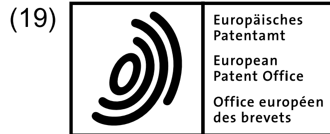
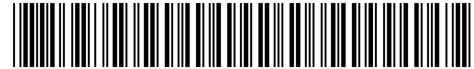
(19) 



(11) **EP 1 183 348 B1**

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
29.06.2011 Bulletin 2011/26

(21) Application number: **00919352.5**

(22) Date of filing: **15.02.2000**

(51) Int Cl.:
C12N 15/12 *(2006.01)* ***C12N 15/52*** *(2006.01)*
C07K 14/47 *(2006.01)* ***C12N 9/00*** *(2006.01)*
C12N 5/10 *(2006.01)* ***C12N 1/21*** *(2006.01)*
A61K 38/17 *(2006.01)* ***A61K 39/00*** *(2006.01)*
C07K 16/30 *(2006.01)* ***C07K 16/32*** *(2006.01)*
C07K 16/40 *(2006.01)* ***A61K 39/395*** *(2006.01)*
A61K 35/12 *(2006.01)* ***C07K 19/00*** *(2006.01)*
C12N 15/62 *(2006.01)* ***C12N 5/00*** *(2006.01)*
G01N 33/68 *(2006.01)* ***C12Q 1/68*** *(2006.01)*
C12N 15/11 *(2006.01)*

(86) International application number:
PCT/US2000/005308

(87) International publication number:
WO 2000/060076 (12.10.2000 Gazette 2000/41)

(54) **COMPOSITIONS FOR THE TREATMENT AND DIAGNOSIS OF BREAST CANCER AND METHODS FOR THEIR USE**

ZUSAMMENSETZUNGEN FÜR DIE THERAPIE UND DIAGNOSE VON BRUSTKREBS UND VERWENDUNG DAVON

COMPOSITIONS POUR LE TRAITEMENT ET LE DIAGNOSTIC DU CANCER DU SEIN ET LEURS PROCEDES D'UTILISATION

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

(30) Priority: **02.04.1999 US 285480**
23.06.1999 US 339338
02.09.1999 US 389681
03.11.1999 US 433826

(43) Date of publication of application:
06.03.2002 Bulletin 2002/10

(73) Proprietor: **CORIXA CORPORATION**
Wilmington, DE 19808 (US)

(72) Inventors:
- **YUQIU, Jiang**
  Kent, WA 98032 (US)
- **DILLON, Davin, C.**
  Redmond, WA 98053 (US)
- **MITCHAM, Jennifer, Lynn**
  Redmond, WA 98052 (US)
- **XU, Jiangchun**
  Bellevue, WA 98006 (US)
- **HARLOCKER, Susan, L.**
  Seattle, WA 98104 (US)

(74) Representative: **Teuten, Andrew John et al**
Sagittarius IP
Taylor House
39 High Street
Marlow, Bucks SL7 1AF (GB)

(56) References cited:
WO-A-00/43420 **WO-A-96/29430**
WO-A-98/33915 **WO-A-99/09155**

EP 1 183 348 B1

Note: Within nine months of the publication of the mention of the grant of the European patent in the European Patent Bulletin, any person may give notice to the European Patent Office of opposition to that patent, in accordance with the Implementing Regulations. Notice of opposition shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

Description

TECHNICAL FIELD

[0001] The present invention relates generally to compositions and methods for the treatment of breast cancer. The invention is more particularly related to polypeptides comprising at least a portion of a protein that is preferentially expressed in breast tumor tissue and to polynucleotides encoding such polypeptides. Such polypeptides and polynucleotides may be used in vaccines for treatment of breast cancer.

BACKGROUND OF THE INVENTION

[0002] Breast cancer is a significant health problem for women in the United States and throughout the world. Although advances have been made in detection and treatment of the disease, breast cancer remains the second leading cause of cancer-related deaths in women, affecting more than 180,000 women in the United States each year. For women in North America, the life-time odds of getting breast cancer are one in eight.

[0003] No vaccine or other universally successful method for the prevention or treatment of breast cancer is currently available. Management of the disease currently relies on a combination of early diagnosis (through routine breast screening procedures) and aggressive treatment, which may include one or more of a variety of treatments such as surgery, radiotherapy, chemotherapy and hormone therapy. The course of treatment for a particular breast cancer is often selected based on a variety of prognostic parameters, including an analysis of specific tumor markers: See, e.g., Porter-Jordan and Lippman, Breast Cancer 8:73-100 (1994). However, the use of established markers often leads to a result that is difficult to interpret, and the high mortality observed in breast cancer patients indicates that improvements are needed in the treatment, diagnosis and prevention of the disease.

[0004] EP 1 144 449 discloses a set of partially overlapping cDNA sequences and polypeptides encoded thereby transcribed from breast tissue. These sequences are described as being useful for diagnosing breast cancer.

[0005] Accordingly, there is a need in the art for improved, methods for the treatment and diagnosis of breast cancer. The present invention fulfils these needs and further provides other related advantages.

SUMMARY OF THE INVENTION

[0006] The present invention provides compounds and methods for the treatment and diagnosis of cancer, such as breast cancer. In one aspect, isolated polypeptides are provided comprising at least a portion of a breast tumor protein or a variant thereof. Certain portions and other variants are immunogenic, such that the ability of the variant to react with protein-specific antisera is not substantially diminished.

[0007] The invention provides an isolated polypeptide comprising at least a portion of a breast tumor protein, wherein the polypeptide comprises an amino acid sequence that is encoded by the polynucleotide sequence recited in SEQ ID NO: 175, or a variant of said polypeptide wherein the variant comprises an amino acid sequence that is encoded by a polynucleotide sequence that has at least 90% identity to a sequence of SEQ ID NO: 175, and the ability of the variant to react with antigen specific antisera is enhanced, unchanged or diminished by less than 50% relative to a polypeptide comprising an amino acid sequence that is encoded by SEQ ID NO: 175; provided that the polypeptide does not have the sequence recited in SEQ ID NO: 176 with the substitution of Asn Ser for Val Ile at positions 316 and 317. In specific embodiments, the inventive polypeptides comprise at least a portion of a tumor antigen that comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 176.

[0008] In related aspects, isolated polynucleotides encoding the above polypeptides, or a portion thereof (such as a portion encoding at least 15 contiguous amino acid residues of a breast tumor protein), are provided. In specific embodiments, such polynucleotides comprise a sequence selected from the group consisting of sequences provided in SEQ ID NO: 175, and variants thereof. The present invention further provides expression vectors comprising the above polynucleotides, together with host cells transformed or transfected with such expression vectors. In preferred embodiments, the host cells are selected from the group consisting of E. coli, yeast and mammalian cells.

[0009] In another aspect, the present invention provides fusion proteins comprising a first and a second inventive polypeptide or, alternatively, an inventive polypeptide and a known breast tumor antigen.

[0010] The present invention provides vaccines. For prophylactic or therapeutic use, comprising at least one of the above polypeptides or a polynucleotide encoding such a polypeptide in combination with an immunostimulant. Pharmaceutical compositions comprising at least one of the above polypeptides, or a polynucleotide encoding such a polypeptide, and a physiologically acceptable carrier, are disclosed. Pharmaceutical compositions and vaccines comprising one or more of the above fusion proteins are also provided.

[0011] Pharmaceutical compositions that comprise: (a) an antibody or antigen-binding fragment thereof that specifically binds to a breast tumor protein; and (b) a physiologically acceptable carrier are disclosed.

[0012] Pharmaceutical compositions comprising: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) a pharmaceutically acceptable carrier or excipient are disclosed. Antigen presenting cells include dendritic cells, macrophages, monocytes, fibroblasts and B cells.

[0013] Within related aspects, the present invention provides vaccines that comprise: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) an immunostimulant.

[0014] In yet another aspect, methods are disclosed for inhibiting the development of breast cancer in a patient, comprising administering an effective amount of at least one of the above pharmaceutical compositions and/or vaccines.

[0015] The present invention further provides, within other aspects, methods for removing tumor cells from a biological sample, comprising contacting a biological sample with T cells that specifically react with a breast tumor protein, wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of cells expressing the protein from the sample.

[0016] Within related aspects, methods are disclosed for inhibiting the development of a cancer in a patient, comprising administering to a patient a biological sample treated as described above.

[0017] Methods are further provided, within other aspects, for stimulating and/or expanding T cells specific for a breast tumor protein, comprising contacting T cells with one or more of: (i) a polypeptide as described above; (ii) a polynucleotide encoding such a polypeptide; and/or (iii) an antigen presenting cell that expresses such a polypeptide; under conditions and for a time sufficient to permit the stimulation and/or expansion of T cells. Isolated T cell populations comprising T cells prepared as described above are also provided.

[0018] Within further aspects, methods for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a T cell population as described above are disclosed.

[0019] Methods for inhibiting the development of a cancer in a patient are disclosed, comprising the steps of: (a) incubating CD4+ and/or CD8+ T cells isolated from a patient with one or more of: (i) a polypeptide comprising at least an immunogenic portion of a breast tumor protein; (ii) a polynucleotide encoding such a polypeptide; and (iii) an antigen-presenting cell that expressed such a polypeptide; and (b) administering to the patient an effective amount of the proliferated T cells, and thereby inhibiting the development of a cancer in the patient. Proliferated cells may, but need not, be cloned prior to administration to the patient.

[0020] The polypeptides disclosed herein may be usefully employed in the diagnosis and monitoring of breast cancer. In one aspect of the present invention, methods are provided for detecting a breast cancer in a patient, comprising: (a) contacting a biological sample obtained from a patient with a binding agent that is capable of binding to one of the above polypeptides; and (b) detecting in the sample an amount of polypeptide that binds to the binding agent; and (c) comparing the amount of polypeptide with a predetermined cut-off value, and therefrom determining the presence or absence of a breast cancer in a patient. In preferred embodiments, the binding agent is an antibody, most preferably a monoclonal antibody.

[0021] In related aspects, methods are provided for monitoring the progression of a breast cancer in a patient, comprising: (a) contacting a biological sample obtained from a patient with a binding agent that is capable of binding to one of the above polypeptides; (b) detecting in the sample an amount of a polypeptide that binds to the binding agent; (c) repeating steps (a) and (b) using a biological sample obtained ftom the patient at a subsequent point in time; and (d) comparing the amounts of polypeptide detected in steps (b) and (c).

[0022] Within related aspects, the present invention provides antibodies, preferably monoclonal antibodies, that bind to the inventive polypeptides, as well as diagnostic kits comprising such antibodies, to inhibit the development of breast cancer. Methods of using such antibodies are also disclosed.

[0023] The present invention further provides, within other aspects, methods for determining the presence or absence of a breast cancer in a patient, comprising the steps of: (a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a breast tumor protein; (b) detecting in the sample a level of a polynucleotide, preferably mRNA, that hybridizes to the oligonucleotide; and (c) comparing the level of polynucleotide that hybridizes to the oligonucleotide with a predetermined cut-off value, and therefrom determining the presence or absence of a breast cancer in the patient. Within certain embodiments, the amount of mRNA is detected via polymerase chain reaction using, for example, at least one oligonucleotide primer that hybridizes to a polynucleotide encoding a polypeptide as recited above, or a complement of such a polynucleotide. Within other embodiments, the amount of mRNA is detected using a hybridization technique, employing an oligonucleotide probe that hybridizes to a polynucleotide that encodes a polypeptide as recited above, or a complement of such a polynucleotide.

[0024] In related aspects, diagnostic kits comprising the above oligonucleotide probes or primers are provided.

[0025] These and other aspects of the present invention will become apparent upon reference to the following detailed description.

BRIEF DESCRIPTION OF THE DRAWING AND SEQUENCE IDENTIFIERS

[0026]

SEQ ID NO: 71 is the determined cDNA sequence of B726P.
SEQ ID NO: 175 is the determined cDNA sequence of B726P-20
SEQ ID NO: 176 is the predicted amino acid sequence of B726P-20
SEQ ID NO: 177 is a PCR primer
SEQ ID NO: 178 is the determined cDNA sequence of B726P-74
SEQ ID NO: 179 is the predicted amino acid sequence of B726P-74
SEQ ID NO: 180 is the determined cDNA sequence of B726P-79
SEQ ID NO: 181 is the predicted amino acid sequence of B726P-79
SEQ ID NO: 463 is a consensus DNA sequence of B726P (referred to as B726P-spliced_seq_B726P).
SEQ ID NO: 464 is the determined cDNA sequence of a second splice form of B726P (referred to as 27490.seq_B726P).
SEQ ID NO: 465 is the determined cDNA sequence of a third splice form of B726P (referred to as 27068.seq_B726P).
SEQ ID NO: 466 is the determined cDNA sequence of a second splice form of B726P (referred to as 23113.seq_B726P).
SEQ ID NO: 467 is the determined cDNA sequence of a second splice form of B726P (referred to as 23103.seq_B726P).
SEQ ID NO: 468 is the determined cDNA sequence of a second splice form of B726P (referred to as 19310.seq_B726P).
SEQ ID NO: 469 is the predicted amino acid sequence encoded by the upstream ORF of SEQ ID NO: 463.
SEQ ID NO: 470 is the predicted amino acid sequence encoded by SEQ ID NO: 464.
SEQ ID NO: 471 is the predicted amino acid sequence encoded by SEQ ID NO: 465.
SEQ ID NO: 472 is the predicted amino acid sequence encoded by SEQ ID NO: 466.
SEQ ID NO: 473 is the predicted amino acid sequence encoded by SEQ ID NO: 467.

DETAILED DESCRIPTION OF THE INVENTION

[0027] As noted above, the present invention is generally directed to compositions and their use for the therapy and diagnosis of cancer, such as breast cancer. The compositions described herein may include breast tumor polypeptides, polynucleotides encoding such polypeptides, binding agents such as antibodies, antigen presenting cells (APCs) and/or immune system cells (*e.g.*, T cells). Polypeptides of the present invention generally comprise at least a portion (such as an immunogenic portion) of a breast tumor protein or a variant thereof A "breast tumor protein" is a protein that is expressed in breast tumor cells at a level that is at least two fold, and preferably at least five fold, greater than the level of expression in a normal tissue, as determined using a representative assay provided herein. Certain breast tumor proteins are tumor proteins that react detectably (within an immunoassay, such as an ELISA or Western blot) with antisera of a patient afflicted with breast cancer. Polynucleotides of the subject invention generally comprise a DNA or RNA sequence that encodes all or a portion of such a polypeptide, or that is complementary to such a sequence. Antibodies are generally immune system proteins, or antigen-binding fragments thereof, that are capable of binding to a polypeptide as described above. Antigen presenting cells include dendritic cells, macrophages, monocytes, fibroblasts and B-cells that express a polypeptide as described above. T cells that may be employed within such compositions are generally T cells that are specific for a polypeptide as described above.

[0028] The present invention is based on the discovery of human breast tumor proteins. Sequences of polynucleotides encoding specific tumor proteins are provided in SEQ ID NO: 175.

BREAST TUMOR PROTEIN POLYNUCLEOTIDES

[0029] Any polynucleotide that encodes a breast tumor protein or a portion or other variant thereof as described herein is encompassed by the present invention. Preferred polynucleotides comprise at least 15 consecutive nucleotides, preferably at least 30 consecutive nucleotides and more preferably at least 45 consecutive nucleotides, that encode a portion of a breast tumor protein. More preferably, a polynucleotide encodes an immunogenic portion of a breast tumor protein. Polynucleotides complementary to any such sequences are also encompassed by the present invention. Polynucleotides may be single-stranded (coding or antisense) or double stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules 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.

[0030] Polynucleotides may comprise a native sequence (ie., an endogenous sequence that encodes a breast tumor protein or a portion thereof) or may comprise a variant of such a sequence. Polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions such that the immunogenicity of the encoded polypeptide is

not diminished, relative to a native tumor protein. The effect on the immunogenicity of the encoded polypeptide may generally be assessed as described herein. Variants preferably exhibit at least about 70% identity, more preferably at least about 80% identity and most preferably at least about 90% identity to a polynucleotide sequence that encodes a native breast tumor protein or a portion thereof. The term "variants" also encompasses homologous genes of xenogenic origin.

[0031] Two polynucleotide or polypeptide sequences are said to be "identical" if the sequence of nucleotides or 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.

[0032] 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 Phylogenes pp. 626-645 Methods in Enzymology vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M. (1989) CABIOS 5:151-153; Myers, E.W. and Muller W. (1988) CABIOS 4:11-17; Robinson, E.D. (1971) Comb. Theor 11:105; Santou, N. Nes, M. (1987) Mol. Biol. Evol. 4:406-425; Sneath. P.H.A. and Sokal, R.R. (1973) Numerical Taxonomy - the Principles and Practice of Numerical Taxonomy, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman. D.J. (1983) Proc. Natl. Acad. Sci. USA 80:726-730.

[0033] Preferably, 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 or 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 nucleic acid bases or 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 identify.

[0034] Variants may also, or alternatively, be substantially homologous to a native gene, or a portion or complement thereof. Such polynucleotide variants are capable of hybridizing under moderated stringent conditions to a naturally occurring DNA sequence encoding a native breast tumor protein (or a complementary sequence). Suitable moderately stringent conditions include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0): hybridizing at 50°C-65°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.

[0035] 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).

[0036] Polynucleotides may be prepared using any of a variety of techniques. For example, a polynucleotide may be identified, as described in more detail below, by screening a microarray of cDNAs for tumor-associated expression (*i.e.*, expression that is at least five fold greater in a breast tumor than in normal tissue, as determined using a representative assay provided herein). Such screens may be performed using a Synteni microarray (Palo Alto, CA) according to the manufacturer's instructions (and essentially as described by Schena et al., Proc. Natl. Acad Sci. USA 93:10614-10619, 1996 and Heller et al., Proc. Natl. Acad Sci. USA 94:2150-2155, 1997). Alternatively, polypeptides may be amplified from cDNA prepared from cells expressing the proteins described herein, such as breast tumor cells. Such polynucleotides may be amplified via polymerase chain reaction (PCR). For this approach, sequence-specific primers may be designed based on the sequences provided herein, and may be purchased or synthesized.

[0037] An amplified portion may be used to isolate a full length gene from a suitable library (*e.g.*, a breast 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.

[0038] For hybridization techniques, a partial sequence may be labeled (*e.g.*, by nick-translation or end-labeling

with ^{32}P) using well known techniques. A bacterial or bacteriophage library is then 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 are then assembled into a single contiguous sequence. A full length cDNA molecule can be generated by ligating suitable fragments, using well known techniques.

[0039] Alternatively, there are numerous amplification techniques for obtaining a full length coding sequence from a partial cDNA sequence. Within such techniques, amplification is generally performed via PCR. Any of a variety of commercially available kits may be used to perform the amplification step. Primers may be designed using, for example, software well known in the art. Primers are preferably 22-30 nucleotides in length, have a GC content of at least 50% and anneal to the target sequence at temperatures of about 68°C to 72°C. The amplified region may be sequenced as described above, and overlapping sequences assembled into a contiguous sequence.

