe Phe Phe Ser Glu Thr Gly Gln Glu Phe Glu
 260 265 270
 Phe Phe Glu Asn Thr Ile Val Ser Arg Ile Ala Arg Ile Cys Lys Gly
 275 280 285
 Asp Glu Gly Gly Glu Arg Val Leu Gln Gln Arg Trp Thr Ser Phe Leu
 290 295 300
 Lys Ala Gln Leu Leu Cys Ser Arg Pro Asp Asp Gly Phe Pro Phe Asn
 305 310 315 320
 Val Leu Gln Asp Val Phe Thr Leu Ser Pro Ser Pro Gln Asp Trp Arg
 325 330 335
 Asp Thr Leu Phe Tyr Gly Val Phe Thr Ser Gln Trp His Arg Gly Thr
 340 345 350
 Thr Glu Gly Ser Ala Val Cys Val Phe Thr Met Lys Asp Val Gln Arg
 355 360 365
 Val Phe Ser Gly Leu Tyr Lys Glu Val Asn Arg Glu Thr Gln Gln Trp
 370 375 380
 Tyr Thr Val Thr His Pro Val Pro Thr Pro Arg Pro Gly Ala Cys Ile
 385 390 395 400
 Thr Asn Ser Ala Arg Glu Arg Lys Ile Asn Ser Ser Leu Gln Leu Pro
 405 410 415
 Asp Arg Val Leu Asn Phe Leu Lys Asp His Phe Leu Met Asp Gly Gln
 420 425 430
 Val Arg Ser Arg Met Leu Leu Leu Gln Pro Gln Ala Arg Tyr Gln Arg
 435 440 445
 Val Ala Val His Arg Val Pro Gly Leu His His Thr Tyr Asp Val Leu
 450 455 460
 Phe Leu Gly Thr Gly Asp Gly Arg Leu His Lys Ala Val Ser Val Gly
 465 470 475 480
 Pro Arg Val His Ile Ile Glu Glu Leu Gln Ile Phe Ser Ser Gly Gln
 485 490 495
 Pro Val Gln Asn Leu Leu Leu Asp Thr His Arg Gly Leu Leu Tyr Ala
 500 505 510
 Ala Ser His Ser Gly Val Val Gln Val Pro Met Ala Asn Cys Ser Leu

ONCF_001_03WO_ST25.TXT

Tyr Arg 515
 530 Ser Cys Gly Asp Cys 520
 535 Leu Leu Ala Arg Asp 525
 540 Pro Tyr Cys Ala
 Trp Ser Gly Ser Ser Cys Lys His Val Ser Leu Tyr Gln Pro Gln Leu
 545 550 555 560
 Ala Thr Arg Pro Trp Ile Gln Asp Ile Glu Gly Ala Ser Ala Lys Asp
 565 570 575
 Leu Cys Ser Ala Ser Ser Val Val Ser Pro Ser Phe Val Pro Thr Gly
 580 585 590
 Glu Lys Pro Cys Glu Gln Val Gln Phe Gln Pro Asn Thr Val Asn Thr
 595 600 605
 Leu Ala Cys Pro Leu Leu Ser Asn Leu Ala Thr Arg Leu Trp Leu Arg
 610 615 620
 Asn Gly Ala Pro Val Asn Ala Ser Ala Ser Cys His Val Leu Pro Thr
 625 630 635 640
 Gly Asp Leu Leu Leu Val Gly Thr Gln Gln Leu Gly Glu Phe Gln Cys
 645 650 655
 Trp Ser Leu Glu Glu Gly Phe Gln Gln Leu Val Ala Ser Tyr Cys Pro
 660 665 670
 Glu Val Val Glu Asp Gly Val Ala Asp Gln Thr Asp Glu Gly Gly Ser
 675 680 685
 Val Pro Val Ile Ile Ser Thr Ser Arg Val Ser Ala Pro Ala Gly Gly
 690 695 700
 Lys Ala Ser Trp Gly Ala Asp Arg Ser Tyr Trp Lys Glu Phe Leu Val
 705 710 715 720
 Met Cys Thr Leu Phe Val Leu Ala Val Leu Leu Pro Val Leu Phe Leu
 725 730 735
 Leu Tyr Arg His Arg Asn Ser Met Lys Val Phe Leu Lys Gln Gly Glu
 740 745 750
 Cys Ala Ser Val His Pro Lys Thr Cys Pro Val Val Leu Pro Pro Glu
 755 760 765
 Thr Arg Pro Leu Asn Gly Leu Gly Pro Pro Ser Thr Pro Leu Asp His
 770 775 780
 Arg Gly Tyr Gln Ser Leu Ser Asp Ser Pro Pro Gly Ser Arg Val Phe
 785 790 795 800
 Thr Glu Ser Glu Lys Arg Pro Leu Ser Ile Gln Asp Ser Phe Val Glu
 805 810 815
 Val Ser Pro Val Cys Pro Arg Pro Arg Val Arg Leu Gly Ser Glu Ile
 820 825 830
 Arg Asp Ser Val Val
 835