[0040] One such amplification technique is inverse PCR (*see* Triglia et al., Nucl. Acids Res. 16:8186, 1988), which uses restriction enzymes to generate a fragment in the known region of the gene. The fragment is then circularized by intramolecular ligation and used as a template for PCR with divergent primers derived from the known region. Within an alternative approach, sequences adjacent to a partial sequence may be retrieved by amplification with a primer to a linker sequence and a primer specific to a known region. The amplified sequences are typically subjected to a second round of amplification with the same linker primer and a second primer specific to the known region. A variation on this procedure, which employs two primers that initiate extension in opposite directions from the known sequence, is described in WO 96/38591. Another such technique is known as "rapid amplification of cDNA ends" or RACE. This technique involves the use of an internal primer and an external primer, which hybridizes to a polyA region or vector sequence, to identify sequences that are 5' and 3' of a known sequence. Additional techniques include capture PCR (Lagerstrom et al., PCR Methods Applic. 1:111-19, 1991) and walking PCR (Parker et al., Nucl. Acids. Res. 19:3055-60, 1991). Other methods employing amplification may also be employed to obtain a full length cDNA sequence.

[0041] In certain instances, it is possible to obtain a full length cDNA sequence by analysis of sequences provided in an expressed sequence tag (EST) database, such as that available from GenBank. Searches for overlapping ESTs may generally be performed using well known programs (*e.g.*, NCBI BLAST searches), and such ESTs may be used to generate a contiguous full length sequence. Full length DNA sequences may also be obtained by analysis of genomic fragments.

[0042] Certain nucleic acid sequences of cDNA molecules encoding portions of breast tumor proteins are provided in SEQ ID NO: 71, 175, 178, 180 and 464-468. The isolation of these sequences is described in detail below.

[0043] Polynucleotide variants may generally be prepared by any method known in the art, including chemical synthesis by, for example, solid phase phosphoramidite chemical synthesis. Modifications in a polynucleotide sequence may also be introduced using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis (*see* Adelman et al., DNA 2:183, 1983). Alternatively, RNA molecules may be generated by *in vitro* or *in vivo* transcription of DNA sequences encoding a breast tumor protein, or portion thereof, provided that the DNA is incorporated into a vector with a suitable RNA polymerase promoter (such as T7 or SP6). Certain portions may be used to prepare an encoded polypeptide, as described herein. In addition, or alternatively, a portion may be administered to a patient such that the encoded polypeptide is generated *in vivo* (*e.g.,* by transfecting antigen-presenting cells, such as dendritic cells, with a cDNA construct encoding a breast tumor polypeptide, and administering the transfected cells to the patient).

[0044] A portion of a sequence complementary to a coding sequence (*i.e.*, an antisense polynucleotide) may also be used as a probe or to modulate gene expression. cDNA constructs that can be transcribed into antisense RNA may also be introduced into cells of tissues to facilitate the production of antisense RNA. An antisense polynucleotide may be used, as described herein, to inhibit expression of a tumor protein. Antisense technology can be used to control gene expression through triple-helix formation, which compromises the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors or regulatory molecules (*see* Gee et al., In Huber and Carr, Molecular and Immunologic Approaches, Futura Publishing Co. (Mt. Kisco, NY; 1994)). Alternatively, an antisense molecule may be designed to hybridize with a control region of a gene (*e.g.*, promoter, enhancer or transcription initiation site), and block transcription of the gene; or to block translation by inhibiting binding of a transcript to ribosomes.

[0045] A portion of a coding sequence, or of a complementary sequence, may also be designed as a probe or primer to detect gene expression. Probes may be labeled with a variety of reporter groups, such as radionuclides and enzymes, and are preferably at least 10 nucleotides in length, more preferably at least 20 nucleotides in length and still more preferably at least 30 nucleotides in length. Primers, as noted above, are preferably 22-30 nucleotides in length.

[0046] 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.

[0047] Nucleotide sequences as described herein may be joined to a variety of other nucleotide sequences using established recombinant DNA techniques. For example, a polynucleotide may be cloned into any of a variety of cloning vectors, including plasmids, phagemids, lambda phage derivatives and cosmids. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors and sequencing vectors. In general, a vector will contain an origin of replication functional in at least one organism, convenient restriction endonuclease sites and one or more selectable markers. Other elements will depend upon the desired use, and will be apparent to those of ordinary skill in the art.

[0048] Within certain embodiments, polynucleotides may be formulated so as to permit entry into a cell of a mammal, and expression therein. Such formulations are particularly useful for therapeutic purposes, as described below. Those of ordinary skill in the art will appreciate that there are many ways to achieve expression of a polynucleotide in a target cell, and any suitable method may be employed. For example, a polynucleotide may be incorporated into a viral vector such as, but not limited to, adenovirus, adeno-associated virus, retrovirus, or vaccinia or other pox virus (*e.g.*, avian pox virus). The polynucleotides may also be administered as naked plasmid vectors. Techniques for incorporating DNA into such vectors are well known to those of ordinary skill in the art. A retroviral vector may additionally transfer or incorporate a gene for a selectable marker (to aid in the identification or selection of transduced cells) and/or a targeting moiety, such as a gene that encodes a ligand for a receptor on a specific target cell, to render the vector target specific. Targeting may also be accomplished using an antibody, by methods known to those of ordinary skill in the art.

[0049] Other formulations for therapeutic purposes include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*i.e.*, an artificial membrane vesicle). The preparation and use of such systems is well known in the art.

BREAST TUMOR POLYPEPTIDES

[0050] Within the context of the present invention, polypeptides may comprise at least an immunogenic portion of a breast tumor protein or a variant thereof, as described herein. As noted above, a "breast tumor protein" is a protein that is expressed by breast tumor cells. Proteins that are breast tumor proteins also react detectably within an immunoassay (such as an ELISA) with antisera from a patient with breast cancer. Polypeptides as described herein may be of any length. Additional sequences derived from the native protein and/or heterologous sequences may be present, and such sequences may (but need not) possess further immunogenic or antigenic properties.

[0051] An "immunogenic portion," as used herein is a portion of a protein that is recognized (*i.e.*, specifically bound) by a B-cell and/or T-cell surface antigen receptor. Such immunogenic portions generally comprise at least 5 amino acid residues, more preferably at least 10, and still more preferably at least 20 amino acid residues of a breast tumor protein or a variant thereof. Certain preferred immunogenic portions include peptides in which an N-terminal leader sequence and/or transmembrane domain have been deleted. Other preferred immunogenic portions may contain a small N- and/or C-terminal deletion (*e.g.*, 1-30 amino acids, preferably 5-15 amino acids), relative to the mature protein.

[0052] 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. An immunogenic portion of a native breast tumor protein is a portion that reacts with such 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). Such immunogenic portions may react within such assays at a level that is similar to or greater than the reactivity of the full length polypeptide. Such screens may generally be performed using methods well known to those of ordinary skill in the art, such as those described in Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988. For 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, ^{125}I-labeled Protein A.

[0053] As noted above, a composition may comprise a variant of a native breast tumor protein. A polypeptide "variant," as used herein, is a polypeptide that differs from a native breast tumor protein in one or more substitutions, deletions, additions and/or insertions, such that the immunogenicity of the polypeptide is not substantially diminished. In other words, the ability of a variant to react with antigen-specific antisera may be enhanced or unchanged, relative to the native protein, or may be diminished by less than 50%, and preferably less than 20%, relative to the native protein. Such

variants may generally be identified by modifying one of the above polypeptide sequences and evaluating the reactivity of the modified polypeptide with antigen-specific antibodies or antisera as described herein. Preferred variants include those in which one or more portions, such as an N-terminal leader sequence or transmembrane domain, have been removed. Other preferred variants include variants in which a small portion (*e.g.*, 1-30 amino acids, preferably 5-15 amino acids) has been removed from the N- and/or C-terminal of the mature protein.

[0054] Polypeptide variants preferably exhibit at least about 70%, more preferably at least about 90% and most preferably at least about 95% identity (determined as described above to the identified polypeptides.

[0055] Preferably, a variant contains 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 hydropathic nature of the polypeptide to be substantially unchanged. Amino acid substitutions may generally 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 hydropathic nature of the polypeptide.

[0056] As noted above, 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.

[0057] Polypeptides may be prepared using any of a variety of well known techniques. Recombinant polypeptides encoded by DNA sequences as described above may be readily prepared from the DNA sequences using any of a variety of expression vectors known to those of ordinary skill in the art. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast, higher eukaryotic and plant cells. Preferably, the host cells employed are *E. coli,* yeast or a mammalian cell line such as COS or CHO. Supernatants from suitable host/vector systems which secrete recombinant protein or polypeptide into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant polypeptide.

[0058] Portions and other variants having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may also be generated by synthetic means, using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be 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.

[0059] Within certain specific embodiments, a polypeptide may be a fusion protein that comprises multiple polypeptides as described herein, or that comprises at least one polypeptide as described herein and an unrelated sequence, such as a known tumor protein. 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 protein or to enable the protein to be targeted to desired intracellular compartments. Still further fusion partners include affinity tags, which facilitate purification of the protein.

[0060] Fusion proteins may generally be prepared using standard techniques, including chemical conjugation. Preferably, a fusion protein is expressed as a recombinant protein, allowing the production of increased levels, relative to a non-fused protein, 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 protein that retains the biological activity of both component polypeptides.

[0061] A peptide linker sequence may be employed to separate the first and the 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 protein 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.

[0062] 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.

[0063] Fusion proteins are also provided that comprise a polypeptide of the present invention together with an unrelated immunogenic protein. Preferably the immunogenic protein is capable of eliciting a recall response. Examples of such proteins include tetanus, tuberculosis and hepatitis proteins (*see,* for example, Stoute et al. New Engl. J. Med., 336: 86-91, 1997).

[0064] Within preferred embodiments, an immunological fusion partner is derived from protein D, a surface protein of the gram-negative bacterium Haemophilus influenza B (WO 91/18926). Preferably, a protein D derivative comprises approximately the first third of the protein (*e.g.*, the first N-terminal 100-110 amino acids), and a protein D derivative may be lipidated. Within certain preferred embodiments, the first 109 residues of a Lipoprotein D fusion partner is included on the N-terminus to provide the polypeptide with additional exogenous T-cell epitopes and to increase the expression level in *E. coli* (thus functioning as an expression enhancer). The lipid tail ensures optimal presentation of the antigen to antigen presenting cells. Other fusion partners include the non-structural protein from influenzae virus, NS1 (hemaglutinin). Typically, the N-terminal 81 amino acids are used, although different fragments that include T-helper epitopes may be used.

[0065] In another embodiment, the immunological fusion partner is the protein known as LYTA, or a portion thereof (preferably a C-terminal portion). LYTA is derived from *Streptococcus pneumoniae,* which synthesizes an N-acetyl-L-alanine amidase known as amidase LYTA (encoded by the LytA gene; Gene 43:265-292, 1986). LYTA is an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LYTA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E. coli* C-LYTA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LYTA fragment at the amino terminus has been described (*see* Biotechnology 10:795-798, 1992). Within a preferred embodiment, a repeat portion of LYTA may be incorporated into a fusion protein. A repeat portion is found in the C-terminal region starting at residue 178. A particularly preferred repeat portion incorporates residues 188-305.

[0066] In general, polypeptides (including fusion proteins) and polynucleotides as described herein are isolated. An "isolated" polypeptide or polynucleotide is one that is removed from its original environment. For example, a naturally-occurring protein is isolated if it is separated from some or all of the coexisting materials in the natural system. Preferably, such polypeptides are at least about 90% pure, more preferably at least about 95% pure and most preferably at least about 99% pure. A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of the natural environment.

BINDING AGENTS

[0067] The present invention further provides agents, such as antibodies and antigen-binding fragments thereof, that specifically bind to a breast tumor protein. As used herein, an antibody, or antigen-binding fragment thereof, is said to "specifically bind" to a breast tumor protein if it reacts at a detectable level (within, for example, an ELISA) with a breast tumor protein, and does not react detectably with unrelated proteins under similar conditions. As used herein, "binding" refers to a noncovalent association between two separate molecules such that a complex is formed. The ability to bind may be evaluated by, for example, determining a binding constant for the formation of the complex. The binding constant is the value obtained when the concentration of the complex is divided by the product of the component concentrations. In general, two compounds are said to "bind," in the context of the present invention, when the binding constant for complex formation exceeds about 10^3 L/mol. The binding constant may be determined using methods well known in the art.

[0068] Binding agents may be further capable of differentiating between patients with and without a cancer, such as breast cancer, using the representative assays provided herein. In other words, antibodies or other binding agents that bind to a breast tumor protein will generate a signal indicating the presence of a cancer in at least about 20% of patients with the disease, and will generate a negative signal indicating the absence of the disease in at least about 90% of individuals without the cancer. To determine whether a binding agent satisfies this requirement, biological samples (*e.g.*, blood, sera, urine and/or tumor biopsies) from patients with and without a cancer (as determined using standard clinical tests) may be assayed as described herein for the presence of polypeptides that bind to the binding agent. It will be apparent that a statistically significant number of samples with and without the disease should be assayed. Each binding agent should satisfy the above criteria; however, those of ordinary skill in the art will recognize that binding agents may be used in combination to improve sensitivity.

[0069] Any agent that satisfies the above requirements may be a binding agent. For example, a binding agent may be a ribosome, with or without a peptide component, an RNA molecule or a polypeptide. 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.

[0070] 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.

[0071] 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.

[0072] Within certain embodiments, the use of antigen-binding fragments of antibodies may be preferred. Such fragments include Fab fragments, which may be prepared using standard techniques. Briefly, immunoglobulins may be purified from rabbit serum by affinity chromatography on Protein A bead columns (Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988) and digested by papain to yield Fab and Fc fragments. The Fab and Fc fragments may be separated by affinity chromatography on protein A bead columns.

[0073] 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.

[0074] 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.

[0075] Alternatively, it may be desirable to couple a therapeutic agent and an antibody via a linker group. A linker

croup 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 increases the coupling efficiency. An increase in chemical reactivity may also facilitate the use of agents, or junctional groups on agents, which otherwise would not be possible.

[0076] 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.

[0077] 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.).

[0078] 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 which provide multiple sites for attachment can be used. Alternatively, a carrier can be used.

[0079] 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.

[0080] A variety of routes of administration for the antibodies and immunoconjugates may be used. Typically, administration will be intravenous, intramuscular, subcutaneous or in the bed of a resected tumor. It will be evident that the precise dose of the antibody/immunoconjugate will vary depending upon the antibody used, the antigen density on the tumor, and the rate of clearance of the antibody.

T CELLS

[0081] Immunotherapeutic compositions may also, or alternatively, comprise T cells specific for a breast tumor protein. 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.

[0082] T cells may be stimulated with a breast tumor polypeptide, polynucleotide encoding a breast tumor 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. Preferably, a breast tumor polypeptide or polynucleotide is present within a delivery vehicle, such as a microsphere, to facilitate the generation of specific T cells.

[0083] T cells are considered to be specific for a breast tumor polypeptide if the T cells 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 breast tumor polypeptide (100 ng/ml - 100 μg/ml, preferably 200 ng/ml - 25 μg/ml) for 3 - 7 days should 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 breast tumor polypeptide, polynucleotide or polypeptide-expressing APC may be $CD4^+$ and/or $CD8^+$. Breast tumor protein-specific T cells may be expanded using standard techniques. Within preferred embodiments, the T cells are derived from either a patient or a related, or unrelated, donor and are administered to the patient following stimulation and expansion.

[0084] For therapeutic purposes, $CD4^+$ or $CD8^+$ T cells that proliferate in response to a breast 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 breast 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 breast tumor polypeptide. Alternatively, one or more T cells that proliferate in the presence of a breast tumor protein can be expanded in number by cloning. Methods for cloning cells are well known in the art, and include limiting dilution.

PHARMACEUTICAL COMPOSITIONS AND VACCINES

[0085] Within certain aspects, polypeptides, polynucleotides, T cells and/or binding agents disclosed herein may be incorporated into pharmaceutical compositions or immunogenic compositions (*i.e.,* vaccines). Pharmaceutical compositions comprise one or more such compounds and a physiologically acceptable carrier. Vaccines may comprise one or more such compounds and an immunostimulant. An immunostimulant may be any substance that enhances an immune response to an exogenous antigen. Examples of immunostimulants include adjuvants, biodegradable microspheres (*e.g.,* polylactic galactide) and liposomes (into which the compound is incorporated; *see e.g.,* Fullerton, U.S. Patent No. 4,235,877). 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). Pharmaceutical compositions and vaccines within the scope of the present invention may also contain other compounds, which may be biologically active or inactive. For example, one or more immunogenic portions of other tumor antigens may be present, either incorporated into a fusion polypeptide or as a separate compound, within the composition or vaccine.

[0086] A pharmaceutical composition or vaccine may contain DNA encoding one or more of the polypeptides as described above, such that the polypeptide is generated *in situ.* As noted above, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral expression systems. 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 nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems 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. In a preferred embodiment, the DNA may be introduced using a viral expression system (*e.g.*, vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective), replication competent virus. Suitable systems are disclosed, 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. Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. The DNA may also be "naked," as described, for example, 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.

[0087] While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions, the type of carrier will vary depending on the mode of administration. Compositions may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous or intramuscular administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactate polyglycolate) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

[0088] Such compositions may also comprise 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, chelating agents such as EDTA or glutathione, adjuvants (e. g., aluminum hydroxide) and/or preservatives. Alternatively, compositions of the present invention may be formulated as a lyophilizate. Compounds may also be encapsulated within liposomes using well known technology.

[0089] Any of a variety of immunostimulants may be employed in the vaccines of this invention. For example, an adjuvant may be included. Most 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, *Bortadella pertussis* or *Mycobacterium tuberculosis* derived proteins. Suitable 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); 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 or interleukin-2, -7, or -12, may also be used as adjuvants.

[0090] Within the vaccines provided herein, the adjuvant composition is preferably designed to induce an immune response predominantly of the Th1 type. High levels of Th1-type cytokines (e.g., IFN-γ, TNF-α, IL-2 and IL-12) tend to favor the induction of cell mediated immune responses to an administered antigen. In contrast, high levels of Th2-type cytokines (e.g., IL-4, IL-5, IL-6 and IL-10) tend to favor the induction of humoral immune responses. Following application of a vaccine as provided herein, a patient will support an immune response that includes Th1- and Th2-type responses. Within a preferred embodiment, in which a response is predominantly Th1-type, the level of Th1-type cytokines will increase to a greater extent than the level of Th2-type cytokines. The levels of these cytokines may be readily assessed using standard assays. For a review of the families of cytokines, see Mosmann and Coffman, Ann. Rev. Immunol. 7: 145-173, 1989.