<210> 21
 <211> 862
 <212> PRT
 <213> Homo sapiens

<400> 21
 Met Arg Met Cys Thr Pro Ile Arg Gly Leu Leu Met Ala Leu Ala Val
 1 5 10 15
 Met Phe Gly Thr Ala Met Ala Phe Ala Pro Ile Pro Arg Ile Thr Trp
 20 25 30
 Glu His Arg Glu Val His Leu Val Gln Phe His Glu Pro Asp Ile Tyr
 35 40 45
 Asn Tyr Ser Ala Leu Leu Leu Ser Glu Asp Lys Asp Thr Leu Tyr Ile
 50 55 60
 Gly Ala Arg Glu Ala Val Phe Ala Val Asn Ala Leu Asn Ile Ser Glu
 65 70 75 80
 Lys Gln His Glu Val Tyr Trp Lys Val Ser Glu Asp Lys Lys Ala Lys
 85 90 95
 Cys Ala Glu Lys Gly Lys Ser Lys Gln Thr Glu Cys Leu Asn Tyr Ile
 100 105 110
 Arg Val Leu Gln Pro Leu Ser Ala Thr Ser Leu Tyr Val Cys Gly Thr
 115 120 125
 Asn Ala Phe Gln Pro Ala Cys Asp His Leu Asn Leu Thr Ser Phe Lys
 130 135 140
 Phe Leu Gly Lys Asn Glu Asp Gly Lys Gly Arg Cys Pro Phe Asp Pro