[0091] Preferred adjuvants for use in eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A (3D-MPL), together with an aluminum salt. MPL adjuvants are available from Ribi ImmunoChem Research Inc. (Hamilton, MT) (see 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. Another preferred adjuvant is a saponin, preferably QS21, which may be used alone or in combination with other adjuvants. For example, an enhanced system involves the combination of a monophosphoryl lipid A and saponin derivative, such as the combination of QS21 and 3D-MPL 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 comprises an oil-in-water emulsion and tocopherol. A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil-in-water emulsion is described in WO 95/17210. Any vaccine provided herein may be prepared using well known methods that result in a combination of antigen, immune response enhancer and a suitable carrier or excipient.

[0092] The compositions described herein may be administered as part of a sustained release formulation (*i.e.*, a formulation such as a capsule, sponge or gel (composed of polysaccharides, for examples that effects a slow release of compound following dministration). Such formulations may generally be prepared using well known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a polypeptides, polynucleotide or antibody dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane. Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release. 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.

[0093] Any of a variety of delivery vehicles may be employed within pharmaceutical compositions and vaccines to facilitate production of an antigen-specific immune response that targets tumor cells. Delivery vehicles include 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.

[0094] Certain preferred embodiments of the present invention use dendritic cells or progenitors thereof as antigen-presenting cells. Dendritic cells are highly potent APCs (Banchereau and Steinman, Nature 392:245-251, 1998) and have been shown to be effective as a physiological adjuvant for eliciting prophylactic or therapeutic antitumor immunity *(see* Timmerman and Levy, Ann. Rev. Med. 50:507-529, 1999). In general, dendritic cells may be identified based on their typical shape (stellate *in situ,* with marked cytoplasmic processes (dendrites) visible *in vitro),* their ability to take

up, process and present antigens with high efficiency, and their ability to activate naïve T cell responses. Dendritic cells may, of course, be engineered to express specific cell-surface receptors or ligands that are not commonly found on dendritic cells *in vivo* or *ex vivo*, and such modified dendritic cells are contemplated by the present invention. As an alternative to dendritic cells, secreted vesicles antigen-loaded dendritic cells (called exosomes) may be used within a vaccine *(see* Zitvogel et al., Nature Med 4:594-600, 1998).

[0095] Dendritic cells and progenitors may be obtained from peripheral blood, bone marrow, tumor-infiltrating cells, peritumoral tissues-infiltrating cells, lymph nodes, spleen, skin, umbilical cord blood or any other suitable tissue or fluid. For example, dendritic cells may be differentiated *ex vivo* by adding a combination of cytokines such as GM-CSF, IL-4, IL-13 and/or TNFα to cultures of monocytes harvested from peripheral blood. Alternatively, CD34 positive cells harvested from peripheral blood, umbilical cord blood or bone marrow may be differentiated into dendritic cells by adding to the culture medium combinations of GM-CSF. IL-3, TNFα, CD40 ligand, LPS, flt3 ligand and/or other compound(s) that induce differentiation, maturation and proliferation of dendritic cells.

[0096] Dendritic cells are conveniently categorized as "immature" and "mature" cells, which allows a simple way to discriminate between two well characterized phenotypes. However, this nomenclature should not be construed to exclude all possible intermediate stages of differentiation. Immature dendritic cells are characterized as APC with a high capacity for antigen uptake and processing, which correlates with the high expression of Fcγ receptor and mannose receptor. The mature phenotype is typically characterized by a lower expression of these markers, but a high expression of cell surface molecules responsible for T cell activation such as class I and class II MHC, adhesion molecules (e.g., CD54 and CD11) and costimulatory molecules (e. g., CD40, CD80, CD86 and 4-1BB).

[0097] APCs may generally be transfected with a polynucleotide encoding a breast tumor protein (or portion or other variant thereof) such that the breast tumor polypeptide, or an immunogenic portion thereof, is expressed on the cell surface. Such transfection may take .. place ex *vivo,* and a composition or vaccine comprising such transfected cells may then be used for therapeutic purposes, as described herein. Alternatively, a gene delivery vehicle that targets a dendritic or other antigen presenting cell may be administered to a patient, resulting in transfection that occurs *in vivo. In vivo* and *ex vivo* transfection of dendritic cells, for example, may generally be performed using any methods known in the art, such as those described in WO 97/24447, or the gene gun approach described by Mahvi et al., Immunology and cell Biology 75:456-460, 1997. Antigen loading of dendritic cells may be achieved by incubating dendritic cells or progenitor cells with the breast tumor polypeptide, DNA (naked or within a plasmid vector) or RNA; or with antigen-expressing recombinant bacterium or viruses (e.g., vaccinia, fowlpox, adenovirus or lentivirus vectors). Prior to loading, the polypeptide may be covalently conjugated to an immunological partner that provides T cell help (e.g., a carrier molecule). Alternatively, a dendritic cell may be pulsed with a non-conjugated immunological partner, separately or in the presence of the polypeptide.

CANCER THERAPY

[0098] In further aspects, the compositions described herein may be used for immunotherapy of cancer, such as breast cancer. Within such methods, pharmaceutical compositions and vaccines are typically administered to a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may or may not be afflicted with cancer. Accordingly, the above pharmaceutical compositions and vaccines may be used to prevent the development of a cancer or to treat a patient afflicted with a cancer. A cancer may be diagnosed using criteria generally accepted in the art, including the presence of a malignant tumor. Pharmaceutical compositions and vaccines may be administered either prior to or following surgical removal of primary tumors and/or treatment such as administration of radiotherapy or conventional chemotherapeutic drugs.

[0099] 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 disclosed herein).

[0100] Within other embodiments, immunotherapy may be passive immunotherapy, in which treatment involves the delivery of agents with established tumor-immune reactivity (such as effector cells or antibodies) 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 (as described above and in U.S. Patent No. 4,918,164) for passive immunotherapy.

[0101] Effector cells may generally be obtained in sufficient quantities for adoptive immunotherapy by growth *in vitro,* as described herein. Culture conditions for expanding single antigen-specific effector cells to several billion in number

with retention of antigen recognition *in vivo* are well known in the art. Such *in vitro* culture conditions typically use intermittent stimulation with antigen, often in the presence of cytokines (such as IL-2) and non-dividing feeder cells. As noted above, immunoreactive polypeptides as provided herein may be used to rapidly expand antigen-specific T cell cultures in order to generate a sufficient number of cells for immunotherapy. In particular, antigen-presenting cells, such as dendritic, macrophage, monocyte, fibroblast or B cells, may be pulsed with immunoreactive polypeptides or transfected with one or more polynucleotides using standard techniques well known in the art. For example, antigen-presenting cells can be transfected with a polynucleotide having a promoter appropriate for increasing expression in a recombinant virus or other expression system. Cultured effector cells for use in therapy must be able to grow and distribute widely, and to survive long term *in vivo*. Studies have shown that cultured effector cells can be induced to grow in vivo and to survive long term in substantial numbers by repeated stimulation with antigen supplemented with IL-2 *(see,* for example, Cheever et al., Immunological Reviews 157:177,1997).

[0102] Alternatively, a vector expressing a polypeptide recited herein may be introduced into antigen presenting cells taken from a patient and clonally propagated *ex vivo* for transplant back into the same patient. Transfected cells may be reintroduced into the patient using any means known in the art, preferably in sterile form by intravenous, intracavitary, intraperitoneal or intratumor administration.

[0103] Routes and frequency of administration of the therapeutic compositions disclosed 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 100 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.

[0104] 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 breast 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.

METHODS FOR DETECTING CANCER

[0105] In general, a cancer may be detected in a patient based on the presence of one or more breast tumor proteins and/or polynucleotides encoding such proteins in a biological sample (for example, blood, sera, 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 such as breast cancer. In addition, such proteins may be useful for the detection of other cancers. The binding agents provided herein generally permit detection of the level of antigen that binds to the agent in the biological sample. Polynucleotide primers and probes may bur used to detect the level of mRNA encoding a tumor protein, which is also indicative of the presence or absence of a cancer. In general, a breast tumor sequence should be present at a level that is at least three fold higher in tumor tissue than in normal tissue

[0106] 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.

[0107] In a preferred 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 breast tumor proteins and portions thereof to which the binding agent binds, as described above.

[0108] 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 I μg, is sufficient to immobilize an adequate amount of binding agent.

[0109] 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 ImmunotechnologyCatalog and Handbook, 1991, at A12-A13).

[0110] In certain embodiment, the assays 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 or 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.

[0111] 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 breast cancer. Preferably, the contact time is sufficient to achieve a level of binding that is 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.

[0112] 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.

[0113] 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.

[0114] To determine the presence or absence of a cancer, such as breast 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 preferred 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 preferred 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.

[0115] 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 μg, 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.

[0116] 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 breast tumor polypeptides to detect antibodies that bind to such polypeptides in a biological sample. The detection of such breast tumor protein specific antibodies may correlate with the presence of a cancer.

[0117] A cancer may also, or alternatively, be detected based on the presence of T cells that specifically react with a breast tumor protein 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 breast 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 breast 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.

[0118] As noted above, a cancer may also, or alternatively, be detected based on the level of mRNA encoding a breast tumor protein 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 breast 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 breast 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 breast tumor protein may be used in a hybridization assay to detect the presence of polynucleotide encoding the tumor protein in a biological sample.

[0119] 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 breast tumor protein that is at least 10 nucleotides, and preferably at least 20 nucleotides, in length. Preferably, oligonucleotide primers and/or probes will hybridize to a polynucleotide encoding a polypeptide disclosed 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 a preferred embodiment, the oligonucleotide primers comprise at least 10 contiguous nucleotides, more preferably at least 15 contiguous nucleotides, of a DNA molecule having a sequence recited in SEQ

ID NOS:1-175, 178, 180 and 182-468. 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).

[0120] One preferred 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.

[0121] In another embodiment, the disclosed compositions 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 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.

[0122] 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.

[0123] As noted above, to improve sensitivity, multiple breast 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.

DIAGNOSTIC KITS

[0124] 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 breast 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.

[0125] Alternatively, a kit may be designed to detect the level of mRNA encoding a breast 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 breast 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 breast tumor protein.

[0126] The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1

ISOLATION AND CHARACTERIZATION OF BREAST TUMOR POLYPEPTIDES

[0127] This Example describe the isolation of breast tumor polypeptides from a breast tumor cDNA library.

[0128] cDNA clones that are over-expressed in breast tumor tissue were isolated from breast cDNA subtraction libraries as follows. Breast subtraction libraries were prepared, as described above, by PCR-based subtraction employing pools of breast tumor cDNA as the tester and pools of either normal breast cDNA or cDNA from other normal tissues as the driver. cDNA clones from breast subtraction were randomly picked and colony PCR amplified and their mRNA expression levels in breast tumor, normal breast and various other normal tissues were determined using the microarray technology described above. Twenty-four distinct cDNA clones were found to be over-expressed in breast tumor and expressed at low levels in all normal tissues tested (breast, brain, liver, pancreas, lung, salivary gland, stomach, colon, kidney, bone marrow, skeletal muscle, PBMC, heart, small intestine, adrenal gland, spinal cord, large intestine and skin). The deter-

mined partial cDNA sequences for one of these clones is provided in SEQ ID NO: 71. Comparison of the sequence with those in the gene bank using the EMBh and GenBank databases (Release 97) found no significant homologies to the sequence of SEQ ID NO: 71.

[0129] Three DNA isoforms for the clone B726P (partial sequence provided in SEQ ID NO: 71) were isolated as follows. A radioactive probe was synthesized from B726P by excising B726P DNA from a pT7Blue vector (Novagen) by a BamHI/XbaI restriction digest and using the resulting DNA as the template in a single-stranded PCR in the presence of [α-32P]dCTP. The sequence of the primer employed for this PCR is provided in SEQ ID NO: 177. The resulting radioactive probe was used to probe a directional cDNA library and a random-primed cDNA library made using RNA isolated from breast tumors. Eighty-five clones were identified, excised, purified and sequenced. Of these 85 clones, three were found to each contain a significant open reading frame. The determined cDNA sequence of the isoform B726P-20 is provided in SEQ ID NO: 175, with the corresponding predicted amino acid sequence being provided in SEQ ID NO: 176. The determined cDNA sequence of the isoform B726P-74 is provided in SEQ ID NO: 178, with the corresponding predicted amino acid sequence being provided in SEQ ID NO: 179. The determined cDNA sequence of the isoform B726P-79 is provided in SEQ ID NO: 180, with the corresponding predicted amino acid sequence being provided in SEQ ID NO: 181.

[0130] Efforts to obtain a full-length clone of B726P using standard techniques led to the isolation of five additional clones that represent additional 5' sequence of B726P. These clones appear to be alternative splice forms of the same gene. The determined cDNA sequences of these clones are provided in SEQ ID NO: 464-468, with the predicted amino acid sequences encoded by SEQ ID NO: 464-467 being provided in SEQ ID NO: 470-473, respectively. Using standard computer techniques, a 3,681 bp consensus DNA sequence (SEQ ID NO: 463) was created that contains two large open reading frames. The downstream ORF encodes the predicted amino acid sequence of SEQ ID NO: 181. The predicted amino acid sequence encoded by the upstream ORF is provided in SEQ ID NO: 469.

Example 2

SYNTHESIS OF POLYPEPTIDES

[0131] Polypeptides may be synthesized on an Perkin Elmer/Applied Biosystems Division 430A peptide synthesizer using FMOC chemistry with HPTU (O-Benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate) activation. A Gly-Cys-Gly sequence may be attached to the amino terminus of the peptide to provide a method of conjugation, binding to an immobilized surface, or labeling of the peptide. Cleavage of the peptides from the solid support may be carried out using the following cleavage mixture: trifluoroacetic acid:ethanedithiol:thioanisole:water:phenol (40:1:2:2:3). After cleaving for 2 hours, the peptides may be precipitated in cold methyl-t-butyl-ether. The peptide pellets may then be dissolved in water containing 0.1% trifluoroacetic acid (TFA) and lyophilized prior to purification by C18 reverse phase HPLC. A gradient of 0%-60% acetonitrile (containing 0.1% TFA) in water (containing 0.1% TFA) may be used to elute the peptides. Following lyophilization of the pure fractions, the peptides may be characterized using electrospray or other types of mass spectrometry and by amino acid analysis.

[0132] From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purposes of illustration, various modifications may be made.

SEQUENCE LISTING

[0133]

<110> Corixa Corporation
Yuqui, Jiang
Dillon, Davin C.
Mitcham, Jennifer L.
Xu, Jiangchun
Harlocker, Susan L.

<120> COMPOSITIONS FOR THE TREATMENT AND
DIAGNOSIS OF BREAST CANCER AND METHODS FOR THEIR USE

<130> 210121.47001PC

<140> PCT
<141> 2000-02-15

<160> 474

<170> FastSEQ for Windows Version 3.0

5

<210> 71
<211> 618
<212> DNA
<213> Homo sapien

10

<400> 71

```
gttgcagtga gctcaagtgt tgggtgtatc agctcaaaac accatgtgat gccaatcatc      60
tccacaggag caatttgttt accttttttt tctgatgctt tactaacttc atcttttaga     120
tttaaatcat tagtagatcc tagaggagcc agtttcagaa aatatagatt ctagttcagc     180
accacccgta gttgtgcatt gaaataatta tcattatgat tatgtatcag agcttctggt     240
tttctcattc tttattcatt tattcaacaa ccacgtgaca aacactggaa ttacaggatg     300
aagatgagat aatccgctcc ttggcagtgt tatactatta tataacctga aaaaacaaac     360
aggtaatttt cacacaaagt aatagatatc atgacacatt taaaataggg cactactgga     420
acacacagat aggacatcca ggttttgggt caatattgta gactttttgg tggatgagat     480
atgcaggttg atrccagaag gacaacaaaa acatatgtca gatagaaggg aggagcaaat     540
gccaagagct ggagctgagg aagatcactg tgaaattcta tgtagtctag ttggctggat     600
gctagagcaa agaggtgg                                                   618
```

15

20

25

<210> 175
<211> 1206
<212> DNA
<213> Homo sapien

30

<400> 175

```
ggcacgagga agttttgtgt actgaaaaag aaactgtcag aagcaaaaga aataaaatca      60
cagttagaga accaaaaagt taaatgggaa caagagctct gcagtgtgag gtttctcaca     120
ctcatgaaaa tgaaaattat ctcttacatg aaaattgcat gttgaaaaag gaaattgcca     180
tgctaaaact ggaaatagcc acactgaaac accaatacca ggaaaaggaa aataaatact     240
ttgaggacat taagatttta aaagaaaaga atgctgaact tcagatgacc ctaaaactga     300
aagaggaatc attaactaaa agggcatctc aatatagtgg gcagcttaaa gttctgatag     360
ctgagaacac aatgctcact tctaaattga aggaaaaaca agacaaagaa atactagagg     420
cagaaattga atcacaccat cctagactgg cttctgctgt acaagaccat gatcaaattg     480
tgacatcaag aaaaagtcaa gaacctgctt tccacattgc aggagatgct tgtttgcaaa     540
gaaaaatgaa tgttgatgtg agtagtacga tatataacaa tgaggtgctc catcaaccac     600
tttctgaagc tcaaaggaaa tccaaaagcc taaaaattaa tctcaattat gccggagatg     660
ctctaagaga aaatacattg gtttcagaac atgcacaaag agaccaacgt gaaacacagt     720
gtcaaatgaa ggaagctgaa cacatgtatc aaaacgaaca agataatgtg aacaaacaca     780
ctgaacagca ggagtctcta gatcagaaat tatttcaact acaaagcaaa aatatgtggc     840
ttcaacagca attagttcat gcacataaga aagctgacaa caaaagcaag ataacaattg     900
atattcattt tcttgagagg aaaatgcaac atcatctcct aaaagagaaa aatgaggaga     960
tatttaatta caataaccat ttaaaaaacc gtatatatca atatgaaaaa gagaaagcag    1020
aaacagaagt tatataatag tataacactg ccaaggagcg gattatctca tcttcatcct    1080
gtaattccag tgtttgtcac gtggttgttg aataaatgaa taaagaatga gaaaaccaga    1140
agctctgata cataatcata atgataatta tttcaatgca caactacggg tggtgctgct    1200
cgtgcc                                                               1206
```

35

40

45

50

<210> 176
<211> 317
<212> PRT
<213> Homo sapien

55

<400> 176

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

<210> 177
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in the Lab

<400> 177
ccaatcatct ccacaggagc 20

<210> 178
<211> 1665
<212> DNA
<213> Homo sapien

<400> 178

```
gcaaactttc aagcagagcc tcccgagaag ccatctgcct tcgagcctgc cattgaaatg     60
caaaagtctg ttccaaataa agccttggaa ttgaagaatg aacaaacatt gagagcagat    120
cagatgttcc cttcagaatc aaaacaaaag aaggttgaag aaaattcttg ggattctgag    180
agtctccgtg agactgtttc acagaaggat gtgtgtgtac ccaaggctac acatcaaaaa    240
gaaatggata aaataagtgg aaaattagaa gattcaacta gcctatcaaa aatcttggat    300
acagttcatt cttgtgaaag agcaagggaa cttcaaaaag atcactgtga acaacgtaca    360
ggaaaaatgg aacaaatgaa aaagaagttt tgtgtactga aaaagaaact gtcagaagca    420
aaagaaataa aatcacagtt agagaaccaa aaagttaaat gggaacaaga gctctgcagt    480
gtgaggtttc tcacactcat gaaaatgaaa attatctctt acatgaaaat tgcatgttga    540
aaaaggaaat tgccatgcta aaactggaaa tagccacact gaaacaccaa taccaggaaa    600
aggaaaataa atactttgag gacattaaga ttttaaaaga aaagaatgct gaacttcaga    660
tgaccctaaa actgaaagag gaatcattaa ctaaaagggc atctcaatat agtgggcagc    720
ttaaagttct gatagctgag aacacaatgc tcacttctaa attgaaggaa aaacaagaca    780
aagaaatact agaggcagaa attgaatcac accatcctag actggcttct gctgtacaag    840
accatgatca aattgtgaca tcaagaaaaa gtcaagaacc tgctttccac attgcaggag    900
atgcttgttt gcaaagaaaa atgaatgttg atgtgagtag tacgatatat aacaatgagg    960
tgctccatca accactttct gaagctcaaa ggaaatccaa aagcctaaaa attaatctca   1020
attatgccgg agatgctcta agagaaaata cattggtttc agaacatgca caaagagacc   1080
aacgtgaaac acagtgtcaa atgaaggaag ctgaacacat gtatcaaaac gaacaagata   1140
atgtgaacaa acacactgaa cagcaggagt ctctagatca gaaattattt caactacaaa   1200
gcaaaaatat gtggcttcaa cagcaattag ttcatgcaca taagaaagct gacaacaaaa   1260
gcaagataac aattgatatt cattttcttg agaggaaaat gcaacatcat ctcctaaaag   1320
agaaaaatga ggagatattt aattacaata accatttaaa aaaccgtata tatcaatatg   1380
aaaaagagaa agcagaaaca gaaaactcat gagagacaag cagtaagaaa cttcttttgg   1440
agaaacaaca gaccagatct ttactcacaa ctcatgctag gaggccagtc ctagcattac   1500
cttatgttga aaatcttacc aatagtctgt gtcaacagaa tacttatttt agaagaaaaa   1560
ttcatgattt cttcctgaag cctgggcgac agagcgagac tctgtctcaa aaaaaaaaaa   1620
aaaaaaagaa agaaagaaat gcctgtgctt acttcgcttc ccagg                   1665
```

<210> 179
<211> 179
<212> PRT
<213> Homo sapien

<400> 179

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

```
Lys Phe Cys Val Leu Lys Lys Lys Leu Ser Glu Ala Lys Glu Ile Lys
    130                 135                 140
Ser Gln Leu Glu Asn Gln Lys Val Lys Trp Glu Gln Glu Leu Cys Ser
145                 150                 155                 160
Val Arg Phe Leu Thr Leu Met Lys Met Lys Ile Ile Ser Tyr Met Lys
                165                 170                 175
Ile Ala Cys
```

<210> 180
<211> 1681
<212> DNA
<213> Homo sapien

<400> 180

```
gatacagtca ttcttgtgaa agagcaaggg aacttcaaaa agatcactgt gaacaacgta     60
caggaaaaat ggaacaaatg aaaaagaagt tttgtgtact gaaaaagaaa ctgtcagaag    120
caaaagaaat aaaatcacag ttagagaacc aaaaagttaa atgggaacaa gagctctgca    180
gtgtgagatt gactttaaac caagaagaag agaagagaag aaatgccgat atattaaatg    240
aaaaaattag ggaagaatta ggaagaatcg aagagcagca taggaaagag ttagaagtga    300
aacaacaact tgaacaggct ctcagaatac aagatataga attgaagagt gtagaaagta    360
atttgaatca ggtttctcac actcatgaaa atgaaaatta tctcttacat gaaaattgca    420
tgttgaaaaa ggaaattgcc atgctaaaac tggaaatagc cacactgaaa caccaatacc    480
aggaaaagga aaataaatac tttgaggaca ttaagatttt aaaagaaaag aatgctgaac    540
ttcagatgac cctaaaactg aaagaggaat cattaactaa aagggcatct caatatagtg    600
ggcagcttaa agttctgata gctgagaaca caatgctcac ttctaaattg aaggaaaaac    660
aagacaaaga aatactagag gcagaaattg aatcacacca tcctagactg gcttctgctg    720
tacaagacca tgatcaaatt gtgacatcaa gaaaaagtca agaacctgct ttccacattg    780
caggagatgc ttgtttgcaa agaaaaatga atgttgatgt gagtagtacg atatataaca    840
atgaggtgct ccatcaacca ctttctgaag ctcaaaggaa atccaaaagc ctaaaaatta    900
atctcaatta tgccggagat gctctaagag aaaatacatt ggtttcagaa catgcacaaa    960
gagaccaacg tgaaacacag tgtcaaatga aggaagctga acacatgtat caaaacgaac   1020
aagataatgt gaacaaacac actgaacagc aggagtctct agatcagaaa ttatttcaac   1080
tacaaagcaa aaatatgtgg cttcaacagc aattagttca tgcacataag aaagctgaca   1140
acaaaagcaa gataacaatt gatattcatt ttcttgagag gaaaatgcaa catcatctcc   1200
taaaagagaa aaatgaggag atatttaatt acaataacca tttaaaaaac cgtatatatc   1260
aatatgaaaa agagaaagca gaaacagaaa actcatgaga gacaagcagt aagaaacttc   1320
ttttggagaa acaacagacc agatctttac tcacaactca tgctaggagg ccagtcctag   1380
cattacctta tgttgaaaaa tcttaccaat agtctgtgtc aacagaatac ttattttaga   1440
agaaaaattc atgatttctt cctgaagcct acagacataa aataacagtg tgaagaatta   1500
cttgttcacg aattgcataa aagctgccca ggatttccat ctaccctgga tgatgccgga   1560
gacatcattc aatccaacca gaatctcgct ctgtcactca ggctggagtg cagtgggcgc   1620
aatctcggct cactgcaact ctgcctccca ggttcacgcc attctctggc acagcctccc   1680
g                                                                   1681
```

<210> 181
<211> 432
<212> PRT
<213> Homo sapien

<400> 181

```
Asp Thr Val His Ser Cys Glu Arg Ala Arg Glu Leu Gln Lys Asp His
1               5                   10                  15
Cys Glu Gln Arg Thr Gly Lys Met Glu Gln Met Lys Lys Lys Phe Cys
            20                  25                  30
```

5

10

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

15

20

25

30

35

40

45

50

55

<210> 463

<211> 3681
<212> DNA
<213> Homo sapiens

5

<400> 463

```
tccgagctga ttacagacac caaggaagat gctgtaaaga gtcagcagcc acagccctgg 60
ctagctggcc ctgtgggcat ttattagtaa agttttaatg acaaaagctt tgagtcaaca 120
cacccgtggg taattaacct ggtcatcccc accctggaga gccatcctgc ccatgggtga 180
tcaaagaagg aacatctgca ggaacacctg atgaggctgc acccttggcg gaaagaacac 240
```

10

15

20

25

30

35

40

45

50

55

```
ctgacacagc tgaaagcttg gtggaaaaaa cacctgatga ggctgcaccc ttggtggaaa 300
gaacacctga cacggctgaa agcttggtgg aaaaaacacc tgatgaggct gcatccttgg 360
tggagggaac atctgacaaa attcaatgtt tggagaaagc gacatctgga aagttcgaac 420
agtcagcaga agaaacacct agggaaatta cgagtcctgc aaaagaaaca tctgagaaat 480
ttacgtggcc agcaaaagga agacctagga agatcgcatg ggagaaaaaa gaagacacac 540
ctagggaaat tatgagtccc gcaaaagaaa catctgagaa atttacgtgg gcagcaaaag 600
gaagacctag gaagatcgca tgggagaaaa aagaaacacc tgtaaagact ggatgcgtgg 660
caagagtaac atctaataaa actaaagttt tggaaaaagg aagatctaag atgattgcat 720
gtcctacaaa agaatcatct acaaaagcaa gtgccaatga tcagaggttc ccatcagaat 780
ccaaacaaga ggaagatgaa gaatattctt gtgattctcg gagtctcttt gagagttctg 840
caaagattca agtgtgtata cctgagtcta tatatcaaaa agtaatggag ataaatagag 900
aagtagaaga gcctcctaag aagccatctg ccttcaagcc tgccattgaa atgcaaaact 960
ctgttccaaa taaagccttt gaattgaaga atgaacaaac attgagagca gatccgatgt 1020
tcccaccaga atccaaacaa aaggactatg aagaaaattc ttgggattct gagagtctct 1080
gtgagactgt ttcacagaag gatgtgtgtt tacccaaggc tacacatcaa aaagaaatag 1140
ataaaataaa tggaaaatta gaagagtctc ctaataaaga tggtcttctg aaggctacct 1200
gcggaatgaa agtttctatt ccaactaaag ccttagaatt gaaggacatg caaactttca 1260
aagcagagcc tccggggaag ccatctgcct tcgagcctgc cactgaaatg caaaagtctg 1320
tcccaaataa agccttggaa ttgaaaaatg aacaaacatt gagagcagat gagatactcc 1380
catcagaatc caaacaaaag gactatgaag aaagttcttg ggattctgag agtctctgtg 1440
agactgtttc acagaaggat gtgtgtttac ccaaggctrc rcatcaaaaa gaaatagata 1500
aaataaatgg aaaattagaa gggtctcctg ttaaagatgg tcttctgaag gctaactgcg 1560
gaatgaaagt ttctattcca actaaagcct tagaattgat ggacatgcaa actttcaaag 1620
cagagcctcc cgagaagcca tctgccttcg agcctgccat tgaaatgcaa aagtctgttc 1680
caaataaagc cttggaattg aagaatgaac aaacattgag agcagatgag atactcccat 1740
cagaatccaa acaaaaggac tatgaagaaa gttcttggga ttctgagagt ctctgtgaga 1800
ctgtttcaca gaaggatgtg tgtttaccca aggctrcrca tcaaaaagaa atagataaaa 1860
taaatggaaa attagaagag tctcctgata atgatggttt tctgaaggct ccctgcagaa 1920
tgaaagtttc tattccaact aaagccttag aattgatgga catgcaaact ttcaaagcag 1980
agcctcccga gaagccatct gccttcgagc ctgccattga aatgcaaaag tctgttccaa 2040
ataaagcctt ggaattgaag aatgaacaaa cattgagagc agatcagatg ttcccttcag 2100
aatcaaaaca aaagaasgtt gaagaaaatt cttgggattc tgagagtctc cgtgagactg 2160
tttcacagaa ggatgtgtgt gtacccaagg ctacacatca aaaagaaatg gataaaataa 2220
gtggaaaatt agaagattca actagcctat caaaaatctt ggatacagtt cattcttgtg 2280
aaagagcaag ggaacttcaa aaagatcact gtgaacaacg tacaggaaaa atggaacaaa 2340
tgaaaaagaa gttttgtgta ctgaaaaaga aactgtcaga agcaaaagaa ataaaatcac 2400
agttagagaa ccaaaaagtt aaatgggaac aagagctctg cagtgtgagg tttctcacac 2460
tcatgaaaat gaaaattatc tcttacatga aaattgcatg ttgaaaaagg aaattgccat 2520
gctaaaactg gaaatagcca cactgaaaca ccaataccag gaaaaggaaa ataaatactt 2580
tgaggacatt aagattttaa aagaaaagaa tgctgaactt cagatgaccc taaaactgaa 2640
agaggaatca ttaactaaaa gggcatctca atatagtggg cagcttaaag ttctgatagc 2700
tgagaacaca atgctcactt ctaaattgaa ggaaaaacaa gacaaagaaa tactagaggc 2760
agaaattgaa tcacaccatc ctagactggc ttctgctgta caagaccatg atcaaattgt 2820
gacatcaaga aaaagtcaag aacctgcttt ccacattgca ggagatgctt gtttgcaaag 2880
aaaaatgaat gttgatgtga gtagtacgat atataacaat gaggtgctcc atcaaccact 2940
ttctgaagct caaaggaaat ccaaaagcct aaaaattaat ctcaattatg cmggagatgc 3000
tctaagagaa aatacattgg tttcagaaca tgcacaaaga gaccaacgtg aaacacagtg 3060
tcaaatgaag gaagctgaac acatgtatca aaacgaacaa gataatgtga acaaacacac 3120
tgaacagcag gagtctctag atcagaaatt atttcaacta caaagcaaaa atatgtggct 3180
tcaacagcaa ttagttcatg cacataagaa agctgacaac aaaagcaaga taacaattga 3240
tattcatttt cttgagagga aaatgcaaca tcatctccta aaagagaaaa atgaggagat 3300
atttaattac aataaccatt taaaaaaccg tatatatcaa tatgaaaaag agaaagcaga 3360
aacagaaaac tcatgagaga caagcagtaa gaaacttctt ttggagaaac aacagaccag 3420
atctttactc acaactcatg ctaggaggcc agtcctagca tcaccttatg ttgaaaatct 3480
taccaatagt ctgtgtcaac agaatactta ttttagaaga aaaattcatg atttcttcct 3540

gaagcctaca gacataaaat aacagtgtga agaattactt gttcacgaat tgcataaagc 3600
tgcacaggat tcccatctac cctgatgatg cagcagacat cattcaatcc aaccagaatc 3660
tcgctctgtc actcaggctg g                                          3681
```

<210> 464
<211> 1424
<212> DNA
<213> Homo sapiens

<400> 464

```
tccgagctga ttacagacac caaggaagat gctgtaaaga gtcagcagcc acagccctgg 60
ctagctggcc ctgtgggcat ttattagtaa agttttaatg acaaaagctt tgagtcaaca 120
cacccgtggg taattaacct ggtcatcccc accctggaga gccatcctgc ccatgggtga 180
tcaaagaagg aacatctgca ggaacacctg atgaggctgc acccttggcg gaaagaacac 240
ctgacacagc tgaaagcttg gtggaaaaaa cacctgatga ggctgcaccc ttggtggaaa 300
gaacacctga cacggctgaa agcttggtgg aaaaaacacc tgatgaggct gcatccttgg 360
tggagggaac atctgacaaa attcaatgtt tggagaaagc gacatctgga aagttcgaac 420
agtcagcaga agaaacacct agggaaatta cgagtcctgc aaaagaaaca tctgagaaat 480
ttacgtggcc agcaaaagga agacctagga agatcgcatg ggagaaaaaa gaagacacac 540
ctagggaaat tatgagtccc gcaaaagaaa catctgagaa atttacgtgg gcagcaaaag 600
gaagacctag gaagatcgca tgggagaaaa aagaaacacc tgtaaagact ggatgcgtgg 660
caagagtaac atctaataaa actaaagttt tggaaaaagg aagatctaag atgattgcat 720
gtcctacaaa agaatcatct acaaaagcaa gtgccaatga tcagaggttc ccatcagaat 780
ccaaacaaga ggaagatgaa gaatattctt gtgattctcg gagtctcttt gagagttctg 840
caaagattca agtgtgtata cctgagtcta tatatcaaaa agtaatggag ataaatagag 900
aagtagaaga gcctcctaag aagccatctg ccttcaagcc tgccattgaa atgcaaaact 960
ctgttccaaa taaagccttt gaattgaaga atgaacaaac attgagagca gatccgatgt 1020
tcccaccaga atccaaacaa aaggactatg aagaaaattc ttgggattct gagagtctct 1080
gtgagactgt ttcacagaag gatgtgtgtt tacccaaggc tacacatcaa aaagaaatag 1140
ataaaataaa tggaaaatta gaaggtaaga accgtttttt atttaaaaat cagttgaccg 1200
aatatttctc taaactgatg aggagggata tcctctagta gctgaagaaa attacctcct 1260
aaatgcaaac catggaaaaa aagagaagtg caatggtcgt aagttgtatg tctcatcagg 1320
tgttggcaac agactatatt gagagtgctg aaaaggagct gaattattag tttgaattca 1380
agatattgca agacctgaga gaaaaaaaaa aaaaaaaaaa aaaa                  1424
```

<210> 465
<211> 674
<212> DNA
<213> Homo sapiens

<400> 465

```
attccgagct gattacagac accaaggaag atgctgtaaa gagtcagcag ccacagccct 60
ggctagctgg ccctgtgggc atttattagt aaagttttaa tgacaaaagc tttgagtcaa 120
cacacccgtg ggtaattaac ctggtcatcc ccaccctgga gagccatcct gcccatgggt 180
gatcaaagaa ggaacatctg caggaacacc tgatgaggct gcacccttgg cggaaagaac 240
acctgacaca gctgaaagct tggtggaaaa aacacctgat gaggctgcac ccttggtgga 300
aagaacacct gacacggctg aaagcttggt ggaaaaaaca cctgatgagg ctgcatcctt 360
ggtggaggga acatctgaca aaattcaatg tttggagaaa gcgacatctg gaaagttcga 420
acagtcagca gaagaaacac ctagggaaat tacgagtcct gcaaaagaaa catctgagaa 480
atttacgtgg ccagcaaaag gaagacctag gaagatcgca tgggagaaaa aagatgactc 540
agttaaggca aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 600
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 660
aaaaaaaaaa aaaa                                                   674
```