ONCF_001_03wo_ST25.TXT

145	Ala	His	Ser	Tyr	Thr	150	Ser	Val	Met	Val	155	Asp	Gly	Glu	Leu	Tyr	Ser	160	Gly
					165						170							175	
	Thr	Ser	Tyr	Asn	Phe	Leu	Gly	Ser	Glu	Pro	Ile	Ile	Ser	Arg	Asn	Ser			
				180							185							190	
	Ser	His	Ser	Pro	Leu	Arg	Thr	Glu	Tyr	Ala	Ile	Pro	Trp	Leu	Asn	Glu			
			195					200					205						
	Pro	Ser	Phe	Val	Phe	Ala	Asp	Val	Ile	Arg	Lys	Ser	Pro	Asp	Ser	Pro			
							215					220							
	Asp	Gly	Glu	Asp	Asp	Arg	Val	Tyr	Phe	Phe	Phe	Thr	Glu	Val	Ser	Val			
						230					235								240
	Glu	Tyr	Glu	Phe	Val	Phe	Arg	Val	Leu	Ile	Pro	Arg	Ile	Ala	Arg	Val			
					245						250								255
	Cys	Lys	Gly	Asp	Gln	Gly	Gly	Leu	Arg	Thr	Leu	Gln	Lys	Lys	Trp	Thr			
				260					265										
	Ser	Phe	Leu	Lys	Ala	Arg	Leu	Ile	Cys	Ser	Arg	Pro	Asp	Ser	Gly	Leu			
								280					285						
	Val	Phe	Asn	Val	Leu	Arg	Asp	Val	Phe	Val	Leu	Arg	Ser	Pro	Gly	Leu			
							295					300							
	Lys	Val	Pro	Val	Phe	Tyr	Ala	Leu	Phe	Thr	Pro	Gln	Leu	Asn	Asn	Val			
						310						315							320
	Gly	Leu	Ser	Ala	Val	Cys	Ala	Tyr	Asn	Leu	Ser	Thr	Ala	Glu	Glu	Val			
						325					330								335
	Phe	Ser	His	Gly	Lys	Tyr	Met	Gln	Ser	Thr	Thr	Val	Glu	Gln	Ser	His			
						340													
	Thr	Lys	Trp	Val	Arg	Tyr	Asn	Gly	Pro	Val	Pro	Lys	Pro	Arg	Pro	Gly			
								360											
	Ala	Cys	Ile	Asp	Ser	Glu	Ala	Arg	Ala	Ala	Asn	Tyr	Thr	Ser	Ser	Leu			
							375					380							
	Asn	Leu	Pro	Asp	Lys	Thr	Leu	Gln	Phe	Val	Lys	Asp	His	Pro	Leu	Met			
						390						395							400
	Asp	Asp	Ser	Val	Thr	Pro	Ile	Asp	Asn	Arg	Pro	Arg	Leu	Ile	Lys	Lys			
					405						410								
	Asp	Val	Asn	Tyr	Thr	Gln	Ile	Val	Val	Asp	Arg	Thr	Gln	Ala	Leu	Asp			
					420														
	Gly	Thr	Val	Tyr	Asp	Val	Met	Phe	Val	Ser	Thr	Asp	Arg	Gly	Ala	Leu			
								440						445					
	His	Lys	Ala	Ile	Ser	Leu	Glu	His	Ala	Val	His	Ile	Ile	Glu	Glu	Thr			
							455					460							
	Gln	Leu	Phe	Gln	Asp	Phe	Glu	Pro	Val	Gln	Thr	Leu	Leu	Leu	Ser	Ser			
						470						475							480
	Lys	Lys	Gly	Asn	Arg	Phe	Val	Tyr	Ala	Gly	Ser	Asn	Ser	Gly	Val	Val			
						485													
	Gln	Ala	Pro	Leu	Ala	Phe	Cys	Gly	Lys	His	Gly	Thr	Cys	Glu	Asp	Cys			
									505										
	Val	Leu	Ala	Arg	Asp	Pro	Tyr	Cys	Ala	Trp	Ser	Pro	Pro	Thr	Ala	Thr			
								520						525					
	Cys	Val	Ala	Leu	His	Gln	Thr	Glu	Ser	Pro	Ser	Arg	Gly	Leu	Ile	Gln			
							535							540					
	Glu	Met	Ser	Gly	Asp	Ala	Ser	Val	Cys	Pro	Asp	Lys	Ser	Lys	Gly	Ser			
						550						555							
	Tyr	Arg	Gln	His	Phe	Phe	Lys	His	Gly	Gly	Thr	Ala	Glu	Leu	Lys	Cys			
						565						570							
	Ser	Gln	Lys	Ser	Asn	Leu	Ala	Arg	Val	Phe	Trp	Lys	Phe	Gln	Asn	Gly			
									585										
	Val	Leu	Lys	Ala	Glu	Ser	Pro	Lys	Tyr	Gly	Leu	Met	Gly	Arg	Lys	Asn			
									600										
	Leu	Leu	Ile	Phe	Asn	Leu	Ser	Glu	Gly	Asp	Ser	Gly	Val	Tyr	Gln	Cys			
							615						620						
	Leu	Ser	Glu	Glu	Arg	Val	Lys	Asn	Lys	Thr	Val	Phe	Gln	Val	Val	Ala			
						630													640
	Lys	His	Val	Leu	Glu	Val	Lys	Val	Val	Pro	Lys	Pro	Val	Val	Ala	Pro			
						645													
	Thr	Leu	Ser	Val	Val	Gln	Thr	Glu	Gly	Ser	Arg	Ile	Ala	Thr	Lys	Val			
									665										
	Leu	Val	Ala	Ser	Thr	Gln	Gly	Ser	Ser	Pro	Pro	Thr	Pro	Ala	Val	Gln			
									680										
	Ala	Thr	Ser	Ser	Gly	Ala	Ile	Thr	Leu	Pro	Pro	Lys	Pro	Ala	Pro	Thr			