<210> 466
<211> 1729
<212> DNA
<213> Homo sapiens

<220>
<221> unsure

<222> (11)
<223> n+A,T,C or G
<221> unsure
<222> (1128)
<223> n=A,T,C or G

<400> 466

```
gaaagttcga ncagtcagca gaagaaacac ctagggaaat tacgagtcct gcaaaagaaa 60
catctgagaa atttacgtgg ccagcaaaag gaagacctag gaagatcgca tgggagaaaa 120
aagaagacac acctagggaa attatgagtc ccgcaaaaga aacatctgag aaatttacgt 180
gggcagcaaa aggaagacct aggaagatcg catgggagaa aaaagaaaca cctgtaaaga 240
ctggatgcgt ggcaagagta acatctaata aaactaaagt tttggaaaaa ggaagatcta 300
agatgattgc atgtcctaca aaagaatcat ctacaaaagc aagtgccaat gatcagaggt 360
tcccatcaga atccaaacaa gaggaagatg aagaatattc ttgtgattct cggagtctct 420
ttgagagttc tgcaaagatt caagtgtgta tacctgagtc tatatatcaa aaagtaatgg 480
agataaatag agaagtagaa gagcctccta agaagccatc tgccttcaag cctgccattg 540
aaatgcaaaa ctctgttcca aataaagcct ttgaattgaa gaatgaacaa acattgagag 600
cagatccgat gttcccacca gaatccaaac aaaaggacta tgaagaaaat tcttgggatt 660
ctgagagtct ctgtgagact gtttcacaga aggatgtgtg tttacccaag gctacacatc 720
aaaaagaaat agataaaata aatggaaaat tagaagagtc tcctaataaa gatggtcttc 780
tgaaggctac ctgcggaatg aaagtttcta ttccaactaa agccttagaa ttgaaggaca 840
tgcaaacttt caaagcagag cctccgggga agccatctgc cttcgagcct gccactgaaa 900
tgcaaaagtc tgtcccaaat aaagccttgg aattgaaaaa tgaacaaaca ttgagagcag 960
atgagatact cccatcagaa tccaaacaaa aggactatga agaaaattct tgggatactg 1020
agagtctctg tgagactgtt tcacagaagg atgtgtgttt acccaaggct gcgcatcaaa 1080
aagaaataga taaaataaat ggaaaattag aagggtctcc tggtaaanat ggtcttctga 1140
aggctaactg cggaatgaaa gtttctattc caactaaagc cttagaattg atggacatgc 1200
aaactttcaa agcagagcct cccgagaagc catctgcctt cgagcctgcc attgaaatgc 1260
aaaagtctgt tccaaataaa gccttggaat tgaagaatga acaaacattg agagcagatg 1320
agatactccc atcagaatcc aaacaaaagg actatgaaga aagttcttgg gattctgaga 1380
gtctctgtga gactgtttca cagaaggatg tgtgtttacc caaggctgcg catcaaaaag 1440
aaatagataa aataaatgga aaattagaag gtaagaaccg tttttttattt aaaaatcatt 1500
tgaccaaata tttctctaaa ttgatgagga aggatatcct ctagtagctg aagaaaatta 1560
cctcctaaat gcaaaccatg gaaaaaaaga gaagtgcaat ggtcataagc tatgtgtctc 1620
atcaggcatt ggcaacagac tatattgtga gtgctgaaga ggagctgaat tactagttta 1680
aattcaagat attccaagac gtgaggaaaa tgagaaaaaa aaaaaaaaa 1729
```

<210> 467
<211> 1337
<212> DNA
<213> Homo sapiens

<400> 467

```
aaaaagaaat agataaaata aatggaaaat tagaagggtc tcctgttaaa gatggtcttc 60
tgaaggctaa ctgcggaatg aaagtttcta ttccaactaa agccttagaa ttgatggaca 120
tgcaaacttt caaagcagag cctcccgaga agccatctgc cttcgagcct gccattgaaa 180
tgcaaaagtc tgttccaaat aaagccttgg aattgaagaa tgaacaaaca ttgagagcag 240
atgagatact cccatcagaa tccaaacaaa aggactatga agaaagttct tgggattctg 300
agagtctctg tgagactgtt tcacagaagg atgtgtgttt acccaaggct gcgcatcaaa 360
```

aagaaataga taaaataaat ggaaaattag aagagtctcc tgataatgat ggttttctga 420
aggctccctg cagaatgaaa gtttctattc caactaaagc cttagaattg atggacatgc 480
aaactttcaa agcagagcct cccgagaagc catctgcctt cgagcctgcc attgaaatgc 540
aaaagtctgt tccaaataaa gccttggaat tgaagaatga acaaacattg agagcagatc 600
agatgttccc ttcagaatca aaacaaaaga aggttgaaga aaattcttgg gattctgaga 660
gtctccgtga gactgtttca cagaaggatg tgtgtgtacc caaggctaca catcaaaaag 720
aaatggataa aataagtgga aaattagaag attcaactag cctatcaaaa atcttggata 780
cagttcattc ttgtgaaaga gcaagggaac ttcaaaaaga tcactgtgaa caacgtacag 840
gaaaaatgga acaaatgaaa aagaagtttt gtgtactgaa aaagaaactg tcagaagcaa 900
aagaaataaa atcacagtta gagaaccaaa aagttaaatg ggaacaagag ctctgcagtg 960
tgagattgac tttaaaccaa gaagaagaga agagaagaaa tgccgatata ttaaatgaaa 1020
aaattaggga agaattagga agaatcgaag agcagcatag gaaagagtta gaagtgaaac 1080
aacaacttga acaggctctc agaatacaag atatagaatt gaagagtgta gaaagtaatt 1140
tgaatcaggt ttctcacact catgaaaatg aaaattatct cttacatgaa aattgcatgt 1200
tgaaaaagga aattgccatg ctaaaactgg aaatagccac actgaaacac caataccagg 1260
aaaaggaaaa taaatacttt gaggacatta agattttaaa agaaaagaat gctgaacttc 1320
agatgacccc tcgtgcc 1337

<210> 468
<211> 2307
<212> DNA
<213> Homo sapiens

<400> 468

attgagagca gatgagatac tcccatcaga atccaaacaa aaggactatg aagaaagttc 60
ttgggattct gagagtctct gtgagactgt ttcacagaag gatgtgtgtt tacccaaggc 120
tacacatcaa aaagaaatag ataaaataaa tggaaaatta gaagggtctc ctgttaaaga 180
tggtcttctg aaggctaact gcggaatgaa agtttctatt ccaactaaag ccttagaatt 240
gatggacatg caaactttca aagcagagcc tcccgagaag ccatctgcct tcgagcctgc 300
cattgaaatg caaaagtctg ttccaaataa agccttggaa ttgaagaatg aacaaacatt 360
gagagcagat gagatactcc catcagaatc caaacaaaag gactatgaag aaagttcttg 420
ggattctgag agtctctgtg agactgtttc acagaaggat gtgtgtttac ccaaggctac 480
acatcaaaaa gaaatagata aaataaatgg aaaattagaa gagtctcctg ataatgatgg 540
ttttctgaag tctccctgca gaatgaaagt ttctattcca actaaagcct tagaattgat 600
ggacatgcaa actttcaaag cagagcctcc cgagaagcca tctgccttcg agcctgccat 660
tgaaatgcaa aagtctgttc caaataaagc cttggaattg aagaatgaac aaacattgag 720
agcagatcag atgttccctt cagaatcaaa acaaaagaac gttgaagaaa attcttggga 780
ttctgagagt ctccgtgaga ctgtttcaca gaaggatgtg tgtgtaccca aggctacaca 840
tcaaaaagaa atggataaaa taagtggaaa attagaagat tcaactagcc tatcaaaaat 900
cttggataca gttcattctt gtgaaagagc aagggaactt caaaaagatc actgtgaaca 960
acgtacagga aaaatggaac aaatgaaaaa gaagttttgt gtactgaaaa agaaactgtc 1020
agaagcaaaa gaaataaaat cacagttaga gaaccaaaaa gttaaatggg aacaagagct 1080
ctgcagtgtg aggtttctca cactcatgaa aatgaaaatt atctcttaca tgaaaattgc 1140
atgttgaaaa aggaaattgc catgctaaaa ctggaaatag ccacactgaa acaccaatac 1200
caggaaaagg aaaataaata ctttgaggac attaagattt taaaagaaaa gaatgctgaa 1260
cttcagatga ccctaaaact gaaagaggaa tcattaacta aaagggcatc tcaatatagt 1320
gggcagctta aagttctgat agctgagaac acaatgctca cttctaaatt gaaggaaaaa 1380
caagacaaag aaatactaga ggcagaaatt gaatcacacc atcctagact ggcttctgct 1440
gtacaagacc atgatcaaat tgtgacatca agaaaaagtc aagaacctgc tttccacatt 1500
gcaggagatg cttgtttgca aagaaaaatg aatgttgatg tgagtagtac gatatataac 1560
aatgaggtgc tccatcaacc actttctgaa gctcaaagga aatccaaaag cctaaaaatt 1620
aatctcaatt atgcaggaga tgctctaaga gaaaatacat tggtttcaga acatgcacaa 1680
agagaccaac gtgaaacaca gtgtcaaatg aaggaagctg aacacatgta tcaaaacgaa 1740
caagataatg tgaacaaaca cactgaacag caggagtctc tagatcagaa attatttcaa 1800
ctacaaagca aaaatatgtg gcttcaacag caattagttc atgcacataa gaaagctgac 1860

```
aacaaaagca agataacaat tgatattcat tttcttgaga ggaaaatgca acatcatctc 1920
ctaaaagaga aaaatgagga gatatttaat tacaataacc atttaaaaaa ccgtatatat 1980
caatatgaaa aagagaaagc agaaacagaa aactcatgag agacaagcag taagaaactt 2040
cttttggaga aacaacagac cagatcttta ctcacaactc atgctaggag gccagtccta 2100
gcatcacctt atgttgaaaa tcttaccaat agtctgtgtc aacagaatac ttattttaga 2160
agaaaaattc atgatttctt cctgaagcct acagacataa aataacagtg tgaagaatta 2220
cttgttcacg aattgcataa agctgcacag gattcccatc taccctgatg atgcagcaga 2280
catcattcaa tccaaccaga atctcgc                                     2307
```

<21> 469
<211> 650
<212> PRT
<213> Homo sapiens

<220>
<221> unsure
<222> (310)
<223> Xaa = Any Amino Acid<221> unsure
<222> (429)
<223> xaa = Any Amino Acid<221> unsure
<222> (522)
<223> Xaa = Any Amino Acid

<400> 469

```
Met Ser Pro Ala Lys Glu Thr Ser Glu Lys Phe Thr Trp Ala Ala Lys
                    5                   10                  15

Gly Arg Pro Arg Lys Ile Ala Trp Glu Lys Lys Glu Thr Pro Val Lys
            20                  25                  30

Thr Gly Cys Val Ala Arg Val Thr Ser Asn Lys Thr Lys Val Leu Glu
        35                  40                  45

Lys Gly Arg Ser Lys Met Ile Ala Cys Pro Thr Lys Glu Ser Ser Thr
    50                  55                  60

Lys Ala Ser Ala Asn Asp Gln Arg Phe Pro Ser Glu Ser Lys Gln Glu
65                  70                  75                  80

Glu Asp Glu Glu Tyr Ser Cys Asp Ser Arg Ser Leu Phe Glu Ser Ser
                85                  90                  95

Ala Lys Ile Gln Val Cys Ile Pro Glu Ser Ile Tyr Gln Lys Val Met
            100                 105                 110

Glu Ile Asn Arg Glu Val Glu Glu Pro Pro Lys Lys Pro Ser Ala Phe
        115                 120                 125

Lys Pro Ala Ile Glu Met Gln Asn Ser Val Pro Asn Lys Ala Phe Glu
    130                 135                 140

Leu Lys Asn Glu Gln Thr Leu Arg Ala Asp Pro Met Phe Pro Pro Glu
145                 150                 155                 160

Ser Lys Gln Lys Asp Tyr Glu Glu Asn Ser Trp Asp Ser Glu Ser Leu
```

```
                    165                 170                 175
Cys Glu Thr Val Ser Gln Lys Asp Val Cys Leu Pro Lys Ala Thr His
                180                 185                 190
Gln Lys Glu Ile Asp Lys Ile Asn Gly Lys Leu Glu Glu Ser Pro Asn
            195                 200                 205
Lys Asp Gly Leu Leu Lys Ala Thr Cys Gly Met Lys Val Ser Ile Pro
    210                 215                 220
Thr Lys Ala Leu Glu Leu Lys Asp Met Gln Thr Phe Lys Ala Glu Pro
225                 230                 235                 240
Pro Gly Lys Pro Ser Ala Phe Glu Pro Ala Thr Glu Met Gln Lys Ser
                245                 250                 255
Val Pro Asn Lys Ala Leu Glu Leu Lys Asn Glu Gln Thr Leu Arg Ala
            260                 265                 270
Asp Glu Ile Leu Pro Ser Glu Ser Lys Gln Lys Asp Tyr Glu Glu Ser
        275                 280                 285
Ser Trp Asp Ser Glu Ser Leu Cys Glu Thr Val Ser Gln Lys Asp Val
    290                 295                 300
Cys Leu Pro Lys Ala Xaa His Gln Lys Glu Ile Asp Lys Ile Asn Gly
305                 310                 315                 320
Lys Leu Glu Gly Ser Pro Val Lys Asp Gly Leu Leu Lys Ala Asn Cys
                325                 330                 335
Gly Met Lys Val Ser Ile Pro Thr Lys Ala Leu Glu Leu Met Asp Met
            340                 345                 350
Gln Thr Phe Lys Ala Glu Pro Pro Glu Lys Pro Ser Ala Phe Glu Pro
        355                 360                 365
Ala Ile Glu Met Gln Lys Ser Val Pro Asn Lys Ala Leu Glu Leu Lys
    370                 375                 380
Asn Glu Gln Thr Leu Arg Ala Asp Glu Ile Leu Pro Ser Glu Ser Lys
385                 390                 395                 400
Gln Lys Asp Tyr Glu Glu Ser Ser Trp Asp Ser Glu Ser Leu Cys Glu
                405                 410                 415
Thr Val Ser Gln Lys Asp Val Cys Leu Pro Lys Ala Xaa His Gln Lys
            420                 425                 430
Glu Ile Asp Lys Ile Asn Gly Lys Leu Glu Glu Ser Pro Asp Asn Asp
        435                 440                 445
Gly Phe Leu Lys Ala Pro Cys Arg Met Lys Val Ser Ile Pro Thr Lys
    450                 455                 460
```

Ala Leu Glu Leu Met Asp Met Gln Thr Phe Lys Ala Glu Pro Pro Glu
465 470 475 480

Lys Pro Ser Ala Phe Glu Pro Ala Ile Glu Met Gln Lys Ser Val Pro
485 490 495

Asn Lys Ala Leu Glu Leu Lys Asn Glu Gln Thr Leu Arg Ala Asp Gln
500 505 510

Met Phe Pro Ser Glu Ser Lys Gln Lys Xaa Val Glu Glu Asn Ser Trp
515 520 525

Asp Ser Glu Ser Leu Arg Glu Thr Val Ser Gln Lys Asp Val Cys Val
530 535 540

Pro Lys Ala Thr His Gln Lys Glu Met Asp Lys Ile Ser Gly Lys Leu
545 550 555 560

Glu Asp Ser Thr Ser Leu Ser Lys Ile Leu Asp Thr Val His Ser Cys
565 570 575

Glu Arg Ala Arg Glu Leu Gln Lys Asp His Cys Glu Gln Arg Thr Gly
580 585 590

Lys Met Glu Gln Met Lys Lys Lys Phe Cys Val Leu Lys Lys Lys Leu
595 600 605

Ser Glu Ala Lys Glu Ile Lys Ser Gln Leu Glu Asn Gln Lys Val Lys
610 615 620

Trp Glu Gln Glu Leu Cys Ser Val Arg Phe Leu Thr Leu Met Lys Met
625 630 635 640

Lys Ile Ile Ser Tyr Met Lys Ile Ala Cys
645 650

<210> 470
<211> 228
<212> PRT
<213> Homo sapiens

<400> 470

Met Ser Pro Ala Lys Glu Thr Ser Glu Lys Phe Thr Trp Ala Ala Lys
5 10 15

Gly Arg Pro Arg Lys Ile Ala Trp Glu Lys Lys Glu Thr Pro Val Lys
20 25 30

Thr Gly Cys Val Ala Arg Val Thr Ser Asn Lys Thr Lys Val Leu Glu
35 40 45

Lys Gly Arg Ser Lys Met Ile Ala Cys Pro Thr Lys Glu Ser Ser Thr
50 55 60

Lys Ala Ser Ala Asn Asp Gln Arg Phe Pro Ser Glu Ser Lys Gln Glu
65 70 75 80

5

Glu Asp Glu Glu Tyr Ser Cys Asp Ser Arg Ser Leu Phe Glu Ser Ser
85 90 95

Ala Lys Ile Gln Val Cys Ile Pro Glu Ser Ile Tyr Gln Lys Val Met
100 105 110

10

Glu Ile Asn Arg Glu Val Glu Glu Pro Pro Lys Lys Pro Ser Ala Phe
115 120 125

Lys Pro Ala Ile Glu Met Gln Asn Ser Val Pro Asn Lys Ala Phe Glu
130 135 140

15

Leu Lys Asn Glu Gln Thr Leu Arg Ala Asp Pro Met Phe Pro Pro Glu
145 150 155 160

20

Ser Lys Gln Lys Asp Tyr Glu Glu Asn Ser Trp Asp Ser Glu Ser Leu
165 170 175

Cys Glu Thr Val Ser Gln Lys Asp Val Cys Leu Pro Lys Ala Thr His
180 185 190

25

Gln Lys Glu Ile Asp Lys Ile Asn Gly Lys Leu Glu Gly Lys Asn Arg
195 200 205

Phe Leu Phe Lys Asn Gln Leu Thr Glu Tyr Phe Ser Lys Leu Met Arg
210 215 220

30

Arg Asp Ile Leu
225

<210> 471

35

<211> 154
<212> PRT
<213> Homo sapiens

<220>

40

<221> unsure
<222> (148)
<Z23> Xaa = Any Amino Acid

<400> 471

45

Met Arg Leu His Pro Trp Arg Lys Glu His Leu Thr Gln Leu Lys Ala
5 10 15

50

Trp Trp Lys Lys His Leu Met Arg Leu His Pro Trp Trp Lys Glu His
20 25 30

Leu Thr Arg Leu Lys Ala Trp Trp Lys Lys His Leu Met Arg Leu His
35 40 45

55

Pro Trp Trp Arg Glu His Leu Thr Lys Phe Asn Val Trp Arg Lys Arg
50 55 60

His Leu Glu Ser Ser Asn Ser Gln Gln Lys Lys His Leu Gly Lys Leu
65 70 75 80

Arg Val Leu Gln Lys Lys His Leu Arg Asn Leu Arg Gly Gln Gln Lys
85 90 95

Glu Asp Leu Gly Arg Ser His Gly Arg Lys Lys Met Thr Gln Leu Arg
100 105 110

Gln Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys
115 120 125

Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys
130 135 140

Lys Lys Lys Xaa Lys Lys Lys Lys Lys Lys
145 150

<210> 472
<211> 467
<212> PRT
<213> Homo sapiens

<220>
<221> unsure
<222> (329)
<223> Xaa = Any Amino Acid

<400> 472

Met Ser Pro Ala Lys Glu Thr Ser Glu Lys Phe Thr Trp Ala Ala Lys
5 10 15

Gly Arg Pro Arg Lys Ile Ala Trp Glu Lys Lys Glu Thr Pro Val Lys
20 25 30

Thr Gly Cys Val Ala Arg Val Thr Ser Asn Lys Thr Lys Val Leu Glu
35 40 45

Lys Gly Arg Ser Lys Met Ile Ala Cys Pro Thr Lys Glu Ser Ser Thr
50 55 60

Lys Ala Ser Ala Asn Asp Gln Arg Phe Pro Ser Glu Ser Lys Gln Glu
65 70 75 80

Glu Asp Glu Glu Tyr Ser Cys Asp Ser Arg Ser Leu Phe Glu Ser Ser
85 90 95

Ala Lys Ile Gln Val Cys Ile Pro Glu Ser Ile Tyr Gln Lys Val Met
100 105 110

Glu Ile Asn Arg Glu Val Glu Glu Pro Pro Lys Lys Pro Ser Ala Phe

115 120 125

Lys Pro Ala Ile Glu Met Gln Asn Ser Val Pro Asn Lys Ala Phe Glu
130 135 140

Leu Lys Asn Glu Gln Thr Leu Arg Ala Asp Pro Met Phe Pro Pro Glu
145 150 155 160

Ser Lys Gln Lys Asp Tyr Glu Glu Asn Ser Trp Asp Ser Glu Ser Leu
165 170 175

Cys Glu Thr Val Ser Gln Lys Asp Val Cys Leu Pro Lys Ala Thr His
180 185 190

Gln Lys Glu Ile Asp Lys Ile Asn Gly Lys Leu Glu Glu Ser Pro Asn
195 200 205

Lys Asp Gly Leu Leu Lys Ala Thr Cys Gly Met Lys Val Ser Ile Pro
210 215 220

Thr Lys Ala Leu Glu Leu Lys Asp Met Gln Thr Phe Lys Ala Glu Pro
225 230 235 240

Pro Gly Lys Pro Ser Ala Phe Glu Pro Ala Thr Glu Met Gln Lys Ser
245 250 255

Val Pro Asn Lys Ala Leu Glu Leu Lys Asn Glu Gln Thr Leu Arg Ala
260 265 270

Asp Glu Ile Leu Pro Ser Glu Ser Lys Gln Lys Asp Tyr Glu Glu Asn
275 280 285

Ser Trp Asp Thr Glu Ser Leu Cys Glu Thr Val Ser Gln Lys Asp Val
290 295 300

Cys Leu Pro Lys Ala Ala His Gln Lys Glu Ile Asp Lys Ile Asn Gly
305 310 315 320

Lys Leu Glu Gly Ser Pro Gly Lys Xaa Gly Leu Leu Lys Ala Asn Cys
325 330 335

Gly Met Lys Val Ser Ile Pro Thr Lys Ala Leu Glu Leu Met Asp Met
340 345 350

Gln Thr Phe Lys Ala Glu Pro Pro Glu Lys Pro Ser Ala Phe Glu Pro
355 360 365

Ala Ile Glu Met Gln Lys Ser Val Pro Asn Lys Ala Leu Glu Leu Lys
370 375 380

Asn Glu Gln Thr Leu Arg Ala Asp Glu Ile Leu Pro Ser Glu Ser Lys
385 390 395 400

Gln Lys Asp Tyr Glu Glu Ser Ser Trp Asp Ser Glu Ser Leu Cys Glu
405 410 415

Thr Val Ser Gln Lys Asp Val Cys Leu Pro Lys Ala Ala His Gln Lys
420 425 430

Glu Ile Asp Lys Ile Asn Gly Lys Leu Glu Gly Lys Asn Arg Phe Leu
435 440 445

Phe Lys Asn His Leu Thr Lys Tyr Phe Ser Lys Leu Met Arg Lys Asp
450 455 460

Ile Leu
465

<210> 473
<211> 445
<212> PRT
<2i3> Homo sapiens

<400> 473

Lys Glu Ile Asp Lys Ile Asn Gly Lys Leu Glu Gly Ser Pro Val Lys
5 10 15

Asp Gly Leu Leu Lys Ala Asn Cys Gly Met Lys Val Ser Ile Pro Thr
20 25 30

Lys Ala Leu Glu Leu Met Asp Met Gln Thr Phe Lys Ala Glu Pro Pro
35 40 45

Glu Lys Pro Ser Ala Phe Glu Pro Ala Ile Glu Met Gln Lys Ser Val
50 55 60

Pro Asn Lys Ala Leu Glu Leu Lys Asn Glu Gln Thr Leu Arg Ala Asp
65 70 75 80

Glu Ile Leu Pro Ser Glu Ser Lys Gln Lys Asp Tyr Glu Glu Ser Ser
85 90 95

Trp Asp Ser Glu Ser Leu Cys Glu Thr Val Ser Gln Lys Asp Val Cys
100 105 110

Leu Pro Lys Ala Ala His Gln Lys Glu Ile Asp Lys Ile Asn Gly Lys
115 120 125

Leu Glu Glu Ser Pro Asp Asn Asp Gly Phe Leu Lys Ala Pro Cys Arg
130 135 140

Met Lys Val Ser Ile Pro Thr Lys Ala Leu Glu Leu Met Asp Met Gln
145 150 155 160

Thr Phe Lys Ala Glu Pro Pro Glu Lys Pro Ser Ala Phe Glu Pro Ala
165 170 175

Ile Glu Met Gln Lys Ser Val Pro Asn Lys Ala Leu Glu Leu Lys Asn
180 185 190

```
Glu Gln Thr Leu Arg Ala Asp Gln Met Phe Pro Ser Glu Ser Lys Gln
        195                 200                 205

Lys Lys Val Glu Glu Asn Ser Trp Asp Ser Glu Ser Leu Arg Glu Thr
    210                 215                 220

Val Ser Gln Lys Asp Val Cys Val Pro Lys Ala Thr His Gln Lys Glu
225                 230                 235                 240

Met Asp Lys Ile Ser Gly Lys Leu Glu Asp Ser Thr Ser Leu Ser Lys
                245                 250                 255

Ile Leu Asp Thr Val His Ser Cys Glu Arg Ala Arg Glu Leu Gln Lys
            260                 265                 270

Asp His Cys Glu Gln Arg Thr Gly Lys Met Glu Gln Met Lys Lys Lys
        275                 280                 285

Phe Cys Val Leu Lys Lys Lys Leu Ser Glu Ala Lys Glu Ile Lys Ser
    290                 295                 300

Gln Leu Glu Asn Gln Lys Val Lys Trp Glu Gln Glu Leu Cys Ser Val
305                 310                 315                 320

Arg Leu Thr Leu Asn Gln Glu Glu Glu Lys Arg Arg Asn Ala Asp Ile
                325                 330                 335

Leu Asn Glu Lys Ile Arg Glu Glu Leu Gly Arg Ile Glu Glu Gln His
            340                 345                 350

Arg Lys Glu Leu Glu Val Lys Gln Gln Leu Glu Gln Ala Leu Arg Ile
        355                 360                 365

Gln Asp Ile Glu Leu Lys Ser Val Glu Ser Asn Leu Asn Gln Val Ser
    370                 375                 380

His Thr His Glu Asn Glu Asn Tyr Leu Leu His Glu Asn Cys Met Leu
385                 390                 395                 400

Lys Lys Glu Ile Ala Met Leu Lys Leu Glu Ile Ala Thr Leu Lys His
                405                 410                 415

Gln Tyr Gln Glu Lys Glu Asn Lys Tyr Phe Glu Asp Ile Lys Ile Leu
            420                 425                 430

Lys Glu Lys Asn Ala Glu Leu Gln Met Thr Pro Arg Ala
        435                 440                 445
```

Claims

1. An isolated polypeptide comprising at least a portion of a breast tumor protein, wherein the polypeptide comprises an amino acid sequence that is encoded by the polynucleotide sequence recited in SEQ ID NO: 175, or a variant of said polypeptide wherein the variant comprises an amino acid sequence that is encoded by a polynucleotide sequence that has at least 90% identity to a sequence of SEQ ID NO: 175, and the ability of the variant to react with antigen specific antisera is enhanced, unchanged or diminished by less than 50% relative to a polypeptide comprising an amino acid sequence that is encoded by SEQ ID NO: 175; provided that the polypeptide does not have the sequence recited in SEQ ID NO: 176 with the substitution of Asn Ser for Val Ile at positions 316 and 317.

2. An isolated polypeptide according to claim 1, wherein the polypeptide is a variant polypeptide comprising an amino acid sequence that is encoded by a polynucleotide having at least 90% identity to the sequence of SEQ ID NO: 175, and the ability of the variant to react with antigen specific antisera is diminished by less than 20% relative to a polypeptide comprising an amino acid sequence that is encoded by SEQ ID NO: 175.

3. An isolated polypeptide according to claim 1, wherein the polypeptide comprises an amino acid sequence that is encoded by the polynucleotide sequence recited in SEQ ID NO: 175.

4. An isolated polypeptide comprising the sequence of SEQ ID NO: 176.

5. An isolated polynucleotide encoding a polypeptide according to claim 1.

6. An isolated polynucleotide encoding a breast tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide comprising the sequence recited in SEQ ID NO: 175.

7. An isolated polynucleotide comprising a sequence recited in SEQ ID NO: 175

8. An isolated polynucleotide comprising a sequence that has at least 90% identity to the sequence recited in SEQ ID NO: 175, and which encodes a polypeptide having the ability to react with antigen specific antisera that is enhanced unchanged or diminished by less than 50% relative to a polypeptide comprising an amino acid sequence that is encoded by SEQ ID NO: 175.

9. An isolated polynucleotide according to claim 8, wherein the polypeptide encoded by that polynucleotide has the ability to react with antigen specific antisera that is diminished by less than 20% relative to a polypeptide comprising an amino acid sequence that is encoded by SEQ ID NO: 175.

10. An isolated polynucleotide complementary to a polynucleotide according to any one of claims 5-9.

11. An expression vector comprising a polynucleotide according to any one of claim 5 - 9.

12. A host cell transformed or transfected with an expression vector according to claim 11.

13. An expression vector comprising a polynucleotide according to claim 10.

14. A host cell transformed or transfected with an expression vector according to claim 13.

15. A vaccine comprising a polypeptide according to claim 1, in combination with an immunostimulant.

16. A vaccine comprising a polynucleotide according to claim 5, in combination with an immunostimulant.

17. An isolated antibody, or antigen-binding fragment thereof, that specifically binds to the portion of a breast tumor protein that comprises an amino acid sequence that is encoded by the polynucleotide sequence recited in SEQ ID NO: 175.

18. A vaccine comprising an antigen-presenting cell that expresses a polypeptide according to claim 1, in combination with an immunostimulant.

19. A vaccine according to claim 18, wherein the antigen-presenting cell is a dendritic cell.

20. A polypeptide according to claim 1 for use in a method for inhibiting the development of a breast cancer in a patient.

21. A polynucleotide according to claim 5 for use in a method for inhibiting the development of a breast cancer in a patient.

22. An antibody or antigen-binding fragment thereof of claim 17 for use in a method for inhibiting the development of a breast cancer in a patient.

23. An antigen-presenting cell that expresses a polypeptide according to claim 1 for use in a method for inhibiting the development of a breast cancer in a patient.

24. A cell according to claim 23, wherein the antigen-presenting cell is a dendritic cell.

25. A fusion protein comprising at least one polypeptide according to claim 1.

26. A fusion protein according to claim 25,wherein the fusion protein comprises an expression enhancer that increases expression of the fusion protein in a host cell transfected with a polynucleotide encoding the fusion protein.

27. A fusion protein according to claim 26, wherein the fusion protein comprises a T helper epitope that is not present within the polypeptide of claim 1.

28. A fusion protein according to claim 26, wherein the fusion protein comprises an affinity tag.

29. An isolated polynucleotide encoding a fusion protein according to claim 25.

30. A vaccine comprising a fusion protein according to claim 25, in combination with an immunostimulant.

31. A vaccine comprising a polynucleotide according to claim 29, in combination with an immunostimulant.

32. A vaccine according to claim 15, 16, 18, 30 or 31, wherein the immunostimulant is an adjuvant.

33. A vaccine according to claim 15, 16, 18, 30 or 31, wherein the immunostimulant induces a predominantly Type I response.

34. A vaccine according to claim 30 or claim 31 for use in a method for inhibiting the development of a breast cancer in a patient.

35. A method for removing tumor cells from a biological sample, comprising contacting a biological sample with T cells that specifically react with a breast tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by the polynucleotide sequence recited in SEQ ID NO: 175, wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of cells expressing the antigen from the sample.

36. A method according to claim 35, wherein the biological sample is blood or a fraction thereof.

37. A method for stimulating and/or expanding T cells specific for a breast tumor protein, carried out ex vivo, comprising contacting T cells with one or more of:

(i) a polypeptide according to claim 1;
(ii) a polynucleotide encoding such a polypeptide; and/or
(iii) an antigen presenting cell that expresses such a polypeptide;

under conditions and for a time sufficient to permit the stimulation and/or expansion of T cells.

38. An isolated T cell population specific for a breast tumor protein according to claim 1 comprising T cells obtainable according to the method of claim 37.

39. A T cell population according to claim 38 for use in a method for inhibiting the development of abreast cancer in a patient.

40. Proliferated CD4+ and /or CD8+ T cells isolated from a patient with at least one component selected from the group consisting of:

(i) a polypeptide according to claim 1;
(ii) a polynucleotide encoding such a polypeptide; or
(iii) an antigen-presenting cell that expresses such a polypeptide and incubated such that T cells proliferate for use in a method for inhibiting the development of a breast cancer in a patient.

41. T cells according to claim 40 for use in a method for inhibiting the development of a cancer in a patient, comprising

the steps of:

(a) cloning at least one proliferated cell; and
(b) administering to the patient an effective amount of the cloned T cells, and thereby inhibiting the development of a breast cancer in the patient.

42. A method for determining the presence or absence of a breast cancer in a patient, comprising the steps of:

(a) contacting a biological sample obtained from a patient with an antibody, or antigen binding fragment thereof, that specifically binds to the portion of a breast tumor protein, that is encoded by the polynucleotide sequence recited in SEQ ID NO: 175; and
(b) detecting in the sample an amount of polypeptide that specifically binds to the antibody and/or antigen binding fragment thereof comparing the amount of polypeptide to a predetermined cut-off value, and therefrom determining the presence or absence of a breast cancer in the patient.

43. A method for monitoring the progression of a breast cancer in a patient, comprising the steps of:

(a) contacting a biological sample obtaining from a patient at a first point in time with an antibody, or antigen binding fragment thereof, that specifically binds to the portion of a breast tumor protein, that is encoded by the polynucleotide sequence recited in SEQ IDNO: 175;
(b) detecting in the sample an amount of polypeptide that specifically binds to the antibody or antigen binding fragment thereof;
(c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and
(d) comparing the amount of polypeptide detected in step (c) to the amount detected instep (b) and therefrom monitoring the progression of the breast cancer in the patient.

44. A method according to claim 42 or 43, wherein the antibody is a monoclonal antibody.

45. A method for determining the presence or absence of a breast cancer in a patient, comprising the steps of:

(a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a breast tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by the polynucleotide sequence recited in SEQ ID NO: 175;
(b) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide; and
(c) comparing the amount of polynucleotide that hybridizes to the oligonucleotide to a predetermined cut-off value, and therefrom determining the presence or absence of a breast cancer in the patient.

46. A method for monitoring the progression of a breast cancer in a patient, comprising the steps of:

(a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a breast tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by the polynucleotide sequence recited in SEQ ID NO: 175;
(b) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide;
(c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and
(d) comparing the amount of a polynucleotide detected in step (c) to the amount detected in step (b) and therefrom monitoring the progression of the breast cancer in the patient.

47. A method according to claim 45 or 46, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a polymerase chain reaction.

48. A method according to claim 45 or 46, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a hybridization assay.

49. A diagnostic kit comprising:

(a) one or more antibodies according to claim 17; and

(b) a detection reagent comprising a reporter group.

50. A kit according to claim 49, wherein the antibodies are immobilized on a solid support.

5

51. A kit according to claim 50, wherein the solid support comprises nitrocellulose, latex or a plastic material.

52. A kit according to claim 49, wherein the detection reagent comprises an anti-immunoglobulin, protein G, protein A or lectin.

10

53. A kit according to claim 49, wherein the reporter group is selected from the group consisting of radioisotopes, fluorescent groups, luminescent groups, enzymes, biotin and dye particles.

54. An oligonucleotide comprising 10 to 40 contiguous nucleotides of a polynucleotide that encodes a breast tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by the polynucleotide sequence recited in SEQ ID NO: 175.

15

55. An oligonucleotide according to claim 54, wherein the oligonucleotide comprises 10-40 contiguous nucleotides recited in SEQ ID NO: 175.

20

56. A diagnostic kit, comprising:

(a) an oligonucleotide according to claim 54; and
(b) a diagnostic reagent for use in a polymerase chain reaction or hybridization assay.

25

Patentansprüche

1. Isoliertes Polypeptid, umfassend wenigstens einen Teil eines Brusttumor-Proteins, wobei das Polypeptid eine Aminosäuresequenz umfasst, die von der in SEQ ID NO: 175 genannten Polynucleotidsequenz codiert wird, oder eine Variante dieses Polypeptids, wobei die Variante eine Aminosäuresequenz umfasst, die von einer Polynucleotidsequenz codiert wird, die wenigstens 90 % Identität mit der Sequenz von SEQ ID NO: 175 aufweist, und die Fähigkeit der Variante zum Reagieren mit antigenspezifischen Antisera im Vergleich zu einem Polypeptid, das eine Aminosäuresequenz umfasst, die von SEQ ID NO: 175 codiert wird, erhöht, unverändert oder um weniger als 50 % abgeschwächt ist; mit der Maßgabe, dass das Polypeptid nicht die in SEQ ID NO: 176 genannte Sequenz mit der Substitution von Asn Ser für Val Ile an den Positionen 316 und 317 aufweist.

30

35

2. Isoliertes Polypeptid gemäß Anspruch 1, wobei das Polypeptid ein variantes Polypeptid ist, umfassend eine Aminosäuresequenz, die von einem Polynucleotid codiert wird, das wenigstens 90 % Identität mit der Sequenz von SEQ ID NO: 175 aufweist, und die Fähigkeit der Variante zum Reagieren mit antigenspezifischen Antisera im Vergleich zu einem Polypeptid, das eine Aminosäuresequenz umfasst, die von SEQ ID NO: 175 codiert wird, um weniger als 20 % abgeschwächt ist.

40

3. Isoliertes Polypeptid gemäß Anspruch 1, wobei das Polypeptid eine Aminosäuresequenz umfasst, die von der in SEQ ID NO: 175 genannten Polynucleotidsequenz codiert wird.

45

4. Isoliertes Polypeptid, umfassend die Sequenz von SEQ ID NO: 176.

5. Isoliertes Polynucleotid, das ein Polypeptid gemäß Anspruch 1 codiert.

50

6. Isoliertes Polynucleotid, das ein Brusttumor-Protein codiert, wobei das Tumorprotein eine Aminosäuresequenz umfasst, die von einem Polynucleotid codiert wird, das die in SEQ ID NO: 175 genannte Polynucleotidsequenz umfasst.

7. Isoliertes Polynucleotid, umfassend eine Sequenz, die in SEQ ID NO: 175 genannt wird.

55

8. Isoliertes Polynucleotid, umfassend eine Sequenz, die wenigstens 90 % Identität mit der in SEQ ID NO: 175 genannten Sequenz aufweist, und das ein Polypeptid codiert, das eine Fähigkeit zum Reagieren mit antigenspezifischen Antisera aufweist, die im Vergleich zu einem Polypeptid, das eine Aminosäuresequenz umfasst, die von SEQ

ID NO: 175 codiert wird, erhöht, unverändert oder um weniger als 50 % abgeschwächt ist.