ONCF_001_03WO_ST25.TXT

690 695 700
 Gly Thr Ser Cys Glu Pro Lys Ile Val Ile Asn Thr Val Pro Gln Leu
 705 710 715 720
 His Ser Glu Lys Thr Met Tyr Leu Lys Ser Ser Asp Asn Arg Leu Leu
 725 730 735
 Met Ser Leu Phe Leu Phe Phe Phe Val Leu Phe Leu Cys Leu Phe Phe
 740 745 750
 Tyr Asn Cys Tyr Lys Gly Tyr Leu Pro Arg Gln Cys Leu Lys Phe Arg
 755 760 765
 Ser Ala Leu Leu Ile Gly Lys Lys Lys Pro Lys Ser Asp Phe Cys Asp
 770 775 780
 Arg Glu Gln Ser Leu Lys Glu Thr Leu Val Glu Pro Gly Ser Phe Ser
 785 790 795 800
 Gln Gln Asn Gly Glu His Pro Lys Pro Ala Leu Asp Thr Gly Tyr Glu
 805 810 815
 Thr Glu Gln Asp Thr Ile Thr Ser Lys Val Pro Thr Asp Arg Glu Asp
 820 825 830
 Ser Gln Arg Ile Asp Asp Leu Ser Ala Arg Asp Lys Pro Phe Asp Val
 835 840 845
 Lys Cys Glu Leu Lys Phe Ala Asp Ser Asp Ala Asp Gly Asp
 850 855 860

<210> 22
 <211> 93
 <212> PRT
 <213> Homo sapiens

<400> 22
 Met Lys Ile Ser Val Ala Ala Ile Pro Phe Phe Leu Leu Ile Thr Ile
 1 5 10 15
 Ala Leu Gly Thr Lys Thr Glu Ser Ser Arg Gly Pro Tyr His Pro
 20 25 30
 Ser Glu Cys Cys Phe Thr Tyr Thr Tyr Lys Ile Pro Arg Gln Arg
 35 40 45
 Ile Met Asp Tyr Tyr Glu Thr Asn Ser Gln Cys Ser Lys Pro Gly Ile
 50 55 60
 Val Phe Ile Thr Lys Arg Gly His Ser Val Cys Thr Asn Pro Ser Asp
 65 70 75 80
 Lys Trp Val Gln Asp Tyr Ile Lys Asp Met Lys Glu Asn
 85 90

<210> 23
 <211> 1462
 <212> PRT
 <213> Homo sapiens

<400> 23
 Met Gly Ala Gln Asp Arg Pro Gln Cys His Phe Asp Ile Glu Ile Asn
 1 5 10 15
 Arg Glu Pro Val Gly Arg Ile Met Phe Gln Leu Phe Ser Asp Ile Cys
 20 25 30
 Pro Lys Thr Cys Lys Asn Phe Leu Cys Leu Cys Ser Gly Glu Lys Gly
 35 40 45
 Leu Gly Lys Thr Thr Gly Lys Lys Leu Cys Tyr Lys Gly Ser Thr Phe
 50 55 60
 His Arg Val Val Lys Asn Phe Met Ile Gln Gly Gly Asp Phe Ser Glu
 65 70 75 80
 Gly Asn Gly Lys Gly Glu Ser Ile Tyr Gly Gly Tyr Phe Lys Asp
 85 90 95
 Glu Asn Phe Ile Leu Lys His Asp Arg Ala Phe Leu Leu Ser Met Ala
 100 105 110
 Asn Arg Gly Lys His Thr Asn Gly Ser Gln Phe Phe Ile Thr Thr Lys
 115 120 125
 Pro Ala Pro His Leu Asp Gly Val His Val Val Phe Gly Leu Val Ile
 130 135 140
 Ser Gly Phe Glu Val Ile Glu Gln Ile Glu Asn Leu Lys Thr Asp Ala

ONCF_001_03wo_ST25.TXT

145	Ala	Ser	Arg	Pro	Tyr	150	Ala	Asp	Val	Arg	Val	155	Ile	Asp	Cys	Gly	Val	160	Leu
					165							170						175	
	Ala	Thr	Lys	Ser	Ile	Lys	Asp	Val	Phe	Glu	Lys	Lys	Arg	Lys	Lys	Arg	Lys	Lys	Pro
				180					185								190		
	Thr	His	Ser	Glu	Gly	Ser	Asp	Ser	Ser	Ser	Asn	Ser							
			195					200						205					
	Glu	Ser	Ser	Ser	Glu	Ser	Glu	Leu	Glu	His	Glu	Arg	Ser	Lys	Lys	Arg	Arg	Arg	Arg
			210					215					220						
	Lys	His	Lys	Arg	Arg	Pro	Lys	Val	Lys	Arg	Ser	Lys	Lys	Arg	Arg	Arg	Lys	Lys	Pro
			225			230						235							240
	Glu	Ala	Ser	Ser	Ser	Glu	Glu	Pro	Arg	Asn	Lys	His	Ala	Met	Asn	Pro	Pro	Pro	Pro
				245						250					255				
	Lys	Gly	His	Ser	Glu	Arg	Ser	Asp	Thr	Asn	Glu	Lys	Arg	Ser	Val	Asp	Val	Val	Val
				260					265						270				
	Ser	Ser	Ala	Lys	Arg	Glu	Lys	Pro	Val	Val	Arg	Pro	Glu	Glu	Ile	Pro	Pro	Pro	Pro
			275					280					285						
	Pro	Val	Pro	Glu	Asn	Arg	Phe	Leu	Leu	Arg	Arg	Asp	Met	Pro	Val	Val	Val	Val	Val
							295					300							
	Thr	Ala	Glu	Pro	Glu	Pro	Lys	Ile	Pro	Asp	Val	Ala	Pro	Ile	Val	Ser	Ser	Ser	Ser
						310					315					320			
	Asp	Gln	Lys	Pro	Ser	Val	Ser	Lys	Ser	Gly	Arg	Lys	Ile	Lys	Gly	Arg	Arg	Arg	Arg
					325					330					335				
	Gly	Thr	Ile	Arg	Tyr	His	Thr	Pro	Pro	Arg	Ser	Arg	Ser	Cys	Ser	Glu	Glu	Glu	Glu
				340					345					350					
	Ser	Asp	Asp	Asp	Ser	Ser	Ser	Glu	Thr	Pro	Pro	His	Trp	Lys	Glu	Glu	Trp	Ser	Ser
			355					360					365						
	Met	Gln	Arg	Leu	Arg	Ala	Tyr	Arg	Pro	Pro	Ser	Gly	Glu	Lys	Trp	Ser	Ser	Ser	Ser
						375						380							
	Lys	Gly	Asp	Lys	Leu	Ser	Asp	Pro	Cys	Ser	Ser	Arg	Trp	Asp	Glu	Arg	Arg	Arg	Arg
						390						395							400
	Ser	Leu	Ser	Gln	Arg	Ser	Arg	Ser	Trp	Ser	Tyr	Asn	Gly	Tyr	Tyr	Ser	Ser	Ser	Ser
				405						410					415				
	Asp	Leu	Ser	Thr	Ala	Arg	His	Ser	Gly	His	His	Lys	Lys	Arg	Arg	Lys	Arg	Arg	Lys