9. Isoliertes Polynucleotid gemäß Anspruch 8, wobei die Fähigkeit des Polypeptids, das von dem Polynucleotid codiert wird, eine Fähigkeit zum Reagieren mit antigenspezifischen Antisera aufweist, die im Vergleich zu einem Polypeptid, das eine Aminosäuresequenz umfasst, die von SEQ ID NO: 175 codiert wird, um weniger als 20 % abgeschwächt ist.

10. Isoliertes Polynucleotid, das zu einem Polynucleotid gemäß einem der Ansprüche 5-9 komplementär ist.

11. Expressionsvektor, umfassend ein Polynucleotid gemäß einem der Ansprüche 5-9.

12. Wirtszelle, die mit einem Expressionsvektor gemäß Anspruch 11 transformiert oder transfektiert ist.

13. Expressionsvektor, umfassend ein Polynucleotid gemäß Anspruch 10.

14. Wirtszelle, die mit einem Expressionsvektor gemäß Anspruch 13 transformiert oder transfektiert ist.

15. Impfstoff, umfassend ein Polypeptid gemäß Anspruch 1 in Kombination mit einem Immunostimulans.

16. Impfstoff, umfassend ein Polynucleotid gemäß Anspruch 5 in Kombination mit einem Immunostimulans.

17. Isolierter Antikörper oder Antigen-bindendes Fragment davon, der spezifisch an den Teil eines Brusttumor-Proteins bindet, der eine Aminosäuresequenz umfasst, die von der in SEQ ID NO: 175 genannten Polynucleotidsequenz codiert wird.

18. Impfstoff, umfassend eine Antigen-präsentierende Zelle, die ein Polypeptid gemäß Anspruch 1 exprimiert, in Kombination mit einem Immunostimulans.

19. Impfstoff gemäß Anspruch 18, wobei die Antigen-präsentierende Zelle eine dendritische Zelle ist.

20. Polypeptid gemäß Anspruch 1 zur Verwendung bei einem Verfahren zum Hemmen der Entwicklung eines Brustkrebses bei einem Patienten.

21. Polynucleotid gemäß Anspruch 5 zur Verwendung bei einem Verfahren zum Hemmen der Entwicklung eines Brustkrebses bei einem Patienten.

22. Antikörper oder Antigen-bindendes Fragment davon gemäß Anspruch 17 zur Verwendung bei einem Verfahren zum Hemmen der Entwicklung eines Brustkrebses bei einem Patienten.

23. Antigen-präsentierende Zelle, die ein Polypeptid gemäß Anspruch 1 exprimiert, zur Verwendung bei einem Verfahren zum Hemmen der Entwicklung eines Brustkrebses bei einem Patienten.

24. Zelle gemäß Anspruch 23, wobei die Antigen-präsentierende Zelle eine dendritische Zelle ist.

25. Fusionsprotein, umfassend wenigstens ein Polypeptid gemäß Anspruch 1.

26. Fusionsprotein gemäß Anspruch 25, wobei das Fusionsprotein einen Expressionsverstärker umfasst, der die Expression des Fusionsproteins in einer Wirtszelle erhöht, die mit einem Polynucleotid, welches das Fusionsprotein codiert, transfektiert ist.

27. Fusionsprotein gemäß Anspruch 26, wobei das Fusionsprotein ein T-Helfer-Epitop umfasst, das in dem Polypeptid gemäß Anspruch 1 nicht vorhanden ist.

28. Fusionsprotein gemäß Anspruch 26, wobei das Fusionsprotein eine Affinitätsmarkierung umfasst.

29. Isoliertes Polynucleotid, das ein Fusionsprotein gemäß Anspruch 25 codiert.

30. Impfstoff, umfassend ein Fusionsprotein gemäß Anspruch 25 in Kombination mit einem Immunostimulans.

31. Impfstoff, umfassend ein Polynucleotid gemäß Anspruch 29 in Kombination mit einem Immunostimulans.

32. Impfstoff gemäß Anspruch 15, 16, 18, 30 oder 31, wobei das Immunostimulans ein Hilfsstoff ist.

33. Impfstoff gemäß Anspruch 15, 16, 18, 30 oder 31, wobei das Immunostimulans eine vorherrschend Typ-I-Antwort induziert.

34. Impfstoff gemäß Anspruch 30 oder 31 zur Verwendung bei einem Verfahren zum Hemmen der Entwicklung eines Brustkrebses bei einem Patienten.

35. Verfahren zum Entfernen von Tumorzellen aus einer biologischen Probe, umfassend das Kontaktieren einer biologischen Probe mit T-Zellen, die spezifisch mit einem Brusttumor-Protein reagieren, wobei das Tumorprotein eine Aminosäuresequenz umfasst, die von der in SEQ ID NO: 175 genannten Polynucleotidsequenz codiert wird, wobei der Schritt des Kontaktierens unter Bedingungen und über eine Zeit durchgeführt wird, die ausreichen, um das Entfernen von Zellen, die das Antigen exprimieren, aus der Probe zu ermöglichen.

36. Verfahren gemäß Anspruch 35, wobei die biologische Probe Blut oder eine Fraktion davon ist.

37. Verfahren zum Stimulieren und/oder Expandieren von T-Zellen, die für ein Brusttumor-Protein spezifisch sind, das ex vivo durchgeführt wird, umfassend das Kontaktieren von T-Zellen mit einem oder mehreren von:

(i) einem Polypeptid gemäß Anspruch 1;
(ii) einen Polynucleotid, das ein derartiges Polypeptid codiert; und/oder
(iii) einer Antigen-präsentierenden Zelle, die ein derartiges Polypeptid exprimiert;

unter Bedingungen und über eine Zeit, die ausreichen, um die Stimulation und/oder Expansion von T-Zellen zu ermöglichen.

38. Isolierte T-Zellen-Population, die für ein Brusttumor-Protein gemäß Anspruch 1 spezifisch ist, umfassend T-Zellen, die gemäß dem Verfahren gemäß Anspruch 37 erhältlich sind.

39. T-Zellen-Population gemäß Anspruch 38 zur Verwendung bei einem Verfahren zum Hemmen der Entwicklung eines Brustkrebses bei einem Patienten.

40. Proliferierte CD4+ und/oder CD8+ T-Zellen, die aus einem Patienten isoliert sind, mit wenigstens einer Komponente, ausgewählt aus der Gruppe, bestehend aus:

(i) einem Polypeptid gemäß Anspruch 1;
(ii) einen Polynucleotid, das ein derartiges Polypeptid codiert; oder
(iii) einer Antigen-präsentierenden Zelle, die ein derartiges Polypeptid exprimiert und derartig inkubiert ist, dass T-Zellen proliferieren, zur Verwendung bei einem Verfahren zum Hemmen der Entwicklung eines Brustkrebses bei einem Patienten.

41. T-Zellen gemäß Anspruch 40 zur Verwendung bei einem Verfahren zum Hemmen der Entwicklung eines Krebses bei einem Patienten, umfassend die Schritte:

(a) Klonieren von wenigstens einer proliferierten Zelle; und
(b) Verabreichen einer wirksamen Menge der klonierten T-Zellen an den Patienten und **dadurch** Hemmen der Entwicklung eines Brustkrebses bei dem Patienten.

42. Verfahren zum Bestimmen des Vorhandenseins oder Fehlens eines Brustkrebses bei einem Patienten, umfassend die Schritte:

(a) Kontaktieren einer von einem Patienten erhaltenen biologischen Probe mit einem Antikörper oder einem Antigen-bindenden Fragment davon, der/das spezifisch an den Teil eines Brusttumor-Proteins bindet, der von der in SEQ ID NO: 175 genannten Polynucleotidsequenz codiert wird; und
(b) Nachweisen der Menge an Polypeptid, das spezifisch an den Antikörper und/oder ein Antigen-bindendes Fragment davon bindet, in der Probe, Vergleichen der Menge an Polypeptid mit einem vorbestimmten Grenzwert

und daraus Bestimmen des Vorhandenseins oder Fehlens eines Brustkrebses bei dem Patienten.

43. Verfahren zum Überwachen des Fortschreitens eines Brustkrebses bei einem Patienten, umfassend die Schritte:

(a) Kontaktieren einer biologischen Probe, die von einem Patienten zu einem ersten Zeitpunkt erhalten wurde, mit einem Antikörper oder Antigen-bindenden Fragment davon, der/das spezifisch an den Teil eines Brusttumor-Proteins bindet, der von der in SEQ ID NO: 175 genannten Polynucleotidsequenz codiert wird;
(b) Nachweisen der Menge an Polypeptid, das spezifisch an den Antikörper oder das Antigen-bindende Fragment davon bindet, in der Probe;
(c) Wiederholen der Schritte (a) und (b) unter Verwendung einer biologischen Probe, die zu einem späteren Zeitpunkt von dem Patienten erhalten wurde; und
(d) Vergleichen der Menge an Polypeptid, die in Schritt (c) nachgewiesen wurde, mit der in Schritt (b) nachgewiesenen Menge und **dadurch** Überwachen des Fortschreitens des Brustkrebses bei dem Patienten.

44. Verfahren gemäß Anspruch 42 oder 43, wobei der Antikörper ein monoklonaler Antikörper ist.

45. Verfahren zum Bestimmen des Vorhandenseins oder Fehlens eines Brustkrebses bei einem Patienten, umfassend die Schritte:

(a) Kontaktieren einer von einem Patienten erhaltenen biologischen Probe mit einem Oligonucleotid, das mit einem Polynucleotid hybridisiert, welches ein Brusttumor-Protein codiert, wobei das Tumorprotein eine Aminosäuresequenz umfasst, die von der in SEQ ID NO: 175 genannten Polynucleotidsequenz codiert wird;
(b) Nachweisen der Menge an Polynucleotid, das mit dem Oligonucleotid hybridisiert, in der Probe; und
(c) Vergleichen der Menge an Polynucleotid, das mit dem Oligonucleotid hybridisiert, mit einem vorbestimmten Grenzwert und daraus Bestimmen des Vorhandenseins oder Fehlens eines Brustkrebses bei dem Patienten.

46. Verfahren zum Überwachen des Fortschreitens eines Brustkrebses bei einem Patienten, umfassend die Schritte:

(a) Kontaktieren einer biologischen Probe, die von einem Patienten erhalten wurde, mit einem Oligonucleotid, das mit einem Polynucleotid hybridisiert, welches ein Brusttumor-Protein codiert, wobei das Tumorprotein eine Aminosäuresequenz umfasst, die von der in SEQ ID NO: 175 genannten Polynucleotidsequenz codiert wird;
(b) Nachweisen der Menge eines Polynucleotids, das mit dem Oligonucleotid hybridisiert, in der Probe;
(c) Wiederholen der Schritte (a) und (b) unter Verwendung einer biologischen Probe, die zu einem späteren Zeitpunkt von dem Patienten erhalten wurde; und
(d) Vergleichen der Menge an Polynucleotid, die in Schritt (c) nachgewiesen wurde, mit der in Schritt (b) nachgewiesenen Menge und **dadurch** Überwachen des Fortschreitens des Brustkrebses bei dem Patienten.

47. Verfahren gemäß Anspruch 45 oder 46, wobei die Menge an Polynucleotid, die mit dem Oligonucleotid hybridisiert, unter Verwendung einer Polymerase-Kettenreaktion nachgewiesen wird.

48. Verfahren gemäß Anspruch 45 oder 46, wobei die Menge an Polynucleotid, die mit dem Oligonucleotid hybridisiert, unter Verwendung eines Hybridisierungsassays nachgewiesen wird.

49. Diagnostisches Kit, umfassend:

(a) einen oder mehrere Antikörper gemäß Anspruch 17; und
(b) ein Nachweisreagens, das eine Reportergruppe umfasst.

50. Kit gemäß Anspruch 49, wobei die Antikörper auf einem festen Träger immobilisiert sind.

51. Kit gemäß Anspruch 50, wobei der feste Träger Nitrocellulose, Latex oder ein Kunststoffmaterial umfasst.

52. Kit gemäß Anspruch 49, wobei das Nachweisreagens ein Antiimmunoglobulin, Protein G, Protein A oder Lectin umfasst.

53. Kit gemäß Anspruch 49, wobei die Reportergruppe ausgewählt ist aus der Gruppe, bestehend aus Radioisotopen, Fluoreszenzgruppen, Lumineszenzgruppen, Enzymen, Biotin und Farbstoffteilchen.

54. Oligonucleotid, umfassend 10 bis 40 zusammenhängende Nucleotide eines Polynucleotids, das ein Brusttumor-Protein codiert, wobei das Tumorprotein eine Aminosäuresequenz umfasst, die von der in SEQ ID NO: 175 genannten Polynucleotidsequenz codiert wird.

5

55. Oligonucleotid gemäß Anspruch 54, wobei das Oligonucleotid 10-40 zusammenhängende, in SEQ ID NO: 175 genannte Nucleotide umfasst.

56. Diagnostisches Kit, umfassend:

10

(a) ein Oligonucleotid gemäß Anspruch 54; und
(b) ein diagnostisches Reagens zur Verwendung bei einer Polymerase-Kettenreaktion oder einem Hybridisierungsassay.

15

Revendications

1. Polypeptide isolé comprenant au moins une partie d'une protéine de tumeur du sein, le polypeptide comprenant une séquence d'acides aminés qui est codée par la séquence polynucléotidique présentée dans SEQ ID N° 175, ou variant dudit polypeptide, le variant comprenant une séquence d'acides aminés qui est codée par une séquence polynucléotidique qui a une identité d' au moins 90 % avec une séquence SEQ ID N° 175, et la capacité du variant à réagir avec des antisérums spécifiques d'antigènes étant renforcée, inchangée ou diminuée de moins de 50 % par rapport à un polypeptide contenant une séquence d'acides aminés qui est codée par SEQ ID N° 175, du moment que le polypeptide n'a pas la séquence présentée dans SEQ ID N° 176, avec le remplacement de Val Ile par Asn Ser sur les positions 316 et 317.

20

25

2. Polypeptide isolé selon la revendication 1, le polypeptide étant un polypeptide variant comprenant une séquence d'acides aminés qui est codée par un polynucléotide ayant une identité d'au moins 90 % avec la séquence SEQ ID N° 175, et la capacité du variant à réagir avec des antisérums spécifiques d'antigène étant diminuée de moins de 20 % par rapport à un polypeptide comprenant une séquence d'acides aminés qui est codée par SEQ ID N° 175.

30

3. Polypeptide isolé selon la revendication 1, le polypeptide comprenant une séquence d'acides aminés qui est codée par la séquence polynucléotidique présentée dans SEQ ID N° 175.

4. Polypeptide isolé comprenant la séquence SEQ ID N° 176.

35

5. Polynucléotide isolé codant pour un polypeptide selon la revendication 1.

6. Polynucléotide isolé codant pour une protéine de tumeur du sein, la protéine de tumeur comprenant une séquence d'acides aminés qui est codée par un polynucléotide comprenant la séquence présentée dans SEQ ID N° 175.

40

7. Polynucléotide isolé comprenant une séquence présentée dans SEQ ID N° 175.

8. Polynucléotide isolé comprenant une séquence qui a une identité d'au moins 90 % avec la séquence présentée dans SEQ ID N° 175, et qui code pour un polypeptide ayant la capacité de réagir avec des antisérums spécifiques d'antigène, qui est renforcée, inchangée ou diminuée d'au moins 50 % par rapport à un polypeptide comprenant une séquence d'acides aminés qui est codée par SEQ ID N° 175.

45

9. Polynucléotide isolé selon la revendication 8, dans lequel le polypeptide codé par ledit polynucléotide a la capacité de réagir avec des antisérums spécifiques d'antigène, qui est diminuée de moins de 20 % par rapport à un polypeptide comprenant une séquence d'acides aminés qui est codée par SEQ ID N° 175.

50

10. Polynucléotide isolé, complémentaire d'un polynucléotide selon l'une quelconque des revendications 5 à 9.

11. Vecteur d'expression comprenant un polynucléotide selon l'une quelconque des revendications 5 à 9.

55

12. Cellule hôte transformée ou transfectée avec un vecteur d'expression selon la revendication 11.

13. Vecteur d'expression comprenant un polynucléotide selon la revendication 10.

14. Cellule hôte transformée ou transfectée avec un vecteur d'expression selon la revendication 13.

15. Vaccin comprenant un polypeptide selon la revendication 1, en combinaison avec un immunostimulant.

16. Vaccin comprenant un polynucléotide selon la revendication 5, en combinaison avec un immunostimulant.

17. Anticorps isolé, ou fragment de liaison à l'antigène de ce dernier, qui se lie d'une manière spécifique à la partie d'une protéine de tumeur du sein qui comprend une séquence d'acides aminés qui est codée par la séquence polynucléotidique présentée dans SEQ ID N° 175.

18. Vaccin comprenant une cellule présentant l'antigène, qui exprime un polypeptide selon la revendication 1, en combinaison avec un immunostimulant.

19. Vaccin selon la revendication 18, dans lequel la cellule présentant l'antigène est une cellule dendritique.

20. Polypeptide selon la revendication 1, pour utilisation dans un procédé pour inhiber le développement d'un cancer du sein chez un patient.

21. Polynucléotide selon la revendication 5, pour utilisation dans un procédé pour inhiber le développement d'un cancer du sein chez un patient.

22. Anticorps ou fragment de liaison à l'antigène de ce dernier de la revendication 17, pour utilisation dans un procédé pour inhiber le développement d'un cancer du sein chez un patient.

23. Cellule présentant l'antigène, qui exprime un polypeptide selon la revendication 1, pour utilisation dans un procédé pour inhiber le développement d'un cancer du sein chez un patient.

24. Cellule selon la revendication 23, où la cellule présentant l'antigène est une cellule dendritique.

25. Protéine de fusion comprenant au moins un polypeptide selon la revendication 1.

26. Protéine de fusion selon la revendication 25, la protéine de fusion comprenant un amplificateur d'expression qui augmente l'expression de la protéine de fusion dans une cellule hôte transfectée avec un polynucléotide codant pour la protéine de fusion.

27. Protéine de fusion selon la revendication 26, la protéine de fusion comprenant un épitope T auxiliaire qui n'est pas présent à l'intérieur du polypeptide de la revendication 1.

28. Protéine de fusion selon la revendication 26, la protéine de fusion comprenant une étiquette d'affinité.

29. Polynucléotide isolé codant pour une protéine de fusion selon la revendication 25.

30. Vaccin comprenant une protéine de fusion selon la revendication 25, en combinaison avec un immunostimulant.

31. Vaccin comprenant un polynucléotide selon la revendication 29, en combinaison avec un immunostimulant.

32. Vaccin selon la revendication 15, 16, 18, 30 ou 31, dans lequel l'immunostimulant est un adjuvant.

33. Vaccin selon la revendication 15, 16, 18, 30 ou 31, dans lequel l'immunostimulant induit une réponse essentiellement de type I.