				420					425						430				
	Glu	Lys	Lys	Val	Lys	His	Lys	Lys	Lys	Gly	Lys	Lys	Gln	Lys	His	Cys	Cys	Cys	Cys
				435					440					445					
	Arg	Arg	His	Lys	Gln	Thr	Lys	Lys	Arg	Arg	Ile	Leu	Ile	Pro	Ser	Asp	Asp	Asp	Asp
						455						460							
	Ile	Glu	Ser	Ser	Lys	Ser	Ser	Thr	Arg	Arg	Met	Lys	Ser	Ser	Cys	Asp	Asp	Asp	Asp
						470					475					480			
	Arg	Glu	Arg	Ser	Ser	Arg	Ser	Ser	Ser	Leu	Ser	Ser	His	His	Ser	Ser	Ser	Ser	Ser
						485					490					495			
	Lys	Arg	Asp	Trp	Ser	Lys	Ser	Asp	Lys	Asp	Val	Gln	Ser	Ser	Leu	Thr	Thr	Thr	Thr
				500					505						510				
	His	Ser	Ser	Arg	Asp	Ser	Tyr	Arg	Ser	Lys	Ser	His	Ser	Gln	Ser	Tyr	Tyr	Tyr	Tyr
								520					525						
	Ser	Arg	Gly	Ser	Ser	Arg	Ser	Arg	Thr	Ala	Ser	Lys	Ser	Ser	Ser	His	His	His	His
								535					540						
	Ser	Arg	Ser	Arg	Ser	Lys	Ser	Arg	Ser	Ser	Ser	Lys	Ser	Gly	His	Arg	Arg	Arg	Arg
						550						555				560			
	Lys	Arg	Ala	Ser	Lys	Ser	Pro	Arg	Lys	Thr	Ala	Ser	Gln	Leu	Ser	Glu	Glu	Glu	Glu
						565									575				
	Asn	Lys	Pro	Val	Lys	Thr	Glu	Pro	Leu	Arg	Ala	Thr	Met	Ala	Gln	Asn	Asn	Asn	Asn
				580					585						590				
	Glu	Asn	Val	Val	Val	Gln	Pro	Val	Val	Ala	Glu	Asn	Ile	Pro	Val	Ile	Ile	Ile	Ile
								600						605					
	Pro	Leu	Ser	Asp	Ser	Pro	Pro	Pro	Ser	Arg	Trp	Lys	Pro	Gly	Gln	Lys	Lys	Lys	Lys
								615					620						
	Pro	Trp	Lys	Pro	Ser	Tyr	Glu	Arg	Ile	Gln	Glu	Met	Lys	Ala	Lys	Thr	Thr	Thr	Thr
						630						635				640			
	Thr	His	Leu	Leu	Pro	Ile	Gln	Ser	Thr	Tyr	Ser	Leu	Ala	Asn	Ile	Lys	Lys	Lys	Lys
					645							650				655			
	Glu	Thr	Gly	Ser	Ser	Ser	Ser	Tyr	His	Lys	Arg	Glu	Lys	Asn	Ser	Glu	Ser	Ser	Ser
									665						670				
	Ser	Asp	Gln	Ser	Thr	Tyr	Ser	Lys	Tyr	Ser	Asp	Arg	Ser	Ser	Glu	Ser	Ser	Ser	Ser
																685			
	Ser	Pro	Arg	Ser	Arg	Ser	Arg	Ser	Ser	Arg	Ser	Arg	Ser	Tyr	Ser	Arg	Arg	Arg	Arg