34. Vaccin selon la revendication 30 ou 31, pour utilisation dans un procédé pour inhiber le développement d'un cancer du sein chez un patient.

35. Procédé pour éliminer des cellules tumorales d'un échantillon biologique, comprenant la mise en contact d'un échantillon biologique avec des cellules T qui réagissent d'une manière spécifique avec une protéine de tumeur du sein, où la protéine de tumeur comprend une séquence d'acides aminés qui est codée par la séquence polynucléotidique présentée dans SEQ ID N° 175, l'étape de mise en contact étant mise en oeuvre dans des conditions et

pendant un laps de temps suffisants pour permettre l'élimination, à partir de l'échantillon, de cellules exprimant l'antigène.

36. Procédé selon la revendication 35, dans lequel l'échantillon biologique est le sang ou une fraction de ce dernier.

37. Procédé pour stimuler et/ou provoquer l'expansion de cellules T spécifiques d'une protéine de tumeur du sein, mis en oeuvre *ex vivo,* comprenant la mise en contact de cellules T avec un ou plusieurs

(i) d'un polypeptide selon la revendication 1 ;
(ii) d'un polynucléotide codant pour un tel polypeptide ; et/ou
(iii) d'une cellule présentant l'antigène, qui exprime un tel polypeptide ;

dans des conditions et pendant un laps de temps suffisants pour permettre la stimulation et/ou l'expansion des cellules T.

38. Population de cellules T isolées spécifiques d'une protéine de tumeur du sein selon la revendication 1, comprenant des cellules T pouvant être obtenues par le procédé de la revendication 37.

39. Population de cellules T selon la revendication 38, pour utilisation dans un procédé pour inhiber le développement d'un cancer du sein chez un patient.

40. Cellules T CD4+ et/ou CD8+ que l'on a fait proliférer, isolées d'un patient avec au moins un composant choisi dans le groupe consistant eu

(i) un polypeptide selon la revendication 1 ;
(ii) un polynucléotide codant pour un tel polypeptide ; ou
(iii) une cellule présentant l'antigène, qui exprime un tel polypeptide, et incubée de telle sorte que des cellules T prolifèrent, pour utilisation dans un procédé pour inhiber le développement d'un cancer du sein chez un patient.

41. Cellules T selon la revendication 40, pour utilisation dans un procédé pour inhiber le développement d'un cancer chez un patient, comprenant les étapes de :

(a) clonage d'au moins une cellule que l'on a fait proliférer ; et
(b) administration au patient d'une quantité efficace des cellules T clonées, de façon à inhiber le développement d'un cancer du sein chez le patient.

42. Procédé pour déterminer la présence ou l'absence d'un cancer du sein chez un patient, comprenant les étapes de:

(a) mise en contact d'un échantillon biologique obtenu d'un patient avec un anticorps ou un fragment de liaison à l'antigène de ce dernier, qui se lie d'une manière spécifique à la partie d'une protéine de tumeur du sein qui est codée par la séquence polynucléotidique présentée dans SEQ ID N° 175 ; et
(b) détection dans l'échantillon d'une quantité du polypeptide qui se lie d'une manière spécifique à l'anticorps et/ou à un fragment de liaison à l'antigène de ce dernier, comparaison de la quantité du polypeptide avec une valeur de coupure prédéterminée, et, à partir de là, détermination de la présence ou de l'absence d'un cancer du sein chez le patient.

43. Procédé pour surveiller la progression d'un cancer du sein chez un patient, comprenant les étapes de :

(a) mise en contact d'un échantillon biologique obtenu d'un patient, à un premier instant, avec un anticorps ou un fragment de liaison à l'antigène de ce dernier, qui se lie d'une manière spécifique à la partie d'une protéine de tumeur du sein qui est codée par la séquence polynucléotidique présentée dans SEQ ID N° 175 ;
(b) détection dans l'échantillon d'une quantité du polypeptide qui se lie d'une manière spécifique à l'anticorps ou à un fragment de liaison à l'antigène de ce dernier ;
(c) répétition des étapes (a) et (b) par utilisation d'un échantillon biologique obtenu d'un patient à un instant ultérieur ; et
(d) comparaison de la quantité de polypeptide détectée dans l'étape (c) à la quantité détectée dans l'étape (b) et, à partir de là, surveillance de la progression du cancer du sein chez le patient.

44. Procédé selon la revendication 42 ou 43, dans lequel l'anticorps est un anticorps monoclonal.

45. Procédé pour déterminer la présence ou l'absence d'un cancer du sein chez un patient, comprenant les étapes de :

5

(a) mise en contact d'un échantillon biologique obtenu d'un patient avec un oligonucléotide qui s'hybride à un polynucléotide qui code pour une protéine de tumeur du sein, la protéine de tumeur comprenant une séquence d'acides aminés qui est codée par la séquence polynucléotidique présentée dans SEQ ID N° 175 ;
(b) détection dans l'échantillon d'une quantité d'un polynucléotide qui s'hybride à l'oligonucléotide ; et
(c) comparaison de la quantité du polynucléotide qui s'hybride à l'oligonucléotide à une valeur de coupure prédéterminée et, à partir de là, détermination de la présence ou de l'absence d'un cancer du sein chez le patient.

10

46. Procédé pour surveiller la progression d'un cancer du sein chez un patient, comprenant les étapes de :

15

(a) mise en contact d'un échantillon biologique obtenu d'un patient avec un oligonucléotide qui s'hybride à un polynucléotide qui code pour une protéine de tumeur du sein, la protéine de tumeur comprenant une séquence d'acides aminés qui est codée par la séquence polynucléotidique présentée dans SEQ ID N° 175 ;
(b) détection dans l'échantillon d'une quantité d'un polynucléotide qui s'hybride à l'oligonucléotide ;
(c) répétition des étapes (a) et (b) par utilisation d'un échantillon biologique obtenu du patient à un instant ultérieur ; et
(d) comparaison de la quantité d'un polynucléotide détecté dans l'étape (c) à la quantité détectée dans l'étape (b) et, à partir de là, surveillance de la progression du cancer du sein chez le patient.

20

47. Procédé selon la revendication 45 ou 46, dans lequel la quantité du polynucléotide qui s'hybride à l'oligonucléotide est déterminée par utilisation d'une réaction en chaîne par polymérase.

25

48. Procédé selon la revendication 45 ou 46, dans lequel la quantité du polynucléotide qui s'hybride à l'oligonucléotide est déterminée par utilisation d'un essai par hybridation.

49. Trousse de diagnostic, comprenant :

30

(a) un ou plusieurs anticorps selon la revendication 17 ; et
(b) un réactif de détection comprenant un groupe rapporteur.

50. Trousse selon la revendication 49, dans laquelle les anticorps sont immobilisés sur un support solide.

35

51. Trousse selon la revendication 50, dans laquelle le support solide comprend de la nitrocellulose, un latex ou un matériau plastique.

52. Trousse selon la revendication 49, dans laquelle le réactif de détection comprend une anti-immunoglobuline, une protéine G, une protéine A ou une lectine.

40

53. Trousse selon la revendication 49, dans laquelle le groupe rapporteur est choisi dans le groupe consistant en les radio-isotopes, les groupes fluorescents, les groupes luminescents, les enzymes, la biotine et les particules de colorant.

45

54. Oligonucléotide comprenant 10 à 40 nucléotides contigus d'un polynucléotide qui code pour une protéine de tumeur du sein, où la protéine de tumeur comprend une séquence d'acides aminés qui est codée par la séquence polynucléotidique présentée dans SEQ ID N° 175.

50

55. Oligonucléotide selon la revendication 54, l'oligonucléotide comprenant 10 à 40 nucléotides contigus présentés dans SEQ ID N° 175.

56. Trousse de diagnostic, comprenant :

55

(a) un oligonucléotide selon la revendication 54 ; et
(b) un réactif de diagnostic pour utilisation dans une réaction en chaîne par polymérase ou dans un essai par hybridation.

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- EP 1144449 A **[0004]**
- WO 9638591 A **[0040]**
- US 4935233 A **[0061]**
- US 4751180 A **[0061]**
- WO 9118926 A **[0064]**
- US 4671958 A, Rodwell **[0076] [0077]**
- US 4489710 A, Spitler **[0077]**
- US 4625014 A, Senter **[0077]**
- US 4638045 A, Kohn **[0077]**
- US 4569789 A, Blattler **[0077]**
- US 4507234 A, Kato **[0079]**
- US 4699784 A, Shih **[0079]**
- US 4429008 A **[0079]**
- US 4873088 A **[0079]**
- US 4735792 A **[0079]**
- US 4673562 A, Davison **[0079]**
- US 5240856 A **[0081]**
- US 5215926 A **[0081]**
- WO 8906280 A **[0081]**
- WO 9116116 A **[0081]**
- WO 9207243 A **[0081]**
- US 4235877 A, Fullerton **[0085]**
- US 4603112 A **[0086]**
- US 4769330 A **[0086]**
- US 5017487 A **[0086]**
- WO 8901973 A **[0086]**
- US 4777127 A **[0086]**
- GB 2200651 A **[0086]**
- EP 0345242 A **[0086]**
- WO 9102805 A **[0086]**
- US 4897268 A **[0087]**
- US 5075109 A **[0087]**
- US 4436727 A **[0091]**
- US 4877611 A **[0091]**
- US 4866034 A **[0091]**
- US 4912094 A **[0091]**
- WO 9602555 A **[0091]**
- WO 9400153 A **[0091]**
- WO 9633739 A **[0091]**
- WO 9517210 A **[0091]**
- WO 9724447 A **[0097]**
- US 4918164 A **[0100]**
- US 5359681 A **[0108]**
- US 20000215 B **[0133]**

Non-patent literature cited in the description

- **Porter-Jordan ; Lippman.** *Breast Cancer,* 1994, vol. 8, 73-100 **[0003]**
- A model of evolutionary change in proteins - Matrices for detecting distant relationships. **Dayhoff, M.O.** Atlas of Protein Sequence and Structure. National Biomedical Research Foundation, 1978, vol. 5, 345-358 **[0032]**
- Unified Approach to Alignment and Phylogenes. **Hein J.** Methods in Enzymology. Academic Press, Inc, 1990, vol. 183, 626-645 **[0032]**
- **Higgins, D.G. ; Sharp, P.M.** *CABIOS,* 1989, vol. 5, 151-153 **[0032]**
- **Myers, E.W. ; Muller W.** *CABIOS,* 1988, vol. 4, 11-17 **[0032]**
- **Robinson, E.D.** *Comb. Theor,* 1971, vol. 11, 105 **[0032]**
- **Santou, N. ; Nes, M.** *Mol. Biol. Evol.,* 1987, vol. 4, 406-425 **[0032]**
- **Sneath. P.H.A. ; Sokal, R.R.** Numerical Taxonomy - the Principles and Practice of Numerical Taxonomy. Freeman Press, 1973 **[0032]**
- **Wilbur, W.J. ; Lipman. D.J.** *Proc. Natl. Acad. Sci. USA,* 1983, vol. 80, 726-730 **[0032]**
- **Schena et al.** *Proc. Natl. Acad Sci. USA,* 1996, vol. 93, 10614-10619 **[0036]**
- **Heller et al.** *Proc. Natl. Acad Sci. USA,* 1997, vol. 94, 2150-2155 **[0036]**
- **Sambrook et al.** Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratories, 1989 **[0038]**
- **Triglia et al.** *Nucl. Acids Res.,* 1988, vol. 16, 8186 **[0040]**
- **Lagerstrom et al.** *PCR Methods Applic.,* 1991, vol. 1, 111-19 **[0040]**
- **Parker et al.** *Nucl. Acids. Res.,* 1991, vol. 19, 3055-60 **[0040]**
- **Adelman et al.** *DNA,* 1983, vol. 2, 183 **[0043]**
- **Gee et al.** Molecular and Immunologic Approaches. Futura Publishing Co, 1994 **[0044]**
- **Paul.** Fundamental Immunology. Raven Press, 1993, 243-247 **[0052]**
- **Harlow ; Lane.** Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory, 1988 **[0052] [0069] [0072] [0106]**
- **Merrifield.** *J. Am. Chem. Soc.,* 1963, vol. 85, 2149-2146 **[0058]**

- **Maratea et al.** *Gene,* 1985, vol. 40, 39-46 **[0061]**
- **Murphy et al.** *Proc. Natl. Acad Sci. USA,* 1986, vol. 83, 8258-8262 **[0061]**
- **Stoute et al.** *New Engl. J. Med.,* 1997, vol. 336, 86-91 **[0063]**
- *Gene,* 1986, vol. 43, 265-292 **[0065]**
- *Biotechnology,* 1992, vol. 10, 795-798 **[0065]**
- **Kohler ; Milstein.** *Eur. J. Immunol.,* 1976, vol. 6, 511-519 **[0070]**
- **Chen et al.** *Cancer Res.,* 1994, vol. 54, 1065-1070 **[0083]**
- **Coligan et al.** Current Protocols in Immunology. Wiley Interscience, 1998, vol. 1 **[0083]**
- Vaccine Design (the subunit and adjuvant approach). Plenum Press, 1995 **[0085]**
- **Rolland.** *Crit. Rev. Therap. Drug Carrier Systems,* 1998, vol. 15, 143-198 **[0086]**
- **Fisher-Hoch et al.** *Proc. Natl. Acad. Sci. USA,* 1989, vol. 86, 317-321 **[0086]**
- **Flexner et al.** *Ann. N.Y. Acad. Sci.,* 1989, vol. 569, 86-103 **[0086]**
- **Flexner et al.** *Vaccine,* 1990, vol. 8, 17-21 **[0086]**
- **Berkner.** *Biotechniques,* 1988, vol. 6, 616-627 **[0086]**
- **Rosenfeld et al.** *Science,* 1991, vol. 252, 431-434 **[0086]**
- **Kolls et al.** *Proc. Natl. Acad. Sci. USA,* 1994, vol. 91, 215-219 **[0086]**
- **Kass-Eisler et al.** *Proc. Natl. Acad. Sci. USA,* 1993, vol. 90, 11498-11502 **[0086]**
- **Guzman et al.** *Circulation,* 1993, vol. 88, 2838-2848 **[0086]**
- **Guzman et al.** *Cir. Res.,* 1993, vol. 73, 1202-1207 **[0086]**
- **Ulmer et al.** *Science,* 1993, vol. 259, 1745-1749 **[0086]**
- **Cohen.** *Science,* 1993, vol. 259, 1691-1692 **[0086]**
- **Mosmann ; Coffman.** *Ann. Rev. Immunol.,* 1989, vol. 7, 145-173 **[0090]**
- **Banchereau ; Steinman.** *Nature,* 1998, vol. 392, 245-251 **[0094]**
- **Timmerman ; Levy.** *Ann. Rev. Med.,* 1999, vol. 50, 507-529 **[0094]**
- **Zitvogel et al.** *Nature Med,* 1998, vol. 4, 594-600 **[0094]**
- **Mahvi et al.** *Immunology and cell Biology,* 1997, vol. 75, 456-460 **[0097]**
- **Cheever et al.** *Immunological Reviews,* 1997, vol. 157, 177 **[0101]**
- **Pierce.** ImmunotechnologyCatalog and Handbook. 1991, A12-A13 **[0109]**
- **Sackett et al.** Clinical Epidemiology: A Basic Science for Clinical Medicine. Little Brown and Co, 1985, 106-7 **[0114]**
- **Mullis et al.** *Cold Spring Harbor Symp. Quant. Biol.,* 1987, vol. 51, 263 **[0119]**
- **Erlich.** PCR Technology. Stockton Press, 1989 **[0119]**

patsnap

专利名称(译)	用于治疗和诊断乳腺癌的组合物及其使用方法		
公开(公告)号	EP1183348B1	公开(公告)日	2011-06-29
申请号	EP2000919352	申请日	2000-02-15
[标]申请(专利权)人(译)	科里克萨有限公司		
申请(专利权)人(译)	Corixa公司CORPORATION		
当前申请(专利权)人(译)	Corixa公司CORPORATION		
[标]发明人	YUQIU JIANG DILLON DAVIN C MITCHAM JENNIFER LYNN XU JIANGCHUN HARLOCKER SUSAN L		
发明人	YUQIU, JIANG DILLON, DAVIN, C. MITCHAM, JENNIFER, LYNN XU, JIANGCHUN HARLOCKER, SUSAN, L.		
IPC分类号	C12N15/12 C12N15/52 C07K14/47 C12N9/00 C12N5/10 C12N1/21 A61K38/17 A61K39/00 C07K16/30 C07K16/32 C07K16/40 A61K39/395 A61K35/12 C07K19/00 C12N15/62 C12N5/00 G01N33/68 C12Q1/68 C12N15/11 G01N33/53 A61K35/14 A61K35/76 A61K38/00 A61K39/39 A61K48/00 A61P35/00 C07K14/82 C12N1/15 C12N1/19 C12N15/09 G01N33/566 G01N33/577		
CPC分类号	C07K14/47 A61K35/12 A61K38/00 A61K39/00 C07K2319/00 C12N9/00 C12Q1/6886 C12Q2600/158		
优先权	09/285480 1999-04-02 US 09/389681 1999-09-02 US 09/433826 1999-11-03 US 09/339338 1999-06-23 US		
其他公开文献	EP1183348A2		
外部链接	Espacenet		

摘要(译)

公开了用于治疗和诊断癌症(例如乳腺癌)的组合物和方法。组合物可包含一种或多种乳腺肿瘤蛋白,其免疫原性部分或编码这些部分的多核苷酸。或者,治疗组合物可包含表达乳腺肿瘤蛋白的抗原呈递细胞,或对表达这种蛋白的细胞特异的T细胞。此类组合物可用于例如预防和治疗诸如乳腺癌的疾病。还提供了基于在样品中检测乳腺肿瘤蛋白或编码这种蛋白的mRNA的诊断方法。


```
gttgcagtga gctcaagtgt tgggtgtatc agctcaaaac accatgtgat gccaatcatc    60
tccacaggag caatttgttt accttttttt tctgatgctt tactaacttc atcttttaga   120
tttaaatcat tagtagatcc tagaggagcc agtttcagaa aatatagatt ctagttcagc   180
accacccgta gttgtgcatt gaaataatta tcattatgat tatgtatcag agcttctggt   240
tttctcattc tttattcatt tattcaacaa ccacgtgaca aacactggna ttacaggatg   300
aagatgagat aatccgctcc ttggcagtgt tatactatta tataacctga aaaaacaaac   360
aggtaatttt cacacaaagt aatagatatc atgacacatt taaaataggg cactactgga   420
acacacagat aggacatcca ggttttgggt caatattgta gactttttgg tggatgagat   480
atgcaggttg atrccagaag gacaacaaaa acatatgtca gatagaaggg aggagcaaat   540
gccaagagct ggagctgagg aagatcactg tgaaattcta tgtagtctag ttggctggat   600
gctagagcaa agaggtgg                                                 618
```