ONCF_001_03wo_ST25.TXT

690 695 700
 Ser Tyr Thr Arg Ser Arg Ser Leu Ala Ser Ser His Ser Arg Ser Arg
 705 Ser Pro Ser Ser Arg Ser His Ser Arg Asn Lys Tyr Ser Asp His Ser
 725 730 735
 Gln Cys Ser Arg Ser Ser Ser Tyr Thr Ser Ile Ser Ser Asp Asp Gly
 740 745 750
 Arg Arg Ala Lys Arg Arg Leu Arg Ser Ser Gly Lys Lys Asn Ser Val
 755 760 765
 Ser His Lys Lys His Ser Ser Ser Ser Glu Lys Thr Leu His Ser Lys
 770 775 780
 Tyr Val Lys Gly Arg Asp Arg Ser Ser Cys Val Arg Lys Tyr Ser Glu
 785 Ser Arg Ser Ser Leu Asp Tyr Ser Ser Asp Ser Glu Gln Ser Ser Val
 805 810 815
 Gln Ala Thr Gln Ser Ala Gln Glu Lys Glu Lys Gln Gly Gln Met Glu
 820 825 830
 Arg Thr His Asn Lys Gln Glu Lys Asn Arg Gly Glu Glu Lys Ser Lys
 835 840 845
 Ser Glu Arg Glu Cys Pro His Ser Lys Lys Arg Thr Leu Lys Glu Asn
 850 855 860
 Leu Ser Asp His Leu Arg Asn Gly Ser Lys Pro Lys Arg Lys Asn Tyr
 865 Ala Gly Ser Lys Trp Asp Ser Glu Ser Asn Ser Glu Arg Asp Val Thr
 885 890 895
 Lys Asn Ser Lys Asn Asp Ser His Pro Ser Ser Asp Lys Glu Glu Gly
 900 905 910
 Glu Ala Thr Ser Asp Ser Glu Ser Glu Val Ser Glu Ile His Ile Lys
 915 920 925
 Val Lys Pro Thr Thr Lys Ser Ser Thr Asn Thr Ser Leu Pro Asp Asp
 930 935 940
 Asn Gly Ala Trp Lys Ser Ser Lys Gln Arg Thr Ser Thr Ser Asp Ser
 945 Glu Gly ser Cys Ser Asn Ser Glu Asn Asn Arg Gly Lys Pro Gln Lys
 965 970 975
 His Lys His Gly Ser Lys Glu Asn Leu Lys Arg Glu His Thr Lys Lys
 980 985 990
 Val Lys Glu Lys Leu Lys Gly Lys Lys Asp Lys Lys His Lys Ala Pro
 995 1000 1005
 Lys Arg Lys Gln Ala Phe His Trp Gln Pro Pro Leu Glu Phe Gly Glu
 1010 1015 1020
 Glu Glu Glu Glu Glu Ile Asp Asp Lys Gln Val Thr Gln Glu Ser Lys
 1025 Glu Lys Lys Val Ser Glu Asn Asn Glu Thr Ile Lys Asp Asn Ile Leu
 1030 1035 1040
 1045 1050 1055
 Lys Thr Glu Lys Ser Ser Glu Glu Asp Leu Ser Gly Lys His Asp Thr
 1060 1065 1070
 Val Thr Val Ser Ser Asp Leu Asp Gln Phe Thr Lys Asp Asp Ser Lys
 1075 1080 1085
 Leu Ser Ile Ser Pro Thr Ala Leu Asn Thr Glu Glu Asn Val Ala Cys
 1090 1095 1100
 Leu Gln Asn Ile Gln His Val Glu Glu Ser Val Pro Asn Gly Val Glu
 1105 1110 1115 1120
 Asp Val Leu Gln Thr Asp Asp Asn Met Glu Ile Cys Thr Pro Asp Arg
 1125 1130 1135
 Ser Ser Pro Ala Lys Val Glu Glu Thr Ser Pro Leu Gly Asn Ala Arg
 1140 1145 1150
 Leu Asp Thr Pro Asp Ile Asn Ile Val Leu Lys Gln Asp Met Ala Thr
 1155 1160 1165
 Glu His Pro Gln Ala Glu Val Val Lys Gln Glu Ser Ser Met Ser Glu
 1170 1175 1180
 Ser Lys Val Leu Gly Glu Val Gly Lys Gln Asp Ser Ser Ser Ala Ser
 1185 1190 1195 1200
 Leu Ala Ser Ala Gly Glu Ser Thr Gly Lys Lys Glu Val Ala Glu Lys
 1205 1210 1215
 Ser Gln Ile Asn Leu Ile Asp Lys Lys Trp Lys Pro Leu Gln Gly Val
 1220 1225 1230
 Gly Asn Leu Ala Ala Pro Asn Ala Ala Thr Ser Ser Ala Val Glu Val

ONCF_001_03WO_ST25.TXT

1235
 Lys Val Leu Thr Thr Val Pro Glu Met Lys Pro Gln Gly Leu Arg Ile
 1250 1240 1245
 Glu Ile Lys Ser Lys Asn Lys Val Arg Pro Gly Ser Leu Phe Asp Glu
 1265 1270 1275 1280
 Val Arg Lys Thr Ala Arg Leu Asn Arg Arg Pro Arg Asn Gln Glu Ser
 1285 1290 1295
 Ser Ser Asp Glu Gln Thr Pro Ser Arg Asp Asp Ser Gln Ser Arg
 1300 1305 1310
 Ser Pro Ser Arg Ser Arg Ser Lys Ser Glu Thr Lys Ser Arg His Arg
 1315 1320 1325
 Thr Arg Ser Val Ser Tyr Ser His Ser Arg Ser Arg Ser Arg Ser Ser
 1330 1335 1340
 Thr Ser Ser Tyr Arg Ser Arg Ser Tyr Ser Arg Ser Arg Ser Arg Gly
 1345 1350 1355 1360
 Trp Tyr Ser Arg Gly Arg Thr Arg Ser Arg Ser Ser Ser Tyr Arg Ser
 1365 1370 1375
 Tyr Lys Ser His Arg Thr Ser Ser Arg Ser Arg Ser Arg Ser Ser Ser
 1380 1385 1390
 Tyr Asp Pro His Ser Arg Ser Arg Ser Tyr Thr Tyr Asp Ser Tyr Tyr
 1395 1400 1405
 Ser Arg Ser Arg Ser Arg Ser Arg Ser Gln Arg Ser Asp Ser Tyr His
 1410 1415 1420
 Arg Gly Arg Ser Tyr Asn Arg Arg Ser Arg Ser Cys Arg Ser Tyr Gly
 1425 1430 1435 1440
 Ser Asp Ser Glu Ser Asp Arg Ser Tyr Ser His His Arg Ser Pro Ser
 1445 1450 1455
 Glu ser ser Arg Tyr Ser
 1460

<210> 24
 <211> 375
 <212> PRT
 <213> Homo sapiens

<400> 24
 Met Leu Leu Phe Leu Leu Ser Ala Leu Val Leu Leu Thr Gln Pro Leu
 1 5 10 15
 Gly Tyr Leu Glu Ala Glu Met Lys Thr Tyr Ser His Arg Thr Met Pro
 20 25 30
 Ser Ala Cys Thr Leu Val Met Cys Ser Ser Val Glu Ser Gly Leu Pro
 35 40 45
 Gly Arg Asp Gly Arg Asp Gly Arg Glu Gly Pro Arg Gly Glu Lys Gly
 50 55 60
 Asp Pro Gly Leu Pro Gly Ala Ala Gly Gln Ala Gly Met Pro Gly Gln
 65 70 75 80
 Ala Gly Pro Val Gly Pro Lys Gly Asp Asn Gly Ser Val Gly Glu Pro
 85 90 95
 Gly Pro Lys Gly Asp Thr Gly Pro Ser Gly Pro Pro Gly Pro Pro Gly
 100 105 110
 Val Pro Gly Pro Ala Gly Arg Glu Gly Pro Leu Gly Lys Gln Gly Asn
 115 120 125
 Ile Gly Pro Gln Gly Lys Pro Gly Pro Lys Gly Glu Ala Gly Pro Lys
 130 135 140
 Gly Glu Val Gly Ala Pro Gly Met Gln Gly Ser Ala Gly Ala Arg Gly
 145 150 155 160
 Leu Ala Gly Pro Lys Gly Glu Arg Gly Val Pro Gly Glu Arg Gly Val
 165 170 175
 Pro Gly Asn Thr Gly Ala Ala Gly Ser Ala Gly Ala Met Gly Pro Gln
 180 185 190
 Gly Ser Pro Gly Ala Arg Gly Pro Pro Gly Leu Lys Gly Asp Lys Gly
 195 200 205
 Ile Pro Gly Asp Lys Gly Ala Lys Gly Glu Ser Gly Leu Pro Asp Val
 210 215 220
 Ala Ser Leu Arg Gln Gln Val Glu Ala Leu Gln Gly Gln Val Gln His
 225 230 235 240
 Leu Gln Ala Ala Phe Ser Gln Tyr Lys Lys Val Glu Leu Phe Pro Asn

ONCF_001_03wo_ST25.TXT

				245					250					255		
Gly	Gln	Ser	Val	Gly	Glu	Lys	Ile	Phe	Lys	Thr	Ala	Gly	Phe	Val	Lys	
			260					265					270			
Pro	Phe	Thr	Glu	Ala	Gln	Leu	Leu	Cys	Thr	Gln	Ala	Gly	Gly	Gln	Leu	
		275					280					285				
Ala	Ser	Pro	Arg	Ser	Ala	Ala	Glu	Asn	Ala	Ala	Leu	Gln	Gln	Leu	Val	
	290				295					300						
Val	Ala	Lys	Asn	Glu	Ala	Ala	Phe	Leu	Ser	Met	Thr	Asp	Ser	Lys	Thr	
305				310						315					320	
Glu	Gly	Lys	Phe	Thr	Tyr	Pro	Thr	Gly	Glu	Ser	Leu	Val	Tyr	Ser	Asn	
				325					330					335		
Trp	Ala	Pro	Gly	Glu	Pro	Asn	Asp	Asp	Gly	Gly	Ser	Glu	Asp	Cys	Val	
			340				345						350			
Glu	Ile	Phe	Thr	Asn	Gly	Lys	Trp	Asn	Asp	Arg	Ala	Cys	Gly	Glu	Lys	
		355					360					365				
Arg	Leu	Val	Val	Cys	Glu	Phe										
	370					375										